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LYMPHATICS OF THE CARDIAC CHORDAE TENDINEAE WITH PARTICULAR CONSIDERATION OF THEIR ORIGIN

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ABSTRACT

We traced the origin of lymphatic capillaries of the chordae tendineae by serial ultrathin sections and electron microscopy of lymphatics identified first by light microscopy at the base of the chordaee tendineae. Most of the lymphatics appear flattened in cross section, ~15 to 20μm and 3 to 5μm in greater and lesser diameters, respectively. Spiny (thorn-like) branchlets (about 10μm in length) randomly projected from the sides of straight lymphatic capillaries and each was composed of a single endothelial cell with marginal zones in apposition to one another. The extreme end of a straight lymphatic capillary terminated blindly and consisted of a single endothelial cell with an ultrastructural appearance that resembled that of the spiny branchlets.

Examination of the heart with atrioventricular valvular failure often reveals fibrous scars in the papillary muscles, a complication perhaps related to a disturbance of lymphatic drainage (1). Using the dye injection method, the lymphatics of the papillary muscles appear denser than other cardiac lymphatics (2), a finding confirmed by examination of resin-embedded specimens (3). Although Uhley et al (2) found cardiac lymphatics originating at the cuspid valve and the valvular ring, we (3) were able to trace lymphatics at this site only to within a few millimeters from the transition of chordae tendineae and papillary muscle toward the cuspid valve.

To resolve this discrepancy, we examined by electron microscopy serial ultrathin sections of the chordae tendineae, adjacent papillary muscles and cuspid valve of the dog.

MATERIALS AND METHODS

Three adult dogs weighing from 10 to 15kg were anesthetized with sodium pentobarbital (15mg/kg B.W.) and the heart isolated. After a catheter of 1.0mm in diameter was inserted into the right and left coronary arteries, 200ml of Ringer's solution was injected to wash out blood followed by 300ml of 2% paraformaldehyde (in 0.1M phosphate buffer solution) for tissue fixation. The apex of the papillary muscles with attached chordae tendineae (6mm in length) were excised, placed in aldehyde fixative for an additional 2 to 3 hours and post-fixed in 1% osmic acid for two hours. Thereafter each specimen underwent dehydration through increasing concentrations of ethanol and embedded in Quetol 651 (Nisshin EM).

1.5μm serial semithin slices were sectioned either transversely or longitudinally from the side of papillary muscle and these were subject to light microscopy after staining with toluidine blue. Semithin slices were continuously sectioned until lymphatics were no longer detectable by light microscopy and then serial ultrathin sections were further taken. These ultrathin sections were stained with uranyl acetate and lead citrate, and examined by electron microscopy (JEM 100B).

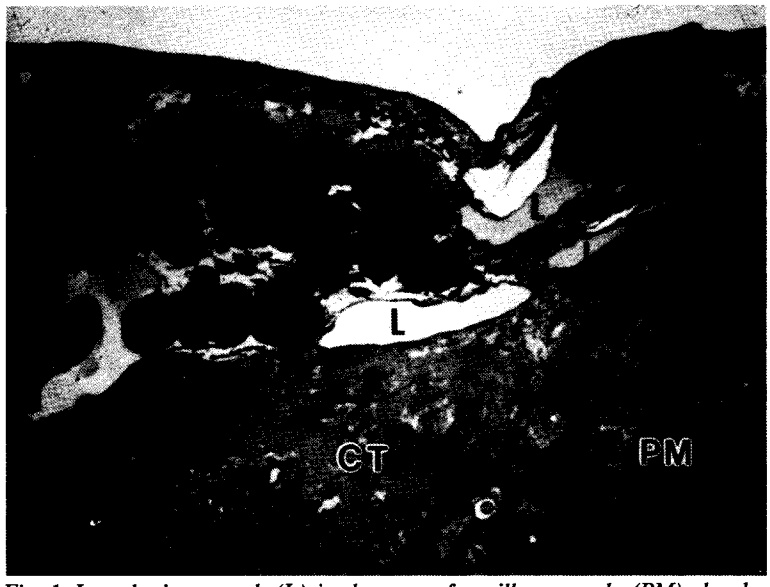

Fig. 1. Lymphatic network (L) in the area of papillary muscle (PM)-chordae tendineae (CT) junction. Toluidine blue stain. x520.

Fig. 2. A lymphatic (L) with valve (K) in beginning area of chordae tendineae. x260.

RESULTS

As we previously described (3), the lymphatic network of the junctional area between the papillary muscle and chordae tendineae was dense beneath the endocardium *(Fig. 1)*. Toward the chordae tendineae, a rich lymphatic network persisted and many displayed intraluminal valves *(Fig. 2)*. On electron microscopy, the lymphatic endothelial cells were thin (about 0.2μm) except around the nucleus and contained few intracytoplasmic organelles. The junctions between endothelial cells were of the overlapping and interdigitating type. A basal lamina was barely detectable on the abluminal endothelial surface although anchoring filaments were

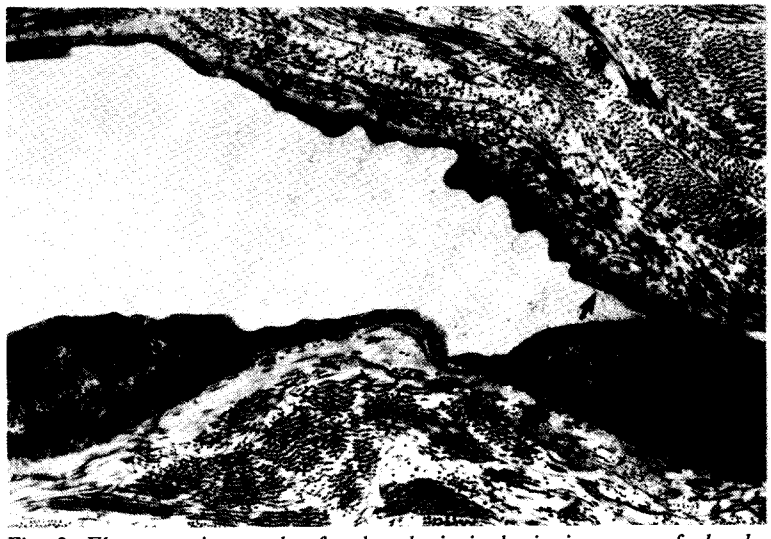

Fig. 3. Electron micrograph of a lymphatic in beginning area of chordae tendineae. Thin endothelial cells are overlapping (arrow). x10,000.

Fig. 4. The smallest lymphatic (L) in chordae tendineae (CT) observable by light microscopy. x660.

evident *(Fig. 3)*. No specific microscopic findings distinguished cardiac lymphatics from lymphatics of other tissues.

Lymphatics were traced continuously from the chordae tendineae-papillary muscle toward the atrio-ventricular valve for about another 500 to 1000μm. Over this distance, the lymphatic lumen became

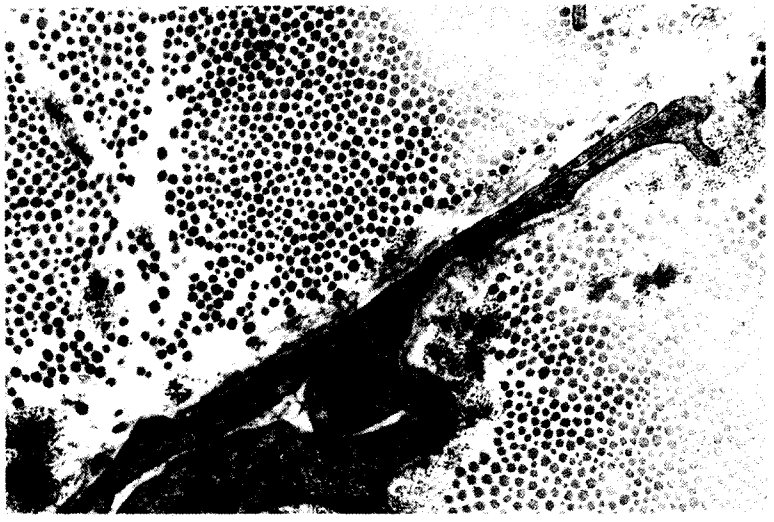

Fig. 5. Apposed margins of lymphatic endothelial cell elongated in one direction. x30,000.

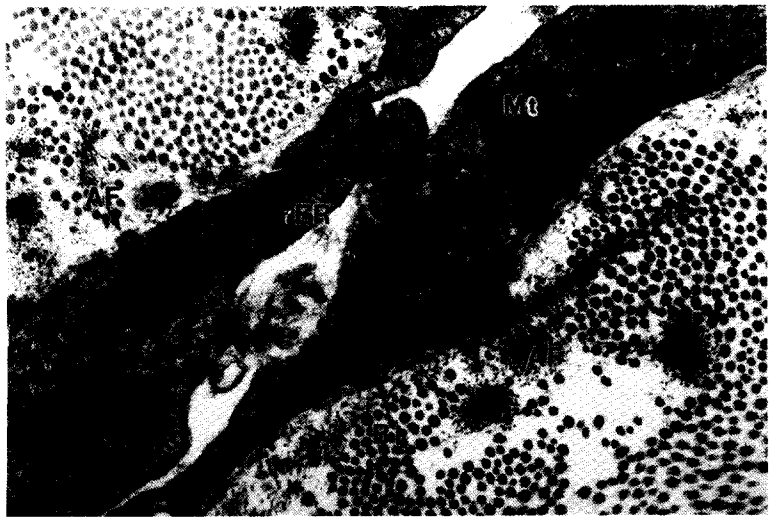

Fig. 6. Electron micrograph of a thickened part of the lymphatic endothelial cell with mitochondria (Mt) and rough endoplasmic reticulum (rER). Anchoring filaments (AF) are visible in the vicinity of the basal surface of the endothelial cell. Poorly developed basal lamina (Bl) is also seen. x30,000.

progressively narrower reaching about 5μm in diameter. This width was the smallest one identified as a lymphatic using light microscopy *(Fig. 4)*. When, however, this tiny lymphatic capillary was examined for another 1000 to 1500μm toward the valve using electron microscopy, a lumen was detectable but was extensively flattened and elongated to one side *(Fig. 5)*. At the far end of this luminal extension, the junction between endothelial cells was primarily in the form of short overlapping and/or interdigitation of the marginal zones of a neighboring endothelial cell. In some areas, adjoining endothelial cells merely faced one another without any junctional sequence for a distance of 2 to 10μm. These endothelial cells were flat (0.15μm), contained pinocytotic vesicles and free ribosome. In oth-

er regions away from the elongated luminal extension, the endothelial cell was thick (0.3 to 0.4μm) with a plethora of cell organelles, including pinocytotic vesicles, free ribosomes, and rough surfaced endoplasmic reticulum. The basal lamina was discontinuous and anchoring filaments were sporadic *(Fig. 6)*.

Sections about 500μm closer toward the valve showed that cell elements in the tissue of the chordae tendineae decreased substantially and collagen fibers were partitioned into small areas (~12μm in diameter) by fibroblasts and their adjoining extension processes. Lymphatics could be observed near and around these fibroblasts *(Fig. 7A)*. Here, the diameter of the lymphatic lumen was about 0.9μm and endothelial cells were 0.3μm thick. Many pinocytotic vesicles were observed at both the luminal and abluminal surfaces of the endothelial cells. There were considerable numbers of free ribosomes in the cytoplasm but other organelles were rarely seen. A basal lamina surrounded more than two-thirds of the abluminal surface of the lymphatic and where the basal lamina was missing, anchoring filaments were found *(Fig. 7B)*. The junction of contiguous endothelial cells was either "short-overlapping" or in direct apposition for a considerable distance.

On getting closer (5μm) to the valve, the diameter of the lymphatic lumen became even narrower (about 0.4μm), and most lymphatics displayed a single endothelial cell, about 0.3μm thick with prominent pinocytotic vesicles. The basal lamina was not discrete at the abluminal surface but anchoring filaments were sporadic, particularly along fibroblast projections *(Fig. 8)*. Advancing another few micrometers toward the valve, the lymphatic lumen just about disappeared, and only cytoplasmic strips of lymphatic endothelial cells were identified next to processes of nearby fibroblasts. In the endothelial cytoplasm, only pinocytotic vesicles were recognized. Anchoring filaments were restricted to the abluminal surface *(Fig. 9)*.

The lymphatics of this region were of such a small dimension that fuller structural delineation was possible only by electron microscopy. Accordingly, we reconstructed the overall organization of the latter (i.e., terminal) part of these lymphatics from serial ultrathin sections *(Fig. 10)*. Most of the lymphatics at the base of the chordae tendineae appeared flattened in cross section, 15 to 20μm and 3 to 5μm in greater and lesser diameters, respectively. Spiny (thorn-like) branchlets (about 10μm in length) randomly projected from the side of a straight lymphatic capillary and each "spine" was composed of a single endothelial cell with marginal

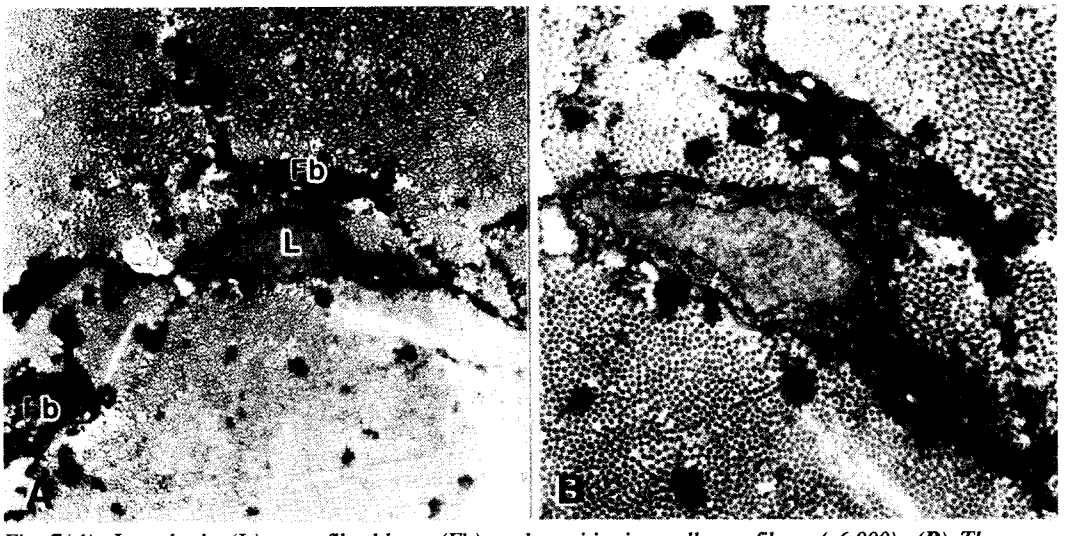

Fig. 7(A). Lymphatic (L) near fibroblasts (Fb) and partitioning collagen fibers (x6,000). (B) The same lymphatic at higher magnification (x15,000).

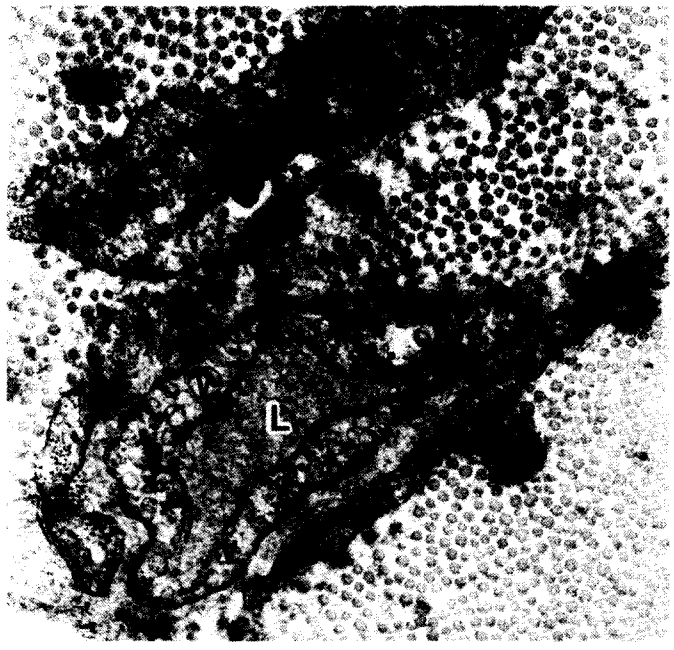

Fig. 8. Lymphatic (L) consisting of one endothelial cell with many pinocytotic vesicles (V). x25,000.

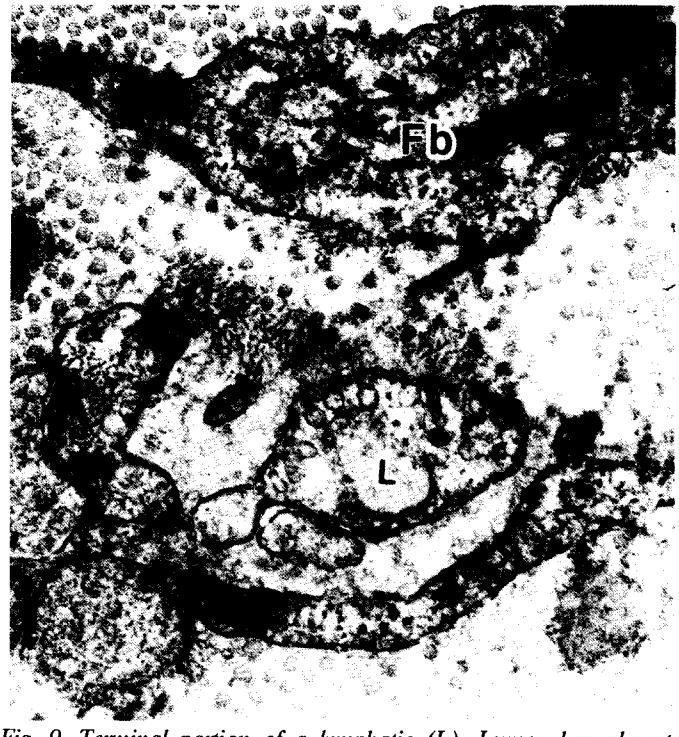

Fig. 9. Terminal portion of a lymphatic (L). Lumen has almost disappeared and only a cytoplasmic strip of the endothelial cell (about 0.5μm) remains along outward processes of a fibroblast (Fb). x30,000.

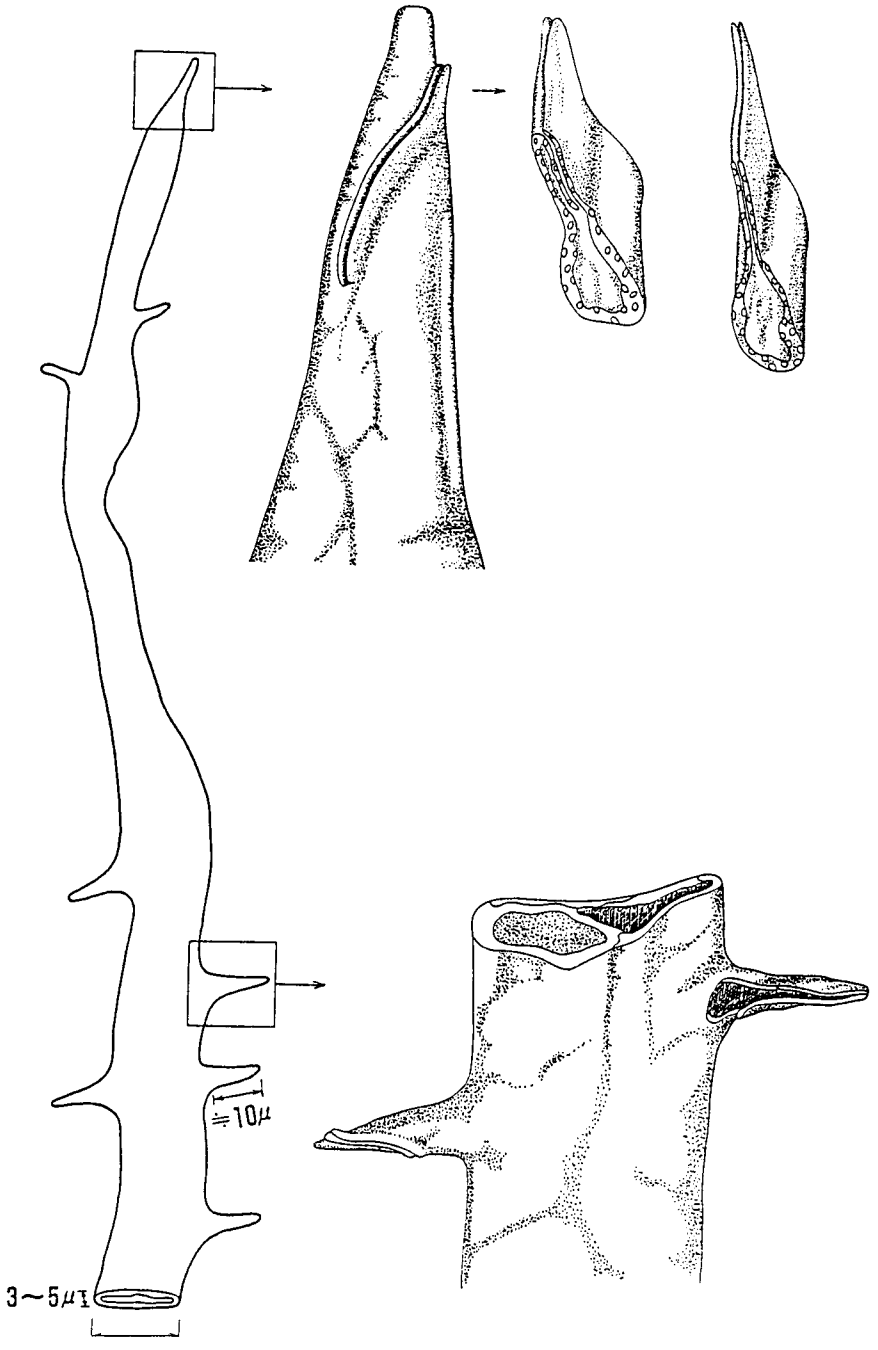

Fig. 10. Lymphatic capillaries reorganized stereoscopically and shown as schematic drawings as reconstructed from electron micrographs.

zones in apposition to each other. The extreme-end of the straight lymphatic capillary consisted of a single endothelial cell and ended abruptly similar to the appearance of the spiny-like branchlet.

Overall, therefore, the peri-valvular lymphatics could be traced about 3mm from the papillary muscles toward the valve into the chordae tendineae where they ended blindly and failed to reach the cuspid valve. Whereas the results detailed represent transverse serial sections, the findings were fully confirmed by examining serial ultrathin sections cut longitudinally along the long axis of the chordae tendineae.

DISCUSSION

This study traced the origin of the lymphatic capillaries of the chordae tendineae by serial ultrathin sections and electron microscopy after initially identifying chordae tendineae lymphatics at its base by light microscopy. Previous light and electron microscopy have simply described lymphatic capillaries as "they ended (began) blindly" (4-6), are saccular with a patent lumen (5,6), or "it [lymphatic capillary] ended (began) with glove-finger-like ending" (7,8). Moreover, it has been suggested from electron microscopy that the most terminal portion is similar to the rest of the lymphatic capillary and that a restricted part of the endothelial cell opens like a "gateway" into the surrounding interstitium (6).

Our findings differed from these earlier studies in that the lymphatic lumen gradually dissipated in width until the lumen disappeared entirely and only endothelial cytoplasmic processes opposing one another remained. Some endothelial cells with a similar appearance projected somewhat like spines into the interstitium near the end of the lymphatic capillaries. These latter findings resemble that seen in lymphatic sprouting (9) in terminal lymphatics described in the growing and/or generating lymphatics (10). Possible interpretations of these data include:

1. Previous microscopic description about the lymphatics of the cardiac chordae tendineae are incorrect or misleading.
2. The anatomic appearance of these lymphatics is applicable only to those of the chordae tendineae or perhaps other tissues with similarly dense collagen fibers.
3. Lymphatics of the chordae tendineae are continuously regenerating or remodeling and the sections illustrated represent the growth process.
4. The ends of the lymphatics of the chordae tendineae are in fact saccular with a wide lumen as previously described, and our processing created an artifactual appearance.

To distinguish among these possibilities, we need to first examine the ends of lymphatics in other tissues by serial ultrathin sections which should resolve the applicability and validity of the findings to other tissues, and, perhaps, also provide insight about blood vessel dynamics and the likelihood of ongoing lymphatic regeneration. The final possibility as to artifacts (#4) seems unlikely in light of the high quality structural appearance of endothelial cells and other adjacent tissue elements indicative of proper preservation of the tissue.

From these findings, we conclude that the lymphatics of the region of the chordae tendineae-papillary muscles begin as blinded ends either from straight lymphatic capillaries or from spiny, thorn-like branchlets.

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