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Microscopic anatomy of the thin-walled vessels leaving the heart of the lobster *Homarus americanus:* anterior lateral arteries

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Abstract. The anterior lateral arteries are paired vessels leaving the anterior end of the lobster (*Homarus americanus*) heart and proceeding to the antennae and eyestalks, the stomach and hepatopancreas, the gonads, and the thoracic and branchial muscles. These vessels have a trilaminar organization, consisting of a tunica interna with elastic fibrils, a tunica intermedia represented by a bilayered cell mass, and a tunica externa with collagen fibrils. In the tunica intermedia, cells flanking the tunica interna (light cells) show less affinity for basic dyes and electron stains than those flanking the tunica externa (dark cells). Each light cell exhibits an irregularly shaped stress fiber (a bundle of closely packed microfilaments) in the region adjoining the tunica interna. Collectively, these bundles have a circumferential or slightly oblique orientation relative to the lumen of the vessel. The role of the stress fibers is unresolved. If they are static structures, they might contribute to the non-linear elasticity shown by lobster arteries. If they generate force, and small bundles of microfilaments do diverge from the stress fibers to enter filamentous mats applied to the plasmalemmata, a coordinated contraction of the cells might reduce the luminal diameter and, thus, retard the flow of hemolymph. Coordination of contraction would have to occur in the absence of nerves and without the benefit of communicating (gap) junctions between the light and dark cells.

Additional key words: F-actin immunohistochemistry, hemolymph vessel, microfilaments

The circulatory system of the lobster *Homarus americanus* H. Milne Edwards 1837 includes a muscular heart in the posterodorsal thorax that pumps hemolymph into seven arteries. These arteries distribute the hemolymph to arterioles, which in turn deposit it into lined capillaries and/or unlined, capillary-sized lacunae. There are no veins in this open circulatory system. Deoxygenated hemolymph collects in irregularly shaped sinuses and gradually returns, by way of the gills, to the pericardial sinus around the heart (Maynard 1960; McLaughlin 1983; Martin & Hose 1995; Wilkens et al. 1997a). Valvular openings (ostia) in the wall of the heart admit hemolymph from the pericardial sinus to the lumen of the organ (Yazawa et al. 1999).

On the basis of wall thickness, the lobster arteries fall into two categories (see McLaughlin [1980:140] for a diagram of the approximate dispositions and the relative sizes of the arteries). The thinner vessels include the paired anterior lateral arteries (antennal arteries), the anterior median artery (ophthalmic artery, cephalic aorta, median aorta, anterior aorta), and the paired hepatic arteries. These vessels have a trilaminar configuration, consisting of an inner lamina of elastic connective tissue, a middle lamina of layered cells, and an outer lamina of collagenous connective tissue (Burnett 1984; Martin & Hose 1995; Wilkens et al. 1997a). Owing to difficulties in locating and recovering these hemolymph vessels, relatively little else is known about their morphologies. The thicker vessels leaving the lobster heart, the dorsal abdominal artery and the sternal artery, are easier to locate and excise owing to their bore, length, and position. The majority of studies on hemolymph vessels in the lobster (Burnett 1984; Shadwick et al. 1990; Davison et al. 1995; Martin & Hose 1995) have used the dorsal abdominal artery.

The goals of our study were to characterize the morphologies of the thin-walled arteries leaving the lobster heart, to evaluate the potential of vascular cells for contraction through the use of F-actin immunohistochemistry, and to gauge the density of nerves to these vessels through application of a classical (methylene blue) staining technique. Coverage

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begins with the histology and ultrastructure of the anterior lateral arteries that supply hemolymph to the antennae and eyestalks, the stomach and hepatopancreas, the gonads, and the thoracic and branchial muscles (McLaughlin 1983; Martin & Hose 1995; Wilkens et al. 1997b). Companion papers will consider the anterior median artery and the hepatic arteries. Portions of the current article have appeared in abstracts (Cavey & Wilkens 1997, 2000).

Methods

Animals and dissection

Lobsters (*Homarus americanus*), weighing 400–600 g, were purchased from a local seafood wholesaler and maintained in recirculating artificial seawater at 12°C. Animals were anesthetized on ice before dissection. Hemolymph was flushed from the vessels by perfusion of the circulatory system with chilled saline (pH 7.6; Cole 1941).

Histology and ultrastructure

The anterior lateral arteries were exposed adjacent to the heart, and the proximal 2–3 cm of each vessel were excised and immersed promptly in a primary fixative (2.5% glutaraldehyde in 0.20 M Millonig's phosphate buffer [pH 7.4] containing 0.14 mol L^{-1} sodium chloride; Cloney & Florey 1968). The arteries were cut transversely into short segments with razor blades, and the segments were transferred to fresh fixative for 45 min at ambient temperature. Specimens were exposed, without rinsing, to a secondary fixative (2% osmium tetroxide in 1.25% sodium bicarbonate buffer [pH 7.2]; Wood & Luft 1965) for 45 min at 0°–4°C. Osmicated specimens were rinsed briefly with demineralized water, dehydrated with a graded series of ethanol, treated with propylene oxide, and infiltrated and embedded in Epon (Luft 1961) or LX-112 epoxy resin (Ladd Research Industries, Williston, VT, USA). The substrates were polymerized for 18 h at 60°C.

Thin sections (1 μm in thickness) were cut with a diamond knife on a Sorvall MT-2B ultramicrotome (DuPont Company, Newtown, CT, USA), affixed to microscope slides by heating, and stained with an alkaline solution of azure II and methylene blue (Richardson et al. 1960). Cover glasses were mounted with high-viscosity immersion oil. Sections were viewed and photographed with a Nikon Optiphot compound microscope (Nikon Corporation, Tokyo, Japan) equipped with planachromatic objective lenses and a Nikon Coolpix 5000 digital camera. The microscope was calibrated with a stage micrometer (100 lines mm^{-1}).

Specimens for transmission electron microscopy were polymerized for an additional 6 h, and ultrathin sections (70 nm in thickness) were cut with a diamond knife on a Sorvall MT-6000 ultramicrotome. Ultrathin sections were collected on naked copper grids and serially stained with aqueous solutions of uranyl acetate (saturated) and lead citrate (Reynolds 1963). The sections were observed and photographed with a Hitachi H-7000 transmission electron microscope (Nissei Sangyo Company, Tokyo, Japan) operated at 75 kV. The microscope was calibrated with a carbon replica of a diffraction grating (2,158 lines mm^{-1}). Electron micrographs were made with Kodak 4489 electron microscope film (Eastman Kodak Company, Rochester, NY, USA).

F-actin immunohistochemistry

Rhodamine phalloidin (Catalog No. R-415; Molecular Probes, Eugene, OR, USA), a fluorescent phallotoxin probe for fibrous actin (Wieland 1986), was applied to spreads of the lobster arteries. Freshly dissected vessels were cut longitudinally with iridectomy scissors, opened onto cover glasses, ringed with petroleum jelly, and flushed with saline. A solution of 3.7% formaldehyde in saline was added to the reservoirs for 10 min. The spreads were subsequently washed with saline for 2 min, permeabilized with 0.1% Triton X-100 in saline for 5 min, washed with saline, incubated in 1% bovine serum albumin in saline for 30 min, and stained with double-strength rhodamine phalloidin stock for 20 min. The spreads were then washed with saline, and the cover glasses were mounted on microscope slides with 1:1 glycerol:saline. As a control, the rhodamine phalloidin stock was omitted from the protocol.

The whole-mounted vessels were viewed and photographed with a Nikon Eclipse TE300 inverted fluorescence microscope. The microscope was calibrated with a stage micrometer (100 lines mm^{-1}). Photomicrographs were made with Kodak Ektachrome 100 film.

Localization of nerves

Freshly dissected vessels were cut longitudinally with iridectomy scissors, spread onto dental wax, and pinned. The spreads were immersed in a dilute staining solution (2–3 drops of 0.1% methylene blue [aqueous] in 150 mL saline) and refrigerated for ~24 h in the dark (Davidson et al. 1998).

Pinned spreads were observed and photographed directly with a Nikon SMZ800 stereomicroscope

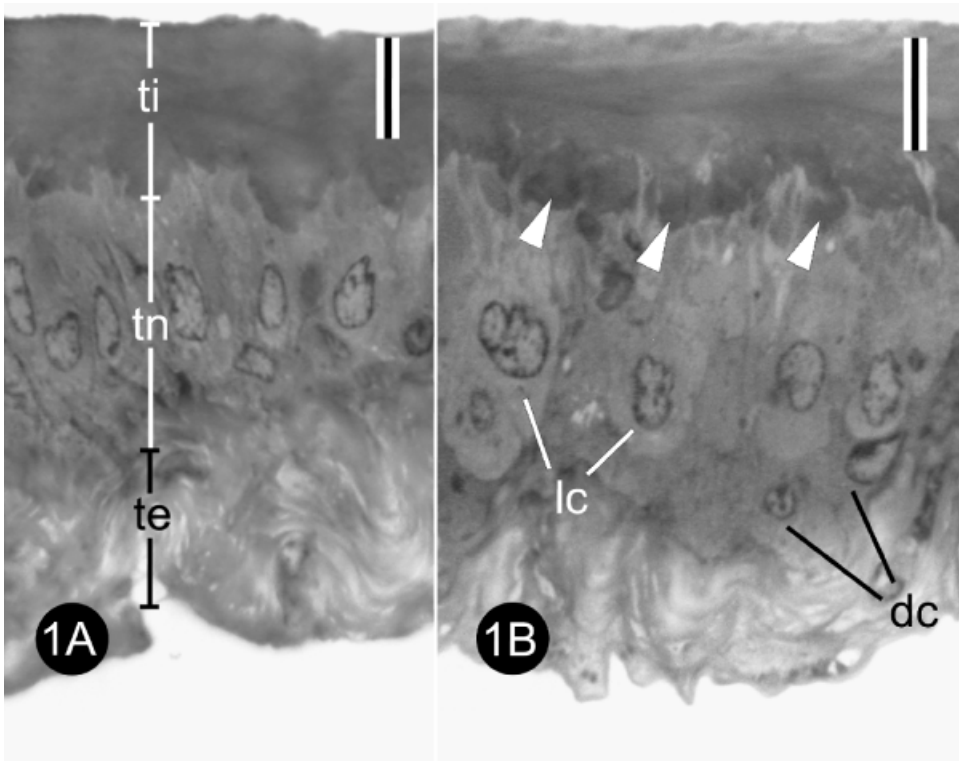

Fig. 1. Histology of the anterior lateral arteries in transverse (**A**) and longitudinal (**B**) planes of section. An artery consists of a tunica interna (ti), a tunica intermedia (tn), and a tunica externa (te). Lateral cells of the tunica intermedia (dc, dark cell) stain more intensely than medial cells (lc, light cell). Stress fibers (arrowheads) are detectable in the exposed ends of the light cells. Scale bars, 10 μm (A,B).

equipped with a Nikon Coolpix 5000 digital camera. The spreads were later retrieved from the dental wax and transferred to microscope slides. Supported cover glasses were added before viewing and photographing the preparations with the compound microscope using Nomarski differential interference-contrast optics.

Image processing and printing

Our digital cameras were set for maximum quality, yielding images that measured 2,560 × 1,920 pixels; images were stored as TIFF (tagged image file format) files with no compression. Negatives generated with the transmission electron microscope and color transparencies taken at the fluorescence microscope were subsequently digitized with a Polaroid SprintScan 45i film scanner (Polaroid Corporation, Cambridge, MA, USA) at a resolution of 600 dpi. Scanned images were also stored as uncompressed TIFF files.

The digital images, regardless of source, were adjusted and printed with "Photoshop CS (8.0) for Windows" software (Adobe Systems, San Jose, CA, USA). Measurements on the digital images were made with "SigmaScan Pro 5.0 for Windows" software (Systat, Point Richmond, CA, USA). Measurements in the text express either a range or a mean ± standard deviation ($n = 50$).

Results

Descriptions in this article pertain to proximal segments of the anterior lateral arteries (within 2–3 cm of the heart and before any major bifurcations). These arteries, like other hemolymph vessels leaving the lobster heart, have a trilaminar organization (Fig. 1). We refer to these laminae as the tunica interna, the tunica intermedia, and the tunica externa. The tunica interna and the tunica externa encompass elastic connective tissue and collagenous connective tissue, respectively. The tunica intermedia is distinguished by a high concentration of cells. The total wall thickness of an anterior lateral artery ranges 49–73 μm, and the thicknesses of its three laminae are 12.7 ± 1.2 μm (tunica interna), 26.0 ± 2.1 μm (tunica intermedia), and 17.9 ± 3.0 μm (tunica externa). Thick histological sections of paraffin-embedded vessels have been

used in determining the radius of an unpressurized anterior lateral artery to be ~1.5 mm (Wilkens et al. 1997a). Anterior lateral arteries are the largest of the thin-walled vessels in terms of radius.

Spreads incubated in dilute methylene blue revealed no nerves in any of the tunics of the anterior lateral arteries.

Tunica interna

The tunica interna has a tripartite organization, consisting of a thin superficial zone next to the lumen, a thick middle zone, and a moderately thick deep zone next to the tunica intermedia (Fig. 2A). Filamentous material is prevalent in the superficial

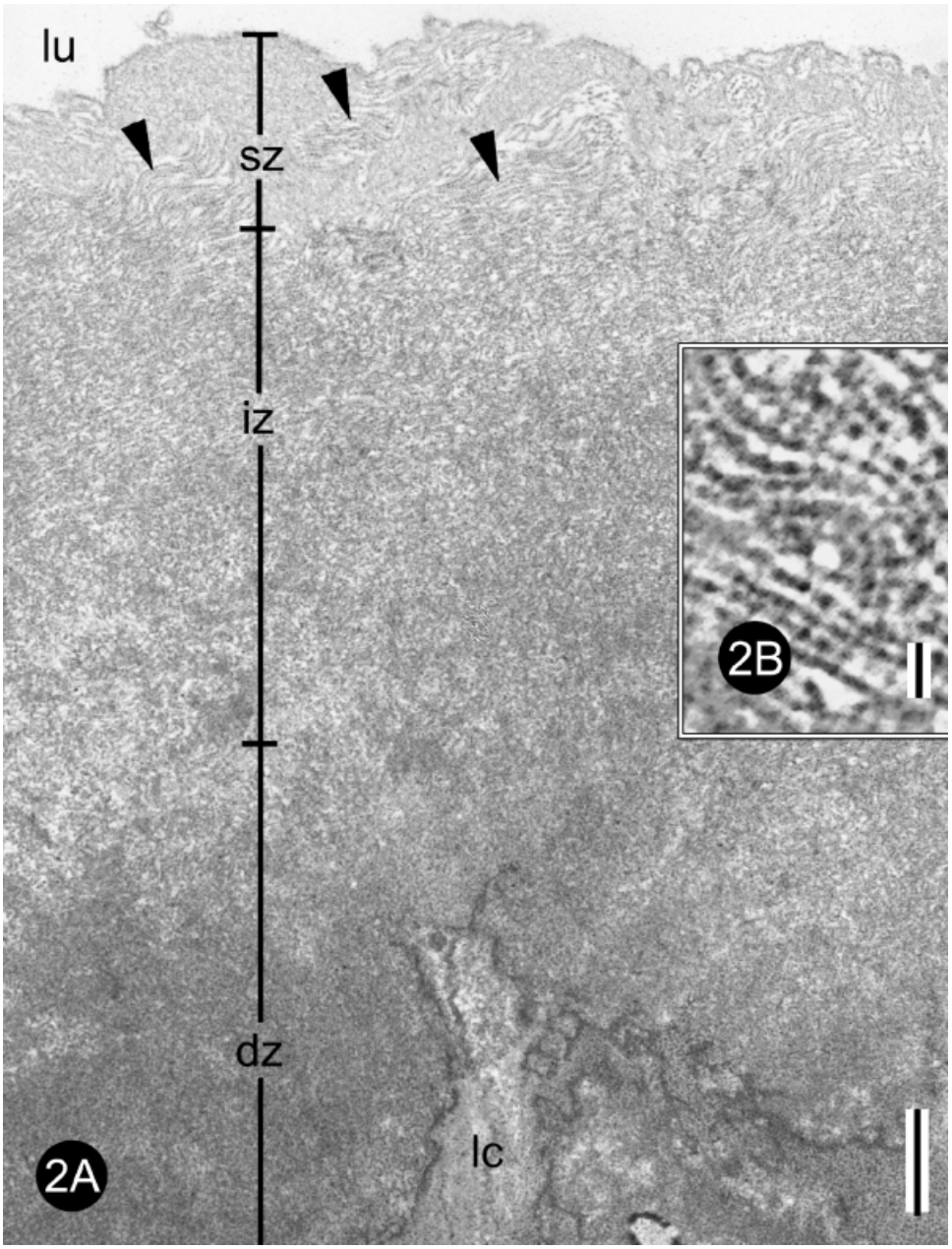

Fig. 2. Ultrastructure of the tunica interna in a transversely sectioned artery. **A.** The superficial (sz) and deep (dz) zones of the connective tissue have a filamentous composition, and the intermediate zone (iz) consists of many transversely sectioned elastic fibrils. Some obliquely oriented fibrils (arrowheads) appear where the intermediate zone meets the superficial zone. The superficial zone adjoins the lumen (lu) of the vessel, and the exposed ends of light cells (lc) in the tunica intermedia send processes into the deep zone. **B.** Elastic fibrils have a ''beaded'' appearance. Scale bars, 1 μm (A), 100 nm (B).

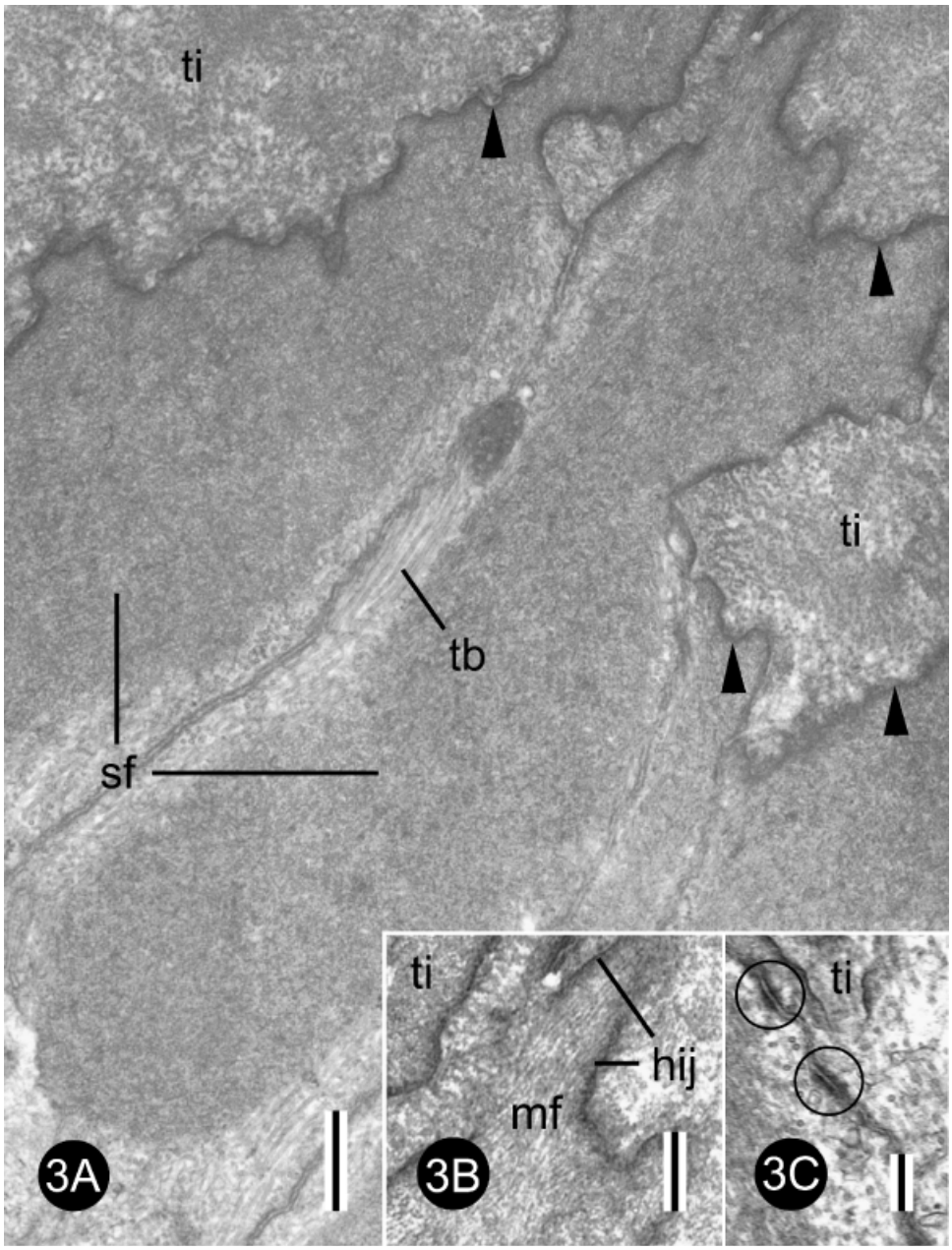

Fig. 3. Ultrastructure of the tunica intermedia in a longitudinally sectioned artery. **A.** Microfilament bundles (sf, stress fiber), surrounded by microtubular sheaths (tb), occupy the exposed ends of light cells. These bundles are easily identified in histological sections; see Fig. 1B. Light cells interdigitate with filamentous material in the deep zone of the tunica interna (ti); see also Fig. 2A. Extensive filamentous mats are applied to the cytoplasmic surfaces of the exposed plasmalemmata (arrowheads). **B.** Microfilaments (mf) span from the stress fiber to the filamentous mat beneath the plasmalemma at the exposed end of a light cell (hij, hemi-intermediate junction). ti, tunica interna. **C.** Bilateral intermediate junctions (open circles) are formed where light cells contact one another. ti, tunica interna. Scale bars, 500 nm (A), 250 nm (B,C).

and deep zones, while delicate elastic fibrils constitute the middle zone. The fibrils tend to orient longitudinally with respect to the primary axis of a vessel. Consistent with earlier reports on the dorsal abdominal artery (Davison et al. 1995), the fibrils have a ''beaded'' appearance (Fig. 2B); their subunits are 22.3 ± 1.7 nm in diameter and 46.7 ± 2.5 nm in length.

Tunica intermedia

The cells of the tunica intermedia are distributed in layers between the tunica interna and the tunica externa. In most sectors of the tunica intermedia, there are two layers of cells; occasionally, one encounters a sector where just a single cell spans from the tunica interna to the tunica externa or a sector where three cells span the distance. Where the tunic is bilayered, medial cells adjoin the tunica interna, and lateral cells adjoin the tunica externa (Fig. 1). The medial cells show less affinity for basic dyes and electron stains than the lateral cells. Hereafter, the medial cells will be called *light cells,* and the lateral cells will be called *dark cells*. There is no morphological evidence of a basal lamina between the tunica interna and the light cells, between the light cells and the dark cells, or between the dark cells and the tunica externa.

Irregularly shaped processes appear on all surfaces of a light cell. Slender processes from the exposed cell surface enter the deep zone of the tunica interna (Figs. 2A, 3A). Broader processes on the other surfaces of a light cell interdigitate with the processes of neighboring light cells and with the bodies and processes of outlying dark cells.

The cytoplasm of a light cell is polarized. A large, irregularly shaped bundle of closely packed filaments (stress fiber) occupies the cytoplasm near the exposed end of a cell (Fig. 3A). The filaments have a diameter of 6.4 ± 1.4 nm, and the orientation of a bundle, like the cell in which it resides, is circumferential or slightly oblique with respect to the arterial lumen (Fig. 3B). When spreads of the anterior lateral arteries are exposed to rhodamine phalloidin, the bundles fluoresce brightly (Fig. 4), providing additional evidence that these filaments are microfilaments. Microtubules, measuring 26.5 ± 1.4 nm in diameter, flank the microfilament bundles (Fig. 3A).

Microfilaments commonly diverge from the main axis of a stress fiber and span to nearby sectors of the plasmalemma. A narrow filamentous mat, measuring 37–43 nm in thickness, abuts the cytoplasmic surface of the plasmalemma on the exposed end of a light cell (Fig. 3A,B). Such contacts have some of the structural attributes of intermediate junctions, so they might be described as *hemi-intermediate junctions*. Focal mats of similar appearance occur on the bounded cell surfaces nearby. Alignment of the mats in adjoining light cells gives the impression of a typically configured intermediate junction (Fig. 3C). The filamentous mats in both locations interact with microfilaments (Fig. 3B), so they could be sites where the cell surface is stabilized or where the tension generated by the stress fiber is exerted on the cell membrane.

The cytoplasm between the stress fiber and the nucleus of a light cell is undistinguished. This region is characterized by cytoplasmic folds that interdigitate with corresponding folds from neighboring light cells (Fig. 5). Small bundles of microfilaments emerge from the stress fiber and enter the cytoplasmic folds. The nucleus of a light cell adjoins the region of cell infolding and interdigitation (Fig. 6). The nucleus is irregularly shaped and indented, and it usually exhibits a pair of nucleoli. A perforated envelope encloses the nucleoplasm. Condensed chromatin adheres to the inner membrane of the nuclear envelope, and evaginations of the outer membrane are continuous with cisternae of the granular (rough) endoplasmic reticulum. One or more Golgi bodies

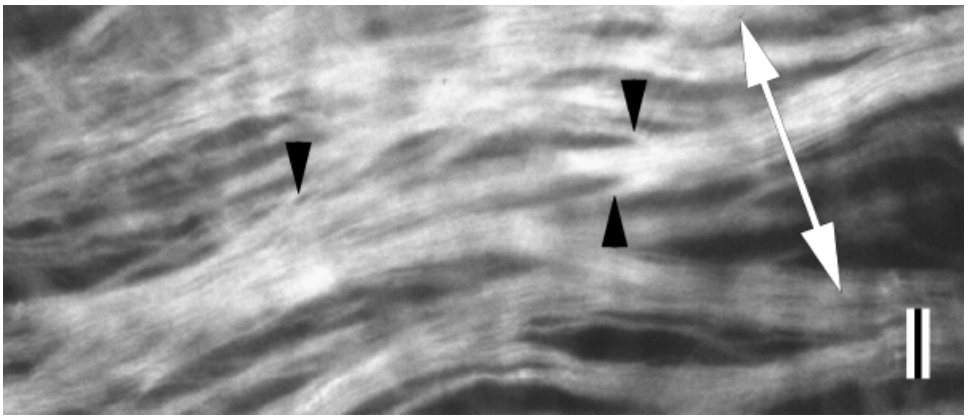

Fig. 4. F-actin immunohistochemistry on an arterial spread. Strong F-actin signals are generated by the stress fibers in the light cells of the tunica intermedia. The primary axis of the vessel is indicated by the double-headed arrow in the photomicrograph. The stress fibers have a circumferential or slightly oblique orientation relative to the lumen. Small fascicles of filaments diverge from the main bundles (arrowheads). Scale bar, 50 μm.

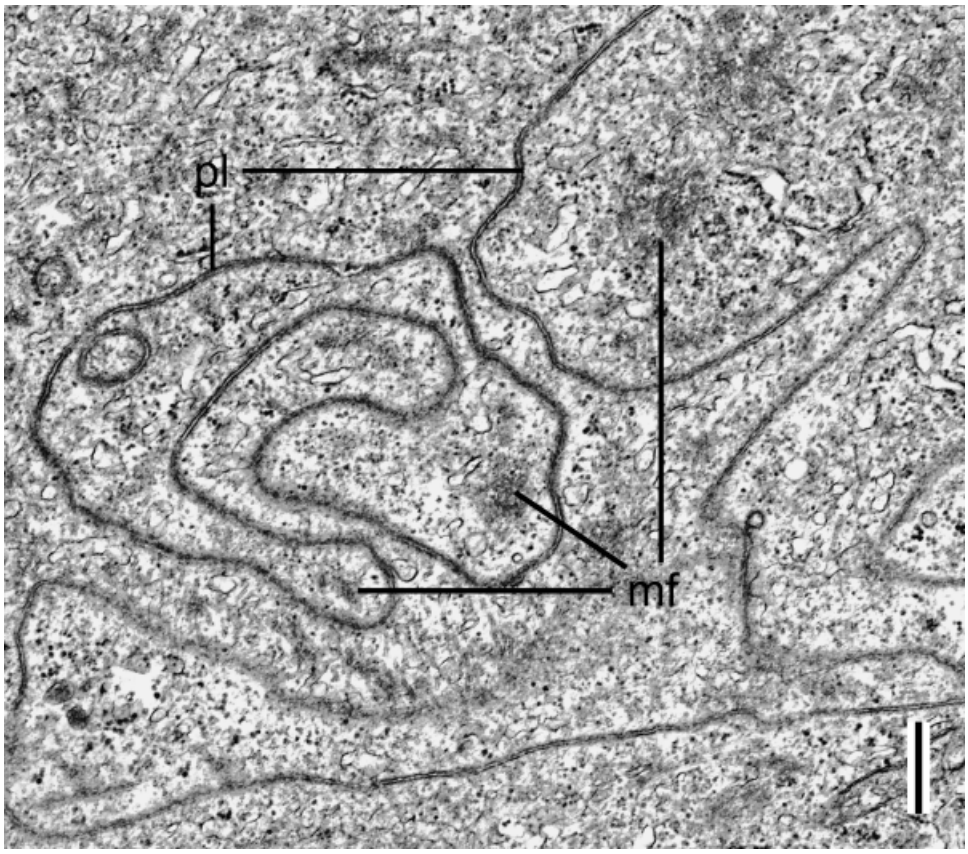

Fig. 5. Ultrastructure of the tunica intermedia in a longitudinally sectioned artery. Infolding and interdigitation of light cells (pl, plasmalemma) are prevalent in the region between stress fiber and nucleus. Small fascicles of microfilaments (mf) diverge from the stress fibers to enter the cytoplasmic folds. Scale bar, 500 nm.

appear in the perinuclear cytoplasm. Lumina of the Golgi cisternae and nearby vesicles contain an electron-dense material, indicating that the organelles are synthetically active. Numerous glycogen granules, spherical and slightly oblong mitochondria with poorly developed cristae, and short cisternae of granular endoplasmic reticulum also occur in the cytoplasm around the nucleus. Large, membrane-bounded vacuoles with heterogeneous contents are sometimes evident.

The deep sector of a light cell bears a number of similarities to the region of cell infolding and interdigitation. Numerous glycogen granules are dispersed in the cytoplasm, along with short cisternae of granular endoplasmic reticulum and small mitochondria. Large, relatively broad processes of a light cell extend outward and interdigitate with the bodies and processes of dark cells (Fig. 7).

The dark cells do not have the pronounced cytoplasmic polarity shown by the light cells (Fig. 8A). The large and irregularly shaped nucleus of a dark cell is located centrally in the cytoplasm. It may exhibit multiple nucleoli, and it shows a prominent layer of heterochromatin along the inner membrane of the nuclear envelope; pillars or plates of condensed chromatin span from one side of the nucleus to the other. The perinuclear cytoplasm of a dark cell contains Golgi bodies, spherical and slightly oblong mitochondria, short cisternae of the agranular (smooth) endoplasmic reticulum, and glycogen granules. An electron-dense substance is also found in the Golgi cisternae and the pericisternal vesicles of a dark cell.

The dark cells, like the light cells, show a paucity of intercellular junctions. The only apparent contacts resemble intermediate junctions. The plasmalemma along the exposed surface of a dark cell shows punctate filamentous mats measuring 46–56 nm in thickness. As in the light cells, such associations between filamentous mats and plasmalemmata may represent hemi-intermediate junctions (Fig. 8B), sites where cytoplasmic microfilaments associate with the unbounded cell membranes. Typical intermediate junctions with a macular configuration are occasionally formed by adjoining dark cells (Fig. 8C).

Short irregularly shaped processes appear on all surfaces of a dark cell. Some processes project both

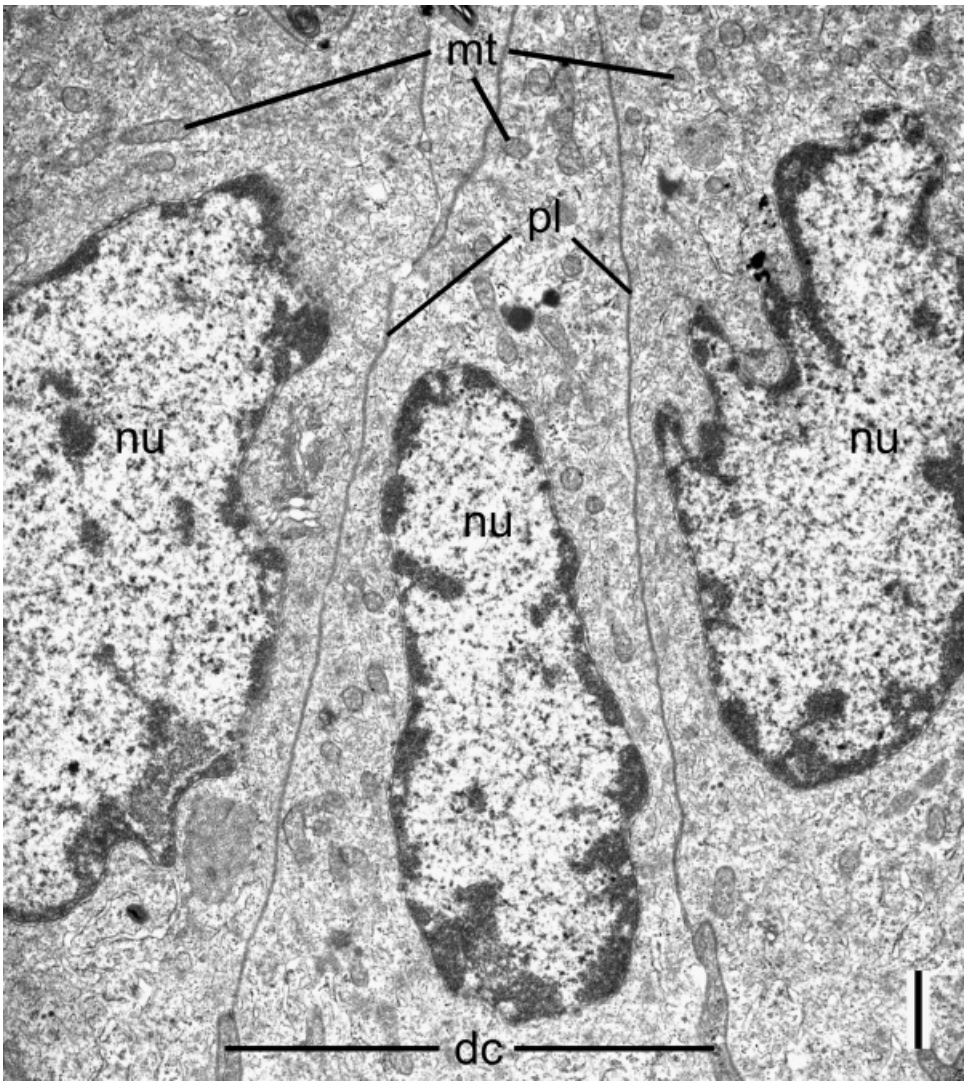

Fig. 6. Ultrastructure of the tunica intermedia in a transversely sectioned artery. The nucleus (nu) of a light cell (mt, mitochondrion; pl, plasmalemma) often shows deep indentations. Note the processes of dark cells (dc) infiltrating between the light cells and penetrating as far as their nuclei. Scale bar, 1 μm.

between and into the adjacent light cells (Figs. 6, 7A), and others interdigitate with corresponding processes from neighboring dark cells. A few slender processes from the exposed surface of a dark cell infiltrate among the collagen fibrils of the tunica externa (Fig. 8B).

Tunica externa

The tunica externa is fundamentally an accumulation of collagen fibrils (Fig. 9A). Fibrils have a diameter of 17.1 ± 0.5 nm and a poorly defined banding pattern (Fig. 9B). The majority of fibrils are longitudinally oriented with respect to the primary axis of a vessel. Lined capillaries and fibroblastic cells are often encountered among the fascicles of collagen fibrils. A fibroblastic cell is fusiform in shape when viewed in ultrathin sections, and it often conforms to the surface contour of a fascicle of collagen fibrils (Fig. 9C). The nucleus of a fibroblastic cell is irregular in shape and may be deeply indented in places. It usually exhibits a pair of nucleoli. The chromatin is considerably condensed, with a thick layer applied to the nucleoplasmic surface of the inner nuclear membrane. Evaginations of the outer nuclear membrane are continuous with cisternae of granular endoplasmic reticulum that tend to accumulate near one pole of the nucleus. Diminutive mitochondria occur throughout the cytoplasm.

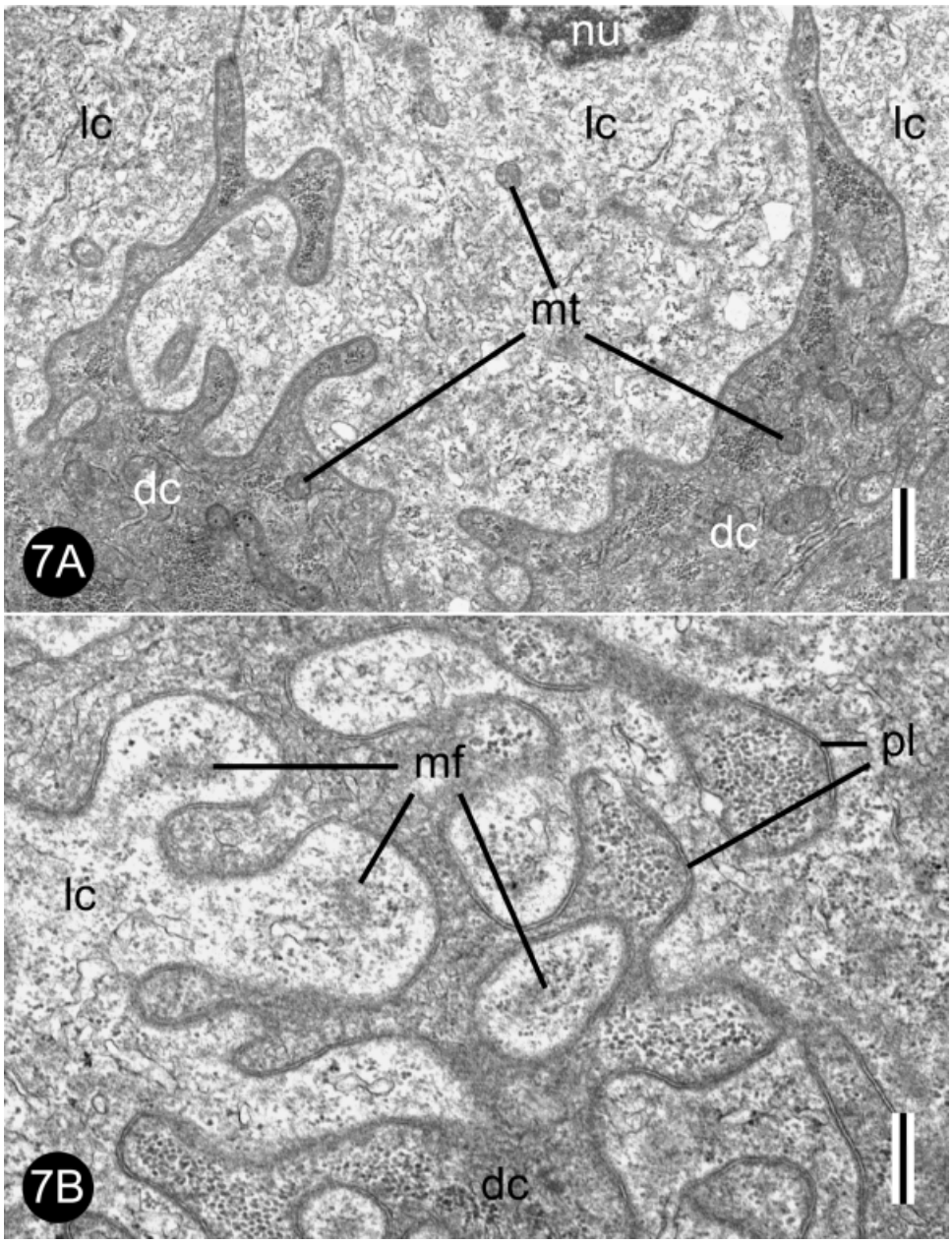

Fig. 7. Ultrastructure of the tunica intermedia in a transversely sectioned artery. **A.** Deep sectors of the light cells (lc) form a convoluted interface with the outlying dark cells (dc). The processes of dark cells, occupying the intercellular spaces, penetrate as far as the nuclear levels of the light cells (nu, nucleus); see also Fig. 6. mt, mitochondrion. **B.** Infolding and interdigitation at the interface of light cells (lc) and dark cells (dc) presumably augment the structural integrity of the bilayered cell mass. Small fascicles of microfilaments (mf) can be detected in some processes from the light cells; similar fascicles would be difficult to identify in processes from the dark cells owing to a higher density of particulate organelles, especially glycogen granules and rosettes, and a lower frequency of membranous cisternae. pl, plasmalemma. Scale bars, 1 μm (A), 500 nm (B).

Discussion

On the basis of wall thickness, the anterior lateral arteries have similar dimensions to the anterior median artery and the paired hepatic arteries. All of these vessels share a trilaminar organization. Unfortunately, the published descriptions of lobster arteries pertain exclusively to the dorsal abdominal artery, the largest vessel in terms of both wall thickness and luminal diameter. Some of the terminology used for

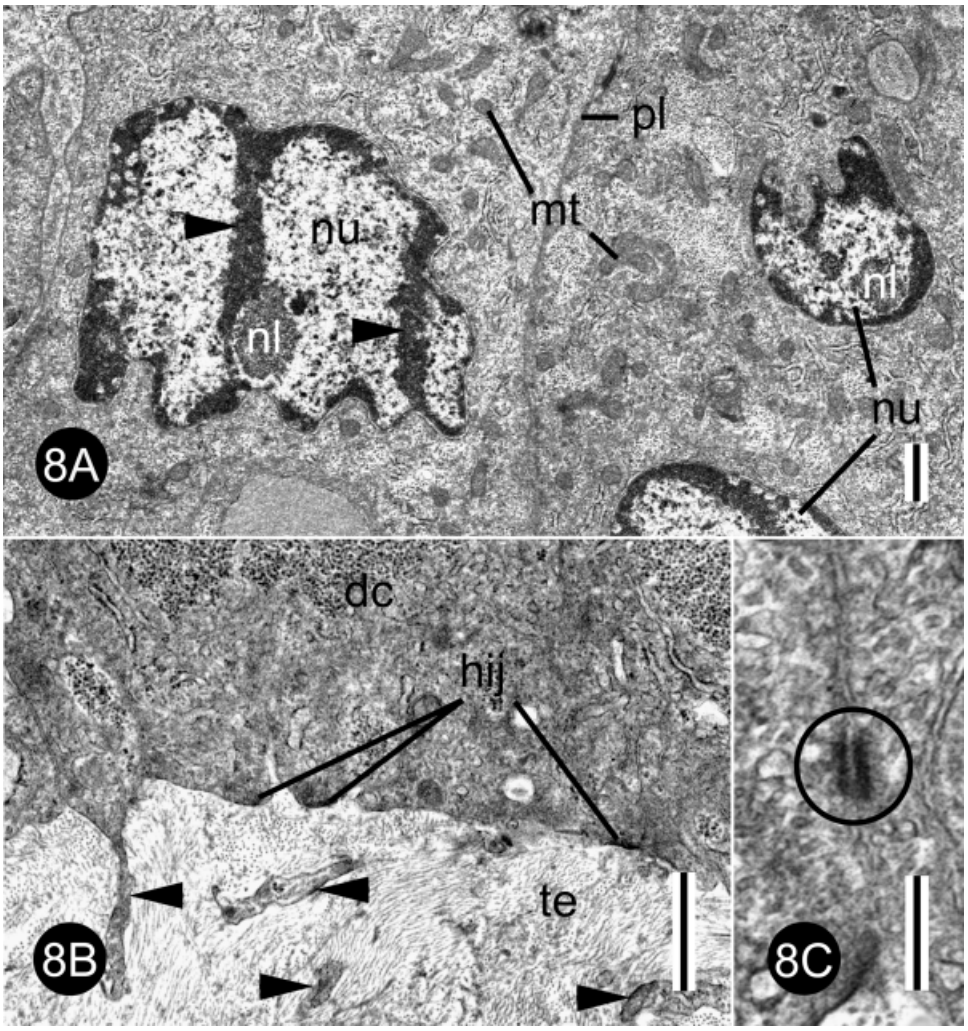

Fig. 8. Ultrastructure of the tunica intermedia in a transversely sectioned artery. **A.** Dark cells (mt, mitochondrion; pl, plasmalemma) exhibit pleomorphic nuclei (nu) with multiple nucleoli (nl), surface indentations, and bars or plates of heterochromatin (arrowheads) spanning across the organelle. **B.** The exposed surfaces of dark cells (dc) send processes (arrowheads) into the collagenous connective tissue of the tunica externa (te). Cytoplasmic microfilaments span to filamentous mats beneath the plasmalemmata, establishing hemi-intermediate junctions (hij) on the exposed surfaces of the dark cells. **C.** Bilateral intermediate junctions (open circle) are formed where dark cells confront one another. Scale bars, 1 μm (A,B), 250 nm (C).

the dorsal abdominal artery, both in the lobster (Burnett 1984; Shadwick et al. 1990; Davison et al. 1995; Martin & Hose 1995) and in other crustaceans (Martin et al. 1989), do not apply to the smaller vessels of *Homarus americanus,* so we have opted to formulate a new descriptive scheme that is better suited to the task. This scheme avoids the use of terms borrowed from the histology of vertebrate tissues and organs that might introduce confusion (e.g., *basal lamina* [''the epithelial contribution to a basement membrane''], *endothelium* [''the simple squamous epithelium lining the circulatory system''], *internal elastic lamina* [''an aggregation of elastic fibers in medium and small arteries''], *external lamina* [''a continuous glycocalyx on all cell surfaces''], and *adventitia* [''connective tissue'']).

Previous studies have emphasized the elastic properties of the tunica interna (Burnett 1984; Martin et al. 1989; Shadwick et al. 1990; Davison et al. 1995; Martin & Hose 1995). The ultrastructure of elastic tissue in this tunic is quite different from that observed in vertebrates. In vertebrates, an elastic fiber consists of slender elastic microfibrils embedded in an amorphous, electron-lucent matrix (elastin). Elastic fibrils of the lobster have a diameter (21–24 nm) that is essentially double that of elastic microfibrils of a vertebrate (10–12 nm), and elastic tissue of the lobster is unipartite, consisting exclusively of ''beaded''

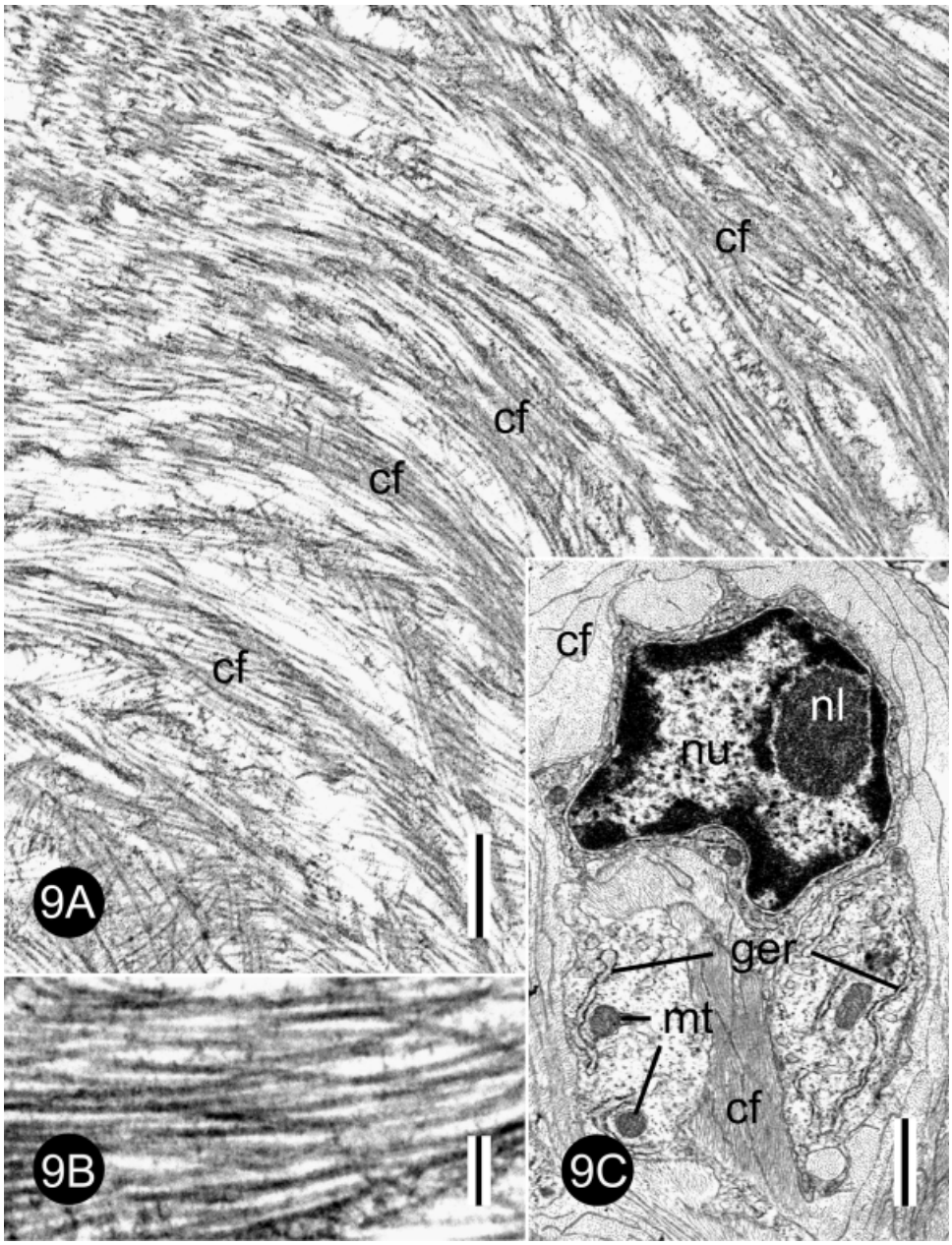

Fig. 9. Ultrastructure of the tunica externa in longitudinally (A,B) and transversely (C) sectioned arteries. **A.** Fascicles of collagen fibrils (cf) tend to parallel the primary axis of a vessel. **B.** Collagen fibrils lack prominent cross-striations. **C.** Fibroblastic cells (ger, granular endoplasmic reticulum; mt, mitochondrion; nl, nucleolus; nu, nucleus) appear throughout the tunica externa, and they tend to conform to the contours of fascicles of collagen fibrils (cf). Scale bars, 500 nm (A), 1 μm (B,C).

fibrils without any amorphous component. The longitudinal orientation of elastic fibrils in the tunica interna of an anterior lateral artery likely aids the propulsion of hemolymph along the vessel. During systole, the elastic tissue of the tunica interna would stretch, accommodating the pulsatile outflow from the heart. During diastole, passive rebound of the elastic tissue would damp the cardiac output while maintaining flow along the main vessel and into smaller branches. A longitudinal orientation of elastic fibrils with respect to the primary axis of the vessel may offer less resistance to hemolymph flow than would be achieved with a circumferential orientation.

Light cells of the tunica intermedia have an irregular interface with the tunica interna. Maintenance of this interface is achieved through interdigitation of

cell processes and the elastic connective tissue, as well as an extensive specialization of the exposed cell surface (hemi-intermediate junction). Such an arrangement reminds one of the condition in vertebrate skin, where projections (papillae) of the dermis indent the overlying epidermis and where basal cells of the epidermis bolster attachment to the dermis through elaboration of hemidesmosomes (Montagna & Parakkal 1974). Reinforcement of the boundary between the tunica interna and the tunica intermedia by interdigitation and junctional specialization suggests a significant degree of distensibility on the part of an anterior lateral artery. Significant distensibility is also reflected in the reserve of membrane present in the processes formed on the bounded surfaces of light and dark cells. The paucity of intercellular junctions between the interdigitated processes is surprising. Except for a modest number of intermediate junctions, no other intercellular contacts are evident. The intercellular spacing within the tunica intermedia is quite narrow, so identification of communicating (gap) junctions is difficult in ultrathin sections. It would be of interest to observe the intercellular regions in freeze-fracture replicas of the tunica intermedia and to view the apposed membranes and intervening spaces in the tunica intermedia from a pressurized artery.

The anterior lateral arteries tend to become stiffer as they are increasingly stretched. Such non-linear elasticity indicates an inextensible component in the vessel wall (Shadwick et al. 1990; Davison et al. 1995; Wilkens et al. 1997a). The collagen fibers in the tunica externa may be responsible for stiffening the wall of the vessel during systole. If the packing density of microfilaments is sufficiently close to exclude myosin molecules, the stress fibers in the light cells might be static elements serving to augment vessel stiffness (Alberts et al. 2002).

With the absence of muscle cells in the tunica intermedia, the anterior lateral arteries might be considered as *capacitance* vessels (Milnor 1990; Wilkens et al. 1997a). Capacitance vessels, owing to elastic components in their walls, are able to reduce the pulsatility of cardiac output while maintaining hemolymph flow during diastole. There is, at least, the potential for the anterior lateral arteries to control their luminal diameters through contraction and relaxation of the stress fibers at the exposed ends of the light cells. The circumferential or slightly oblique orientation of the bundles is appropriate for decreasing/increasing the luminal diameter, there is a strong parallel arrangement of the constituent microfilaments within a bundle that would facilitate interaction, and there are structural associations between microfilaments from the bundles and the nearby sectors of plasmalemmata.

If contractility is the purpose of the microfilaments in the light cells (Katoh et al. 1998), the anterior lateral arteries might qualify as *resistance* vessels and, thus, be allied more closely with the dorsal abdominal artery. (The dorsal abdominal artery is the only known crustacean vessel to incorporate muscle cells into its tunica intermedia.) Resistance vessels, owing to the presence of muscle cells in their walls, have the ability to control luminal diameter and, thus, actively regulate the flow of hemolymph. The present study illustrates that the absence of discrete muscle cells is not necessarily diagnostic of a capacitance vessel, because force generation to modify luminal diameter could just as well be vested in a network of microfilaments as in a network of myofilaments.

It is known that an anterior lateral artery can alter its resistance to hemolymph flow if perfused with saline containing FLMRamide-related peptide F1 or F2, proctolin, or glutamic acid (Wilkens 1997). Are these vasoactive substances acting on the light cells, inducing contractions of their stress fibers, or is the control over vascular resistance localized elsewhere? Using dilute methylene blue on vascular spreads, we have sampled a relatively large area of the wall of an anterior lateral artery. The apparent absence of nerves to the tunica externa and the tunica intermedia is consistent with the finding that the anterior lateral arteries fail to respond to electrical stimulation (Wilkens 1997). If contraction is regulated by circulating substances, it would explain the coordination of contraction between light cells and obviate the need for communicating junctions between those cells. The orientation of the microfilament bundles is suggestive of a role in the control of luminal diameter. Vascular resistance must also take into account the valves situated between the heart and the vessels exiting from it (Kuramoto et al. 1992). Cardioarterial valves are present where the anterior lateral arteries leave the heart, but arterial valves are not apparent at the branch points along distal segments of the anterior lateral arteries. Arterial valves do appear at the side branches of the dorsal abdominal artery (Davidson et al. 1998), so branch points of the anterior lateral arteries warrant closer examination.

Our observations indicate that the wall of an anterior lateral artery has a simple organization, consisting of a cell bilayer sandwiched between elastic and collagenous connective tissues, and there is a curious absence of basal/external laminae segregating the cells from the connective-tissue elements. This fundamental design also holds for the other thin-walled vessels of *H. americanus* (M.J. Cavey, K.S.

Chan, & J.L. Wilkens, unpubl. data). The next report in this series will deal with the anterior median artery supplying hemolymph to the brain, the antennae, and the eyestalks. The anterior median artery is the only thin-walled vessel bearing nerves, and a number of subtle, but intriguing differences distinguish it from the anterior lateral arteries, including bundles of interspersed microfilaments and microtubules in the medial cells of the tunica intermedia and a reticulum of fibroblastic cells in the tunica externa.

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