Microvascular hematocrit and red cell flow in resting and contracting striated muscle

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KLITZMAN, BRUCE, AND BRIAN R. DULING. Microvascular hematocrit and red cell flow in resting and contracting striated muscle. Am. J. Physiol. 237(4): H481-H490, 1979.—Microvascular hematocrit and its possible relation to oxygen supply were systematically examined. We studied the red cell volume fraction (hematocrit) in arterial blood and in capillaries under a variety of circumstances. Control capillary hematocrit averaged 10.4 ± 2.0% (SE) and arteriolar (14.2 μm ID) hematocrit averaged 13.9 ± 1.2% in cremaster muscles of pentobarbital-anesthetized hamsters. Carotid artery hematocrit was 53.2 ± 0.6%. The low microvessel hematocrit could not be entirely explained by a high red cell flux through arteriovenous channels other than capillaries (shunting). Hematocrit was not only low at rest, but varied with physiological stimuli. A 1-Hz muscle contraction increased capillary hematocrit to 18.5 ± 2.4%, and maximal vasodilation induced a rise to 39.3 ± 9.5%. The quantitative relations between capillary red cell flux, arterial hematocrit, and total blood flow could be explained by a two-element model of microvascular blood flow that incorporated a relatively slow-moving plasma layer (1.2 μm). Such a model would generate a low microvessel hematocrit and might reduce the diffusion capacity of individual capillaries, but would not reduce time-averaged red cell flux or alter steady-state vascular oxygen supply.

A NUMBER OF STUDIES using various techniques have shown that the hematocrit in microvessels is low compared to the hematocrit in the large vessels (7, 16, 22, 23, 29, 30, 33). Although it is frequently assumed that the low microvessel hematocrit reflects shunting of red blood cells around the microvessels, the etiology of the hematocrit reduction has not been determined experimentally, nor is there a comprehensive theory to explain the microvessel hematocrit. Not only is the microvascular hematocrit low, but there are reports that microvessel hematocrit may vary with vasomotion (7, 24, 29). The low and variable microvascular hematocrit may be an important physiological determinant of tissue oxygen supply if the microvessel hematocrit accurately reflects red cell flux. There has been little investigation of the possible physiological role of low microvessel hematocrits and no systematic measurements have been made in striated muscle. The purpose of this study was to measure microvessel hematocrit in striated muscle, to determine if microvessel hematocrit varies under physiological circumstances, and to evaluate the possible role of hematocrit variation in the physiological regulation of oxygen delivery.

METHODS

General

Thirty-two male golden hamsters weighing 106 ± 5 g (mean ± SE) were used for this study. They were anesthetized with sodium pentobarbital (60 mg/kg ip), and a tracheostomy was performed. The left femoral vein was cannulated and normal saline was infused at 0.0034 ml/min to replace fluid losses. The infused contained 0.18 mg/ml of sodium pentobarbital, which maintained the anesthetic level of the animal throughout the experiment. Deep esophageal temperature was maintained at 37-38°C.

Preparation

The cremaster muscle preparation, as described by Baëz (2) and Kozlov (28), with the adaptations of Gorczynski et al. (18) was used. The muscle was freed from the scrotum, incised longitudinally, separated from the testis and epididymis, cleared of connective tissue, and pinned over a Lucite pedestal. The muscle was continuously superfused with a bicarbonate-buffered physiologic salt solution that was equilibrated with a gas containing 5% CO₂-95% N₂ and warmed to maintain tissue temperature at 34°C.

The muscle was transilluminated with a xenon arc lamp and observed with a Leitz Labolux II microscope, a Colu video camera, a Sony 3650 videotape recorder, and a Conrac video monitor. Total magnification was ×1180 from tissue to video monitor. A time reference (± 0.01 s, Odetics) was recorded on each video field. Controlled increases in muscle oxygen demand were produced by inducing twitch contractions. Muscles were stimulated by a Ag-AgCl cathode on the proximal portion of the muscle and a similar anode encircling the distal portion. Stimuli were square waves (0.02 ms duration) adjusted to a voltage adequate to produce vigorous twitch contractions (6-18 V). Motor nerves are stimulated at intensities well below the threshold for vasoconstrictor fibers, and therefore stimulation was probably limited to motoneurons and striated muscle cells (26).

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**Measurement Methods**

**Vascular diameters and lengths.** Diameters were measured by recording a voltage that was proportional to the separation of two video lines positioned over the TV image of the inner walls of the vessels. This system was accurate to ± 1 μm, as described previously for arterioles under experimental conditions identical to those of this study (18). Vessel segment lengths were measured using this same system.

**Oxygen tension.** Microelectrodes were used to measure the PO₂ of the superfusate 0.3-1.2 mm above the tissue (41). The superfusate PO₂ was elevated from 8.3 ± 0.5 to 40.0 ± 1.2 mmHg by adding oxygen to the equilibration gas, thereby reducing the demand for vascular oxygen supply.

**Hematocrit.** The method chosen for measurement of hematocrit (see Fig. 1) was first suggested by Barbee and Cokelet (3) and used by Schmid-Shönbein and Zweifach (33). In the absence of a better description of vascular geometry, they assumed that microvessels were cylindrical. It was further assumed that the mean corpuscular volume of the red cells was known and was constant throughout the peripheral circulation, and that there was free movement of plasma and cells throughout the vessel lumen. From these assumptions it follows that

\[
\text{hematocrit (\%) } = \frac{nV}{\pi r^2 L} \times 100
\]

where \(n\) = number of red cells in a vessel segment, \(V\) = mean corpuscular volume of red cells (μm³), \(r\) = vessel radius (μm), and \(L\) = length of a vessel segment (μm) with \(n\) red cells.

The mean corpuscular volume was determined in vitro by dividing the measured centrifugal hematocrit by the number of red cells in a known volume of blood. A Coulter model Z particle counter was used to determine the number of red cells in a volume of blood. The counter was calibrated immediately prior to use with a blood sample of known hematocrit and measured cell numbers per unit blood volume. In a small sample, red cell volumes were measured by dividing hematocrit by a hemocytometer count of red cells per unit volume. The two measurements were in good agreement. The mean corpuscular volume of the hamster red cells (taking a single value for each animal) averaged 59.0 ± 0.8 (SD) μm³, which was considerably less than previously reported (e.g., 72.4 ± 4.2 (SD) μm³; Ref. 34). Reasons for this discrepancy are not known.

The limits on the accuracy of the method were estimated as described by Bevington (5). From the above equation, the sum of the partial contributions to error in the hematocrit would be

\[
\text{dHct} = \frac{\partial Hct}{\partial n} \Delta n + \frac{\partial Hct}{\partial V} \Delta V + \frac{\partial Hct}{\partial r} \Delta r + \frac{\partial Hct}{\partial L} \Delta L
\]

We replaced the differentials by the finite errors that we took as reasonable values.

- \(n\) = 8 red cells \(\Delta n = ± 1\) cell
- \(V = 59 \mu m^3\) \(\Delta V = ± 1.6 \mu m^3\) (2 SD)
- \(r = 2.53 \mu m\) \(\Delta r = ± 0.5 \mu m\)
- \(L = 200 \mu m\) \(\Delta L = ± 1 \mu m\)

**FIG. 1.** Schematic illustration of the two methods used for estimation of capillary hematocrit in vivo; plug flow method and constant red cell volume method (used in this study). Also shown are the errors inherent in each method, calculated in hematocrit units.
All signs were chosen so as to maximize $\Delta Hct$, which yielded a maximum estimated error in the computed hematocrit of 4.2% (Hct units); the measured hematocrit was 11.7% (Hct units). Thus, the possible error in the accuracy of this method was 36% of the measured value. Although the fractional maximal error is large, it is better by severalfold (using the same error analysis method) than previous methods used to estimate microvascular hematocrit in vivo (see Fig. 1) (23, 24).

The optical method for hematocrit determination was validated with the use of rectangular glass microcuvettes (Vitro Dynamics; lumen = $18.5 \times 216.0 \mu m$; length = 4 cm) filled with dilute suspensions of washed hamster red cells. (Suspensions that showed either lysis or hemolysis were eliminated.) The hematocrit was determined by the optical method described above and, following this, the microcuvettes were centrifuged and the hematocrit again determined in the conventional manner (volume fraction of packed red cells). A linear regression of the optically measured hematocrit on the conventionally measured hematocrit yielded a correlation coefficient of 0.995, using the least-squares method (27). Optical measurement of hematocrits greater than 12% was prohibited due to the amount of red cell overlap in the 18.6-\(\mu m\) tube. It should be noted that the good agreement between the two methods implies an accurate measurement of red cell volume.

The optical method was used to measure capillary and arteriolar hematocrits in vivo. In vessels larger than capillaries, counting of red cells was possible only when the blood was stationary, due to the high red cell velocity and subsequent smearing of the video image. Arteriolar hematocrits could be determined, however, by occluding an unbranched segment of the arteriole very rapidly between two micropipettes separated by approximately 500 \(\mu m\). (An observation site was chosen in the center of the occluded segment.) Overlapping red cells could be separated by use of a third micropipette to agitate the contents of the arteriole. Compression of the arteriole under the occlusion pipettes and the subsequent elimination of red cells from that segment of the arteriole caused reductions in vessel hematocrit within 30 \(\mu m\) of the pipettes. Vessel hematocrit in the central portion of the occluded segment of the arteriole appeared unaffected by the vessel compression and was assumed to be representative of preocclusion hematocrit.

Red cell velocity. Elapsed time between events recorded on videotape could be determined from the recorded video time data. The velocity of red cells in the capillaries was measured by choosing a capillary segment and dividing its length by the time required for red cells to traverse the segment.

Regional blood flow. In 10 animals, regional blood flow measurements were performed using radionuclide-labeled microspheres. A cannula was prepared by narrowing the first 25 mm of a length of PE-100 tubing to 250 \(\mu m\) (OD) by pulling over a flame. The cannula was inserted through the right carotid artery into the left ventricle, and the tip position was estimated by monitoring blood pressure during insertion.

The methods of microsphere handling suggested by Heymann et al. (20) were followed. Approximately 300,000 microspheres labeled with \(^{111}\)Ce (3M Co.) (diam 15.0 ± 0.1 (SE) \(\mu m\)) were suspended in 0.25 ml of a 10% dextran solution and injected over 15 s into the left ventricle. (The cannula was flushed with 0.5 ml isotonic saline.) During injection, a reference blood sample was continuously withdrawn from the cannulated left femoral artery at a rate of 23.3 \(\mu l/min\) for a period beginning 30 s before and ending 1 min after the injection. Two minutes after the injection the animal was killed with an overdose of sodium pentobarbital, and tissues were dissected with no further treatment. Gamma radiation (100-130 keV) of the blood (\(cpm_{blood}\)) and tissue (\(cpm_{tissue}\)) samples was determined in a Beckman Biogamma II gamma-counting spectrometer system. The regional blood flows were calculated by the following formula:

\[
\text{blood flow (ml·min}^{-1}·100 \text{g}^{-1}) = \frac{(cpm_{tissue} \times 23.3 \mu l/min)/(cpm_{blood} \times \text{tissue weight})}{\text{Optical}}
\]

Capillary shunting. Evaluation of the significance of measured capillary hematocrits was dependent on accurate knowledge of the fraction of red cells shunting around the observed capillaries. Two methods of evaluation were used: 1) direct observation of capillaries, and 2) fractional passage of 15-\(\mu m\) microspheres through the microcirculation.

Direct observation evinced no arteriovenous pathways larger than capillaries in the cremaster but, in some vessels of capillary size, hematocrit was not measurable due to cell overlap or high velocity of red cells. The majority of these channels eventually branched into capillaries in which hematocrit was measurable, but a few vessels passed to venules without branching into vessels in which hematocrit could be measured. The fraction of such vessels was determined by counting.

The second method used for the quantitation of shunt fraction involved the injection of radionuclide-labeled microspheres. A glass micropipette (~80 \(\mu m\) OD) was inserted into one of the small (200 \(\mu m\)) cremasteric veins, and all of the blood flowing into the vein was collected. During the collection of the venous effluent, \(^{111}\)Ce microspheres were injected into the left ventricle. By comparing the relative gamma emissions of the venous effluent and the tissue drained by the cannulated vein, an estimate of the fraction of total cremasteric blood flow traveling through arteriovenous shunts (large enough for the 15-\(\mu m\)-diameter spheres to pass) was obtained.

Approximately 0.1% of the gamma activity of the microsphere solution was due to \(^{141}\)Ce that leached out of the spheres into the suspension fluid. The venous effluent collected and assayed for gamma activity was corrected for the free \(^{141}\)Ce, since most of it could have traveled through capillaries.

RESULTS

The hematocrit in the small arteries and veins (100-200 \(\mu m\)) of the resting cremaster was measured by withdrawing and centrifuging a blood sample. The hematocrit in these vessels was similar to that found in the large vessels, i.e., small artery hematocrit = 49.4 ± 1.1% and small vein hematocrit = 50.8 ± 1.0% (SE).

Hematocrits measured in capillaries of resting cremas-
ter muscles using the optical method are shown in Fig. 2. (Internal capillary diameters averaged 5.1 ± 0.1 (SE) μm, and did not change with experimental conditions.) Under control conditions, the capillary hematocrits averaged 10.4 ± 2.0% (SE), compared with a carotid artery hematocrit of 53.2 ± 0.6% in these hamsters. As the superfusate PO₂ was raised from 8 to 40 mmHg, thereby reducing the demand on vascular oxygen delivery, the capillary hematocrit fell to 6.3 ± 1.8%, as shown in Fig. 2.

Capillary hematocrit was also measured during increases in tissue oxygen demand induced by muscular contraction. The hematocrit was significantly increased to 18.5 ± 2.4% (P < 0.05) during muscular contraction (Fig. 2). As with the resting muscle, increasing the superfusate PO₂ led to a reduction in the hematocrit during contraction.

The hematocrits in arterioles (14.2 ± 1.2 μm mean ID) averaged 13.9 ± 1.2% in the resting muscle (Fig. 3). This was slightly higher than the capillary hematocrit, but was not statistically different at the 5% confidence level. Hematocrit in larger arterioles could not be accurately measured due to red cell overlap.

The low capillary hematocrits were not strictly the result of some inherent inability in the method to detect high hematocrit since, when the muscle was superfused with 0.1 mM adenosine for several minutes in conjunction with 8 Hz stimulation of the muscle, capillary hematocrit increased to 39.8 ± 9.5% (as shown in Fig. 3). The hematocrit in these same capillaries at rest was 10.9 ± 3.6%, and during contraction at 1 Hz was 22.1 ± 7.9%. The maximum hematocrit thus approached, but was still below, the large vessel hematocrit of 50%.

**Blood Flow**

Regional blood flow was measured primarily to estimate the red cell flow to the cremaster muscle. Results from the blood flow determinations are listed in Table 1. Cardiac output averaged 220 ± 35 ml·min⁻¹·kg⁻¹ with a mean body weight of 107 ± 4 g. Mixing of the spheres appeared adequate because the bilateral flow determinations differed by 6 ± 3% (range 1-14%) in tissues unaffected by cannulation or dissection. Our results for cremasteric blood flow agree well with previous reports of blood flow to "prepared" skeletal muscles (13, 26, 36).

Blood flow measured in the prepared, resting cremaster muscle with superfusate PO₂ of 8 mmHg was twice that in the undissected cremaster. Contraction of the cremaster led to a tripling of blood flow. This agrees well with the tripling of gastrocnemius muscle blood flow seen with moderate treadmill exercise of conscious dogs (12).
**Red Cell Mass Balance**

The red cell flow rate through the cremasteric artery should equal the red cell flow rate through the capillaries in the steady state if no high hematocrit shunts exist. Red cell flows through the small artery to the resting and contracting cremaster muscles (3.8 ± 1.1 and 10.6 ± 3.2 ml·min⁻¹·100 g⁻¹, respectively) were estimated by multiplying the hematocrit of the arterial blood (49%) by the measured blood flow.

The estimate of red cell flow through the capillaries was more involved than the estimate for the artery. The average rate of red cell passage through the capillaries was determined by videotape analysis in the same muscles that were used to determine total blood flow (before microsphere injection). At rest, the average red cell flow per capillary (508 red cells/min × 5.9 × 10⁻¹¹ ml/red cell = 3.00 × 10⁻⁶ ml red cells/min) was multiplied by the number of capillaries per 100 g of muscle, as determined previously (410 capillaries/mm² + 0.50 mm/capillary = 1.0 × 10⁻⁵ 100 g/mm³ = 7.82 × 10⁷ capillaries/100 g). The resulting estimate of red cell flow through the capillaries was 2.4 ± 1.5 ml red cells·min⁻¹·100 g⁻¹. There was no significant difference between the estimated red cell flow through the arteries or through the capillaries of the resting muscle (0.3 < P < 0.4). Similarly, the estimated red cell flow through the contracting muscle arteries was 10.6 ± 3.2 ml red cells·min⁻¹·100 g⁻¹ compared to 10.2 ± 1.7 for the capillaries. Thus, red cell mass balance seems to be consistent between the arterial and capillary measurements.

**Red Cell Velocity**

The average red cell velocities in open capillaries of resting and contracting muscle at the two different values of superfusate PO₂ are shown in Fig. 4. Control red cell velocity through the capillaries in our study (0.21 mm/s) was slightly below the previously reported values for other tissues, e.g., 0.38 mm/s for cat sartorius (25) and 0.38 mm/s for rabbit tenuissimus (38).

The vascular response to elevation of environmental PO₂ has been demonstrated by other investigators for various tissues (10, 17, 32, 38). Although the increases in velocity induced by striated muscle contraction were significant (paired t test, P < 0.05), they did not differ as the superfusate PO₂ was altered (Fig. 4). The magnitude of this contraction-induced increase in velocity was similar regardless of superfusate PO₂ (velocity increase = 0.25 ± 0.11 (SE) mm/s at 8 mmHg; 0.22 ± 0.09 mm/s at 40 mmHg).

**Shunting**

Much of our analysis of red cell flow depends on an accurate assessment of the possible a-v pathways for blood flow. Therefore, a number of microscopic fields (n = 68) were chosen randomly from seven cremasters and the arteriovenous channels examined. Hematocrit could not be measured in 20 of 338 vessels of capillary size due to technical limitations. However, of the 20, only 2 did not eventually branch into channels in which the hematocrit would have been measured in other fields of view. Thus, only 0.6% of vessels of capillary dimension would have been excluded from measurements and thereby might have contributed to shunting of red cells. We were able to measure the red cell velocity in these channels; they averaged 392.7 ± 78.8 μm/s. This was 80% faster than the velocity of red cells in the other capillaries. Since hematocrit cannot exceed 100%, no more than 2.2% of total red cell flow (0.006 × 1.80 × 2) could have passed through these channels. Other investigators have also noted a lack of noncapillary arteriovenous pathways in the cremaster (2, 18, 28, 35).

Following intra-arterial injection, 15-μm microspheres appeared in cremasteric venous blood in a concentration equal to 0.5% of the tissue sample. Combining the esti-

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**TABLE 1. Regional blood flow using labeled microspheres**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Blood Flow, ml·min⁻¹·100 g⁻¹</th>
<th>Unilateral Wet Wt, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissected right cremaster (rest, low PO₂)</td>
<td>7.7 ± 2.3</td>
<td>149 ± 13</td>
</tr>
<tr>
<td>Dissected right cremaster (contracting, low PO₂)</td>
<td>21.6 ± 6.4</td>
<td>164 ± 16</td>
</tr>
<tr>
<td>Intact left cremaster</td>
<td>3.3 ± 0.5</td>
<td>192 ± 12</td>
</tr>
<tr>
<td>Kidney*</td>
<td>503.9 ± 44.1</td>
<td>630 ± 22</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>4.9 ± 1.7</td>
<td>214 ± 10</td>
</tr>
<tr>
<td>Biceps brachii*</td>
<td>4.1 ± 0.5</td>
<td>121 ± 7</td>
</tr>
<tr>
<td>Check pouch</td>
<td>3.7 ± 0.9</td>
<td>333 ± 13</td>
</tr>
<tr>
<td>Cerebrum,* abundant white matter</td>
<td>35.7 ± 4.3</td>
<td>377 ± 8</td>
</tr>
<tr>
<td>Lungs*</td>
<td>49.2 ± 7.2</td>
<td>496 ± 82</td>
</tr>
<tr>
<td>Ventricles</td>
<td>307.1 ± 79.2</td>
<td>323 ± 24</td>
</tr>
</tbody>
</table>

Values are means ± SE with number of observations in parentheses. * Agreement between left and right sides was excellent, with a mean difference of 6 ± 3%.
mated amount of shunting through the two different sizes of vessels yields an estimate of total arteriovenous shunting of blood around the observed capillary bed of less than 1.5% of the total blood flow to the cremaster muscle.

**DISCUSSION**

Our results show that microvascular hematocrit is low and that it varies in response to physiological stimuli. These findings confirm and extend a variety of reports of low microvessel hematocrit, using both direct and indirect methods (7, 16, 22, 23, 29, 30, 33). The average hematocrit of the entire vasculature, as determined by dilution of plasma and red cell indicators, is consistently lower than the hematocrit of blood drawn from large vessels, and the ratio of mean vascular hematocrit to large vessel hematocrit in a variety of species ranges from 0.74 to 0.94 (16, 20). The fact that this ratio is consistently less than 1.0 suggests that there is a real difference between the mean vascular hematocrit and the large vessel hematocrit and that an excess volume of plasma exists somewhere within the vascular system (16).

In addition to indicator dilution methods, observation of microvessels has led to a variety of reports of low microvessel hematocrits. Very early reports of low capillary hematocrits in frogs (7, 29) have been confirmed by a variety of modern observations. Johnson (23) reported a "hematocrit index," to which one can apply the formula used in our study (100 nV/πr²L) to estimate the absolute value of the hematocrit in cap mesenteric microvessels. An average capillary hematocrit value of 10% is the result of such a calculation. A method similar to the one used in our study has been used previously by Schmid-Schönbein and Zweifach (33) to measure hematocrits averaging 17% in the rabbit omentum microvessels, and by Lipowsky and Zweifach (30), who reported capillary hematocrits averaging 8.23 ± 0.44% (SD) in cat mesentery.

Not only is microvessel hematocrit much lower than the large vessel hematocrit, but our data and earlier findings show that the microvessel hematocrit may vary with physiological stimuli. Microvessel hematocrit increased or decreased depending on the stimulus intensity of the spinal nerves (7) and increased after stimulation of striated muscle (7, 29). Spontaneous cyclic variations in capillary hematocrit have also been reported (24).

Given that the microvessel hematocrit is low and variable, it is important to consider whether or not microvessel hematocrit may be an important independent variable which is a determinant of tissue oxygenation. To assess the possible physiological importance of our findings, three general questions may be asked: 1) Is the low hematocrit simply a reflection of a measurement error or artifact? 2) Does the low microvessel hematocrit reflect the existence of a population of channels that permit shunting of red cells around capillaries? 3) Is the microvessel hematocrit low because of red cell dilution by fluid within the microvessels?

**Analysis of the Method of Hematocrit Determination**

The measurement technique we have used is indirect, and potential sources of artifact or systematic error might have a major impact on our findings. Our error analysis indicates that the measurement error per se should not exceed 4% (hematocrit units). Thus, measurement error could explain small variations in hematocrit, but not the large variations observed.

Two other facts argue against major measurement error. First, the test of the method using micropipettes showed close agreement between the optical estimation of red cell volume fraction and the actual volume of packed red cells. Second, when maximal dilation of the microcirculation was induced, the hematocrit rose to a value approaching systemic hematocrit. These facts argue that the method used for determining hematocrit of blood in capillaries should be in error by no more than a few percent. If we assume that the method is valid, then the reduction of microvascular hematocrit below large vessel hematocrit must be explained either by shunting of red cells past the observed microvessels or by intravascular dilution of red cells.

**Role of Shunting in Reducing Microvessel Hematocrit**

The most straightforward means by which capillary hematocrit might be reduced would be to permit a high fraction of red cells to bypass or shunt the capillaries. Because only capillaries were observed, red cells flowing through these shunts would not be measured. This appears not to have been a major contribution to our findings since three methods of estimation of red cell flow around the capillaries yield similar low values. First, the similarity of calculated red cell flows through the capillaries (2.4 ± 1.5 at rest; 10.2 ± 1.7 ml.min⁻¹.100 g⁻¹ during contraction) and through the feed arteries (3.8 ± 1.1 at rest; 10.6 ± 3.2 during contraction) provides evidence against the existence of shunts. Second the insignificant appearance of microspheres in the venous effluent of the cremaster following arterial injection casts doubt on the existence of large (> 15 μm) arteriovenous shunts. Third, direct observation of the microcirculation revealed that capillary-size shunts are too few to allow for significant shunting of red cells.

A word of caution regarding the microscopic observation should be inserted. Our observations were usually limited to the first and second muscle layers, and it could be that small shunts existed in the deepest muscle layer of the cremaster. Such shunts, had they existed, could have provided a pathway for high hematocrit blood around the capillary bed, thereby leaving low hematocrit blood for perfusion of the observed microvessels. This seems unlikely, however, because calculated red cell flows were similar for both the capillary and the arterial levels. Since we find no error in the methodology and no significant red cell shunting, it is likely that the low capillary hematocrit reflects red cell dilution by some mechanism.

**Role of Intravascular Red Cell Dilution**

Logically, the red cells might be diluted by fluid absorbed from the environment or by a component of plasma that is relatively slow moving within the microvessels. The first of these seems unlikely on quantitative grounds. If we assume a capillary hydrostatic pressure of 29 cmH₂O, a plasma oncotic pressure of 19 cmH₂O, and...
a capillary filtration coefficient of $0.001 \mu m^{-1} \mu m^{-2} \cdot s^{-1} \cdot cmH_2O^{-1}$ (35), we estimate that the microvessels would absorb fluid at only 1% of the measured flow rate ($77 \mu l \cdot min^{-1} \cdot 100 g^{-1}$), which is far too little to explain the 80% reduction in hematocrit observed in some cases. Therefore, it seems appropriate to consider intravascular dilution of red cells by some local plasma component as the source of the low microvascular hematocrit.

A peripheral layer of plasma is created in blood vessels by the physical exclusion of red cells from the perimural space and by the tendency of red cells to migrate toward the center of the vessel (11). These phenomena tend to create a fluid "core" that contains both red cells and plasma in varying concentrations and a surrounding annulus of pure plasma. A cell-poor layer has been observed near the vessel wall, and it was estimated that a layer such as that observed would reduce mean optical hematocrit in the vessel to about 25% from a core hematocrit of 50% (4, 6, 42). In addition, the thickness of in vitro plasma layers in some cases has been observed to be dependent on flow velocity (21).

Although we have no information on the thickness of the plasma layer in the capillaries we observed, one can estimate the thickness of an annular plasma layer that would be required to completely explain the observed reduction in hematocrit from 50% (large vessel) to 10% (capillary). For a capillary diameter of 5 \mu m and a capillary hematocrit equal to one-fifth of the systemic hematocrit, the required plasma layer thickness would be 1.4 \mu m, because the hematocrit reduction would be proportional to the ratio of the cross-sectional area of the core to the cross-sectional area of the vessel.

A different estimate for the thickness of the plasma layer can be obtained from the data presented here and an estimate of capillary density from data in a previous report (27). The plasma layer can be estimated from the difference between the anatomic cross-sectional area and the area available for blood flow per capillary, which will be calculated by dividing the mean capillary blood flow by the mean blood velocity through the capillaries. We obtain mean capillary blood flow as the product of the measured blood flow, $7.7 \times 10^{-3} cm^3 \cdot min^{-1} \cdot 100 g^{-1}$, the specific gravity, 1.055 (31), and the average capillary length, 0.0498 cm, divided by the number of open capillaries per unit tissue cross section, $4.11 \times 10^4/cm^2$. This computation yields an average mean blood flow through a single capillary of $9.84 \times 10^{-6} cm^3/ min$. As will be shown below, the ratio of red cell velocity to mean blood velocity must lie between 1.0 and 2.0. The measured red cell velocity was $212 \mu m/s$, and thus, the mean blood velocity through the capillaries must lie between 0.64 and 1.27 cm/min. Dividing the estimated flow through each capillary by the range of mean blood velocities yields a range for the effective area available for blood flow through a capillary of $7.75 \times 10^{-8}$ to $1.55 \times 10^{-7} cm^2/capillary$. The radii associated with these areas are 1.57 and 2.22 \mu m. The measured anatomic capillary radius averaged 2.53 \mu m, and therefore, the plasma layers predicted from these data would range from 0.31 to 0.96 \mu m. Thus, both of the above estimates are consistent with the existence of a thin plasma layer in the microvessels, which could lead to a difference between the effective hematocrit and the optical hematocrit.

A second well-known characteristic of microvessel flow may contribute to the low microvessel hematocrit. Red cell velocity in small tubes exceeds plasma velocity (9, 11) and the ratio of red cell velocity to mean blood velocity must be between 1 and 2. The value of 2 arises from the fact that, in flowing Newtonian fluids, the centerline velocity equals twice the bulk velocity. A lower limit of 1 would be achieved with plug flow, in which boluses of plasma are trapped between red cells. The ratio of red cell velocity to mean blood velocity has been estimated to be 1.75 in microvessels (11) and has been found empirically to be 1.6 in small tubes (15). Consistent with the observed velocity ratio between cells and whole blood is the fact that the ratio of red cells to plasma is also greater than 1.0 in the microvessels. Values of approximately 1.4 have been observed both in vitro and in vivo (14, 37).

Mass is conserved in the microcirculation, and we find no evidence of shunting (see above). It follows that plasma and red cells must pass all cross sections through the vascular bed at a rate equal to the entry rate. However, red cell velocity is systematically larger than plasma velocity. If equal flow rates through the vasculature are to be maintained, any difference in velocities between red cells and plasma must be reflected by an inverse difference in volume fraction in the tube. Thus, differential mean velocities of red cells and plasma imply changing hematocrits; because red cell velocity is higher, hematocrit must be lower.

We are thus in a position to propose that the low capillary hematocrits we observe are largely or entirely the result of two factors: 1) a red cell-plasma velocity difference, and 2) a relatively slow-moving plasma annulus. (A similar model has been suggested previously for flow in small glass tubes (42).) A quantitative examination of the combined effects of a dilute peripheral plasma layer and a red cell velocity/plasma velocity ratio on capillary hematocrit is shown in Fig. 5. (Equations of Fig. 5 are derived in the APPENDIX.) The figure shows that our measured value for capillary hematocrit in the resting muscle (10%) could be completely explained by the existence of a 1.2-\mu m plasma layer and a red cell/plasma velocity ratio of 1.6 within the core. Furthermore, the observed change in capillary hematocrit with muscular contraction could be explained by a change in the plasma layer of less than 0.4 \mu m, which would not be detectable with the light microscope and illuminating wavelength (436 nm) used in this study.

From Fig. 6, it appears that the plasma layers needed to explain the observed capillary hematocrits are relatively large. It must be emphasized that the estimate is subject to a variety of uncertainties in the data used to compute it and that it serves only as a first approximation. It demonstrates that our data are reconcilable with the idea of very low apparent microvessel hematocrits existing in vascular systems that have minimal shunting, but careful future quantitation of relevant variables is required. In particular, the estimate of capillary density we have used is one that was obtained by indirect meth-
function lies in the relation between hematocrit and oxygen-carrying capacity of the blood. It is tempting to conclude from our data that the hematocrit changes we have observed in response to elevated oxygen supply and to muscular contraction are functional and that these changes are an accurate reflection of the oxygen supply capacity of the blood. The relation between hematocrit and oxygen capacity is likely to be more complex, however.

In some tissues such as the mesentery, and especially the carotid body, the microvessel hematocrit is very low and much or most of the oxygen may be supplied by the plasma (1, 23). In these tissues, however, red cells are likely to be shunted around the microvascular bed. In striated muscle, we can find no evidence that red cells are shunted, and thus they must flow through the capillaries.

In the steady state it seems logical that capillary hematocrit per se need not be a major determinant of oxygen delivery. Under normal circumstances, the bulk of oxygen entering a tissue is in red cells, and red cell flow is directly proportional to blood flow. Thus, oxygen supply to the tissues is directly proportional to red cell flow. It follows that red cell flow or flux, not hematocrit, is the major determinant of oxygen supply to striated muscle cells, and that equal oxygen supply rates can be achieved by a low hematocrit–high velocity flow or by a high hematocrit–low velocity flow (19).

The effect of a low microvascular hematocrit on oxygen supply to tissue during non-steady-state conditions is not clear, however. If the net red cell flow through the microvessels is held constant and only capillary hematocrit changes, then the effect of an altered microvascular hematocrit will be to alter the number of red cells in the vessel segment and the dwell time of cells in the capillary. In non-steady-state conditions, the time dependence of red cell passage may make actual intravascular hematocrit (red cells per unit length) a contributing variable to tissue oxygen supply. This might be of particular importance, for example, in muscle where contraction frequencies generate transients of the same order of magnitude as the red cell transit times through capillaries.

One additional point can be made regarding the effect of the hypothesized plasma layer on diffusion of oxygen between red cells and tissue. The layer of plasma near the vessel wall will diminish the effective radius of the vessel to that of the free-flowing core of the vessel, not the anatomic radius. The magnitude of this effect can be roughly estimated by the Krogh equation (29), which predicts that reductions of the capillary radius by 0.6 \( \mu \text{m} \), and by 1.2 \( \mu \text{m} \) due to the addition of the plasma layer will increase the blood-tissue oxygen tension difference required to supply oxygen to the average muscle cell by 18% and 42%, respectively. Thus, in addition to reducing microvascular hematocrit, small plasma layers might be of modest importance in considerations of oxygen supply to tissue.

In summary, we find a relatively low microvessel hematocrit, which changes in response to physiological stimuli. A major contribution to the generation of this low hematocrit is hypothesized to be a slow-moving plasma layer on the order of 1 \( \mu \text{m} \) thick. Physical phenomena

Possible Physiological Effects of Red Cell Flow, Plasma Layers, and Microvessel Hematocrit on Oxygen Supply

The potential importance of hematocrit to microvessel
that might be involved in the formation of the plasma layer remain unidentified, and definitive estimates of the thickness of the plasma layer under physiological conditions must be made. The possible importance of the hypothesized plasma layer to tissue oxygen supply emphasizes the necessity for quantitative estimates of the plasma layer thickness in this and other systems where a thorough knowledge of oxygen supply is desired.

APPENDIX

Influence of Radial Velocity Differences and Plasma Layer Thickness on Oxygen Hematocrit in Microvessels

Definition of Terms

CD = capillary density (number/mm² tissue cross section)
Hct = hematocrit of blood in vessel core (r)
Hctm = hematocrit of blood in large vessel feeding cremaster (s)
Hcte = hematocrit of blood in entire vessel = optical hematocrit (r')
L = mean capillary length (mm)
l = length of a vessel segment (mm)
n/I = number of RBC per vessel segment length
Q = blood flow per unit volume of tissue (mm³ blood/s per mm³ tissue)
Qh,m = flow of RBC per unit volume of tissue (mm³ RBC/s per mm³ tissue)
Qh = flow of plasma per unit volume of tissue (mm³ plasma/s per mm³ tissue)
V = vessel radius (µm)
ν = mean corpuscular volume (mm³/RBC)
νp = plasma flow per capillary (µm³ plasma/s per mm³ tissue)
δP = plasma layer thickness (µm)
γ = ρp/ρw

Equations

A) blood flow = Q
Qh,m = Q - Hctm
Qh = Q(1 - Hctm)
B) RBC flow per capillary = νh,m Vh,m n/I
plasma flow per capillary = νp(π(r - δ)(1 - Hct))
C) Without shunting, mass must be conserved.
arterial Qh,m = total capillary Qh
(Qh,m = (Q(1 - Hctm))/ Resident, Vh,m)

From Eqs. 1 and 2

Hct = \left( \frac{1}{1/Hctm} \right)

Solving for Hct,

Hct = \left( \frac{1}{1/(1/Hctm)} - 1 \right) + 1

If we now assume that Hct = 50%, then

Hct = \left( \frac{1}{1/(δ/r)} \right)

Equation 5 was used for Fig. 5.

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REFERENCES

17. GORCZYNSKI, R. J., AND R. R. DULING. Role of oxygen in arteriolar functional vasodilation in hamster striated muscle Am. J. Physiol

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