A structural basis for the aortic stress–strain relation in uniaxial tension

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Abstract

A constitutive law that includes three analytical expressions was recently proposed to approximate the low, physiologic, and high-stress parts of the aortic stress–strain relation in uniaxial tension, consistent with the biphasic nature of the aortic wall under passive conditions. This consistency, and the fact that previous phenomenological uniaxial laws have only indirectly been related to vessel wall structure, motivates the investigation of the structural basis underlying the newly proposed three-part constitutive law. For this purpose, longitudinally oriented aortic strips were fixed in Karnovsky’s solution, while subjected to various pre-selected levels of uniaxial tensile stress. Light microscopy examination disclosed that the elastic lamellae gradually unfolded at low and were almost straight at physiologic and high stresses, while collagen fibers reoriented in the longitudinal axis at low, started uncoiling at physiologic, and straightened massively at high stresses. In the circumferential sections, the elastic lamellae and the circumferentially distributed collagen bundles remained wavy at all levels of longitudinally applied stress. These microstructural changes suggest that elastin becomes load-bearing at low, and collagen at physiologic but mostly at high stresses, so that the first and third parts of the constitutive law are in turn due to the presence of elastin and collagen alone, and the second due to both elastin and collagen. The structural basis of this constitutive law allows physically significant interpretation of its parameters, offering insight into how the aortic microstructure determines the macromechanical response.

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1. Introduction

The aorta does not serve only a conduit function, but, through its mechanical properties, plays important roles moderating pressure and flow in the entire cardiovascular system (Boudoulas and Wooley, 1996; Nichols and O’Rourke, 1990). It is a large-caliber vessel, whose mechanical response is predominantly attributed to the medial layer, because this is the thickest layer in cross-section and the most organized one. Previous studies (Wolinsky and Glagov, 1964, 1967; Clark and Glagov, 1979, 1985) have shown that the aortic media contains concentric cylindrical lamellae, which serve as building blocks of the aortic wall. These include two fenestrated sheets of elastin (Roach, 1983; Song and Roach, 1983, 1985; Roach and Song, 1988), separated by vascular smooth muscle cells, collagen, and ground substance. Elastin and collagen are the principal components to determine the passive mechanical properties of the

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aortic wall (Roach and Burton, 1957; Glagov and Wolinsky, 1963; Cox, 1978), whereas smooth muscle cells are responsible for the active mechanical properties and the production of extracellular matrix (Dobrin and Rovick, 1969; Cox, 1976).

It is well appreciated today that the aorta displays a nonlinear stress–strain response of increasing stiffness. Recently, a phenomenological constitutive law, consisting of three analytical functions, was proposed (Sokolis et al., 2002) to describe the low, physiologic, and high-stress parts of this response upon uniaxial tension. It is still rewarding to know how the three-part shape of the stress–strain response is influenced by the interaction of different passive tissue components, because only then can physical interpretation to the constitutive parameters be given; the more so, since previous uniaxial constitutive laws in the arterial mechanics literature have only indirectly been related to the aortic wall structure.

Accordingly, the aim of the present study was the investigation of the structural basis underlying the aortic stress–strain response in uniaxial tension. Our attention was restricted to the newly proposed three-part constitutive law, still the structural basis presented here may also be applicable to previous phenomenological laws, presumably allowing physically based interpretation of their parameters. Further, it offers insight into how the aortic microstructure determines the macromechanical response, and may be employed for the improvement of existing structural and phenomenological laws that will embody detailed considerations of the aortic wall composition and architecture.

In particular, this study was concerned with the sequence of changes occurring in the network of elastic lamellae and collagen fibers of the medial layer in response to progressive stressing, and with showing how these changes relate to the three parts of the recently proposed constitutive law. It was found that during the first part of relatively low aortic stiffness at low stresses, the elastic lamellae straighten and collagen fibers become aligned along the stress axis, whereas during the second part of increased stiffness at physiologic stresses, the elastic lamellae are straight and the realigned collagen fibers start to straighten. In the third part referring to high stresses and characterized by very high stiffness, the elastic lamellae are straight and compacted, and collagen fibers straighten massively. The parameters of the three-part constitutive law, thus, admit direct physical interpretation; those associated with the first part stand as microstructural indices of the state, content, and elastic modulus of elastin, those associated with the third part as the respective indices of collagen, and those associated with the second part as indices of both elastin and collagen.

2. Materials and methods

2.1. Surgical procedures and specimen preparation

Thirty-five healthy white male New Zealand rabbits weighing 3.450–3.750 kg were studied according to the guiding principles of the American Physiological Society and the Greek Presidential Decree 160/1991, issued after the European Union Directive 609/1986. The animals were sacrificed with an intravenous overdose of sodium thiopental. The thoracic cavity was entered through a median sternotomy, and the aorta was recognized. A segment of the vessel between the second and sixth pair of intercostals was isolated with two ligatures. The aortic segment including the ligatures was then resected, and excess adipose tissue was removed up to the adventitia. During subsequent histological examination of the vessels, the adventitial layer was apparent in all stained sections.

The length of the descending thoracic aorta between the two ligatures was measured with a micrometer before and after excision, and the in situ stretch $l_{\text{in situ}}$ was calculated as the ratio of in situ to excised length (Han and Fung, 1995). The aortic segment was opened with an incision between the intercostal arteries, and parallel-sided specimens 3.1 cm long by 0.5 cm wide were obtained along the longitudinal direction of the vessel. These were transferred to physiologic saline at room temperature (22°C), until the initiation of mechanical testing.

2.2. Uniaxial tension

Uniaxial tension of the aortic specimens was performed in the direction of their long axis using a Vitrodyne V1000 Universal Tester (Liveco Inc, Burlington, VT, USA) within 2 h from excision, as previously described (Sokolis et al., 2002; Angouras et al., 2000). The strips were held in the grips of the apparatus, lined with sandpaper to prevent slippage, and immersed in a saline bath at 37°C. The lower grip remained firm, while the upper one was attached to the actuator that stretched the strips to maximum extension at a quasi-static rate of 10 μm/s, then released them to the original position. To reduce hysteresis and acquire steady-state data (Bergel, 1961; Fung, 1967, 1973), the tissue was preconditioned by subjecting it to 10 cycles of loading up to maximum extension and unloading to zero. The tensile load versus extension measurements from the loading phase of the ninth cycle did not differ significantly from those of the tenth cycle. The latter were recorded with a sampling frequency of 50 Hz, and used in the analysis of data. They were assumed to represent the mechanical properties of the aortic tissue under negligible smooth muscle tone (Cox, 1978).
Data analysis involved the assessment of tensile stress, strain, and elastic modulus (Fung, 1990; Humphrey, 2002). Lagrangian stress $T$ was determined as the ratio of applied load to the original width and thickness of the specimens under zero load. Strain $\varepsilon$ was calculated by subtracting unity from the stretch ratio, the latter being the deformed length of the specimens divided by their original length at zero load. Elastic modulus $M$ was defined as the tangent modulus, namely as the first derivative of stress with respect to strain.

For the evaluation of stress and strain, the strips at zero load were considered to be in the zero stress state (Vaishnav and Vossoughi, 1987; Fung and Liu, 1989), their original dimensions measured when tensile load began to register after preconditioning. Their original length was determined by measuring with the tensile-testing device the distance between the grips, and their original width and thickness by reading via a laser scan micrometer (LS-3100, Keyence Corp, Osaka, Japan), with resolution of 1 $\mu$m, the width and thickness at four equidistant locations along their length and averaging those values.

### 2.3. Fixation under stress

Following preconditioning, the aortic specimens were extended at 10 $\mu$m/s to discrete strain levels, corresponding to successive points on the stress–strain curve. At a desired level, the actuator was stopped, and the specimen was allowed to stress relax for 15 min. Grips and specimen were then immersed in Karnovsky’s solution (3% glutaraldehyde and 1% formaldehyde in phosphate buffer 0.1 M, pH 7.4) to preserve the stressed state of the tissue.

Preliminary studies showed that specimens, remaining in the fixative for 90 min up to a day, did not retain their stressed length upon release from stress, but recovered 5 to 30% of the strain, depending on the level of applied stress, and still retained compliance. But then, since the strain range of the third part of the stress–strain response is just over 30% (see Fig. 1a for a typical result), fixation for a few hours would only allow investigating the microstructure of the aortic tissue up to the very beginning of the third part, provided that the specimens would be subjected to the highest strain levels of the third part during fixation.

To minimize the effect of incomplete fixation, specimens subjected to physiologic and high stresses were soaked in the fixing agent for 1 month, leading to a significant reduction in tissue shortening upon stress relief, from 0 to 15%. These were removed from the grips of the tensile-testing device after stress relaxation at a given strain, clamped to tongue depressors, and transferred to the fixative. One end was clamped first, and the free end pulled, stretching the specimen to the extension it had on the tensile-testing device. The free end was next clamped to the depressor, to maintain the specimen at the stretched dimension.

After fixation over the selected time period, i.e. 90 min for specimens fixed under low stresses and 1 month for specimens fixed under physiologic and high stresses, strain was measured upon stress release. This was assumed to be the strain of the aortic tissue in the histological sections. The entire stress range from zero to very high levels, close to aortic rupture, was investigated using a sufficiently large number of strips, each fixed for a given time period at a pre-selected level of strain. The experimental protocol included strips that were not subjected to preconditioning, and to which no stress was applied. These fully relaxed specimens were put in the...
fixative for 90 min immediately after resection, and served for reference. Some specimens were precondi-
tioned only, without further application of stress. These were fixed in Karnovsky’s solution for 90 min, to investigate the effect of preconditioning on the histological structure of the vessel wall.

2.4. Histological studies

Subsequent to fixation, samples from the stretched specimens were embedded in paraffin, sectioned at 5 μm, and stained with Verhoeff’s elastica to identify elastin and Sirius red (0.1% Sirius red F3BA dissolved in saturated picric acid, pH 2.0) to identify collagen. To avoid end effects caused by mechanical clamping, samples were taken from the center of the strips. Several blocks were sectioned serially, and studied in steps at 10 to 20-section intervals. The configuration of elastin and collagen fibers was examined in longitudinal and circumferential sections of the aortic wall.

Histological sections were taken of stressed specimens that remained in the fixing agent for a few hours, and compared with those of neighboring specimens from the same aortic segment that were stored in the fixing agent for 1 month; upon stress relief, the strain was the same in both specimens, within the range of low and physiologic levels. No appreciable differences were observed in the structure of elastin and collagen at each stress level examined, but the long fixation time caused appreciable de-staining of cell nuclei and ground substance. This validated the use of specimens after 1 month of fixation for the investigation of the elastin and collagen fiber organization under physiologic and high stresses.

3. Results

3.1. Three-part constitutive law

A typical example of a stress–strain curve from an aortic specimen is shown in Fig. 1a. This curve may be conveniently considered in three parts. The initial section of the curve for stresses less than 40 kPa formed part I, the middle section of the curve between 40 and 120 kPa was part II, and the steep final section for stresses higher than 120 kPa represented part III. These are expressed in mathematical terms as

\[ T = (T_{II} + a/b) e^{k(c - \varepsilon_{II})} - a/b, \quad \varepsilon_{II} \leq \varepsilon \leq \varepsilon_{II} \]  

Part II, 

\[ T = (T_{II} + c/d) e^{k(c - \varepsilon_{II})} - c/d, \quad \varepsilon_{II} \leq \varepsilon \leq \varepsilon_{II} \]  

Part III, 

\[ M = kT^{q}, \quad 0 \leq T \leq T_{I} \]  

Part I, 

\[ M = a + bT, \quad T_{I} \leq T \leq T_{II} \]  

Part II, 

\[ M = c + dT, \quad T_{II} \leq T \leq T_{I} \]  

Part III.

Symbols \( b \) and \( d \) represent the slopes of the linear segments for parts II and III of the elastic modulus–stress curve, \( a \) and \( c \) and their intercepts on the stress-axis, whereas \( k \) and \( q \) express the nonlinear relation of part I. The transition points, which constitute the limits of the three parts, are defined by stresses \( T_{I} \) and \( T_{II} \), and strains \( \varepsilon_{I} \) and \( \varepsilon_{II} \), respectively. \( T_{I} \) and \( \varepsilon_{I} \) indicate the highest stress and strain to which the aortic specimens were subjected.

We estimated for the descending thoracic aorta an in situ longitudinal strain \( \varepsilon_{situ} \) of \( 32.9 \pm 3.1\% \) (Mean ± SEM), which lies within the limits of part II of the stress–strain curve. According to this estimate, normal physiologic pressures cause stresses in part II of the curves, and therefore constitutive parameters \( a \) and \( b \) are necessary for the study of the elastic properties at physiologic stress levels, whereas \( k \) and \( q \), and \( c \) and \( d \), in turn, are necessary for the study at low and high levels. Parameters \( k \), \( a \), and \( c \), whose units are those of stress, may be considered from the structural perspective as indices of the inherent stiffness of the aortic wall, independent of the applied stress. In contrast, the non-dimensional parameters \( q \), \( b \), and \( d \) are indicative of vessel stiffening under progressive stressing.

3.2. Effect of mechanical stress on the microarchitecture of the aortic wall

Figs. 2 and 3 are representative histological sections of the aortic wall, showing the deformational changes of elastin fibers that took place under the action of a variety of stress levels. Under zero stress, the elastic lamellae were wavy and crumpled, oriented along both the longitudinal and circumferential directions of the aortic wall. Preconditioning did not affect the spatial distribution, orientation, and waviness of the lamellae. Subsequent to preconditioning, the effect of longitudinally applied stress was to unfold first, followed by extension and compaction of the elastic lamellae at higher stresses (Fig. 2). The onset of these changes was
dependent on the three discrete parts of the stress–strain response.
Starting from the unstressed state, progressive stressing caused the undulating form of the elastic lamellae to sequentially straighten, until the end of part I when all lamellae were nearly taut. Raising stress to parts II and III of the stress–strain response caused extension and compaction of the lamellae. Deformational changes were restricted to the longitudinal sections of the aortic wall. No such changes were noted in the circumferential sections (Fig. 3).

The microscopic arrangement of collagen fibers within the aortic media, fixed while subjected to various stresses, is shown in Figs. 4 and 5. The corrugated appearance and disarrayed orientation of collagen fibers was clearly observed in the longitudinal sections of the unstressed aortic wall. In the transverse sections, collagen was seen as long wavy bundles with mainly circumferential orientation, and as short corrugated fibers with no consistent arrangement. The configuration of collagen did not change after preconditioning, as was the case with the elastic lamellae. Application of progressively higher stresses caused some collagen fibers to reorient towards the direction of stress, straighten, and become closely aligned in parallel arrays (Fig. 4). Changes in configuration paralleled those of the elastic lamellae, intimately corresponding to the three parts of the stress–strain response.

At the end of part I, all collagen remained corrugated, but tended to be more oriented towards the stress axis, compared to the unstressed state. During part II, collagen fibers continued to reorient and some started to uncoil, until by the end of this part, a higher proportion of fibers had reoriented along the stress axis and a few of them were even straight. As the aortic tissue was stressed into part III, more fibers were steadily uncoiling, and well into part III, the majority of fibers were almost straight and densely packed. At this very high stress level, individual fibers presented a greater length compared to their convoluted state in the unstressed aortic wall. The same was apparent at the beginning of part III, since a large number of collagen fiber convolutions were already straightened out. The occurrence of microstructural changes varied through the thickness of the aortic media, with changes in the outer media preceding those in the inner layers. Progressive stressing in the longitudinal direction had no effect on the circumferentially arranged collagen bundles (Fig. 5).
4. Discussion

In a previous paper (Sokolis et al., 2002), we employed a three-part constitutive law, via a power and a bi-exponential function, to approximate the nonlinear stress–strain response of the aortic wall in uniaxial tension, following the exponential and bi-exponential laws proposed in chronological order by Fung (1967, 1973) and Hayashi (1993) (Hayashi et al., 1981). The aim of this work was to report optical microscopy observations that would relate the three-part constitutive law to the aortic microstructure.

4.1. Histological changes of the aortic wall under uniaxial stress

Our histological findings suggest that progressive stressing had a three-phase effect on the elastic lamellae and collagen fibers. Low stresses caused straightening of the lamellae, while physiologic and high stresses caused extension and compaction. Collagen fibers reoriented in response to low levels, started to straighten in response to physiologic levels, and straightened extensively as well as compacted in response to high levels.

The present findings are in line with the histological observations by Glagov and Wolinsky (1963) (Wolinsky and Glagov, 1964) and by Clark and Glagov (1979, 1985) of the aortic components fixed at distending pressures between 0 and 250 mmHg. These authors found that the elastic lamellae were straight above diastolic pressure, and collagen, while randomly arranged and coiled at low pressures, became oriented circumferentially but remained coiled at higher pressures. Similar observations were reported more recently for the carotid artery by Dobrin (1999), who noted that the lamellae were nearly straight and load-bearing at sub-diastolic pressures. Unlike our study, the above-mentioned ones examined microstructural changes of the vessels only in the circumferential direction.

Our findings agree also with those of Roveri et al. (1980), who used X-ray diffraction to investigate the orientation of collagen under uniaxial stress in the thoracic aorta. They observed, with regard to molecular rather than fiber orientation, that collagen molecules aligned along the direction of stress even for very small deformations, with their number increasing at higher stress levels. Our findings extend those of Samila and Carter (1981), who measured folding of the elastic lamellae and collagen fibers in human carotid arteries. Similar to our study, they used strips that were stretched to various degrees, then fixed and stained for elastin and collagen, but unlike our study, the strips were circumferentially oriented, not preconditioned, and folding was examined only in the circumferential direction up to
40% strain, most probably because of limitations imposed by incomplete fixation at higher strain levels. They noted minimal changes in collagen fiber folding throughout the tested range of stretch, and a quick unfolding of the lamellae, with minor changes occurring beyond 10% strain.

The onset of deformational changes varied throughout the thickness of the aortic wall, and there are several lines of evidence suggesting that this may be related to the variable pattern of the media from the intimal to the adventitial side. In particular, previous studies by Song and Roach (1983, 1985) have shown that the internal elastica lamina and the next few layers of medial elastin are fenestrated sheets, whereas the layers on the adventitial side of the media form a fibrous network, and among them are contained transitional layers between sheets and fibers. Furthermore, Clark and Glagov (1985) noted that the elastic lamellae, smooth muscle cells, and collagen all deviated from a predominantly circumferential toward a longitudinal orientation in the outer media, ascribing this phenomenon to a significant axial tensile stress component in this region, due to tethering of the aorta in surrounding tissues.

We observed in Figs. 3 and 5 that the longitudinally applied stress had no effect on the circumferentially arranged elastic lamellae and collagen bundles. However, knowing that the elastic lamellae are in the form of sheets at least on the intimal side of the media, we expected them to be deformed by uniaxial stress in all directions. The fact that they were unaffected by stress in circumferential sections indicates first that they were fibrous, and second that fibers orthogonal to stress were not attached to non-orthogonal ones (Fig. 6). The fibrous appearance of the elastic lamellae under stress may be explained by previous scanning electron microscope studies (Sherebrin et al., 1982; Roach and Song, 1988), which have indicated that the elastic lamellae suffer a change in form when subjected to strains of even 10%, from a membrane-like to a more fibrous structure.

In contrast, the circumferentially oriented collagen bundles were not expected to deform, considering the model of the aortic wall reported by Clark and Glagov (1979, 1985). They examined the microstructure of the aortic wall under in situ perfusion fixation, during which the stresses exerted on the circumferential and longitudinal directions were comparable and within the physiologic range. Their histological studies provided...
evidence of two distinct sets of collagen, one in the form of bundles with a predominantly circumferential direction, and another in the form of a pericellular matrix with interlaced fibrils. It is expected that, upon removal of the stresses from both directions, the two sets of collagen will maintain their orientation. The distinct arrangement of collagen is in fact discerned in the longitudinal and circumferential sections of Figs. 4a and 5a under zero stress. Because the strips were tested uniaxially, the influence of fibers oriented off the axis of applied stress would be reduced, and fibers orthogonal to the stress axis would have no influence in resisting deformation, unless they were connected to non-orthogonal elements. That was evidently the case with the interlaced fibrils of the pericellular matrix, which, under the action of longitudinal stress, were found straightened along both the radial and longitudinal directions, whereas it was not at all the case with the thicker collagen bundles that maintained a circumferential orientation and a wavy configuration (Fig. 6).

The effect of preconditioning on the structural properties of the aortic tissue has not been previously assessed. From the mechanical viewpoint, it is known that the aortic tissue should be cycled a number of times before it exhibits repeatable behavior; and further that stabilization occurs only within the extension range to which a specimen is subjected during cycling. It is also known that the aortic tissue undergoes a permanent elongation following preconditioning, which Sherebrin et al. (1982) attributed, without microstructural verification, to a variety of mechanisms, such as fiber fracture and reorientation, as well as irreversible stretching and thinning of the lamellae. This study disclosed that preconditioning had no influence on the orientation and spatial distribution of the elastic lamellae and collagen bundles, but no definite conclusions were drawn regarding the mechanism of lamellae stretching and thinning, and additionally the role of the ground substance was not examined. This phenomenon, therefore, remains an open issue for further study.

Regarding fixation under stress, we found that the aortic tissue cannot be fixed in a few hours at any pre-selected stress, shortening significantly upon stress relief prior to histological processing. Our finding is consistent with the study of Fung and Sobin (1981), who first documented the incomplete fixation of elastin by conventional aqueous fixatives, demonstrating its effect in histological preparations of pulmonary alveoli (Sobin et al., 1982). As a partial solution to the problem of incomplete fixation, we found that storage in the fixative over long periods of time resulted in solid specimens, which shortened comparatively less, thereby allowing characterization of the aortic microstructure under high stresses.
4.2. Structural basis of the three-part constitutive law

The stress–strain and elastic modulus–stress curves of the aortic strips demonstrated the previously reported three-part form, shown in Fig. 1. Initially, there was a fairly rapid increase in strain for small increments in stress, referring to low values of elastic modulus for the aortic wall. Terminally, minor degrees of strain resulted from relatively large increases in stress, characterized by very high elastic modulus values. Between the two extremes laid a middle part representing the change from the first to the last part of the curves.

We found that the rabbit thoracic aorta experienced a 33% retraction in length, when dissected from the surrounding tissues, in agreement with the study by Han and Fung (1995) of the regional and species differences in longitudinal strain between porcine and canine aortas. The addition of an extra 1% strain to this value due to blood pressure gives an estimate of the in vivo strain in the longitudinal direction (Humphrey, 2002; Nichols and O’Rourke, 1990), suggesting that the aorta normally operates in part II of the stress–strain relation, while parts I and III include low and high stresses, respectively.

Evidently, the mechanical state modeled by the three-part constitutive law does not represent the in vivo state. The tissue in vivo is in a homeostatic state that is disrupted once the tissue is removed from the body. It is only after preconditioning that the in vitro mechanical response becomes repeatable. Following Fung (1973), the preconditioned state of the arterial tissue in vitro was regarded as most representative of its in vivo homeostatic state.

Direct histological examination of the aortic structure during uniaxial tension that was undertaken in the present study indicated that part I of the stress–strain relation, corresponding to low stresses, involves the unfolding of elastin fibers; this finding is in partial contrast to several mathematical models for the arterial wall (Armentano et al., 1991, 1995; Bank et al., 1996;
Raghavan et al., 1996; Wuyts et al., 1995), which considered the elastic fibers to be straight at zero up to low stresses, ascribing the nonlinearity of the constitutive law entirely to collagen. In addition, part III, corresponding to higher stresses, is the direct result of straightening and aligning of the disarrayed collagen fibers in the direction of stretch, whereas part II, which refers to physiologic stresses, involves both elastin and collagen. Such a division seems justified by previous differential digestion studies (Roach and Burton, 1957; Minns et al., 1973; Hoffman et al., 1977; Dobrin and Canfield, 1984), which showed that the initial part of the stress–strain relation is removed by elastase, and the final part by collagenase, with hyaluronidase eliminating the amorphous matrix and causing a displacement of the final part of the relation to higher strains.

However, inspection of the structural model for the aortic wall proposed by Clark and Glagov (1979, 1985) suggests that smooth muscle cells may have a role in transmitting passive stresses. These authors have described the aortic media as consisting of elastic lamellae and fibers, collagen bundles with a predominant circumferential direction, and smooth muscle cells bound together in series by their basement membranes and a matrix of interwoven collagen fibrils. Evidently, their description implies in biomechanical terms that some of the collagen fibrils act in series with smooth muscle cells, so that stresses are shared by collagen and smooth muscle tissue, and furthermore that this smooth muscle-collagen network is arranged in parallel with parallel elastin and collagen elements (Silver et al., 2003).

In the following, the adventitia is considered mechanically irrelevant due to its loose structure, and the presence of ground substance and endothelial cells is ignored, since their elastic moduli are much less than that of elastin. Knowledge of the volume fractions $W_E$, $W_C$, and $W_{CS}$ of the parallel elastin and collagen elements, and of the series arrangement of collagen and smooth muscle, in addition to their corresponding elastic moduli $M_E$, $M_C$, and $M_{CS}$, allows determination of the composite stiffness $M$ of the aortic tissue (Jones, 1975)

$$M = f_E W_E M_E + (f_C W_C M_C + f_{CS} W_{CS} M_{CS})$$

(3)

Parameters $f_E$, $f_C$, and $f_{CS}$ are recruitment functions, representing the fractions of elastin and collagen in the parallel elements, and of collagen in the series arrangement with smooth muscle, that have been aligned, straightened out, and are bearing stresses. The first term in Eq. (3) characterizes the nonlinear elasticity of elastin, and the second and third terms that of collagen. These terms form the passive constitutive law, assuming that smooth muscle makes no active contribution to the mechanical properties under passive conditions.

Histological evidence from this study suggests that the parallel collagen element remains crimped in all three parts of the stress–strain relation, so that $f_C$ tends to zero, and the composite stiffness $M$ of the aortic tissue in the longitudinal direction is

$$M = f_E W_E M_E + f_{CS} W_{CS} M_{CS}$$

(4)

where the first term refers to the component of elastic lamellae in the longitudinal direction. Collagen in the series element is crimped in part I, so that $f_{CS}$ is zero, and Eq. (4) becomes

$$M = f_E W_E M_E, 0 \leq \varepsilon \leq \varepsilon_1 \text{ Part I},$$

(5)

representing the contribution of elastin. The pericellular matrix of collagen fibrils, surrounding the smooth muscle cell basement membrane, is progressively engaged in part II following extension of the elastic lamellae, so that Eq. (4) becomes

$$M = f_E W_E M_E + f_{CS} W_{CS} M_{CS}, \varepsilon_1 \leq \varepsilon \leq \varepsilon_{II} \text{ Part II},$$

(6)

by virtue of the fact that all lamellae are taut in part II, i.e. $f_E = 1$. The majority of interlaced fibrils are recruited in part III, but since the stiffness of elastin is much less than that of the series network of collagen and smooth muscle, i.e. $M_E \ll M_{CS}$, Eq. (4) is expressed as

$$M = f_{CS} W_{CS} M_{CS}, \varepsilon_{II} \leq \varepsilon \leq \varepsilon_f \text{ Part III}.$$  

(7)

Finally, at very high stress levels, the totality of collagen is engaged, i.e. $f_{CS}$ approaches unity, and Eq. (7) is recast as $M = W_{CS} M_{CS}$.

The constitutive parameters may therefore be physically interpreted as follows: Dimensionless parameters $b$, $d$, and $q$ determine how rapidly wall stiffness increases in turn at low, physiologic, and high stresses, and may be regarded microstructurally as indices of the configuration, content, and elastic modulus of elastin for low stresses, of both elastin and collagen for physiologic stresses, and of collagen for high stresses. A high value of $d$ suggests that collagen becomes recruited more rapidly, and so does a high value of $q$ for elastin, implying that the distribution of waviness becomes smaller, making the fibers more uniform in their crimping. In contrast, $a$, $c$, and $k$, whose units are those of stress, are scaling factors, and on that account, the higher the values of $b$, $d$, and $q$, the lower the values of $a$, $c$, and $k$. At last, transition strains $\varepsilon_1$ and $\varepsilon_{II}$ denote the strain levels, associated with the transition from one part to another, microstructurally standing as indices of the degree of waviness of elastin and collagen. Then, a decrease in $\varepsilon_{II}$ correlates to a shift in the heel of the stress–strain response towards the origin, signifying that collagen has become less wavy.

Our mechanical data support such a microstructural interpretation of the proposed three-part law. It is worth noting in the elastic modulus–stress curves of Fig. 1 that, upon correction of the upper value of elastic moduli in
part I for the volume fraction of elastin in the rabbit thoracic aorta, this value is roughly associated with the elastic modulus of pure elastin (Lake and Armeniades, 1972; Aaron and Gosline, 1981). Further, correction of the upper value of elastic moduli in part III for the volume fraction of collagen gives a value, which is at least an order of magnitude less than that of pure collagen (Canfield and Dobrin, 1987; Shadwick, 1990; Silver et al., 1992), consistent with the presence of serial connections between collagen and smooth muscle.

In conclusion, the newly proposed three-part constitutive law, being an extension of pioneering work by Fung (1967, 1973) and Hayashi (1993) (Hayashi et al., 1981), is advantageous in comparison to earlier ones, which were derived purely from phenomenological treatments of the experimental data, not embodying considerations of the arterial wall composition and architecture. These laws contained parameters that did not possess direct physical meaning, but were mainly related to the specific form of the constitutive law, hence offering only general comparative information. The abovementioned deficiencies were surpassed herein by analyzing the stress–strain relation in three parts that refer to low, physiologic, and high stresses, and by assigning distinct microstructural interpretation to the constitutive parameters of each part.

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