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Elastic Arteries in Invertebrates: Mechanics of the Octopus Aorta

Abstract. *The aorta of the octopus,* Octopus dofleini, *is a highly distensible, elastic tube. The circumferential elastic modulus increases with inflation in the physiological range from about 10^4 to 10^5 newtons per square meter. Rubber-like fibers have been isolated, apparently for the first time, from the aorta of an invertebrate. These fibers have an elastic modulus, like elastin, of about 4×10^5 newtons per square meter and are present in sufficient quantity to account for the elastic properties of the intact vessel under physiological conditions. Thus the circulatory system of an invertebrate animal provides an "elastic reservoir" (much like that of the vertebrate system), which increases the efficiency of the circulation.*

The passive elasticity of blood vessels is a fundamental feature of the vertebrate circulatory system. Major arteries provide an elastic reservoir, which is distended during cardiac systole and maintains positive blood flow to the peripheral vessels while the heart refills (*1*). The pulsatile flow of blood from the heart is thus transformed into a steady flow through the capillaries, thereby increasing the efficiency of the circulation (*1*). In the vertebrates, the rubber-like protein elastin is responsible for the high extensibility and elasticity of the artery wall under physiological pressures (*2*). Elastin, however, has been found exclusively in the vertebrates, and its appearance coincides with the evolution of the advanced circulatory systems in these animals (*3*). The inherent advantages of elastic reservoirs has led us to suspect that elastic arteries also exist in many invertebrates. If this is so, then the elasticity must be based on a material that is different from elastin. Histological studies have suggested that "elastic fibers" occur in the blood vessels of many invertebrates (*4*). However, there are no reports on the mechanical properties of any invertebrate blood vessel, nor have the presumptive elastic fibers ever been isolated and tested mechanically.

From observations on the blood pressure of the cephalopod *Octopus dofleini*, Johansen and Martin (*5*) concluded that the circulatory system contained an elastic component. We, therefore, investigated the mechanical properties of the aorta of this invertebrate by in vitro inflations of arterial segments and found that the aorta is indeed a highly extensible elastic tube. Pressure-radius data gathered from these tests were used to construct a circumferential stress-extension curve for the artery wall [Fig. 1a, (i)]. Like most soft biological tissues the artery wall of the octopus exhibits a nonlinear (J-shaped) stress-extension curve, in which the incremental elastic modulus (*2*), that is, the stiffness, increases continuously with extension ratio (λ, where λ is the ratio between the extended length L and the starting length L_0), particularly in the physiological range of stress [Fig. 1a, (i)]. J-shaped stress-extension curves are found for all vertebrate arteries [for example, see Fig. 1a, (ii)]; this appears to be a design feature of highly extensible pressure vessels that prevents rupture (*6*). In vertebrate arteries, the low modulus region of the stress-extension curve is dominated by elastin (*7*). Similarly, we suspected, in the octopus, that an undescribed elastic protein might be present to provide the low modulus region of the stress-extension curve seen in Fig. 1a, (i). We have subsequently isolated and performed direct mechanical tests on an extracellular, rubber-like protein that we believe is responsible for the elasticity of the octopus aorta under physiological pressures.

Examination of the aorta of *O. dofleini* by light and electron microscopy revealed several tissue layers: an inner layer of circular muscles, a central layer of longitudinal muscle, and, outermost, a loose collagenous sheath. In addition, a layer of presumptive elastic fibers (5 to 7 μm in diameter) lines the vessel lumen and is called the internal elastica (IE)

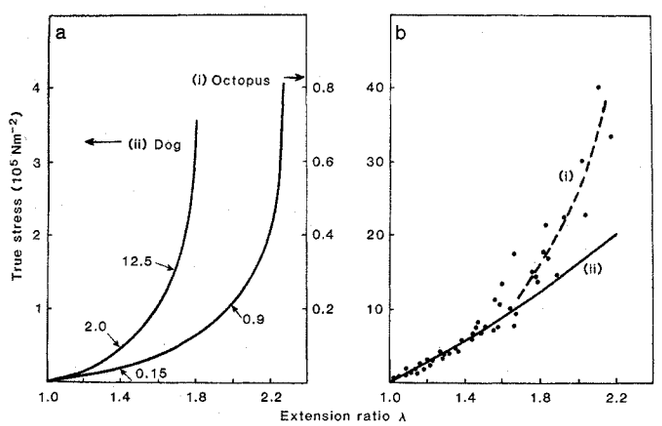

Fig. 1. Plots of true stress (force per cross-sectional area) against extension ratio (λ, ratio of extended length to starting length). (a) Comparisons of in vitro inflations of whole artery segments from (i) *Octopus dofleini* with (ii) the dog carotid, a typical vertebrate artery (*14*). The stress axis is on the right side for the octopus and on the left side for the dog. In both cases the stress and extension ratio are calculated for the circumferential direction. Both curves are nonlinear and the incremental elastic modulus [as defined by Bergel (*2*)] increases continuously with λ. The upper and lower limits of extension under average physiological pressures (18 to 40 mmHg in the octopus, 60 to 120 mmHg in the dog) are indicated by the arrows on the curves along with the corresponding values of the incremental elastic modulus at these extensions; this modulus has the units of stress (10^5 Nm^{-2}). The lower values of stress and modulus in the octopus aorta compared to those in the dog artery reflect the difference in pressures that are normally experienced by these two animals. (b) Results from force-extension tests on native elastic fibers taken from the internal elastica (IE) of the octopus aorta (i). These tests were performed on force transducers similar to that shown in Fig. 2b (*8*). Up to 60 percent extension ($\lambda = 1.6$) the data fit the predicted curve for an ideal Gaussian rubber (ii) plotted from the equation stress $= G(\lambda^2 - \lambda^{-1})$, where G, the elastic modulus, is equal to 4.6×10^5 Nm^{-2}. At extensions above 60 percent, the data diverge from the ideal case as the elastic fibers begin to exhibit non-Gaussian behavior that is typical of natural rubbers when stretched to large extensions. The octopus elastic fibers have the extensibility and modulus of a rubber-like material.

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(Fig. 2a). Thinner elastic fibers (about 1 μm in diameter) that arise from the IE lie between muscle cells throughout the entire thickness of the artery wall.

To demonstrate that the fibers are highly extensible and rubber-like, we performed mechanical tests on isolated samples. Fibers from the IE were isolated by dissection; no chemical treatment was required. When stretched, the fibers developed tension and snapped back immediately on release. The samples could be tested repeatedly to large extensions with no apparent damage.

To quantify the elastic properties, small bundles of these fibers were mounted and stretched on a micro-force transducer attached to a polarizing microscope (*8*) (Fig. 2, b and c). Results from force-extension tests are shown in Fig. 1b. Up to $\lambda = 1.6$ (that is, extended by 60 percent), the data follow the predictions of the kinetic theory of rubber elasticity for an ideal (Gaussian) rubber with elastic modulus $G = 4.6 \times 10^5$ Nm^{-2} [Fig. 1b, (ii)]. This value of modulus is similar to that for lightly cross-linked synthetic rubbers as well as for the protein rubbers elastin (*9*), resilin (*10*), and abductin (*11*). However, at large extensions all real rubbers diverge from the Gaussian prediction, becoming stiffer as the limit of deformability of the molecular network is approached (*9, 12*). For the octopus elastic fibers the non-Gaussian behavior appears at extensions greater than $\lambda = 1.6$. Although most synthetic rubbers do not exhibit non-Gaussian properties at such small extensions, this behavior is characteristic of both resilin (*10*) and elastin (*9*).

The theory of rubber elasticity is based on the presence of an isotropic, cross-linked network of kinetically free random-coil molecules (*12*). Octopus elastic fibers are optically isotropic and became birefringent only when stretched (Fig. 2, d and e), a property consistent with the random network model. The presence of covalent cross-linking in the network is indicated by the insolubility of the elastic fibers in strong protein denaturing agents such as 98 percent formic acid, 8*M* guanidine hydrochloride with 1 percent mercaptoethanol, 6*M* urea, and 3*M* ammonium sulfate. The elastic fibers can only be dissolved by hydrolysis of the peptide bonds. Further, the mechanical properties of fibers treated with formic acid are virtually identical to those in Fig. 1b. Thus, on the basis of the mechanical, optical, and chemical properties, we conclude that these fibers are indeed rubber-like.

Table 1. Amino acid composition of elastic fibers from the aorta of *Octopus dofleini* (residues/1000 residues).

Amino acid	Whole artery extract	Purified IE
Aspartic acid*	97.7	90.6
Hydroxyproline	0	0
Threonine	60.5	64.4
Serine	74.1	71.8
Glutamic acid†	133.4	121.1
Proline	52.9	54.9
Glycine	77.6	85.0
Alanine	75.6	71.7
Cystine	8.5	7.4
Valine	52.3	61.9
Methionine	19.8	21.0
Isoleucine	48.0	59.6
Leucine	84.3	73.5
Tyrosine	25.2	36.2
Phenylalanine	35.7	42.3
Histidine	19.1	21.0
Lysine	70.8	68.3
Arginine	56.3	45.8

*Or asparagine. † Or glutamine.

Hydrolyzates of formic acid purified IE (*13*), as well as the total residue from whole arteries extracted in formic acid, were analyzed on a Beckman 118C amino acid analyzer (Table 1). The whole artery extract contains the IE and the intermuscular elastic fibers plus any other insoluble proteins. The amino acid compositions of the whole artery extract and the purified IE are almost identical, an indication that formic acid removes all material other than the cross-linked protein in the elastic fibers. The octopus elastic protein is distinctly different from the other known protein rubbers: elastin (*3*), resilin (*10*), and abductin (*11*). Most striking is the relatively low glycine content.

Formic acid extractions indicate that the elastic fibers make up 5 percent of the dry weight of the artery wall. Collagen, extracted by autoclave solubilization, makes up 30 percent of the dry weight. Since muscle cells contain more water than connective tissue fibers, we estimate that the elastic fibers occupy about 3 percent of the hydrated volume of the artery wall, collagen about 20 percent, and the muscle cells the remaining 77 percent. The IE is laid down with the fibers predominantly in the longitudinal direction (the artery changes length as well as diameter when inflated), while the other elastic fibers run throughout the muscle layers as a multidirectional array. If as little as one third of the total elastic fiber content supports load in the circumferential direction, then the entire

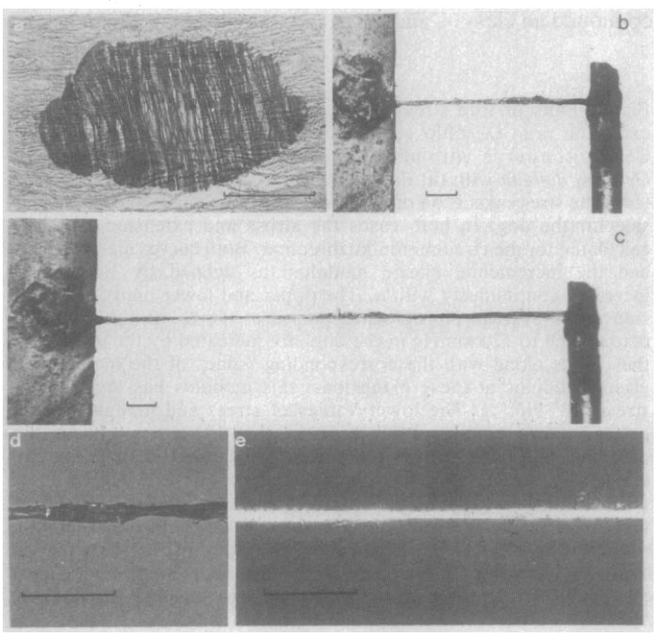

Fig. 2. (a) A section of the aorta of *Octopus dofleini* cut tangential to the luminal surface and viewed under the light microscope. A portion of the internal elastica (IE), stained with aldehyde fuchsin (*15*), is seen as a network of fibers with vertical orientation lying over circular muscle cells in the horizontal direction. (b) A small sample of elastic fibers from the IE is mounted in distilled water on the force transducer described in (*8*). The fibers are attached, on the right, to the free end of a glass cantilever (15 mm long, 95 μm in diameter) and, on the left, to a movable glass plate. The sample is unstretched. (c) By sliding the plate the sample is stretched to $\lambda = 2.5$ (that is, extended to 2.5 times the resting length). When the tension is released, the fibers return immediately to the starting length demonstrating the long-range, reversible elasticity of the fibers. (d) A sample of unstretched elastic fibers seen under polarized light. The angle between the polarizers is reduced from 90° to 88° in order to allow a small amount of background illumination for the photograph. The fibers are not birefringent here. (e) The same as (d) but with the sample stretched by 100 percent. Now the elastic fibers are highly birefringent. Scale bars represent 100 μm.

circumferential wall stress under physiological pressures must be carried by approximately 1 percent of the wall material. The physiological range of extension ($\lambda = 1.4$ to 2.0) corresponds to wall stresses of 5×10^3 to 2×10^4 Nm^{-2} [Fig. 1a, (i)]. Inspection of Fig. 1 shows that, at similar extension ratios, the stress in isolated fibers is at least 100 times greater than the stress in the whole artery wall. In other words, the elastic fibers are present in sufficient quantity to account for the passive elasticity of the aorta under physiological pressures. At higher extensions the artery wall stiffens greatly, presumably a result of the recruitment of collagen fibers. Thus, the parallel arrangement of elastic fibers and collagen accounts for the mechanical properties of both octopus and vertebrate arteries.

Our study presents direct evidence of elasticity in the circulatory system of an invertebrate and describes a new rubber-like protein as the basis for this elasticity. Clearly, elegantly designed elastic arteries are not found only in vertebrates. Considering the functional benefits of elastic arteries and the widespread distribution of histologically identified "elastic fibers" (*4*), we believe that elastic blood vessels occur in many other invertebrates. Indeed, the presence of some sort of elastic reservoir may be a fundamental component of any circulatory system powered by a pulsatile pump. Of particular note is the extensive variation in amino acid composition among the protein rubbers (elastin, resilin, abductin, octopus elastic fibers), which suggests that each protein arose independently during evolution; and we suspect that other elastic tissues will contain as yet undescribed and distinct protein rubbers. Since there probably will not be enough "stretchy" names for all the protein rubbers, we propose a naming system based on organism and tissue type; therefore, the protein described here is called octopus arterial elastomer.

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8. This apparatus is modified from T. Weis-Fogh and W. B. Amos [*Nature (London)* **236**, 301 (1972)]. The sample is mounted with one end attached to a slender glass cantilever of uniform diameter and the other end attached to a movable glass plate (Fig. 2b). The sample is then stretched by sliding the plate (Fig. 2c). Tensile force in the sample is calculated from the deflection of the cantilever, while extension is determined by measuring changes in the spacing of surface features on the sample. All measurements are made with a Wild filar micrometer eyepiece on a Wild M21 polarizing microscope. We estimate the error to be within ± 5 percent. Since the fibers are rubbery only when hydrated, the samples are covered in distilled water at all times.
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13. Because the IE is insoluble and mechanically unaltered in formic acid we used this as a treatment to prepare chemically pure samples. This same method is used to isolate and purify elastin from vertebrate tissues [B. L. Rasmussen *et al.*, *Anal. Biochem.* **64**, 255 (1975)].
14. In this figure, the curve for the dog data is redrawn from R. H. Cox, *Am. J. Physiol.* **231**, 420 (1976).
15. The IE was shown in *O. vulgaris* by V. C. Barber and P. Graziadei [*Z. Zellforsch. Microsk. Anat.* **77**, 162 (1967)] but was described as a basement membrane. We found that the IE stained positively with aldehyde fuchsin [M. L. Cameron and J. E. Steele, *Stain Technol.* **34**, 265 (1959)], a technique that also stains elastin, and we confirmed other reports on cephalopod arteries that no elastic fibers were visualized in sections treated with conventional elastin stains (*3*, *4*).
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The Permeability of Plant Cell Walls as Measured by Gel Filtration Chromatography

Abstract. *The permeability of plant cell walls to macromolecules may limit the ability of enzymes to alter the biochemical and physical properties of the wall. Proteins of molecular weight up to 60,000 can permeate a substantial portion of the cell wall. Measurements of wall permeability in which cells are exposed to hypertonic solutions of macromolecules may seriously underestimate wall permeability.*

The permeability of the plant cell wall to macromolecules is of particular importance because it determines the ability of enzymes, extracellular glycoproteins, and polysaccharides to penetrate and alter the cell wall. Enzymes may play an important role in many cell wall processes such as cell wall biosynthesis (*1*), the dissolution of the cell wall by plant pathogens (*2*) or by the plant itself during fruit ripening (*3*) or leaf abscission (*4*), and growth-hormone-induced cell wall loosening (*5*).

Until recently, it was assumed that the cell wall is freely permeable to large macromolecules. In a recent report Carpita *et al.* (*6*) suggested that only small macromolecules (radius, 16 to 19 Å) could rapidly penetrate the cell wall of a variety of plant cell types. This size corresponds to a globular protein with a molecular weight of approximately 17,000. These results cast serious doubt on the possibility of enzyme involvement in cell wall biochemistry, since most enzymes are too large to penetrate pores of such small dimensions.

Carpita *et al.* (*6*) measured the ability of extremely high concentrations of macromolecules to penetrate through the cell wall. Using gel filtration chromatography, we have reexamined the permeability of the cell wall. With this technique it is possible to detect permeation of macromolecules into the cell wall matrix at much lower concentrations. These conditions should more closely imitate those to which plants are exposed in nature. Using proteins of known size, we calibrated a column packed with isolated cell wall fragments and determined the degree to which the proteins can penetrate the cell wall matrix. Our results show that proteins much larger (molecular weight, 40,000 to 60,000) than would be predicted from the work of Carpita *et al.* (*6*) can penetrate a substantial portion of the cell wall space. Using techniques analogous to those of Carpita *et al.* (*6*), we have established one reason why they might have underestimated the permeability of the cell walls they studied.

Cell walls were isolated from the hypocotyls of 7-day-old dark-grown bean (*Phaseolus vulgaris* L.) seedlings. Frozen hypocotyl segments were ground to a fine powder in a Sorvall Omnimixer cooled in a Dry Ice–ethanol bath. The powder was allowed to thaw, and attached cytoplasm was released by further disruption with a Blackstone ultrasonic probe (in 40-ml portions) for 30 seconds at 200 W. The slurry was diluted to 600 ml with deionized water and allowed to settle until two layers appeared

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