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MICROCORROSION CASTS IN THE MICROCIRCULATION OF SKELETAL MUSCLE

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Abstract

The authors review the contribution of microcorrosion cast studies towards clarifying the structure of skeletal muscle microcirculation.

Former studies performed on naturally contracted muscles show the presence of a primary and a secondary arterial network and a capillary network. At the level of the capillary network pericyte imprints are present. Muscles characterized by different types of metabolism show different features of the capillary pattern. Other authors have affirmed that the extended muscle is characterized by long and straight capillaries, while the contracted one features clusters of vessels all around a muscle fiber.

The authors have made the present observations in order to determine how the capillary pattern of muscles with different metabolism is modified by extension and shortening of the muscle belly.

The capillary pattern observed appears very similar to that observed in former studies. The differences between the oxidative and the glycolytic muscle are evident in every condition of the muscle belly.

These data confirm the theory that there is a permanent endogenous difference in microcirculation between oxidative and glycolytic muscle, determined by muscle fiber metabolism.

KEY WORDS: Corrosion casting; capillary bed; muscle (skeletal); microcirculation; pericytes; arterioles; venules; arteries; veins; blood vessels

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Introduction

In the field of research on skeletal muscle microcirculation, scanning electron microscopy (SEM) microcorrosion casts were first employed to study extraocular muscles both in the monkey and in the human newborn (20,28).

In extraocular muscles, capillaries run parallel to the supposed direction of muscles and are grouped in bundles of about 300 microns in width. Capillaries are anastomosed about every 200 microns by short branches, about 50 microns long, running on different planes.

During the eighties, research teams employing SEM microcorrosion casts paid much more attention to the general architecture of skeletal muscle microcirculation.

Research performed by means of traditional techniques did not always agree on the subdivisions of the microvascular network (21,29,22,23, 24,25,4, 14,5,16). On the other hand, since 1874 (18) some authors described differences of trend and frequency of anastomoses in the capillary network of different muscles (25,13,1,2,19).

In our previous works (6,7,8) we studied systematically the subdivisions of the microvascular tree and the arrangement of the capillary network in the pectineus, in the tibialis anterior and in the soleus muscles in the rat. This allowed us to compare two fast twitch, mostly glycolytic, muscles (the pectineus and tibialis anterior) with a slow twitch, oxidative muscle (the soleus) (3).

In the pectineus, 40 micron wide arterioles, called "primary", run parallel to capillaries. The "secondary" arterioles, 20 microns wide in lumen, stem from the primary ones. Their trend is, on the whole, transverse to capillaries. The secondary vessels give off 10 micron wide arterioles at sharp angles. They run mostly parallel to the capillaries. Sometimes, they are dilated up to 20 microns. The dilatation was more often sited at a curve of the vessel. The 10 micron

wide arterioles divide dichotomously into two 55 micron long trunks, having the same diameter as the originating vessel. At the origin of 10 micron wide arteriole casts, it is very often possible to see a limited reduction in diameter or even a groove, like the imprint of a sphincter (6,7).

These observations are mostly consistent with a model of microvascular bed developed by Stingl (24,25), but different in that we also observed a 55 micron long, 10 micron wide precapillary arteriole.

The capillary network develops from the arterial tree by means of subsequent tuning fork division (10, 6, 7, 8). Capillary casts showed an average 4 micron diameter.

In the tibialis anterior and pectineus muscle the capillaries are straight, sometimes tortuous. They show rather straight transverse anastomoses of different lengths, more frequently towards the venous end of the capillaries (4,6,7, 8). On the other hand, the soleus muscle is characterized by tortuous capillaries, called "main capillaries", running on the whole along the main axis of the muscle belly.

These give origin to capillary loops connected at both ends with the same main capillary. Transverse capillary anastomoses are more frequent, longer and more tortuous than in tibialis anterior and pectineus. A group of main capillaries with their collateral circle and anastomoses form a cage around a space devoid of vessels in which is located the muscle fiber.

Narrowing of the casts are often found at certain sites. They are present at the origin of transverse anastomoses and at the venular extreme of capillaries. In the soleus muscle they are also found at the origin of collateral circles of main capillaries.

These narrowings extend for about 3-6 microns along the cast. In most of the cases they appear well delimited having the shape of a round or groove-like imprint. Sometimes, the feature appears as a progressive tapering. But in no case is the whole circumference of the cast marked by an imprint. These aspects are caused by the presence of a pericyte over the capillary endothelium (8).

The asymmetry of the imprint copes well with transmission electron microscopic observations made by other authors (26).

Also, "in vivo" observations, in rat and rabbit muscles (14,15), and immunofluorescent studies, in human skeletal muscle, visualized small vessels and pericytes along capillaries and at capillary branching sites (27).

Moreover, recent studies on in situ immunoperoxidase localization indicated that tropomyosin is present in capillary and postcapillary venule pericytes in relatively high concentration (11).

Because of their contractility and localization, pericytes could play an important role in regulating microshunts between two capillaries or between two tracts of the same capillary. They can open collateral pathways or modify locally the capillary blood pressure (8).

Postcapillary venules show an inner diameter of 12 microns. They rise from two or more capillaries joining together. A single capillary can reach a postcapillary venule almost perpendicularly. Sometimes,a major venous trunk can be flowed into by a capillary (4,7).

Potter and Groom and Groom et al. (17,9) performed their studies on capillary diameters and geometry on rat gracilis and gastrocnemius muscles using a modified Batson resin to obtain SEM microcorrosion casts. They studied the gastrocnemius muscle both in "in situ" passive full extention and full shortening and the gracilis and gastrocnemius muscles in a free to shorten position.

They observed that the diameter of capillary casts in different position does not show significant differences.

Comparing extended with passively and actively shortened gastrocnemius muscles they observed that in the fully extended muscle the capillaries have a long and straight trend and run parallel to muscle fibers. When the muscle is at its shortest in vivo length the capillaries form a tightly meshed network of sinuous vessels, covering an increased fraction of the fiber surface and this enhances diffusional interaction between adjacent capillaries.

For these reasons they affirmed that the Krogh's model (12) for O_2 transport from blood to tissue, based on a single capillary supplying a tissue cylinder,is not applicable to a contracted muscle.

Consequently, they proposed a diffusion model of a cylindrical muscle fiber supplied by a uniform peripheral lining of capillaries.

As stated before, our observations, performed in naturally contracted muscles, showed extremely different patterns of capillary networks in tibialis anterior and soleus muscles. For this reason, we have checked the variations of these patterns by studying glycolytic and oxidative muscles in a state of passive full extension and passive full shortening.

We studied both tibialis anterior and soleus muscles since they are well typified histochemically, ultrastructurally and physiologically and they show different characteristics.

In fact, the tibialis anterior is a fast

twitch muscle. It mainly depends on anaerobic pathways and sustains less prolonged work load than the soleus muscle.

On the contrary, the soleus is a slow twitch, mainly oxidative muscle (3).

Materials and Methods

Male adult Wistar rats, weighing about 150-200 g., were anesthetized with an ether oxygen blend.

The aorta and inferior vena cava were prepared and a catheter 0.6 mm in diameter was introduced into the artery almost up to the iliac fork and fixed by means of two "0" silk ligatures.

The vascular bed was rinsed using heparinized 0.9% saline solution with 1% carbocaine. At the same time, the inferior vena cava was opened in order to allow the outflow of perfusing fluids.

At this point, when absolutely no phenomenon connected with rigor mortis was present, the right posterior foot and leg were bent while the opposite foot and leg were extended. This position was maintained during every other further phase of the experiment. Successively, a 1% solution of glutaraldehyde in 0.1M cacodylate buffer at pH 7.3 was perfused for 1 min.,in order to fix the endothelium.

Mercox CL2R resin mixed with a standard amount of its catalyzer, was injected at room temperature until complete filling of the vascular bed. Time was left for polymerization. Then tibialis anterior and soleus muscles were excised.

The specimens were macerated in a 15% NaOH solution at room temperature for 24h. The samples were rinsed in distilled water and then in 5% trichloroacetic acid solution in order to free the casts from tissue remnants. Finally, they were frozen in distilled water and freeze dried.

The samples were dissected out and small pieces, 5 mm large, were obtained. The specimens were fixed to stubs, covered with gold in an Edwards Sputterer and observed by a Cambridge 150 SEM, operating at 15-20 kV.

Observations were performed at different levels and depths in the muscle belly.

Results and Discussion

Both in tibialis anterior and soleus muscles primary and secondary arterioles are evident (Fig.1). In turn, their 10 micron wide branches give off capillaries, after a dichotomous division (Fig. 2).

The capillary networks show the features described before, both in the extended and in shortened muscles.

In the extended tibialis anterior, parallel capillaries with a rarely tortuous trend are present (Fig. 3). Straight transverse anastomoses of different lengths were also observed. At their extremities, these trunks often show imprints due to the presence of pericytes.

In the shortened tibialis anterior, the arrangement of capillary network is almost the same as in the extended muscle. The only difference is in the fact that all the capillaries show a certain degree of tortuosity (Fig. 4).

In the extended soleus muscle the capillary network appeared almost identical to what we observed in our previous studies (Fig. 5).

The main capillary showed a lower degree of tortuosity. On the other hand, the complete structure of the capillary cage is readily recognized, although the space between two transverse anastomoses is slightly increased and the distance between two main capillaries is reduced.

In the shortened soleus we saw the typical arrangement of the capillary network as described before, with the same degree of tortuosity as in the naturally contracted muscle (Fig. 6).

In no case did capillary network in one type of muscle show the peculiar features of the other type.

Our observation showed that, although a difference exists between capillary network patterns in extended and shortened muscles, the main features of tibialis anterior and soleus microcirculation remain constantly evident.

It must be pointed out that observations performed at different levels and depths along the muscle belly lead to the same conclusion.

These data confirm the theory that between glycolytic and oxidative muscle microcirculation there is a permanent endogenous difference determined by muscle fiber metabolism.

Such a difference is neither exclusively nor primarily restricted to the degree of tortuosity of capillaries. As we showed in soleus muscle, long loops and tortuous transverse interconnections, connected with longitudinally arranged main capillaries, allow an extensive encasing of the muscle fibers either in a relaxed or in a contracted position.

For these reasons we cannot accept the general rule that in any muscle exists, during relaxation, a straight arrangement of capillaries, as opposed to a downright compact clustering of capillaries and their anastomoses around the contracted muscle fibers. Moreover, we believe that, although the metabolism of muscle fibers determines a typical permanent structure of the microvascular network, its range of variation in compactness is due to other factors: the shape, the

length, the cross section and the type of origin and insertion of the muscle belly.

The concurrence of these factors could explain the enormous difference in the compactness of capillary network in the shortened and in the extended gastrocnemius as found by Potter and Groom (17). However, as the gastrocnemius muscle does not have a uniform population of fibers, especially if its medial and lateral heads are compared, a special care should be taken in evaluating the results obtained. In fact, artifacts could occur by a sampling from not precisely defined areas of the muscle belly.

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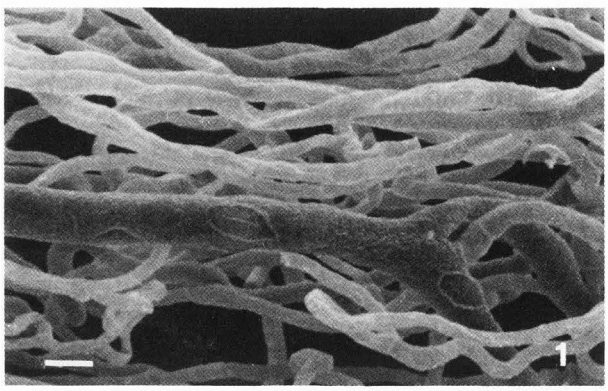

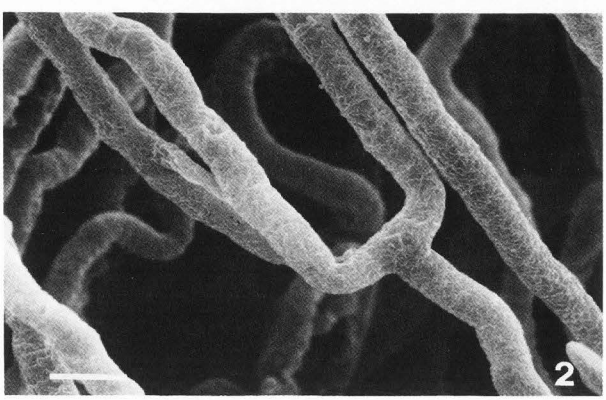

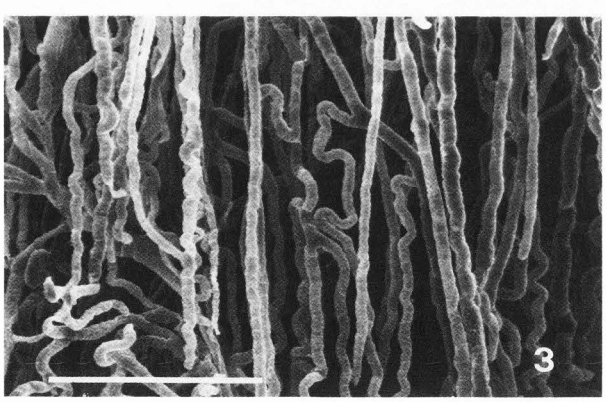

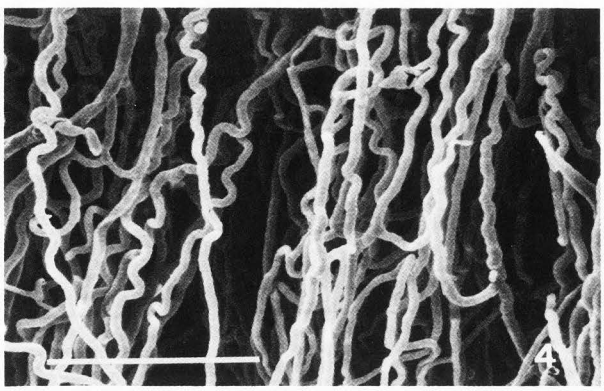

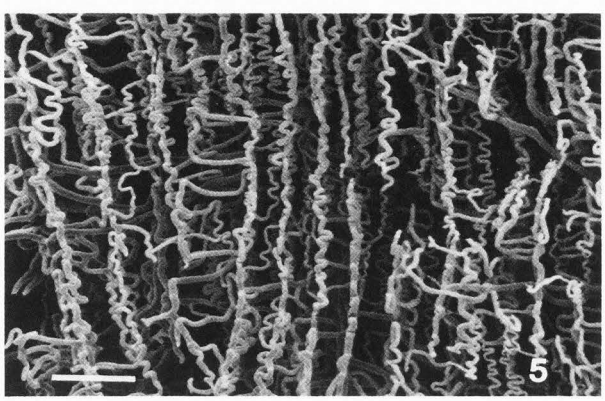

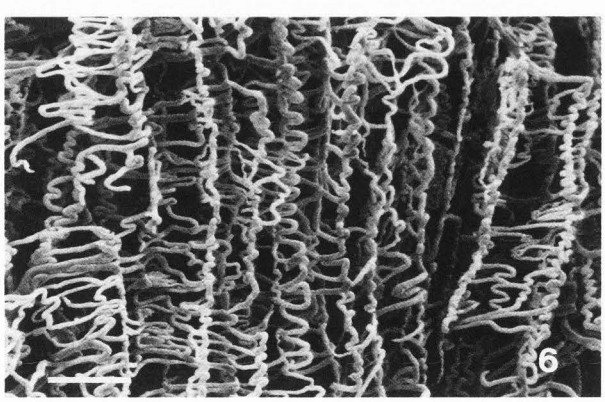

Fig. 1: Extended tibialis anterior. A secondary arteriole surrounded by straight capillaries is evident; Bar = 10 μm.

Fig. 2: Extended tibialis anterior. Capillary tuning fork division; Bar = 10 μm.

Fig. 3: Extended tibialis anterior.Capillary vessels showing a mostly straight trend;Bar = 100 μm.

Fig. 4: Shortened tibialis anterior. Capillary vessels showing a tortuous trend; Bar = 100 μm.

Fig. 5: Extended soleus. Notice the capillary cage structure. Longitudinal main capillaries show a low degree of tortuosity; Bar = 100 μm.

Fig. 6: Shortened soleus. Notice more tortuous main capillaries. Bar = 100 μm.

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Discussion with Reviewers

A. Castenholz: Did you also observe in casts of the muscular vascular system those structures described by us as "plastic strips" (Scanning Electron Microsc. 1983;I:161-170. Mikroskopie - Wien-39:95-106, 1982), which imitate the pericytes and smooth muscle cells in the microvascular area as positive figures ?
Authors: With this particular technique we have never been able to observe any "plastic strips".

A. Castenholz: Is there any difference between the type of muscle you examined in this study and shortened and stretched muscles you described in previous studies with respect to the commonly narrowed sphincter zone of arteriolar casts ?
Authors: We observed that passive shortening and stretching of the muscle belly does not change the diameter of the sphincter zones of arteriolar casts, when compared with what we observed in spontaneously contracted muscles.

H.D. Geissinger: Are the oxidative fibers in either soleus or tibialis anterior muscles surrounded by tortuous capillaries and glycolytic fibers by "straight" capillaries, or the other way around ? Is it possible to prove either concept definitely by experiment ?
Authors: No consistent correlation has yet been found between type of muscle fiber and its localization within the muscle belly of these two particular muscles. For this reason we correlate the various patterns of microvascular bed with the average metabolism of the muscle. We believe that a positive direct correlation between muscle fiber types and patterns of capillary beds could be checked by the same technique we employed. But one should take into consideration a muscle characterized by a constant, definite distribution of muscle fibers.

H.D. Geissinger: What does the blood supply to intermediate fibers look like ?
Authors: Their blood supply looks like what we described in the soleus muscle, according to the definition of "intermediate fibers" also accepted by other authors (see Sillau AH, Banchero N. Pleugers Archiv 370:227-232, 1977).