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A. Castenholz
University of Kassel

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INTERPRETATION OF STRUCTURAL PATTERNS APPEARING ON CORROSION CASTS OF SMALL BLOOD AND INITIAL LYMPHATIC VESSELS

A. Castenholz

Dept. of Human Biology,
University of Kassel
Heinrich-Plett-Str. 40,
D-3500 Kassel, West Germany

Phone number: (0561) 804 4356

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Abstract

Structures imprinted on the corrosion casts of blood and lymphatic microvessels are examined by correlative scanning electron microscopy of corroded casts and fixed tissue. Replicas of the endothelial nuclear portions and cell boundaries and the plastic bands surrounding the casts of terminal blood vessels are demonstrated. In small lymphatics the replication of valve structures, interendothelial open junctions and direct prelymphatic connections is shown. The different imprint patterns are examined in regard to their morphological significance and under methodical aspects.

KEY WORDS: Corrosion casts, small blood vessels, initial lymphatics.

Introduction

Since the first attempt to employ polymerizing media in connection with the corrosion cast technique in the early seventies (Murakami 1971) the method has become an important measure to examine the vascular organization of different organs by scanning electron microscopy. The technique with its various modifications described in detail by Nowell and Lohse (1974), Hodde and Nowell (1980), Hodde (1981) and Lametschwandtner et al. (1984) has made it possible to show a microvascular area on a three-dimensional scale due to the extraordinary range of magnification and the great depth of field of the scanning electron microscope. Although the main application for the casting method has been till now the analysis of the organization of the vasculature of certain organs (Miodónski et al. 1981) it also provides the possibility to evaluate the spatial relations between different types of vessels and between vascular structures and tissue components. On the other hand it could be demonstrated by many investigators that carefully produced plastic casts exhibit surfaces with a high replication quality. Consequently, such preparations also allow one to identify a special kind of vessel by the analysis of the relief indented into the cast surface by the vascular endothelium. This has been first recognized by Miodónski (1976) and has been confirmed in correlative SEM studies of casts and fixed tissue by Hodde et al. (1977). Further information on the vascular morphology can be obtained from the plastic structures surrounding the luminal cone of injected vessels (Anderson and Anderson 1978, Kardon and Kessel 1979, Castenholz et al. 1982, Castenholz 1983 a,b, 1987 a,b). In the present paper the structural pattern of the casts of microvessels was examined. Terminal blood and initial lymphatic vessels were analyzed by correlating the corrosion casts and the tissue preparations with respect to previous studies (Castenholz 1983a, 1983b, 1984, 1985).

Materials and Methods

Corrosion casts and tissue preparations of 22 rats (Wistar strain), 3 guinea pigs and 3 tree shrews (Tupaia Belangeri) were examined in this

study. Adult animals of both sexes were investigated in each species and tissue from various organs as tongue, skeletal muscle, skin, lymph nodes, Peyer's plaques found special consideration. The casting medium, Mercox R (Vilene Hospital, Tokyo Japan) or Plastoid R (Röhm Comp. Darmstadt, W. Germany), was injected into the left ventricle or into the jugular vein to fill the blood microvascular system from the venous side. Casting of lymphatic vessels was possible by interstitial injections of the resin into the connective tissue of the tongue and skin. Mercox and Plastoid were prepared according to the instructions of the producers. In some cases Mercox was diluted with methyl methacrylate (methacrylic acid methyl ester, Merck-Schuchardt, W. Germany) in the volume ratio of 3:1. The procedure of preparing the animal, corrosion of the tissue, drying and cleaning of the specimens are reported in detail in previous papers (Castenholz 1983a, 1986). For tissue preparations the anaesthetized animal was perfused with Ringer's solution from the heart and fixed with 2.5 % glutaraldehyde. The dehydrated and dried tissue (critical point method) was sputtered with gold like the corrosion casts, fixed on stubs with conduction carbon and examined in the AMR 1200 microscope (Leitz, W. Germany) at an accelerating voltage of 15 and 25 kV.

Basic Morphology of Small Vessels

The small vessels examined had diameters in a range of a few to maximally 100 µm. In the blood circulation one microcirculatory unit (fig. 1) consisted of small arteries (50 - 100 µm), arterioles (30 - 50 µm), terminal arterioles (30-10 µm), capillaries (4 - 10 µm), venules (10 - 30 µm), collecting venules (30 - 50 µm), and small veins (above 50 µm). The initial lymphatics are arranged in a plexus-like manner with finger-like outpocketings (fig. 2). Their diameters varied widely between a few and 60 µm. Precollector channels draining the lymph from the initial lymphatics exhibit diameters up to 100 µm and more (the structural features of blood and lymphatic microvessels were described by Scharrer 1940, Castenholz 1970, Fujiwara and Uehara 1984, Hammersen 1971, Rhodin 1962, 1974; for microvascular terminology see Baez 1977). The arteriolar system is furnished with a complete or discontinuous layer of smooth muscle cells surrounding the endothelium. The venules and capillaries are wrapped by an incomplete layer of pericytes (Rouget 1873). The pericytes in the collecting venules are replaced by smooth muscle cells. The basic morphology of blood capillaries and initial lymphatics (lymphatic capillaries) is depicted in figures 3 and 4. Special structural conditions with constricted lumina reveal the initial segments of small arterioles and capillaries arising from terminal arterioles. In these "sphincter areas" individual groups or single elements of smooth muscle cells appear, which may have a special controlling function for the local blood flow (Chambers and Zweifach 1944, Rhodin 1967, Fulton 1970, McCuskey 1971).

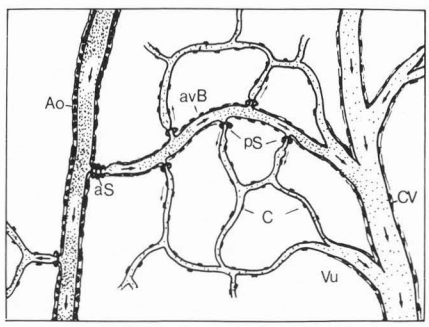

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Fig. 1. Diagram showing the microvascular system of blood circulation (principle of distribution after Chambers and Zweifach 1944).
Ao - arteriole
aS - arteriolar sphincter
avB- arterio-venous bridge (metarteriole)
pS - precapillary sphincter
C - capillary
Vu - venule
CV - collecting venule
Direction of blood flow indicated by arrows.

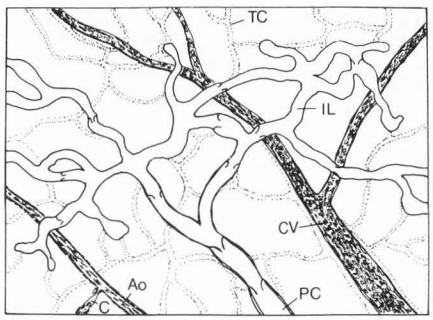

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Fig. 2. Diagram of the initial lymphatic system.
IL - initial lymphatic plexus with outpocketings
PC - precollector channel
TC - tissue channels (prelymphatics)
Ao - arteriole
C - capillary
CV - collecting vein

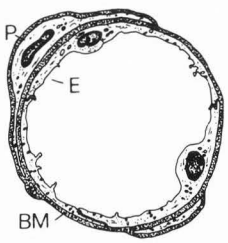

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Fig. 3. Diagram illustrating the basic morphology of a blood capillary
E - endothelium
BM - basal lamina
P - pericyte

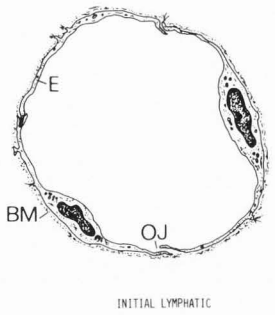


Fig. 4. Diagram demonstrating the basic morphology of an initial lymphatic
E - endothelium
OJ - open junction
BM - basal lamina with anchoring fibers

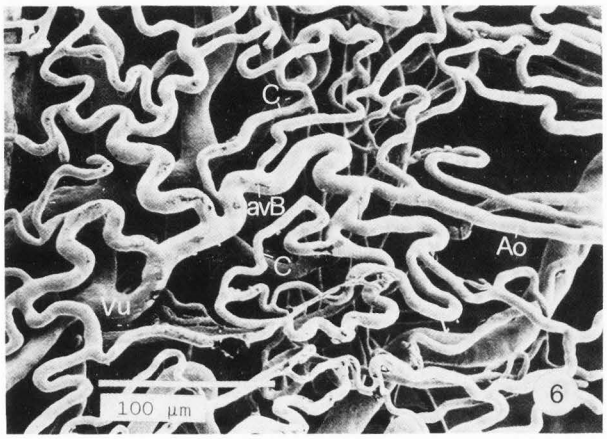


Fig. 6. Corrosion cast of connective tissue of rat showing a vascular distribution after Chambers and Zweifach (1944).
Ao - arteriole
avB- arteriolo-venous bridge
C - capillaries
Vu - venules

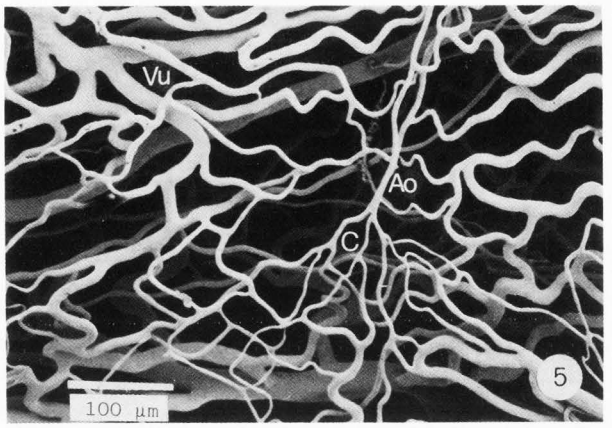


Fig. 5. Corrosion cast of the terminal vascular system of skeletal muscle tissue (hypodermis, rat). Vascular distribution according to the "classic principle" can be seen.
Ao - terminal arteriole
C - capillaries, Vu - venules

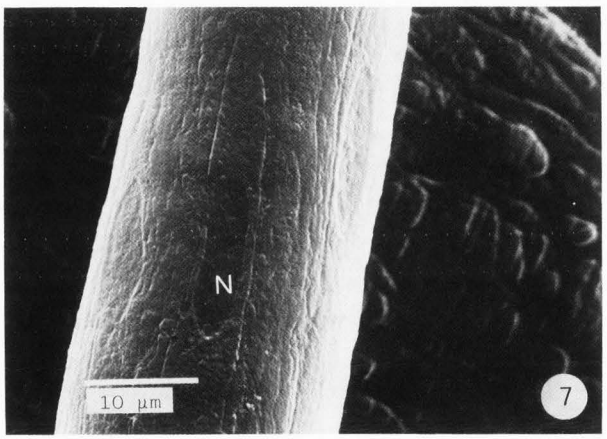


Fig. 7. Cast of a small arteriole (mesentery, rabbit); note the oval-shaped depressions of the endothelial nuclei (N) and the system of fine fissures created by the endothelial boundaries.

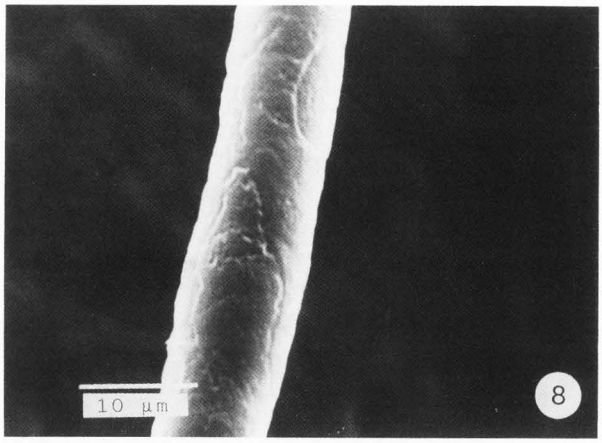
Evaluation of the Casts

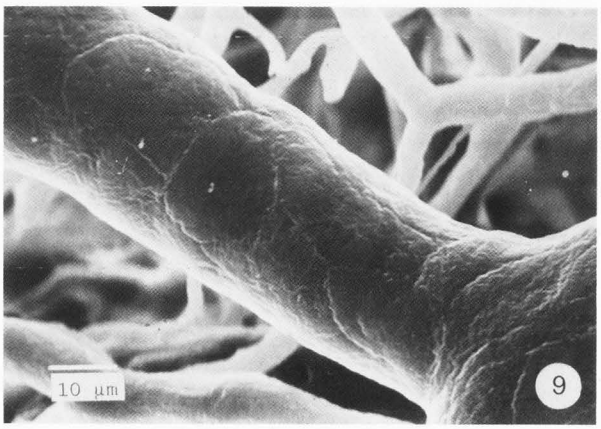
Blood Microvessels

In most casts of a vascular system in the blood circulation bigger arteries and veins can be distinguished by comparing their dimensions, patterns of ramification, and surface features. The diameter of an artery is usually smaller than a corresponding vein. In addition, the origins of small arteries and arterioles are constricted. This phenomenon can be traced to the capillary level, but it is not found on the venous side. The cast surface of arteries and arterioles often reveals a coarse pattern of longitudinal foldings and ruffles produced by a corresponding profile of the endothelium. It occurs in a state of vascular constriction, whereas the cast surfaces of the veins, obviously less stressed, remain smooth.

Different vessels can be identified as arterioles, capillaries, and venules tracing them from the feeding arteries to the draining veins. In our corrosion casts the vascular distribution patterns of both the "classic type" (fig. 5) and that of Chambers and Zweifach (1944) (fig. 6) were recognizable.

More information on the type of vessel can be obtained from the replicated structures on the cast surface. Such structures consist of different imprints created by the prominent nuclear zones as well as by the boundaries of the endothelial cells. In arteriolar vessels the nuclear depressions are longish and oval-shaped (fig. 7). The replicated endothelial borderlines of arterial vessels are composed of slender, elm-leaf-shaped figures. In capillaries they consist of broader rhombic or polygonal figures (fig. 8) and patterns of irregular broad figures are discernible on the cast surface of small venules and veins (fig. 9). The endothelial nuclei cre-





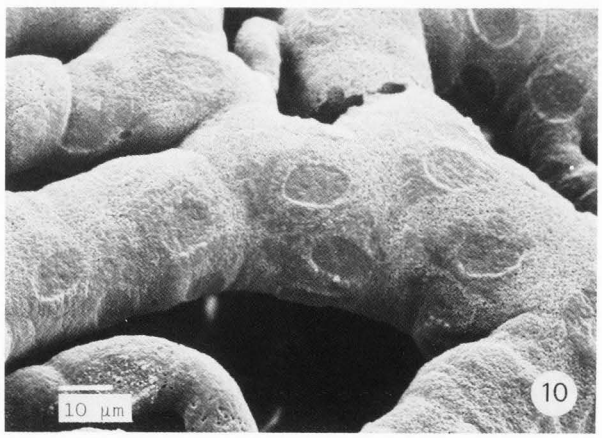


Fig. 8. Cast of a blood capillary (mesentery, rabbit). The imprint of the endothelial boundaries is well visible.

Fig. 9. Cast of a venule (lymph node, rat). The endothelial borderline system has been replicated. Nuclear depressions cannot be detected.

Fig. 10. Cast of a venule system (small intestine,guinea pig). Note the roundish depressions of the endothelial nuclei. Replicas of the endothelial borderline system are not detectible on this preparation.

Fig. 11. Cast of a small arteriole with twig (brain, rat). The initial segment of the smaller arteriole is narrowed and encircled by circular plastic bands of Mercox. At the origin of the twig a group of broader bands appears corresponding to the sphincter muscles located at this area.

Fig. 12. Cast of a capillary (musculature, tongue, rat). The lumina cone of these vessels is partly wrapped by slender and branched plastic structures imitating pericytic cells.

Fig. 13. Cast of a precapillary sphincter (brain, rat). At the site where the capillary (C) branches off from the terminal arteriole (Ao) the luminal cast is clearly narrowed and encircled by a spiral plastic band corresponding to the sphincter muscle.

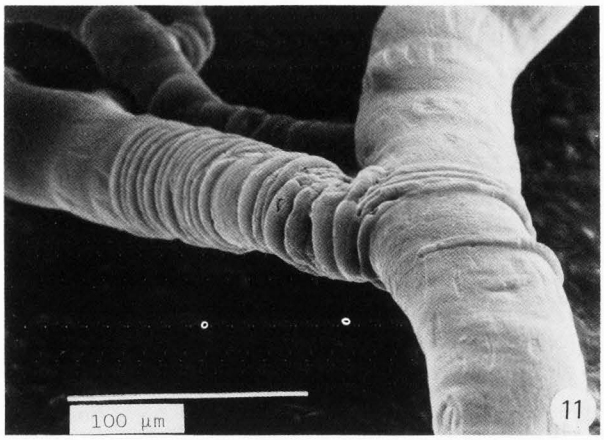
Fig. 14. Cast of a precapillary sphincter (brain, rat). Deep oval-shaped depressions occur at the site, where the capillary (C) arises from the terminal arteriole (Ao). The depressions are interpreted as created by the bulging endothelial cells of the sphincter zone.

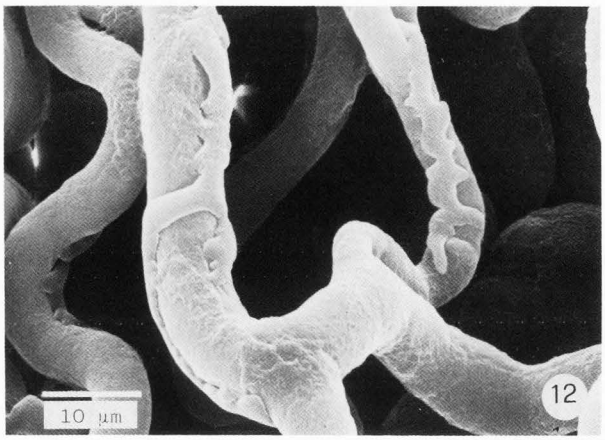
ate on casts of bigger venules round or slightly oval imprints (fig. 10). Results similar to what we have found in our cast preparations have been reported in other organs and species (Lametschwandtner et al. 1976, Hodde et al. 1977, Miodónski et al. 1981).

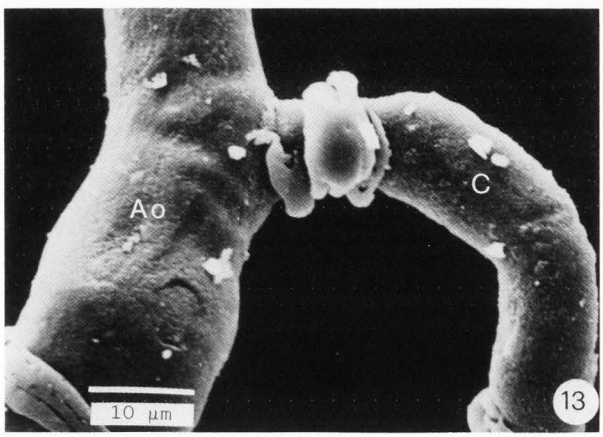
The casts of arterioles are frequently surrounded by circular or spiral structures of Mercox or Plastoid (fig. 11). Around the casts of capillaries these resin bands or strips assume a more irregular branched shape (fig. 12) and their narrowed sphincter area is wrapped by a single spiral or simply branched plastic structure (fig. 13). In that area also deep depressions of the endothelial nuclei are visible if the spiral elements are lacking (fig. 14). Those findings agree with the observations of Rhodin (1967), who demonstrated by the transmission electron microscope that the nuclear portions of the endothelial cells strongly protrude just in the area of a precapillary sphincter. On casts of arterioles, sometimes a pattern of fine circular notches can be detected which obviously is produced by the smooth muscle cells.

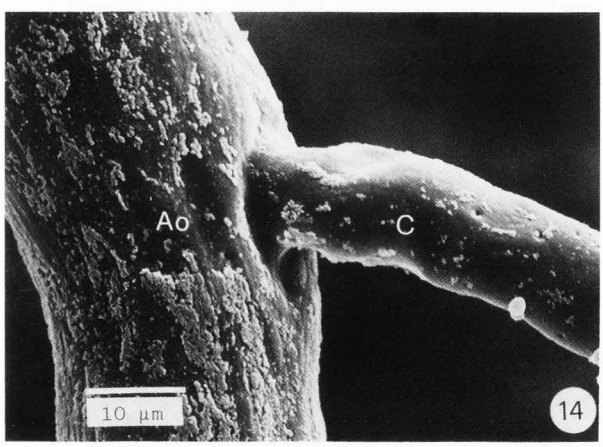
Although the exact nature of the plastic structures around the luminal casts has been controversially assessed in the literature we demonstrated in a correlative light-, transmission- and scanning electron microscopic study that casting resin penetrates and replaces the myocytes and pericytes in the subendothelial layer and so imitated the morphological features of these cells (figs. 15a, b) (Castenholz et al. 1982, Castenholz 1983a).

On the venous side of the terminal blood circulation the plastic bands or strips are seldom to be found. Casts of postcapillary venules (high endothelial venules) of lymphatic tissue such as lymph nodes and Peyer's patches show another phenomenon that is characteristic for this kind of vessel. The cast surface exhibits a coarse









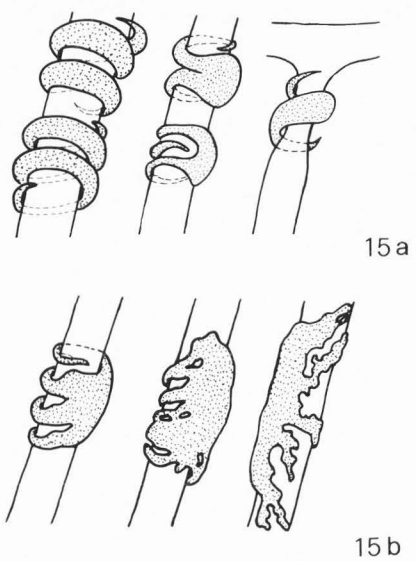
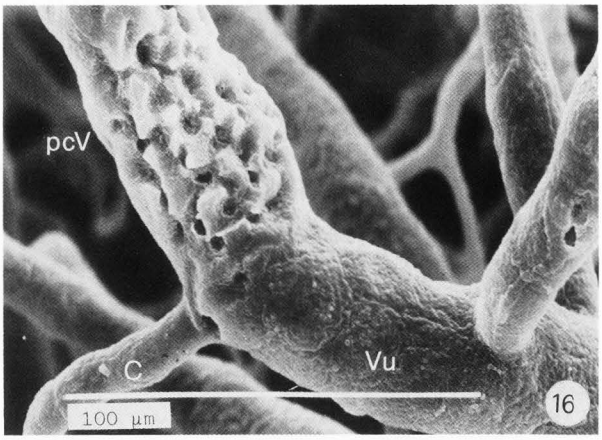


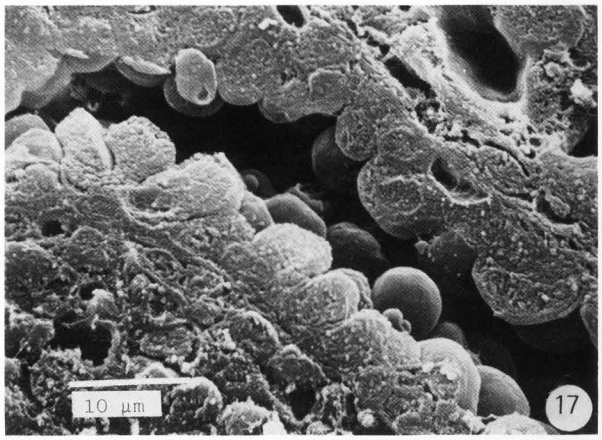
Fig. 15. Diagram showing the various shapes of plastic bands surrounding arteriolar vessels (a) and capillary (b) in corrosion casts. The plastic structures correspond well regarding shape and location to the myocytic and pericytic elements found in tissue preparations of the vessels (from Castenholz 1983a).

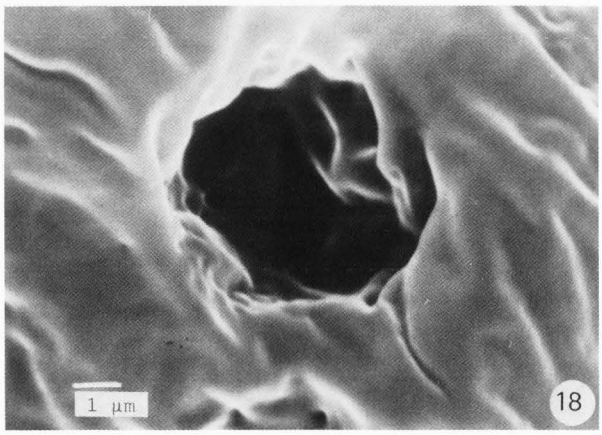
pattern of deep notches and high crests. At many places deep roundish holes appear (fig. 16). Zones with such a wrinkled profile are sharply marked against the joining distal venules with smooth luminal surfaces. In tissue preparations the endothelium of postcapillary venules of lymphatic organs consists of high cells, whose apical part deeply extends into the lumen (fig. 17). Many leukocytes, most of them lymphocytes, are found sticking on the luminal side of the endothelium, some of them being in a state of diapedesis. These special features of the high endothelial venules are truly replicated in the cast. Holes, as one is shown in figure 18, are obviously created by the sticking cells (fig. 19). Similar findings on postcapillary venules have been reported by Cho and DeBruyn 1979, Yamagushi and Schoefl 1983a, b, and by Steeber at al. 1987.

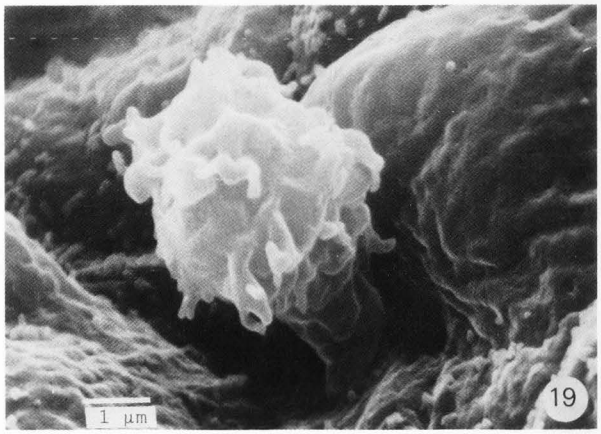
Small Lymphatics

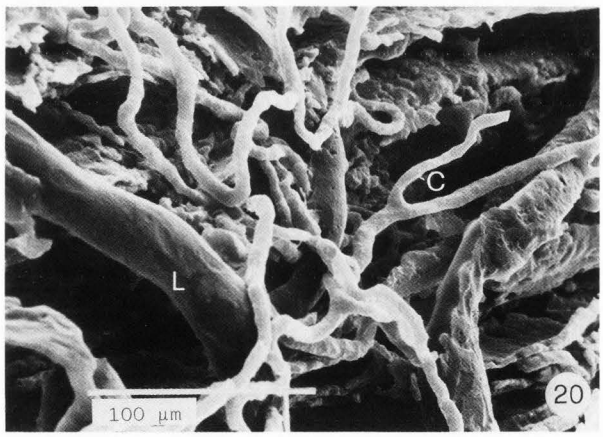
Other than blood capillaries fine lymphatics can be filled with stains or resin after injection of these substances into the interstitial tissue. For the corrosion cast technique











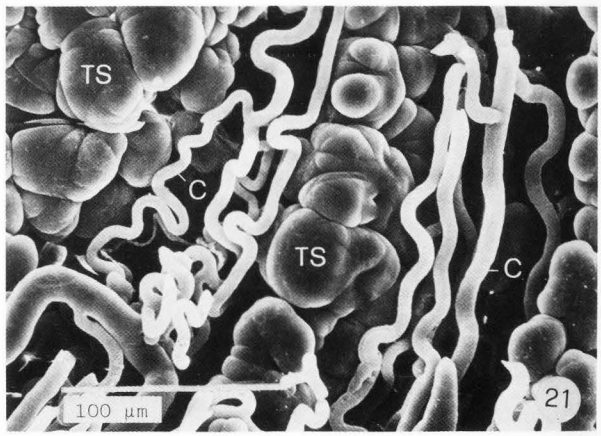
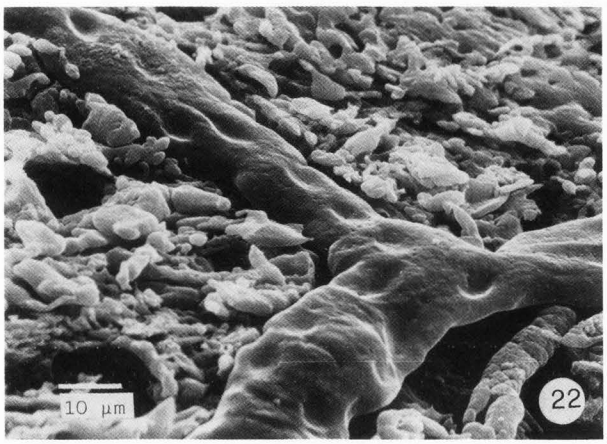
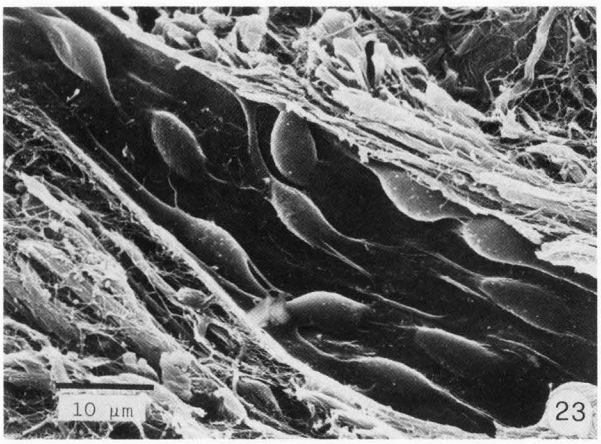


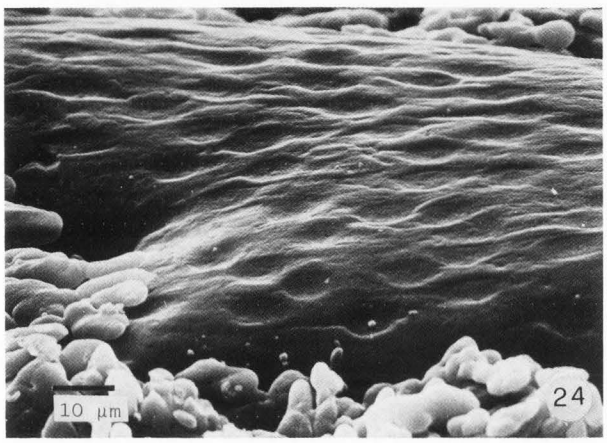
Fig. 16. Cast showing a sectional area of the venule system of a lymph node (rat). Note the rough surface relief appearing in the area of a postcapillary (high endothelial) venule (pcV). The distal venule (Vu) and the capillaries (C) exhibit a smooth cast surface, on which only the pattern of endothelial border lines becomes visible.

Fig. 17. High endothelial postcapillary venule as represented in a cut plane of a tissue preparation. Note the high endothelial layer of this type of vessel (lymph node, rat). The apical segment of the endothelial cells bulge strongly into the lumen.

Fig. 18. At high magnification hole-like depressions can be visualized in the cast of high endothelial postcapillary venules (lymph node, guinea pig). The phenomenon is obviously due to a sticking cell on the endothelial surface (see fig. 19).







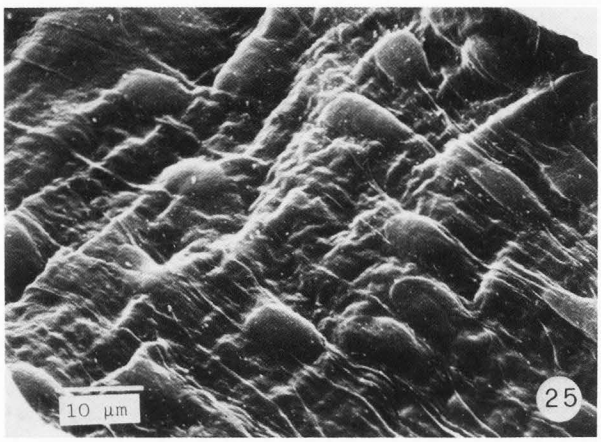


Fig. 19. Leucocyte in a state of diapedesis fixed on the endothelial surface of a high endothelial postcapillary venule (tissue preparation, lymph node guinea pig).

Fig. 20. Corrosion cast of connective tissue (subepithelial zone, tongue, rat). The tissue has been injected by arterial as well as by interstitial application of Mercox. Thus, both vascular systems that of terminal blood vessels and the initial lymphatics have been filled up. Note the regular smaller diameters of the blood capillaries (C) in comparison with the wider and irregular casts of the lymphatics (L).

Fig. 21. Representation of the blood capillary system together with the interstitial spaces of skeletal muscle tissue (tongue, rat). Mercox was applied into the blood circulation as well as into the interstice. The topographical relations between the capillaries (C) and the surrounding tissue spaces (TS) are well recognizable in this preparation. The muscle fibers originally were situated in the spaces now solely occupied by their supplying capillaries.

Fig. 22. Cast of an initial lymphatic (tongue, rat). Deep and shallow impressions created by the nuclear zones of the endothelial cells appear on the surface of the cast. From some depressions small notches extend indicating cellular branches of single endothelial cells.

Fig. 23. View on the endothelium of an initial lymphatic (tongue, rat). The rough profile of the lymphatic endothelium produced by prominent and branched endothelial cells becomes very distinct in this tissue preparation.

Fig. 24. Cast of a precollector channel (tongue, rat). Some nuclear depressions appear on the surface of the cast.

Fig. 25. View on the luminal surface of a precollector channel (tongue, rat). Slight protrusions appear in the zone of the endothelial nuclei. Tissue preparation, compare fig. 24.

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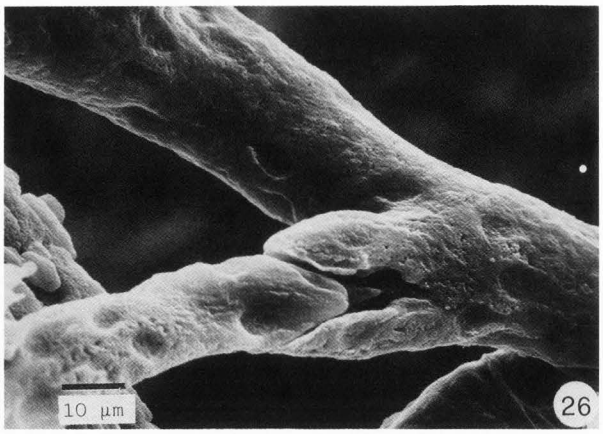
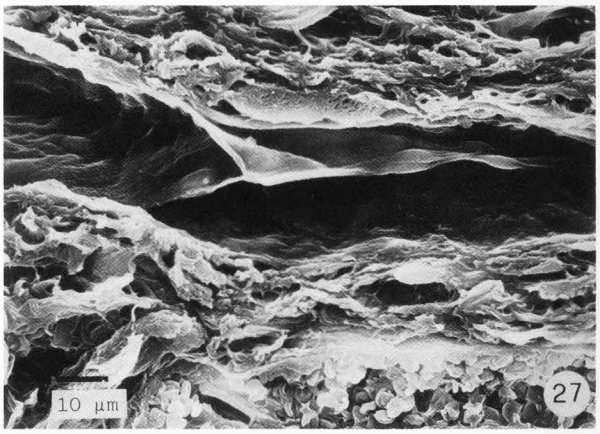
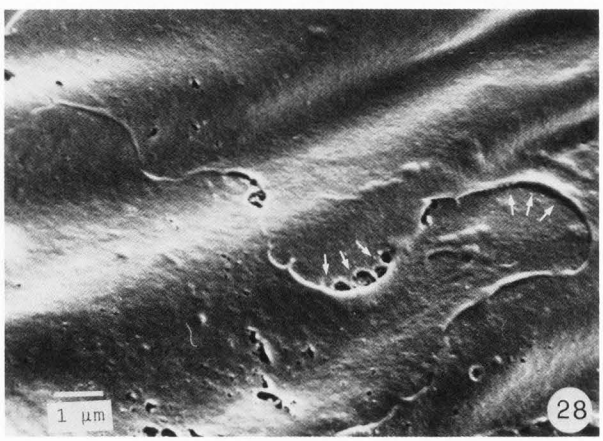
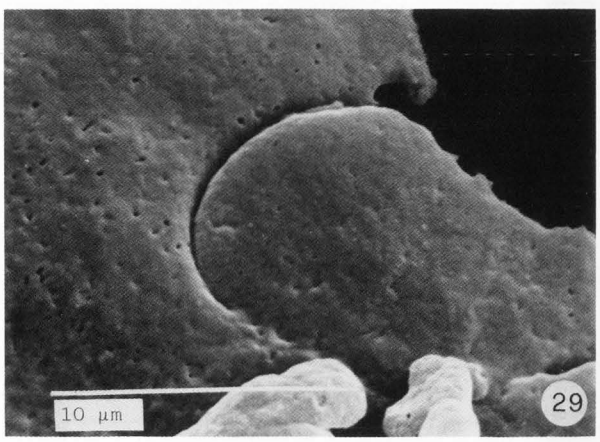
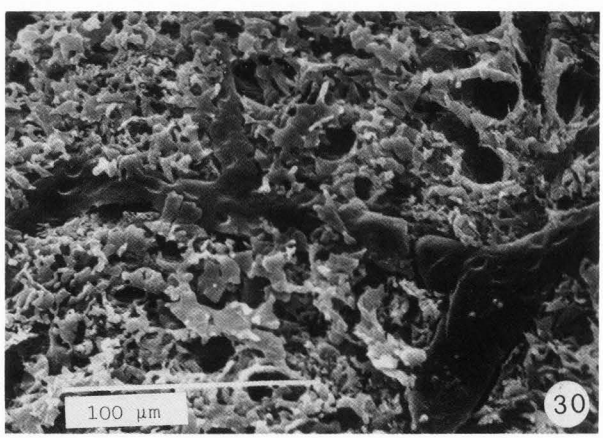
in scanning electron microscopy good results have been obtained using methacrylate and other resins as the injecting media (Kobayashi et al, 1976, Castenholz 1984, 1985, 1986, Ohtani 1984, 1987, Ohtani and Ohtsuka 1985, Ohtani et al. 1986, Ohtani and Murakami 1986). If one considers special morphological features of the small lymphatics, it is not difficult to distinguish these from the cast patterns of small blood vessels. This becomes obvious in casts with a double filling of both the blood and lymphatic vascular system (fig. 20). On the other hand in such preparations non-organized tissue spaces and prelymphatic channels as occurring in the connective tissue between striated muscle fibers in the tongue are easy to differentiate from the structures of blood capillaries (fig. 21).

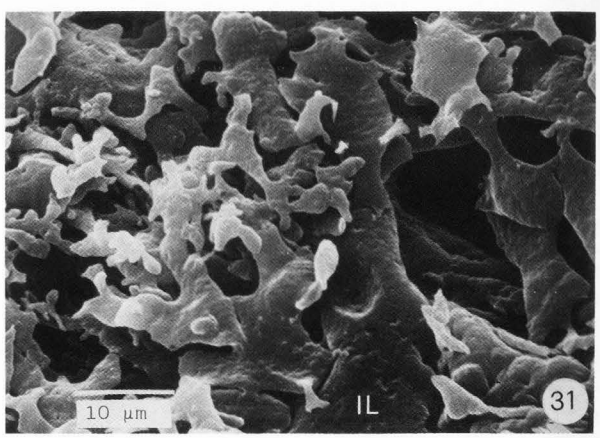












Characteristic features of casts of initial lymphatics are the greater and widely varying diameters (15 - 50 µm) of the structures in comparison with blood capillaries. Typical for initial lymphatics is the irregular pattern of deep oval depressions (figs. 22, 23). Some imprints reveal elongated notches originating from trough-shaped depressions that are created by the cellular processes the lymphatic endothelial cells are richly provided with. The pattern of nuclear depressions appearing on casts of precollector lymphatic vessels is more regular, which enables them to be distinguished from the initial lymphatics (figs. 24, 25). Deep sharply marked fissures are also a characteristic element of casts of small lymphatic vessels. Although fissures are found in the initial lymphatics they are more frequent in the precollectors. Bicuspid valves create a V-shaped pattern into the cast (figs. 26, 27).

The endothelial boundaries become occasionally replicated on the cast surface of small

Fig. 26. Deep V-shaped notches corresponding to a valve structure are a frequent phenomenon of the initial lymphatic (cast, tongue, rat).

Fig. 27. Tissue preparation showing a valve structure in the lumen of an initial lymphatic (tongue, rat). Compare fig. 26.

Fig. 28. At high magnification the wavy endothelial border-line system of initial lymphatics can be represented (tongue, rat). At some sites the cytoplasm of neighbouring cells overlaps forming pocketing-like structures with open junctions (↙). Tissue preparation.

Fig. 29. Oval-shaped notches appear at some sites on casts of initial lymphatics. Such structures obviously correspond to the pocketing areas located along the endothelial borderline system in tissue preparations (compare fig. 28).

Fig. 30. Cast of connective tissue of the subepithelial zone (tongue, rat) showing an initial lymphatic together with the system of interstitial spaces. In retrograde tracing the vessel to the tissue spaces it can be demonstrated that the vessel continues without any interposed membrane into the irregularly formed spatial structures of the connective tissue.

Fig. 31. Enlarged sectional area from fig. 30 showing the transitorial zone between the cast of the initial lymphatic (IL) and that of the tissue spaces.

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lymphatics. Sometimes oval-shaped indentations or protrusions can be detected, which relate to the wavy endothelial cell boundary of the initial lymphatics (fig. 28). In the course of an endothelial boundary zones of simple edge-to-edge contact change with those of cytoplasmic overlappings which form pocketings with open junctions (fig. 4). On some casts these interendothelial pocketings are replicated as oval-shaped sharp protrusions or notches (fig. 29). From such areas occasionally small plastic bridges extend into the paravascular space and fuse with cast structures of the tissue indicating extravasation of the resin through the open junctions in the endothelium. In some preparations the cast pattern of initial lymphatics can backwardly be traced to the labyrinth of the tissue spaces (figs. 30, 31). Such a finding obviously does not agree with the current meaning on the morphological features of the fine lymphatics, which are considered to be lined by a continuous endothelium. Thus, the question of an open prelymphatic-lymphatic interface is renewed by the scanning electron microscopic observations.

Comments

This paper examines by means of corrosion casts the fine structure of the vessels of the terminal blood circulation and the initial lymphatic system. For an exact morphological interpretation of the casts, however, a high replication quality of the specimens is necesssary. Thus, special media such as Mercox and careful preparative procedures need to be applied. Furthermore, the investigator must be well informed of the cytological, histological and functional properties of the vessels to be studied. Such knowledge is derived from correlative observations of fixed tissue using the light and scanning electron microscope. In some cases a further examination of the tissue by means of the transmission electron microscope may be necessary. Great attention should also be paid to the differences of vascular structures in different organs. Finally, artefacts have to be distinguished from replicated structures that correspond to the true cytological conditions of the vessels at time of injection.

Important morphological structures may include all imprints of the cast surface such as those produced by the endothelial cells like nuclear depressions and boundary structures. The morphological significance of the "plastic strips" surrounding the luminal cones of some vessels are still a subject of discussion. They appear predominantly on casts of arterioles and capillaries imitating the muscular and pericytic elements in this vascular area. But it remains uncertain till now, whether the cells are completely or only in parts replicated by the strips.

Also the mode of filling of the initial lymphatics with resin after interstitial injection is unknown. One meaning is that the resin may invade the vessels' lumen through the numerous small open junctions of the lymphatic endothelium (Wenzl-Hora et al. 1987). It is also conceivable that the casting medium fills the vessel via a direct prelymphatic-lymphatic connection which possibly exists in different places of the connective tissue. A direct communication between prelymphatic spaces or channels and true lymphatics has been described in the mesentery (Hauck 1973, Hauck et al. 1987) and could recently be seen also in living and fixed tongue tissue (Castenholz 1989). Thus, in our opinion the initial lymphatics are filled from the interstice via such preformed or artificial tissue-vessel communications.

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Discussion with Reviewers

A. Miodónski: Did the author try to inject the lymphatics of other organs or tissues than specified in this paper, e. g., of the diaphragm where arrangement between mesothelial and lymphatic endothelial cells forms circular pores leading directly to the lymphatic vessels?
Author: This particular tissue area has not yet been examined by us.

A. Lametschwandtner: You used diluted and undiluted Mercox for casting tongue and skin lymphatics. Did you find any differences in respect to ease, completeness or morphology of the casted lymphatics if using different viscous injection media in your present or past work or are you aware of such differences from published work of other authors?
Author: We prefer to apply diluted Mercox for casting of terminal blood vessels from the venous side, because the injection pressure should be kept moderate in supplying veins. The high liquid resin, after our experience, more easily reaches the finest venules and often also the capillaries. Good results can be obtained with diluted Mercox in embryonic tissue, too. Thus, we succeeded in getting good casts of whole organs in rat embryos during prenatal development.