Brief Review

An Examination of the Measurement of Flow Heterogeneity in Striated Muscle

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Transcapillary exchange of small solutes is dependent on blood flow; exchange is also dependent on the uniformity with which the inflowing blood is distributed among the exchange vessels. A nonuniform or “heterogeneous” distribution of flow among the vessels of an organ has been invoked to explain such diverse phenomena as flow-limited muscular performance,1 suboptimal capillary transport of small solutes,2 pathology of hypotension,3,4 and heterogeneous tissue Po2 distributions.5 Thus, it is apparent that a clear delineation of the significance of flow heterogeneity.

DEFINITIONS. Flow is the convective movement of blood, red cells, or plasma through the vasculature (often referred to as bulk flow). The vasculature is composed of many parallel flow pathways between artery and vein, and flow heterogeneity means simply that the total inflow is not distributed identically among the perfused vessels. In the case of perfectly homogeneous perfusion, all vessels would receive 1/n of the total inflow, where n is the number of any class of vessels arranged in parallel. To the extent that some vessels receive more and some less flow than their appropriate fraction of the total, the flow is judged to be heterogeneous. Following prior discussion, we refer to such heterogeneity as the relative dispersion of the flow.6

The relative dispersion can only be accurately assessed using a combination of two statistical parameters: an index of the central tendency of the data and an index of the dispersion. The mean flow is a measure of the former, and the standard deviation is a measure of the latter. The quotient of the two, the coefficient of variation, is a convenient measure of the relative dispersion or the true heterogeneity of flow.

It must be appreciated that the relative dispersion will not accurately reflect the range of flows among vessels in a heterogeneously perfused tissue. The range or total dispersion of flow is subject to two determinants: the relative dispersion (defined above) and the absolute value of the total flow entering the vasculature. The combined magnitude of the two sources of flow dispersion is often expressed by a single measurement, the standard deviation, which we refer to as the absolute dispersion of the flows.

The importance of making the definitions and distinctions between the two aspects of dispersion and heterogeneity can be appreciated by considering the result of increasing total flow into a vascular bed with constant relative dispersion (true heterogeneity). The standard deviation of the flow will increase in direct proportion to the mean flow, and the increase in standard deviation might be misinterpreted as an increase in the inequality of flow distribution among the vessels of the network. Such a misinterpretation is forestalled, however, by computing the coefficient of variation.
Since both mean and standard deviation increase in proportion to the change in total flow, the coefficient of variation will remain constant if there is a constant fractional distribution of flow among the microvessels, i.e., constant relative dispersion. Alternatively, at a constant level of mean flow, fractional flow distribution might be altered by redistributing flow among patent vessels, and the coefficient of variation will show the change in relative dispersion.

Other important information regarding flow dispersion may be gained through an analysis of the histograms describing the sample distribution. In principle, it is possible that the coefficient of variation could remain constant during an experimental intervention while the shape of the sample distribution would change, indicative of a redistribution of flow. This possibility would be manifested as a change in the shape of the histograms.5,7,8

The importance of distinguishing between the absolute and the relative dispersions in an examination of microvascular flow stems from the fact that the three variables—total flow through the organ, the number of vessels perfused, and the fractional distribution of flow among perfused channels—may be altered as semi-independent variables by physiological controls and pathological events each with different implications for function and each requiring separate analysis if vascular control is to be fully understood.

We turn now to a comparison of the various methods used to assess flow heterogeneity and to some comments on their strengths and weaknesses. Many of the comparisons are summarized schematically in Figure 1, which shows the relation of the various flow measurements to each other; the relations among such factors as transit times (Sections I and II), volume, and flow in the various vessel segments (Sections I and II); and to observations using in vivo microscopy (Section III).

Tissue deposition indicators. Several different indicators are deposited in the tissue in proportion to the rate at which they are convected into a region by the inflowing blood, e.g., tritiated water, antipyrene, and microspheres. A common means of assessing perfusion heterogeneity is based on the use of such indicators followed by careful dissection of the tissue into small samples and subsequent estimation of flow in

\[ \bar{t} = \frac{1}{F} \left[ \sum V_a + \sum V_c + \sum V_v \right] \]  
(Eqn. 1)

**Figure 1.** Schematic illustration of different aspects of the measurement of flow heterogeneity. Section I illustrates a group of capillaries in which the capillary perfusion heterogeneity is estimated from the transit time of an intravascular reference indicator; \( t \) for each capillary is proportional to \( V/F \) (volume/flow) and the mean transit time for the group is the flow-weighted average transit time. Section II illustrates the paired use of an intravascular reference indicator and a diffusible indicator to determine the capillary exchange capacity, \( PS \). Note that the region in which exchange occurs (stippled area) is not confined to the same portions of the vasculature as is traced by the reference indicator (hatched area). Most important, the transit time for the reference indicator is influenced both by the flow heterogeneity and by the volume heterogeneity in precapillary and postcapillary volumes (Equation 1 and text). Section III shows a measurement that might be made with in vivo microscopy. Red cell transit times can be converted to transit times measured in Section I only by flow weighting the individual transit times. A microsphere is shown lodged in an arteriole to emphasize that the sphere distribution need not be equivalent to either the distribution described by the tracers or that described by direct observation.
each bit of tissue from the quantity of indicator trapped. Although these techniques provide a direct measurement of flow heterogeneity, they are limited in spatial resolution. Counting statistics make accuracy inversely proportional to the tissue sample size; the size of tissue in which heterogeneity can be detected is dependent on the specific activity of the indicator and the amount of indicator that can be placed in the tissues. This limit is typically reached with a mass of tissue that contains many capillaries, arterioles, and venules, thus making it difficult to know how accurately microvessel flow may be traced by the tissue deposition method. Note also that microspheres probably trace flow heterogeneity in arterioles, where they are lodged in Figure 1, but not necessarily the flow heterogeneity of the exchange vessels.

**Visual observation of flow patterns.** The flow distribution of blood within an organ can be directly visualized using x-ray observation of contrast media or in vivo microscopy. Because of our interest in microvessel function and flow heterogeneity, we focus on the latter technique. Estimates of microvessel flow are derived from the observation of red cells or the motion of labelled plasma or other visible indicators placed within the vasculature. The most important attribute of these techniques is that the main exchange microvessels can be placed under direct observation and that flow heterogeneity can be assigned to specific microvascular segments (Figure 1, Section III).

While providing precise information about capillary function and perfusion, in vivo microscopy is also limited in many ways. Even though velocity dispersion in individual microvessels can be accurately measured, flow can be estimated only by assuming that red cell velocity has a constant relation to the mean velocity and to the capillary cross section (Figure 1, Section III), assumptions that have yet to be validated in the in vivo situation and which may be in doubt.

Perhaps the most limiting aspect of the direct observation method is that it is usually applied to specialized tissues that are either superficial or quite thin and may not be representative of tissues studied by other techniques. These tissues are exposed and often superfused with solutions of poorly defined gas composition. As a result, experiments based on in vivo microscopy may be more likely to be associated with tissue disturbance and altered vascular perfusion patterns. Whereas it has been shown that total muscle flow need not be markedly altered if the tissue is prepared with adequate care, there is no such demonstration for possible changes in flow distribution or heterogeneity.

In addition to flow heterogeneity, transit-time heterogeneity can also be measured using in vivo microscopy in conjunction with labelled cells or plasma. This is of some significance, since the indicator dilution techniques (described below) are based on the evaluation of vascular transit-time measurements (Figure 1; Section III, Equation 1). Since the indicator dilution techniques can be carried out on intact undisturbed tissues, the possibility of making transit-time measurements on a microvessel level provides an opportunity for making direct comparisons between the two techniques; however, discretion must be exercised in relating the two types of measurement. Measurement of the average time required for red cells to pass from an arteriole to a venule and of the statistical distribution of such individual transit times is not the same as the mean transit time determined with an intravascular dilution indicator, even if data are obtained on the microcirculation. This follows from the fact that the average of the transit times for individual cells is not flow weighted. In this study, the time the red cells are in the capillaries is designated the sojourn time. To convert the average sojourn time to the more commonly used mean transit time, i.e., to a measure comparable to that obtained by studying indicator washout patterns, the red cell sojourn times must be flow weighted for each flow path, a calculation only rarely made.

Microcirculatory data have also been used to estimate transit time by dividing the total flow by the estimated number of capillaries among which the flow is distributed and then assuming values for the capillary size and red cell velocity. Such a calculation assumes that the number of parallel paths through which blood flows is well defined. This is not always true, however, since even the definition of capillary is equivocal. Furthermore, the computation is likely to yield improbable results.

Perhaps most important in attempting to reconcile data obtained by microvascular observation with those obtained by indicator dilution measurements is the fact that, even if the flow paths were accurately defined, microvessel observation can only yield data on the heterogeneity of transit times among the smallest vessels, and this need not correspond to a measurement of transit time through the organ, which includes passage through many classes of vessels of all sizes.

An indirect estimate of microvessel flow heterogeneity, yielding a measure intermediate in character between that obtained with the tissue-deposition indicators and with in vivo microscopy, has been made by studying the sequence of capillary filling of microvessels in fixed tissue sections following timed injections of a visible indicator such as carbon black or fluorescent dye. Inherent in this measurement is the thought that the indicators accurately trace flow patterns by their appearance times. This technique extends the spatial limitation of the deposition indicators to smaller tissue regions and can be performed on a wide variety of tissues where direct observation is impossible. The usefulness of the method is severely limited, however, in that it is highly dependent upon preservation of the geometry during preparation of tissue slices and upon the thickness of the histological section.

The most important concern regarding this method is that it is impossible to distinguish true flow heterogeneity from spatial heterogeneity. The method depends on selecting an appropriate sample time after injection. At short times, only the vessels with high flows will be filled. Only as sample times are extended will all vessels patent at a given time be perfused.
However, as the sample times become long to ensure complete filling, vessels that were unperfused at the time of injection will have time to open and, thus, the measurement will inaccurately reflect the instantaneous flow distribution in the organ. The relation of these facts to cyclical vasomotor phenomena will be discussed below.

**Indicator Dilution and Intravascular Tracers.** Direct measurement of regional or microvessel flow heterogeneity is often impossible, and one must therefore resort to indirect estimates of flow heterogeneity or to inferences about heterogeneity based on measurements of extraction fraction or transit times for various tracers. The most appropriate means for the evaluation of flow heterogeneity is through an examination of the venous outflow pattern of an indicator confined to an intravascular space. A bolus of such a tracer is smeared during passage through the vasculature by several processes, including intravascular dispersion due to velocity gradients within the vessels and passage of portions of the bolus through flow paths with different transit times. The net result produces a complex venous outflow curve that contains historical information on all the processes that led to dispersion of the indicator. If the outflow curves can be deconvoluted, it is theoretically possible to obtain measures of the magnitude of heterogeneities in flow.

There are three fundamental problems, often unappreciated by the nonexperts in the field, that limit the ability of these methods to describe flow heterogeneity in the microvessels in a way that might be related to more direct measurements of flow dispersion. First, a critical oversight is commonly made by ignoring the fact that heterogeneities in transit time for a reference indicator are the composite of heterogeneities in both flow and volume, not flow alone (Figure 1, Equation 1). Since there is no independent method to assess the volume of the vascular compartments in which the flow heterogeneity occurs, the transit-time measurement can never be used to estimate directly anything other than the combined heterogeneity of the two parameters. Obviously this issue provides a potential focus for important interactions between investigators studying the microcirculation directly and those using the indicator techniques. Until regional variations in vascular volume are more fully explored, estimates of flow heterogeneity from transit-time data must be accepted with serious reservations.

**Diffusible indicators.** In view of the comments to follow, it is reasonable to assert that measurement of the capillary exchange of diffusible indicators is not a currently acceptable means of assessing the degree of flow heterogeneity. However, this type of measurement has provided much of the historical basis for assuming the existence of flow heterogeneity, and thus its significance and limits should be discussed. Theoretically, the degree of heterogeneity can be estimated from the flow dependence of the capillary exchange of small solutes. One of the earliest assertions that flow heterogeneity existed was derived from the theoretical prediction that the capillary exchange capacity for small solutes in a heterogeneously perfused organ should asymptotically approach a maximum as the flow was increased. It is now clear, however, that understanding the significance of these measurements and relating them to the microcirculation depends on the realization that even under optimal conditions the measurement of the exchange of diffusible indicators yields no estimate of the flow heterogeneity per se. Rather, some estimate of the ratio of the permeability-surface-area product (PS) to the flow (F) can be determined. Unfortunately, as will be discussed below, there is no unequivocal means by which the individual components of the ratio can be independently analyzed. One would hope that the three microvascular parameters P, S, and F might be distinguished by direct measurement using in vivo microscopy but, to date, technical limitations have completely prevented this.

A second problem related to the analysis of microvascular heterogeneities with diffusible tracers arises from the fact that the specific vessels across which solute flux occurs and among which one wishes to study flow heterogeneity cannot easily be related to the transit-time distribution. The capillaries are not the only exchange vessels, and differences in transit times for the intravascular indicator are not solely caused by differences in the flow through the capillaries. Rather, transit time is the aggregate of all the delays incurred in all vessel segments (arterioles, capillaries, and venules) from inflow to outflow (Figure 1, Equation 1). The volume of the venous compartment is larger than that of the other segments, and therefore the transit time through this portion of the vasculature will dominate the characteristics of the outflow pattern. This problem has been dealt with in the case of intravascular indicators by assuming a proportionality between large vessel transit time and capillary transit time, but there is no evidence that such a proportionality exists. Our unpublished observation of the flow patterns in a number of capillary beds with in vivo microscopy suggests that it does not. A second means of handling this problem assumes no volume heterogeneity and that all heterogeneity is in the flow parameter, an equally unlikely assumption. Hence, the heterogeneities in transit time detected at a venous sampling site cannot be presumed to yield specific information about the heterogeneities in capillary flow per se.

Even if an experimental measurement of transit times could be restricted to a measurement of the capillary transit time, there is an inescapable limitation to the use of the indicator dilution techniques in that all three determinants of capillary exchange—PS, F, and vascular volume (V)—may be heterogeneously distributed within the capillaries. Transit time for an intravascular indicator is determined by the ratio of V to F (see Figure 1, Section I), whereas transcapillary exchange is determined by the ratio of PS to F. Were all capillaries homogeneous with respect to V/F, they need not be homogeneous with respect to PS/F because the surface area of a capillary varies linearly with its diameter, whereas its volume varies with the square of
its diameter. Consequently, precise measurement of capillary transit time of a diffusible indicator in comparison with the transit time of a reference indicator need not yield a unique deconvolution of the components of the transit times into heterogeneities of PS and V, let alone of F. These problems are discussed more fully by Bassingthwaite and Goresky and Bass and Robinson.

**Heterogeneity—Spatial and Temporal**

We have discussed issues related to largely steady-state analysis of the heterogeneity of flow distribution among perfused vessels extant within a tissue. However, not all vessels within a tissue are perfused at any moment, thus leading to spatial heterogeneity of perfusion. Such heterogeneity will have a major impact on the microvascular transport capacity, but it must be maintained distinct from flow heterogeneity and analyzed separately.

While the subject of this review is flow heterogeneity, it should be recognized that spatial heterogeneity also contributes importantly to estimates of flow heterogeneities measured with the different methods. The volume of tissue in which flow is detected may be quite different and, in some cases, is undetermined (Table 1). This raises an important issue regarding sampling and comparison of data between laboratories, since the heterogeneities that are observed with one sampling technique may be undetectable with another. For example, heterogeneities in uptake of diffusible indicators would likely pass undetected in most in vivo microcirculatory experiments since the sampling window for an average microscopic observation is usually 0.4 mm × 0.7 mm and only 50–100 μm deep, while the sample size used to study uptake of tracer water by Paradise et al was 0.2 ml or roughly equal to a sample 6-mm square. Only by exhaustive, repeated sampling of the muscle surface would a microcirculatory observation ever delineate even this relatively small-scale heterogeneity detected by the tracer methodology.

The distinction between spatial heterogeneity and flow heterogeneity may become blurred by the presence of spontaneous cyclic vasomotion or temporal heterogeneity. Since all vessels need not oscillate in synchrony, the degree of heterogeneity may shift in time. The effect of cyclic vasomotion on exchange is complex and consideration is beyond the scope of this work. However, the presence of cyclic vasomotion will have different effects on various measurement techniques. This is related to the fact that each of the methods used in the assessment of flow distribution is subject to significant and different sampling errors as a result of temporal and spatial variations in flow. Va- somotion, which is common in the microcirculation, leads to cyclic variations in flow and its distribution. Therefore, the sample interval used to obtain data will influence the degree of heterogeneity recorded. As the sample interval becomes long relative to the period of oscillation, flow will appear to become more uniform.

Without details on the frequency of vasomotion and the sampling interval in most experiments, the importance of this cannot be fully assessed. However, a few observations are pertinent. The in vivo observation techniques involve essentially instantaneous samples, since they are commonly obtained from stop-motion playback of video recordings. The indicator dilution methods and the microsphere technique integrate the flow over a period that is usually several seconds, as determined by the dispersion of the indicator between the injection and sample sites. When using the visible indicators of capillary flow and subsequent histological examination, equilibration times must be long enough to ensure that the indicator traces all patent flow paths. This requirement implies a long equilibra-

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**Table 1. Measured Perfusion Heterogeneities in Literature by Sample Size, Method, Index, and Species**

<table>
<thead>
<tr>
<th>Tissue sample size (mm²)</th>
<th>Method</th>
<th>Index of heterogeneity</th>
<th>Reference</th>
<th>Species tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 × 10³</td>
<td>Number of microspheres in histological cross-section</td>
<td>None given</td>
<td>33</td>
<td>Cat triceps</td>
</tr>
<tr>
<td>1.5 × 10²</td>
<td>A-V transit times</td>
<td>None given</td>
<td>36</td>
<td>Cat gastroc. and sartorius</td>
</tr>
<tr>
<td>9 × 10²</td>
<td>Microspheres</td>
<td>c.v. = 1.07*</td>
<td>25</td>
<td>Dog heart</td>
</tr>
<tr>
<td>2 × 10⁹ to 2 × 10²</td>
<td>Tissue [THO]</td>
<td>c.v. = 0.62* (vasoconstriicted)</td>
<td>39</td>
<td>Dog heart</td>
</tr>
<tr>
<td>1 × 10⁵</td>
<td>None given</td>
<td>c.v. = 0.36</td>
<td>22</td>
<td>Rat skeletal muscle</td>
</tr>
<tr>
<td>1 × 10²</td>
<td>Number of ink-filled capillaries in histological sections</td>
<td>c.v. = 0.28†</td>
<td>40</td>
<td>Rabbit leg muscle</td>
</tr>
<tr>
<td>3 × 10^{-3}</td>
<td>NADH fluorescence in myocardium</td>
<td>None given</td>
<td>42</td>
<td>Rat heart</td>
</tr>
<tr>
<td>3 × 10^{-3}</td>
<td>Direct observation of capillary rbc’s</td>
<td>c.v. = 0.56</td>
<td>54</td>
<td>Hamster cremaster</td>
</tr>
</tbody>
</table>

*Standard deviation estimated using width of outflow fraction curves at 50% of peak height.*
†Estimated at t = 30 seconds; c.v. (coefficient of variation) = 50/×.
tion time, which means the elements of the vasculature that were closed at the time of the initial injection may open, thereby yielding artificially high perfusion fractions and apparent uniformity. Careful experimentation is required to determine how cyclic vasomotion contributes to overall flow heterogeneity.

Summary. The foregoing comments indicate that there is no perfect method for measuring flow heterogeneity; each has its own strengths and weaknesses. Each may measure a different aspect of vascular function, and only by integrating the results obtained by several methods and by appreciating their unique characteristics can a meaningful analysis of vascular heterogeneity be conducted.

Heterogeneity—Magnitude and Control

The following is a compilation of a variety of reports on the nature and control of heterogeneous perfusion in the vasculature. The sampling is not meant to be exhaustive, but to indicate the sources of our understanding of the existence of heterogeneity and the nature of the data in light of the foregoing comments on the strengths and limitations of the methodology.

Length Scale of Heterogeneity Measurements. A sampling of reports of flow heterogeneity in striated muscle is presented in Table 1, with the data presented in decreasing order of the probable size of the vascular element in which the flow heterogeneity was detected. The table indicates the method of measurement and, where reported, a quantitative index of the variability in flow. This organization allows us to present measurements of flow heterogeneities in tissue samples ranging over 7 orders of magnitude. We assume that as the problem of flow heterogeneity is more clearly understood it may be possible to establish quantitative relations between the size of a tissue element perfused and a particular vascular element or metabolic process responsible for the heterogeneity.

The inhomogeneities with the largest scale reflect variations in bulk flow to different parts of the tissue, e.g., between proximal and distal portions of a skeletal muscle or from apex to base and endocardium to epicardium in cardiac muscle. Although the causes of heterogeneities at this level are undetermined, they are likely to reflect differences in the anatomy or behavior of the larger feed vessels and may also reflect regional differences in metabolism.

Flow heterogeneities are also reported among different tissue components in the muscle. Barlow et al., Lindbom, and Sparks and Mohrman all report that flow is distributed heterogeneously between connective tissue and myocytes. Furthermore, there appear to be differences in the flow control in the two tissue types. Such heterogeneities are likely to be partially responsible for multicomponent indicator washout curves seen in this tissue. As a result, flows to connective tissue will be difficult to distinguish from those to heterogeneously perfused portions of the muscle.

Note in Table 1 that the measurement of heterogeneity based on indicator washout cannot be related to a particular element of tissue. This is because both volume and flow heterogeneities from all parts of the tissue contribute to the time course of the washout, and only aggregate heterogeneity can be assessed.

Perfusion heterogeneities with dimensions from a few cubic millimeters to a cubic centimeter are commonly reported. Paradise et al. found that the uptake of tritiated water by the dog extensor digitorum longus muscle was nonuniform, an observation which may be partly due to preparative trauma. Heterogeneities of similar magnitude were found using radioactive microspheres.

In observations that might be related to flow heterogeneity, Steenbergen et al. observed patterns of hypoxic foci that developed when the oxygen content of the perfusate supplying Langendorff hearts was reduced. These foci, whose presence were demonstrated by an increase in NADH fluorescence on the surface of the heart, appeared in discrete patches (approximately 0.5 mm), suggesting a heterogeneous perfusion or, at least, a heterogeneous distribution of the inflowing oxygen. However, their results are also compatible with small-scale heterogeneities in tissue oxygen consumption.

A smaller scale of flow heterogeneity (10⁻² mm) is suggested by data obtained with histological examination of sections made from muscles fixed following timed injection of visible indicators. Capillary filling is patchy, and small groups of capillaries fill more rapidly with indicator than their neighbors, suggesting heterogeneous inflow of the dye.

There is anatomical and functional evidence to support our opinion that the regions of heterogeneous perfusion with dimensions of cubic millimeters are attributable to the behavior of single arterioles that, by opening and closing, can control flow simultaneously in groups of capillaries. This view of microvessel organization, based on the regulation of the behavior of groups of capillaries, suggests a lower limit to the size of tissue whose perfusion can be regulated and to the degree of control of perfusion heterogeneity. However, only preliminary studies of the participation of these units in control have been carried out. (This view of capillary flow control and heterogeneity may have some clinical significance. If flow to a small group of capillaries is controlled by a single arteriole, then occlusion of the vessel might be expected to produce a local area of tissue necrosis. Factor and Sonnenblick report that small localized foci of tissue necrosis occur in certain types of cardiomyopathies, with a size similar to that of the groups of capillaries filled by injection indicators. Furthermore, it has been proposed that Duchenne’s muscular dystrophy may be based on a similar vascular disorder.)

The smallest scale upon which flow heterogeneities might be examined is at the level of single microvessels (Table 1, bottom). In the past 20 years, there have been many observations on red cell flow patterns in microcirculation, but relatively few have been conducted in a manner that allows quantitative assessment of the magnitude of perfusion heterogeneity. Capillary flow has not been measured directly but has been in-
Flow Heterogeneity in Striated Muscle

Defered from red cell velocity. The coefficients of variation of measured red cell velocity that have been reported suggest a fairly large degree of microvessel flow heterogeneity, with values ranging from 30–60%. Where velocity histograms have been published, they show substantial dispersion and are often skewed toward low velocities.

Flow need not be homogeneous along the length of an individual capillary because there are frequent cross-connections between adjacent capillary segments through which blood can flow. As blood moves along a capillary and passes from one segment to another, there should be step changes in flow at the bifurcations where cross-connections originate that would cause longitudinal heterogeneity in capillary segment flow. No one has yet made a systematic study of this possibility by making serial measurements along the length of the various flow paths in striated muscle.

Control of flow heterogeneity. Is it possible to alter the capillary exchange capacity by making local, neural, or endocrine mechanisms? This possibility has not been investigated in detail, but there is abundant evidence that has been interpreted to indicate that bulk flow into a tissue and the functional distribution of flow within the tissue may be affected independently by physiological control processes. Little effort has been devoted to an evaluation of these reports in the light of current understanding of the accuracy of the measurement techniques. There are few data showing control of heterogeneity based on direct observation of the vasculature, assessment of the behavior of microspheres or tissue deposition indicators, or use of intravascular indicators.

The potential for differential control of total flow and distribution (heterogeneity) is important because in principle it is possible to alter the capillary perfusion heterogeneity and the net capillary exchange by changing the number of locations. To the extent that venous flow were uniformly distributed, the observation that an increase in arterial oxygen tension in human subjects, induced by elevating the fraction of oxygen in the inspired gas, produced little change in mean tissue PO2 while broadening the tissue PO2 histogram and producing a fall in tissue PO2 in a significant number of locations. To the extent that venous blood oxygen saturation can be taken as an index of local flow, the observation by Monroe et al that vasodilation causes a decrease in the heterogeneity of venular oxygen saturation in the myocardium supports the proposal that heterogeneity is physiologically controlled. In a number of experimental preparations, raising the total oxygen input to a tissue by elevating perfusion pressure or arterial oxygen content has been reported to increase oxygen consumption. The cause for this increase is not clear, but it has been observed under conditions in which the venous oxygen saturation and blood flow are high enough to make it unlikely that capillary blood flow would have been limiting if the flow were uniformly distributed.
Measurements of capillary red cell flow by microscopic observation provide the most direct means of assessing microvascular flow dispersion, but such measurements have only rarely been made during experimental interventions, when vascular control mechanisms could be studied. Vetterlein and Schmidt showed that infusion of acetylcholine caused a decrease in the velocity of red blood cells in marginal capillaries in striated muscle at a time when total flow into the muscle increased. Their finding suggests that vasodilation induced either massive capillary recruitment or increased capillary flow heterogeneity. The significance of this observation is compromised by the fact that marginal portions of the tissue were observed, and thus the vessels studied may have been damaged or not representative of the tissue as a whole. Burton and Johnson found that the velocity distributions of red cells in capillaries of cat sartorius muscle were broadened during reactive hyperemia, and Damon and Duling reported that the standard deviation of velocities sampled in capillaries of the hamster anterior tibialis muscle increased during muscular contraction. Sarelius et al also reported that the standard deviation of the velocity; over a sevenfold change in blood flows.

Examination of Table 2 shows a high degree of parallelism between the mean velocity and the standard deviation of the velocity; over a sevenfold change in mean velocity, the coefficient of variation varies by less than 2. The constancy of the coefficient of variation suggests that the relative dispersion of the flow among the microvessels was invariant in the face of these changes in vascular tone. The simple interpretation is that the increased standard deviation was predominantly the result of an increase in total flow through the system, not an alteration in the distribution of flow among the vessels.

### Table 2. Means, Standard Deviations, and Coefficients of Variation of Capillary Red Blood Cell Velocities in Striated Muscles

<table>
<thead>
<tr>
<th>Muscles*</th>
<th>Mean (μm/sec)</th>
<th>Standard deviation (μm/sec)</th>
<th>Coefficient of variation</th>
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<td>53</td>
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<tr>
<td>431</td>
<td>284</td>
<td>0.66</td>
<td>8</td>
<td>8</td>
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<tr>
<td>548</td>
<td>412</td>
<td>0.75</td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td>675</td>
<td>302</td>
<td>0.45</td>
<td>51</td>
<td>51</td>
</tr>
</tbody>
</table>

* Tibialis anterior, hamster<sup>51</sup>; cremaster, hamster<sup>54</sup>; tenuissimus, rabbit<sup>51</sup>; and spinotrapezius, rat<sup>51</sup>

These findings suggest that for the metabolic stimuli reported here, capillary flow heterogeneity may not be under physiological control. Rather, it appears that the fractional distribution of flow among the capillaries is stable. The constancy of flow dispersion, even with maximal vasodilation, suggests that flow distribution may depend on undefined aspects of vascular function that are inherent in the structure and rheology of microcirculation. It is possible that a 60% coefficient of variation represents a minimal value for perfusion heterogeneity in capillaries perfused with blood and that the heterogeneity is determined by basic patterns of red cell flow in capillaries. It is interesting to note in Table 1 that a similar trend is suggested in whole organ experiments. The data show rather small ranges for the coefficient of variation, much smaller than the range of blood flows.

Data in Table 2 were chosen to reflect heterogeneities at the level of the capillaries, and since they were all obtained using in vivo microscopy, they reflect the behavior of relatively small sample volumes. Other nonuniformities in perfusion observed in whole organ experiments may reflect nonuniform perfusion on a larger scale than the capillary. This possibility would imply the existence of integrative vasomotor control in relatively large vessels. We must emphasize that our focus here is on flow heterogeneity and does not address possible heterogeneity of spatial distribution of perfused capillaries. However, it is apparent that spatial heterogeneity must also contribute to the limits of exchange.

An interesting aspect of this problem is that functional dilation is usually accompanied by an increase in the number of capillaries containing flowing red cells. Since the process of capillary recruitment takes place
while maintaining a constant coefficient of variation in capillary flow (Table 2), we infer that the capillary recruitment during vasodilation is accomplished in such a way that the flow through the population of newly recruited capillaries experiences the same fractionation as does that passing through the capillaries perfused in the control condition. It is unknown whether recruitment of capillaries with a constant degree of flow dispersion occurs or how this is accomplished. The possibility that capillary recruitment is related to arteriolar closure was deduced from observations of capillary recruitment patterns by Honig et al. Their data, as well as ours, are consistent with the idea that capillaries are recruited in small functional groups or units and that the heterogeneity of flow among these groups is the source of the relatively constant dispersion. This possibility could be easily explored in striated muscle by direct measurement of flow at the origin of these units, a measurement yet to be made under well-defined conditions.

**Effect of Heterogeneity on Microvessel Function**

**Eff ect on solute exchange.** While the foregoing suggests that perfusion heterogeneity of the capillaries may not be metabolically regulated, it does not detail how a given level of heterogeneity might affect capillary exchange. We have used the fact that the coefficients of variation shown in Table 2 are constant over a variety of experimental circumstances to obtain a quantitative estimate of the functional significance of changes in flow, capillary density, and absolute dispersion. We assumed that the same degree of flow heterogeneity would be reflected in the capillaries of the dog gracilis, a tissue in which all the necessary data were available. Based on published values for PS and capillary surface area at rest and during exercise, we made a simple calculation to assess the effects of a constant level of heterogeneity on capillary transport at resting blood flow and during muscle stimulation at 4 Hz. We used the heterogeneity estimate to predict the spread of clearances around the average values. For simplicity, clearances (C) were normalized to the existing PS. On transition from resting to 4 Hz contraction, the calculation predicted that the average C/PS would increase by 156% when all capillaries were perfused identically. Assuming a heterogeneous distribution of flow with a coefficient of variation of 60% we computed a reduction in the capillary exchange capacity of 8% from the ideal case at rest, but only 1% following 4 Hz stimulation. Thus, the effect of flow heterogeneity did appear to be less during muscle work, but this is the result of the nonlinearities in the relation between flow and exchange. More important is the fact that the observed heterogeneity seems to have a rather small effect on capillary exchange.

Renkin has utilized a more complex model of capillary exchange, as described by Bass and Robinson, to compute the degree of heterogeneity of PS/F that would be required to explain the flow dependence of extraction observed in striated muscle. His computation indicates that a coefficient of variation of about 90% would be required, i.e., greater by 30% than we observed in microcirculation. The inequality between the coefficient of variation estimated by Renkin and that observed in vivo studies suggests that the heterogeneities traced out by the indicator diffusion methods may well reflect the combined results of both macro- and microscale heterogeneities. However, we recognize that our assessment is a very simple one and that final conclusions will require fitting the presently available data on capillary flow heterogeneity to more complex exchange models.

**Capillary heterogeneity and oxygen transport.** We can also make a very simple estimate of the effect of capillary perfusion heterogeneity on tissue oxygenation with an analysis similar to one proposed some years ago by Kety, taking advantage of apparent constancy of capillary flow heterogeneity (Table 2). If we assume that the capillary can be treated as a cylinder supplying a uniform annulus of tissue, that the affinity of the tissue enzymes for oxygen is high relative to the oxygen tension in the tissue and blood, and that blood flow velocity is constant along the length of the vessel, then oxygen content will fall linearly from the arteriolar to the venular end of the capillary, with a slope proportional to the ratio of the metabolic rate to the blood flow (Figure 2). This linear fall in capillary oxygen content will continue until tissue Po2 falls

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**Figure 2.** Estimate of the effect of heterogeneous flow on capillary oxygenation in resting (top) and working (bottom) striated muscle. It was assumed that the capillary oxygen content falls linearly along the capillary with a slope equal to the ratio of the tissue oxygen consumption to the flow. The coefficient of variation of capillary flow was assumed to be 0.60. Capillary oxygen contents for flow 1 standard deviation above and below the mean are shown by the dotted lines.
below that required for mitochondrial oxidative phosphorylation. For the sake of this discussion, we assume that the oxygen consumption is a zero-order process.

We used literature data for the dog gracilis muscle for flows varying from 5 ml/min/100 g at rest to 75 ml/min/100 g during 4 Hz stimulation. We chose oxygen consumptions of .22 and 12.8 ml/min/100 g at rest and during 4 Hz stimulation. Assuming that the coefficient of variation of capillary blood flow is equal to 0.60, we examine the fall in oxygen content in capillaries perfused at the mean blood flow and those perfused at the mean flow plus or minus one standard deviation of flow. The predicted diminution of capillary blood oxygen content along the length of the capillary is shown for the resting muscle in Figure 2 (top panel) and for a muscle contracting at 4 Hz in Figure 2 (bottom panel). The lower panel shows that, for the working muscle, the oxygen content falls to zero in capillaries that are perfused at mean flow less one standard deviation of flow. Thus, even at one standard deviation from the mean velocities that we observed, it appears that oxygen supply could be rate limiting for oxygen uptake by the tissue. Many capillaries would naturally have even slower velocities.

It must be emphasized that both this analysis of capillary oxygen gradients and the preceding one on clearance heterogeneities are based only on an examination of the expected effects of flow heterogeneities in capillaries. The critical factor in the clearance calculation is the ratio of PS to F in a given capillary. In our analysis, we have ignored systematic variations in permeability and/or capillary geometry, which clearly could alter our predictions. There are no systematic studies comparing velocity and capillary permeability, the absence of which must provide a spur for new measurements. Similarly, heterogeneities in capillary length or in flow path could in principle offset changes in flow heterogeneity and obviate the analysis carried out above. Length measurements in the microcirculation do show a fair degree of heterogeneity. The critical issue is the ratio of the path length to the flow velocity, and Kitzman and Johnson report that there is no correlation between the two variables. Also of importance is data on path length change during vasodilation. The available data suggest that the change in flow path is minimal, but there is a great need for more detailed exploration of the relations between various heterogeneities in microvessels.

Relations between Flow and Hematocrit. Our initial interest in the problem of microvessel flow heterogeneity was prompted by the often ignored fact that flow dispersion may indirectly influence tissue oxygenation by inducing heterogeneities in red cell distribution and capillary hematocrit. Knowledge of capillary hematocrit is also critical to the interpretation of tracer estimates of heterogeneity. The potential importance of changes in capillary hematocrit may be appreciated from the fact that microvessel hematocrits in striated muscle are consistently less than systemic and vary from 5 to 37% as a function of vasomotor state. Thus, if the reported capillary hematocrits accurately reflect the red cell flux, the delivery of red cells to the capillaries may be increased by as much as sevenfold through a redistribution of blood flow and red cells within the tissue.

Contribution of Capillary Flow Heterogeneity to Low Capillary Hematocrit. While it is clear from in vitro measurements that heterogeneous flow can induce heterogeneities in hematocrit, none of the efforts to relate heterogeneous capillary flow to low capillary hematocrit have been entirely successful. Kanzow et al attempted to use histograms to explain the low number of microvessel hematocrits within microcirculation, with the discovery that hematocrit reductions as a result of heterogeneous branching were inadequate to explain the data. Some amount of red cell shunting had to be added to the model to reconcile theory and observation, but no red cell shunts have been observed in striated muscle.

Coccelet pointed out that the effects of heterogeneities on microvessel tube hematocrit might be compounded over successive bifurcations leading to large effects on capillary hematocrit. He concluded that heterogeneous flows might thereby explain the findings on capillary tube hematocrit. However, his model predicted that there would be a significant population of microvessels with zero hematocrit, and, in spite of efforts to detect such vessels, they do not appear to exist in significant numbers in tissues in which low capillary hematocrits are reported. The data shown in Table 2 provide a unique opportunity to evaluate the hypothesis that capillary hematocrit may be related to dispersion of capillary flow. As pointed out above, capillary hematocrit increases with vasodilation, which leads to the expectation that, if the heterogeneity hypothesis is valid, greater flow heterogeneity should be associated with a reduction in hematocrit. The data in Table 2 make it obvious that the absolute dispersion of capillary velocity increases and that the relative dispersion in velocity is unchanged during vasodilation, a time when the hematocrit increases. Thus, the measurements of velocity dispersions in the microvasculature are inconsistent with the hypothesis that the capillary hematocrit changes because of altered flow dispersion.

A pivotal measurement that should be taken in order to deepen our understanding of the relations among flow heterogeneity, capillary rheology, and microvessel hematocrit is the discharge hematocrit of individual microvessels. Of particular interest would be the hematocrit in discharge hematocrit. The need for this measurement stems from the fact that it will provide the unique link between flow heterogeneity, red cell flux, and tube hematocrit. Because of the small size, reactivity, and flow patterns in microvessels, it has been difficult to make such measurements, but the literature does contain one report in which undefined renal "microvessels" were punctured with micropettes and blood samples were withdrawn, yielding an average value for the microvessel samples equal to the systemic hematocrit. Similar measurements have been made in this laboratory on vessels as small as...
Flow Heterogeneity in Striated Muscle

9 μm in diameter with an equivalent result, i.e., that discharge hematocrits in both arterial and venous microvessels are equal to arterial hematocrits. If these measurements prove to be representative of microcirculation as a whole, they strongly imply that low capillary hematocrits are not the result of flow heterogeneity within the microcirculation but rather that low capillary hematocrit results from some other aspect of microvascular function.10,87

Summary

This review leads us to a number of conclusions and suggestions for further study. First, we find wide differences in the meaning of flow heterogeneity, arising as a result of the different methods used. These differences will have to be reconciled to form a comprehensive view of the role of heterogeneity in determining vascular function.

Second, in the future, the meaning of heterogeneity must be clearly defined and related to a particular microvascular component, and it is imperative that the differences in scale of heterogeneity be appreciated when comparing data from various laboratories. These heterogeneities have different implications for function, and failure to distinguish among them leads to confusion.

Third, the degree to which perfusion heterogeneity is regulated in the microcirculation remains in doubt. Reports of variations in flow heterogeneity in response to physiological stimuli are for the most part based on highly questionable indirect methods.

Fourth, the heterogeneity that can be demonstrated at the capillary level within striated muscle does not appear to be large relative to the capacity for the microcirculation to exchange most diffusible solutes. Thus, the inferences regarding heterogeneity, as evidenced by diffusible indicators, are likely to be the result of different preparations, damage to the preparations, or perhaps large-scale heterogeneities in the tissue. An alternate possibility would be that the heterogeneity occurs at the microvascular level but reflects some other aspect of microcirculatory function, such as length or hematocrit heterogeneities, but not flow heterogeneities.

Fifth, flow heterogeneity within microvessels implies important consequences for capillary exchange and tissue oxygenation. Heterogeneities of velocity of a magnitude comparable to those observed by direct visualization of microcirculation can clearly produce reductions in oxygen supply to small tissue regions of a degree that may limit oxygen delivery, and thereby, tissue function.

Sixth, flow heterogeneity may also influence capillary hematocrit and/or red cell spacing by producing cell separation at bifurcations and a resultant reduction in mean capillary tube hematocrit. There is as yet no agreement on why and how these hematocrits influence tissue oxygenation and function. Although several hypotheses are advanced to explain the distribution of blood flow and red cells within microcirculation, each lacks a critical experimental test at present.

Further studies must concentrate on three aspects of the measurement of capillary flow heterogeneity: 1) additional quantitative studies on the degree of perfusion heterogeneity within the vasculature, both on the macroscopic and microscopic scale; 2) complementary simultaneous measurements of flow, vessel geometry, permeability, and volume to describe precisely the process of capillary exchange; and 3) assessment of flow heterogeneity at microvessel bifurcations with concurrent measurements of blood flow and red cell flux.

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