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E. Gaudio
State University of Rome "La Sapienza"

L. Pannarale
State University of Rome "La Sapienza"

A. Caggiati
State University of Rome "La Sapienza"

G. Marinozzi
State University of Rome "La Sapienza"

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A THREE-DIMENSIONAL STUDY OF THE MORPHOLOGY AND TOPOGRAPHY OF PERICYTES IN THE MICROVASCULAR BED OF SKELETAL MUSCLE

E. Gaudio[*], L. Pannarale, A. Caggiati, and G. Marinozzi

Dept. of Anatomy, State University of Rome "La Sapienza",
Via A. Borelli 50, 00161 Rome, Italy

Abstract

Digested tissue specimens and corrosion casts of rat soleus and tibialis anterior muscles were employed for this Scanning Electron Microscopy (SEM) study. The shape, morphology, and position of pericytes were compared to corresponding imprints on the cast surfaces. Pericytes, observed in digested tissue specimens, showed a typical morphological pattern: a central body with two primary processes that run along the capillary in opposite directions. From these primary processes, secondary ones arise and often encircle the vessel almost completely.

On the surface of corrosion casts, roundish imprints were found in the microvascular tree at the same level where digested tissue specimens showed the presence of pericyte bodies. Along and around the cast surface, shallow grooves reproduced the course of the primary and secondary processes. The peculiar tridimensional arrangement of pericytes at the level of capillary bifurcations underlines their role in red cell flow regulation. However, if the mechanical linkage of the pericytes to the endothelium and their contractability is taken into account, additional roles of these perivascular cells may be hypothesized.

*Address for Correspondence and Present Address:
Eugenio Gaudio,
State University of "L'Aquila", Dept. Exptl. Med.
S. Sisto, 67100 L'Aquila, Italy
                    Telephone: (39 862) 4311
                    FAX: (39 862) 600213

Introduction

The morphology and topography of pericytes in the microvascular tree of the skeletal muscle is a very interesting topic because of its morphofunctional implications. Several studies on skeletal muscle pericytes have been made by different techniques. In vivo studies showing evidence of interruption of erythrocyte flow at certain levels have suggested that pericytes can contract (Zweifach and Metz, 1955; Stingl, 1976; Myrhage and Hudlicka 1976). Furthermore, immunohistochemical studies have indicated the presence of contractile proteins in these cells (Joyce et al., 1985 a,b) as well as their strong mechanical connection to the endothelium by fibronectin plaques (Courtoy and Boyles, 1983).

The Transmission Electron Microscope (TEM) has demonstrated the complex ultrastructural relationship between pericyte processes and the endothelium (Tilton et al. 1979 a). Moreover, TEM has been used to demonstrate pericyte contractility and its effect on the capillary lumen and endothelium (Tilton et al., 1979 b). However, it remains difficult to evaluate the pericyte's overall size and branching pattern by the techniques mentioned above. Also, by these methods, it is not easy to identify exactly the different positions of pericytes in the capillary network as a whole. In order to study the position of the pericytes in the capillary bed, Williamson et al. (1980) developed an immunofluorescence technique based on staining of the vascular basement membrane. This permits visualization of small vessels in unsectioned tissue. Yet, this method is limitated by the observation of thick specimens by light microscopy. Moreover, the preparation of these specimens includes the manual distention of tissue fragments thereby possibly causing an unnatural distortion of the vascular tree.

Another technique that can be used to study the capillary bed is Scanning Electron Microscopy (SEM) of corrosion casts and of digested tissue

specimens. Corrosion casts allow one to follow the tridimensional arrangement of a microvascular network (Murakami, 1971; Nowell and Lohse, 1974; Groom et al., 1982; Potter and Groom, 1980,1983). Secondly, corrosion casts provide indirect information on the structure of the vascular wall. In fact, it is possible to recognize the different endothelial shapes from the various imprints constantly present on the surface of the venous and arterial vessels (Myrhage and Hudlicka, 1976). Moreover, the localized tapering of the cast has in certain cases been, related to the presence of sphincter-like structures (Gaudio et al., 1984; Pannarale et al., 1986; Gaudio et al., 1989). The consistent presence of certain imprints on the capillary casts has been attributed to the presence of pericytes on the basis of similar observations made by other researchers who, however, used different techniques (Gaudio et al., 1985).

Another feature of corrosion casts that could provide some information on the vascular wall has been known as "plastic strips". This aspect has been thoroughly studied by Castenholz and his coworkers in different tissues (Castenholz, 1980; 1983 a,b; 1989; Castenholz et al., 1982). "Plastic strips" are resin extravasations occuring in the space occupied in vivo by perivascular cells (Castenholz et al., 1982). However, not all perivascular cells are replicated, and plastic strips can be obtained only in small arteries and arterioles and only rarely at the capillary level (Castenholz et al., 1982).

On the other hand, the SEM observation of chemically digested specimens allows the tridimensional study of the surface morphology of the vascular and perivascular cells (Mazanet and Franzini-Armstrong 1980,1982; Mazanet et al. 1979; Shotton et al. 1977; Maggioni et al. 1989). Moreover, this method shows the tridimensional relationship of blood vessels with neighboring structures. Nevertheless, this technique does not give a precise idea of the overall vascular pattern, and not all of the perivascular cells are preserved during digestion (Holley and Fahim, 1983).

The present study is based on the comparison of corrosion casts and digested tissue specimens. Its aim is to evaluate properly the surface features of capillary corrosion casts and to trace pericytes through the whole vascular bed. The study of the tridimensional arrangement of these cells and the consideration of their position can help us to elucidate their function.

Materials and methods

Ten adult male Wistar rats weighing
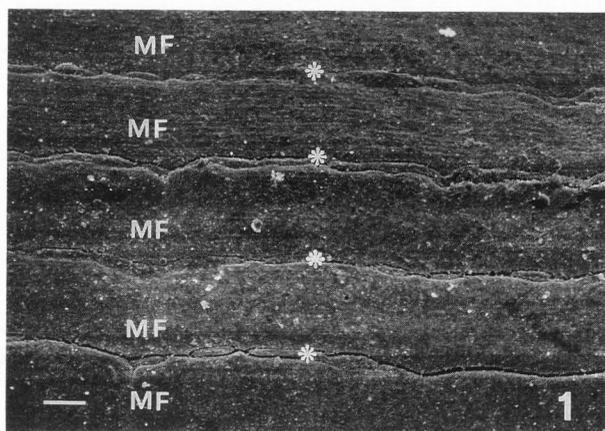
Fig. 1: Digested tissue specimen of tibialis anterior. In the spaces between muscle fibers (MF) parallel capillaries (*) can be observed. Bar= 10 micrometers.

approximately 150-200 g. were used for this study. Tibialis anterior and soleus muscle specimens obtained from different animals were prepared using the two different techniques. The animals were anesthetized with an ethyl-ether oxygen mixure.

Samples for KOH digestion were prepared as follows (Maggioni et al., 1989). After rinsing the vascular bed for 30 seconds with 20 ml. of cacodylate buffer (0.1 M, at 7.3 pH), fixation was performed by perfusing, via the abdominal aorta, the lower limbs for 3 minutes with a 2.5% glutaraldehyde solution buffered to pH 7.3 in cacodylate (0.1 M) (Miller et al., 1982). The muscles were then excised and kept in the same fixative for 24 h at 4*C. After washing for two hours, a partial digestion of the tissue was achieved by immersing the samples in a 30% potassium hydroxide aqueosus solution at 60*C for 10-20 minutes. Afterwards, the specimens were washed for 2 hours in running tap water, postfixed in 1% osmium tetroxide for 2h at room temperature, dehydrated in ethanol and critical point-dried in liquid carbon dioxide. The dried specimens were coated with gold (in an EDWARD's sputtering device) and examined in a Cambridge 150 SEM at 15-20 kV.

Corrosion casts were prepared as follows. The aorta and inferior vena cava were dissected free and a catheter 0.8 mm in diameter was introduced into the aorta almost up to the iliac bifurcation and secured by means of two "circular" silk ligatures.

The vascular bed was rinsed with heparinized saline (Hodde et al., 1977; Miodonski and Jasinski, 1979) supplemented with 1% carbocaine (Gaudio et al., 1982). At the same time, the inferior vena cava was opened in order to allow

Fig. 2: Digested tissue specimen. Insert: corrosion cast. C=capillary; P=pericyte body; arrows= pericyte processes; CC=capillary cast; *=traces on the cast. Bar= 1 micrometer; insert Bar=10 micrometers.
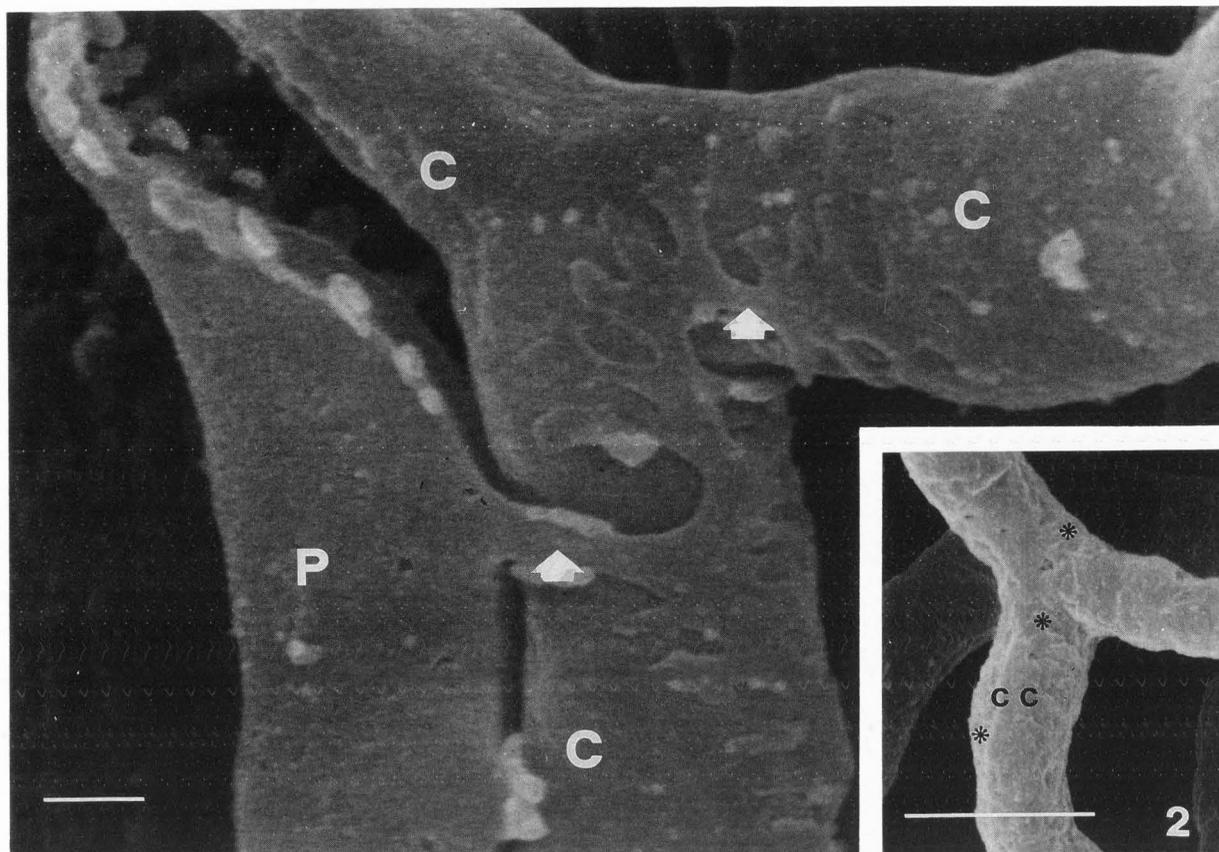
the outflow of perfusion fluid.

After washing out of the blood (less than 3 min) a "prefixation" (Lametschwandtner et al., 1984) was performed. The vascular bed was perfused for 1 min with a 1% solution of glutaraldehyde in 0.1M cacodylate buffer at pH 7.3 (Gaudio et al., 1982; Pannarale et al., 1986), at a flow rate of 20 ml/min.

Finally, the vascular system was filled with Mercox CL2R (20 mg of catalyzer per 2 ml of base compound) at room temperature. Resin injection flow was 15 ml/min. Injected animals were left at room temperature for at least 24 h to allow for polymerization. Then the tibialis anterior and soleus muscles were excised and macerated in a 15% NaOH solution at room temperature for 24h. Afterwards, samples were rinsed in distilled water, cleaned in 5% trichloroacetic acid (Miodonski and Bar, 1987), washed again in distilled water and freeze-dried (Lametschwandtner et al., 1984).

For further study with the SEM, specimens were dissected under a stereomicroscope. The small samples obtained, (approximately 5 mm in diameter) were attached to stubs by means of "Silver Dab" compound, coated with gold in an Edwards sputter coater and observed in a Cambridge 150 SEM at 15-20 hV (Gaudio et al., 1982).

Results

KOH digestion of skeletal muscle samples permitted the direct observation of the surface microanatomy of the microvascular bed and of the myofibers. These cells appeared to be cylindrical in shape and ran parallel to the long axis of the muscle. Arteriolar and venular vessels are rarely seen between muscle fibers; such vessels are more easily distinguished within the connective endomysial tissue. Numerous capillaries were observed running parallel to the myofibers (Fig. 1); these capillaries were frequently connected by means of short, straight capillaries. Because of the chemical digestion, neither the intercellular stroma nor the pericapillary connective tissue was visible. A few sparse fibroblasts lay near the vessels. These cells had a cell body and three or four main processes having a regular outline. At higher magnification, the capillaries resembled as a cylindrical tube on which were pericytes were present (Fig.2).
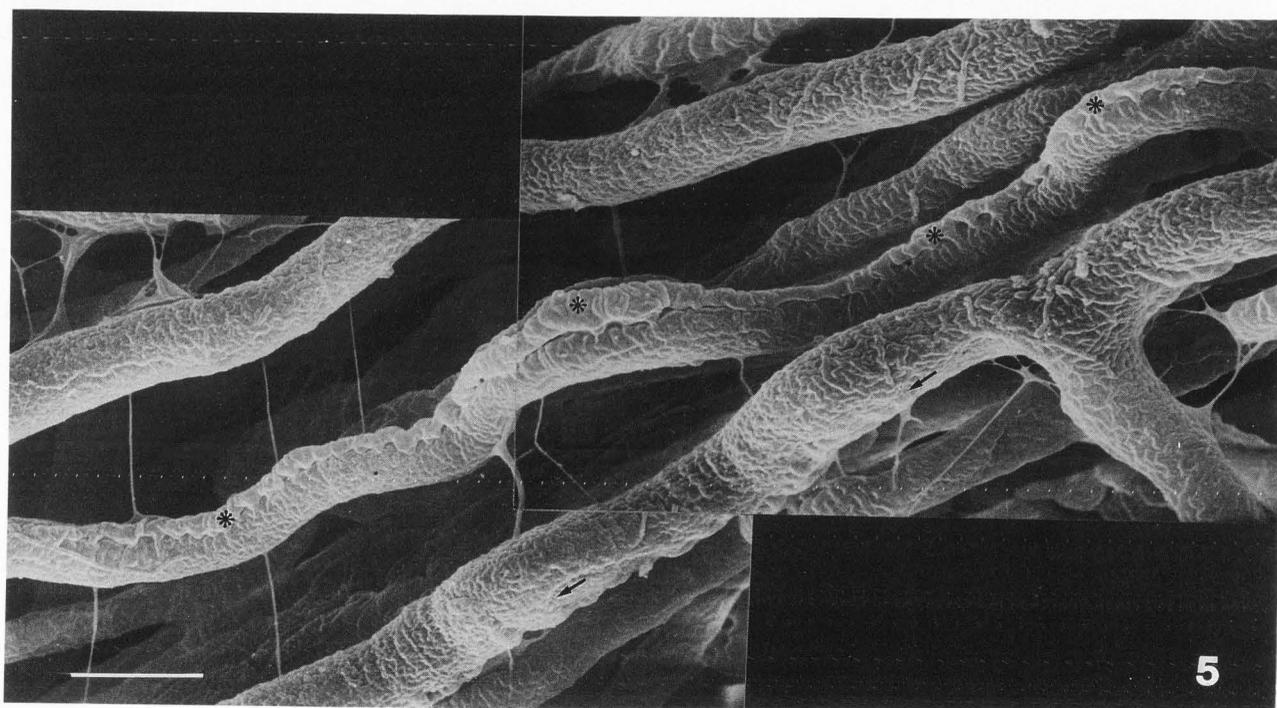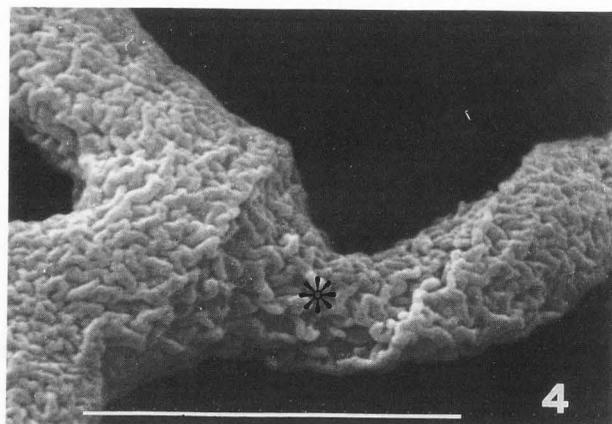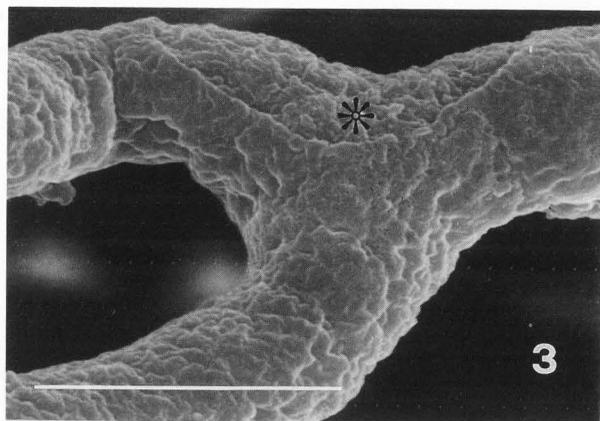
Fig. 3: Corrosion cast; *=shallow round imprint at the level of capillary bifurcation. Bar= 10 micrometers.

Fig. 4: Corrosion cast; *=round "punched out" imprint at the level of a capillary bifurcation. Microphotograph taken of the same sample shown in Gaudio et al., (1985). Bar= 10 micrometers.

Fig. 5: Corrosion cast of the tibialis anterior; micrograph taken in a zone showing also tissue remnants. *= body and processes of a tissue remnant; arrows=longitudinal groove on a capillary cast. Bar= 10 micrometers.

Pericytes showed a typical cytoplasmic arrangement: from the central body two processes arose (primary processes) which run along the capillary in opposite directions. These processes were up to 60 micrometers in length and approximately 1.5 micrometers wide. Generally, these primary processes were closely attached to the capillaries along their entire course (Fig. 2). Numerous secondary processes arose from the primary ones. These secondary processes ran perpendicularly to the main axis of the vessel. The length of these processes varied greatly even within the same specimen (from 0.5 to 5 circa micrometers), as we could roughly estimate from their extension around the capillary wall.

SEM observation of long tracts of longitudinal capillaries revealed the presence of

at least one pericyte on the tract between two transverse anastomoses. Its body diameter was, approximately, 10 micrometers. The pericytes observed on the longitudinal capillaries possessed two very long primary processes that arose from opposite poles of the cell body. Pericytes located at the capillary bifurcations could possess more than two primary processes which, emerging from the central body, were directed radially towards each capillary branch.

SEM of digested specimens consistently revealed the presence of one or more pericytes at the capillary bifurcations. The primary processes arising from the pericytes located at the capillary bifurcations were wider and thicker than those running on the longitudinal capillaries. Such processes frequently encircled the microvessel almost completely. Some of the pericytes close to the capillary bifurcations frequentely had to a cytoplasmic projection that bridged directly a parallel vessel. The study of corrosion casts showed the presence of longitudinal capillaries and of their side branches. The surface of the cast had a typical, regularly wavy surface. If a capillary is followed along its course between two side branches, it is possible to observe the presence of a very shallow groove along the cast. Inside the groove, the typical wavy aspect of the cast surface is somewhat changed in orientation. This furrow can be followed, sometimes, for up to 60 micrometers along the cast. The width of the groove generally measured about 3 micrometers but it could be enlarged around the cast giving the impression of a round patch.

More complex surface features could be observed at the level of the origins of the side branches. There we could see the same kind of groove that we found along the longitudinal tract of the casts. But, in this case, the groove sometimes extended from the longitudinal capillary to the side branch. Moreover, at the level of the side branch origins we could find round imprints. These imprints could be as shallow as the above mentioned grooves (Fig. 3) or could appear much deeper, as if punched out by a hemispheric body (Fig. 4) .

At the level of the branching point different kind of imprints were generally associated or two grooves could be present along the same side branch. Circular imprints tended to be wider than longitudinal ones. At the origin of the side branches as well as at the venular end, it was often possible to see a homogeneous tapering of the cast or an almost circumferential groove around the cast.

A small number of casts showed some uncorroded tissue remnants. These remnants appeared to be lying on the surface of the cast
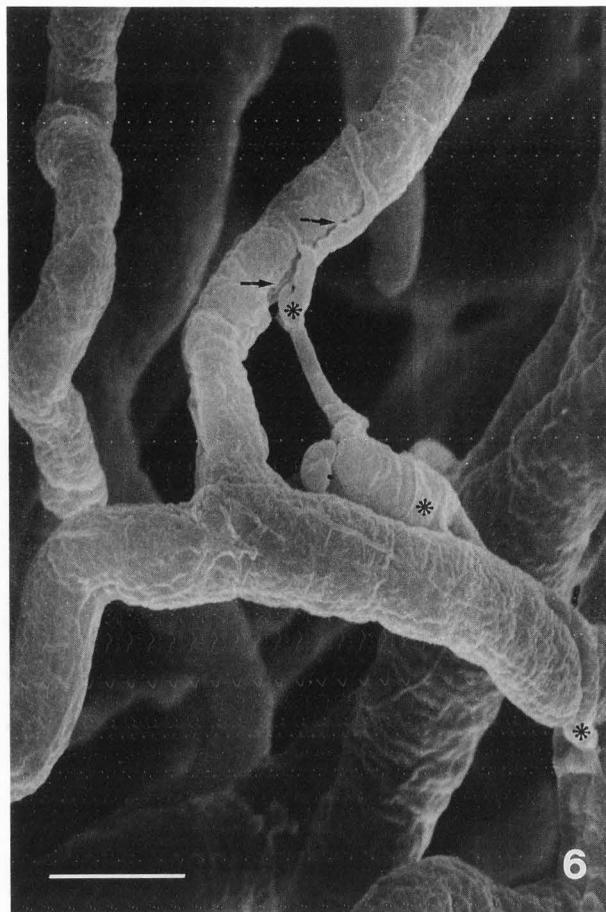
Fig. 6: Corrosion cast of a capillary bifurcation; micrograph taken in a zone also showing tissue remnants. *= body and processes of a tissue remnant. Note the close relation of the grooves on the cast (arrows) and the processes of the uncorroded stucture. Bar= 10 micrometers .

and did not have the typical wavy surface of the cast. Some remnants had a peculiar shape and relation to certain specific sites of the cast. These structures were elongated, about 4 micrometers in width, and up to 120 micrometers in length. They had a wavy border with edentations of different lengths extending around the cast (Fig. 5). They were always located in close proximity to the round imprints or grooves on the cast mentioned above, but most often a space between them could be observed. Wherever the structures gave off a process around the vessel a groove could always be observed on the cast surface. Sometimes, at the level of a capillary cast bifurcation, the structures appeared to recede from the capillary nook and to be taut between the two branches (Fig. 6). Some showed an enlarged central body from wich thinner

parts arose.

Discussion

The technique proposed by Maggioni et al. (1989) for the preparation of KOH digested-tissue specimens has proved to be a valuable tool for the study of the tridimensional morphology of pericytes. In fact, this method has provided morphological data that are very similar to those obtained by Mazanet and Franzini-Armstrong (1982), although it has required much less technical effort. Comparison of the morphology of the perivascular cells that we have observed with the description of pericytes given by others, that studied them in the rat by SEM and TEM (Mazanet and Franzini-Armstrong, 1982; Tilton et al. 1979a,b), confirmed that the cells we observed are pericytes.

The position and arrangement of the pericytes along and between capillaries were in agreement with the observations made by fluorescent microscopy in human skeletal muscle by Williamson et al. (1980). Nevertheless, in our specimens we observed a much tighter adhesion of the pericyte bodies and primary processes to the vascular walls. This main difference is probably caused by the tissue processing method employed by Williamson et al. (1980), who used autopsy material, teased apart in order to be spread for observation.

The frequency of pericytes can not be studied by this or similar digestion techniques. In fact, a variable degree of pericyte loss during corrosion is always present (Mazanet and Franzini-Armstrong, 1982; Holley and Fahim, 1983). Moreover, the presence of the skeletal muscle myofibers prevents the observation of the whole circumference of the vessel, and thus possibly hides perivascular cells.

Corrosion casts have interesting traces on their surfaces when properly prepared and attentively observed. It must be emphasized the imprints we found were so shallow that they could have been easily overlooked during observation or lost during cast processing.

According to our own and others' experience (Lametschwandtner et al., 1984) a series of parameters must be taken into account to get the best surface details. First of all, vascular bed washing by means of heparinized saline (Hodde et al. 1977; Miodonski and Jasinski, 1979) also containing a vasodilator (Gaudio et al., 1982) is important both for a complete filling and for avoiding cast inclusions of blood cells.

Moreover, short "prefixation" (Lametschwandtner et al., 1984) of the vascular wall has been found to be important for identification of endothelial imprints.

Corrosion times are also important. The specimens must be exposed to the basic and acid solutions only for the time necessary to remove tissue remnants, and no longer. Sputtering of specimens must not be excessive either. Finally, observation parameters must be selected in order to optimally visualize the very shallow cast surface details.

In trying to interpret such traces on the cast, we find it quite difficult to attribute them to the endothelium. In fact, endothelial cell nuclei are known to leave imprints on the surfaces of corrosion casts of arteries and veins (Miodonski et al., 1976), of metarterioles (Gaudio et al., 1989) and of capillaries (Shimizu and Ujie, 1978; Lametschwandtner et al., 1984). But these imprints never exceed 15 micrometers in length (data obtained from measurements on the micrographs of the papers mentioned above) and they always have a round or oval shape. Moreover, in sites where endothelial nuclear imprints are found, they show the same frequency as shown by endothelial cells observed by transmission electron microscopy of serial sections (Shimizu and Ujie, 1978).

The traces that we described as grooves along the capillary vessels can not be compared with these imprints with regard to their shape, dimensions or frequency. The shape and dimension of the "punched out" or "round patch" traces is comparable to that of the endothelial nuclear imprints as described by others. However, they do not appear as regularly and frequently spaced as the endothelial cells. Moreover, if both types of traces were left by capillary endothelial cells, then they both should have the same morphology.

The only other cellular component of the capillary wall that could possibly leave a trace on the cast is the pericyte. In fact, it is known from the work of Kendall and Eissman (1980) that the casting medium tends to form indentations where the capillary wall is attenuated. At the level of the pericyte the capillary wall is not only thicker (Tilton 1979a,b) but is also considered more rigid (Cogan et al., 1961). Moreover, many authors have underlined the intimate structural association between pericytes and endothelium (Rhodin, 1968, 1974; Weibel, 1974; Matsusaka, 1975; Forbes et al., 1977; Ryan et al., 1979; Wallow and Burnside, 1980; Mazanet and Franzini-Armstrong, 1982) and the strong mechanical linkage (Courtoy and Boyles, 1983) between them. Thus we can reason that, when the resin is injected, the wall of the vessel tends to be homogeneously distended by the resin. At the level of pericyte attachment, however, the capillary wall is thicker and more rigid and therefore less

stretched than in the neighboring areas. This leads to the outlining of the pericyte attachment point on the surface of the cast.

It is quite difficult to compare the dimensions in the digested tissue specimens with those in Mercox corrosion casts because of the unequal and variable shrinkage percentages of the two types of specimen (Boyde, 1978; Weiger et al., 1982; Lametschwandtner et al., 1984). However, we can try to compare the position and the extension of pericytes around and along the capillaries with the imprints we found on the capillary casts. Different kinds of imprints can be found on the cast because different parts of the pericyte happen to be in close contact with the endothelium. The most extended traces are the "grooves" along the course of a capillary between two transverse anastomoses. Similarly, the pericytes showing the longest processes are those with the same position. Moreover, the pericytes in these areas have less extended secondary processes around the vessel, and the grooves do not tend to extend circumferentially.

At the level of the capillary bifurcation on the casts the pericytes tend to be arranged in a more complex way, as shown in digested tissue specimens. Processes are often extended circumferentially. Thus, at the origin of the side branches of the cast we very often find a groove or localized tapering of the cast. At the same level, both the imprints and the processes themselves are wider than at any other site. Moreover, the presence of more than one pericyte at a branching point corresponds to the presence of different imprints at the same bifurcation of the cast.

Depending on different geometrical factors the pericyte body appears to give different signs of its presence. It can produce a real buckling of the vascular wall, changing dramatically the shape and the diameter of the cast, or it can give rise to a shallow imprint that barely influences capillary diameters. This is the case when the pericytes show only a few thin processes along the vessels. Of course, due to its larger mass the pericyte body is the part that is more likely to cause a "choking point".

If the correlations mentioned above are taken into account, it is reasonable to consider the tissue remnants observed on the casts as due to pericytes. Their morphology resembles pericytes very closely and the imprints they cover are exactly the same as those we have studied. Their partial loss of contact with the cast could be explained by the differences in shrinkage of tissue and casts.

The perivascular orientation and close connection of primary and secondary processes entwined around the capillaries at the branching

points can be almost certainly related to a role in the regulation of erythrocyte flow (Tilton et al., 1979a,b). Certainly the importance of this role is different in the tibialis anterior and in the soleus muscle. In fact, in the latter we observed a different pattern of microvascular bed with many more branching points at capillary level and connections with metarterioles (Pannarale et al., 1986; Gaudio et al., 1989). On the other hand, the purpose of pericyte processes running along the vessels or bridging between two vessels can not be flow regulation, because of their mechanical linkage with the endothelium (Courtoy and Boyles, 1983). We believe that one or more of the following hypotheses could be evaluated.

1) The pericytes are merely a mechanical support (Cogan et al. 1961): there is nothing against this, but such a theory does not explain the purpose of their contractility.

2) The contraction of the pericytes processes avoids excessive narrowing of vessels during muscular contraction. This hypotesis could explain the functional role of bridging processes and their contractility.

In fact, these processes are not present in a non-contractile tissue such as the retina (Williamson et al., 1980).

3) Contraction of the longitudinal processes of pericytes could cause a buckling of the capillary wall (Mazanet and Franzini Armstrong, 1982) or increase the tortuosity of the vessel (Courtoy and Boyles, 1983). This would certainly affect blood flow. This is the only hemodynamic interpretation of the longitudinal processes.

4) Pericytes can stretch intercellular junctions, thus playing a role in vascular permeability, according to Miller and Sims (1986). This hypothesis would require that the pericyte processes are not bridging between two adjacent endothelial cells. Perhaps we can find such a situation in the retina, but not in skeletal muscle.

We believe that a direct comparison between the tridimensional morphology of pericyte and the surface features of the corrosion casts can help in a better interpretation of some shallow traces often neglected in the routine observation of casts.

References

Boyde A (1978) Pros and cons of critical point drying and freeze drying for Scanning Electron Microsc. 1978; II; 303-314.

Castenholz A (1980) Scanning electron microscopy of myocytes and pericytes in terminal blood vessels of the rat. Microvasc. Res. 19, 395.

Castenholz A, Zoltzer H, Erhardt H (1982) Structures imitating myocytes and pericytes in corrosion casts of terminal blood vessels. A methodical approach to the phenomenon of "plastic strips" in SEM. Mikroskopie 39, 95-106.

Castenholtz A, (1983a) Visualization of periendothelial cells in arterioles and capillaries by scanning electron microscopy of ultrasound treated and plastoid injected brains in rats. Scanning Electron Microsc. 1983; I, 161-170.

Castenholz A, (1983b) The outer surface morphology of blood vessels as revealed in scanning electron microscopy in resin cast, non-corroded tissue specimens. Scanning Electron Microsc. 1983; IV, 1955-1962.

Castenholz A, (1989) Interpretation of structural patterns appearing on corrosion casts of small blood and initial lymphatic vessels. Scanning Microscopy, 3, 315-325.

Cogan DG, Toussaint D, and Kuwabara T (1961) Retinal vascular patterns. IV. Diabetic retinopathy. Archs. Ophthal. 66, 366-378.

Courtoy P and Boyles J (1983) Fibronectin in the microvasculature: localization in the pericyte-endothelial interstitium. J. Ultrastruct. Res. 83, 258-273.

Forbes MS, Rennels MC, and Nelson E (1977) Ultrastructure of pericyte in mouse heart. Amer. J. Anat. 149, 47-70.

Gaudio E, Pappalardo G, Pannarale L, Pintucci S, Resta S, Ripani M (1982) Three-dimensional observations (by SEM) on the microcirculation of adenocarcinoma of the recto-sigmoid junction in man. It. J. Surg. Sci. 12, 221-225.

Gaudio E, Pannarale L, Marinozzi G (1984) A tridimensional study of microcirculation in skeletal muscle. Vasc. Surg 18: 372-381.

Gaudio E, Pannarale L, Marinozzi G (1985) An SEM corrosion cast study on pericyte localization and role in microcirculation of skeletal muscle. Angiology 36 458-464.

Gaudio E, Pannarale L, Marinozzi G (1989) Corrosion casts in the microcirculation of skeletal muscle. In "Cells and Tissues: A Three Dimensional Approach by Modern Techniques in Microscopy", Motta P.M. ed, Alan R. Liss Inc., New York,443-450.

Groom AC, Ellis CG, Potter RF (1982) Tortuosity of capillaries in contracted skeletal muscle studied by corrosion casts. Microvasc. Res. 23: 255-256.

Hodde KC, Miodonski A, Bakker C, Veltman WAM (1977) SEM of microcorrosion casts with special attention on arterio-venous differences and application to the rat's cochlea. Scanning Electron Microsc. 1977; II, 477-484.

Holley JA, Fahim MA (1983) Scanning electron microscopy of mouse muscle microvasculature. Anat. Rec., 205, 109-117.

Joyce NC, Haire MF, Palade GE (1985 a) Contractile proteins in pericytes. I. Immunoperoxidase localization of tropomyosin. J. Cell. Biol., 100, 1379-1386.

Joyce NC, Haire MF, Palade GE (1985 b) Contractile proteins in pericytes. II. Immunocytochemical evidence for the presence of two isomyosins in graded concentrations. J. Cell. Biol., 100, 1387-1395.

Kendall MW, Eissman E (1980) Scanning electron microscopic examination of human pulmonary capillaries using a latex replication method. Anat. Rec.196, 275-283.

Lametschwandtner L, Lametschwandtner U, Weiger T (1984) Scanning electron microscopy of vascular corrosion casts-techiniques and application. In: Scanning, Electron Microsc. 1984; II, 663-695.

Maggioni A, Caggiati A, Macchiarelli G (1989) Scanning electron microscopy of microvessels and perivascular cells in different organs after KOH digestion.In "Cells and Tissues: A Three-Dimensional Approach by Modern Techniques in Microscopy", Motta P. ed.,Alan R. Liss Inc., 469-474.

Matsusaka T (1975) Tridimensional views of the relationship of pericytes to endothelial cells of capillaries in the human choroid and retina. J. Electron Microsc. 24, 13-18.

Mazanet R, Reese BF, Franzini-Armstrong C, Reese TS (1979) SEM of satellite cells and their response to muscle fiber injury. J. Cell. Biol. 83, 383a.

Mazanet R, Franzini-Armstrong C. (1980) SEM of vascular pericyte in rat red muscle. J. Cell. Biol. 87, 259a.

Mazanet R and Franzini-Armstrong C (1982) Scanning electron microscopy of pericytes in rat red muscle. Microvasc. Res. 23, 361-369.

Miller BG, Woods RI, Bohlen HG, Evan AP (1982) A new morphological procedure for viewing microvessels: a scanning electron microscopic study of the vasculature of the small intestine. Anat. Rec. 203: 493-503.

Miller FN, Sims DE (1986) Contractile elements in the regulation of macromolecular permeability. Fedn. Proc.45: 84-88.

Miodonski AJ, Hodde CK, Backer C (1976) Rasterelektronenmikroskopie von Plastik-korro-sion-praphareten: Morphologische Unterschiede zwischen Arterien und Venen. Beitr Elektronenmikroskop Direktabb Oberfl (Munchen) 9: 435-442.

Miodonski A, Jasinski A (1979) SEM study of microcorrosion casts of the vascular bed in the skin of the spotted salamander, Salamandra

salamandra L. Cell. Tiss. Res. 196, 153-162.

Miodonski AJ, Bar T (1987) Arterial supply of the choriocapillaris of anuran amphibians (Rana temporaria, Rana esculenta). Scanning electron microscopic study of microcorrosion casts. Cell Tissue Res. 249: 101-109.

Murakami T (1971) Application of the scanning electron microscope to the study of the fine distribution of the blood vessels. Arch. histol. Jap. 32, 445-454.

Myrhage R and Hudlicka O (1976) The microvascular bed and capillary surface area in rat extensor hallucis propius muscle (EHP). Microvasc. Res. 11: 315-323.

Nowell JA and Lohse CL (1974) Injection replication of the microvasculature for SEM. Scanning Electron Microsc.; 267-274.

Pannarale L, Gaudio E, Marinozzi G (1986) Microcorrosion casts in the microcirculation of skeletal muscle. Scanning Electron Microsc. 1986; III: 1103-1108.

Potter RF and Groom AC (1980) Corrosion casts of the microvasculature in heart and skeletal muscle, studied by scanning electron microscopy. The Physiologist 30: 90.

Potter RF and Groom AC (1983) Capillary diameter and geometry in cardiac and skeletal muscles studied by means of corrosion casts. Microvasc. Res. 25: 68-84.

Rhodin JAG (1968) Ultrastructure of mammalian venous capillaries, venules, and small collecting veins. J. Ultrastruct. Res. 25, 452-500.

Rhodin JAG (1974) "Histology: A Text and Atlas", Oxford Univ. Press. New York pp. 331-370.

Ryan US, Ryan JW, and Whitaker C (1979) How do kinins affect vascular tone? Adv. Exp. Med. Biol. 102A, 237-291.

Shimizu K and Ujie K (1978) Structure of Ocular Vessels. Igaku-shoin - Tokyo. p. 126.

Stingl J (1976) Fine structure of precapillary arterioles of skeletal muscle in the rat. Acta Anat. 96: 196-205.

Tilton RG, Kilo C, Williamson JR (1979a) Pericyte-endothelial relationships in cardiac and skeletal muscle capillaries. Microvasc. Res. 18, 325- 335.

Tilton RG, Kilo C, Williamson JR, Murch DW (1979b) Differences in pericyte contractile function in rat cardiac and skeletal muscle microvasculatures. Microvasc. Res. 18, 336-352.

Wallow IH, Burnside B (1980) Actin filaments in retinal pericytes and endothelial cells. Invest. Ophthal. Vis. Sci. 19, 1433-1441.

Weibel ER (1974) On pericytes, particularly their existence on lung capillaries. Microvasc. Res.8, 218-235.

Weiger T, Lametschwandtner A, Adam H (1982) Methylmethacrylate and Mercox CL in the scanning electron microscopy of corrosion casts. Mikroskopie 39, 187-197.

Williamson JR, Tilton RG, Kilo C, Yu S (1980) Immunofluorescent imaging of capillaries and pericytes in human skeletal muscle and retina. Microvasc. Res. 20, 233-241.

Zweifach BW and Metz DB (1955) Selective distribution of blood through the terminal vascular bed of the mesenteric structure and skeletal muscle. Angiology 6: 282-290.

Discussion with Reviewers

A. Castenholz: What lends force to the supposition of the authors that the longish structures embracing the luminal casts in figure 5 are "remnants" of pericytes? Do they regard them as organic residues of this type of cell? In the brain circulation of the rat we could show that similar structures surrounding arterioles and capillaries habe been produced by leakage of the resin. So we consider them as pure resin deposits ("plastic strips") within the outer vascular wall and not as incompletely corroded tissue components. Such an interpretation was mainly supported by the fact that these peculiar structures are as sensitive against the electron beam focused on them as the resin of the luminal casts. Moreover, plastic strips imitating pericytes exhibit quite smooth surfaces, no inclusions of cellular material, and frequently assume bloated plump shapes uncommon for true pericytes.

Have you any explanation for the fine thread-like structures stretching between the vascular cast in Fig. 5?

Authors: The kind of structures that we have considered tissue remnants (e.g. the thread-like structures that you have observed in Fig. 5) can only be osserved in samples that showed other signs of incomplete corrosion under the dissecting microscope, before sputtering, or by electron microscopy. In fact, your description of "plastic strips" in your work and in your question does not correspond to what we observed. The "tissue remnants" we describe do not show smooth surfaces. On the other hand, they do show a shape that is, in most cases, very close to that of "true" pericytes as seen in digested tissue specimens.

The structures we describe receded from capillary nooks, because they appeared taut between two branches and because of their position, can not be interpreted as intramural extravasations, but can easily be considered as tissue remnants that have shown a different

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degree of shrinkage in comparison to that of the cast.

A. Lametschwandtner: There are very few casting studies indeed which mention imprint patterns of pericytes. What are the reasons for this fact? Did authors overlook them or is this due to differences in the casting procedures used by different authors or due to differences in pericyte number location behaviour etc. in different tissues examined so far? Please comment.
Authors: We suspect that in many cases, researchers have overlooked the traces of perivascular cells on the surfaces of their casts. Perhaps, such signs have also been interpreted in the past as being related to endothelium. We believe that a reliable interpretation of these traces can only be achieved by comparing corrosion cast observations with other observations made through the use of different techniques. In this respect, we believe that digested tissue specimens are the most useful complementary tool.

A. Lametschwandtner: Fixation of skeletal muscle with 2.5% glutaraldehyde often results in changes of the muscle tone. Please comment upon the state the muscle you have studied with the two different techniques actually were in.
Authors: The muscle fibers were left free to contract under the effect of the fixative solution. A former study of ours (Pannarale et al., '86) demonstrated that the different microvascular patterns of muscles characterized by different metabolisms did not change their main features in extended and shortened muscle bellies.

A. Lametschwandtner: You describe "tissue remnants" in Fig. 5 and 6. What make you sure to term these structures "tissue remnants" and not "casted perivascular structures", i.e. structures from resin? In Fig. 5 processes of these structures gruadually change into the casted vessel.
Authors: See answer to Dr. Castenholz. Concerning the apparent continuity of the "remnants" with the cast, this is most likely due to a microscopic build-up of uncorroded material all over the cast. In fact, since the perivascular cell remnants seem present, it is unlikely that the basal membrane and endothelium could be completely corroded.

A. Lametschwandtner: From Fig. 1 one gets the impression that a) capillary diameters are very small (around 3 um) and b) capillaries ondulate. Please comment.
Authors: We must admit that Fig. 1 does not lend itself to performing measurements of vessels because muscle fibers partially hide capillaries and because of the low photographic magnification.
Capillaries in skeletal muscles are not thought to be straight: in most cases, they show an ondulate trend along a straight direction.

A. Miodonski: The fragment of the cast (bifurcation point) shown on the Fig. 4 exhibits, from the all presented, rough, strongly wrinkeled surface. Please comment.
Authors: The wrinkeled surface of the cast shown on Fig. 4 is in our opinion an enhancement of the more common surface pattern of our casts. In any case we consider it within the average.

A. Miodonski: Why on the Fig. 1 the described transverse anastomoses between capillaries are not visible. Please comment.
Authors: Transverse anastomoses are not seen in Fig 1 because they were absent on that side of the fiber bundle. In the tibialis anterior, it was easy to observe capillaries without transverse anastomoses on one side.