Arteriolar Control of Capillary Cell Flow in Striated Muscle
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This study tests the hypothesis that capillary perfusion is controlled in groups rather than at the level of the individual capillary. We measured cell flux (using cells labeled with substituted tetramethyl rhodamine isothiocyanate, XRTTC) and vessel diameter in adjoining arterioles of the terminal vasculature of hamster cremaster muscle (Nembutal, 70 mg/kg i.p.) during rest and hyperemia (10^{-4} M adenosine). In terminal arterioles (TAs), 32 of 68 vessels showed cell flux increases from rest to hyperemia exceeding 25 times (i.e., 47% of TAs were relatively unperfused at rest). In vessels feeding TAs (TAFs), 33 of 95 (34%) were relatively unperfused at rest. Cell flux heterogeneity in TAFs decreased significantly by 27% from rest to hyperemia; the corresponding decrease (16%) in TAs was not significant. Thus, unperfused TAFs are present in a proportion which reflects capillary recruitment in hamster cremaster (Sarelius et al., Am J Physiol 1981;241:H317) while TAs are not, and TAFs independently modulate flow distribution distally while TAs do not. The data therefore support the conclusion that TAFs control cell flow in the distal microvasculature. Analysis of normalized ranked maximal diameters showed that TAFs unperfused at rest tend to be the smaller vessels at any tissue site. (Circulation Research 1989;64:112-120)

Sinc the time of Krogh, the concept of the capillary as the smallest independently controlled unit of capillary perfusion has been widely accepted.

However, recent measurements of cell flux in capillaries arising from a common terminal arteriole have shown that, within these groups of capillaries, recruitment does not occur. In these experiments, if a feeding terminal arteriole had flow, all the capillaries arising from it also had flow. During hyperemia, the rate of perfusion through the capillary network increased, but no new flow paths were recruited. This is an apparent discrepancy with earlier data in which capillary recruitment was observed across the entire cremaster muscle. These observations suggest that capillary recruitment might be coordinated in groups and thus support the concept of “functional precapillary sphincters”—a hypothesized behavior of the terminal vasculature.

Therefore, the current investigation is focused on the possibility that capillary perfusion is coordinated in groups. Anatomically, capillaries have been shown to occur in modular, overlapping networks, and recent observations made by Slaaf et al suggest that the perfusion of large numbers of capillaries arising from a common feed might be coordinated. Given the occurrence of capillary recruitment and the potential for capillaries to be arranged and perfused in a modular fashion, we hypothesized that primary control of capillary blood flow is achieved proximal to true capillaries by the coordinated behavior of the arteriolar microvasculature. We measured erythrocyte flux and vessel diameter in terminal arterioles and in the group of vessels from which terminal arterioles arise. The study was designed to determine whether either of these arteriolar groups played a significant role in control of capillary blood flow and to determine if the controlling vessels could be identified by appropriate anatomical correlates.

Materials and Methods

Preparation
Male golden hamsters (Charles River, Wilmington, Massachusetts) (85–100 days old) were used. Each animal was anesthetized with sodium pentobarbital (Nembutal, Abbott Laboratories; 70 mg/kg i.p.). The trachea was intubated to insure a patent airway. The right jugular vein was cannulated for the injection of fluorescent erythrocytes; arterial blood pressure was monitored via a left femoral arterial catheter. Saline containing 10 mg/ml sodium pentobarbital was infused (0.56 ml/hr) via a left...
femoral venous catheter to maintain anesthesia and compensate for respiratory and renal fluid losses. Deep body temperature was maintained at 37–38° C by a thermostated warming coil. Arterial pressure was recorded with a Statham P23Gb pressure transducer and Gould Brush 220 pen recorder (Cleveland, Ohio).

The right cremaster was prepared and superfused for in vivo microscopy as described elsewhere.3,4 During dissection, care was taken to cut and lay out the muscle in a manner that presented the same general arteriolar network geometry. Occasionally, anatomical variations necessitated cutting one of the large arcade vessels; this did not result in a distinguishable difference in the data. The tissue was allowed to stabilize for 1 hour before data were taken.

The overall state of each muscle preparation was assessed before the start of each experimental protocol. The presence of vasoactive tone was confirmed in two to three randomly selected arterioles in all preparations by observation of brisk dilation in response to local application of 10^{-4} M adenosine; oxygen sensitivity was demonstrated by observation of vasoconstriction in response to transient equilibration of the suffusate with gas containing 10% oxygen. In the studies of terminal arterioles, the extensive testing of vessel reactivity to adenosine and norepinephrine, which was necessary to identify the terminal arterioles (see Vessel Nomenclature), did not allow time for confirmation of oxygen sensitivity. These vessels were responsive to 10^{-7} M norepinephrine, and the oxygen sensitivity of the arterioles that fed them (as well as that of some observed terminal arterioles) was confirmed in subsequent experiments.

Spontaneous vasomotion of significant frequency and amplitude is not commonly observed in these cremaster preparations. When vasomotion was evident, it was of relatively small amplitude (approximately 10–20% of peak diameter) and had a periodicity of 5–10 seconds.

Erythrocytes from a separate donor animal were labeled with substituted tetramethyl rhodamine isothiocyanate (XRITC) as described previously.3,9 Labeled erythrocytes were used at circulating fractions of 0.81±0.04% for terminal arteriolar observations and 0.089±0.004% for observations of terminal arteriolar feed vessels; the fraction was measured precisely by flow cytometry of toe clip blood obtained at the conclusion of each experiment.10 Labeled cell normalcy was confirmed as previously described.3,9

Vessel Nomenclature

Arterioles were identified using both topologically and functionally based descriptors. Topologically defined vessels included the main tissue feed arterioles, the arcade arterioles, and the transverse arterioles (Figure 1). These vessels were identified according to the connections at their proximal ends. The tissue feed arterioles, such as the central feed arteriole (Figure 1), arose directly from the arterial feed to the tissue. The vessels that bifurcated from the tissue feed arterioles, and that generally anastomosed amongst themselves, were designated arcade arterioles (Figure 1). A small fraction of these vessels were not seen to form true arcades but interdigitated with similar vessels and occupied the same general geometric location as arcades in other preparations. Transverse arterioles, by definition, arose directly from the arcade or arcade-type vessels.
Functionally defined vessels included terminal arterioles (TAs) and terminal arteriole feed vessels (TAFs) and were identified according to their distal connections. A TA was defined as the most distal arteriolar segment to respond actively to topical application of $10^{-4}$ M adenosine and/or $10^{-3}$ M norepinephrine. A segment was defined as the section of vessel between two bifurcations. Infrequently, a segment was active for only about 30 $\mu$m from its origin and then appeared to be a true capillary. These segments were not included as TAs.

Two corollaries of this definition of a terminal arteriole are 1) vessels bifurcating from a terminal arteriole are capillaries, and 2) if both vessels arising from the bifurcation of a segment with an active vasomotor response are themselves capable of an active response, then the parent vessel is not a terminal arteriole.

Some of the terminal arterioles identified according to this definition are marked by asterisks in Figure 1 to clarify the definition of TAFs. A TAF was defined as a vessel that gave rise directly to terminal arterioles (or, occasionally, to a capillary). That is, it had distal to itself only one order of vessel capable of an active vasomotor response. In Figure 1, vessels that were TAFs include 4A, 5A, 3D, 8A, 9A, 7C, and 10A.

It is important to note that a vessel that had been topologically defined as a transverse arteriole may have also been a TA or TAF based on its functional definition. In Figure 1, vessel 15A was both a transverse arteriole and a TA.

**Experimental Protocols**

For each of the protocols described below, experiments were performed on alternate preparations at one of two tissue sites. We chose to focus on two specified sites in order to reduce the contribution of anatomically related variability. Observations of two sites, rather than one, allowed us to evaluate the generality of our findings. The arcade arteriole feeding site I was located just distal to the first major bifurcation of the central feed artery; site II began at the proximal end of the last major arcade arteriole to bifurcate from the central feed arteriole before it passed across the edge of the Plexiglas pedestal.

**Terminal arteriole studies.** In 16 hamsters, site I or site II was chosen for observation in alternate experiments. If the site to be observed in a particular experiment was not available, the other site was recorded. During the initial equilibration of the tissue 0.06 ml of packed XRITC-labeled cells were injected. With a $\times3.5$ objective (n.a., 0.09), the measurement site was recorded while $10^{-4}$ M adenosine was applied topically to enable identification of the vessels closest to the capillaries that dilated. The first branch of the first transverse arteriole of the site that appeared to have four or more TAs arising from it was chosen as the potential measurement site. This site was examined at higher magnification to confirm the identity of the TAs. This was done by recording the site during transient vasodilation with topical $10^{-4}$ M adenosine, making a sketch of the constituent vessels, then identifying each TA of the site by testing for reactivity to adenosine and/or norepinephrine. Testing was initiated approximately 30 minutes after completion of the tissue preparation and continued for up to 45 minutes. The tissue was then allowed to equilibrate for 5 minutes to permit complete washout of the vasoactive agents.

Cell flux and vessel diameter recordings were made on those vessels (up to a maximum of five) that were identified as TAs by the above criteria. All of the TAs recorded in a given experiment were fed by the same TAF. Terminal arterioles were chosen for measurement without regard to the flow rate through them.

With a $\times25$ objective (n.a., 0.60), labeled cell flux was recorded at each vessel for 20–30 seconds. After the completion of flux recording, any vessel with a cumulative labeled cell flux of less than five cells in the 30-second period was recorded again for 20–30 seconds, during transillumination with 436 nm light, to permit determination of cell flux from the entire erythrocyte population. If a vessel had no flow, it was recorded for the full 30 seconds.

Upon completion of the flux recording, the diameters of the TAs were recorded with a $\times55$ objective (n.a., 0.80) while transilluminating with 580 nm light. Subsequently, the flux measurements were repeated. The cell flux values reported are the averages of the two recordings.

When the recording of the resting tissue was completed, $10^{-4}$ M adenosine was added to the superfusate, and erythrocyte flux and vessel diameter were recorded again at the same site. If the labeled cell flux in any TA was too high to resolve, the fluxes in the vessels that it fed were recorded, and the terminal arteriolar flux was calculated as the sum.

**Transverse arteriole studies.** In each of 14 hamsters, after tissue preparation, 0.05 ml of an eight-fold dilution of packed XRITC-labeled cells were injected, and the appropriate measurement site was chosen for observation as in the terminal arteriole experiments. Adenosine ($10^{-4}$ M) was applied topically, and the first five transverse arterioles of the arcade vessel were recorded, including enough vessels downstream of each transverse arteriole to identify a few TAs arising from each. The TAFs that arose from each transverse arteriole were then determined by identifying the TAs that they fed. As with the terminal arteriole experiments, a maximum of 75 minutes postpreparation was allowed for vessel identification. Ten minutes were then allowed for the washout of vasoactive agents before recording was initiated.

Labeled cell flux was recorded for 20–30 seconds, with the $\times25$ objective, in each of the TAFs arising from the transverse arterioles (or in the
transverse arteriole itself, if it was a TAF or TA). Any vessels that had a cumulative flux of fewer than two labeled cells in the 30-second period were recorded again with 436 nm transillumination to allow cell flux to be determined from the total erythrocyte population. If the total cell flux in a vessel was too high, it was recorded in the vessels fed by it and summed. Zero fluxes were recorded for 30 seconds. The diameters of all vessels, down to the level of the TAF, were then recorded with the ×55 objective. Cell flux recording was then repeated, starting at the opposite end of the measurement site. Cell flux and diameter were then recorded during hyperemia as before.

Measurements

Erythrocyte flux (cells/sec) was determined during videotape playback by counting the number of labeled red cells passing a vessel cross-section in a predetermined time interval and dividing by the labeled-cell fraction and time interval. In transilluminated vessels, flux was determined by dividing the total number of cells counted by the counting time interval. Vessel diameter was measured directly off the video monitor during videotape playback, with a videotaped stage micrometer for reference. Accuracy was ±0.5 μm.

There was no significant difference between the cell flux values recorded before or after the recording of vessel diameter (paired t test). This was true for measurements at either tissue site under both experimental conditions, as well as when the data from the two sites were combined.

Estimates of the relative precision of cell flux measurements were obtained. In the terminal arteriole studies, mean cell flux precisions were 9.9% and 4.8% for resting and hyperemic tissues, respectively. The relative precision of the cell flux estimates made in the transverse arteriole experiments was 18.5% for the resting tissue and 8.6% during hyperemia.

Experimental Analyses

These experiments were designed to identify the vessels controlling capillary cell flow. Such vessels should fulfill two criteria. 1) An appropriate proportion of the vessel group should be relatively unperfused at rest (one-third for cremaster muscle, based on observed capillary recruitment). 2) Each of the vessels should be capable of altering capillary cell flow in a manner that is substantially independent of the influence of a more proximal vessel. Relatively unperfused vessels have a large cell flux increment from rest to hyperemia. Cell flux increment was calculated in two ways. First, the increment was calculated as the ratio of hyperemic cell flux to resting cell flux; the cell flux increment among the vessels examined was compared by analyzing the frequency distribution of the cell flux increment data. Second, relative changes in cell flux (and also in diameter) were obtained by expressing the difference between resting and hyperemic values as a fraction of maximum.

Independent control of cell flux within a group of vessels is indicated by heterogeneous flow behavior among vessels fed by the same parent vessel. Heterogeneity of flux was estimated by determining the coefficient of variation (CV) of cell flux for vessels sharing a common parent. Thus, vessels were considered to be independently controlling cell flux if the CV of cell flux among vessels of a common parent decreased significantly from rest to hyperemia. In other words, during rest, when active control of separate capillary groups will be most evident, one might expect to see significant variations in cell flux between individual arterioles arising from a common feed. During passive hyperemia, when active control is abolished, the variability in observed cell flux between the arterioles should decrease.

We defined relatively unperfused arterioles in terms of the capillary flow arising from them. Thus, by using the criterion that the estimated average resting cell flux for "unperfused" capillaries be less than 2 cells/sec and from the average number of capillaries per TA (19 caps/TA) or per TAF (104 caps/TAF), we defined both expected minimal cell flows and minimal cell flux increments from rest to hyperemia (>25 times) as criteria for identifying unperfused vessels.

Data are presented as mean±SEM. Group means were compared with paired or unpaired t tests. Significance was assessed at the 95% confidence level.

Results

Terminal arteriolar cell flux increment data are shown in Figure 2. The data from both measurement sites are shown in panels A and B, respectively. Neither measurement site contains the proportion of perfused and relatively unperfused vessels that would be expected from capillary recruitment data if TAs were the vessels controlling capillary cell flow. Of the total of 68 vessels observed, 36 were relatively unperfused (group mean resting cell flux, 9.3±1.9 [SEM] cells/sec), and 32 were classified as perfused (group mean resting cell flux 195±36.2 cells/sec). The distinction between perfused (low cell flux increment) and unperfused (high cell flux increment) TAs is evident between the two measurement sites rather than within them, with over 80% of the vessels at site 1 being relatively unperfused.

Cell flux increments in TAFs are illustrated in Figure 3. The data show that these vessels fulfill the first criterion for identification of the controllers of capillary perfusion in hamster cremaster because the data from the two sites combined (panel C) show that approximately one-third of the TAFs are relatively unperfused at rest. Each measurement site contains both a population of TAFs that showed a relatively small cell flux increment with hyperemia and a tail to the population distribution representing vessels with increasingly greater cell flux.
Figure 2. Terminal arteriolar cell flux increment (flux_{hyp} / flux_{rest}), where flux is in cells/sec. Observations were made at two tissue sites (see text). Panel A, Site I; panel B, Site II.

Figure 3. Terminal arteriole feed vessel cell flux increment (as in Figure 2). Panel A, Site I; panel B, Site II; panel C, both the sampled tissue sites combined.
cell flux in each functional group of vessels (e.g., all TAs) to the average flux in its feed vessel. We determined the average number of vessels with flow that originated from a given vessel by dividing the average parent vessel flux by the average flux of the distal vessels. It was assumed that all vessels had flow during hyperemia, and therefore the flow quotient obtained from the hyperemia data reflected the anatomical number of vessels arising from a particular parent vessel type. A similar quotient in the resting tissue would indicate that, on average, the anatomically defined number of vessels was perfused by a given parent vessel. A reduced quotient would suggest that some of the downstream vessels were shut off, reflecting independent control among these vessels.

The combined data from the two sites showed that the number of perfused TAs per TAF remained constant from hyperemia to rest, being 5.6 and 5.7 TAs per TAF, respectively. Thus, the results of this analysis indicate that TAs do not control capillary cell flux independently of TAFs because the number of perfused TAs remained unchanged.

The cell flux and diameter data for perfused and unperfused TAFs are given in Table 2. The unperfused TAFs included vessels from both measurement sites and represented 12 of the 14 experiments. These vessels had an average resting cell flux of 104±25 cells/sec. This was significantly less than the resting cell flux in the perfused TAF group, which averaged 827±106 cells/sec. Nevertheless, the maximum cell flux (during hyperemia) through the relatively unperfused TAFs was not significantly different from that through the perfused TAF group; it averaged 6,640±880 and 7,080±720 cells/sec, respectively.

Unperfused TAFs were found at both measurement sites, although more were present at site I (n=23) than at the site II (n=9). Overall, the perfused TAFs did not occupy any particular regions of the arteriolar network. They represented TAFs that were themselves transverse arterioles, as well as some that were side branches and terminal bifurcations of transverse arterioles. In two experiments, both from site I, all of the TAFs that were observed were unperfused.

The unperfused TAFs had a significantly smaller average resting diameter (4.6±0.6 μm) and significantly greater average normalized diameter increment (78.7±2.3 μm) than the perfused TAF group, whose values averaged 7.6±0.4 and 65.5±1.7 μm, respectively. The mean maximum diameter for the unperfused group (20.4±1.1 μm) appeared to be systematically less than that of the perfused TAFs (22.3±0.8 μm), but the difference was not statistically significant.

The distribution of diameters between perfused and unperfused TAFs was analyzed for individual tissue sites by ranking the vessels relative to the maximum diameter (D_max). These were then averaged across all experiments. The average minimum and maximum possible percentile rank was also calculated. The results show that, with a minimum possible average percentile of 29.2±4.3% and a maximum of 85.5±4.2%, the average percentile of D_max of the unperfused vessels was 45.5±6.0%, which was significantly different from both the minimum (p<0.05) and maximum (p<0.001). This

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<th>Table 1. Cell Flux Heterogeneity</th>
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<td>Terminal arterioles</td>
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<td>Site I</td>
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<tr>
<td>CV(%)</td>
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<tr>
<td>59±22</td>
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<td>(10)</td>
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<tr>
<td>Site II</td>
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<td>CV(%)</td>
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<td>91±12</td>
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<td>(8)</td>
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<tr>
<td>Combined sites</td>
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<tr>
<td>CV(%)</td>
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<td>73±14</td>
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<td>(18)</td>
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CV, coefficient of variation; hyp, hyperemia.
*Number of observations. Data are given as mean±SEM.
*Significantly different from rest to hyperemia.

<table>
<thead>
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<th>Table 2. Terminal Arteriolar Feed Vessel Cell Flows and Diameters</th>
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<tr>
<td>Cell flux (cells/sec)</td>
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<tr>
<td>Hyperemia</td>
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<tr>
<td>Rest</td>
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<tr>
<td>Relative unperfused vessels</td>
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<tr>
<td>104±25*</td>
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<tr>
<td>(32)</td>
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<td>Perfused vessels</td>
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<td>827±106</td>
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<td>(63)</td>
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Diameter (μm)
Hyperemia                    |
Rest                         |
Relative unperfused vessels  | 78.7±2.3* |
4.6±0.6*                     | 20.4±1.1  |
(32)                         | (32)     |
Perfused vessels             | 65.5±1.7 |
7.6±0.4                      | 22.3±0.8 |
(63)                         | (63)     |

*Normalized cell flux or diameter increment; \( \frac{X_{\text{HYP}} - X_{\text{REST}}}{X_{\text{REST}}} \times 100 \), where X is equal to cell flux or diameter.
*Number of observations. Data are given as mean±SEM.
*Significantly different from perfused vessel group.
shows that while the mean $D_{\text{max}}$ of the unperfused TAFs was not significantly different from that of the perfused TAFs, in terms of ranking within the group, unperfused TAFs tended to be the smaller (though not always the smallest) TAFs at a given tissue site.

Discussion

Control of Capillary Cell Flow

This study supports the hypothesis that the terminal arteriole feed vessels (TAFs) are the primary controllers of capillary erythrocyte flow in hamster cremaster muscle. That is, these vessels appear to be the ones that most directly determine whether a specified group of capillaries is perfused in the resting tissue. We found that approximately one-third of the TAFs across the tissue were relatively unperfused at rest, which reflected the ratio of unperfused to perfused capillaries found previously by Sarelius et al. in the same tissue. Further, analysis of the heterogeneity of cell flux changes showed that TAFs arising from the same parent vessel were each capable of independently controlling cell perfusion within the more distal ramifications of the arteriolar network. Terminal arterioles did not appear to be regulating cell flux independently of their TAF, nor were perfused and unperfused TAs present in the appropriate ratios. They therefore could not be the primary determinants of capillary perfusion, although our data do not rule out a significant role for TAs in distributing flow within the unit primarily controlled by a TAF. Furthermore, control of capillary flow by TAFs does not preclude a mechanism by which TAFs and the cell flux through them are influenced by higher levels of similarly organized control or by more global control mechanisms, such as neurally mediated changes in peripheral resistance.

Several investigators have suggested that capillary cell flow is controlled proximal to the capillary networks. Honig et al. inferred from the spatial distribution of erythrocyte-filled capillaries in dog gracilis muscle that capillary flow was controlled by small arterioles. Gorczynski et al. found no evidence for precapillary sphincters in hamster cremaster that could control the perfusion of individual capillaries and suggested that control of capillary erythrocyte flow might reside in the terminal arterioles, although these vessels were not rigorously defined.

Lindbom and Arfors suggested that, in rabbit tenuissimus, the terminal arterioles ultimately control capillary flow, in that they respond more sensitively than transverse arterioles to various stimuli (e.g., raised superfusate $P_O_2$). While Lindbom and Arfors did not discriminate between the different vasoactive segments of terminal arterioles, Eriksson and Lisander showed in the cat tenuissimus that the terminal arteriole, as both they and Lindbom and Arfors defined it, was not the last vaso-

active segment before the capillaries. The tenuissimus terminal arteriole that they described appears to be analogous to the TAFs defined in the present work. Slaaf et al. concluded that the vasomotion cycle of rabbit tenuissimus terminal arterioles (which they call first order side branches, FOS) dominated the flow pattern in the capillaries to which they ultimately gave rise. The FOS of Slaaf et al. appear analogous to the terminal arterioles of Lindbom and Arfors, and, hence, to our TAFs. An important conclusion from consideration of our data and the above-cited work is that, in two different muscles and three species, capillary flow control can be related to the same group of vessels, independent of the varying degrees of cyclic vasomotion in these preparations.

Sarelius, studying hamster cremaster muscle, and Lund et al. working with hamster tibialis anterior muscle, both inferred that capillary flow might be organized in groups under the control of terminal arterioles (defined as in the present study), but their observations were also consistent with control by some other precapillary vessel group, not necessarily TAs. Finally, Delashaw and Duling suggested, based on observations of groups of hamster tibialis anterior capillaries made during perfusion with fluorescent albumin, that the site of control of capillary perfusion was either the capillary feeding arteriole or some more proximal vessel.

The Pathway for Flow

This study has shown, through direct observation of erythrocyte flux, that TAFs are the most distal vessels which actively control cell flow into capillary networks. It is clear that the microvascular groups under the control of TAFs are not strictly "on" (all capillaries flowing during normal resting rates) or "off" (completely unperfused). Rather, hemodynamics and microvascular architecture appear to modulate perfusion. In resting cremaster, cells tend to flow predominantly down central pathways, bypassing side branches (authors' unpublished observations), presumably due to hemodynamic considerations. As flow within a group of vessels decreases, sidebranching capillaries may become unperfused passively. A central pathway also has been documented for leukocyte flow through the cremaster by Blixt et al. In addition, Lindbom and Arfors showed that when central arterial pressure was reduced below 50 mm Hg, the number of perfused capillaries arising from a single branch of terminal arteriole was reduced, although the lowest cell velocity they recorded in a capillary regarded as flowing is over 100 $\mu$m/sec, which is the average resting cell velocity measured in hamster cremaster in this laboratory. This highlights the important question of what should be regarded as flowing or nonflowing.

Parenthetically, note that the relative cell flux increase measured in the arterioles observed in the current experiments does not indicate that total
tissue cell flow increased by this magnitude. Preliminary experiments showed that cell flux increased only twofold to fourfold from rest to hyperemia (10⁻⁴ M adenosine) in the arcade arterioles of the network. These vessels appear to be central pathways for flow on the whole-tissue level. The relative increase in flow across the whole tissue is likely to be intermediate to what was measured in arcade arterioles and the more distal vessels.

**Individual Coordination of Capillary Groups**

Analysis of the maximum diameters of TAFs showed that it was generally the smaller TAFs of a given tissue site that were unperfused at rest. This is consistent with a hypothesis that the closure of vessels in the resting tissue is mediated by vascular smooth muscle mechanics. Greensmith and Duling and Sleek and Duling showed that the vascular smooth muscle (VSM) of an arteriole becomes convoluted as the arteriole constricts. When this occurs, a smaller percentage of the VSM myofilaments are in an orientation which can support vessel tangential stress and generate the force necessary for constriction. As the VSM of the smaller TAFs may be in a more favorable geometry to support wall stress, these vessels may react with greater sensitivity to a common vasoconstrictor signal and may be more likely than larger TAFs to stop or severely reduce perfusion. If true, this would represent an architectural design of the microvasculature which allows for differential perfusion of vessels based on their anatomical size and permits a degree of control over perfusion distribution based on the geometrical arrangement of large and small vessels.

Lindbom and Arfors suggested that reduced capillary flow is brought about by a uniform increase in the resistance of all terminal arterioles, rather than by the closure of particular vessels. This is in contrast to the data presented here, which showed that some TAFs are relatively unperfused at rest. This may indicate tissue-specific differences. However, Lindbom and Arfors did not measure cell flux in terminal arterioles, but only in capillaries. Furthermore, it is clear that they did not observe directly the terminal arterioles that supplied the capillaries whose cell flux was measured. Their inference is therefore likely to be based on the assumption that uniformity of capillary flow reflects uniformity of terminal arteriole perfusion. This supposes a particular arrangement among capillaries arising from different terminal arterioles (e.g., there is no overlap and so capillary flow at a particular tissue location is indicative of the flow in the most adjacent terminal arteriole). The data of Lund et al indicate that there is a considerable amount of overlap among capillaries arising from different terminal arterioles. Discrete control of flow within groups of capillaries may not be discernible unless capillary network organization is considered in the analysis of flow control. Therefore, an alternative explanation for the observations of Lindbom and Arfors is that tenueissimus terminal arteriolar cell flux may be as heterogeneous as the TAF cell flux that is reported here for cremaster, but that this heterogeneity could not be resolved, due to experimental design.

**Consequences for Understanding Blood-Tissue Exchange**

Krogh suggested that the capillary was the basic unit for exchange of oxygen. He hypothesized that control of capillary perfusion lay at the level of the individual capillary and that a portion of the capillaries within the tissue were constricted and thus unperfused in resting muscle; release of constriction increased the density of perfused capillaries during periods of hyperemia. Our data suggest that this is not the case. Rather, the data indicate that capillary perfusion is coordinated by the vessels immediately preceding the terminal arterioles. This organization might in turn lead to significant nonuniformities in tissue perfusion. While temporal heterogeneity in blood-tissue exchange capacity will clearly be influenced by such mechanisms as capillary intermittency or vasomotion, our data highlight the possibility of spatial nonuniformity. We hypothesize that this could be minimized by architecturally related mechanisms. For example, individual capillary networks might be arranged so that perfused and unperfused networks overlap. This could be achieved by an appropriate spatial distribution of TAFs, where differences in TAF function might in turn be due to differences in maximum diameter (and hence, behavior) or to differences in sensitivity. It will be important in future studies to test such ideas as these, to determine the extent of perfusion heterogeneity and the basis for its control.

**Acknowledgment**

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