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SCANNING AND TRANSMISSION ELECTRON MICROSCOPY OF RABBIT HEART LYMPHATIC CAPILLARIES

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ABSTRACT

The ultrastructural characteristics of the lymphatic capillaries of the rabbit heart were examined by scanning and transmission electron microscopy (SEM and TEM) using similar technical preparation. SEM defined the interrelationships between endothelial cells including the pocket-like structures formed by overlapping between cells and the corresponding intercellular bridges. Except for rare spindle cells apposed on the luminal surface of the endothelium seen only on SEM, these features were confirmed by TEM. On the abluminal side of the lymphatic capillary wall, a rich network of filaments and fibrils was detectable using both ultrastructural techniques. These two modalities (SEM and TEM) complement one another in defining the microanatomy of the lymphatic capillary.

The lymphatics of the heart and the ultrastructural characteristics of the lymphatic capillaries have been examined using light and transmission electron microscopy (TEM) (1-4). In the heart, the "anchoring filaments" connecting the endothelial wall to an interstitial network of fibrils have been carefully described (2). Other important information has been supplied by TEM on the intricate junctional complexes between the endothelial cells (2-4) and on the intramural channels connecting the capillary lumen to the interstitium (4). Nonetheless, photomicrographs obtained by TEM are at times inadequate to understand the irregular shape of the lymphatic capillaries and the functional significance of certain intricate features. Utilization of serial sections has previously helped to explain some of these phenomena (4).

Scanning electron microscopy (SEM) has also proved useful for studying the ultrastructure of the lymphatic capillaries in many organs, including the irregular luminal surface of the endothelium and the filaments and fibrils surrounding the lymphatic capillary wall (5-14). Moreover, SEM has depicted the protrusion of branched or spindle cells on the luminal surface of the endothelium and its relationship to varying conditions of lymphatic contraction (6-9). These cells, however, have not been verified by TEM.

Most SEM studies have previously been carried out after injection of fixative into the heart interstitium to enhance lymphatic dilatation and thereby facilitate their visualization. Different fixative pressures have also been previously used for determining the varied morphology of the lymphatic wall during interstitial edema (6-10). In this study, however, for purposes of comparison, we fixed the tissue samples for SEM and TEM using the same preparative technique so as to permit valid interpretation of morphologic comparisons between the two ultrastructural methods.

MATERIALS AND METHODS

Four adult rabbits (2.5-3kg body weight) were anesthetized with sodium nembutal. The hearts were removed and fixed by retrograde perfusion via the aorta with a mixture of paraformaldehyde (2%) and glutaraldehyde (2.5%) in sodium cacodylate buffer 0.1M (pH 7.4) (15) for 15 minutes. The initial perfusion pressure was 110mmHg and the fixative flow was 40ml/min.

After perfusion, 20 fragments of tissue were excised from the subepicardial regions of both ventricles. The samples were reimmersed in the fixative mixture for three hours at 4°C and then post-fixed in 1.33% osmium tetroxide in collidine buffer for two hours at 4°C.

Because of identical fixation technique, the samples for TEM and SEM comparison studies were taken from neighboring areas of the subepicardium of both ventricles. For TEM, the specimens were dehydrated and embedded in epoxy resin. The ultrathin sections were stained with an aqueous solution of orcein (2%) and then with uranyl acetate and lead citrate. For SEM, the samples were dehydrated in alcohol, placed in amyl acetate and then critical-point dried. After spattering with gold-palladium the blocks were examined. On each TEM and SEM examined block, at least one lymphatic capillary was present.

RESULTS

Our investigations on SEM and TEM samples were confined to the subepicardium of the ventricular walls where the cardiac lymphatic network is more extensive (3). The lymphatic capillaries were irregularly shaped with a highly attenuated wall delimiting a wide lumen sometimes filled with floccular material. These characteristics distinguished lymphatic capillaries from blood capillaries which had a thicker wall and an empty lumen after fixative perfusion.

On SEM, the luminal surface of the lymphatic capillaries exhibited oval-shaped, large, smooth bulges corresponding to the nuclear zones of endothelial cells *(Fig. 1)*. On occasion, smaller, more subtle protrusions arose from the endothelial wall and extended into the lumen. Other cellular protrusions of non-nuclear zones were also seen in TEM sections as luminal infoldings of the cytoplasmic profile *(Fig. 2a-b)*.

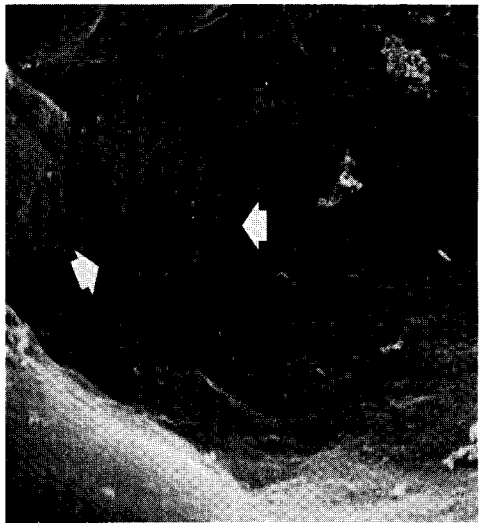

Fig. 1. SEM luminal surface of a cardiac lymphatic capillary. The oval bulge (arrowheads) corresponds to the nuclear zone of an endothelial cell (4500x).

On frontal view of the luminal surface of the lymphatic capillaries, the cellular contours were easily identified. They had irregular borders indented by the surfaces of adjacent cells. Generally these contacts were characterized by simple edge to edge adherence between endothelial cells *(Fig. 2a)*. Sometimes a limited portion of endothelial cell cytoplasm protruded over an adjacent cell to form a pocket-like structure extending into the lumen *(Fig. 3a)*. These SEM features corresponded to cytoplasmic endothelial cell projections extending into the lumen as seen by TEM in transverse sections of the lymphatic capillaries *(Fig. 3b)*.

On the luminal surface of the lymphatic capillaries, there were also long and subtle cytoplasmic extensions that arose from one endothelial cell, protruded into the lumen and abutted against

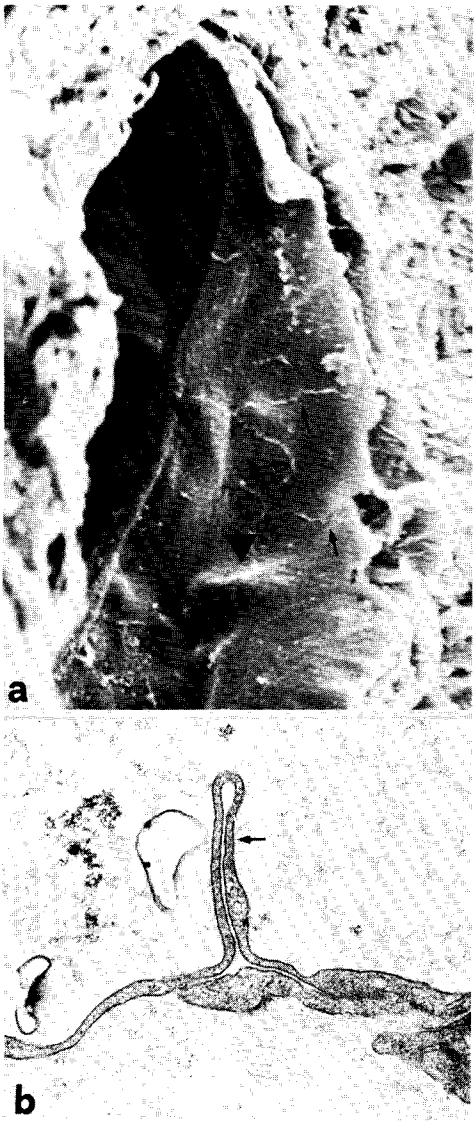

Fig. 2. ***a****. SEM luminal surface with a cardiac lymphatic capillary. Note the irregular, indented cellular contour (arrows). A subtle cytoplasmic infolding is seem to protrude into the lymphatic lumen (arrowhead) (2000x).* ***b****. TEM appearance of a thin luminal infolding (arrow) of an endothelial cell (2000x).*

another endothelial cell. These "bridges" connected different regions at a distance from the endothelial wall *(Fig. 4a)*. Using TEM, these structures were also commonly seen as thin cytoplasmic projections joining one endothelial cell to another *(Fig. 4b)*.

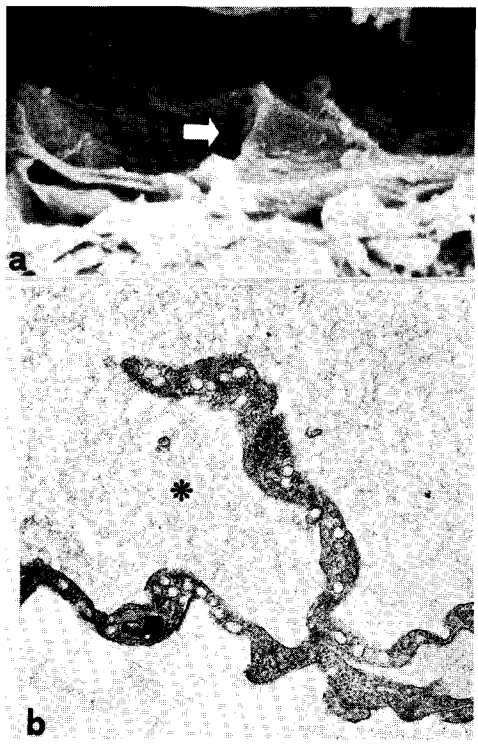

Fig. 3. ***a****. SEM of a pocket-like structure (arrow) formed by two overlapping endothelial cells (7000x).* ***b****. TEM image of a cellular edge overlapping a neighboring endothelial cell. This feature probably corresponds to a sagittal section of a pocket-like structure (*) (15000x).*

Prominent cells were also detected on the luminal surface of the lymphatic capillaries. Its cellular type was characterized by a fusiform, bulging body with two or more fine branches. These cells were generally single and were not seen in all lymphatic capillaries. They differed from flat endothelial cells, were not part of the endothelial layer, but were apposed to endothelium and protruded into the lumen *(Fig. 4a)*. Similar structures were not seen in TEM sections.

The abluminal surface of the lymphatic capillaries were covered by a network of variously arranged thin fibrils. Some attached to the endothelial wall or seemed to encircle the wall. Among the thinnest fibrils, there were other thicker

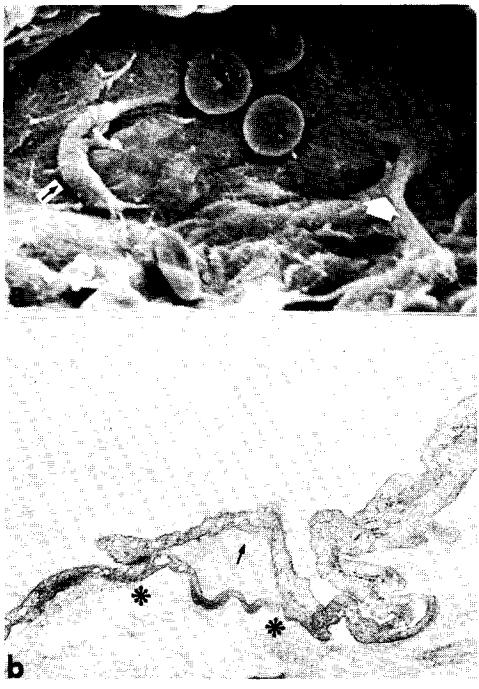

*Fig. 4. **a.** A cytoplasmic protrusion (white arrowhead) arises from an endothelial cell and abuts another at its edge. This structure appears to act as a bridge joining two endothelial cells. A spindle cell (black arrow) adheres to the luminal endothelial layer (4000x). **b.** TEM photomicrograph of a transverse section of a fine endothelial bridge (arrow) seen joining one cell to another. In this overlapping structure, the endothelial cells adhere only at the edges of the "bridge" (*) (15000x).*

fibers which extended into the interstitium *(Fig. 5a-b)*. These filaments and fibrils resembled "anchoring filaments." Collagen and elastic fibrils were also common in the interstitium around lymphatic capillaries as seen by TEM *(Fig. 5c)*.

DISCUSSION

Most studies on the morphology of lymphatic capillaries have been carried out either solely by TEM or by SEM in different organs, or under varying functional conditions of the tissues. Few investigations (5,13) have used both techniques simultaneously to examine lymphatic capillaries of a single organ. Similarly, many (1-4) have investigated the lymphatic capillaries of the heart using TEM but not simultaneously with SEM.

In the current studies, samples were taken from adjacent areas of the subepicardium of the cardiac ventricles after perfusion with fixative and then processed for scanning (SEM) and transmission electron microscopy (TEM). In these specimens, pocket-like structures and intercellular bridges of the lymphatic capillary wall were apparent and their complex three-dimensional properties were defined by comparing the photomicrographs of "frontal" SEM with transverse sections on TEM. The cytoplasmic edges protruding into the lymphatic capillary lumen as seen by TEM were confirmed on SEM as cellular pocket overlapping of an adjacent endothelial cell and very fine cytoplasmic "bridges" extending from one endothelial cell to another.

The spindle-shaped cells seen in apposition on the luminal surface of the lymphatic capillary on SEM were not verified by TEM. Whereas they may be a special cell, they are uncommon and, accordingly, their importance in lymphatic vasomotion (6,8) remains unclear.

SEM failed to delineate clefts or "open junctions" between neighboring endothelial cells or in the overlapping regions of the endothelium shaped "pockets." These data support the concept that under physiologic conditions of normal interstitial pressure, open junctions are not a conspicuous feature in the intercellular adjoining structures of the cardiac lymphatic capillary wall (4). Moreover, these findings agree with the observation that the number of lymphatic capillary "open junctions" markedly increase when the interstitium is perfused at high pressure with fixative (6).

Three types of intercellular junctions have been disclosed by TEM: "end-to-end," "overlapping," and "interdigitating" (1-4). Use of transverse sections of lymphatic capillaries help elucidate the complexity of the interdigitations among multiple cytoplasmic branches of adjacent

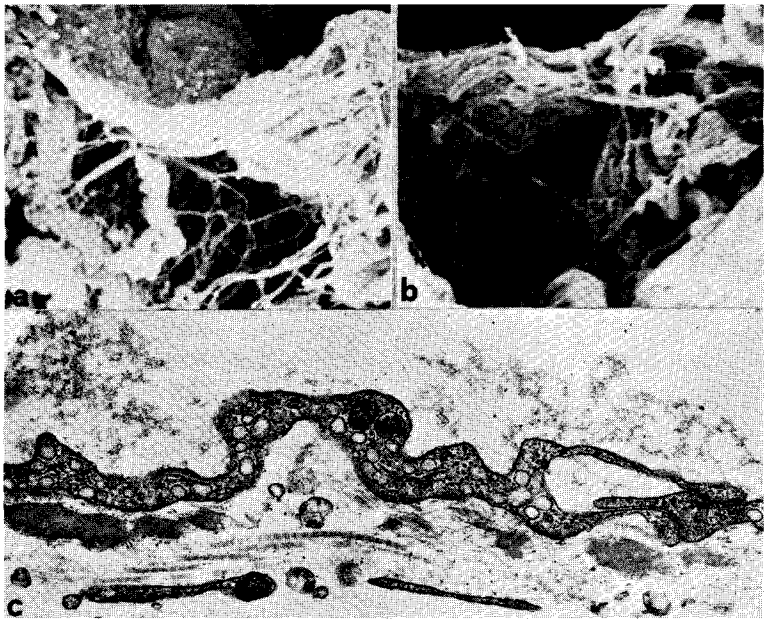

*Fig. 5. **a-b**. SEM features of the abluminal side of the wall of a cardiac lymphatic capillary. A network of filaments and fibrils extends from the lymphatic wall to the interstitium (4700x). **c**. Photomicrographs (TEM) demonstrating anchoring filaments, collagen, and elastic (orcein stained) fibrils that surround the endothelial wall (20000x).*

endothelial cells. SEM is more suitable for defining cellular borders of the end-to-end and overlapping junctions. However, SEM is not as useful for defining the intricate interdigitating adjoining areas that are not entirely visible on the luminal or the abluminal surfaces of the lymphatic capillary wall.

Intramural channels that connect the vascular lumen to the interstitium described in the lymphatic capillaries of the heart by TEM (4) were not observed with SEM despite using the same fixative technique.

The dense network of filaments and fibrils connecting the outer surface of the lymphatic capillaries with the surrounding interstitium is also clearly seen with SEM. In fact, the three-dimensional appearance provides noteworthy features of the spatial relationships of the various fibrillar components. Defining their exact nature and composition, however, probably awaits histochemistry and immunohistochemistry.

Overall, these studies indicate that SEM is particularly suitable for visualizing the luminal surface of the lymphatic capillaries. SEM clarifies the properties and significance of specific morphologic structures not detectable by TEM. Conversely, TEM using cross-sections of the lymphatic vessels allows better definition of the complex intramural anatomy of the lymphatic capillary wall itself. Together, these two modalities complement one another in defining the ultrastructure of lymphatic capillaries.

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