Augmented Tissue Oxygen Supply during Striated Muscle Contraction in the Hamster

Relative Contributions of Capillary Recruitment, Functional Dilation, and Reduced Tissue PO₂

Bruce Klitzman, David N. Damon, Richard J. Gorczynski, and Brian R. Duling
From the Department of Physiology, School of Medicine, University of Virginia, Charlottesville, Virginia

SUMMARY. To investigate the relative contributions of alterations in blood flow, capillary density, and tissue PO₂ to elevated oxygen delivery in working muscle, we conducted experiments on the suffused hamster cremaster muscle, using in vivo microscopic techniques. Muscle PO₂ was measured during striated muscle twitch contraction at 1 Hz. Tissue oxygenation was changed by using suffusion solutions equilibrated with 0%, 5%, 10%, 21%, or 50% oxygen. Contraction caused an increase in capillary density (capillary recruitment), whose magnitude was related to the equilibration gas and, thus, to the suffusate PO₂. Capillary recruitment first increased as the oxygen content was raised, peaked with 10% oxygen, and then diminished with higher oxygen content. Arteriolar functional dilation was also observed; when oxygen was raised above 21%, dilation was decreased. The data suggest that oxygen supply is increased primarily by arteriolar conductance changes with low suffusion solution oxygen (0% to 5%), and by capillary recruitment and increased PO₂ gradients above 10% oxygen. When vasomotor tone was increased by addition of norepinephrine to the suffusion medium, the changes observed were similar to those observed when oxygen was increased. Therefore, we propose that the altered microvascular responses during vasoconstriction are a function of vascular tone rather than the levels of tissue PO₂. A model is proposed which may partially explain the relations among vascular tone, functional dilation, and capillary recruitment. Our data also suggest that tissue PO₂ may not be precisely regulated about a narrowly defined set point in this striated muscle but that, instead, tissue PO₂ is a dependent variable controlled by the integrated effects of capillary recruitment, functional vasodilation, and altered metabolism. (Circ Res 51: 711-721, 1982)

THE oxygen consumption of working striated muscle is greater than that of resting muscle, and tissue oxygen delivery is usually augmented to match the increase in oxygen demand that occurs in the working muscle. The coordination of vascular oxygen delivery and tissue oxygen consumption is not fully understood, but at least three factors are known to contribute to the increased ability of the microcirculation to supply oxygen to working muscle. These are: arteriolar dilation, which increases flow; capillary recruitment, which reduces diffusion distances within the tissue; and decreased tissue PO₂, which steepens oxygen diffusion gradients (Krogh, 1918; Gray et al., 1967; Granger et al., 1976; Gorczynski and Duling, 1978; Gorczynski et al., 1978; Honig et al., 1980).

The relative contributions of these elements have been reported to vary with different experimental manipulations, such as sympathetic stimulation (Renkin and Rosell, 1962; Lewis and Mellander, 1962), application of humoral agents (Jarhult, 1971; Altura, 1973; Vetterlein and Schmidt, 1976; Katz, 1978) and muscle contraction (Granger et al., 1976; Honig et al., 1980). Also, relative effects on flow and capillary exchange capacity during exercise may vary with the level of muscular work. Renkin et al. (1966) and Appelgren (1972) have reported that, with progressive increases in muscle work, capillary exchange capacity may reach a maximum and then begin to decline while conductance continues to increase. Capillary recruitment may also follow a much different time course than does the flow increase in a working muscle (Honig et al., 1980). In addition, it is reported that the level of tissue oxygenation may influence the vasomotor response. With a low venous PO₂, oxygen supply increments depend primarily on increased blood flow. In contrast, with high venous PO₂, augmentation of oxygen supply is accomplished to a larger extent by capillary recruitment and a decrease in the diffusion distance, accompanied by an increase in oxygen extraction from the blood (Granger et al., 1976).

Differential responses of arterioles and capillaries are not easy to reconcile with the anatomy of the microcirculation of striated muscle since direct microscopic examination of the origins of the capillaries has failed to disclose any form of precapillary sphincters which might permit independent control of arterioles and capillaries (Wiedeman et al., 1976). Rather, in this tissue, control of capillary patency and flow seems to reside either in the terminal arterioles (Er-
iksson and Myrhage, 1972; Gorczynski et al., 1978) or, possibly to a lesser extent, in the postcapillary vasculature (Klitzman and Johnson, 1982).

Examination of pressure distributions in the microcirculation leads us to the conclusion that the vascular resistance is probably distributed over the entire vascular bed, including the terminal arterioles and large vessels (Fronek and Zweifach, 1975; Bohlen et al., 1977). One must therefore ask how it is possible to exert differential control of capillaries and arterioles when the effector for such control is to some extent the same for both microcirculatory elements—that is, the smooth muscle of the terminal arterioles.

Those data which appear to show differential control of flow and capillary density have been derived from indirect methods for assessing capillary density: measurement of fluid filtration (Granger et al., 1976), measurement of small molecular weight solute extraction by the capillaries (Renkin et al., 1966), or histological presence of red cells in the capillaries (Honig et al., 1980). We examined some of these relations in a superfused microcirculatory preparation.

In vivo microscopic examination of transilluminated muscle provides a tool for directly observing the postulated interactions between capillary recruitment and arteriolar dilation in effecting the delivery of oxygen to the muscle. Furthermore, net tissue oxygen delivery can be manipulated as an independent variable in a microcirculatory preparation by altering the concentration of oxygen in a suffusion solution. In addition, tissue PO2 may be measured at precisely defined locations to determine the net effects of altered supply and demand. The purposes of this investigation were to determine the relationships between capillary and arteriolar responses during muscle contraction and to determine the contribution of tissue PO2 changes to oxygen delivery. Our findings led us to propose a physical mechanism for the interaction between arteriolar diameter and capillary recruitment and to suggest that tissue PO2 is not a precisely regulated variable.

Methods

Animals and Perfusion Solutions

Male golden hamsters (100 to 150 g) were anesthetized with an i.p. injection of sodium pentobarbital (70 mg/kg). Deep esophageal temperature was maintained at 38°C by conductive heating. Tracheal and femoral venous catheters were inserted. Supplemental normal saline containing 9.0 mg/ml sodium pentobarbital was infused at 0.49 ml/hr, which maintained a stable level of anesthesia and hydration. Animals prepared in this fashion display stable cardiovascular and microcirculatory function for several hours, as evidenced by stable microvessel tone and reactivity, as well as stable femoral arterial blood pressure.

The cremaster muscle was prepared as described by Gorczynski et al. (1978). It was pinned as a flat sheet on a Lucite pedestal and suffused (approximately 5 ml/min) with bicarbonate-buffered physiological saline with the following composition (mM): NaCl, 131.9; KCl, 4.7; CaCl2, 2.0; MgSO4, 1.2; and NaHCO3, 20.0, at pH 7.35–7.40. The saline was equilibrated with gas nominally containing 5% CO2, various percentages of O2, and the balance N2.

The muscle was transilluminated using light from a xenon arc lamp, monochromatically filtered at 436 nm and viewed through a Leitz Laborlux II microscope equipped with a Cohu television camera, Conrac video monitor, and Sony EV 3650 videotape recorder. A time reference, generated by an Odetics digital timer accurate to 0.01 sec, was recorded on each video field. The magnification of the image was 1180X from tissue to TV monitor screen.

Oxygen tensions of solutions and tissues were measured with recessed-tip oxygen microelectrodes (Whalen et al., 1967) with outside tip diameters of 2–5 μm. For suffusate PO2 measurements, the oxygen microelectrode was positioned in the solution 0.3 to 1.5 mm above the muscle. For muscle PO2 measurements, the oxygen microelectrode was positioned equidistant from the venous end of a pair of capillaries, 50 to 150 μm below the muscle surface, a region previously shown to reflect the minimum tissue PO2 (Gorczynski and Duling, 1978). Electrodes were calibrated against N2 and room air at 34°C and measurements were discarded if the calibrations before and after an experimental series differed by more than 10%. Tissue PO2 was also measured several minutes after the circulation was arrested to obtain a minimum PO2 of the superfused muscle as an internal check on the zero reference for the oxygen electrode.

Muscle Stimulation

Phasic contractions of the striated muscle were used to increase tissue oxygen demand. Contraction of the muscle for 1 minute was induced by square wave pulses 0.02–0.05 msec in duration, at 6–18 V, and 1 Hz. These pulses were applied across two Ag-AgCl macroelectrodes; the cathode was positioned across the narrow proximal portion of the cremaster, and the anode encircled the distal portion. The amplitude of the stimulus voltage was adjusted to produce twitch contractions yielding maximal displacement of the tissue. In most experiments, the stimulation voltage was increased 20% above the level producing maximal contraction.

Kjellmer (1965) has shown that motor nerves may be stimulated at intensities well below threshold for vasconstrictor fibers. However, more recently, vascular responses related to stimulation of autonomic nerves have been reported by Hirst (1977), but not when low frequency (1 Hz) electric field stimulation was used. Thus, we assume that autonomic neurons were not likely to have been stimulated.

In a few experiments, small isolated groups of muscle fibers were stimulated using 3 to 5 NaCl-filled micropipettes to deliver electrical pulses directly to the muscle fibers (0.1 to 1.0 msec at 4 Hz) as described previously (Gorczynski et al., 1978).

Measurement of Capillary Density

Capillary density was estimated by counting the number of capillaries containing flowing red cells which intersected a 250-μm long reference line on the TV monitor. Fields for observation of capillaries were not confined to any particular portion of the cremaster, with the exception of a band of tissue approximately 2 mm wide at the tissue edge which was rarely observed because of the presence of damaged regions. Within the area studied, regions of obvious damage or regions free of capillary flow were excluded from study.

The reference line was positioned perpendicular to the
skelatal muscle fiber axis and, thus, to most capillaries. Capillary density data were recorded in units of number of capillaries per line (#/250 μm line) and #/mm² as described previously (Klitzman, 1979). Number of capillaries per mm² tissue cross-section was estimated from the depth of field of the optical system and the length of the reference line. The reference line and the depth of field of the objective were assumed to form a plane, normal to the long axis of the capillaries, with the dimensions 0.25 mm × 0.05 mm = 0.0125 mm². Dividing the number of capillaries with red cell flow intersecting this plane by the area of the plane yielded an estimate of capillary density with the units of #/mm². This method should yield data similar to those obtained by sectioning the tissue perpendicular to the capillaries and counting the number of capillaries in the cross-section.

Measurement of the capillary density in contracting muscle was not practical because of excessive tissue movement. Therefore, estimates for the steady state contraction values of capillary density were obtained during the first 10 seconds after stimulation. We observed little change during this brief interval in either the number or the rate of perfusion of capillaries, and therefore assumed steady state values.

Measurement of Arteriolar Diameter

Arteriolar diameters were measured on-line by positioning two parallel lines, generated by a modified Colorado Video Image Analyzer, over the inner walls of the vessels (Gorczynski et al., 1978). An output voltage proportional to the separation of the two lines was recorded. The system was accurate to ± 1 μm. Arteriolar studies included third- and fourth-order vessels, and values reported here are the diameters at the end of 1 minute of stimulation.

Application of Vasoreactive Agents

Since exposure to oxygen induces vasosconstriction, responses observed might have been due to either altered oxygen delivery or to vasoconstriction. To evaluate the specificity of oxygen, we used topical application of norepinephrine (NE) to induce a degree of vasosconstriction equivalent to elevating the susffusion solution oxygen from 0% to one of the higher oxygen content gases. Appropriate NE concentrations were achieved in the susffusion solution by infusing 10⁻⁴ M NE into the line that carried the susffusion solution to the tissue surface. The vasoconstrictor effect of susffusion solutions equilibrated with 5 and 10% oxygen tended to decrease the capillary density significantly in both the resting and contracting muscle.

Functional Dilation of the Arterioles

As nearly as could be ascertained, capillary density increased within 15-30 seconds after the initiation of 1 Hz stimulation and reached a steady state within 45 seconds. Within 15-30 seconds after the stimulus ended, capillary density began to decrease, and it had returned to control after 60-120 seconds.

The capillary densities observed with the different susffuse PO₂ levels during rest and contraction are graphed in Figure 1A. Elevation of the susffuse PO₂ tended to decrease the capillary density significantly in both the resting and contracting muscle.

Figure 1B shows the effect of oxygen on the capillary recruitment; i.e., the difference in capillary density between the contracting and the resting state. An enhanced capillary recruitment during muscle contraction was observed as solution oxygen contents were increased up to 10% oxygen. However, as PO₂ was elevated further, capillary recruitment diminished strikingly.

Maximal capillary density was estimated by adding 10⁻⁴ M adenosine to the 0% oxygen susffuse during 8 Hz twitch contraction. This caused an increase in capillary density to 9.1 ± 0.4/line or 728 ± 33/mm² (ss). Therefore, the capillary densities observed during 1 Hz contraction were submaximal.

Functional Dilation of the Arterioles

Arteriolar diameters during striated muscle contraction are presented in Figure 2. When exposed to
Figure 1. Effects of altered suffusion solution oxygen content on capillary density. Measurements made at rest (O) and during 1 Hz stimulation of the muscle (●) are shown in panel A. Capillary densities in the contracting muscles are significantly different from the resting muscles in all solutions except 50% O₂. The magnitude of the increment in capillary density during the contraction (capillary recruitment) is shown in panel B. At each point, the data represent the difference between the rest and the contraction capillary density. Data points are means ± se; numbers in parentheses are numbers of observations. The maximum capillary density is shown as the dashed line in the upper part of panel A.

The lowest suffusate PO₂ (12 mm Hg, see Table 1) the average arteriolar diameter was 18.6 ± 1.7 μm and ranged from 10.9 to 28.8 μm. The maximal diameters for these arterioles, determined under the same conditions as maximum capillary density, averaged 40.7 ± 2.4 μm. Elevation of the suffusate PO₂ caused a reduction in the diameter of arterioles at rest and during contraction (Fig. 2A). The functional dilations (differences between the arteriolar diameters in the resting and in the contracting muscle) are shown in Figure 2B. The magnitude of the functional dilation is diminished in the presence of the highest suffusate PO₂.

Relative conductances in arteriolar segments of the cremaster muscle were estimated roughly from our measurements of arteriolar diameter (conductance is assumed to be proportional to diameter raised to the fourth power; see Discussion) and these are shown in Figure 3. The estimated relative conductances were reduced significantly as the superfusate PO₂ was elevated, both at rest and during contraction. The change in conductance with exercise is also diminished as solution oxygen is elevated (panel B). It is interesting to note that, whereas the diameter change in Figure 2B is not influenced by changes in suffusion solution oxygen from 0 to 21%, the estimated conductance change is influenced significantly at all oxygen levels (Fig. 3B). This reflects the fact that the same arteriolar diameter increment is induced from a smaller resting diameter as solution oxygen content is elevated.

Table 1

<table>
<thead>
<tr>
<th>Oxygen tension (mm Hg)</th>
<th>Percent oxygen in equilibration gas</th>
<th>Norepinephrine suffusion*</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0%</td>
<td>5%</td>
</tr>
<tr>
<td>Solution</td>
<td></td>
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</tr>
<tr>
<td>0%</td>
<td>12.0 ± 2.3</td>
<td>42.5 ± 2.7</td>
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<tr>
<td>(21)</td>
<td>(19)</td>
<td>(18)</td>
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<tr>
<td>Tissue (rest)</td>
<td></td>
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<tr>
<td>14.8 ± 2.6</td>
<td>24.4 ± 2.2</td>
<td>37.4 ± 6.0</td>
</tr>
<tr>
<td>(23)</td>
<td>(25)</td>
<td>(26)</td>
</tr>
<tr>
<td>Tissue (contraction)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.2 ± 2.2</td>
<td>14.2 ± 2.6</td>
<td>22.3 ± 3.4</td>
</tr>
<tr>
<td>(23)</td>
<td>(25)</td>
<td>(26)</td>
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<tr>
<td>Change in PO₂†</td>
<td></td>
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<tr>
<td>5.7 ± 1.0</td>
<td>10.2 ± 1.5</td>
<td>19.3 ± 2.6</td>
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<td>(23)</td>
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<td>(26)</td>
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Data are expressed as means ± se. Numbers in parentheses = number of observations.

* Norepinephrine suffusion used to mimic O₂ induced contraction (Fig. 2). Solutions containing norepinephrine were equilibrated with 0% O₂ and solution PO₂ was approximately as in other experiments.

† Contraction minus rest.
To assure that the arteriolar dilations which we observed were not a complex response induced by pressure redistribution secondary to upstream or downstream microvessel dilation, we stimulated small groups of muscle fibers, using microelectrodes. From previous experiments, we knew that a local stimulus frequency of 4 Hz causes a dilation similar to that obtained with whole muscle stimulation at 1 Hz (Gorzynski et al., 1979). Figure 4 shows the results of the localized stimulation experiments. The vessels constricted when the suffusate PO₂ was elevated, both at rest and during muscle contraction, but the functional dilation during contraction was significantly smaller (paired t-test, \( P < 0.05 \)) when vessels were exposed to 5% oxygen than when exposed to 0% oxygen. The reduced functional dilation with elevated oxygen was seen only with much higher percentages of oxygen in the case of whole cremaster stimulation (Fig. 2B).

**Specificity of Oxygen**

Elevated suffusate PO₂ caused a significant reduction of capillary density and arteriolar diameter both prior to and during muscle contraction (Figs. 1, 2, and 4). Therefore, the effects of altered suffusate PO₂ on capillary recruitment or arteriolar dilation might have been either the specific result of altered availability of oxygen to the tissues or a nonspecific result of the PO₂ could be mimicked by application of norepinephrine or by oxygen.

**Tissue PO₂**

Tissue PO₂ was measured, both as an index of the efficacy of the regulatory process, and as one parameter which determines tissue oxygen diffusion gradients. In Table 1, tissue PO₂ measurements are presented as a function of the oxygen content of the gas equilibrated with the suffusion solution. Also, the PO₂ measurements taken during application of norepinephrine in a 0% oxygen solution are shown for the resting and working muscles.
Elevation of the suffusate PO2 caused an elevation of the tissue PO2 in both the resting and contracting muscles as a result of a greater flux of oxygen from solution to tissue. The tissue PO2 in the working muscle was somewhat less sensitive to suffusion gas composition than the PO2 in the resting muscle. This fact was reflected in a fall in tissue PO2 during contraction which was proportional to the resting tissue PO2. This relationship is reflected in Figure 7, where the fall in tissue PO2 during contraction increases as a function of the resting tissue PO2 prior to muscle stimulation. It should be noted that the proportionality between resting tissue PO2 and the contraction-induced decrease in tissue PO2 appears to extend even to observations made during application of norepinephrine (lower left section of Fig. 7, triangles). Application of norepinephrine in a concentration sufficient to mimic 10% oxygen suffusion caused a statistically significant reduction in the change in PO2 observed during contraction, when compared to the response observed in 0% oxygen.

Discussion

In this study, we provide direct evidence that the relative contribution of different microvascular elements to control of tissue oxygen supply may vary, depending on the initial state of vascular tone and not necessarily on the level of tissue oxygenation. We find that, during striated muscle stimulation, the change in diameter (Figs. 2, 4, and 5) and estimated conductance (Fig. 3) is less in constricted vessels than in dilated vessels. By inference, we assume that this would have been reflected in a smaller functional hyperemia had we measured flow. In contrast, the capillary recruitment is first increased by elevations of solution oxygen content, and only at very high oxygen is the recruitment reduced (Figs. 1 and 6).

To compare our findings on arteriolar diameter with previous reports using flow measurement, we assumed a simple, direct proportionality between conductance and the fourth power of the arteriolar diameter. We recognize that this assumption has been only partially validated (Lipowsky et al., 1980), and may not apply in all cases. Furthermore, we have assumed that total conductance is determined primarily by the diameter of the arterioles (Fronk and Zweifach, 1975) but it is clear that, under some conditions, other vascular segments can also influence total vascular conductance (Abboud, 1972). With these cautions in mind, however, some insights may be obtained from the computation.
Our findings support other reports that a low tissue PO_{2} (presumably causing a more diluted initial state) is a common feature of a more precise balance between flow and oxygen demand in striated muscle (Stainsby, 1962; Jones and Berne, 1965; Granger et al., 1976; Sullivan and Johnson, 1981). However, the results are not consistent with the idea that the level of tissue oxygenation per se is the main determinant of the regulatory pattern. The data suggest that it is not the level of tissue oxygenation that determines the vasomotor response but, rather, the state of vascular tone. The data on which this conclusion is based are shown in Figures 2, 4, and 5, and in Table 1. When suffusate PO_{2} was elevated, the tissue received additional oxygen from the solution, tissue PO_{2} was elevated, and less oxygen would have been required from the vascular route to meet tissue metabolic needs. Associated with this was a vasoconstriction induced by local regulatory processes (Figs. 2, 4, and 5). Norepinephrine, applied in a dose chosen to mimic the arteriolar constriction induced by oxygen, also caused reduced functional dilation, but resting tissue PO_{2} decreased (Table 1). Thus, oxygen and norepinephrine had identical effects on the initial vascular tone of the arterioles, but opposite effects on the tissue oxygenation. However, constrictions induced by both agents reduced the magnitude of the functional dilation. Thus, diminished functional dilation was correlated with a reduction of initial (resting) arteriolar diameter, not with a reduction in the initial tissue PO_{2}.

The parallel between vasomotor tone (rather than tissue oxygenation) and vascular response to exercise is emphasized further by the data of Figures 1 and 6. The degree of capillary recruitment during constriction of the muscle was related to the initial capillary density, whether constriction was induced by oxygen or by norepinephrine. Both constrictions caused a reduction in the capillary density of resting muscle and both augmented capillary recruitment during exercise, despite opposite effects on tissue oxygenation (Table 1). Thus, these data also suggest that the initial state of the vasculature is the common feature determining a regulatory response, not the level of tissue oxygenation.

Our observations on both the functional dilation of arterioles and on capillary recruitment during stimulation emphasize the need for considering the initial state of the vasculature in evaluating the vascular response to an experimental intervention. A number of other experimental findings show that the initial resistance, capillary density, and subsequent vasomotor response are related in complex ways (Emerson et al., 1973; Myers et al., 1975; Granger et al., 1976), and these facts emphasize that analysis of vascular function must be carried out under defined initial conditions of the vasculature and, wherever possible, constant initial conditions.

In this regard, it should be noted that the capillary reserve as well as the degree of capillary recruitment are highly dependent on the level of tissue oxygenation (Fig. 1) and on the level of vasomotor tone (Figs. 1 and 6) at the time of stimulation. Therefore, discrepant reports on capillary reserve and recruitment (Krogh, 1919; Gorczynski et al., 1978; Lindbom et al., 1980; Sarelius et al., 1981; Hudlicka et al., 1982) should be reexamined in this light. It may be that these disparities reflect only differences in the state of the vascular bed at the time of study.

The mechanism that links the magnitude of a functional dilation and the initial vascular diameter is unknown. It may be that our findings reflect the fact that the smooth muscle of the constricted arterioles operates at different points on its stress-strain curve (Johnson, 1968; Gore, 1972; Herlihy and Murphy, 1973), and thus arteriolar reactivity is changed. Gore (1972) has proposed that vasomotor tone may alter the initial wall stress in relation to some optimal stress level and thereby change the magnitude of the diameter response to a stimulation. Alternatively, the initial constriction may be interpreted to have altered the initial length of the contractile elements rather than wall stress, and this may have been the key factor in reducing the subsequent response. Further experimental work in this regard is necessary, as the role of arteriolar length-tension relations in vascular reactivity remains an almost unexplored area at this time. It is clear, however, that under controlled conditions, the initial state of an arteriole can determine arteriolar reactivity (Baez et al., 1967; Gore, 1972).

The diminished functional dilation induced by vasoconstriction (Figs. 2, 4, and 5) might also reflect a true alteration in microvessel sensitivity as a result of the accumulation of some metabolite during the period of reduced flow associated with the vasoconstriction. Our data are insufficient to assess this possibility.

In the absence of precapillary sphincters, it must be assumed that the shift of response dominance between arterioles and capillaries shown in Figures 1 and 2 reflects in some way the activity of arterioles (Eriksson and Myrhave, 1972; Gorczynski et al., 1978; Lindbom et al., 1980). It might be proposed that the different effects on capillary perfusion and total flow resistance may derive from the fact that a significant fraction of the total resistance lies in arteriolar vessels from which capillaries do not originate; that is, second- and third-order arterioles (Fronek and Zweifach, 1975). Since capillaries in this preparation originate largely from third- and fourth-order arterioles, the findings reported here might to some extent reflect opening and closing of third- and fourth-order vessels in response to the various stimuli applied, or what might be termed “arteriolar recruitment.” If the fourth-order vessels represent only a small part of the total resistance, then closure of a fraction of them would not greatly affect total resistance (Mayrovitz et al., 1977). Our findings might then reflect higher sensitivity of fourth-order vessels to exercise than of other arterioles. Final conclusions on this possibility will require a detailed analysis of comparative activities of the arterioles at all levels of the arteriolar tree under one set of conditions.

We can conclude from our data that regulation of
capillary density by closure of terminal arterioles is not the sole factor involved in the responses we observe, however. Several facts lead us to this conclusion. First, a greater sensitivity of the terminal arterioles to functional dilation was not observed in earlier work with this preparation by Gorczynski et al. (1978). Second, over the low end of the PO2 range shown in Figures 1 and 2, closure of arterioles was an infrequent observation, and, thus, a segmental analysis would not likely produce a major alteration in the conclusions reached here. Another argument against closure of terminal arterioles as the sole determinant of capillary density is the fact that many of the vessels observed in the present work were parent vessels for capillaries. We often observed that red cells stopped entering individual capillaries originating from an arteriole when flow in the parent arteriole was still brisk, and while red cells continued entering capillaries upstream and downstream from the unperfused capillary. It thus is necessary to reconcile the facts that: (1) terminal arterioles need not close to regulate capillary flow; (2) terminal arterioles appear to contribute significantly to vascular resistance; and (3) flow control seems to be at least qualitatively different from capillary density control.

There is some evidence that capillaries from amphibians may contract. (Weigelt et al., 1981) but this has not been observed in mammals. Occlusion of vessels by either red cells or white cells is another possible means for blocking capillary flow (Staaf and Wiederhielm, 1982; Schmidt-Schoenbein and Engler, 1982), but it is not obvious that these effects should vary in proportion to the state of arteriolar tone. It is also possible that the differential responses of capillary recruitment and functional dilation are related to shifting hydrostatic pressure gradients across the capillaries as a result of changes in arteriolar resistance. Data are not available to assess this possibility.

On the basis of recent preliminary observations made on in vitro isolated cannulated arterioles (Duling et al., 1981; R. Dacey, personal communication), we can propose a hypothetical explanation for an interaction between arterioles and capillaries which is not usually considered. It is occasionally possible to observe the orifice of small microvessels which branch from a cannulated arteriole; the orifice diameter varies with the diameter of the parent arteriole. An example of measurements made on such a preparation is shown in Figure 8. These data were obtained from a maximally dilated vessel and, thus, represent purely passive behavior of the orifice of a microvessel as a function of the internal diameter of the parent vessel (terminal arteriole and capillary, for example). As would be expected, as the internal diameter of the parent vessel increased, the size of the orifice increased as well, although nonlinearly. This observation has suggested to us a mechanism for regulation of capillary flow which is diagrammed in Figure 9.

Suppose that, in the maximally dilated state, most capillary origins are large enough to permit red cells to enter with a minimum amount of deformation (capillary perfusion = 100%). As the arterioles contract, the diameters of the capillary orifices decrease progressively, requiring greater deformation of red cells entering the capillary. Ultimately, some of the capillary orifices will become too small for red cells to enter. Assuming some population distribution of red cell size and capillary orifice size, the reduction in the number of capillaries perfused should be progressive and graded.

In Figure 9 we have approximated the shape of the regulation between orifice diameter and arteriolar diameter shown in Figure 8. The drawing is scaled so that, as maximal arteriolar diameter is approached, all of the capillaries originating from the arteriole are assumed to be perfused. Analysis of Figure 9 allows one to predict some of the interrelations which we have shown between arteriolar diameter and capillary density. Three different resting (R) and contracting...
(C) diameters are plotted on the hypothetical curve. It is assumed for this case that the functional dilation causes the same increase in diameter regardless of the initial diameter of the vessels (Fig. 2B, low PO2 range). The predicted increments in capillary density corresponding to the changes in diameter are indicated by the solid vertical lines for each of the rest-contraction transitions. Because of the shape of the curve, the theoretical increment in capillary density is smallest at the upper and the lower regions of the curve and largest in the center. Note that this is the relation expected from Figures 1 and 2. Thus, at least qualitatively, the apparent interaction between capillaries and arterioles could be explained by very simple geometric considerations or by what might be called a "passive sphincter" mechanism.

Although the analysis proposed in Figure 9 is obviously a simplification, it serves two useful purposes. First, it emphasizes that there is likely to be a relation of the general form shown in Figures 8 and 9 which will determine, in part, how arteriolar diameter and capillary density interact. Second, it partially obviates the need for a precapillary sphincter as classically defined, which has not been found, while allowing capillary density to change somewhat independently of arteriolar flow. Figure 9 also highlights the fact that a complex interaction observed between microvessel elements need not reflect precise feedback between the two separate elements and the tissue. Instead, it may be that our findings represent an intrinsic behavior pattern generated in part simply by the anatomy of the microcirculation.

Oxygen delivery to contracting striated muscles may be increased by at least three processes: increased flow, increased capillary density, and decreased tissue PO2. Our findings lead to the conclusion that the degree of participation of each of these in regulation depends on the initial state of the vasculature. At high levels of tone, the changes in capillary density and tissue PO2 are large, whereas, with low levels of tone, the change in flow predominates. The efficacy of these processes in matching supply to demand may be estimated by examining Table 1 and Figure 7. First, it is apparent, in Table 1, that in no case is regulation of tissue PO2 perfect. Elevation of suffusate PO2 causes vasoconstriction and reduced capillary density, thereby reducing oxygen input to the system via vascular sources, but this does not prevent a rise in tissue PO2, either in the resting or in the contracting muscle. Above 21% oxygen, the tissue PO2 begins to rise steeply with elevations in suffusate oxygen and, in fact, in this region the tissue PO2 change is not different than would be observed in an unperfused cremaster muscle (Klitzman, 1979). This might be expected, however, since in this region the vessels are so greatly constricted by the elevated oxygen that diameter (Fig. 2) and blood flow (Lindbom et al., 1980) to striated muscle may be reduced almost to zero. In this context, it should be noted that the range of suffusate PO2's between 0 and 21% oxygen include the range of physiological oxygen tensions.

When the striated muscle contracts, the tissue PO2 falls in all cases (Fig. 7), thus demonstrating that the combination of functional dilation and capillary recruitment is inadequate to meet the elevated oxygen needs of this tissue without a reduction in tissue PO2. However, the reduced PO2 is functional in a sense, as this steepens the diffusion gradient for oxygen and increases the tissue oxygen delivery.

If the cremaster were unperfused and the resting oxygen consumptions were the same at all levels of tissue oxygenation, then diffusion theory would predict that the magnitude of the decrease in tissue PO2 should be the same, regardless of the initial PO2. The magnitude of the reduction should be directly proportional to the ratio of the oxygen consumption to the Krogh diffusion coefficient (Jacobs, 1967; Klitzman, in press). We have carried out a few experiments with very high oxygen compositions (21%, 50%, 95% oxygen) and these appear to fit the predictions of diffusion theory (unpublished observations; Klitzman et al., in press).

It is therefore somewhat curious that the contraction-induced fall in tissue PO2 was closely related to the initial tissue PO2 (Fig. 7). This relation appeared to hold for both the experiments in which tissue PO2 was raised by changes in suffusion solution oxygen, and those in which tissue PO2 was lowered by application of norepinephrine (Fig. 7, shaded area). A similar, though weaker, correlation has been observed to extend across animals of various ages (Proctor et al., 1981). This finding is remarkable because it is not what would be predicted from simple diffusion theory, as mentioned above.

These facts are worth noting, since a possible explanation for the behavior shown in Figure 7 is that the increase in oxygen consumption of the cremaster muscle during stimulation is proportional to the ambient PO2, i.e., the consumption of oxygen by the working muscle might be supply limited. This possibility should be examined in future work, as it would substantially complicate our understanding of the regulatory process.

At the low end of the PO2 range, which should be of greater physiological relevance, the slope relating tissue PO2 and suffusate oxygen content is more shallow (Table 1). This end of the range shows the combined effects of increasing blood flow and decreasing diffusion distance (Figs. 1 and 2) as the solution PO2 falls. In contrast to earlier work (Duling, 1972), tissue PO2 was not constant either in the resting or in the contracting muscles as the suffusate PO2 was altered. Thus, either the muscles were incapable of regulating PO2 in the face of the altered solution PO2, or alternatively, tissue PO2 is poorly controlled in this striated muscle. It should be pointed out that our results were obtained from preparations which were stimulated at relatively low frequencies and on a muscle which has a high population of glycolytic fibers (Sarelius, Maxwell, Gray, Duling, unpublished observations). The work load and associated oxygen consumptions at 1 Hz are likely to have been low, and
this may have influenced our findings. It would be of interest to explore the interactions reported here at higher work loads and in more oxidative tissues.

The results shown in Table 1 also provide some hints about the nature of the oxygen-linked feedback system relating vascular responses and tissue oxygenation. First, the data suggest that it may be inappropriate to consider tissue PO2 a regulated variable in the idealized sense of a proportional or integral control model with a predetermined set point (Granger and Shepherd, 1979). This follows from the fact that tissue PO2 is not fixed and held at a precise level as oxygen availability changes. Rather, the tissue-vessel feedback seems to buffer the change in PO2 instead of seeking a single value of tissue PO2 (i.e., a set point). Furthermore, the tissue PO2 is not held constant during either norepinephrine suffusion or muscle contraction. The vessels clearly possess the capacity to respond to tissue oxygenation in both cases, but there is no real evidence that a fixed reference tissue PO2 is sought. This concept is quite consistent with a previous observation made on the cheek pouch microcirculation during alterations in sulfamate oxygen and carbon dioxide (Duling, 1973).

Thus, we feel that the data are consistent with the idea that there is a feedback between tissue oxygenation and vascular tone, but the feedback simply increases vessel tone as tissue PO2 rises; it does not seek a particular level of tissue oxygenation. It may be more appropriate to view the smooth muscle of the arteriole as analogous to a summing amplifier, with tissue PO2 being one of a multitude of neural and hormonal inputs acting in concert to determine overall vascular behavior.

The second fact which emerges from consideration of the data in Table 1, in combination with the data showing the arteriolar sensitivity to oxygen in Figure 2, is that the vasoconstrictor response does not appear to be explicable on the basis of some critical tissue element hovering on the brink of anoxia (Thews, 1960). Vasoconstriction was seen over the full range of tissue PO2. The location chosen for our electrodes in these studies was a point at which the minimum PO2 in the tissue was observed, and thus, we can conclude that the muscle circulation can be influenced by oxygen at striated muscle cell PO2 levels as high as 30-40 mm Hg. Isolated mitochondria appear to be limited by oxygen availability only at PO2's less than 1 mm Hg (Chance, 1969), and thus, simple limitation of oxygen availability as a factor in regulation is unlikely to occur until the solution oxygen falls below 5%. Even in the 0% oxygen solution, a gradient of almost 15 mm Hg would have to exist between the electrode measuring site and the mitochondria to lower PO2 at the cytochrome chain to the required critically low values. Three plausible explanations for these data may be advanced. First, some chemical process other than the cytochromes may be acting in the feedback between oxygen and vessel tone. Second, the critical PO2 for mitochondria in intact skeletal muscle may be different than is thought (Jobis, 1972; Wilson et al., 1979). Third, there may be steep local gradients in PO2 within the cells, for example, due to clustering of mitochondria (Hoppeler et al., 1981) or unexpectedly low diffusion coefficients within the cells (Longmuir et al., 1978). We cannot distinguish these possibilities, and future experiments aimed at answering these questions more directly are needed.

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The present address for Dr. Klitzman is: Department of Physiology and Biophysics, School of Medicine, Louisiana State University at Shreveport, Shreveport, Louisiana 71130.


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INDEX TERMS: Arteriole • Capillary density • Hyperemia • Microcirculation • Vascular smooth muscle • Set point
Augmented tissue oxygen supply during striated muscle contraction in the hamster. Relative contributions of capillary recruitment, functional dilation, and reduced tissue PO2.

B Klitzman, D N Damon, R J Gorczynski and B R Duling

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