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INITIAL LYMPHATICS — MORPHOLOGY AND FUNCTION OF THE ENDOTHELIAL CELLS

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ABSTRACT

In considering lymph formation, the function of the initial lymph sinus is usually considered a passive process. The cells, however, of the initial lymphatics hold a key position in absorbing fluid from the interstitial space. The present study using rats and guinea pigs in different days of the estrus cycle or pregnancy examined by light, scanning electron, and transmission electron microscopy suggests that the forming and closing of the open-junction formations is an active component of the lymphatic endothelium. Open-interface structures represent a further entry into initial lymphatic pathways. The existence of "endothelial cellular buds" probably act structurally to build elements of the initial lymph sinus. In short, the endothelium of the initial lymphatic sinus appears to be a structure of utmost flexibility able to respond promptly to increased amounts of lymph fluid transported from the interstitial space.

The typical endothelium of the initial lymphatics has a circumference similar to an oak leaf. Besides regular cell-cell connections, there are zones of overlapping endothelial segments forming an inlet valve. Currently, most investigators consider that the initial lymphatic function is passive in absorbing tissue fluid (1-5). When fluid filtration by blood capillaries increases, the tissue fiber system stretches by way of the "anchoring filaments," which connect with the attenuated basement membrane of the initial lymphatics. As a result, the lumen of the initial lymphatics enlarges and the inlet valves of the open-junction separate thereby facilitating an increased uptake of tissue fluid (1-3).

Nonetheless, the range of lymph production depends on the number of open-junction formations, which in several experiments have yielded variable results. For example, in the tunica vascularis of the uterus of rats or guinea pigs in the diestrous phase of the sexual cycle, distinctly fewer open-junctions are visible compared with estrus or during pregnancy.

When the amount of tissue fluid exceeds the transport capacity of lymphatics, edema ensues. Since edema seldom appears in daily life despite the great demands on lymph transport, it has been suggested that initial lymphatics may not be solely passive structures.

Accordingly, we examined the following questions:

1. Is the number of open-junction formations of initial lymphatics and pre-collectors a constant value?
2. Are other mechanisms acting as pressure depending inlet valves?
3. Can new initial lymphatics develop on demand as required; that is, does an adaptive growth phenomenon exist?

MATERIALS AND METHODS

This study used rats (Wistar strain,

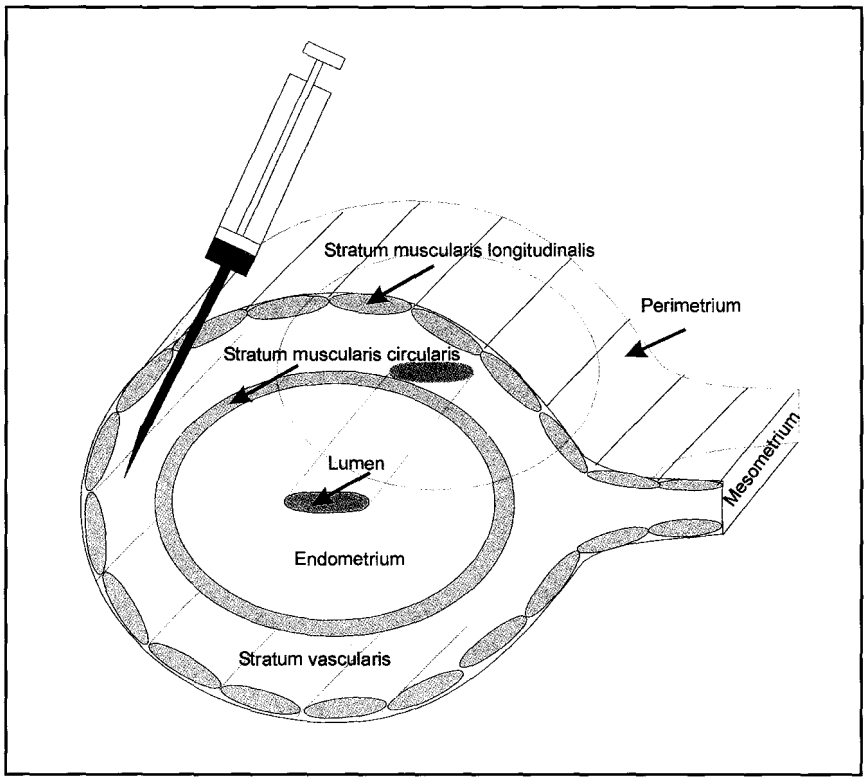

Fig. 1. Schematic cross section of the uterus of Rattus *or* Cavia *depicting localization for interstitial perfusion (5).*

Hannover) and guinea pigs of our own breeding stock on different days of the estrus cycle or pregnancy. After narcotization and killing of the animal, the uteri were prepared in situ and interstitially perfused in the tunica vascularis of the antimesometrial side *(Fig. 1)*. Two steps of solutions were used, first a chloride-free isotonic medium (Na_2SO_4, 3.3%) and secondly silver nitrate (0.5% in double distilled water) at about a 30 cm water column. Subsequently, the uteri were removed, fixed in formaldehyde (4% in double distilled water), and irradiated with UV light for 5 minutes at room temperature. 12 hours later the fixative was washed out four times in double distilled water. The uteri were dehydrated in alcohol and then cleared in xylene. With a razor blade, parts of the uteri were cut in slices of 100 µm, covered with DePeX® and examined by light microscopy. The other parts of the uteri were embedded in paraffin, cut in slices of 10, 50, and 100 µm by a microtome and then examined in stained or unstained condition by light microscopy (stains used: nuclear red, H&E, Goldner, Azan). Control slices without silver nitrate perfusion completed the study.

Glutardialdehyde was used instead of formaldehyde in a second examination. After perfusion with or without silver nitrate, the specimens were dehydrated, dried at critical point, mounted on stubs with conducting carbon, sputtered with platinum and examined with a scanning electron microscope (field-emission SEM, Hitachi S-4000) at acceleration voltages of 5 and 10 kV, respectively.

To gain insight into the structure of the cytoskeleton three specimens were perfused interstitially with a buffer (6) at a 20 cm water column. Subsequently, 0.1% Triton-X-100

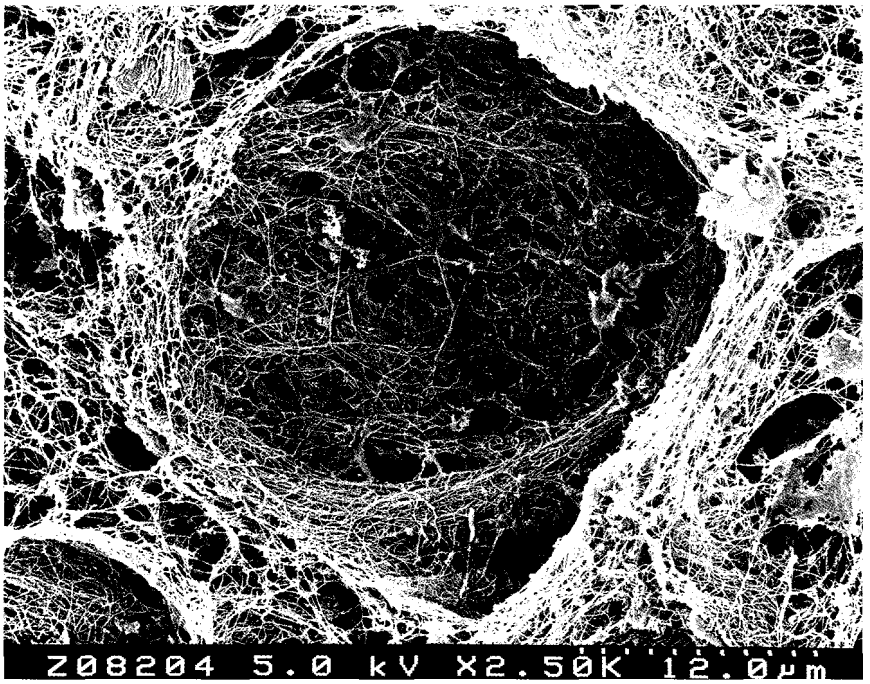

Fig. 2. Space of approximately 24 x 24 µm in the connective tissue of the tunica vascularis surrounded by collagen fibers. Rattus, *6th day of pregnancy, lateral of an embryonic field, SEM (5).*

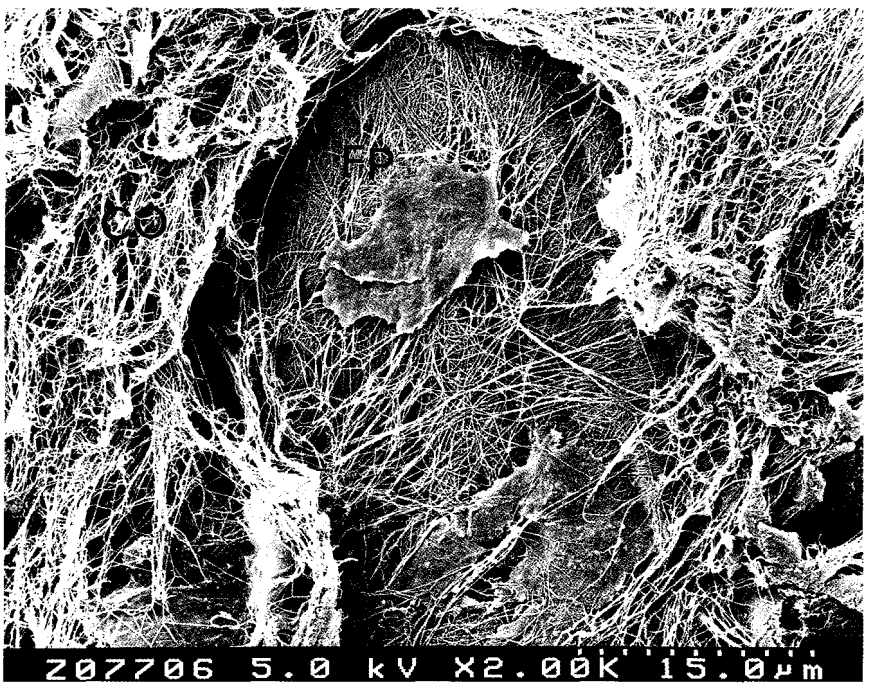

Fig. 3. Space of approximately 30 µm in the connective tissue of the tunica vascularis surrounded with the typical fiber structure and some single cells. FP = filopodia, CO = collagen fibers. Rattus, *17th day of pregnancy, lateral of an embryonic field, SEM (5).*

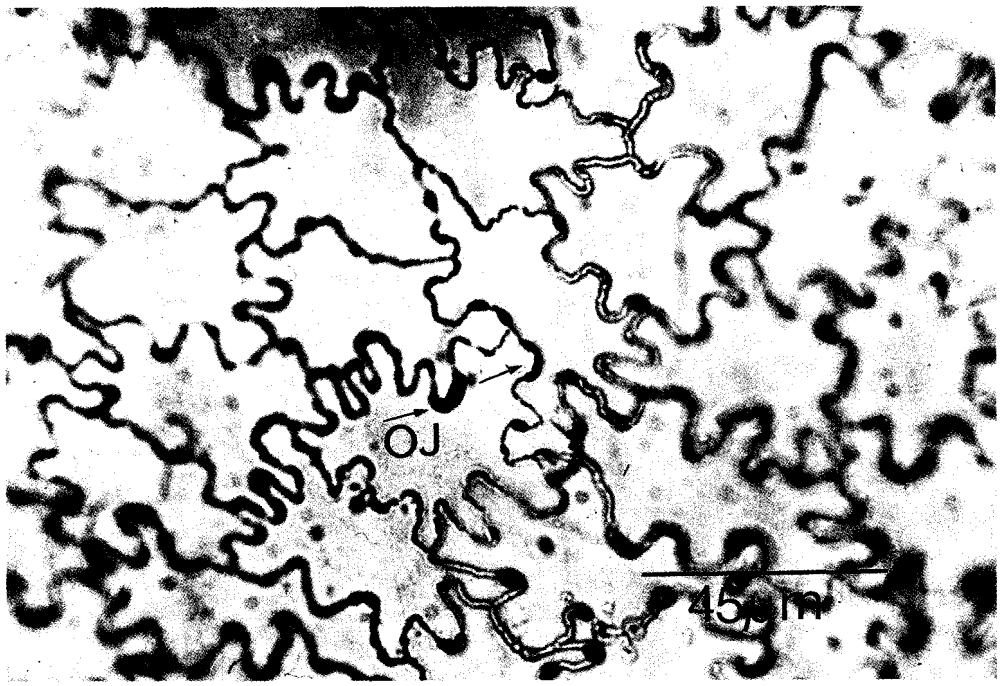

Fig. 4. Oak-leaf pattern of an initial lymphatic in the inner layer of the tunica vascularis. OJ = open-junction formation, Rattus, *diestrus, silver impregnation, LM (4).*

was applied for 30 minutes in buffer, the specimens washed in pure buffer, fixed in 0.25% glutardialdehyde solution, and then prepared for SEM. Control specimens for examination were interstitially perfused only with Sorensen buffer (pH 7.2) followed by glutardialdehyde (2.5%) and prepared for SEM.

For transmission electron microscopy (TEM), the uteri were perfused interstitially with Sorensen buffer (pH 7.2) and glutardialdehyde (2.5%) successively. Specimens with edges of 1 mm length were cut, post fixed in glutardialdehyde overnight and then with OsO_4, dehydrated in acetone up to 70%, contrasted with phosphotungstic acid and uranyl acetate, dehydrated up to 100% and embedded in Vestopal®. Ultrathin sections were examined by TEM (E9 S, Carl Zeiss).

RESULTS

General Observations

During interstitial perfusion of $AgNO_3$ or glutardialdehyde, the solution spread in the tunica vascularis and was transported mainly through lymphatics *(Fig. 1)*. The results were deposits of silver, contrasting especially the cell-cell junctions of the blood and lymph vessel endothelium. By light microscopy, in the cleared slides one can see dark lines at the cell borders. In lymph vessels, these are so typical that the differentiation between lymph and blood vessels was clear.

Tissue Channels

Following the interstitial perfusion of the tunica vascularis with glutardialdehyde, in SEM regular spaces with diameters of 25 up to 100 µm and larger were visible *(Fig. 2,3)*, lined only by a network of fibers with meshes up to 1.2 µm *(Fig. 2)*. Other spaces were lined incompletely with endothelial-like, widely-spaced cells *(Fig. 3)*. These cells exhibited

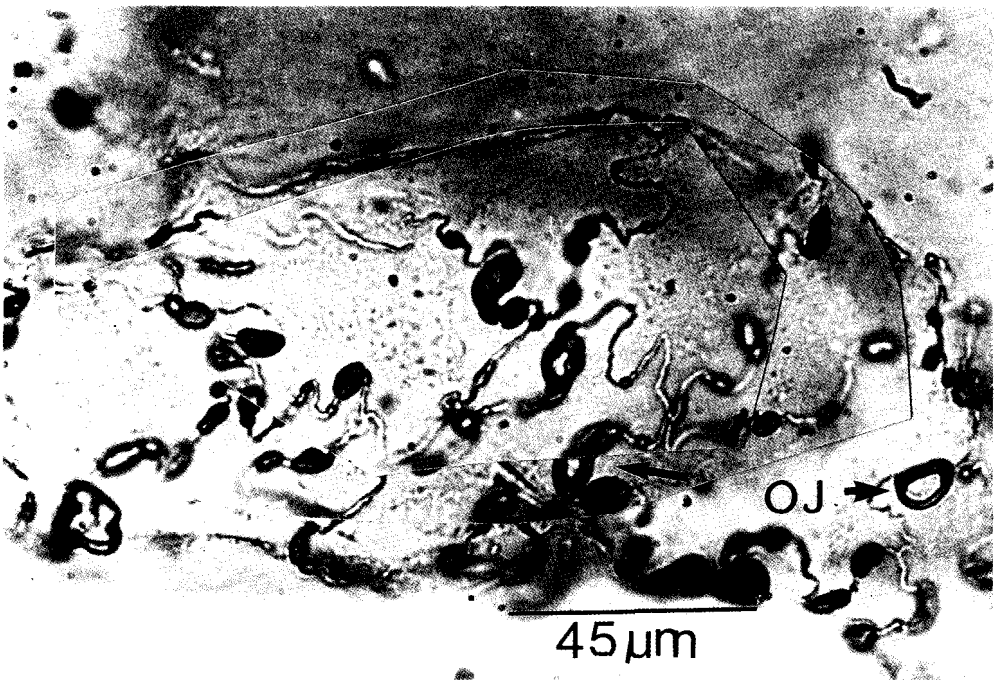

Fig. 5. Initial lymphatic. Numerous open-junction formations (OJ), partly exhibiting a double-lined system after treatment with silver nitrate. LM, mounting of three levels of sharpness. Cavia, *estrus, LM (4).*

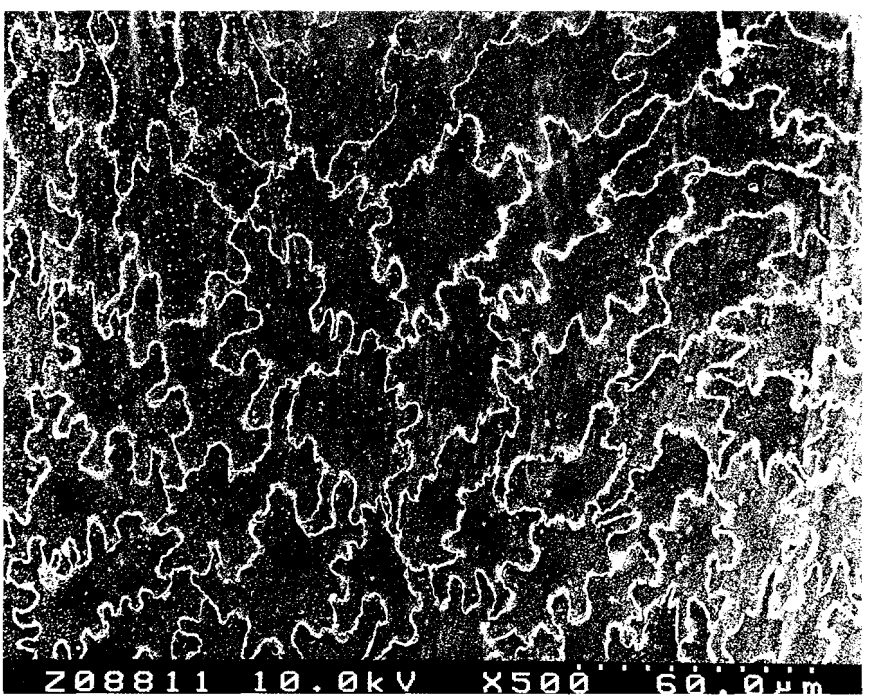

Fig. 6. Oak-leaf pattern of an initial lymphatic in the inner layer of the tunica vascularis, lateral of an embryonic field. Rattus, *18th day of pregnancy, silver method, SEM (4).*

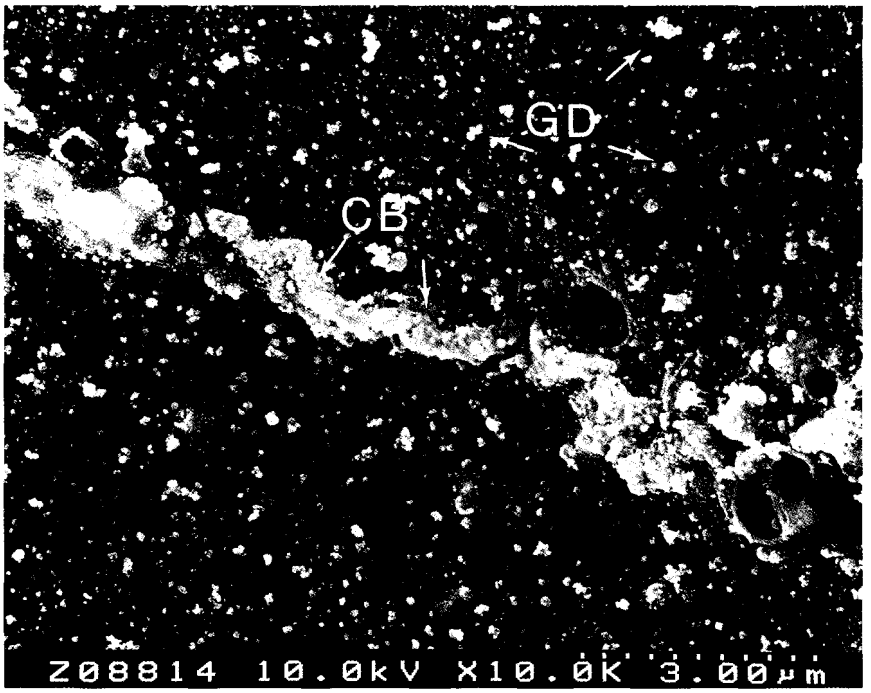

Fig. 7. Cell borders (CB) of initial lymphatic endothelium in the tunica vascularis, lateral of an embryonic field. On the cell surface fine granular deposits (GD). Silver method. Rattus, *18th day of pregnancy, SEM (4).*

diameters of 14 x 7 µm and possessed 3 µm inlets between short processes of 3-4 µm. These filopodes were connected with the collagen fiber system of the connective tissue. These spaces were most frequently visible in estrus and post-estrus and pregnancy, and much less in the inter-estrus and pre-estrus phase.

Initial Lymphatics

The typical endothelial cell of initial lymphatics possessed the circumference similar to an oak leaf *(Fig. 4-6)*. Approximately 500 cells existed per mm^2. One cell extended over approximately 2000 μm^2. The cell diameters were 60 x 30 µm, the diameter of the inlets about 4-8 µm. In SEM the cell borders were raised, several about 500 nm. Finely dispersed granules lay on the cell surface of lymphatics perfused with silver nitrate, the cell borders of which were closely covered with these granules. *(Fig. 7)*.

Neighboring cells of the initial lymphatics demonstrated numerous open-junction formations after silver impregnation, visible in light microscopy as dark oval or circular structures, partly exhibiting double rings *(Fig. 5)* along the cell borders. In estrus and pregnancy the number of open-junction formations was distinctly greater than in diestrous *(Fig. 4,5)*. Partial pores were visible on the cell surface, a typical appearance of an open-junction formation one can see in SEM *(Fig. 8)*. The cell borders of neighboring cells overlapped each other to form an inlet valve. Each cell possessed up to 15 inlet valves common with its neighbor. The number of open-junction formations was about 3750/mm^2 (15 x 500/2). Examining in SEM an initial lymphatic from the backside *(Fig. 9)*, the fiber texture of the sparse basal membrane is visible with single filaments of a diameter of 20-30 nm. Interwoven in this network there were anchoring filaments with a diameter of 50-60 nm radiating in the connective tissue

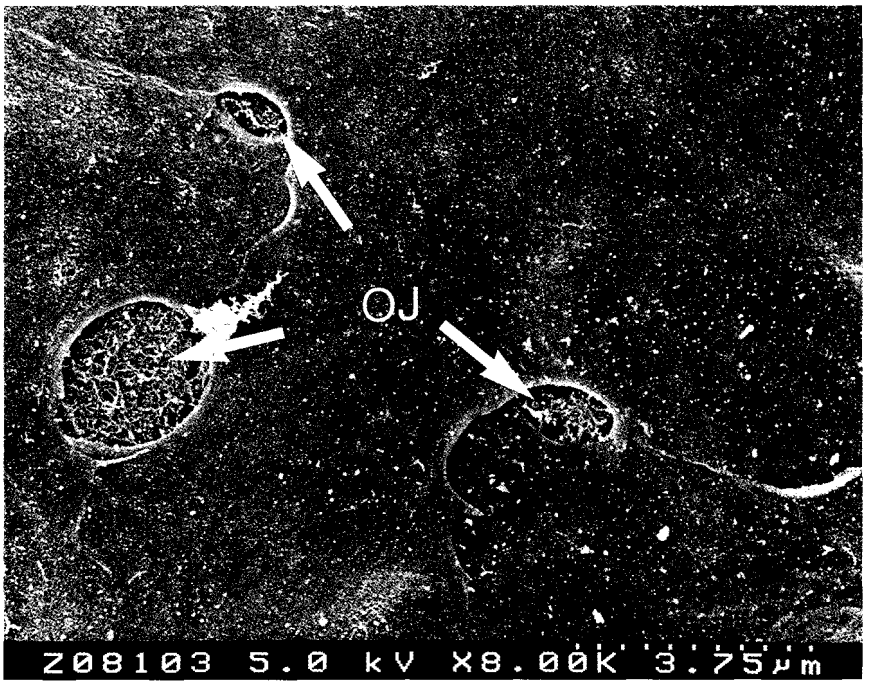

Fig. 8. Cell border system of the initial lymphatic including three open-junction formations (OJ) with different openings (OJ). Cavia, *estrus, SEM (4).*

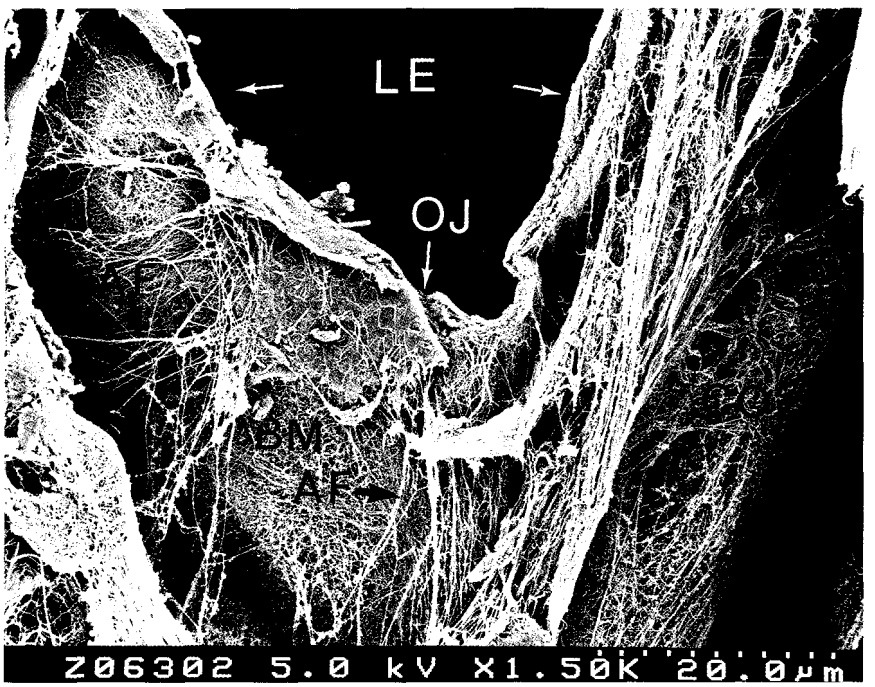

Fig. 9. Endothelium of an initial lymphatic viewed from the abluminal side. LE = endothelial cell, OJ = open-junction formation, AF = anchoring filaments, BM =basement membrane, CO = collagen fibers of the connective tissue. Rattus, *13th day of pregnancy, SEM (5).*

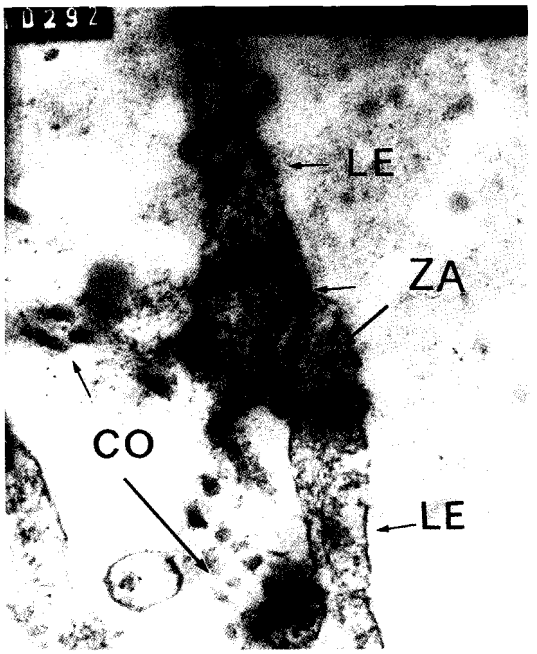

Fig. 10. Overlapping zone of two endothelial cells of an initial lymphatic in the basal endometrium. LE = lymphatic endothelium, ZA = zonulae adherentes, CO = collagen fibers. Cavia, *estrus. TEM, 47,500x (4).*

around the initial lymphatic. On the cut edge of this specimen an open-junction formation was visible, but, in contrast to the area beside this structure, lesser or no filaments of basement membrane existed. Even TEM examination showed open-junction formations of initial lymphatics in the tunica vascularis underlain by collagen fibers.

Outside of the open-junction formations, the endothelial cells of the initial lymphatics were connected with zonulae adherentes. In TEM *(Fig. 10)*, the overlapping zone of two cells had a length of about 3 µm. Between the basal side of one cell and the apical side of another, two zonulae adherentes existed.

Following treatment with Triton-X-100, elements of the cytoskeleton were visible in SEM. Above the nucleus, its texture was relatively compact with a mesh about 75 nm wide. In contrast, in the perinuclear zone it was wider and partly formed bundles, partly existing of single filaments with a mesh about 150-750 nm wide. Along the cell border was regularly seen a broad band of filaments (DPB = dense peripheral band) of the cytoskeleton with a diameter of 150-300 nm *(Fig. 11a,11b)*. The open-junction formation in this SEM figure *(Fig. 11a)* was divided into two parts of 1.5 µm with a filopodium of 2 µm of length and a width of 150 nm up to 500 nm contacting the neighboring cell. In the open space between the two cells, the underlying basement membrane was visible. The network of the cytoskeleton in initial lymphatics was more homogenous in the perinuclear region, where it was directed in length by the cells of the pre-collectors *(Fig 12)*.

Despite almost completely demonstrable lymphatics in the uterus, in the luminal two thirds of the endometrium lymphatics could not be visualized either by light microscopy or electron microscopy. Initial lymphatics were demonstrable in the basal endometrium only in primi- or multiparae rats. Here, in the junction zone between endometrium and tunica muscularis circularis, initial lymphatics existed with a diameter of 50-90 µm, partly branched and apparently beginning to taper into the endometrium *(Fig. 13,14)*. Lymphatics of the inner tunica vascularis mostly had a radial course, some portions were slightly enlarged up to the tunica muscularis circularis *(Fig 15)*.

A particularity was the bud-like cell formation of an initial lymphatic at the bases of the endometrium *(Fig. 16)*, demonstrable in only one uterus of a gravid rat beside an embryonic field. In SEM this structure extended from an initial lymphatic, consisting of circularly arranged spindle-shaped endothelial cells. The cell diameter was about 3 µm, the length about 12-14 µm. A basement membrane was not detectable in SEM.

DISCUSSION

Tissue Channels

With proper injection, the methods applied for the demonstration of lymphatic

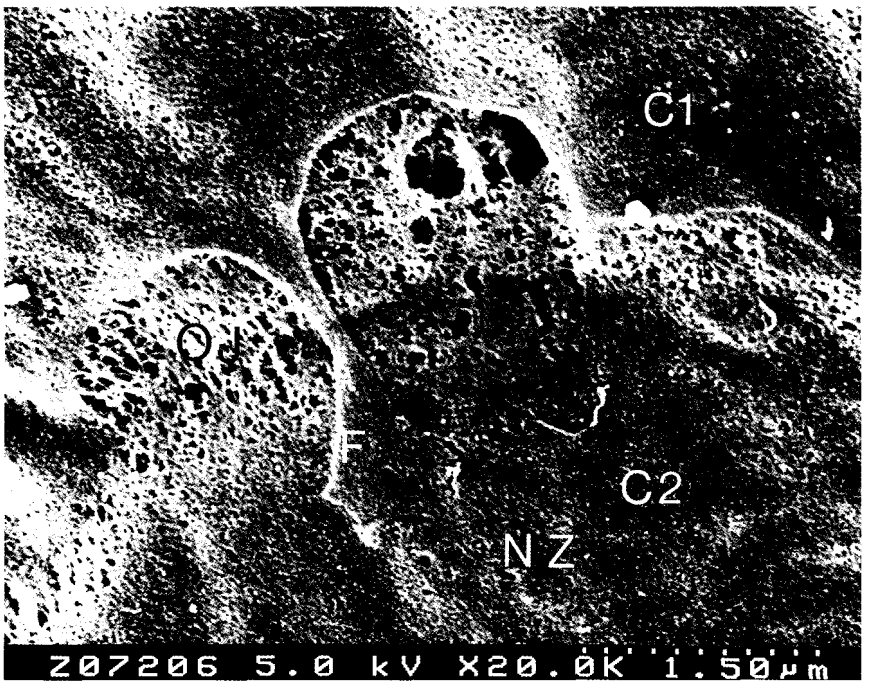

Fig. 11a. Open-junction formation of an initial lymphatic, Triton-X-100 method. OJ = open-junction formation, F = filopodium, NZ = nucleus zone, C1 = cell 1, C2 = cell 2. Rattus, *pre-estrus, SEM (4).*

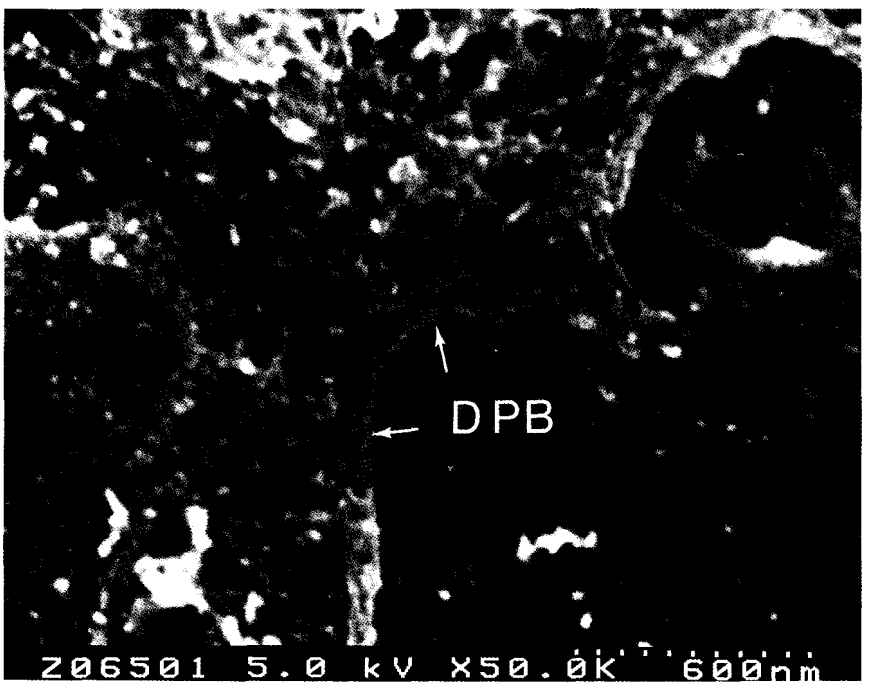

Fig. 11b. Endothelium of an initial lymphatic, zone of an open-junction formation, Triton-X-100-method. The plasmalemma of the superior cell is removed completely. Parallel to the cell border a bundle of filaments (DPB) in the cell meshes builds up from microfilaments. Rattus, *pre-estrus, SEM (4).*

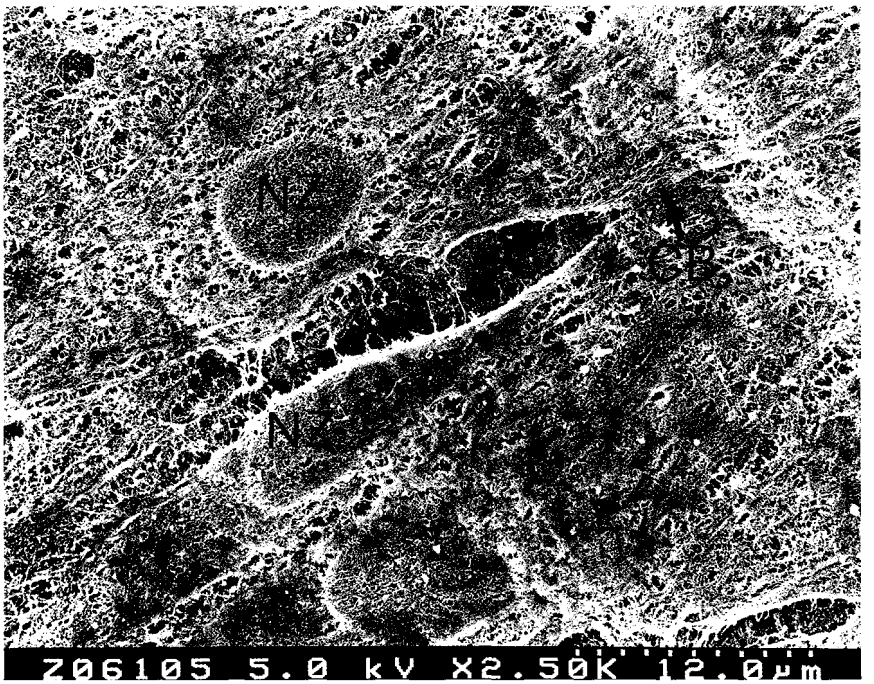

Fig. 12. Endothelium of a pre-collector after treatment with Triton-X-100. The plasmalemma of the cells is completely removed, only elements of the cytoskeleton are visible. Filamentous structures running preferentially in longitudinal direction of the endothelial cells. NZ = Nucleus zone, CB = Cell border. Rattus, *13th day of pregnancy, SEM (5).*

cell borders consistently show all lymphatics. If lymphatics are not seen in a zone which is otherwise well-marked, there are reliably no lymphatics present. Of significance, initial lymphatics in the luminal two thirds of the endometrium were absent in all examined slides (7,8). Thus, the existence of initial lymphatics in the luminal two thirds of the endometrium can be excluded. This is a feature of the uterus in context with the tolerance of the allogenic fetus and sperm. In the literature there exist different statements about the existence of lymphatics in the endometrium of *Rattus* and *Cavia*. Most conclude that there are no lymphatics (9-11) but Fabian (12-14) describes a subepithelial fine network of "lymphatic capillaries" with branches in the deeper endometrium, which are connected to larger efferent lymphatics in the basal endometrium. She mainly used patent blue to reach this conclusion. A disadvantage of her method, however, is that nothing can be concluded about histological structures along the spreading pathway of the dye. Thus, these structures could either be "tissue channels" (see below) or lymphatics. In correspondence with others mentioned in this study, neither in *Rattus* nor in *Cavia* were lymphatics demonstrable in the luminal two thirds of the endometrium.

In the absence of endometrial lymphatics in *Rattus* and *Cavia*, a tissue structure must exist which is responsible for the clearance of cyclic (estrogen-stimulated) edemas. These edemas arise from increased permeability of the epithelium of the subepithelial endometrial blood vessel plexus corresponding to variations in hormone concentration (15,16). In principle, there may exist two perhaps mutually functional mechanisms: on the one hand a particular adaptation of the venous capillary of the endometrial blood vessels, and on the other hand the so-called "tissue channels." In gel-interstitium, several authors

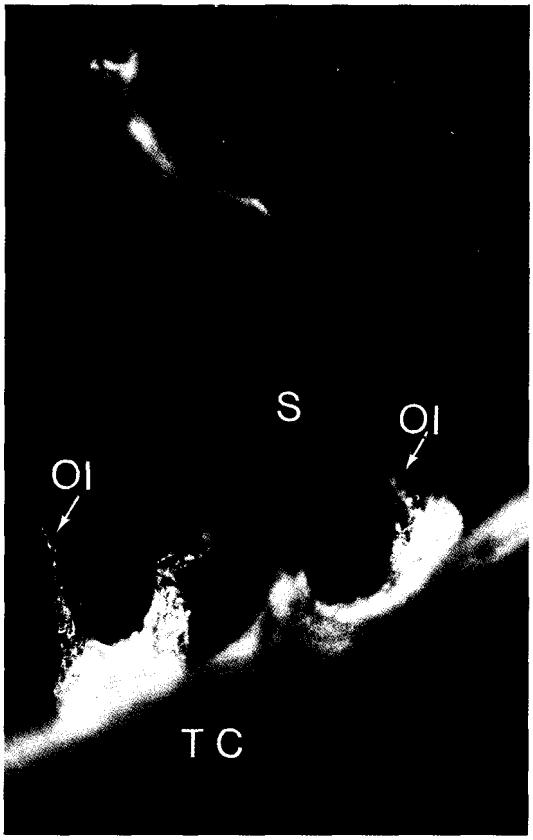

Fig. 13. Initial lymphatics and open-interface formations (OI) near the endometrial basis, visible in dark field illumination. S = stromal elements of the basal endometrium, TC = tunica muscularis circularis. Cavia, *estrus, silver method, LM, dark field (5).*

describe the existence of branched finer and larger channels and spaces filled with aqueous or wateriness phase of the extracellular matrix (17-21). Von Recklinghausen (22) and Kihara (23) describe these structures as "Saftkanälchen" (little juice channels), Hauck (19) as "tissue channels" or "low-resistance-pathways," underlining their function of facilitating the transport of molecules and particles through the interstitium. This pre-lymphatic system normally contains about 1% of free fluid otherwise bound on the gelatinous phase of the extracellular matrice (24).

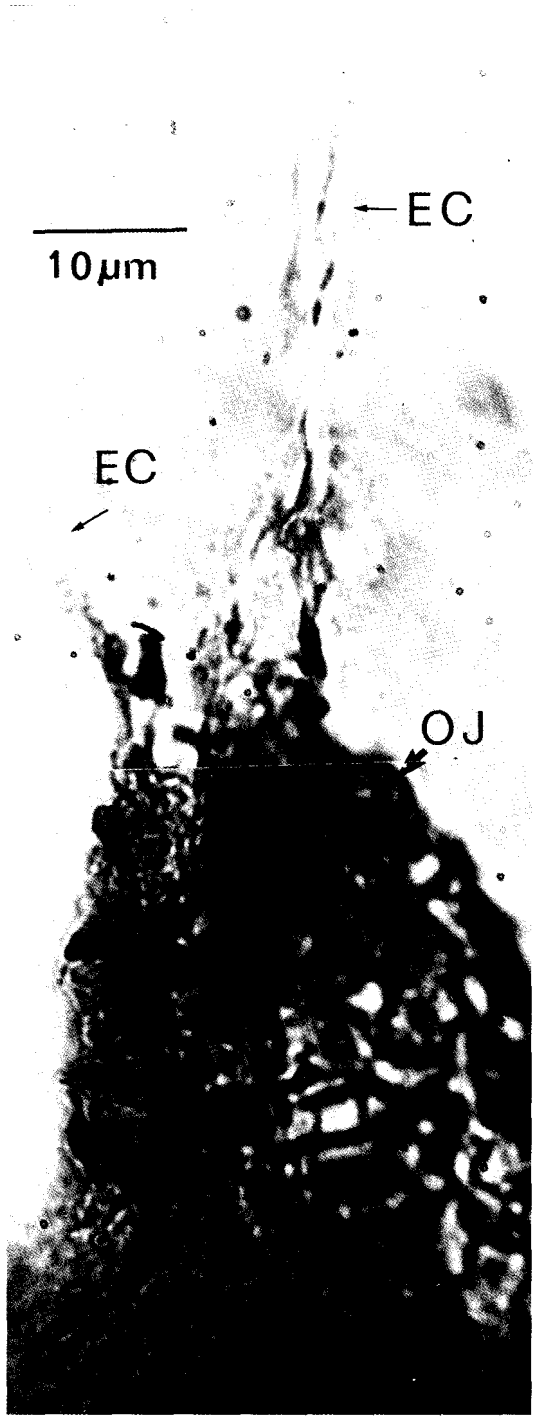

Fig. 14. Open-interface formation near the endometrial basis. EC = endothelial cell, OJ = open-junction formation. Cavia, *silver method, LM, bright field, mounting of several photographs (5).*

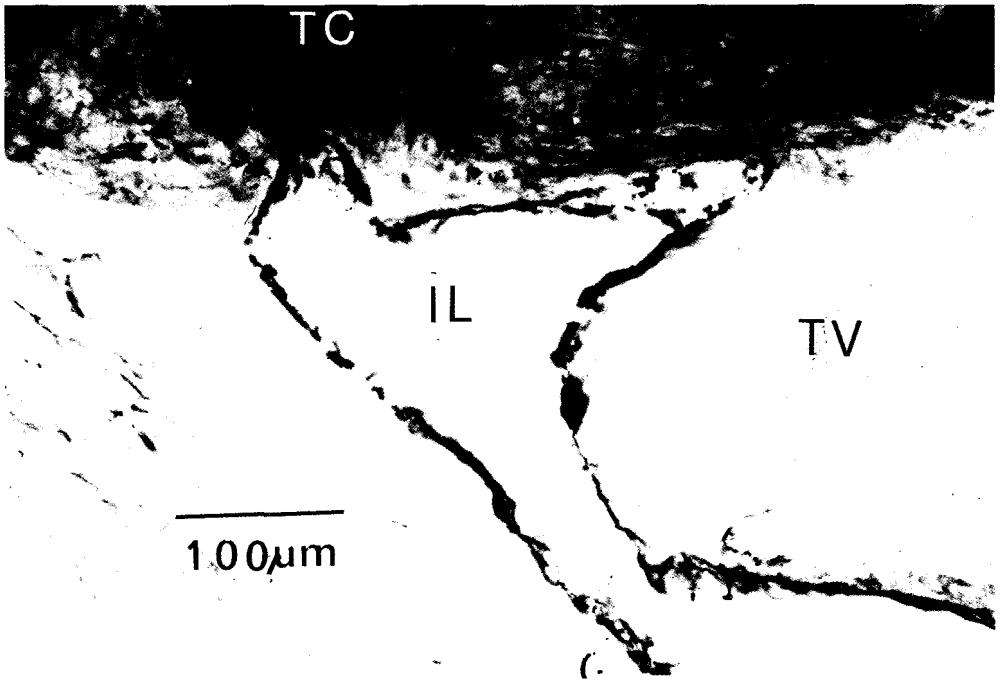

Fig. 15. Initial lymphatic (IL) in the abluminal part of the tunica muscularis circularis (TC). TV = tunica vascularis. Cavia; *estrus, silver method, LM (5).*

Fig. 16. Basal endometrium. Circularly arranged spindle-shaped cells (SZ) at the end of an initial lymphatic (IL). Rattus, *6th day of pregnancy, lateral of an embryonic field. SEM (5).*

The drainage of endometrial interstitial fluid from the luminal region of the endometrium could reach these low resistance pathways (19). Such endometrial tissue spaces to facilitate transport of tissue fluid should theoretically exist in the basal endometrium, but have not been described up to now. Only Fabian referred to this phenomenon in her studies (12-14). In the tunica vascularis we demonstrated such spaces in SEM *(Figs. 2,3)*. After interstitial perfusion, discrete spaces were visible only lined by the fiber system of the connective tissue. *(Fig. 2)*. Single flattened cells are partly found along the border *(Fig. 3)*, which apparently connect with the fiber system of the connective tissue. The outer border of these cells shows many similarities with endothelial cells of the initial lymphatics, but nevertheless, it forms a close layer of cells. Morphologically, these spaces are tissue channels with a diameter of a few up to 50 µm and more, building a system of connections. In native tissue these spaces may be filled with free fluid. Such tissue channels can also be visualized in the mesentery of *Cavia porcellus* (20, 21).

Although it is possible to view the tissue channels as an artificial structure due to interstitial perfusion, it is nonetheless very clear that the injected fluid does not spread homogenously in the tissue. There exist regions where the fluid can be transported without noticeable resistance towards the initial lymphatics. These are termed "low-resistance-pathways" by Hauck (19). Remarkably, these tissue channels are frequently visible in estrus, post-estrus and pregnancy but less often in the inter- and pre-estrus phase. Thus, a functional adaptation to a higher "lymphatic load" is reasonable.

Initial Lymphatics, General Endothelial Cell Borders

Under light microscopy, it is possible to differentiate between initial lymphatics, pre-collectors and collectors by applied silver staining (7,25). Whereas initial lymphatics consist of endothelial cells with an oak-leaf shape, collector endothelium is rhomboid shaped and in pre-collectors both forms are visible. Moreover, many trabeculae exist in the lymphatic space of the pre-collectors. In SEM one sees the patterns of the cell borders well both with or without silver staining. With silver staining, fine granular deposits of metallic silver are visible on the cell surface with an increased presence of deposits along the cell borders, partly in conglomerates. The cell borders distinctly bulge up to approximately 500 nm *(Fig. 7)*. A possible interpretation of this phenomenon is reduced shrinking (~5-8%) during preparation for SEM, due to silver deposits. In the region of the cell borders, the silver deposits "compensate" for this shrinking and bulge formation appears.

Initial Lymphatics, Zonulae Adherentes

Up to now, there is no logical explanation for the characteristic selective silver deposits along the cell borders of the endothelium after contact with a solution of silver nitrate (26). Nonetheless, the cell borders of initial lymphatics are well-marked in light microscopy and SEM. With light microscopy a double-lined system is often visible at the cell borders which continues into the open-junction formations, thus producing double-marked ring structures *(Fig. 5)*. With TEM, the zones of cell-cell contacts show two electron dense structures over a distance of 300 nm. Casley-Smith (27), Leak (28) and others describe this contact zones as desmosomes, partly as tight junctions. The present study points out that the silver deposits correlate with these cell-cell contacts. However, due to the closed lines of the silver deposits these cell-cell contacts cannot be desmosomes; they must be zonulae adherentes. These zonulae adherentes, on the one hand, serve as anchoring structures for the dense peripheral filament band (DPB) of the cytoskeleton (29) and, on the other hand, they are the structural basis for the contact

between neighboring cells. The intercellular cleft is about 15 nm wide. In the cell membrane there exist transmembrane molecules (E-Cadherin and N-Cadherin). The neighboring catherins are connected with each other in the intercellular space by means of calcium ions (Ca^{2+}). These ions are perhaps the reason for the selective marking of the cell borders with silver. Within the cell, transmembrane molecules are bound to protein linkers on the actin filaments of the DPB (30). This DPB is a dense 150-300 nm broad band following the circumference of the cell, built up by cytoskeletal filaments and clearly visible after application of the Triton-X-100 method (6). In endothelial cells of blood venules, Wong and Gottlieb (31) determined actin, myosin, tropomyosin, α-actinin, and vinculin as components of the DPB. Thus, this DPB band is able to contract. The cell border is the region where most cellular performances occur, and it is likely that the DPB controls cellular formation. The cellular skeleton, visible after application of the Triton-X-100 method, shows a distinct differentiation between the endothelium of initial lymphatics and pre-collectors *(Fig. 11,12)*. This finding likely indicates that different mechanical conditions exist for endothelial cells in specific formations. In the initial endothelium the mechanical forces act from all directions, whereas in the endothelium of pre-collectors they occur preferentially in direction of the lymph stream and at right angles to the stream.

Initial Lymphatics, Basement Membrane

In contrast to blood capillaries, the basement membrane of the initial lymphatics is sparse and reduced to a few reticular fibers *(Fig. 9)*. Lauweryns and Cornillie (32,33) also observed interruptions in the basement membrane and its absence over long distances. At higher microscopical magnifications, a thin layer of fine reticular fibers is seen on the outer surface of the endothelium. The reticular fiber network is a system of meshes with a diameter of about 150 nm, sometimes several µm, allowing particles to pass through this membrane. In this network, bigger collagen fibers are interwoven (so-called "anchoring filaments") (28,34), radiating into the surrounding connective tissue. Solito and co-authors (35) describe these anchoring filaments as elastic fibers based on immunohistochemical methods (HB 8). They propose that this elastic fiber system probably produces a force that helps transport the lymph towards the collectors. Further studies on the molecular nature of the anchoring filaments are necessary to confirm this assumption.

Initial Lymphatics, Open-Junction Formations

Beneath the zonulae adherentes, single cells of the initial lymphatics possess inlets where neighboring cells overlap one another *(Fig. 8)*, thus forming inlet valves (2,3). These open-junction formations are U-shaped zones, partly stabilized with filopodia *(Fig. 11)*. The "pockets" disappear during dilation of the vessel resulting in small clefts or round openings. The diameter of an open-junction formation is from 3-6 µm. In an undilated condition, a 1 µm wide luminal cleft exists for the free entrance of fluids and particles from the interstitial space. As each cell has up to 15 inlet valves and in initial lymphatics 500 endothelial cells per mm^2 exist, a total number of approximately 3750/mm^2 inlet valves likely exist. Thus, 2.3% of the surface of an undilated initial lymphatic is part of the inlet zone. Dilation can expand part of inlet zones up to 7% and more and assures an easy removal of the "lymphatic load" under normal conditions.

Initial Lymphatics, Theory of an Adapted Number of Open-Junction Formations

The above-mentioned silver lines visible in light microscopy often show a double line system. The uninterrupted ring-shaped silver-line system in the open-junction formation

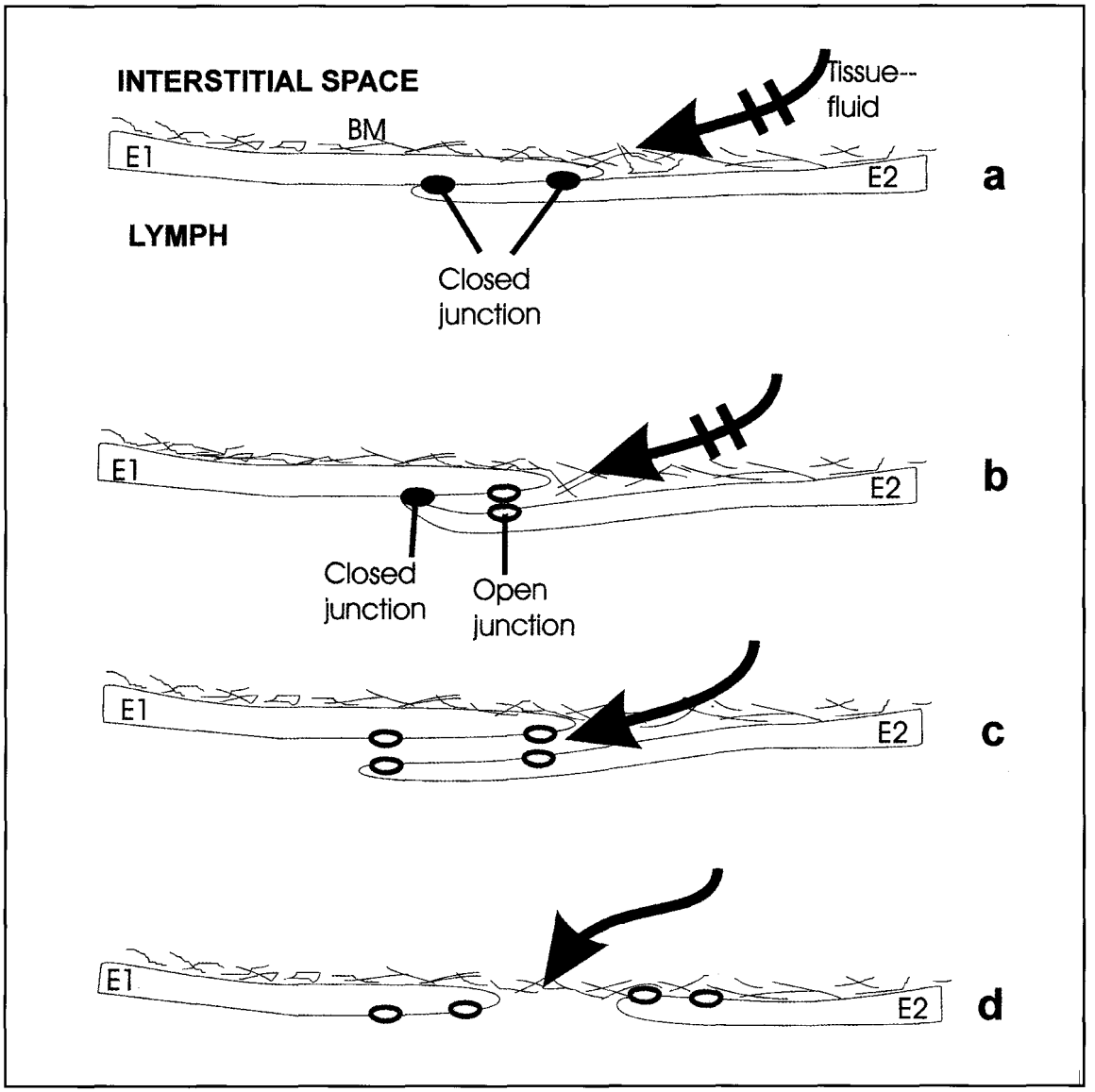

Fig. 17. Theory for the interpretation of the double ring respective double line systems of the initial lymphatics after silver impregnation. Dark filled circles: zonulae resp. maculae adherentes; Light open circle: preformed contact structures, a site where the greatest amount of silver deposits occurred (4). BM=Basement Membrane.

may be interpreted in the way that elements of the zonulae adherentes remain in this zone. In that context, it is remarkable that the number of open-junction formations in estrus and pregnancy is apparently increased in comparison with inter-estrus. This phenomenon is also demonstrable in uteri of swine (35). A possible conclusion is that due to the cadherin molecules, the open-junction formation can be transformed in a short time into a closed junction with zonulae adherentes and vice versa *(Fig 17)*. Our finding that the changing number of open-junction formations is an active process of the initial lymphatic endothelium provides new insight into the functions of these lymphatic vessels. Charac-

terized previously as passive conduits, these endothelial cells could qualify as an active controlling element in lymph production depending upon the actual situation in the connective tissue.

Returning to the zonulae adherentes, another aspect is worth mentioning. Epithelial as well as endothelial cells are described as basal, apically polarized cells. In the epithelium zonulae adherentes they exist only in the apical part of the cell (30). In the initial lymphatic endothelium the zonulae adherentes connects the apical part of one cell with the basal part of another cell *(Fig. 10)*. Whereas this arrangement suggests a relatively low grade of differentiation of these endothelial cells, it also suggests a higher flexibility and adaptability. In venules, cell-cell connections of the endothelium are described as dynamically reacting structures (36,37). The process of building up and regressing takes place within minutes so that blood cells can migrate readily through the endothelium. Similar mechanisms are probably involved in the endothelium of initial lymphatics forming open- or closed-junction structures relative to the actual interstitial fluid requirements. The reversible closing of the cell-cell contacts could considerably influence the capacity of transporting fluids from the interstitial space into the initial lymphatics. How this phenomenon is regulated in more detail is not as yet known. Two key questions arise from this hypothesis: what chain of signals releases the opening or closing of open-junction formations and how can these processes be influenced to reduce edema? Perhaps this process could be influenced by medicaments that support lymph production.

Initial Lymphatics, Open-Interface Formations

In the above-mentioned "tissue channels," tissue fluid is transported toward the initial lymphatics, where specific structures are often visible – the "open-interface formations." In the junction zone between basal endometrium and the tunica muscularis circularis there exist more or less of these formations arising from initial lymphatics, branching and tapering into the endometrium *(Fig. 13,14)* (7,25). The endothelial cells of this formation *(Fig. 14)* show a distinct space between them with prolongation into the interstitial space of the endometrium (7,25). Such open-interface formations have been postulated by Hauck (19,38,39), and the present study verifies this morphological structure in the uterus. Studies by our group in other organs like the tongue and mesentery (3,8) substantiate a regular occurrence of open-interface formations. Even others (32,33,40,41) describe structures in the basal endometrium corresponding to these open-interface formations. In this way, we demonstrate a direct connection between tissue channels draining interstitial fluid of the endometrium and the initial lymphatics. In its function as a pressure relief valve, greater amounts of tissue fluid, as for example in estrus, can be transported to the initial lymphatics, suggesting that the open-interface formation is able to respond to a greater lymph load.

In the junction zone between endometrium and muscularis, we were also able to demonstrate in SEM a sprout-like formation arising from an initial lymphatic *(Fig. 16)*. This arrangement may be able to build up new open-interfaces or new initial lymphatics within a short time under the different conditions of estrus cycles or pregnancy. Along these lines, the findings of others on lymphatic regeneration are of interest (42-44). Although this structure may only exist in the uterus, others describe similar cyclic changes of the lymphatic system of the uterus (41,45-47).

CONCLUSIONS

This study supports that initial lymphatics are lymphatic vessels with distinct features and readily distinguished from blood capillaries. Initial lymphatics have relatively

wide and variable lumina, their endothelial cells possess a typical oak-leafed shape and are extremely flattened. The basement membrane is remarkably attenuated and anchoring filaments interweave and radiate into the surrounding connective tissue. Typical elements are the open-junction formations, valve-like structures, and trabecular endothelial processes (3). TEM of others support the active components in lymph production performed by initial lymphatics (48). All these features attest to a vessel formation for which the term "capillary" is unsuitable. Because of the numerous similarities with "sinusoids," the expression "initial lymph sinus" is proposed (1,49), which is supported by findings in the present study.

The endothelium of initial lymphatics is characterized as a flexible structure with the ability to respond to higher tissue fluid demands and the need to transport more lymph. These facts lead to the following hypothesis *(Fig. 17)*.

1. Normally, there is a dynamic fluid balance and the lymphatic capacity is sufficient to transport interstitial fluid.
2. Higher lymphatic loads promote an increased diameter of the initial lymphatics and the open-junction formations are widely dilated.
3. Minimal edema (lasting a few hours?) promotes an increased number of open-junction formations of the initial lymphatics.
4. Widening of the open-interface formations or the proliferation of these structures from an endothelial bud may be regarded as a result of chronic edema. These buds may also be the structural basis for the organization of new initial lymphatics.

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