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THE ENDOTHELIUM OF INITIAL LYMPHATICS DURING POSTNATAL DEVELOPMENT OF THE RAT TONGUE

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Abstract

The luminal surface of the subepithelial lymphatic plexus in the tongue of rats was investigated with SEM at different stages of postnatal development. In newborn and infant animals prominent and branched endothelial cells exhibit a conspicuous phenomenon producing a very irregular inner profile of the vascular wall. Among these cells the spindle-shaped type proves to be an essential component of the valve structures already found in few-day-old animals. There are also prominent cells with a polygonal appearance resembling histioblasts which form with their manifold processes, that partly extend into the lumen, an interlacing cellular pattern. The special morphological characteristics of the endothelium of the initial lymphatics already occurring in early postnatal development point to several particular functional activities such as controlled permeability, contractility, phagocytotic property, and demonstrate the exceptional position of this kind of vessel among the structures of the lymph- and blood-draining system.

KEY WORDS: Endothelium, Initial Lymphatics, Postnatal Development, Rat, Tongue, Scanning Electron Microscopy

Introduction

The endothelium of the initial lymphatics is generally considered a continuous layer of thin cells with an outline pattern resembling oak leaves[8, 10, 13]. The picture of these particular cells, mainly derived from light microscopic preparations after silver impregnation, has been enriched in the last decades by transmission electron microscopic (TEM) findings giving information particularly on the fine structural features of their cytoplasm and the nature of their intercellular junctions[3, 9, 11, 16].

In previous papers we described the surface morphology of the initial lymphatics as seen in scanning electron microscopy (SEM)[4-6], but we could observe that the lymphatics of the subepithelial plexus in the rat tongue were not furnished throughout with a regular pattern of thin endothelial cells. There were also places, where the lymphatic endothelium distinctly deviated from a regular cellular arrangement displaying prominent cells with various shapes. These peculiar cells, most of them equipped with different processes, suggest that they may reflect a special functional state such as the state of cytoplasmic contraction evoked by an experimental edema.

In the meantime we could also see those outstanding endothelial cells in the initial lymphatics in specimens in which the increased interstitial pressure, as used in our previous studies, did not seem to be the determining factor. This situation induced us to engage in the present SEM study to examine the question whether the prominent and branched cells in the lymphatic endothelium already occur in the early stages of ontogenetic development or whether they have to be considered a sign of special adaptability to a momentary process. Thus we hoped to get information on both the cellular

differentiation of the lymphatic endothelium in newborns and infants and on the significance of the prominent, branched cells, in particular.

Materials and Methods

Forty-two rats (Wistar) of both sexes weighing 6 to 160 g (corresponding to an age of two to fifty days) were used in this study. All animals were anesthetized intraperitoneally with Nembutal® from CEVA GmbH, Bad Segeberg, W. Germany (15 mg/kg body weight). The tongues of the animals were prefixed in situ by interstitial injection with 2.5% glutaraldehyde via a fine hypodermic needle (0.4 x 19 mm) inserted into the tongue body. During this procedure, which lasted 15-20 minutes, the injection pressure was kept constant at a level of 30 cm H_2O by an irrigator device. A precise description of the apparatus is given elsewhere[4]. Thereafter the animal was killed by a cut through the heart, the tongue dissected and postfixed in the same fixation fluid for 48 hours.

The fixed organ was washed in phosphate buffer (pH 7.3) and dehydrated in a graded series of alcohol. The dehydrated tongues were cut into thin sections by hand with a razor blade under the stereomicroscope. Cutting was done exactly in the plane parallel to the surface of the lateral and lower side of the tongue to get horizontal sections especially through the subepithelial connective tissue where the plexus with the initial lymphatics are embedded (compare fig. 1). The sections were transferred to Freon 11 and 13, dried in the critical point apparatus, mounted on stubs with carbon and sputtered with gold. The preparations were examined in the Leitz AMR 1200 scanning electron microscope at an accelerating voltage of 25 kV.

Results

In all the stages of postnatal development investigated by us, a well developed subepithelial lymphatic plexus can be demonstrated in the rat tongue (fig. 1). All vessels of this vascular area are furnished with a layer of endothelial cells, even shortly after the birth of the animals. Apart from some single erythrocytes and other free cells, the lumen of the lymphatics appears empty. The inner endothelial surface of the initial vessels, whose luminal width ranges between 15 and 30 µm, exhibits a quite rough profile, which hardly changes in postnatal life. The irregularity of the lymphatic endothelium in young animals is caused by cells of various shapes with markedly prominent portions (fig. 2). A flat formation of endothelial cells with a regular pattern of serrate borders, as seen in adult animals[4], can seldom be recognized in newborn and infant animals. Only collecting vessels (precollectors) with diameters of more than 30 µm already show a flat endothelial lining in early postnatal development (fig. 3). However, the endothelium of this vessel type is composed of broad, rhomboid-shaped cells with only slight nuclear protrusions and by no means corresponds to the more polygonal cells characterizing the flat endothelium of the initial lymphatics in adults (fig. 4).

The different variations of the highly prominent cells in the early lymphatic endothelium are clearly distinguishable with SEM. We were not able to determine an essential difference between the previously described prominent cells in adults and those in newborns and infants.

Very conspicuous is a spindle-shaped cell. It has a slender, oviform body and two long, tapering processes. It frequently appears as a constructive element together with a growing or well established valve (fig. 5). Several cells of this kind particularly tend to unite in clusters and strongly extend into the lumen, like a crest. If such figures occur in pairs on opposite sides of the vascular wall a bicuspid valve type may develop. The process of valve building in our preparations can first be recognized on day 5 after birth. It especially takes place in precollecting vessels with wide lumina, but also in some of the smaller initial vascular structures.

The lips of the well established valves occurring in later stages of postnatal development have a sheet-like appearance so that the single spindle cell elements cannot be differentiated further or are only recognizable in both angles of the valve slit where the free valve lips touch the vascular wall (fig. 6).

Another type of prominent cell seen in the early initial lymphatic endothelium has a multiple-branched, or stellate shape, with a bulging central part from which several long or short processes arise (fig. 7). Some of its processes extend from the vascular wall to the lumen, which they sometimes cross when their broadened endings join again with the endothelial layer (figs. 8, 9). Occasionally the whole lumen of the initial lymphatics is traversed by one cellular process. Together with the cytoplasm of another endothelial cell, the branched prominent

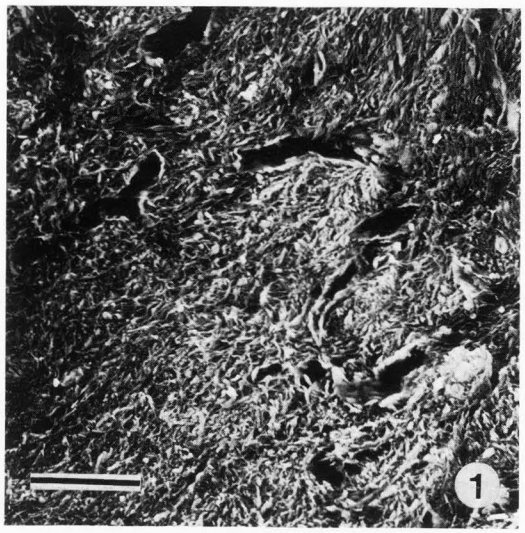
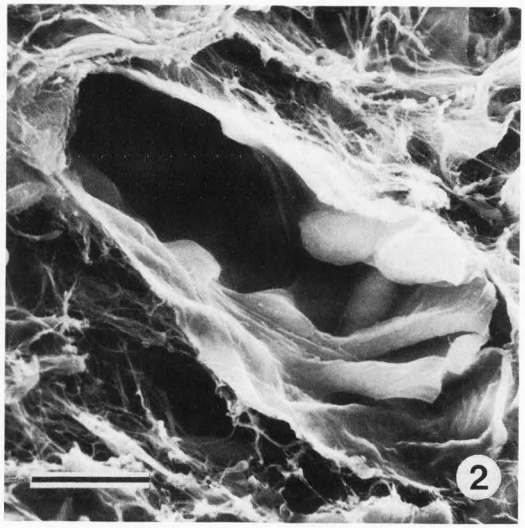
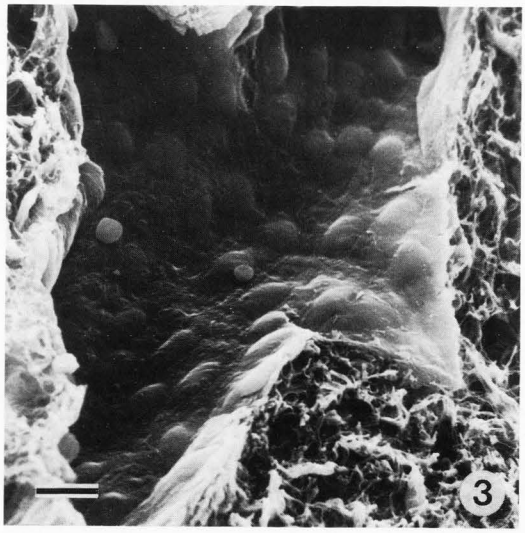


Fig. 1. Scanning electron micrograph of a horizontal section through the subepithelial connective tissue of the tongue in newborn rat. The lymphatic plexus consisting of initial segments and precollectors is widened due to interstitial injection of fixation fluid in the living state and can thus be well visualized. All SEM micrographs shown in the following figures are taken from this topographic area. Bar = 10 µm.

Fig. 2. Cross section of an initial lymphatic in a six-day-old rat. The inner endothelial surface exhibits an irregular profile caused by strongly protruding nuclear portions and different processes of the lining cells. Bar = 10 µm.

Fig. 3. View on the lumen of a precollecting lymphatic vessel in a six-day-old rat. Bar = 10 µm.





cells build manifold overlapping structures. At some sites a wavy intercellular line with pocketings becomes obvious. This is described as a characteristic feature of the flat lymphatic endothelial formation in adults[4].

Accumulations of prominent, branched cells produce a very irregular endothelial layer in which numerous processes often fuse to an interlacing syncytium. In such an area many round or oval-shaped openings occur between touching cytoplasmic flaps. The largest openings reach diameters of 4 µm. They are outside covered by a dense, fine fibrous network of the basement membrane. Such a diaphragm is sometimes partly or completely lacking so that a free communication between the vascular lumen and the interstitial space exists (fig. 9). At higher magnifications no remarkable differences can be determined between the various cell types. The surface of most cells appears smooth apart from a scarce pattern of knot-like microvilli spreading over the cell.

Some cells with a more globular shape and with few blebs on their surface are sticking on the vascular wall, thus having contact with the endothelium by one or few processes. Some of them suggest to be migrating cells exhibiting a stage of vascular diapedesis.

Discussion

Although TEM and SEM have elucidated the fine structural features of the initial lymphatics there are still open questions regarding the morphology and physiology of this vascular area.

One major point in the discussion about the initial lymphatics certainly refers to the cellular differentiation of this kind of vessel. In a previous study[4] it was obvious that the lymphatic endothelium exhibits a heterogeneous nature due to cells which deviate from the regular pattern of flat and polygonal cells with serrate outlines.

One light microscopic study has already discussed different types of endothelial cells - festoon-like, irregular, and spindle-shaped cells - lining the initial lymphatic endothelium[1]. Their occurrence has been interpreted as a functional phenomenon evoked under the conditions of an aseptic inflammation. With SEM we were able to distinguish between the flat, polygonal endothelial cells and the special outstanding cellular elements with a spindle-like or multiple-branched appearance. The present investigation carried out on young rats shows that such cells line the initial lymphatics in the early stage of postnatal development and even become determining elements for their luminal profile. Thus these vessels in newborns have similar morphological characteristics to those previously described in adults. We might interpret these findings as a sign of early cellular differentiation of the initial lymphatic endothelium.

The spindle-shaped prominent cell mainly proves to be an element of primordial or definite valves ('exit valves'[3]). Concerning the functional significance of the various multiple-branched cells no definite statements can be made at present. Most of them have a great similarity with the cells of the histioblast type in SEM. The fact that these cells remain an integrating element of the initial lymphatic endothelium during the whole period of postnatal life and that they are also found together with a regular, flat endothelial formation in adults suggests that they are not only precursors of the flat polygonal cells but should also be considered specialized components with their own functional properties.

Independent of this principal question we must suppose that in zones where the initial lymphatic endothelium is predominantly composed of irregularly shaped, branched cells the vascular permeability is not as much controlled by a system of open junctions with an 'inlet valve' function[3] as it is in zones with regularly arranged flat cells where a backflow of the lymph into the interstitium is hindered. On the other hand the lymphatic endothelium already displays under normal conditions several openings in early life, some of them even without any fibrous diaphragm (fig. 9), which in adult animals become only visible after a high increase of the interstitial pressure[4]. Therefore the vascular permeability of initial lymphatics in early postnatal development could be much higher than in later life.

In experimental vascular research irregularities of the endothelial contours similar to those recognizable in the present study have been described to be functional efficiency like migratory and motile activity[7, 14]. Those activities are performed during the sprouting of the endothelium, during phagocytotic and cytopoetic functions, and during

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Fig. 4. Higher magnification of the vessel shown in fig. 3. A regular pattern of rhomboid-shaped endothelial cells can be recognized. Bar = 10 µm.

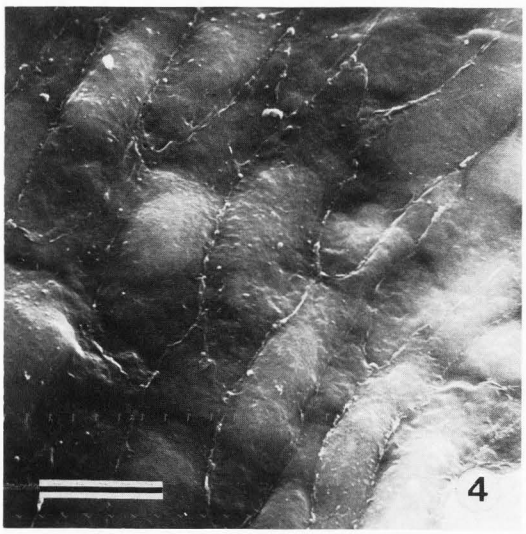
Fig. 5. Two groups of spindle-shaped cells - one already providing firm elements for the valves, one still in loose contact with it. Initial lymphatic of an infant rat (fourteen days after birth). Bar = 10 µm.

Fig. 6. Initial lymphatic of a ten-day-old rat with a valve of the bicuspid type. In the angle where both lips of the valve arise, this tissue is built of groups of prominent spindle-shaped cells. Bar = 10 µm.

Fig. 7. Sectional area of the luminal surface of an initial lymphatic in a fourteen-day-old rat. A multiple-branched and prominent endothelial cell is represented whose processes form small recesses and a large pocketing. Bar = 10 µm.

Fig. 8. Sectional area of the inner endothelial surface of an initial lymphatic in a six-day-old rat. In this micrograph two strongly prominent and branched cells are depicted whose different portions interlace. A wavy intercellular border system with out-pocketing structures can be seen on the left side of the picture (↙). Bar = 10 µm.

Fig. 9. A long, slender process of a prominent cell of an initial lymphatic extends into the lumen and connects again with its broadened ending with the endothelium. Some openings appear in the course of the intercellular line (↙). In one opening a hole (↙↙) is noticeable in the fine fibrous network of the outer basement membrane (six-day-old rat). Bar = 10 µm.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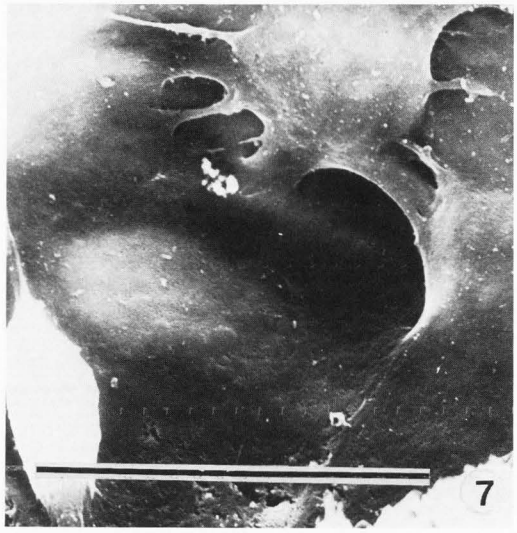
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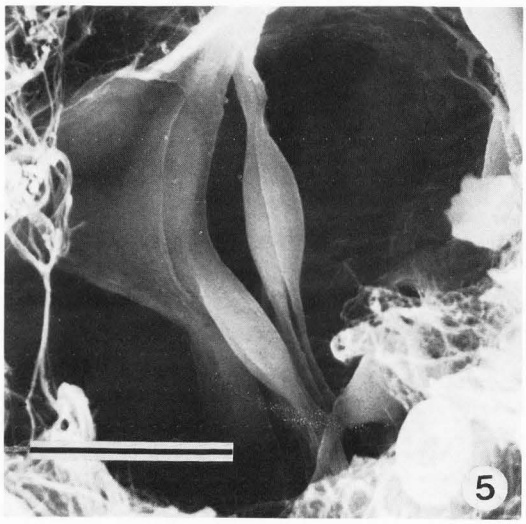
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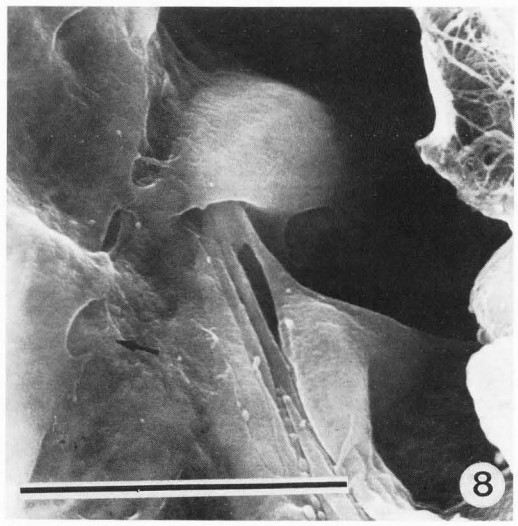
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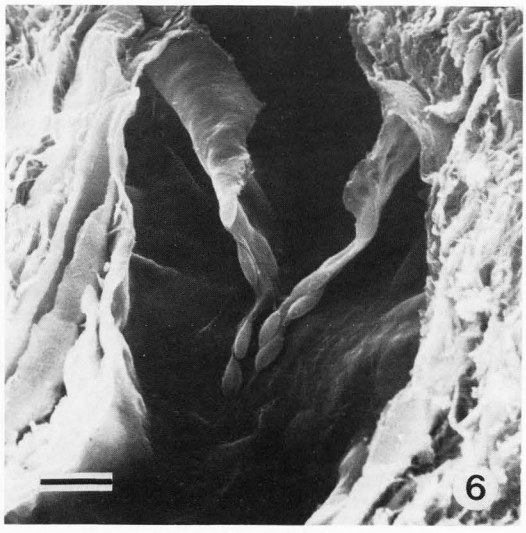
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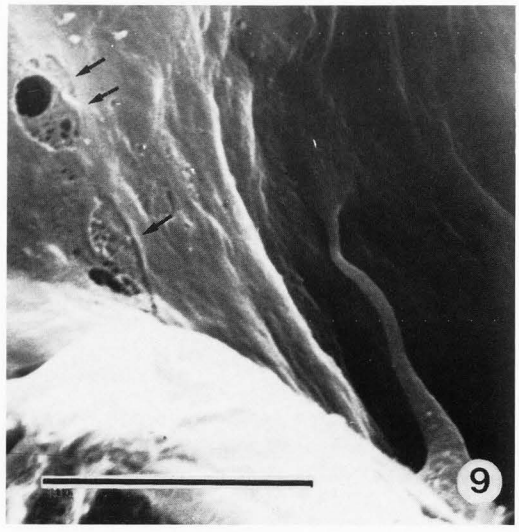
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local contractions of the vascular wall. The possibility of a contractile behaviour of the lymphatic endothelium has already been pointed out by us in a previous study[4].

TEM observations of initial lymphatics exposed to carbon particles have revealed that the lymphatic endothelium indeed possesses phagocytotic properties[2, 11]. Similar observations have already been made by Shdanow[15] forty years ago with the light microscope. He saw that after the application of carbon into the peritoneal sac the carbon particles are absorbed by the wall of the small and medium-sized lymphatics.

The functions mentioned here in connection with the endothelium of the initial lymphatics point to the exceptional position of this tissue among the structures of the vascular system. Hence its morphological features as well as its special efficiency already occurring in early developmental stages have been compared by some investigators[12, 15] with the outstanding structures of the liver sinusoids and other constituents of the reticuloendothelial system (RES). Such considerations are confirmed by the present SEM results.

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References

1. Aminova GG (1963). Die Erforschung des Lymphkapillarendothels und der Gefäße des Diaphragmas beim Kaninchen, Arch. Anat. Histol. u. Embr. 44, 81-90.
2. Casley-Smith JR (1964). Endothelial permeability - the passage of particles into and out of diaphragmatic lymphatics, Quart. J. Exp. Physiol. 49, 365-383.
3. Casley-Smith JR (1967). The fine structure, properties and permeabilities of the lymphatic endothelium, in: New Trends in Basic Lymphology, Proc. of a Symp. held at Charleroi (Belgium) on July 11-13, 1966, Colett JM, Janlet G, Schoffeniels E (eds.), Birkhäuser Verlag, Basel/Stuttgart, 19-39.
4. Castenholz A (1984). Morphological characteristics of initial lymphatics in the tongue as shown by scanning electron microscopy, Scanning Electron Microsc. 1984; III: 1343-1352.
5. Castenholz A (1984). Strukturbild und Wirkungsweise der 'Initialen Lymphbahn', Zeitschr. f. Lymph., Bd. VIII, 2, 55-64.
6. Castenholz A (1985). The demonstration of lymphatics in casts and fixed tissue with the scanning electron microscope, in: The Initial Lymphatics. New Methods and Findings, International Symp. Zurich 1984, Bollinger A, Partsch H, Wolfe JHW (eds.), G. Thieme/Thieme-Stratton Inc., Stuttgart/New York, 75-83.
7. Clark ER, Clark EL (1935). Observations on changes in blood vascular endothelium in the living animal, Am. J. Anat. 57, 385-438.
8. Courtice FB (1980). The lymphatic circulation, in: Structure and Function of the Circulation, Vol. 2, Schwartz CJ, Werthessen NT, Wolf S (eds.), Plenum Press, New York, 487-602.
9. Földi M, Casley-Smith JR (1983). The structure and functioning of the blood vessels, interstitial tissues, and lymphatics, Schattauer, Stuttgart/New York, 111-112.
10. Kampmeier OF (1969). Evolution and comparative morphology of the lymphatic system, C.C. Thomas, Springfield, Ill., 17-55.
11. Leak LV (1970). Electron microscopic observations of lymphatic capillaries and structural components of the connective tissue-lymph interface, Microvasc. Res. 2, 381-391.
12. Magari S (1962). Grundlagen und neue Ergebnisse der Erforschung des Lymphgefäßsystem, Z. naturwiss.-med. Grundlagenforsch. (Basel) 1, 4-37.
13. Rusznyák J, Földi M, Stabó G (1969). Lymphologie, Physiologie und Pathologie der Lymphgefäße und des Lymphkreislaufes, Gustav Fischer Verlag, Stuttgart.
14. Schoefl GJ (1963). Studies on inflammation. III. Growing capillaries: their structure and permeability. Virchows Arch. Abt. A. Path. Anat. 337, 97-141.
15. Shdanow DA (1935). Über einige histophysiologische Eigentümlichkeiten der Wand von Lymphgefäßen, Anat. Anz. (Jena) 79, 431-440.
16. Yoffey JM, Courtice FC (1980). Lymphatics, Lymph and Lymphomyeloid Complex, Academic Press, London/New York.

Discussion with Reviewers

R. Albrecht: Has the author attempted infusing a suspension of heavy metal particles, such as tantalum, before fixation in order to determine, by BSE imaging, the degree of phagocytosis exhibited by the different cell types?
Author: In the last year we have started a study in which the uptake of metal particles such as nickel, gold, and silver by the lymphatics in the rat tongue is examined. The tissue infused with these metal suspensions will be evaluated by the EDX system of our

scanning electron microscopy. Definite results are still outstanding.

R. Albrecht: Does the author see any free macrophage or lymphocyte like cells within the lumina of the developing lymphatics?
Author: Yes, we observed in some infant rats many globular cells with diameters between 6 and 10 µm furnished with many blebs on their surfaces and short and plump processes sticking on the vascular wall of some precollecting vessels. In smaller lymphatic vascular structures this phenomenon can seldom be detected in postnatal development. A definite classification of this cell type - microphage or lymphocyte - is, however, not possible with SEM.

J.R. Casley-Smith: You say that the irregularity and lack of closure of the initial lymphatic endothelium will cause much higher permeability of these vessels, compared with that of the initial lymphatics of the adults. I would doubt this statement in this form; rather, I would suggest that this lack of closable 'inlet valves' would make the initial lymphatics of the young animals much less efficient. That is, they would not seal as well as the initial lymphatics in the adults. Their inwards permeability would be similar, but they would also be very permeable to macromolecules passing out during tissue compression, i.e. they would be even more leaky than the adults.
A.C. Nelson: What is the physiological significance of the holes in the endothelial lining of lymphatics, and are these holes modified during the development of mature valve structures?
Author: In order to definitely decide about the influence of the vascular permeability of initial lymphatics in young animals and to compare it with that in adults further functional and transmission electron microscopic studies are necessary. My assumption for a relatively high permeability of the early lymphatic endothelium is only based on SEM findings representing the endothelium in that stage of development as many irregular cellular units and several open communications to the interstitial space. But I agree with you that this situation may predominantly influence the outward permeability of these vessels.

A.C. Nelson: How can one be reasonably certain that the tissue under study is lymphatic rather than circulatory?
Author: In tongue tissue fixed by interstitial injection the lymphatics get dilated. By this phenomenon and other features such as thin wall, empty lumen, varying diameter and valve structures it is possible to distinguish clearly this kind of vessel from the blood vessels in the same region. The latter are mostly filled with blood cells. Moreover, the classification of the lymphatics was confirmed by comparative light and transmission electron microscopic studies made by us.

A.C. Nelson: The corrosion casting technique could reveal interesting features of the three-dimensional lymphatic vessel structure. Does the author feel that vascular casting is possible in the rat tongue system, and if so, what questions could be addressed using the casting technique in conjunction with the present studies?
Author: In a previous study[4] we could already gather some experience in corrosion casting technique for the representation of lymphatic structures in adult animals. We also used this method for the representation of pre- and postnatal vascular lymphatic structures. The results will be published elsewhere. The intention of our present study was rather to describe the cellular differentiation of the lymphatic endothelium from the luminal side than to show the three-dimensional aspect of the subepithelial lymphatic plexus in this organ. For this special purpose the casting technique used in our previous study proved to be very useful. In early stages of postnatal development the commencement of the valve building process and the localization of valve structures in the initial lymphatics can well be recognized in casts and thus helped us to get further information on this process.

J.G. Walmsley: Could the author please document which control studies or published procedures were used to fix and prepare the tissue for scanning? In particular, questions related to three steps in the procedure should be clarified. What is meant by interstitial injection of the fixative and how does such injection preserve structure and shape of the lymphatic wall and lumen? Since Lee et al. (J. Micros. 120: 85-91, 1980) have shown that only specific fixation solutions can be used to preserve vascular cell volume, could we be informed of the composition of the glutaraldehyde solution and, furthermore, can we be reassured that there are no significant changes in the cell shape and volume? Similarly, what is the exact procedure used for critical point drying and does it effect cell shape?
Author: The method of interstitial injection of fluids and fixatives into the tongue tissue is described in detail elsewhere[4]. The injected fluids applied by us - physiological solution and glutaraldehyde - penetrate the tongue

tissue and enter into the lumen of the lymphatics. We believe that these fluids reach the lymphatic endothelium of the subepithelial lymphatics within a few seconds after injection so that in case of the fixative the preserving effect takes place rapidly. The interstitial injection also furnishes a 'clean' inner lymphatic surface because all lymph residues of the vessels are removed by it.
We used in our experiments a solution of 2.5% glutaraldehyde buffered with Sörensen (pH 7.2). The influence of the fixative on the cell volume could not easily be estimated because certain morphological changes cannot be excluded. This statement is also valid for other fixative solutions which more or less interfere in the normal cytoplasmic conditions of the living cell. If our fixative is used for the perfusion of arterial blood vascular system the endothelium of the terminal blood vascular system exhibits nowhere cells with those morphological characteristics, as they can be seen in the lymphatics treated with the same fixative solution. Therefore we regard the occurrence of spindle-shaped and multiple-branched cells in our study as a significant phenomenon which also exists in the living vessel and is not only produced by this fixation procedure.
Drying of our specimens was done by the critical point method with Freon 13. So far, we have not yet investigated air-dried specimens. A comparison with other drying techniques is therefore not possible.

J.G. Walmsley: Is it perfectly clear which structures are lymphatics and which ones are blood vessels? It is stated that some single erythrocytes are seen in lumen. Is it possible that these might be flushed venous vessels?
Author: The answer of how to distinguish lymphatics from blood vessel structures is given in the above discussion with Nelson. Single erythrocytes can sometimes be seen in the lumen of the lymphatics. Their occurrence in these vascular structures does not justify regarding them as blood vessels.