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STRUCTURAL AND FUNCTIONAL PROPERTIES OF INITIAL LYMPHATICS IN THE RAT TONGUE: SCANNING ELECTRON MICROSCOPIC FINDINGS

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ABSTRACT

The luminal and outer wall morphology of the initial lymphatics in the rat tongue were demonstrated using scanning electron microscopy (SEM) after tissue perfusion with pressures up to 300 torr, topical heat and histamine administration. The findings emphasize the structural and functional importance of the reticular fiber network of the basement lamina (in contradistinction to single "anchoring filaments") as a supportive framework for lymphatic endothelium and a major regulatory role in tissue volume control. Increased tissue pressure was associated with dilatation of initial lymphatics which was maximal at ~ 60 torr. Higher pressures (up to 300 torr) did not damage the vessels. This vasodilatory response of the initial lymphatics was even more evident when tissue swelling was hindered by externally applied plaster "bandage". Under SEM, protruding and branched cells were conspicuous in otherwise flat lymphatic endothelium. These cells may have contractile properties and with pronounced dilatation, thermal injury and application of histamine these cells probably contract, thereby creating large gaps at the site of open junctions.

Initial lymphatics play a key role in the early formation of lymph. Light and electron microscopy have previously shown that these vessels exhibit special morphological features which facilitate lymph fluid uptake. Lymph formation, however, is a complex process that also includes the interstitium with its tissue spaces, fibrous matrix, and terminal blood vessels. Therefore, function of initial lymphatics is more properly understood in terms of interaction with all these tissue elements or in effect, as a morphological and functional unit (1).

A variety of hypotheses of lymph formation have been proposed (2), but it is only in recent years that electron microscopy has provided a clearer understanding of entry of fluid into initial lymphatics. Perhaps the most striking finding of transmission electron microscopy (TEM) has been the large number of "open junctions" in the lymphatic endothelium and the cytoplasmic overlappings covering them in the shape of simple valves. This structural arrangement has helped explain the high permeability of initial lymphatics and their ability to act as a one-way drainage system. A crucial role for fluid passage through the open junctions has been ascribed to the so-called "anchoring filaments" (3-7), which are connected to the surrounding interstitium and insert on the outer leaflet of the lymphatic endothelium.

Only meager information is available on the existence and morphology around initial lymphatics of a basement membrane, a structural feature considered important to the outer wall of blood capillaries. No detailed study has been made of possible functional significance of a basement membrane in initial lymphatics with most investigators simply contending that the basement membrane in this area is either missing or discontinuous (7,8). Whereas scanning electron microscopy

(SEM) has confirmed many light and transmission electron microscopic observations, in some respects SEM has led to new insight into the structural organization of initial lymphatics and adjacent interstitium. As SEM is ideally suited to examination of organ and cell surfaces, earlier studies were directed at the luminal surface of lymphatic vessels, and especially the borderline system and the various cell types in the initial lymphatic endothelium. In conjunction with corrosion cast techniques it has been possible to show a branching pattern of the initial lymphatic plexus and prelymphatic spaces (9-13). Other SEM studies revealed early lymphatic structures in pre- and post-natal stages of ontogenetic development (14,15). In this paper SEM findings of the initial lymphatic system in the rat tongue are further examined. By studying the outer wall morphology of these vascular structures we aim to explore in more detail the reticular fiber system surrounding the initial lymphatics including the branched and protruding cells on the luminal side and how they relate to fluid migration into the initial lymphatic. As in our earlier studies, rat tongue was examined after interstitial perfusion with saline and fixation under abnormally high pressure. In this state of artificial edema, initial lymphatics display wide and empty lumina and are readily visualized by SEM as to basic morphology and topographical relationship to the surrounding tissue. In selected experiments the influence of extremely high interstitial pressure (up to 300 torr) was tested. The questions posed were: 1) Can the extremely thin lymphatic endothelium withstand high pressure without damage? 2) How do tongue lymphatics respond when subjected to high interstitial pressure, but swelling of the tongue is restricted by external compression? 3) Are lymphatics collapsed or dilated in the latter situation? Finally, the rat tongue was subjected to mild and intense heat and after administration of histamine (high protein edema) the structural response of the initial lymphatics was determined.

MATERIALS AND METHODS

The tongues of 35 adult male Wistar rats were examined. Rats were anesthetized intraperitoneally with pentobarbital for operative manipulations. Interstitial perfusion and fixation of the tongue was carried out as described previously (9,11).

Increased interstitial (hydrostatic) pressure

The needle inserted into the tongue was connected via a three-way stopcock to two irrigation bottles, one filled with 0.9% saline and the other with 2% buffered glutaraldehyde. The perfusion pressure was adjusted to the desired level by simply raising the bottles to the appropriate height with a stable support device. Pressures to 300 torr were generated by two mercury manometers with an inflatable bulb, each connected to an irrigation bottle. In some instances the tongue was encased in a plaster "cuff" to restrict swelling while the interstitial pressure was raised. Liquid plaster was carefully inserted into the mouth with a spatula around the tongue after needle insertion. The perfusion pressure was thereafter raised after plaster hardening. Saline perfusion lasted about 20-30 min and was followed by 30 min glutaraldehyde perfusion at the same pressure. After sacrificing the rat (by exsanguination) the prefixed tongue was squeezed by a clamp on the base, dissected free from the mouth, and placed into 2% glutaraldehyde for further fixation for two days.

Thermal experiments

In these studies a mild thermal injury to the tongue was produced using heated Ringer's solution (48-52 °C) and the tongue interstitium perfused at a pressure of 30 torr. The tissue was then fixed with glutaraldehyde through a separate injection. In other experiments hot water (54 °C) was dripped onto the tongue surface over 5-10 minutes. This maneuver induced a thermal "burn" of greater intensity. As hot water dripped, the tongue was perfused with saline and thereafter with glutaraldehyde. These experiments, too, were carried out under a perfusion pressure of 30 torr.

Application of histamine

Histamine-saline solution (10, 50, and 200mg/dl) was perfused into the tongue. Per-

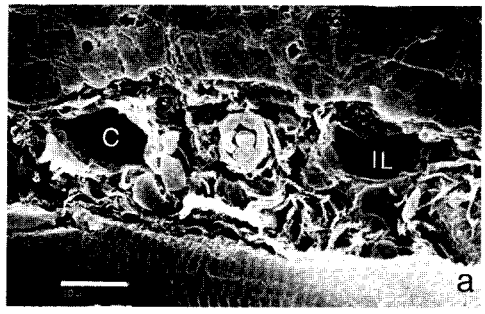


Fig. 1

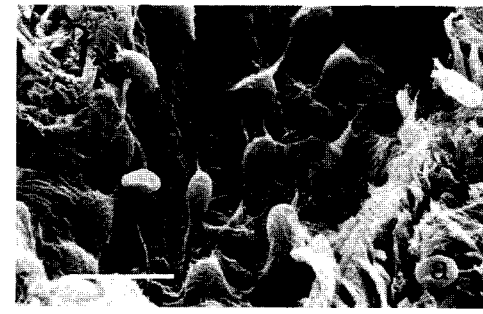


Fig. 2

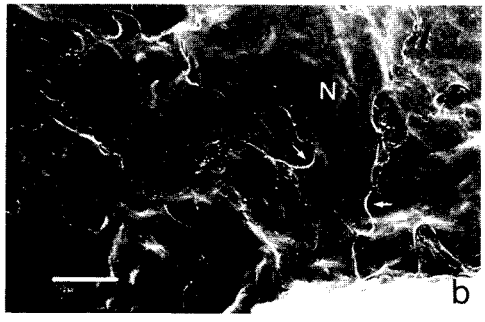


Fig. 1. a) Stromal tissue of the rat tongue interstitially perfused with saline and glutaraldehyde at a pressure of 35 torr. Two initial lymphatics (IL) and one blood capillary (C) are visible. Note the thin vascular wall and the dilated state of the lymphatics. Bar = 10μm. b) View on the luminal surface of an initial lymphatic with the typical flat pavement of broad cells. Note the wavy borders with numerous overlappings forming "pockets" (arrows). N = nuclear zone of an endothelial cell. Bar = 10μm.

Fig. 2. a) Protruding endothelial cells form the irregular profile of the luminal surface of an initial lymphatic. Tongue tissue fixed at a perfusion pressure of 150 torr. Bar = 10μm. b) Branched cell of an initial lymphatic. The processes of the cell extend to the "pockets" of the interendothelial borders. Bar = 10μm.

fusion pressures were 10, 20, and 30 torr and the perfusion time was 30 min for each experiment. Interstitial fixation with glutaraldehyde was done using the same perfusion apparatus.

SEM preparation and examination

The fixed tongue was transferred into phosphate buffer and dehydrated in 30-100% ethanol. In 70% ethanol sections were made (hand razor blade) of the subepithelial zone of the lateral and lower tongue portion. The cutting plane corresponded approximately to the plane of the surface, thus generating flat sections to facilitate visualization of the initial lymphatic plexus of the subepithelial zone. From each specimen some cross-sections were also made. The completely dehydrated sections were dried by the critical point method (Freon 13 as a transitional fluid), mounted on stubs with conducting carbon, sputter-coated with gold, and viewed in the AMR 1200 (E. Leitz, Wetzlar) scanning electron microscope at an accelerating voltage of 25kV.

RESULTS

The luminal surface of the initial lymphatics (also see 9,10)

With tongue tissue fixed under slightly increased interstitial pressure, the initial lymphatic plexus of the subepithelial connective tissue exhibited wide and empty lumina. Lymphatics were readily distinguished from terminal blood vessels which had a thicker wall and were characteristically filled with blood cells *(Fig. 1a)*. Scanning electron micrographs of the inner endothelial surface of the

initial lymphatics frequently showed a typical pavement pattern consisting of broad and flat cells with wavy borders *(Fig 1b)*. Apart from small microvilli the endothelial surface appeared smooth; only the nuclear zones were slightly prominent. Along the endothelial borders, a simple fusion of the cytoplasm between adjacent cells alternated with those where the cytoplasm overlapped and formed structures like flat "pockets". In the base of the pockets open junctions (normally not visible in SEM) completed a conduit between the tissue and lumen. Thus, each open junction together with the overlapping cytoplasmic flaps represented a simple valve (inlet valve).

The total length of the endothelial borders of an initial lymphatic in the rat tongue (related to a surface area of a square millimeter) was calculated as up to 12mm. Two-thirds of the border occupied the pockets of the open junctions with an entrance width of approximately 5-8μm. Thus, there are 1000-1600 of these interendothelial conduits per mm^2. Deviating from this pattern of flat endothelial cells, other sites demonstrated protruding and branched cells on the luminal side of the initial lymphatic *(Fig. 2a)*. In shape they resembled histioblasts more than endothelial cells. Two kinds of branched cells were clearly identified: those with a spindle-like perikaryotic zone from which two long and small processes extended in opposite directions and others with a more polymorphic cell body giving off short processes (lamellipodae) in different directions *(Fig. 2b, 3a)*. The spindle-shaped elements tended to unite in clusters which commonly connected to a bicuspid valve (exit valve) or primordial valve. Occasionally, elongated processes of the branched cells arose from the endothelial sheet to cross the vascular lumen over a comparatively short or longer distance. The broadened endings of the processes fused with

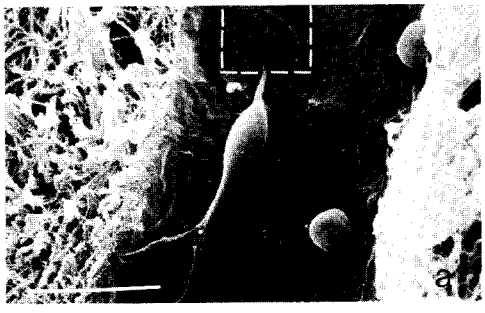


Fig. 3

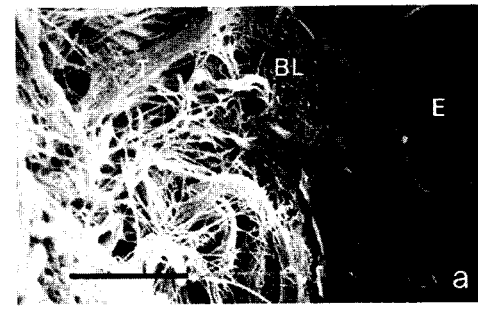


Fig. 4

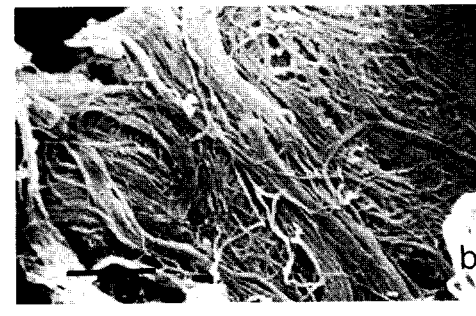


Fig. 3. a) Branched cellular element of an initial lymphatic with three slender processes extending with flap-like protrusions to an endothelial pocket. Bar = 10μm. b) enlarged sectional area of Fig. 3a *showing an endothelial pocket (arrows). Bar = 10μm.*

Fig. 4. a) A flat section of an initial lymphatic showing the luminal endothelial surface (E) together with the reticular sheath of the basement lamina (BL) and tissue fibers (T). Bar = 5μm. b) View on the reticular structure of the basement lamina covering the lymphatic endothelium from the outside. Single small fibrils and strands of fibrils are interwoven to form a delicate meshwork. Bar = 2μm.

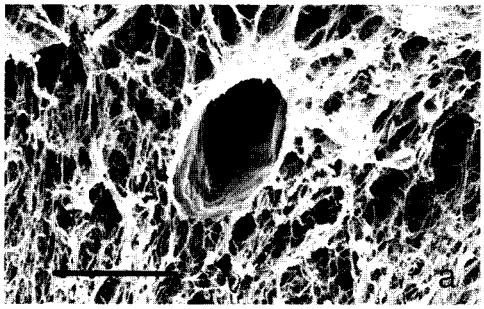


Fig. 5

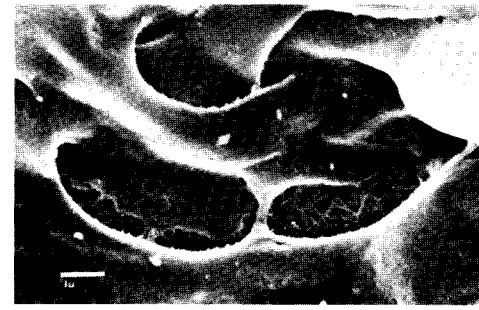
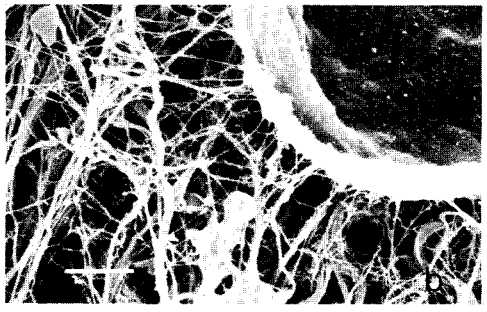


Fig. 6



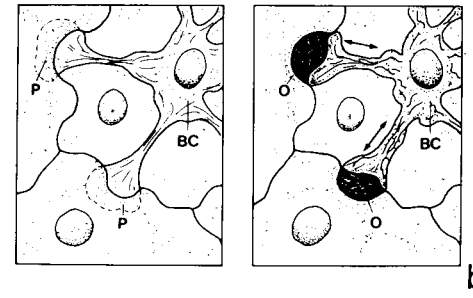


Fig. 5. a) Photomicrograph showing fixation of an initial lymphatic in the loose connective tissue by a network of radially oriented fibers. Tongue tissue perfused at a pressure of 60 torr. Bar = 50μm. b) Numerous fine single fibers and large fiber bundles connecting the tissues to the reticular sheath of the basement lamina around the initial lymphatics. Bar = 10μm.
Fig. 6. a) Tongue tissue fixed at an interstitial pressure of 100 torr showing broad openings along the endothelial borderlines at the sites where in a less-dilated state of the lymphatic "pockets" are found. Note the reticular membrane covering each opening from the outside of the endothelium. Bar = 1μm. b) Schematic drawings illustrating the mechanism by which the open junctions in the lymphatic endothelium are widened by a branched contractile cell (BC). Instead of pockets (P, left figure) openings (O, right figure) occur by retraction of the cellular processes.

the endothelium and often overlapped an endothelial pocket *(Fig. 3b)*.

Sometimes seemingly in a state of diapedesis, free cells were occasionally found in the lumen of initial lymphatics. This phenomenon has been conspicuous in early ontogenetic development (14,15) and in the rats after thermal injury and administration of histamine *(see below)*. Residues of lymph as fine filament or granular masses coated the inner surface of the initial lymphatics, especially when not perfused or perfused at very low pressure. Sometimes a small quantity of granular material adhered to the lymphatic endothelium at sites close to the intercellular borders as if a viscous substance (protein) was fixed just as it entered the lymphatic lumen.

The outer wall morphology of the initial lymphatics

The abluminal surface of the initial lymphatic endothelium was not easily seen in SEM because of coverage by a thin layer of fine fibers. This layer is part of the basement lamina. (From electron microscopic and histochemistry the "basement membrane" or "basement lamina" is precisely defined nowadays. According to Rhodin (16) the basement membrane consists of two parts: a thin basal lamina facing the cell membranes of related cells; and a network of reticular and collagenous fibrils blending with adjacent connective tissue fibrils. Fawcett (17) confines the term "basement lamina" to the endothelial cell membrane immediately underlying "lamina rara" and an outer "lamina densa" with 4nm thin filaments. The reticular fibrils of 50- 55nm enclosing both layers are no longer considered part of the basement membrane.) Amorphous material indicative of

ground substance was not detectable with certainty on SEM. The fibers, most about 100nm and smaller, interlaced to form a delicate meshwork *(Fig. 4a)*. At some sites, larger fibrous strands were traced running in different directions through the membrane. Side-by-side arranged bundles of small fibers with an undulating course were also frequently seen *(Fig. 4b)*.

Light microscopy (stained sections) showed that most of the fibers were reticular. There were also elastic fibers interwoven into the reticular pattern (13). Light microscopy also revealed a small alcian blue positive zone around the lymphatic endothelium which suggested that a small amount of ground substance existed in the outer wall of the initial lymphatics. Specimens in which the sheet of reticulum fibrils was partly stripped from the lymphatic endothelium revealed that single small fiber elements were in fact attached to the outer surface of the endothelium and corresponded to the "anchoring filaments" as seen by TEM. In these preparations, however, fibrils failed to demonstrate discrete insertion near an open junction, but had multifold connections to tissue fibers by single and multiple filaments *(Fig. 5a)*. This predominantly interconnecting fiber system was clearly visible in tissue exposed to high perfusion pressure and resembled the spokes on a bicycle wheel *(Fig. 5b)*. Light microscopy suggested that the radial fiber system consisted primarily of collagen and elastic fibers. Further away from the lymphatic these radial fibers fused with the fiber bundles in the tissue.

Perfusion experiments with high pressures

In the tongue interstitially perfused and fixed under high pressure, the initial lymphatic plexus of the subepithelial zone and the precollectors were uniformly and markedly dilated. This dilatation was already evident at interstitial perfusion pressures as low as 10 torr and became more distinct at higher pressures.

With lymphatic dilation the interendothelial pockets were diminished by shortening of the overlapping structures and most disappeared at pressures greater than 60 torr. At the site of these pockets, openings develop which were essentially identical to the open junctions aggressively challenged under these artificial conditions *(Fig. 6a)*. From the outer wall each opening was covered by the reticular structure of the basement lamina thereby forming a sieve-like diaphragm between the interstitial space and the lumen.

The heterogenous cytology of the initial lymphatic endothelium was highly conspicuous in widely dilated vessels. Spindle-shaped, irregularly branched cells were readily distinguished from broad and flat cells. The distinct impression was that some processes of branched cells retracted as part of the pockets as depicted in the schematic drawings *(Fig. 6b)*.

Tongue volume also increased after perfusion under high pressure and the degree of swelling depended upon the pressure applied *(Fig. 7a)*. Histometric measurement carried out on cross sections of rat tongues interstitially perfused with various pressures showed that tongue volume reached a maximum at 60 torr *(Fig. 7b)*, indicating that the dilating response of the initial lymphatics was not augmented at pressures beyond that level. It should also be noted that at very high interstitial perfusion pressures up to 300 torr there was a remarkable lack of direct damage to the tongue initial lymphatics. In other words, the structural morphology of the lymphatics was similar at very high and low perfusion pressures. On the other hand, in the tongue where swelling was largely circumvented by a noncompressible "cuff" (plaster bandage) interstitial perfusion pressures up to 150 torr and higher still induced lymphatic dilatation in some areas *(Fig. 8)*. The dilator response seemed more consistent in the tongue precollectors and more variable in the subepithelial initial lymphatics.

Thermal experiments

Moderate warming of the rat tongue by interstitial perfusion of warmed saline (48-52 °C) at a pressure of 30 torr induced swelling of the tongue in excess of that expected with similar perfusion using 37 °C saline. The widened tissue spaces were partly filled with granular masses of presumably protein-rich exudate and numerous free cells, most of them blood elements. The lymphatics

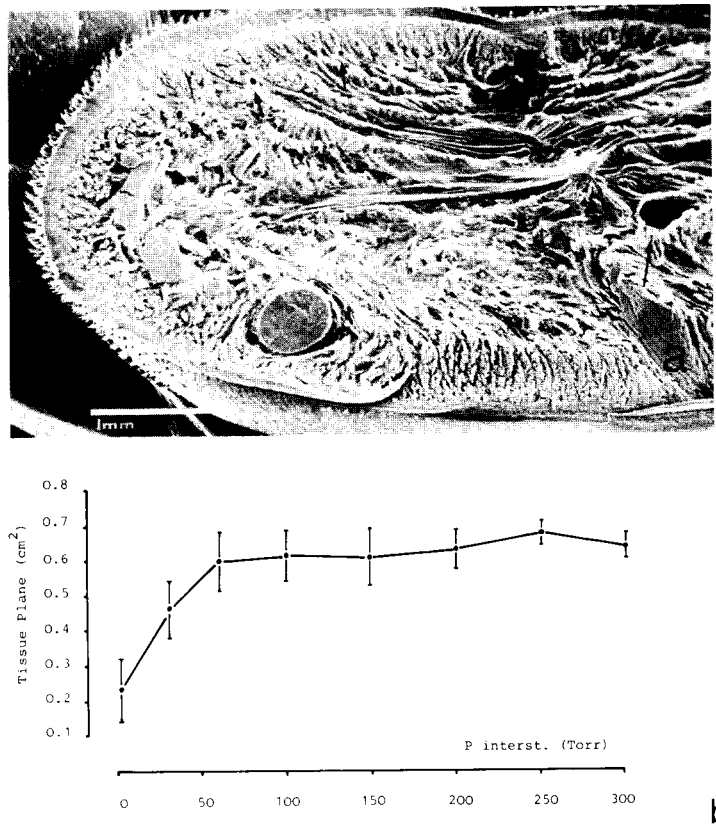


Fig. 7. a) Cross-section of a swollen rat tongue (artificial edema) perfused and fixed at a pressure of 100 torr. The stromal tissue between muscle fibers appears stretched and widely separated. The lymphatics are extremely dilated (arrows). Bar = 1mm. b) Diagram showing the relationship between the plane of a cross-section of the rat tongue and different interstitial perfusion pressures. The histometric data refer to a cross-section area in the middle part of the tongue.

(initial and precollectors) of the warmed tongue exhibited wide lumina *(Fig. 9a)*. In some lymphatics a fibrous or granular material coated the luminal surface as residues of lymph. In vessels with undisturbed luminal surface knobby microvilli and occasionally large blebs were seen *(Fig. 9b)*. Single or groups of blood cells, many of them sticking on the endothelium were also found in "warmed tongue". With greater thermal injury (dripping of 54°C warmed saline) tongue swelling was correspondingly more intense and the effects on precollectors and initial lymphatics further exaggerated. The tissue was densely filled with granular masses of a viscous exudate and a plethora of blood cells which probably extravasated from small blood vessels *(Fig. 10a)*. At some sites the lymphatic endothelium was disrupted and lumen cells were commonly in a state of diapedesis *(Fig. 10b)*. In some lymphatics large gaps developed after severe thermal injury.

Application of histamine

A 10-200mg% solution of histamine interstitially instilled into the tongue at perfusion pressures of 10-30 torr produced swelling which was greater than with similar perfusion without histamine. The lymphatics

in these experiments also demonstrated wide lumina. As a distinct phenomena after histamine application, however, numerous leukocytes were found adherent especially to the luminal surface of dilated small veins or venules *(Fig. 11a)*. Single cells and cell groups (lymphocytes, erythrocytes) were also attached to the lymphatic endothelium and sometimes granular material partly filled the vascular lumen. Large gaps were demonstrable along the endothelial borders of some lymphatics and the cytoplasmic surface was covered with knobby microvilli *(Fig. 11b)*. These findings were most notable in the tongue treated with 200mg% histamine solution and were less prominent after lower doses of histamine.

DISCUSSION

Most previous explanations on the role of initial lymphatics during lymph formation, examine the ability of these fine structures to act as a one-way drainage system and to alter their shape according to unique tissue attachment properties involving "anchoring filaments or fibrils" (18-22). Presumably, as interstitial pressure rises these filaments stretch and "pull" the lymphatics open. Because the filaments insert primarily at an open junction a direct controlling action on the width of the open junctions has also been ascribed (3-5,7,23,24). This filament hypothesis needs to be reconsidered in light of the outer wall morphology of the initial lymphatics. For example, are these fine filaments with diameters less than 100nm capable of transmitting strong mechanical forces from tissue to thin lymphatic endothelium without damage? Are all open junctions supplied with such filaments or do some lack these connections? If unconnected, how do these junctions operate to change shape?

To address these questions one must grasp the fiber organization in connective tissue. In this regard, fibrils as small as "anchoring filaments" can likely only attain a

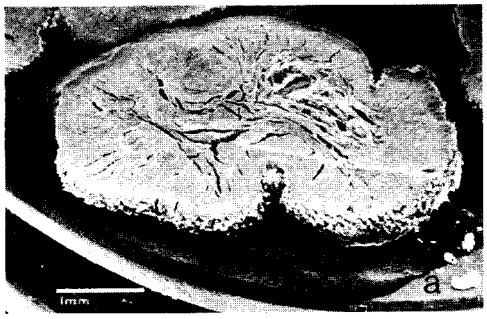


Fig. 8

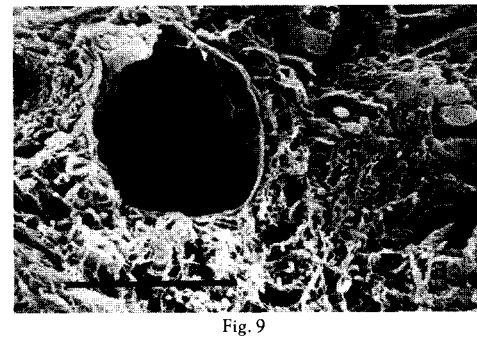
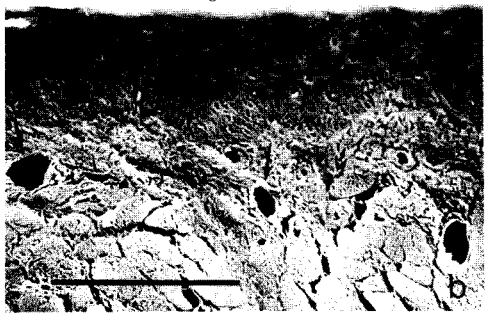


Fig. 9



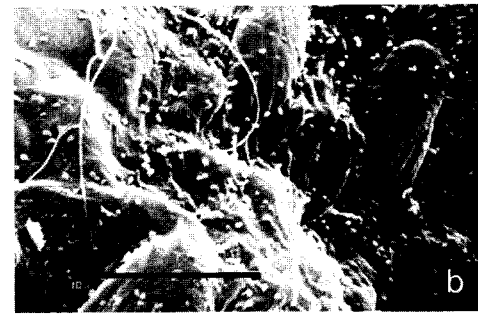


Fig. 8. a) Cross-section of a rat tongue interstitially perfused at a pressure of 150 torr, but where swelling was restricted by a plaster bandage. Note the tissue has maintained a dense and normal structure. Bar = 1mm. b) Distinctly dilated lymphatics (arrowheads) in the subepithelial zone of a preparation shown in Fig. 8a. *Bar = 10μm. Fig. 9. a) Subepithelial connective tissue of the tongue in an edematous state after interstitial perfusion with warm saline (48°C). Note the wide lumen of the initial lymphatic and the numerous cells within the tissue. Bar = 100μm. b) Numerous "knobby" microvilli on the luminal surface of an initial lymphatic after mild heating of the tongue. Bar = 10μm.*

Fig. 10

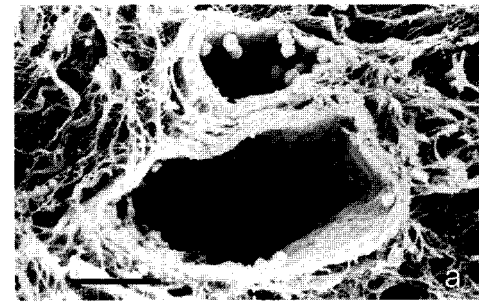
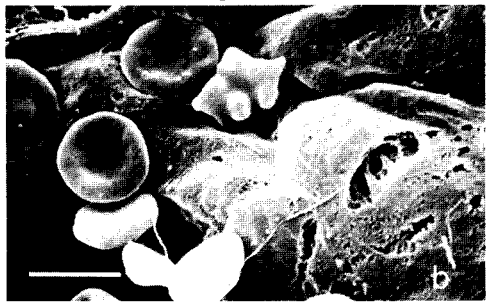


Fig. 11



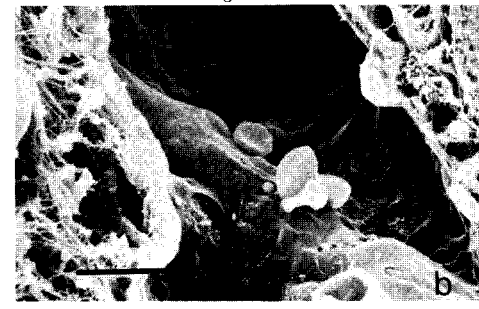


Fig. 10. a) Stromal tissue with extensive edema after thermal injury by dripping warm water (52 °C) on the tongue surface. Widened tissue spaces are filled with a granular material of a protein-rich exudate. Bar = 20μm. b) Numerous blood cells are seen in the lumen of the initial lymphatics, some of them in a state of diapedesis after intense heating. At multiple sites the endothelial sheet is torn indicating severe damage. Bar = 5μm.

Fig. 11. a) Connective tissue of the subepithelial zone of the tongue with two venules after interstitial application of 50mg% histamine solution. There is slight edema. Note the "leucocyte sticking phenomenon" in the vessel lumens. Bar = 25μm. b) Initial lymphatic with large interendothelial openings and a few blood cells in the lumen. The tissue was interstitially perfused with 50mg% histamine at a pressure of 20 torr. Bar = 10μm.

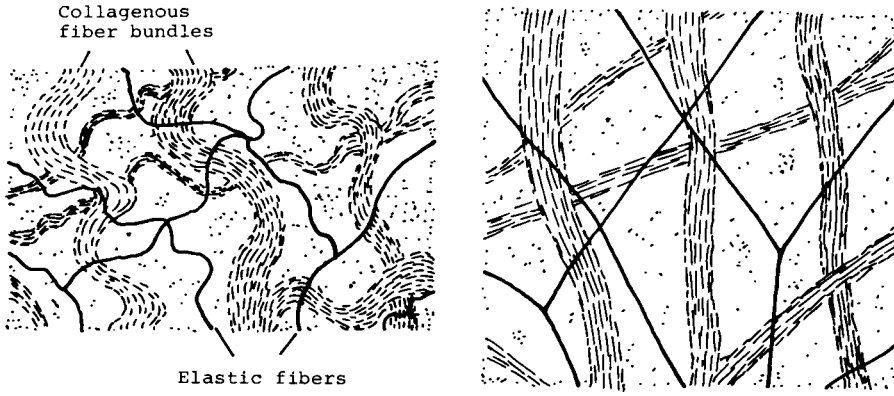


Fig. 12. General arrangement of the collagenous and elastic fibers in the loose connective tissue at normal (a) and high interstitial pressure (b).

subordinate role. Tissue stromal fibers are generally arranged in an architectural hierarchy with coarse collagenous fibers of the capsule and larger septa blending into smaller collagen bundles which finally divide into fine reticulum enveloping organ subunits and around each cell (16,25). Interposed throughout are elastic fibers *(Fig. 12a)*. These fibers of different sizes, arrangement and composition, interdigitate in such a way that when fiber tension occurs in one part, other portions are affected comparably. Thus, tissue pressure elevation that develops after an increase of interstitial fluid volume are transmitted uniformly throughout the connective tissue and fiber tensions adjust simultaneously *(Fig. 12b)*. This functional principle has special significance for initial lymphatics, which have extraordinary attachment within connective tissue by the reticular structure of the basement lamina and radial fibers. Accordingly, after an increase in tissue fluid volume, tension forces are transmitted evenly throughout the adjacent tissue. The reticular layer of the basement lamina around the initial lymphatics represents the final link of this stretched tissue and transfers the tension equally to the thin lymphatic endothelium which in turn conforms to changes in shape and structure passively. This concept based on the morphologic relationship between endothelium and basement lamina is shown in conjunction with a schematic diagram of the "anchoring filament" hypothesis *(Fig. 13)*. When the basement membrane is stretched, interendothelial junctions simultaneously open and thereby accommodate more fluid and solute influx. Conversely, with diminution in fiber tension the reticular layer of the basement lamina retracts, and the lymphatic endothelium shrinks. In this situation, the overlappings broadly cover the junctions and thereby reduce lumen size. The fundamental steps of interactions between initial lymphatics and terminal blood vessels based on the functional properties of the tissue fiber system during lymph formation are enumerated in *Fig. 14*. The structural relationship between the tissue fiber system and the initial lymphatics under normal and elevated interstitial pressure is schematically outlined in *Fig. 15*.

The special adherence of the initial lymphatics to the tissue fiber system not only provides a reasonable explanation for accommodation of tissue fluid volume shifts, but also explains how injury to thin-walled lymphatic vessels is averted under extremely high pressure as exemplified in the rat tongue with tissue pressures as high as 300 torr. Here, the basement lamina acts as a protective sheath for the thin lymphatic endothelium similar to the resistance to fluid expansion provided by coarse collagen bundles within an organ capsule and throughout the tissue elements. In contrast, if only single and weak

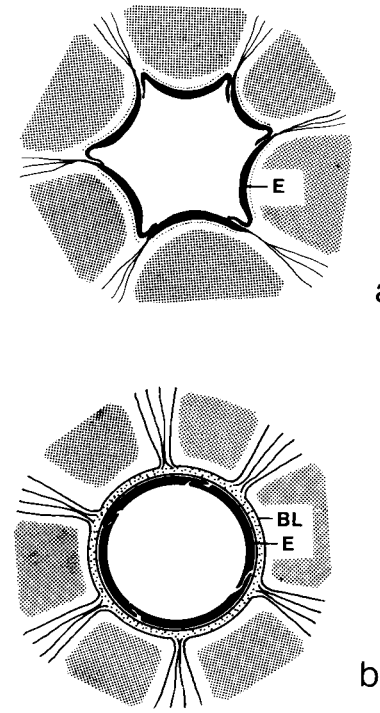


Fig. 13. Diagram showing the effect of radially oriented filaments of fibers on an initial lymphatic in edematous tissue. a) Situation according to the "anchoring filament" hypothesis. The filaments insert directly at the endothelium (E) near the open junctions and pull the endothelium outward at a few sites (modified after Casley-Smith, 1980). b) Situation according to the "basement lamina" concept based on SEM. Tissue radial fibers are connected with the reticular sheath of the basement lamina (BL) which uniformly stretches the lymphatic endothelium.

"anchoring filaments" were responsible for transmitting the tensions, it is likely that disruption of the tenuous endothelium would occur.

Where tongue swelling was restricted by firm external compression during high interstitial pressure, a dilation of lymphatics also developed suggesting that lymph transport was not retarded but even facilitated. This response may explain the favorable benefit of compression bandages in the management of peripheral lymphedema (26).

SEM further suggests that during lymphatic dilation the overlapping cytoplasm bordering each open junction in the initial lymphatics is pulled more in the plane of the endothelium and less outward as commonly assumed. This phenomenon with differing degrees of lymphatic dilation are shown in *Fig. 16*. Note that with extreme dilation the open junctions are no longer covered by overlapping structures and therefore lose valve competence. At this stage, both the interstitial space and the lymphatic lumen are in direct contact with the widened diaphragm of the basement lamina. Whereas the basement membrane of blood capillaries functions like filters to prevent egress of large molecules and particles from the bloodstream, such a role for lymphatic basement lamina is more limited with filtering only for coarse particles and larger cell aggregates. Thus, under high tissue pressure the reticular fiber system stretches the membrane open and the large gaps seem capable of accommodating macromolecules and cellular elements.

An unresolved issue is whether there is an active control mechanism in lymph formation by the lymphatic vessel itself. Previously, we proposed that protruding and branched cells in the lymphatic endothelium may have contractile capability (9,10). The observations of the present studies whereby the processes of these cells are commonly found in close proximity to the interendothelial open junctions conforms to this viewpoint and supports a direct influence on the width of these junctions *(see Fig. 3)*. Thus, contractile activity of the branched cells may be stimulated by stretching of the lymphatic endothelium, tissue warming, and drugs. Casley-Smith and Bolton (27) postulated that lymphatic endothelium contracts after histamine administration and the findings of large gaps in lymphatic endo-

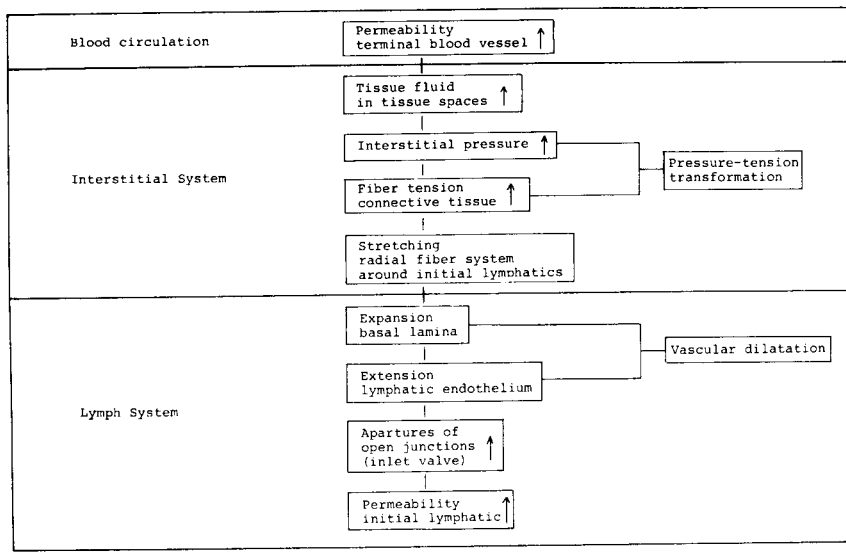


Fig. 14. Functional steps of lymph formation in the blood-tissue-lymph interface.

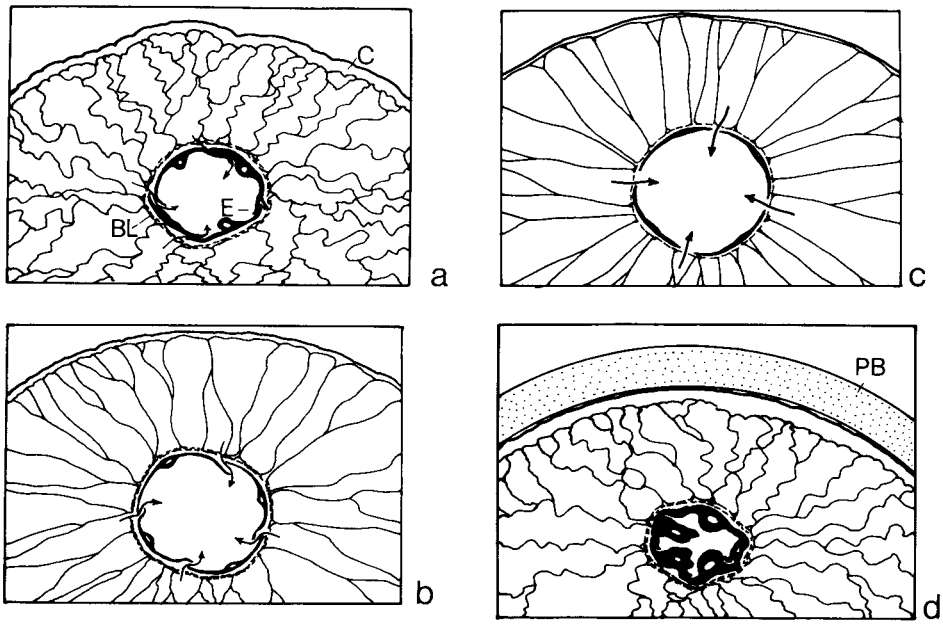


Fig. 15. Schematic drawings illustrating the relationship between an initial lymphatic and the fiber system of an organ under different tissue pressures. a) Normal. C = organ capsule, BL = basement lamina, E = endothelium. Arrows indicate the sites of the lymphatic interendothelial open junctions. b) moderately increased interstitial pressure. The tissue fiber system and the reticular layer of the basement lamina are stretched. The apertures of the open junctions are widened, but valve function is intact. c) greater interstitial pressure. The tissue fiber system and the reticular layer of the basement lamina are maximally "stressed". The open junctions are maximally enlarged, and valve function is incompetent. d) high interstitial pressure with restricted swelling by external compression (PB). The fibers of the tissue are scarcely stretched, the initial lymphatic is not compressed and lymph movement into the lumen is not impaired.

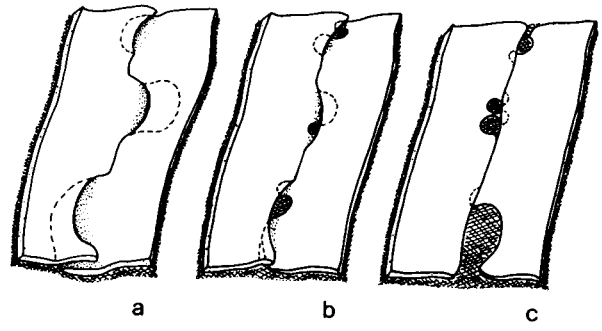


Fig. 16. Endothelial border at three different levels of interstitial pressure. a) Normal. b) Moderately increased pressure. c) High pressure. With elevated tissue pressure the reticular fibrils of the basement lamina stretch, the lymphatic endothelium dilates, and the "pockets" of the endothelial border become shortened and eventually disappear as widening of the open junctions occurs (from Castenholz, 9).

thelium in our studies after histamine conform to this hypothesis. A similar phenomenon after histamine is seen in the "stomata" of dilated venules (28).

Three mechanisms of lymph formation (i.e., hydrostatic, oncotic, or reticular) have been proposed, although the hydrostatic mechanism ostensibly dominates in most tissues (29). It is also suggested that the initial lymphatics act as tiny hydraulic pumps to create a hydrostatic suction force on tissue fluid, the formation of which in turn is by a large number of valve-like slits in the endothelium. SEM in combination with high interstitial pressure experiments as carried out in this study provides striking ultrastructural support for this functional concept of the tissue- lymphatic interface in lymph formation.

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