

LYMPH VESSELS OF THE RABBIT HEART: DISTRIBUTION AND FINE STRUCTURE IN VENTRICLES

C. Marchetti, P. Poggi, A. Calligaro, A. Casasco

Institute of Istology and General Embriology
University of Pavia, Italy

ABSTRACT

We examined the distribution and fine morphology of small lymph channels in the rabbit heart, and particularly in the ventricular myocardium. Light microscopy showed many small subendocardial lymphatics in both ventricles and especially in the subendocardium of papillary muscles and right side of the septum. Electron microscopy revealed that the endothelial ultrastructure of these lymphatics was similar at different sites. The cytoplasm exhibited frequent adluminal expansions and contained numerous micropinocytotic vesicles opening both into the lumen and surrounding interstitium. Bundles of microfilaments, commonly arranged in a parallel fashion, were also visible. Between laminations of the endothelial wall were relatively dense, finely granular material. Intercellular contacts were characterized by cell superimpositions and interlockings, specialized junctions and occasionally maculae adherentes. The basal membrane was discontinuous and often of variable thickness. In the surrounding connective tissue, thin filaments appeared adjacent to the abluminal surface of the endothelial wall. Although the fine structure of the walls of small lymphatics in the rabbit ventricular myocardium was similar to that of small lymphatics at other sites, their distribution varied from other mammalian species.

A lymphatic network in the heart has been described in several mammalian species. The first investigations (1-5) used injecting dyes such as India ink or Evans blue directly into large lymph channels or at various loci within the heart. These techniques displayed both macroscopic and

microscopic evidence of lymphatic capillaries throughout the heart but most prominent in the ventricles.

A rich subepicardial lymphatic network has consistently been found in both ventricles (1, 2, 4-8), but the presence of lymph capillaries in myocardium and subendocardium is controversial. Indeed, some (1-3, 9-11) report an extensive lymphatic network in the subendocardium and myocardium in dog, pig and man, while others (12-14) fail to detect subendocardial lymphatics in these species.

More recently (8), labeling with dye and fixation by perfusion in the dog heart have revealed epicardial, myocardial and subendocardial lymphatics in both ventricles. In these studies, attention focused primarily on ultrastructural features of lymph capillaries.

Others (7) examining the entire thickness of the ventricular walls and of the septum in the mouse heart, confirm the presence of a rich subepicardial lymphatic network in the left ventricle. On the other hand, they detect only a few lymph channels in the outer half of the myocardium and in the subendocardial region of the right portion of the septum, and virtually no lymphatics in the right ventricle.

Thus far, there have been no data on lymphatics in rabbit heart. We, therefore, systematically studied the extent and distribution of the lymphatic network in this species with special emphasis on the ventricular walls.

MATERIALS AND METHODS

Adult rabbits of both sexes weighing 2.5-3 Kg underwent general anesthesia with sodium nembtal. The hearts were removed and perfused retrograde through the aorta with a fixation solution (glutaraldehyde-paraformaldehyde, 2.5%-2% mixture in 0.1

M sodium cacodylate buffer, pH 7.4 (Karnovsky, modified) (15).

Fifteen minutes after beginning perfusion, the hearts were isolated and small fragments of the whole right ventricle, of the epicardial, myocardial and endocardial regions of the left ventricle, of the interventricular septum and of the papillary muscles from both ventricles were sampled.

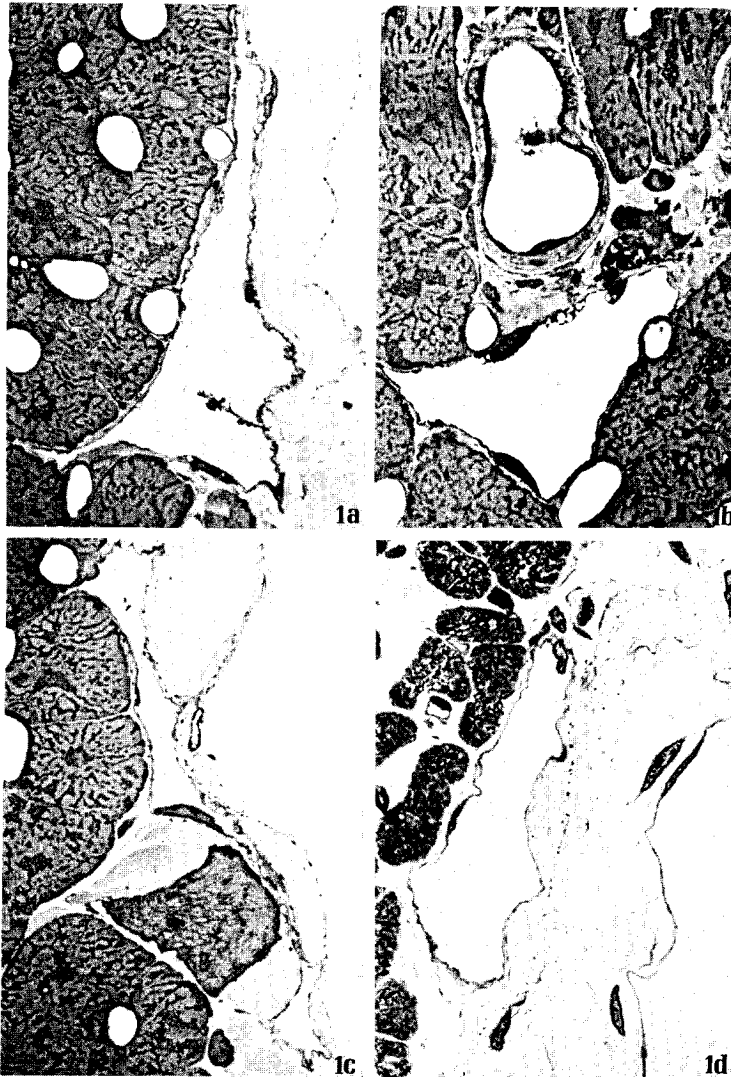


Fig. 1: Light microscopy. Semithin sections of ventricular wall. Large, irregularly shaped lymph vessels, filled with flocculent material (Toluidine blue x1000). A: subepicardium of right ventricle. B: myocardium of left ventricle. C: right subendocardium of interventricular septum. D: subendocardium of right papillary muscle.

The specimens were then immersed into the same fixative for three hours for 4°C and post-fixed in OsO₄ in collidine buffer for 1½ hours at 4° C, dehydrated and embedded in epoxy resin.

Semithin sections of the fixed specimens were taken at different levels along the ventricular walls to determine the extent of the lymphatic network by light

microscopy.

Subsequently, ultrathin sections (about 800-1000 Å) were prepared and stained with uranyl acetate and lead citrate for electron microscopy.

RESULTS

Light Microscopy: The perfusion

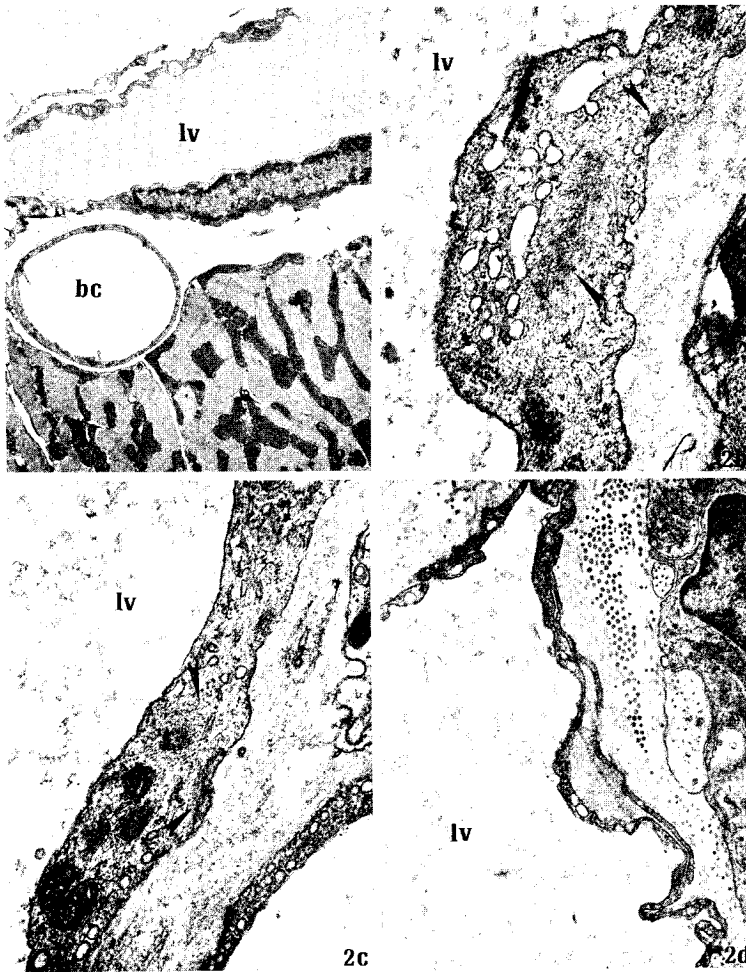


Fig. 2: Electron microscopy. A: lymphatic vessel (lv) containing a dense precipitate near a blood capillary (bc) distended and completely empty (x5600). B: endothelial cells of lymph vessel (lv) wall: the cytoplasm contains multivesicular bodies, micropinocytotic vesicles, thin filaments (arrow) small mitochondria and ribosomes (x25,000). C: lymph vessel (lv) wall: superimposed cytoplasmic processes of adjacent endothelial cells. They delimit a sac filled with moderate electron dense material. Collagen fibrils and thin filaments in the surrounding connective tissue (x 18,000).

technique allowed ready distinction between lymph and blood vessels. Whereas blood channels uniformly exhibited a well distended wall without intraluminal content, lymph capillaries were consistently filled with fine, flocculent matter (coagulated lymph).

The lymphatic capillary network was not uniformly distributed in the ventricular walls. In the subepicardial connective tissue of both ventricles and between the most superficial myocardial cells and mesothelial leaflet, the lymphatic network was diffuse and composed of many lymph vessels with large lumina (Fig. 1A). Towards the inner side of the ventricular wall, lymphatics were less extensive with only rare lymph vessels detectable in the interstitial connective tissue (Fig. 1B). In the subendocardium connective tissue of both ventricular walls lymphatic capillaries were rare.

The interventricular septum also showed an inhomogeneous distribution of lymphatic channels: in the right subendocardial region lymphatics were extensive (Fig. 1C), whereas in the corresponding left side they were virtually absent.

Finally, in the papillary muscles of both ventricles, lymph capillaries were localized in the subendocardial connective tissue (Fig. 1D). The network extended from the base of the papillary muscle throughout the tendon cords to the valve cusps.

No important differences were noted in the microscopic structure of lymphatics in different myocardial regions. Their caliber resembled that of small veins rather than blood capillaries. Their shape was generally irregular with narrowings and dilations often seemingly adjusting to surrounding interstitial connective tissue. Their walls were quite thin and indented with adluminal protrusions. The nuclei were usually elongated and thin, but occasionally roundish and often protruded into the lumen.

The epicardial and endocardial lymph vessels were on one side, adjacent to abundant connective tissue and on the other side to myocardium and blood capillaries of the interstitial connective tissue. Deeper

lymphatics were generally contained within abundant connective tissue along with blood capillaries and venules. At this site, moreover, lymphatic capillaries appeared connected to cardiac muscle cells through interposition of thin connective laminae.

Electron microscopy: Ultrastructural features of lymphatics in the various areas examined were similar. The endothelial wall was thin, irregular, with frequent scallopings; it was thickened corresponding with nuclei where the cytoplasm

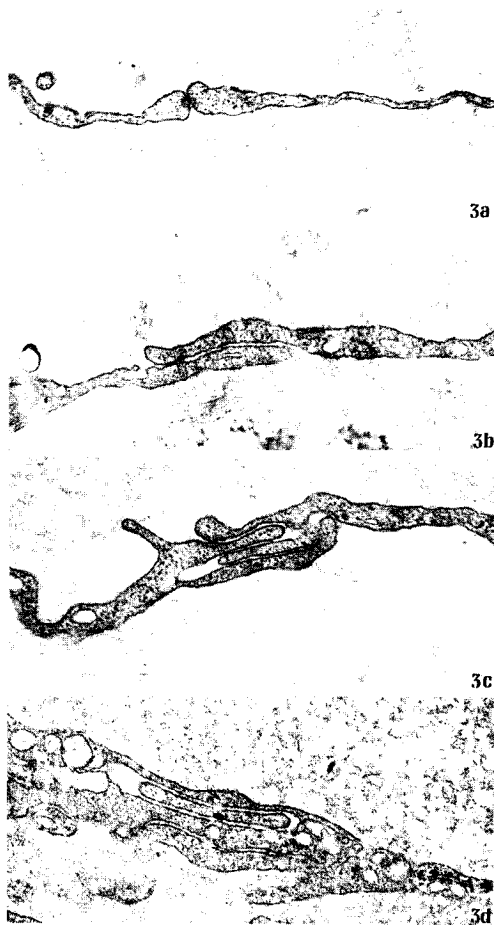


Fig. 3: Relationships between contiguous endothelial cells. A: end-to-end adhesion with desmosome-like junction. B: overlapping of cytoplasmic cell processes. C: fork-like interlockings among several superimposed cytoplasmic projections.

was also more abundant (Fig. 2A).

The endothelial cell membrane was often invaginated to form small micropinocytotic vesicles on both abluminal and adluminal sides. The cytoplasm contained small, dense matrix mitochondria, cisternae of the rough endoplasmic reticulum and free ribosomes, a small Golgi apparatus and rarely multivesicular bodies and lysosomes. In the areas where the endothelium was thinnest, the cytoplasm contained free ribosomes, vesicles and rare mitochondria. Thin filaments were always present, mostly collected into small, parallel bundles or variously intermingled, and occasionally inserting into the lymphatic wall. Such filaments were occasionally discrete and entered the thin projections of cytoplasm protruding into the lumen (Figs. 2B, C).

Relationships between contiguous endothelial cells were variable: desmosome-like or maculae adherentes-like (Fig. 3A) specialized junctions; often cell interlockings. Cytoplasmic projections, often relatively large and usually thin and tortuous were variously superimposed: they were arranged in parallel (Fig. 3B), or limited sacs filled with finely granular, moderately electron dense material (Fig. 2D). A more complex pattern was occasionally seen, with multiple, tortuous, fork-like interlockings among several superimposed processes (Figs. 3C, D). Specialized junctions could be found in adhesion areas.

The basal lamina of variable thickness was discontinuous, unlike that of the adjacent blood capillaries. The lymph vessel outer connective tissue was usually abundant, as also shown by light microscopy, and contained many collagen fibrils also collected into large variously intermingled bundles and elastic fibers.

Between the collagen fibrils and the lymph vessel wall was a network of thin anchoring filaments. Some were connected with the endothelial wall or the basal membrane, while others appeared connected to adjacent collagen fibrils. Where the connective tissue was less abundant and the lymph vessel was closer to myocardial cells and

blood capillaries, thin filaments extended from the lymph vessel wall to the sarcolemma and to the blood vessel wall.

In the interstitial connective tissue, fibroblasts with long, thin elongations, often parallel to the lymph vessel wall occasionally showed a tortuous, complex pattern. Small bundles of nerve fibers were also common.

DISCUSSION

The rabbit heart shows a nonuniformly distributed lymphatic network throughout the thickness of the ventricular walls. Lymph vessels are particularly abundant in the subepicardial region, less common in the subendocardium and virtually absent in the innermost areas of the myocardium. Lymph vessels are also found in the papillary muscles, in the interventricular septum and in the subepicardial right ventricle. Small lymph vessels are consistent and numerous in areas rich in connective tissue such as the subepicardium. On the other hand, in the innermost myocardium where muscle cells are closely opposed and in the subendocardium where connective tissue is less abundant, a lymphatic network is sparse.

With regard to ultrastructure, lymph capillaries of rabbit heart ventricles are similar to those described previously in hearts of other mammalian species (7, 8). They usually exhibit an irregular shape and a thin wall. The endothelium is indented and rich in adluminal protrusions which often interlock thereby interconnecting adjacent endothelial cells. The presence of sacs limited by thin cytoplasmic processes and containing moderately dense material are also noteworthy. The basal membrane is characteristically discontinuous and many thin filaments anchor the adluminal endothelial wall to the surrounding connective tissue rich in collagen fibers.

Based on these findings, there is general agreement concerning the fine morphology of small lymph vessels in the heart of the rabbit and other mammals. Differences are related to lymphatic localization and

distribution in the ventricular walls and not to morphologic appearance.

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