

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

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ABSTRACT

The distribution and fine structure of lymph capillaries in the atria of the rabbit heart were examined. A lymph capillary network extends into both the right and left atria but its distribution is not uniform. It is developed in the subepicardium but not in the myocardium and subendocardium of the atrial wall; overall it is less extensive than the corresponding network in the ventricles. In the atrial conduction system and in particular the sino-atrial node and the crista terminalis, the lymph network is more widely distributed; small lymph capillaries are also detected between the myocardial cells of the conduction system. A large lymph channel consistently runs along the medial side of the crista terminalis. The morphologic characteristics of atrial lymph capillaries include irregular shape, thinness of the endothelial wall, superimpositions and interlockings of the cells in the contact areas with complex junctional processes, thin and discontinuous basal membrane.

Knowledge about the lymphatic network draining the mammalian heart is still rudimentary and controversial. Some workers (1-7) have detected a lymphatic network in both atria by labeling lymphatics after injecting the myocardial interstitium or lymphatics directly with a vital dye. There is general agreement by these workers that lymphatics in the atria are less extensive than in the ventricles and are largely restricted to the subepicardium.

Some investigations focus on possible spatial and functional relationships between the lymphatic network and the conduction system. Golab (4,8) described lymph capillaries in the interventricular septum, in the sinoatrial and atrioventricular nodes and in the bundle of His in both human and pig hearts. These anatomic results, however, are somewhat at variance with those of Eliska (2,9,10) in human and dog hearts. Others (11,12) have tried to link the conduction system of the heart to its lymphatic drainage, to explain functional disturbances.

In contrast, Patek (13) and Bradham (14) readily visualized a ventricular lymphatic network using India Ink but were unable to detect atrial lymphatics with this same method. It is noteworthy, that these earlier anatomic studies were confined to delineating medium and larger-size lymphatic channels, or to ultrastructure of heart lymph capillaries in the ventricles (15,16), where the lymphatic network is more abundant. To date, there have been no ultrastructural studies of the atrial lymph capillaries.

Previously (17) we described the extent and distribution of lymphatics in the ventricles of the rabbit heart using light and electron microscopy. Now we focus on the distribution and ultrastructure of atrial lymph capillaries. We also examined lymph drainage of the atrial conduction system and in particular the sinoatrial nodal region and the crista terminalis, a specific pathway of internodal conduction within the right atrium.

MATERIALS AND METHODS

Hearts of adult rabbits weighing 2.5-3 kg were examined. After induction of general anesthesia, the heart was removed and immediately "fixed" by retrograde perfusion through the aorta with modified Karnovsky fixative solution (glutaraldehyde 2.5%-Paraformaldehyde 2%, mixed in 0.1 M sodium cacodylate buffer, pH 7.4) (18).

Perfusion was performed for 15 minutes and then fragments of the left and right atrium were isolated. In the right atrium, wall fragments from the sinoatrial nodal region and from the crista terminalis were also removed. Fixation was continued by immersion of the fragments into the same mixture for three hours at 4°C and thereafter in OSO_4 - collidine buffer for 1 1/2 hours at 4°C. Specimens were then dehydrated and embedded in epoxy resin. 0.5-0.7 μ semithin sections, taken at different levels along the whole atrial wall, were cut and stained with toluidine blue. After observation at light microscopy, 800-1000 \AA ultrathin section were obtained, stained with uranyl acetate and lead citrate and observed under a ZEISS EM 109 electron microscope.

RESULTS

Light microscopy: Observations of semithin sections, taken at different levels on the specimens of various atrial regions, allowed identification of the presence and extent of the lymph capillary network draining the atrial wall. The fixative perfusion technique clearly differentiated lymph from blood vessels as previously shown in studies on the lymphatic network of rabbit ventricles (17).

In both atria lymph capillaries were seen, but the network was considerably less extensive than in the ventricle. An extremely scanty network of small lymph channels extended throughout the whole subepicardial regions of the right and left atrial walls (Fig. 1A), delimiting the epicardium from the myocardial cells. Rarely, some lymphatics existed between the outer myocardial cells surrounded by thin connective tissue. Because the atrial wall is very thin we were able with

serial sections to examine and compare, at the same time, the epicardial, myocardial and endocardial regions. Lymph capillaries were not found, however, either between the inner myocardial cells nor in the subendocardial region.

The conduction system also showed an inhomogeneous distribution of lymph vessels. In the area of the sinoatrial node the atrial wall was extremely thin and lymph capillaries were localized to the epicardium and subepicardium (Fig. 1B); some lymphatics were occasionally seen between small myocardial cells (Fig. 1C). Subepicardial lymph capillaries were more extensive than in the rest of the atrium, and were characterized by fairly large lumina compared, for example, to smaller myocardial lymph vessels. In the crista terminalis, the thickness of the wall was greater than in the

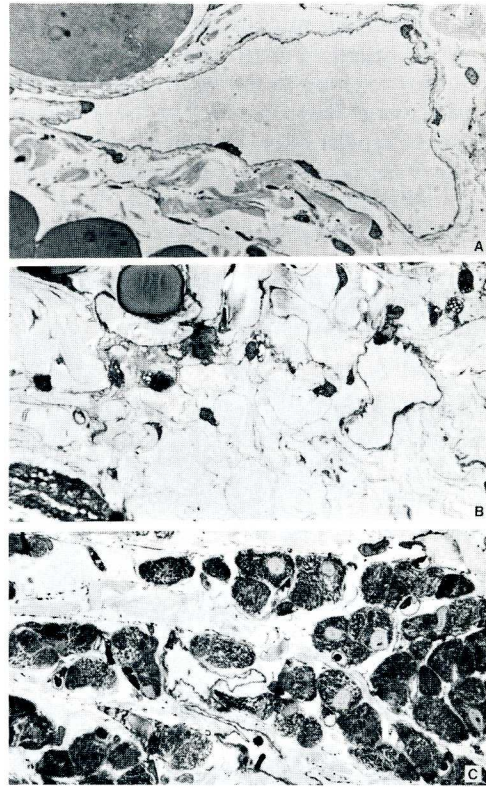


Fig. 1: Light microscopy of atrial lymph vessels (x800)
 A — right atrium subepicardium
 B — Sino-atrial node subepicardium
 C — Sino-atrial node myocardium

surrounding atrium. Here, the lymph network was similar to that in the subepicardium of the sinoatrial node, but the network also extended far inward between myocardial cells (Fig. 2). There were no lymph vessels in the subendocardium of the crista. A fairly large and consistent lymph channel coursed along the medial side of the crista in its full extent (Fig. 3). At this site the atrial wall was comparatively thin and the lymph vessel coursed from the subepicardium to the subendocardium.

In each area examined the lymph capillaries of the subepicardium were larger than channels within the myocardium. The atrial lymph capillaries were commonly tortuous and branched (Fig. 2). The lymphatic wall characteristically, was irregular, very thin with adluminal protrusions, and the channels were surrounded by interstitial connective tissue.

Electron microscopy: The ultrastructural features of atrial lymph capillaries were similar to those in the ventricles (17). The wall was uniformly very thin, including the widest capillaries of the subepicardium and was formed solely by endothelial cells with an adherent, thin, discontinuous basal lamina. Around these capillaries was a close-

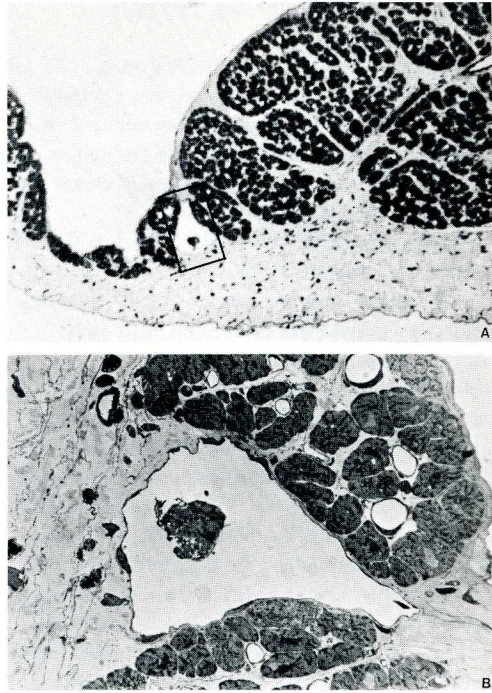


Fig. 3: A — Light microscopy of the crista terminalis region. A large lymph vessel runs along the medial side of the crista (square) (x250)
B — High magnification of the lymph vessel showed in A (x800)

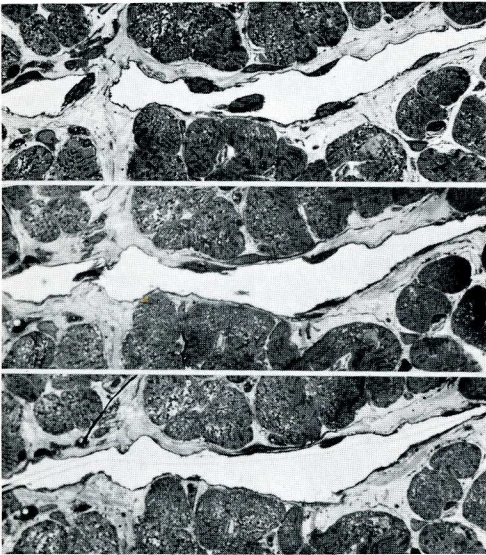


Fig. 2: Light microscopy of serial sections of a myocardial lymphatic of the crista terminalis. Note the branching and winding nature of the channel (x800)

ly woven network of anchoring filaments. More externally, collagen fibers, often in bundles, and elastic fibers were evident in the adjacent connective tissue. This lining connective sheath was uniformly present and also surrounded the inner lymph capillaries.

The endothelial cells often displayed thin and bizarre-shaped adluminal projections. The contact areas between contiguous cells were characterized by interlockings and superimpositions of cytoplasmic projections; they were often large, tortuous with a complex arrangement. Specialized junctions were seen in adhesion areas.

The cell membrane uniformly displayed invaginations to form micropinocytotic vesicles on both the abluminal and the adluminal sides; the cytoplasm contained clear free vesicles, similar to or greater than the micropinocytotic vesicles; homogeneous bodies were also present.

Overall the atrial lymph capillaries were

extremely thin, the endothelial cells contained many organelles: small dense mitochondria, free ribosomes, rough endoplasmic reticulum, thin filaments in parallel or variously arranged, and small Golgi complexes most characteristically in the perinuclear region (Fig. 4).

DISCUSSION

These studies demonstrate and confirm the presence of a lymph capillary network in both atria of the mammalian (rabbit) heart. This network, however, is not uniformly distributed; rather it is developed only in the subepicardium and specifically not in the myocardial and subendocardial areas. In comparison to the ventricles, the lymphatic

capillary network of the atria is much less extensive. In general, these observations extend and conform to other workers using different animal species (1-7). The divergent lymphatic pattern between the cardiac atria and ventricles probably relates to the varied thickness of the heart wall. Thus, in the region of the crista terminalis, where the atrium is thicker, lymph capillaries are more numerous and are also detectable between the myocardial cells. A similar pattern exists in the ventricles where lymphatic capillaries are more numerous in the thicker portions and less prominent in the thinner regions (17). Morphologically, atrial lymph capillaries are similar to those in the ventricles: irregular shape, thin endothelial wall, complex junctional processes, discontinuous basal

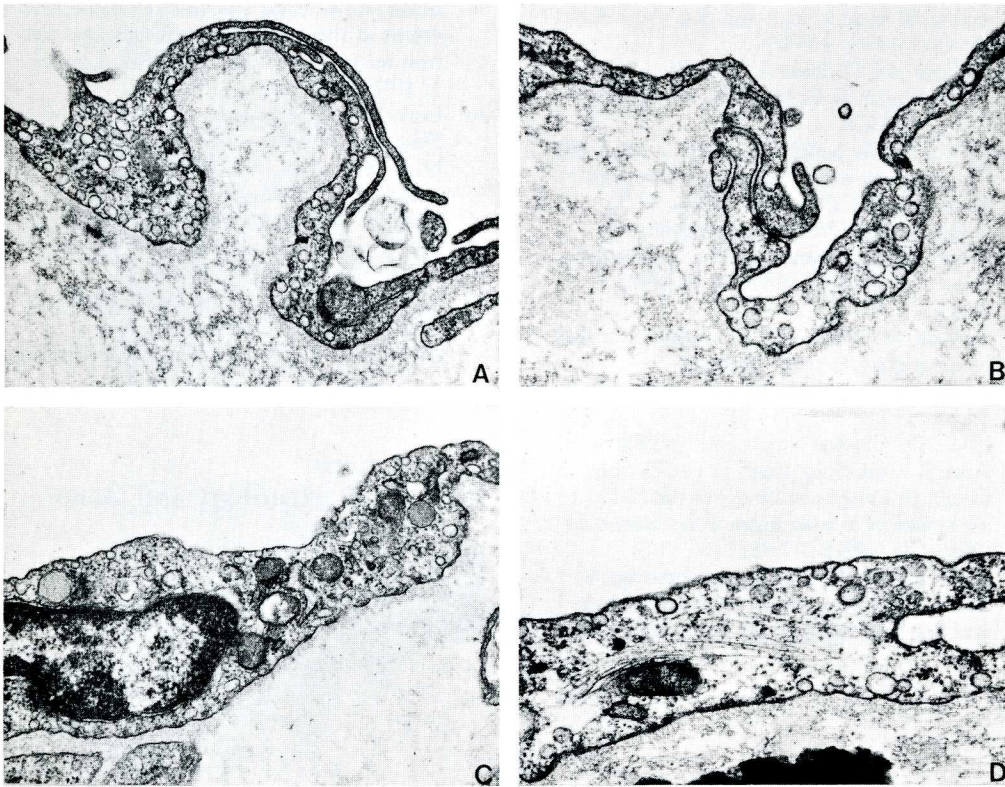


Fig. 4: Ultrastructural features of atrial lymph capillaries.

A — Adluminal superimposed projections of the endothelial wall. Micropinocytotic and clear vesicles in the cytoplasm (x20,000)

B — Adhesion area of contiguous cells with specialized junctional complexes (x30,000)

C — Perinuclear region of an endothelial cell with mitochondria, ribosomes, homogeneous bodies (x20,000)

D — Endothelial cell cytoplasm with micropinocytotic vesicles, thin filaments, ribosomes (x30,000)

membrane, rich bounding connective tissue.

On the sinoatrial node and the crista terminalis the "conductive tissue" has a broader network of lymphatics compared with the rest of the atrium. In fact, small lymph capillaries are also located deeply between the myocardial cells of the conduction system. Nonetheless, it remains overly speculative to attribute dysfunction (e.g. arrhythmias) to these patterns and further pathologic and experimental studies are needed.

REFERENCES

1. Aagaard, OC: Les vaisseaux lymphatique du coeur chez l'homme et chez quelques mammiferes. Levin and Munksgaard, Copenhagen, 1924.
2. Eliska, O, M Eliskova: Lymph drainage of sinu-atrial node in man and dog. *Acta Anat.* (Basel) 3 (1976), 418-428.
3. Eliskova, M, O Eliska: Lymph drainage of the dog heart. *Folia Morphol.* (Warsz) 22 (1974), 320-323.
4. Golab, B: The lymphatic vessels of the heart. The subendocardial and muscle networks. *Folia Morphol.* (Warsz) 12 (1961), 47-53.
5. Polikarpov, LS: Individual variability of lymph out flow paths from the human heart. *Arkh. Anat. Gistol. Embriol.* 62 (1972), 48-55.
6. Servelle, M, J Andrieux, C Cornu, et al: Les lymphatiques du coeur injections perioperatoires. *Arch. Mal Coeur* 60 (1967), 89-106.
7. Ullal, SR: Cardiac lymph and lymphatics. *Ann. R. Coll. Surg. Engl.* 51 (1972), 282-298.
8. Golab, B: Lymphatic vessels of the conducting system of human heart. *Folia Morphol.* (Warsz) 36 (1977), 317-322.
9. Eliska, O, M Eliskova: Lymph drainage of the conduction system of the heart in man and dog (sino-atrial node). In: *Lymphology*, Proceedings of the VIth International Congress, Prague, 1977, edited by P Malek, V Bartos, H. Weissleder and MH Witte, pp. 21-23 Georg Thieme Verlag, Stuttgart.
10. Eliska, O, M Eliskova: Lymphatic drainage of the ventricular conduction system in man and in the dog. *Acta Anat.* 107 (1980), 205-213.
11. Rossi, L: Case of cardiac lymphangitis with atrioventricular block. *Br. Med. J.* 2 (1965), 32-33.
12. Uhley, HM, SE Leeds, MA Sung: The subendocardial lymphatics of the canine heart. *Am. J. Cardiol.* 29 (1972), 367-371.
13. Patek, RP: The morphology of the lymphatics of the mammalian heart. *Am. J. Anat.* 64 (1938), 203-249.
14. Bradham, RR, EF Parker, BA Barrington, et al: The cardiac lymphatics. *Ann. Surg.* 71 (1970), 899-902.
15. Boger, A, W Hart: Distribution of the lymph vessels in the mouse heart. A light and electron microscopic study. *Basic Res. Cardiol.* 72 (1977), 510-529.
16. Leak, LV, A Schannahan, H Scull, WM Daggett: Lymphatic vessels of the mammalian heart. *Anat. Rec.* 191 (1978), 1983-202.
17. Marchetti, C, P Poggi, A Calligaro, A Casasco: Lymph vessels of the rabbit heart: distribution and fine structure in ventricles. *Lymphology* 18 (1985), 90-95.
18. Karnovsky, MJ: A formaldehyde fixative of high osmolarity of use in electron microscopy. *J. Cell Biol.* 27 (1965), 137a-138a.

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