THE STRUCTURE AND PHYSICAL PROPERTIES OF INVERTEBRATE AND PRIMITIVE VERTEBRATE ARTERIES

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Summary

Light and electron microscopy and in vitro inflation experiments were conducted on the aortae of three different invertebrate species: the lobster Homarus americanus, the horseshoe crab Limulus polyphemus and the whelkBusycon contrarium. Inflation experiments were also performed on the aortae of two species of primitive vertebrates, the sea lamprey Petromyzon marinus and the Atlantic hagfish Myxine glutinosa. The inflation experiments demonstrated similar overall biomechanical properties in each case, despite the existence of differences in tissue structure. The vessels were compliant at low strains, but demonstrated nonlinear elasticity, increasing in stiffness as strains increased; this property could act as protection against artery wall rupture. The vessels of the lamprey, hagfish and lobster are capable of acting as fairly efficient elastic reservoirs and of smoothing blood flow during circulation as they had low hysteresis values (13–18%). The aortae of the horseshoe crab and whelk, if performing this function, have much higher energy losses, up to more than 30% per cycle.

The microscopy studies of the aortae of the lobster, horseshoe crab and whelk revealed tissue structures which differ widely from each other as well as from the structures of the lamprey and hagfish. None of these arteries contained elastin, but all contained fibrillar material which differed in appearance, size and arrangement between species. These materials were conjectured to contribute to the elastic properties of the tissue.

Key words: lamprey, hagfish, lobster, whelk, horseshoe crab, aorta, artery, elasticity, mechanical properties, microfibrils.

Introduction

The mechanics of vertebrate arteries have been quite well-studied in comparison with that of invertebrates. The large arteries of vertebrates have been shown to act as elastic reservoirs, reducing the pulsatility of blood flow to the peripheral circulatory system (Shadwick, 1992). During systole, the artery inflates and stores energy elastically, most of which is regained when the vessel passively recoils during diastole. This elastic behaviour smooths the blood flow at peripheral vessels by reducing the initial pressure pulse and providing continued blood flow during diastole. This smoother flow reduces hydraulic power requirements and shear stresses on the walls of blood vessels. These vessels are also nonlinearly elastic, becoming stiffer the more they are stretched, so that they are protected against rupture at high blood pressures. This is due to the fact that the arteries are composite structures, containing both elastin and collagen as well as a cellular component (Shadwick, 1992). At low strains, the collagen fibres are slightly kinked and are not directly stressed, so the behaviour of the tissue is due mostly to the compliant elastin fibres. At higher stresses, the collagen fibres, having an elastic modulus about 1000 times greater than that of elastin, are straightened and begin to become directly stressed. This causes a large increase in stiffness (Gosline and Shadwick, 1983).

Much less is known about the mechanics and tissue structure of invertebrate arteries. However, in vessels from animals with highly developed circulatory systems, including lobsterHomarus americanus and crab Cancer magister (McMahon and Burnett, 1990), octopus Octopus dolfini (Wells, 1978) and squidNototodarus sloani (O’Dor, 1989), nonlinear stress–strain curves have also been observed (Gosline and Shadwick, 1983; Shadwick et al. 1990). This characteristic is also evident in the horseshoe crabLimulus polyphemus (Vreugdenhil and Redmond, 1987). In studies considering both mechanical and blood pressure data, the nonlinearity of the stress–strain curve again begins over the physiological pressure range, indicating that the arteries are...
probably performing both elastic and protective functions similar to those in vertebrates (Shadwick, 1992). Invertebrate arteries, like those of higher vertebrates, are also composite structures, usually containing muscle tissue and collagen (Shadwick, 1992). Elastin is not found in invertebrates, but seems to be replaced by some other connective tissue, probably elastic in nature (Elder, 1973; Shadwick, 1992). This tissue can differ in nature and arrangement between species. In the octopus aorta, it has been given the name octopus arterial elastomer or OAE (Shadwick and Gosline, 1985). The dorsal aorta of the lobster contains a fibrillar given the name octopus arterial elastomer or OAE (Shadwick and Gosline, 1985). The dorsal aorta of the lobster contains a fibrillar material in the middle lamella, with individual fibrils having a diameter of about 25 nm (Shadwick et al., 1990). In two primitive vertebrates, the sea lamprey Petromyzon marinus and the Atlantic hagfish Myxine glutinosa, the aorta again contains microfibril-based tissue (Wright, 1984), this time with individual microfibrils 11–17 nm in diameter.

A study by DeMont and Wright (1993) found no evidence of nonlinearly elastic behaviour in the ventral aorta of the lamprey. This could mean either that this species uses an alternative method of dealing with the problem of vessel rupture or that rupture is not a problem because of blood pressures that are lower than in most higher vertebrates. It is also possible that the pressures used in their experiments were not high enough to cause an increase in stiffness.

The present paper surveys the mechanical behaviour of the arteries of several major invertebrate and lower vertebrate phyla to provide more information about whether nonlinear elasticity is universal among these animals and why. It attempts to understand their functional mechanical behaviour using information on the composite structure of the vessels obtained from microscopy studies. The properties of these tissues under different temperature conditions are also examined. Most invertebrates are poikilotherms, so their body temperature varies with ambient temperature. Their important functional biomaterials might, therefore, be expected to display stability over a wide range of temperatures. Changes in the behaviour of artery material due to temperature changes could have a profound impact on the circulatory dynamics of the animal by influencing pressure-wave propagation characteristics and possibly by increasing the pulsatility of blood flow or by allowing rupture to occur as a result of changes in stiffness.

### Materials and methods

#### Animals

Experiments were conducted on arterial samples from several different invertebrate species: the lobster Homarus americanus Milne-Edwards (N=5; mass 0.623±0.100 kg, mean ± s.d.; obtained locally), the horseshoe crab Limulus polyphemus L. (N=3; mass 0.766±0.188 kg; Gulf Specimen Marine Lab, Panacea, Florida, USA) and the giant left-handed whelk Busycon contrarium Conrad (N=3; body mass varying between approximately 0.300 and 0.600 kg; Gulf Specimen Marine Lab, Panacea, Florida, USA). Two species of primitive vertebrates were also used, the sea lamprey Petromyzon marinus L. (N=5; mass 0.772±0.042 kg; New Germany, Nova Scotia) and the Atlantic hagfish Myxine glutinosa L. (N=3; approximate mass 0.15 kg, Huntsman Marine Science Center, New Brunswick). All specimens were maintained and killed according to the guidelines set by the Canadian Council of Animal Care. The lobsters, crabs and whelks were killed by slowly raising their body temperature to 40 °C. The lamprey and hagfish were anaesthetised by immersion in 0.5 % tricaine methanesulphonate (MS-222) and killed by decapitation.

Tests were performed on segments of the ventral aorta of the lamprey and hagfish, the dorsal abdominal artery of the lobster, the anterior aorta of the horseshoe crab and the aorta of the whelk. Two female Zucker lean rats Rattus norvegicus (Charles River, St Constant, Quebec), both with a mass of 0.20 kg, were anaesthetized with 60 mg kg⁻¹ sodium pentobarbital and killed by pneumothoracotomy, and segments of the thoracic and abdominal aortae were used as controls for histochemical stains. All aortic segments were dissected out immediately and prepared for light and electron microscopy or for mechanical testing.

#### Mechanical testing

Short artery segments immediately adjacent to the heart, 0.02–0.05 m long, were dissected and mounted on the testing apparatus. Both ends of the segment were tied with surgical silk thread to blunted syringe tips, and the segment was stretched to its approximate in vivo length, measured during dissection. One syringe tip was blocked, while the other was connected using polyethylene tubing to a pressure transducer and linear infusion syringe pump (Harvard Apparatus 55-2226), similar to Shadwick (1992). The external diameter of the artery was recorded continuously using a video dimension analyzer (VDA, Instruments for Physiology and Medicine model 303) and video camera (JVC TK-S300) attached to a dissecting microscope. The VDA signal was collected on a digitizing oscilloscope (Hewlett Packard 54501A), as was the intraluminal pressure recorded by the transducer, and these data were downloaded to a microcomputer (HP Vectra QS16). A water bath (NESLab RTE-111) was used to keep the saline solution at a constant temperature (±0.5 °C).

Each artery was subjected to a series of tests. First, each segment was slowly inflated and deflated over a period of 50 s, using the syringe pump, in a series of 2–3 conditioning cycles. This inflation speed was used because of limitations imposed by the oscilloscope, which could collect a maximum of 50 s (512 points) of data at one time, although longer cycles of 2–3 min would have been desirable. Previous work on vertebrate arteries has shown that conditioning is necessary for reproducible results in mechanical tests (Gosline and Shadwick, 1983). The initial inflation pressure was above zero so that the vessels were rounded and wrinkles and flattening were eliminated, but low enough so that they were essentially unstrained in the circumferential direction. Each segment then underwent another series of slow inflation–deflation cycles, again using the syringe pump, twice with one cycle over a period of 50 s, twice with two cycles over 50 s, and once with three cycles over 50 s. These tests were performed at a water
isotropic vessel tethered at both ends, given by:

$\Delta r = \frac{Pr}{h}$, (1)

where $P$ is the intraluminal pressure in Pascals, $r_i$ is the internal radius in m at that particular pressure, and $h$ is the corresponding wall width in m. The internal radius and instantaneous wall width were calculated by assuming that the total cross-sectional area of the wall was constant. Circumferential strain ($\varepsilon_c$) is given by:

$\varepsilon_c = \frac{\Delta R_{mw}}{R_{mw0}}$, (2)

where $R_{mw}$ is the mid-wall radius in m, and $R_{mw0}$ is the unstressed mid-wall radius in m, again assuming total cross-sectional area to be constant (Gibbons and Shadwick, 1989). Each inflation–deflation curve was plotted as stress and strain. The tangential circumferential elastic modulus ($E_c$) for an isotropic vessel tethered at both ends, given by:

$E_c = 0.75(\Delta \sigma_c/\Delta \varepsilon_c)$, (3)

was calculated from the slope of the relatively straight, low-strain portion of the resulting curve using a simple regression analysis. The pressure range of this portion included the lower end of the physiological pressure range of the species being tested. This regression was sometimes performed at both low and high strain values to quantify nonlinearity, as the modulus will increase with increasing strains.

A printed graph of the pressure–volume curve for each inflation–deflation cycle was used to calculate hysteresis. A planimeter (Gelman Instrument Co.) was used in each trial to make measurements of the areas underneath the inflation and deflation curves on the stress–strain plots; hysteresis was calculated as the ratio of the difference between the two areas to the area of the inflation curve.

**Data analysis**

The minimum radius at the beginning of the inflation cycle was taken as the value of the external radius of the artery ($R_e$) while uninflated and unstressed. Using this unstressed radius, along with the unstressed wall thickness, pressure and external radius for each data point, the circumferential wall stress and strain for each pair of pressure and diameter data points could be calculated. Circumferential stress ($\sigma_c$) is given by:

$\sigma_c = \frac{Pr}{h}$,

where $P$ is the intraluminal pressure in Pascals, $r_i$ is the internal radius in m at that particular pressure, and $h$ is the corresponding wall width in m. The internal radius and instantaneous wall width were calculated by assuming that the total cross-sectional area of the wall was constant. Circumferential strain ($\varepsilon_c$) is given by:

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**Statistical analyses**

Means and standard deviations of the tangential elastic modulus and hysteresis values were calculated for each species at 5, 10 and 20°C. The means of the tangential elastic moduli from the static data were compared at the three temperatures, using one-way analysis of variance (ANOVA, $P=0.05$); this process was repeated for the hysteresis data. In cases where significant differences did exist between species, Tukey’s test was performed to test for significant differences between each pair of individual means. Finally, the regressions of the temperature–modulus and temperature–hysteresis plots were examined using one-way ANOVA ($P=0.05$) to test whether temperature changes influenced modulus and hysteresis.

**Microscopy**

Tissues for light microscopy were fixed in either Bouin’s fluid or 10% isotonic buffered formalin for 24–48 h, dehydrated in an ethanol series, cleared in xylene and embedded in Tissue Prep (Fisher Scientific). The tissue was orientated such that transverse and longitudinal sections could be cut.

Serial 6 µm thick sections were mounted on glass slides and stained with Verhoeff’s iodine–iron haematoxylin for fully developed elastic fibres (Montes, 1992), standard Weigert’s resorcin–fuchsin for demonstrating elaunin fibres (Montes, 1992), modified Weigert’s technique for demonstrating oxytalan fibres and lamprey and hagfish ventral aorta which are known, from ultrastructural studies, to contain only oxytalan-like fibres (Wright, 1984; DeMont and Wright, 1993).

Positive controls for elastic fibre staining consisted of sections of rat aorta which are know to give positive reactions for the elastic, elaunin and oxytalan stains and lamprey and hagfish ventral aorta which are known, from ultrastructural studies, to contain only oxytalan-like fibres (Wright, 1984; DeMont and Wright, 1993).

Aortae from the lobster, horseshoe crab and whelk were fixed for electron microscopy in either 2.5% glutaraldehyde in sea water or 2.5% glutaraldehyde in 0.1 mol l⁻¹ cacodylate buffer at pH 7.3 for 24 h at room temperature (20°C). They were then rinsed in sea water or 0.1 mol l⁻¹ cacodylate buffer and post-fixed for 1 h in 2% OsO₄ in either sea water or cacodylate buffer at room temperature (20°C). All tissues were then washed in sea water or buffer, dehydrated in an ethanol series and propylene oxide and embedded in Epon/Araldite. Sections (0.5 µm thick) were mounted on glass slides and stained with 1% Toluidine Blue and lead citrate and were examined and photographed using a Hitachi-600 electron microscope operated at 75 kV. Dimensions of specific structures were measured from 20 representative examples of the structures in at least five micrographs at 95 000× magnification.

**Results**

**Mechanical properties**

All of the arteries, with the exception of those of the whelk,
Table 1. Mean tangential circumferential elastic modulus $E_c$ and hysteresis values for different species under different temperature conditions

<table>
<thead>
<tr>
<th>Animal</th>
<th>Temperature ($°C$)</th>
<th>$10^4 \times E_c$  (N m$^{-2}$)</th>
<th>Hysteresis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamprey</td>
<td>5</td>
<td>6.3±0.6 (5)</td>
<td>17.3±2.0 (6)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5.3±1.1 (29)</td>
<td>17.0±4.0 (24)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5.2±1.3 (13)</td>
<td>18.0±5.7 (23)</td>
</tr>
<tr>
<td>Hagfish</td>
<td>5</td>
<td>5.1±1.5 (26)</td>
<td>15.6±6.9 (26)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>5.3±2.2 (16)</td>
<td>12.7±3.8 (16)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4.6±1.0 (12)</td>
<td>13.0±4.6 (12)</td>
</tr>
<tr>
<td>Lobster</td>
<td>5</td>
<td>4.2±0.8 (6)</td>
<td>18.0±1.1 (2)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2.7±0.7 (16)</td>
<td>13.6±5.7 (15)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.2±0.9 (23)</td>
<td>13.7±4.3 (20)</td>
</tr>
<tr>
<td>Horseshoe crab</td>
<td>10</td>
<td>9.2±2.5 (6)</td>
<td>24.8±6.3 (6)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3.3±2.3 (8)</td>
<td>27.6±4.8 (8)</td>
</tr>
<tr>
<td>Whelk</td>
<td>5</td>
<td>14.8±7.2 (2)</td>
<td>24.3±7.7 (7)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>16.7±9.8 (12)</td>
<td>21.8±7.2 (16)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>11.2±4.0 (12)</td>
<td>30.9±8.8 (11)</td>
</tr>
</tbody>
</table>

Values are means ± s.d. (N).

Table 2. Increases in circumferential elastic modulus with increases in pressure for different species at 10°C

<table>
<thead>
<tr>
<th>Species</th>
<th>Low pressure</th>
<th>High pressure</th>
<th>Factorial increase</th>
<th>Pressure range (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamprey</td>
<td>4.0×10³</td>
<td>2.5×10⁶</td>
<td>63</td>
<td>1.0–16.0</td>
</tr>
<tr>
<td>Hagfish</td>
<td>5.3×10³</td>
<td>1.6×10⁶</td>
<td>30</td>
<td>1.8–15.0</td>
</tr>
<tr>
<td>Lobster</td>
<td>5.4×10⁴</td>
<td>8.0×10⁵</td>
<td>15</td>
<td>1.4–8.0</td>
</tr>
<tr>
<td>Whelk</td>
<td>2.7×10⁴</td>
<td>2.7×10⁵</td>
<td>10</td>
<td>1.2–6.5</td>
</tr>
<tr>
<td>Horseshoe crab</td>
<td>1.3×10⁴</td>
<td>1.6×10⁵</td>
<td>12</td>
<td>0.5–2.0</td>
</tr>
</tbody>
</table>

showed elastic behaviour upon dissection, recoiling to a shorter length than had been measured in vivo. However, the degree of recoil was not measured for comparison with the in vivo length. Results from the mechanical testing (mean values ± s.d. for the tangential elastic modulus and hysteresis values for each species, at three different temperatures) are given in Table 1. At physiological pressure ranges, the moduli of the aortae of each species at different temperatures were found to be of the order of $10^4$ N m$^{-2}$, with the exception of that of the whelk, which was of the order of $10^5$ N m$^{-2}$. The lobster, lamprey and hagfish showed fairly small energy losses per inflation–deflation cycle (hysteresis), between 13 and 18 %, while the horseshoe crab and whelk were much less efficient, only returning approximately 21–30 % of the inflation energy. Fig. 1 shows examples of the pressure–volume curves used to generate the hysteresis data; the area underneath the upper (inflation) curves represents the energy required to inflate the arteries, while the area under the lower curves represents the energy recovered through passive recoil. The differences in hysteresis are found by comparing the areas between these curves. The mean resting blood pressure values or ranges are indicated on the curves. Blood pressure values for the hagfish and lobster are taken from Prosser (1991), values for the horseshoe crab are from Vreugdenhil and Redmond (1987) and the range for the lamprey is given in DeMont and Wright (1993). No blood pressure data were available for the giant whelk Busycon contrarium. Systolic pressure of the whelk Busycon canaliculatum is estimated by Smith (1985) as being approximately 900 Pa, but this is not based on direct measurements, but projected from knowledge of other gastropod species, and so is not plotted in Fig. 1.

Nonlinearity was clearly present in almost all of the stress–strain curves and occurs most clearly and most dramatically in the cases where the artery was inflated to high pressures. This effect was most pronounced in the lamprey and hagfish, where the modulus (slope of the stress–strain curve) rose quite steeply when compared with the more moderate increases in the moduli of the lobster and horseshoe crab (Fig. 2A). In all cases, the increase in stiffness can be seen to begin at pressures not far above the resting mean blood pressure of each animal, which is indicated on each curve. The distensibility of the tissues from the different species is also illustrated here; the lobster vessel was able to expand the furthest, while the horseshoe crab showed intermediate strains and the lamprey and hagfish showed the least increase in radius of these four animals. The whelk aorta was quite different in this respect, increasing in stiffness almost as much as the horseshoe crab, but over a strain range of only 0–0.25 (Fig. 2B; this curve appears qualitatively different from that of the horseshoe crab because of differences in scale). The circumferential elastic moduli at both the low- and high-pressure ends of these curves are given in Table 2, along with the factorial increase in modulus between these two extremes and the pressure ranges to which the vessels were subjected.

At each temperature, significant differences were found between the means of the moduli of different species. The relationships between these means, given by Tukey’s test, are shown in Table 3; all species which were not significantly different from each other are underlined in a group; all the members of each underlined group show significant differences from members of the other groups. Significant differences were also found between the hysteresis means (Table 4). The whelk, horseshoe crab, or both, showed slightly different properties from the other three species in many cases.

Temperature had no significant effects (within the experimental range of 5–20°C) on hysteresis in any of the species tested. The tangential elastic modulus was also unaffected in most species; effects were significant only for the horseshoe crab because of differences in scale). The circumferential elastic moduli at both the low- and high-pressure ends of these curves are given in Table 2, along with the factorial increase in modulus between these two extremes and the pressure ranges to which the vessels were subjected.

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Temperature had no significant effects (within the experimental range of 5–20°C) on hysteresis in any of the species tested. The tangential elastic modulus was also unaffected in most species; effects were significant only for the horseshoe crab, where the elastic modulus decreased with increasing temperature ($F_{calc,1,12}=11.97$, $F_{0.05,1,12}=4.75$; $P<0.05$).

**Microscopy**

**Lobster**

The wall of the dorsal abdominal aorta consists of three distinct layers (Fig. 3A). Closest to the lumen is an acellular...
internal lamina (2–7 μm thick). A thick middle lamina contains many large fibroblasts between a dense network of anastomosing fibres. Bundles of obliquely orientated striated muscle are present within the middle lamina immediately adjacent to the internal lamina. An external lamina of dense connective tissue, rich in granulated cells, forms the outermost layer.

The internal lamina and fibres of the middle lamina contained oxytalan-like fibres, indicated by their staining with the modified Weigert’s technique (Fig. 3A). No elastin-containing fibres were present in the aorta wall, as demonstrated by the absence of any staining with either Verhoeff’s iodine–iron haematoxylin or standard Weigert’s resorcin–fuchsin. Acid proteoglycans were present in the

Fig. 1. Pressure–volume inflation–deflation curves for aortae from five different species at 10 °C. (A) Lamprey, (B) hagfish, (C) lobster, (D) horseshoe crab and (E) whelk. Mean resting blood pressures are shown by the open squares; two squares indicate an estimated range. Relative volume is given by $V/V_0$, where $V$ is the instantaneous volume of the vessel and $V_0$ is the volume at the pressure at which the inflation–deflation cycle was started.
internal lamina, between muscle cells beneath the internal lamina, and were most abundant in the external lamina. The internal lamina and fibres of the middle and external laminae were PAS-positive.

At the electron microscope level, the acellular internal lamina is a fibrous matrix composed of a mixture of unbranched fibrils that have a diameter of 20–35 nm and a dense meshwork of fine, branched filamentous material (Fig. 3B). The fibrils, in longitudinal profile, appear to have a beaded appearance with a periodicity of 40–52 nm (periodicity measured as the distance from the leading edge of one bead to the leading edge of the next bead; Fig. 3B,C). These fibrils are the primary components of the extracellular matrix of the middle lamina. The majority of fibrils are oriented at oblique angles to the short and long axes of the blood vessel wall, such that when they are viewed in cross section and longitudinal section they appear to be predominantly in a longitudinal arrangement. Many of the fibrils are aligned laterally to form fibres that course between and around the cells within the middle lamina.

The extracellular matrix of the external lamina consists of fine filamentous material, similar to that found in the internal lamina, and thin collagen fibrils, 12–15 nm in diameter and with poorly defined striations (Fig. 3D).

**Horseshoe crab**

The wall of the anterior aorta initially appears, at the light microscopic level, to be quite similar to that of the lobster aorta (compare Figs 2A and 3A). However, there are subtle differences in all three layers compared with the lobster vessel wall. The acellular internal lamina is very thin. The majority of the wall consists of a thick middle lamina of dense fibres.

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**Table 3. Interspecific differences with respect to mean tangential elastic modulus at different temperatures**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Species groupings</th>
<th>F-statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Lamprey Hagfish Lobster Whelk</td>
<td>$F_{\text{calc},3,37}=26.4^*$</td>
</tr>
<tr>
<td>10</td>
<td>Lobster Lamprey Hagfish Horseshoe crab Whelk</td>
<td>$F_{\text{calc},4,72}=26.4^*$</td>
</tr>
<tr>
<td>20</td>
<td>Lamprey Hagfish Horseshoe Lobster crab</td>
<td>$F_{\text{calc},4,63}=32.1^*$</td>
</tr>
</tbody>
</table>

Members of underlined groups show no significant differences with respect to others in the group, but show significant differences with respect to the members of other groups.

* indicates significantly different ($P<0.05$) $F$-statistics calculated using one-way ANOVA.

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**Table 4. Interspecific differences with respect to mean hysteresis at different temperatures**

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Species groupings</th>
<th>F-statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Whelk Lobster Lamprey Hagfish</td>
<td>$F_{\text{calc},3,36}=6.89^*$</td>
</tr>
<tr>
<td>10</td>
<td>Lamprey Hagfish Lobster Whelk Horseshoe crab</td>
<td>$F_{\text{calc},4,70}=10.9^*$</td>
</tr>
<tr>
<td>20</td>
<td>Lamprey Hagfish Lobster Whelk Horseshoe crab</td>
<td>$F_{\text{calc},4,62}=18.9^*$</td>
</tr>
</tbody>
</table>

Members of underlined groups show no significant differences with respect to others in the group, but show significant differences with respect to the members of other groups.

* indicates significantly different ($P<0.05$) $F$-statistics calculated using one-way ANOVA.

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Fig. 2. (A) Stress–strain inflation curves for the aortae of four different species at 10 °C, showing increases in slope (modulus). Mean resting blood pressures are marked on each curve. (a) Lamprey (filled circle), (b) hagfish (triangle), (c) lobster (square) and (d) horseshoe crab (open circle). (B) Stress–strain inflation curve for the aorta of the whelk, also showing an increase in slope (modulus). This curve was plotted separately as the increase occurs over a much smaller strain range than for the other four species.
that form interconnecting, undulating lamellae. Fibroblasts and thick striated muscle cells are dispersed throughout the lamina between the fibrous lamellae. The striated muscle is orientated longitudinally and obliquely. The external lamina consists of loose connective tissue containing numerous granulated cells.

The internal lamina and fibres of the middle lamina stained positively using modified Weigert’s technique, indicating the presence of oxytalan-like fibres (Fig. 4A). No regions of the aorta stained for elastin-containing fibres. Only the internal and external laminae stained for acid proteoglycans. The internal lamina, fibres of the middle lamina and components of the external lamina were PAS-positive.

Ultrastructurally, the thin internal lamina and the extracellular matrix of the middle lamina consist of fibrils and of filamentous material, similar to that observed in the lobster (Fig. 4B,C). The fibrils are 20–25 nm in diameter and have a beaded appearance, with no obvious periodicity. Within the middle lamina, many of the fibrils are densely packed together.
to form coiled fibres (Fig. 4C,D). Groups of coiled fibres are
arranged to form larger undulating lamellae that are separated
by filamentous material and fibroblasts or striated muscle
cells.

The loose connective tissue of the external lamina consists
of collagen fibrils and fine filamentous material.

**Whelk**

The wall of the whelk aorta appears quite different from that
of the lobster and horseshoe crab (Fig. 5A). There is no
obvious internal lamina and the majority of the wall thickness
is composed of a dense, irregular arrangement of smooth
muscle cells (oriented circumferentially, longitudinally and
obliquely) surrounded by thin fibres. Fibroblasts and granulated cells are scattered between the muscle cells. An external lamina of loose connective tissue lacks the fibres associated with the muscle layer.

The fine fibres associated with the smooth muscle throughout the main portion of the aorta wall stained positively using modified Weigert’s technique (Fig. 5A); no elastin-containing fibres were present. All extracellular material in the aorta wall stained for acid proteoglycans. Only the smooth-muscle-associated fine fibres stained positively with PAS.

At the electron microscopic level, the extracellular matrix surrounding and between smooth muscle cells contained collagen fibrils 20–45 nm in diameter with obvious cross-striations, branched fine filamentous material and aggregates

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Fig. 5. (A) Light micrograph of a transverse section of whelk aorta stained using modified Weigert’s technique. Positively stained fibrous material surrounds smooth muscle cells (sm), which contribute to most of the wall thickness. The external lamina (el) contains no positively stained fibres. Lumen (l). Scale bar, 30 μm. (B) Electron micrograph showing a portion of a smooth muscle cell (sm) and the extracellular matrix containing electron-dense fibres (fi). Scale bar, 800 nm. (C) Electron-dense fibres (fi) of the extracellular matrix. Note the region of the fibres that shows a distinct periodicity (p). Scale bar, 320 nm. (D) Higher magnification of an electron-dense fibre from C showing the prominent striations in one region (arrowheads). Scale bar, 164 nm.
Measurements of the elastic modulus gave values below $10^4 \text{Nm}^{-2}$ for the octopus aorta, consistent with those for other invertebrate species. The slightly lower than normal vertebrate values, these results are in agreement with previously reported values (29±7%: Vreugdenhil and Redmond, 1987) and for the lobster is only slightly lower than the value of 20% found by Shadwick et al. (1990). Again, for the lamprey, our results were much lower than those obtained by DeMont and Wright (1993), who give values as high as 50%. These values, especially for the lamprey, hagfish and lobster, mean that the bulk of the expansion energy would be returned during recoil, pushing the blood along in the vessel; very little energy would be lost as heat.

It is the combination of these two properties, the ability to expand and store elastic strain energy and the ability to recoil with minimal loss of this energy, which enables the arteries of higher vertebrates to help to smooth the blood flow in the peripheral circulatory system. All of the vessels tested showed these properties to a significant extent, suggesting that in these animals the aortae perform the same function, smoothing pressure pulses from the heart. This analogy is strongest in the lamprey and hagfish, which have closed circulatory systems and tissue architectures which closely parallel those of higher vertebrates. Despite the fact that the other animals we studied have a partially open circulatory system and have tissue architectures very different from those of higher vertebrates, the presence of these properties seems to indicate that pulsatility of blood flow is still a physiological problem for these animals, which can be solved, at least in part, by the use of the blood vessels as elastic reservoirs.

The observation that stiffness increases at high strains implies that these organisms may also be protected from artery wall rupture, as is true for higher vertebrates (Gosline and Shadwick, 1983). Increases in circumferential elastic modulus by at least an order of magnitude, which were observed in all five species, would help to prevent the vessels from bursting at higher pressures. This is further supported by the observation that the increase in stiffness begins within, or just above, the normal physiological pressure range, as can be seen in Fig. 2A. The presence of nonlinear elasticity in the lobster agrees with the findings of Shadwick et al. (1990). DeMont and Wright (1993) attributed the lack of nonlinearly elastic behaviour in the lamprey ventral aorta to reduced amounts of collagen, but it is likely that the pressures required to observe such behaviour were not reached in their study, as nonlinearity is clearly demonstrated here for the same species. The peak pressure in their study was 5.7 kPa, while significant increases in stiffness do not seem to occur until pressures of about 6–8 kPa are reached.

The general lack of temperature effects on the elastic modulus indicates that the aorta wall material provides a stable mechanical response over a wide range of temperatures. The decrease in the modulus of the horseshoe crab vessel at higher temperatures could mean that the physical structure of the aorta of this species contains some component, absent in the other species, which is affected by temperature.

As in vertebrates, the mechanical behaviour of the artery wall seems to be dependent on structure. The structure of the...
vessels of the species studied, however, varied drastically both from the typical vertebrate arrangement and from each other. Vertebrate artery tissue contains smooth muscle in a regular configuration (DeMont and Wright, 1993), but the tissue of the whelk contained smooth muscle in a random arrangement, and that of the lobster and horseshoe crab contained only striated muscle. The striated muscle in the lobster was confined to an area adjacent to the internal lamina. A structure similar to that of the lobster was observed in the shrimp *Sicyonia ingentis* (Martin et al. 1989) and the California spiny lobster *Panulirus interruptus* (Burnett, 1984).

One common factor found in all species except the whelk was the presence of fibrillar tissue, mainly in the internal and medial laminae, although the fibres were not identical in each species. The fibrils found in the lobster and horseshoe crab bear a strong resemblance to the microfibrils found in the extracellular matrix of agnathan and mammalian tissues, although they have a larger diameter. Mammalian microfibrils have a diameter of about 10–12 nm (Montes, 1992), compared with 11–17 nm for those found in the lamprey and hagfish (Wright, 1984) and between 20 and 35 nm for the fibrils found in the lobster and horseshoe crab. The beaded appearance of the fibrils, and the distinct periodicity in the case of the lobster (although shorter than that found in mammalian fibrils), suggests extensibility. The periodicity of mammalian microfibrils has been shown to change from 52 nm in unstressed tissue to 75 nm in stressed tissue (Keene et al. 1991). Aggregates of these fibrils form fibres in the lobster and the horseshoe crab aortae which are quite similar to oxytalan fibres in vertebrates; the first phase in the development of elastic fibres (Montes, 1992). Fibrils approximately 25 nm in diameter were found oriented predominantly in a circumferential direction in the dorsal abdominal aorta of the lobster by Shadwick et al. (1990), although a beaded appearance and periodicity were not mentioned. Fibrils 10–20 nm in diameter have also been described (Martin and Hose, 1992) as being oriented roughly parallel to the circumference of the ophthalmic artery of the shrimp (no species given) but, again, no note was made of a beaded appearance or periodicity. Beaded filamentous elements of invertebrate elastic fibres have been previously described (Elder, 1966). Another comparative study by Elder (1973) found spirit-blue-positive (non-collagenous) fibres in the anterior dorsal artery of the horseshoe crab, and electron microscopy revealed lamellae of longitudinally oriented fibres. Elder (1973) concluded that invertebrate elastic fibres most closely resemble oxytalan fibres, a conclusion previously drawn by Argaud (1908, 1909). The electron-dense fibres found in the whelk are the most unusual; no previous reference to them or to a material similar in appearance in the whelk, or in other invertebrates, could be found. The striated patterning in portions of the fibres is not dissimilar to that of collagen, with a smaller periodicity, but the fibres in the whelk vessel stained positively for oxytalan-like fibres, not for collagen. The pattern is also reminiscent of elastoidin, a collagen-like protein found in the fins of chondrichthyens and teleosts, appearing as large fibrils 1–5 μm in width and with a cross-striation periodicity of 67 nm (Mari-Beffa et al. 1989).

As the wall structures of the arteries of the lamprey and hagfish are very close to those of more advanced vertebrates, and as their mechanical responses are similar to each other and to those of higher vertebrates, it is likely that the corresponding tissue components behave in the same manner. No elastin is present (Wright, 1984), but the microfibrillar-based elastic tissue observed in these species would presumably be a compliant material similar to elastin, employed at lower extensions and providing the vessel with the capacity for expansion and recoil. This provides further support for the theory that these fibres are extensible. The stiffer collagen bundles, interspersed throughout this tissue, would be likely to be employed in carrying the load at higher extensions.

The dorsal abdominal artery of the lobster, although also containing both a microfibrillar material and a collagenous material, has these two components arranged in a different manner, with the collagenous layer in the external lamina. However, it still shows a nonlinear stress–strain curve which mirrors that of higher vertebrates, suggesting that it, too, could contain both an extensible and a stiffening component. This was also suggested by Shadwick et al. (1990). Again, the major elastic tissue component seems to be the oxytalan-like fibrillar material, found both in the internal and middle laminae. It seems likely that this tissue would provide the compliance of the vessel at low distensions. The outer collagen layer might contribute to the stiffness at higher pressures, being stressed at higher extensions.

A similar situation is found for the horseshoe crab, but other factors are also present. The internal lamina would probably contribute less to the overall properties of the vessel than in the lobster, as it is smaller and fibrils are found in a different configuration in the middle lamina. The presence of large undulating lamellae formed from coiled fibres might contribute to nonlinear elasticity in a manner similar to collagen fibres in vertebrates. As the horseshoe crab aorta begins to expand, these lamellae would not be directly stressed, but would merely be straightened and would offer little resistance to expansion. Once straightened, the fibres making up the lamellae would become stressed in response to further expansion, providing an increase in stiffness. These fibres are coiled, so straightening them might result in uncoiling, stretching them out to a more linear arrangement. Once this occurred, then the fibrils themselves could begin to be directly stretched, giving a further increase in stiffness. This effect would also be complemented by the stiffness of the collagen of the outer lamina, which might again give added resistance at higher extensions.

The most difficult case to interpret is that of the whelk, as the fibrillar material could not be identified, and very little can be assumed about its properties. The distensibility of the whelk aorta was markedly less than that of the other species, an observation best explained by the presence of collagen fibrils interspersed within the smooth muscle. Only a relatively small degree of nonlinearity was found in the whelk vessel. Like the
lobster and horseshoe crab, this could be due to contributions from the collagen in the external lamina at higher extensions. The aorta has an extremely large diameter (several millimetres in width) and might be capable of carrying blood flow sufficient for the requirements of the animal without significant wall extension.

Although there were large differences between the wall architecture of many of the species, the aortae demonstrated the same gross mechanical properties: extensibility at low pressures and stiffness at high pressures and the return of a large portion of the energy stored during inflation, sufficient for them to work as efficient elastic reservoirs which damp pressure oscillations. All of the vessels studied also showed nonlinear elasticity, becoming stiffer at high deformations. The extensibility, in most cases, seems to be provided by a fibrillar tissue serving a role similar to that of elastin in vertebrates. It is also possible that nonlinear elasticity is an intrinsic property of these microfibrils, as occurs in octopus arterial elastomer. Mechanical tests of individual fibrils could provide valuable information for the interpretation of the mechanics of the artery as a whole. Investigation of this problem is currently under way. More information about the unknown materials that are likely to be present in the whelk tissue would also aid in understanding its mechanical behaviour.

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