Po2 measurements in the microcirculation using phosphorescence quenching microscopy at high magnification

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Golub AS, Pittman RN. Po2 measurements in the microcirculation using phosphorescence quenching microscopy at high magnification. Am J Physiol Heart Circ Physiol 294: H2905–H2916, 2008. First published March 28, 2008; doi:10.1152/ajpheart.01347.2007.—In phosphorescence quenching microscopy (PQM), the multiple excitation of a reference volume produces the integration of oxygen consumption artifacts caused by individual flashes. We analyzed the performance of two types of PQM instruments to explain reported data on Po2 in the microcirculation. The combination of a large excitation area (LEA) and high flash rate produces a large oxygen photoconsumption artifact manifested differently in stationary and flowing fluids. A LEA instrument strongly depresses Po2 in a motionless tissue, but less in flowing blood, creating an apparent transmural Po2 drop in arterioles. The proposed model explains the mechanisms responsible for producing apparent transmural and longitudinal Po2 gradients in arterioles, a Po2 rise in venules, a hypothetical high respiratory rate in the arteriolar wall and mesenteric tissue, a low Po2 in lymphatic microvessels, and both low and uniform tissue Po2. This alternative explanation for reported paradoxical results of Po2 distribution in the microcirculation obviates the need to revise the dominant role of capillaries in oxygen transport to tissue. Finding a way to eliminate the photoconsumption artifact is crucial for accurate microscopic oxygen measurements in microvascular networks and tissue. The PQM technique that employs a small excitation area (SEA) together with a low flash rate was specially designed to avoid accumulated oxygen photoconsumption in flowing blood and lymph. The related scanning SEA instrument provides artifact-free Po2 measurements in stationary tissue and motionless fluids. Thus the SEA technique significantly improves the accuracy of microscopic Po2 measurements in the microcirculation using the PQM.

oxygen consumption; oxygen tension; oxygen gradient

MEASUREMENT OF OXYGEN TENSION (PO2) IN INDIVIDUAL MICROVESSELs AND IN MICROSCOPIC VOLUMES OF Tissue USING A SOLELY OPTICAL TECHNIQUE represents an attractive approach for physiological research. The phosphorescence quenching method opened the way for noninvasive measurements of the concentration of dissolved oxygen in living organs isolated from atmospheric oxygen. The initial reports of the phosphorescence quenching method (69, 74) demonstrated that it was a robust in vitro and in vivo technique for measurement of Po2. Extension of the method to microscopic measurements of Po2 provided crucial advantages compared with the conventional microelectrode technique (52, 53, 56, 58, 75). However, conditions inside microscopic volumes of tissue and individual microvessels (smaller than 100 μm ID) are different from those in macroscopic volumes containing multiple vessels of different types and diameters. Thus the transition of the phosphorescence quenching method to high-magnification biomicroscopy requires further investigation and analysis aimed to improve its accuracy and reliability.

In phosphorescence quenching microscopy (PQM), the excitation volume is formed by the intersection of a cone of excitation light, coming out of the objective lens or condenser, with a tissue volume or vascular segments containing the dissolved phosphorescent probe. In thick tissues and vessels the depth of excitation is limited by light absorption and scattering. The volume from which the phosphorescence emission is collected is referred to as the sampled, detection, or reference volume. It can be limited by the size of the excitation spot, by a diaphragm in the light collection-detection path, and by the penetration depth of the excitation light, all parameters that are dependent on the instrument design. Since the probe [usually Pd-meso-tetra (4-carboxyphenyl) porphyrin, Pd-MTCCPP, or its derivatives] is conjugated with albumin, the results obtained during the time between probe injection into the bloodstream and its extravasation from the circulation should be attributed specifically to the intravascular compartment (44, 56). In skeletal muscle, phosphorescence from the extravascular space was not detected even 35 min after injection of the probe (44). In tumor and mesenteric microvessels, more rapid extravasation of the probe provides a detectable concentration in the interstitium around the microvessels after 15–20 min (5, 58, 63), which allows Po2 measurements in the tissue outside a vessel (29, 58, 67). Thus in tissues with more leaky microvessels, measurements of intravascular Po2 become problematic, since the phosphorescence signal collected from the vessel region contains a combination of intra- and extravascular components. Another problem with perivascular Po2 measurements is caused by steep local Po2 gradients, whereby the phosphorescence signal contains components with a range of lifetimes. Such heterogeneous signals require more complicated methods of data analysis for recovery of the Po2 distribution in the sampled volume (22, 24, 30, 42, 70, 75). These analytical methods demand a high signal-to-noise ratio that cannot be achieved by single-flash excitation, because of the microscopic size of the volume of interest and limitations on excitation light intensity and probe concentration. Thus multiple excitation flashes combined with averaging of the decay curves has been employed to obtain an acceptable quality of the signal (56, 69). To achieve this goal, previous investigators have also increased the probe concentration from ~1 μM to 0.5–1 mM (56, 69, 74). The intensity of the excitation light was also increased by

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one to two orders of magnitude by focusing light with the microscope objective lens, used as an epi-illumination condenser, and employing higher flash energy (56, 69). The combined effect of augmentation of these two parameters on the concentration of the excited triplet state of the probe could be more than a factor of $10^4$. Originally, the oxygen consumption by the phosphorescence quenching method under microscopic conditions was estimated to be 60 nM/min (0.04 nM/flash) (69). Increasing this value by a factor of $10^4$ may result in a consumption of 0.4 μM per flash (~0.25 mmHg/flash) and thus potentially becomes a serious problem in the microscopic application of the method in measurements involving multiple excitations of the same sampled volume.

During the last decade research teams working with different types of PQM instruments have achieved a consensus about the significance of oxygen consumption by the method in microscopic PO2 measurements (6, 42). They also agree that the magnitude of the photoconsumption has to be estimated for conditions where PQM is applied in a microscopic environment (6, 21, 42). There is also concurrence on the limitation of excitation energy density and probe concentration needed to keep the magnitude of the photoconsumption artifact below 1 mmHg (6, 21, 42, 46). Considering the importance of the parameters involved in the PQM measurement procedure (probe concentration, energy density, flash rate, size of the excitation and detection areas, and temperature of the object), they all should be determined and reported. Control of all these factors is important for reliability and reproducibility of results.

Surprisingly, the experimental results obtained with the different types of PQM instruments on the same objects, with the same probe concentration and excitation energy density, are substantially discrepant (7, 21, 26, 43, 60, 63, 73). Reported data on longitudinal and transmural PO2 gradients, tissue PO2, rate of oxygen consumption in connective tissue and the vascular wall, and PO2 in capillaries and venules in the rat mesentery are so dissimilar that they support absolutely different concepts of oxygen transport to tissue (7, 20, 21, 26, 43, 63, 73). It is demonstrable that the source of these discrepancies is related to the difference in the design of the employed PQM instruments and specific features of the measuring procedures. Understanding the source of the discrepancies and finding criteria for verification of the experimental results require a comparative analysis of the design and performance of the types of PQM instruments employed in the studies.

**Oxygen Consumption by PQM**

The oxygen probe employed in the phosphorescence quenching method generates singlet oxygen on irradiation (15). A brief excitation light pulse generates excited triplet states of Pd-MTCCPP, which in turn convert a fraction of the dissolved oxygen molecules into a reactive form, which irreversibly reacts with organic molecules abundant in plasma and tissue (6, 14, 32, 40, 42, 69). Throughout the text we will use the term “photoconsumption” for the photoactivated disappearance of oxygen from the excitation region. The amount of dissolved oxygen consumed by a single flash depends on the probe concentration, energy density of the excitation light pulse, concentration of dissolved oxygen, and concentration of organic molecules available for photooxidation. The PO2 decrease, $\Delta P$, caused in the excitation volume by a single flash also depends on the solubility of oxygen in the medium.

The levels of probe concentration and excitation energy density are usually selected empirically to keep $\Delta P < 1$ mmHg at the highest PO2s, considering this an acceptable error in physiological studies (6, 21, 42). The in vitro probe concentration used in initial reports of the phosphorescence quenching method (69) was based on an optical density of 0.1, yielding $\sim 0.3$ μg/ml for Pd-MTCCPP. The probe concentration in plasma used in the original microscopic application of the method was 0.75 mg/ml (56). In many publications since then the typical concentration of the probe in plasma has been in the range 0.5 to 1 mg/ml.

The oxygen consumption in plasma, caused by the flash of a conventional xenon lamp (0.5 J/flash, 430 ± 30 nm excitation filter), delivered by standard epi-illumination optics (objective ×40) to the microscopic field (50 pJ·μm$^{-2}$·flash$^{-1}$) and at a probe concentration 0.5 mg/ml, was found in a test experiment to be 0.1–0.3 mmHg/flash at a PO2 of 80 mmHg (0.1–0.4% of actual PO2) (42). Tsai et al. (63) in their test with a higher flash energy (1.5 J/flash, 420 nm excitation filter) obtained an oxygen consumption of 0.02 mmHg/flash at a PO2 of 37 mmHg (0.05%). It was recently demonstrated that the photoconsumption in this latter test was underestimated by at least 10-fold (21, 61). New tests of photoconsumption performed by the same laboratory under the microscopic conditions resulted in values of 0.39–0.84 μM/flash (~0.3–0.6 mmHg/flash or 0.4–0.8% at a PO2 of 72 mmHg) (6). Previously published values of PO2 photoconsumption by the method at an energy density of 81 pJ·μm$^{-2}$·flash$^{-1}$ were in the range 0.15–0.28 mmHg at a PO2 of 68 mmHg (0.2–0.4%) (20). A similar range of oxygen photoconsumption could be expected in instruments that employed a flash lamp at 0.72 J/flash and energy density in the focal plane of 30–90 pJ·μm$^{-2}$·flash$^{-1}$ (46, 51). Thus we can assume that the typical flash energy density used for intravascular and interstitial PO2 measurements is below 100 pJ·μm$^{-2}$·flash$^{-1}$. Under these conditions the magnitude of photoconsumption is in the range of 0.3–0.6 mmHg/flash at 60 mmHg (0.5–1% of actual PO2). To illustrate the disturbances caused by photoconsumption, we will use typical values of PO2 decrease per flash for instruments with flash lamp excitation sources or lasers with the same energy density in the object plane. To reflect the inequality of the probe concentration in plasma and interstitium, we will calculate examples with 1% photoconsumption per flash in plasma and 0.5% in the tissue. The magnitude of the photoconsumption artifact caused by other values of $k$ can be calculated by using the proposed equations.

Because of photoconsumption of the small fraction of dissolved oxygen by each flash and multiple excitation flashes, the oxygen concentration in the excitation region progressively decreases, so that the measured PO2 value is lower than the actual one (6, 42). To evaluate the effect of accumulated photoconsumption on the results of PO2 measurements, we developed a simple model of the PQM measurement procedure. It is important to notice that the accumulation of photoconsumption is negative and results in progressive reduction of PO2 in a sampled volume during the excitation light pulse train.

Registration of oxygen disappearance from a sealed illuminated volume containing the phosphorescent probe provided information on the rate of accumulated photoconsumption (6, 40, 42). The oxygen disappearance curves have a rate of
decline strongly dependent on PO2. On that basis we assume that first-order kinetics can be used to describe the process of accumulated photoconsumption.

**Photoconsumption Error in a Stationary Fluid**

The plot in Fig. 1 demonstrates the effect on measured PO2 due to multiple excitations produced in a stationary medium (agarose gel film with immobilized Pd-MTCPP) in which the sampled volume accumulates oxygen consumption for a number of excitation pulses \( N = F \cdot T \), where \( F \) is the flash rate and \( T \) is the total duration of the flash illumination period. The accumulated photoconsumption in this stationary medium can be estimated by the following simple model. The PO2 decrement \( (\Delta P) \) caused by a single light pulse can be presented by first-order kinetics:

\[
\Delta P = kP
\]

where \( P \) is the PO2 in the medium before the excitation flash and \( k = \Delta P/P \) is the photoconsumption coefficient representing the decrement in PO2 caused by a single flash.

Under this assumption the PO2 in the detection area before and after the train of \( N \) excitation flashes will be denoted by \( P_0 \) and \( P_N \), respectively. Thus the magnitude of the photoconsumption artifact can be expressed as \( P_N/P_0 \). The PO2 drop caused by the \( i \)th flash \((i = 1 \ldots N)\) in the sampled volume is \( \Delta P_i = k \cdot P_{i-1} \), where \( k \) is the photoconsumption coefficient and \( P_{i-1} \) is the PO2 in the sampled volume before the \( i \)th flash:

\[
P_i = P_{i-1} - \Delta P_i = P_{i-1} - k \cdot P_{i-1} = P_{i-1}(1 - k)
\]

Thus the measured PO2 in the sampled volume in the detection region after \( N \) flashes is

\[
P_N = P_0(1 - k)^N
\]

Fig. 1. Effect on measured PO2 due to accumulated oxygen consumption by the phosphorescence quenching microscopy method, recorded in an agarose gel film containing the phosphorescence probe Pd-MTCPP. The successful fitting of the experimental data with Eq. 4 demonstrates the validity of the assumption of first-order kinetics. In this in vitro test the PO2 was measured with the phosphorescence microscope in a thin film of gel (0.2 mm thick) placed between a slide and a coverslip. A square area (40 \( \times \) 40 \( \mu \)m) was excited by flash illumination (lamp FX-249 at rate 10 Hz and excitation band 420 \( \pm \) 30 nm). To obtain the entire photoconsumption curve during a short time period, the oxygen consumption artifact was deliberately enhanced by using a probe concentration of 1 mg/ml Pd-MTCPP and energy of 4 J/flash, 2 and 8 times higher than the typical values used in vivo experiments (0.5 mg/ml and 0.5 J/flash). The photoconsumption rate determined from parameter fitting using Eq. 4 was \( k = 0.13 \) or 13 times higher than the typical values used for in vivo measurements. 

**Large Excitation Area and Small Excitation Area Instruments**

The performance of a phosphorescence quenching microscope applied to PO2 measurements in moving media (e.g., flowing blood, lymph) significantly depends on its design principle, especially on the size of excitation and detection areas in the object plane. In particular, there are limitations on maximum and minimum of linear velocity of the sampled volume, which contains the excited phosphorescence probe. If the medium is moving too fast, the recorded phosphorescence decay will be distorted and truncated because the light emitted by the sampled volume is moving out of the detection area. If it is moving too slowly, the reference volume will receive multiple excitation flashes and the measured PO2 will be underestimated because of accumulated photoconsumption.

The measuring procedure in a PQM instrument will be analyzed in models related to the size of concentrically arranged excitation and detection areas, with radii \( R \) and \( r \) (see Figs. 3 and 4). The large circle could be as wide as the entire microscopic field and the small circle could be as small as allowed by the diffraction limit of the microscope optics. The vessel under study crosses both areas through their common center. The sampled volume of fluid inside the vessel is limited by a circle of radius \( r \), equal to the size of the detection area. This volume moves along the vessel with velocity \( V \). Existing PQM instruments can be divided into two distinct types, based on the size of their excitation and detection areas (see Figs. 3 and 4). In large excitation area (LEA) instruments the large circle of radius \( R \) is the excitation area and the small circle of radius \( r \) is the detection area. Conversely, in the small excitation area (SEA) instrument the large circle of radius \( R \) is the detection area and the small circle of radius \( r \) is the excitation area.

The photoconsumption in a stationary medium can also be expressed via the flash rate and duration of the excitation period:

\[
P_N/P_0 = (1 - k)^F
\]

The in vitro photoconsumption test presented in Fig. 1 was specially designed to obtain the entire oxygen disappearance curve for a relatively small number of flashes. For that purpose the probe concentration in the agarose gel film and the excitation flash energy were set much higher (two and eight times, respectively) than typical values used for in vivo measurements. Equation 3 provides a good fit for the experimental data, confirming the validity of the proposed first-order kinetics of photoconsumption. The coefficient \( k = 0.13 \) determined by the curve fitting indicates that photoconsumption in this test produced a large decrease in PO2 in the reference volume.

However, even a small value of \( k \), considered as typical, can produce a substantial decrement in PO2 for stationary measurements, if the number of applied flashes is large. The plot of PO2 decrease caused by a pulse train of 90 flashes (as was used in Ref. 63) for different values of \( k \) was calculated according to Eq. 4 and presented in Fig. 2. The PO2 readings for typical values of \( k \) for tissue and plasma \((k = 0.005 \& 0.01)\) were 64 and 40%, respectively, of the actual PO2 values. Thus the accumulated photoconsumption may be responsible for the low apparent PO2 measured in microscopic volumes of tissue and in microvessels with arrested blood flow.
The difference between the two types of PQM instruments, LEA and SEA, will become clear when their performance is analyzed in regard to the velocity of the sampled volume.

The maximum velocity ($V_{\text{max}}$) of the sampled volume that allows undistorted recording of the decay curve is limited by the radius of the excitation (in LEA) or detection (in SEA) areas and the duration of the recorded phosphorescence decay curve. If the boundary between excited and nonexcited probe in a LEA instrument moves too fast toward the central detection area, the recorded phosphorescence decay curve (collected from the moving column of fluid) will be distorted and truncated. In a SEA instrument, signal truncation may happen when the sampled volume excited in the central light spot moves too fast to the edge of the detection area. The transit time for the excited region is equal to the duration of the recorded phosphorescence decay curve, which can be taken as seven times the longest lifetime, as recommended for exponential analysis with a signal-to-noise ratio of 1,000 (30). The longest lifetime ($\tau_0$) of the phosphorescent probe occurs in the absence of oxygen; thus $7\tau_0$ can be taken as the limiting time for the determination of $V_{\text{max}}$. The most remote sampled volume in this situation moves from the edge of the excitation area to the detection circle in a LEA instrument and from the central excitation circle to the edge of detection area in a SEA instrument, in both cases for a distance ($R - r$). Thus the maximum velocity $V_{\text{max}}$ limitation is the same for both LEA and SEA instruments:

$$V_{\text{max}} = (R - r)/7\tau_0$$  \hspace{1cm} (5)

For a LEA instrument with objective magnification $\times 40$ (63), where $R = 70 \, \mu m$, $r = 10 \, \mu m$ (along the flow line), and $\tau_0 = 600 \, \mu s$, the maximum velocity for acquiring an undistorted decay curve is 14 mm/s. In SEA instrument with a $\times 40$ objective, $R = 290 \, \mu m$ and $r = 3-4 \, \mu m$ (20, 21); the maximum velocity is $\sim 68 \, mm/s$. Since the PO$_2$ in microvessels is significantly higher than zero (so $\tau < \tau_0$), the upper limit of velocity for PO$_2 \gg 0$ is much higher than that for PO$_2 = 0$. Thus it is unlikely that the maximum velocity can be a source of error in microcirculatory PO$_2$ measurements by both LEA and SEA instruments, if the condition $R \gg r$ is valid. When these radii are not significantly different, $R = 10 \, \mu m$ and $r = 5 \, \mu m$ as in the work reported in Ref. 49, then $V_{\text{max}} = 1.2 \, mm/s$ (at PO$_2 = 0$). Application of that instrument to 1A arterioles with PO$_2 = 40\text{--}70 \, \text{mmHg}$ ($\tau \approx 71\text{--}43 \, \mu s$) increases $V_{\text{max}}$ to 10--17 mm/s, still below the linear velocity in these types of microvessels, $\sim 30 \, mm/s$ (13).

Two other combinations of the area sizes, with equal sizes of excitation and detection areas ($R = r$) both large or both small are excluded from further analysis. The reason is that they can be used only for motionless media, since it follows from Eq. 5 for $R = r$ that $V_{\text{max}} = 0$. An instrument in which both areas are large could be used to reduce the energy density and increase the quality of the signal in a stationary tissue PO$_2$ measurement with low spatial resolution. The case in which excitation and detection areas are both small can be used for confocal tissue PO$_2$ measurements. However, either of the PQM systems with $R = r$ cannot be recommended for application to measurements with a moving sample volume.

**Critical velocity.** The main source of error for PO$_2$ measurements in flowing fluids occurs in the application of an instrument to a medium moving slower than a minimum velocity determined by the instrument design. The limitation of minimum velocity bounds in both types of PQM instrument is based on different criteria. When the velocity ($V$) of the sampled fluid in a microvessel (e.g., plasma, lymph) is high

The main source of error for PO$_2$ measurements in flowing fluids occurs in the application of an instrument to a medium moving slower than a minimum velocity determined by the instrument design. The limitation of minimum velocity bounds in both types of PQM instrument is based on different criteria. When the velocity ($V$) of the sampled fluid in a microvessel (e.g., plasma, lymph) is high
enough, each flash of light excites a separate portion of the moving fluid, so that the averaged curve contains the same small error of photoconsumption (ΔP) as that caused by a single flash. At a velocity below the critical value \( V < V_{cr} \), the same sampled volume undergoes multiple excitations. In that case the \( \text{PO}_2 \) decrease in the sampled volume will accumulate and depend on the number of absorbed excitation light pulses. Accordingly, at a certain flash rate (F) the averaged decay curve will be affected by the accumulated artifact of photoconsumption, to a degree inversely related to the velocity.

The magnitude of the accumulated consumption artifact in multiple excitation measurements depends on the combination of the excitation area radius and flash rate. For a LEA instrument no multiple excitations of a sampled volume of radius \( r \) take place, if this volume moves from the edge of the excitation area to the detection area during the time between two consecutive flashes (see Fig. 4) with a critical velocity of

\[
V_{cr}^{\text{LEA}} = (R + r)F
\]  

To prevent multiple excitations of the same sampled volume in a SEA instrument, the excited volume of radius \( r \) has to move a distance \( 2r \) during the same time, yielding a critical velocity of

\[
V_{cr}^{\text{SEA}} = 2Fr
\]

Comparison of the expressions Eqs. 6 and 7, for \( R \gg r \), demonstrates that \( V_{cr} \) for a SEA instrument is always lower than that for a LEA instrument. For instance, the LEA configuration \( R = 70 \, \mu\text{m}, r = 10 \, \mu\text{m}, \text{and} \, F = 30 \, \text{Hz} \) (63) yields \( V_{cr} = 2400 \, \mu\text{m/s} \), whereas \( V_{cr} \) for a SEA instrument with the same parameters, \( r = 10 \, \mu\text{m} \) and \( F = 30 \, \text{Hz} \) would be \( V_{cr} = 600 \, \mu\text{m/s} \).

When the size of the excitation and detection areas, \( R \) and \( r \), are selected, the flash rate \( F \) cannot be taken as arbitrary because of the bounds on \( V_{cr} \) set by Eqs. 6 and 7 (and vice versa). LEA instruments described in previous publications (46, 51, 63) had \( V_{cr} = 3, 2.4 \) and 2.7 mm/s, respectively, which exceeded the typical velocities in many classes of microvessels of the studied organs. Thus the \( \text{PO}_2 \) measurements obtained with LEA instruments in these classes of vessels should have been affected by the accumulated oxygen consumption of the method itself. Even the SEA type instrument with an excitation spot \( r = 5 \, \mu\text{m} \) and \( F = 20 \, \text{Hz} \) (48) has \( V_{cr} = 200 \, \mu\text{m/s} \), which requires reduction of the flash rate for application to some capillaries.

In addition, the penetration depth in blood in the Soret band (~420 nm) of excitation light is small because of the strong absorbance by hemoglobin (~50% absorption for a path length of 7 \( \mu\text{m} \)). Thus most of the phosphorescence signal in large arterioles and venules is emitted by peripheral plasma layers, whose linear velocity is lower than the mean velocity in a vessel. Therefore the accumulated oxygen consumption artifact in a LEA instrument could take place even if the mean blood velocity in a vessel is higher than \( V_{cr} \).

Because of kinetic limitations (68) the photoconsumption in plasma cannot be effectively compensated by oxygen release from red blood cells (RBCs). Since the half-time of RBC deoxygenation is ~0.15 s (68), there is no time between flashes at 30–80 Hz to release enough bound oxygen to compensate for the photoconsumption artifact. However, oxygen release from RBCs can substantially replenish oxygen in the plasma compartment at flash rates below 5 Hz.

**Advantages of SEA Instruments**

The large excitation area and high flash rate in LEA instruments prevent the replenishing, by diffusion from surrounding volumes, of oxygen consumed in the sampled volume. The relationships between the size of the excitation area and the time \( t = 1/F \) required to replenish 90% of the reduced \( \text{PO}_2 \) in the center of the excitation area can be obtained from the analysis of oxygen diffusion into a cylinder (9):

\[
t_{90} = 0.54R^2/D_{O2}
\]

where \( D_{O2} \) is the diffusion coefficient for oxygen in the medium. Thus, in connective tissue at 37°C where \( D_{O2} = 1.04 \cdot 10^{-5} \, \text{cm}^2/\text{s} \) (1), a wide excitation area \( R = 70 \, \mu\text{m} \) limits the flash period to 2.5 s, much longer than the time interval between flashes at a rate of 30–50 Hz used in typical LEA instruments (46, 63). In contrast, the combination of instrumental parameters \( r = 5 \, \mu\text{m} \) and \( F = 20 \, \text{Hz} \) in a SEA instrument (48) yields \( t_{90} = 13 \, \text{ms} \), shorter than the time between flashes of 50 ms. In that case a substantial amount of the oxygen consumed by photooxidation can be replenished by diffusion from an adjacent vessel or tissue. In this respect, the relationship between LEA and SEA instruments is similar to that between oxygen electrodes with a large tip diameter and microelectrodes (12, 25, 47). Nevertheless, this diffusional compensation in a SEA instrument also cannot be effective for a long pulse train (except when the spot is in close proximity to a vessel that is a source of oxygen). Multiple excitations will deplete the oxygen from the adjacent tissue, so that the accumulated photoconsumption in a stationary medium will be reduced but not fully compensated. For that case a SEA instrument provides the possibility to overcome this problem by scanning the tissue in discrete steps with the excitation light spot (21), thereby avoiding multiple illumination of the same volume.

The SEA design has other advantages that make it superior compared with LEA instruments. A small excitation area prevents the unnecessary illumination and photoconsumption in the volume of fluid surrounding the area of \( \text{PO}_2 \) measurements. A wide detection area provides for better light collection by the photodetector. The critical velocity in a SEA instrument is low, allowing measurements in practically all types of microvessels without the accumulated photoconsumption artifact. For example, the SEA instrument used in Ref. 20 had \( V_{cr} = 77 \, \mu\text{m/s} \), and it could be reduced even more by using a smaller excitation spot and lower flash rate.

The combination of a small excitation area, a low flash frequency, and scanning the object with the excitation spot employed in the SEA method allows for \( \text{PO}_2 \) measurements in stationary tissue or slowly flowing fluids without the accumulated photoconsumption of oxygen. The only type of photoconsumption error in the SEA technique is single-flash consumption, which has a small, systematic magnitude that can be determined in a test and then used to correct the results of \( \text{PO}_2 \) measurements. That is why the following analysis of measurement errors in flowing and stationary fluids pertains mainly to a LEA instrument that is likely to exhibit the artifact.
Accumulated Photoconsumption in Moving Media at $V < V_{cr}$

For a sampled volume of fluid moving in a LEA instrument from the edge of the excitation area to the small detection window in its center (see Fig. 4), the transit time is $t = (R - r)/V$ and the number of excitation flashes received by the same volume during its transit from the edge of the excitation area to the detection area is

$$N = F \cdot t = (R - r) \cdot F/V \quad (9)$$

Combining Eqs. 3 and 9, the measured PO$_2$ in the sampled volume after $N$ flashes is

$$P = P_0(1 - k)^N = P_0(1 - k)^{(R-r)/V} \quad (10)$$

and the PO$_2$ underestimation can be expressed as

$$\frac{P}{P_0} = (1 - k)^{(R-r)/V} \quad (11)$$

The plots in Fig. 5 demonstrate the dependence of the underestimation of PO$_2$ on plasma velocity for $V < V_{cr}$. These curves were obtained according to Eq. 11 for the typical cases of $k = 0.005–0.01$ and $V_{cr} = 2.4$ mm/s. The parameters of the LEA instrument used for calculations were taken from published data ($R = 70 \mu m$, $r = 10 \mu m$, $F = 30$ Hz; Refs. 35, 66). Experimental profiles of PO$_2$ as a function of $V$ were obtained with this instrument in arterioles and venules of the hamster skinfold chamber preparation at microvascular velocities below $V_{cr}$ (35). Because of accumulated photoconsumption, the PO$_2$ measurements in large and small microvessels could yield different PO$_2$s at the same actual PO$_2$ because of differences in linear velocities (see Fig. 5). The longitudinal PO$_2$ gradients in microvascular networks obtained with LEA instruments could be in part artifactual because of a failure of the LEA instrument for $V \ll V_{cr}$. However, the PO$_2$ reduction in moving fluid remains less than 10% for $1 > V/V_{cr} > 0.1$ and at velocities below 0.05 $V_{cr}$ does the PO$_2$ underestimation become large (Fig. 5). The shape of the experimental curves PO$_2$ vs. $V$ in Ref. 35 is similar to those in Fig. 5, indicating the presence of the accumulated photoconsumption artifact.

Predictions of Results of LEA Measurements for Microcirculatory PO$_2$

The analysis of LEA instrument performance in regard to dependence on velocity of the sampled volume, flash rate, and duration of the excitation pulse train can be used to make predictions about PO$_2$ measurements in the microcirculation. The predictions are based on the intrinsic artifact of accumulated photoconsumption for $V \ll V_{cr}$ and are not related to the actual PO$_2$ distributions in the vessels and tissue. The predictions are listed below:

1) The same PO$_2$s measured in large and small microvessels are expected to appear as two different values because of blood velocity differences between the vessels. The observed PO$_2$ difference between large and small arterioles could be interpreted as a longitudinal PO$_2$ gradient caused solely by high oxygen losses in arterioles (see Eq. 11 and Fig. 5).

2) Two equal PO$_2$s in flowing blood and in stationary tissue are expected to be measured as substantially different values. The observed PO$_2$ values measured inside and outside a vessel could be interpreted as a large transmural PO$_2$ gradient (see Eqs. 4 and 11 and Fig. 5).

3) The artifactual transmural PO$_2$ drop could be attributed to extremely high oxygen consumption in the vascular wall. This interpretation could lead to the concept that the vascular wall itself is the main oxygen sink in an organ.

4) The oxygen consumption in a tissue around an arteriole, determined from analysis of PO$_2$ profiles perpendicular to the vessel, is expected to be exaggerated because of the addition of photoconsumption to normal tissue respiration (see Eq. 4).

5) The PO$_2$ underreading in lymphatic microvessels is expected to be large because of the low velocity ($V \ll V_{cr}$) and low oxygen capacity of lymph (see Eqs. 10 and 11 and Fig. 5).

6) The rate of oxygen disappearance from rapidly arrested blood flow in arterioles is expected to be faster than that in the blood without illumination because of the oxygen consumption by the method itself (see Eqs. 3 and 4 and Fig. 2).

7) The increase of the linear velocity of blood on its way from capillaries to large venules is accompanied by a decrease of the photoconsumption artifact (Fig. 5). This may create an apparent rise of venular PO$_2$ in contrast to the transmural PO$_2$ gradients in these microvessels. The finding of a PO$_2$ rise in venules is expected in different organs because of the typically low velocities in these microvessels ($V \ll V_{cr}$) (see Eq. 11 and Fig. 5).

8) The PO$_2$ in capillaries and the PO$_2$ drop along a capillary will be strongly reduced by photoconsumption, since the blood velocity in a capillary is much lower than $V_{cr}$ in a LEA instrument. These results could lead to the conclusion that the role of capillaries in the supply of oxygen to tissue is insignificant (see Eq. 11 and Fig. 5).
9) The PO2 in the smallest microvessels could be found to be lower than in the surrounding tissue because of a combination of low velocity ($V \ll V_c$) and higher intravascular probe concentration. These findings could lead to the conclusion that capillaries, the smallest arterioles and venules can take oxygen from the surrounding tissue (see Eqs. 4 and 11; Figs. 2 and 5).

10) The tissue PO2 distribution is expected to appear more homogeneous than the actual one. The tissue PO2 range using a LEA instrument is compressed according to Eq. 4 and Fig. 2. Another factor compressing the PO2 range is the colocalization of LEA instruments (33, 35, 46, 51, 56, 63). A compilation of the microcirculation has been based heavily on results from and interpretations of high concentrations of the probe and oxygen in the perivascular region with the result that high perivascular PO2s are reduced to a greater degree than lower PO2s because of a higher local coefficient of photoconsumption.

Experimental Data Obtained with LEA Instruments and Their Interpretations

During the last decade the discourse on oxygen transport in the microcirculation has been based heavily on results from LEA instruments (33, 35, 46, 51, 56, 63). A compilation of results on PO2 in the microcirculation obtained with LEA instruments is presented in Table 1. These data on PO2 distribution in the microcirculation have been used to develop a novel concept of oxygen transport to tissue (16, 18, 26, 29, 51, 60, 62, 63, 65, 66). According to this concept, 1) arterioles are the main supplier of oxygen to the tissue; 2) capillaries deliver oxygen only to the capillary wall endothelium; 3) arteriovenous capillary PO2 differences are virtually nonexistent and capillaries are in virtual equilibrium with the tissue; 4) tissue PO2 is generally homogeneous; 5) the vascular wall is a dominant oxygen sink and oxygen consumption in the vascular wall is hundreds of times higher than in the surrounding tissue, protected by this mechanism from the high oxygen content of blood; 6) the arteriolar wall oxygen consumption can increase further during vasoconstriction; 7) there is an oxygen flux from the tissue to the small arterioles and capillaries; 8) PO2 rise in the venules exists because of oxygen flow from the extravascular tissue into the venules; and 9) terminal lymphatic vessels have the lowest PO2 in the microvasculature.

A comparison of experimental results obtained with LEA and SEA instruments for similar tissues and vessels is presented in Table 1. The results obtained with the two types of PQM devices are distinctly different; measurements obtained with LEA instruments support the above novel concept of oxygen transport whereas none of the SEA measurements do. Thus there is an opportunity to compare these two sets of results and discuss them in terms of the comparative analysis of performance of LEA and SEA instruments.

Longitudinal PO2 Gradient in Arterioles

The decrease of PO2 and hemoglobin oxygen saturation from large arterioles to capillaries in different organs was analyzed in the comprehensive review of Tsai et al. (65). Most of the results on blood oxygenation in microvessels, available in literature, are expressed as a difference of PO2S or the hemoglobin oxygen saturation or PO2 in vessels of different diameter rather than in terms of longitudinal gradient.

The experimental data on longitudinal oxygen losses in the rat’s microcirculation are limited. The first polarographic measurements of the PO2 drop of ~10 mmHg between feeding arterioles and arterial capillaries were made in the rat cremaster muscle (10). In resting splanchnic muscle the perivascular PO2 measured with a microelectrode decreased from 50 to 28 mmHg between first-order arterioles (mean internal diameter $d = 38 \mu m$) and capillaries (39). In cerebral cortical arterioles, studied with microelectrodes, the PO2 decrease between 1A and 5A arterioles ($d = 45$ and 8 $\mu m$) was 81 to 62 mmHg (71). The highest longitudinal PO2 drop in that study was found between the arterial and venous ends of capillaries, 58 and 41 mmHg, respectively. The longitudinal oxygen saturation gradient in capillaries was estimated in this work to be as high as 78%/mm. Spectroscopic measurements of the oxygen saturation drop along intestinal 2A arterioles ($d = 31 \mu m$) resulted in a longitudinal saturation gradient of 11.5%/mm (2). The longi-

### Table 1. Discrepancies between results on microcirculatory PO2 measured with LEA and SEA instruments

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LEA, large excitation area; SEA, small excitation area.
tudinal gradient calculated from the results of PQM PO₂ measurements in mesenteric arterioles \( (d = 23 \, \mu m) \) was 24%/mm (63). This large magnitude of longitudinal oxygen losses is surprising in arterioles surrounded by a thin sheet of connective tissue.

PO₂ measurements in mesenteric arterioles of rats performed with the SEA technique found no statistically significant PO₂ decrease between the largest and smallest arterioles (20). Mean PO₂ in the largest arterioles in mesenteric “windows” \( (d = 28 \, \mu m) \) was 66 mmHg and in the smallest arterioles \( (d = 12 \, \mu m) \) it was 67 mmHg. The significant PO₂ drop of 16 mmHg was found in the mesentery between the smallest arterioles and venules \( (d = 12 \text{ and } 13 \, \mu m) \) that corresponded to decline in 14% oxygen saturation. This result is supported by the reported oxygen saturation difference of \( \sim 13\% \) between arterioles and venules \( (d = 20 \text{ and } 28 \, \mu m) \) in the rat mesentery measured using Raman spectroscopy of hemoglobin (59).

For measurements with a LEA instrument, the longitudinal decrease in PO₂, caused by vessel wall and tissue oxygen consumption, coincides with the trend caused by photoconsumption. The error of the measured PO₂ is mostly pronounced in the smallest microvessels (Fig. 5). The magnitude of the artifactual longitudinal PO₂ gradient for \( V < V_{cr} \) depends on the difference of velocities between the points of measurement selected along the arteriolar tree. It can be seen from Eq. 11 and Fig. 5 that the contribution of this artifact to the longitudinal PO₂ gradient is strongest for velocities under 300 \( \mu m/s \) \( (V < 0.1 \, V_{cr}) \), which is characteristic for capillaries in resting muscle (27). The LEA measurements of PO₂ in microvessels of the hamster skinfold chamber give cause for concern, since all studied venules, capillaries, and many arterioles had velocities below \( V_{cr} = 2.4 \, mm/s \) determined for this instrument (34, 35). In the mesenteric microvasculature, the terminal arterioles, capillaries, and most of the venules also have velocities (45) that are lower than \( V_{cr} (2.4-3 \, mm/s) \) estimated for the employed LEA instruments (46, 51, 63). Comparison of the PO₂-vs.-velocity plots in Ref. 35 and Fig. 5 indicates that substantial oxygen losses from microvessels happened at much higher velocities \( (1-2 \, mm/s) \) than those predicted by Eq. 11. One of the possible explanations of this enhanced effect could lie in the low velocities of peripheral plasma layers providing most of the phosphorescence signal in microvessels. The analysis shows that the accumulated photoconsumption could be a cause for the low PO₂ and longitudinal PO₂ gradients in the smallest microvessels at \( V < V_{cr} \). Thus the determination of the physiological oxygen losses in the microvascular network requires a careful accounting for the contribution of the accumulated photoconsumption artifact.

**Longitudinal PO₂ Gradient in Venules**

Experimental evidence for PO₂ and oxygen saturation rise in venules has been found using polarographic microelectrodes (39), hemoglobin spectroscopy (37, 38, 54), and PQM (18, 29, 31, 46, 51). However, in other experiments employing the same measuring techniques no significant PO₂ and oxygen saturation rise in venules was found (2, 20, 55, 71). The large transmural PO₂ drop found in venules \( (12 \, mmHg; \text{ Ref. 17}) \) supports a fall in the blood PO₂ from capillaries to large venules, rather than its rise. Several hypothetical mechanisms were proposed to explain the reported PO₂ rise in venules, including the oxygen uptake from surrounding tissue (18, 51), diffusional shunting between paired vessels (18, 37, 38, 51), and heterogeneity of blood flow and oxygenation in capillaries (39, 46). Arteriovenous shunting of blood was found to be smaller than 1.5% and, therefore, cannot explain the reoxygenation of blood in venules (36). Mathematical modeling showed that the contribution of the countercurrent diffusion between paired vessels was estimated as \( \sim 10\% \), which is also not enough for the explanation of the high venous PO₂ (3). The capillary convection around paired vessels could facilitate oxygen exchange between arterioles and venules (8, 72).

However, the reported PO₂ rise in venules can be explained by the alternative mechanism related to a stronger photoactivated PO₂ depression in the smallest venules compared with the larger ones. The similarity between the experimental dependence of PO₂ vs. \( V \) in venules (34, 35) and the curves predicted by Eq. 11 (Fig. 5) indicate that the apparent PO₂ rise in venules rather could be because of different magnitudes of the photoconsumption artifact in venules of different diameter than to uptake of oxygen from the surrounding tissue.

The PO₂ profiles obtained with a SEA instrument in the mesenteric microcirculation do not support the concept of increasing PO₂ in venules (20). No statistically significant difference was found between PO₂ = 50 and 54 mmHg in the smallest \( (d = 13 \, \mu m) \) and largest venules \( (d = 33 \, \mu m) \), respectively. Thus one can suggest that the reported U-shape of the microvascular PO₂ profiles, with equal PO₂ in arteriole and venular branches of the curve, can be attributed to the performance of a LEA instrument for \( V < V_{cr} \).

**PO₂ Measured in a Stationary Medium**

**Tissue PO₂ distribution.** Early PQM studies of tissue PO₂ profiles in periarteriolar tissue regions reported relatively low interstitial PO₂ and a rapid decrease of PO₂ with distance from a nearby arteriole (63). In the rat mesentery the PO₂ was found to be reduced from 30 mmHg at the arteriolar wall to zero at 60–80 \( \mu m \) away from arterioles. Recent measurements of tissue PO₂ in the hamster skinfold chamber provided evidence of low tissue PO₂ = 25 mmHg that is distributed more homogeneously (64). Several factors work in concert to reduce tissue PO₂ and its heterogeneity, when determined with a LEA instrument:

1) The accumulated photoconsumption of oxygen in stationary interstitial fluid (Eq. 4, Fig. 2) reduces mean tissue PO₂ and the width of its distribution. The contribution of the accumulated photoconsumption to tissue PO₂ measurements can be estimated from Eq. 4 for parameters of the LEA instrument used by Tsai et al. (63). For small \( k = 0.005-0.01 \), the measured PO₂ value in the stationary tissue is reduced to 64–40% of the actual interstitial PO₂ level and the whole range of tissue PO₂ is compressed in the same proportion (Fig. 2).

2) The colocalization of higher concentrations of the probe and oxygen in the vicinity of vessels causes a higher photoconsumption in these areas and reduces the measured PO₂ heterogeneity.

3) The application of a monoeponential fitting model to analyze the heterogeneous signal (35, 64), collected from square areas with sides from 20 to 80 \( \mu m \), leads to substantial underestimation of the mean PO₂ (24).
4) Using a 50-μs fitting delay (33, 64) in the analysis of heterogeneous phosphorescence decay curve acts as a short lifetime filter that removes components with \( \text{PO}_2 > 60 \text{ mmHg} \) from the analyzed composite decay curve (23, 30).

\( \text{PO}_2 \) measurements in capillaries and the smallest arterioles and venules are special because of their size and low linear velocity of blood. Generally, the accumulated photoconsumption is greater in stationary perivascular tissue than inside the vessel with fast moving blood (Eqs. 4 and 11, Figs. 2 and 5). The number of excitation light pulses in a LEA instrument absorbed by a portion of a flowing blood is usually lower than that in a stationary tissue. However, when blood velocity is numerically lower than \( R - r \), a sampled volume of blood receives the same number of pulses as the stationary tissue (See Eq. 9). Under this condition the difference of the probe concentration between plasma in a vessel and in the interstitial fluid will play a major role in the reduction of the intravascular \( \text{PO}_2 \), so the apparent \( \text{PO}_2 \) in tissue could be equal or even higher than that in capillaries. This paradox was reported in several experimental studies and reviews, leading to the conclusion that there is a virtual \( \text{PO}_2 \) equilibrium between capillaries and tissue (16, 19, 29, 62). Evidence has also been presented that \( \text{PO}_2 \) is higher than in capillaries and the smallest microvessels, so that the oxygen flux is directed from the tissue to these microvessels (6, 18, 19). The mean \( \text{PO}_{28} \) found in tissue and capillaries of hamster skinfold were 22 and 13.7 mmHg, respectively (6). These measurements were made at different flash rates, 80 Hz for capillaries and 30 Hz for tissue, so that the number of flashes delivered to a slowly moving blood could be even higher than that received by a sampled volume of stationary tissue. Results such as these have been presented as support for the idea that the role of capillaries in the oxygen supply to tissue is negligible.

Accounting for the accumulated photoconsumption expected with a LEA instrument can provide an alternative explanation for the capillary-tissue \( \text{PO}_2 \) paradox as a combined effect of low velocity and high probe concentration in capillaries. This mechanism can be illustrated by analysis of a hypothetical case, at a capillary velocity \( V \leq 60 \mu \text{m/s} \), \( F = 30 \text{ Hz} \), \( R = 70 \mu \text{m} \), \( r = 10 \mu \text{m} \), and typical \( k \) is for plasma (0.01) and tissue (0.005), the \( \text{PO}_2 \) in plasma is reduced to 67% and \( \text{PO}_2 \) in tissue reduced to 74% of its actual value (according to Eqs. 4 and 11).

Tissue oxygen consumption by the PQM method itself, added to the tissue respiration, is expected to distort measured radial \( \text{PO}_2 \) profiles around arterioles, which have previously been used for determination of tissue respiration (63). The reported oxygen consumption in mesenteric tissue was 240 nl \( \text{O}_2 \cdot \text{cm}^{-3} \cdot \text{s}^{-1} \) (63), which is surprisingly high for a thin layer of connective tissue. The tissue \( \text{PO}_2 \) measured with the LEA method was found to be near zero at the distance of 60–80 μm from the arteriolar wall (63), whereas the \( \text{PO}_2 \) profiles determined with a scanning SEA instrument showed high \( \text{PO}_2 = 44 \text{ mmHg} \) at the distance of 180 μm from an arteriole (21). Oxygen consumption by the mesentery, determined with the scanning SEA method was only 60–68 nl \( \text{O}_2 \cdot \text{cm}^{-3} \cdot \text{s}^{-1} \). The contribution of photoconsumption in stationary tissue to the total oxygen consumption could be evaluated from Eq. 4 for conditions of tissue measurements performed in the mesentery by Tsai et al. (63). Parameters used for this estimation are \( \text{PO}_0 = 60 \text{ mmHg} \), \( k = 0.005 \), \( F = 30 \text{ Hz} \), \( T = 3 \text{ s} \), and oxygen solubility in connective tissue, \( \alpha = 1.4 \text{ nmol} \cdot \text{cm}^{-3} \cdot \text{mmHg}^{-1} \) (1). The \( \text{PO}_2 \) decrease in the sampled volume for the 3 s excitation period is shown in Fig. 2. The \( \text{PO}_2 \) decrease in the tissue for 3 s of excitation, predicted from Eq. 4, is 22 mmHg. The rate of photoconsumption can be converted, using Henry’s law for \( \alpha = 1.4 \text{ nmol} \cdot \text{cm}^{-3} \cdot \text{mmHg}^{-1} \), to the artificial oxygen consumption rate of 220 nl \( \text{O}_2 \cdot \text{cm}^{-3} \cdot \text{s}^{-1} \), close to the difference between oxygen consumption in the mesentery obtained with the LEA and scanning SEA instruments. This example demonstrates that using the LEA methodology may yield an oxygen consumption by the method that is higher than the actual respiration rate.

The oxygen loss from mesenteric arterioles with arrested flow, independently measured with two different LEA instruments (7, 43), showed a 10-fold difference in measured lifetimes of the oxygen depletion (126 and 12.8 s) from arterioles of similar diameters (46 and 58 μm). The conditions of the measurements (probe concentration and Xe-flash lamp illumination of the entire vessel) were the same except for the flash rates: 1 Hz and 10 Hz, respectively. However, the lifetimes of oxygen disappearance in arterioles, expressed as the number of flashes, were the same: 128 and 126 flashes. The recent photoconsumption test (6) performed in a stationary plasma sample sealed in a quartz cuvette resulted in a lifetime of \( \text{PO}_2 \) depletion of ~1.6 s at 80 flashes/s (for low light excitation), or the same number of flashes. This rate of oxygen elimination because of photoconsumption for that test can be obtained from Eq. 4 for the reaction \( k = 0.008 \) that is within the typical range of \( k = 0.005–0.01 \). The coincidence of the rate of oxygen elimination from an arteriole with arrested flow and the rate of accumulated photoconsumption in vitro indicates that almost all of the oxygen losses from the stationary blood in microvessels in both these experiments (7, 43) was because of the accumulated photoconsumption artifact. Thus the contribution of vascular wall respiration to the oxygen disappearance from an arteriole was negligible in both cases.

The \( \text{PO}_2 \) in large lymphatic microvessels measured with a LEA instrument was found to be 22–25 mmHg (26). This low \( \text{PO}_2 \) was inconsistent with the observation, made in the same work, of intensive vascularization of these lymphatic vessels. Results of \( \text{PO}_2 \) measurements in lymphatic vessels with the SEA method yielded a \( \text{PO}_2 \) of 76 mmHg, even higher than that in small arterioles located in the mesenteric windows. These lymphatic vessels were located in close proximity to transit arteries and enclosed in adipose tissue with a dense capillary network. Previously reported measurements of \( \text{PO}_2 \) in mesenteric lymph, made with a polarographic technique in dogs, found that the \( \text{PO}_2 \) exceeded 50 mmHg (11), supporting results obtained with the SEA instrument. High oxygen consumption caused by the LEA instrument in the tissue and lymph appears to be the main factor leading to the low measured \( \text{PO}_2 \) in lymphatic microvessels.

**Vessel Wall Oxygen Consumption**

Application of PQM instruments to measurements in both arterioles and adjacent tissue resulted in the discovery of a large transmural \( \text{PO}_2 \) drop (48–50, 63). The existence and magnitude of this transmural \( \text{PO}_2 \) drop can be explained by the difference in accumulated photoconsumption in blood moving in the arteriole and in the stationary tissue around it. Since the
linear velocity in arterioles is higher than 300 μm/s, the predicted PO2 underestimation (see Fig. 5 and Eq. 11) should be smaller than 5% (at k = 0.01, F = 30 Hz, and T = 3 s, as in Ref. 63) or less than 3 mmHg for an actual arteriolar PO2 = 60 mmHg. At the same time the PO2 decrease in tissue (k = 0.005), according to Eq. 4 and Fig. 2, is predicted to be 22 mmHg. The calculated transmural PO2 drop of 19 mmHg is the same as that measured in Ref. 63. This result demonstrates that the reported transmural PO2 drop in arterioles could be due mainly to the PO2 measurements both in flowing blood and the stationary interstitial fluid with the LEA instrument.

We have designed two experiments to verify the existence of a large transmural PO2 drop in arterioles of the rat mesentery (20, 21). Animals and mesenteric tissue were maintained under the same conditions in both experiments. In both cases the probe was administered to the compartment of interest (i.e., either intra- or extravascular). In an experiment with tissue PO2 measurements, the localization of the probe in the interstitial compartment was ensured by the direct application of the probe to the tissue. Accurate PO2 measurements in a stationary medium were performed by the SEA technique with a scanning excitation spot that prevented multiple excitation of the same sampled volume (21). In experiments with intravascular PO2 measurements, the probe confinement inside of microvessels was ensured by limiting the time period of measurements to 35 min after the probe was injected into the circulation (20). Intravascular PO2 was measured with a SEA instrument at V > Vc (77 μm/s) with an estimated single-flash oxygen consumption coefficient k = 0.002–0.004. Independent measurements of intra- and periarteriolar PO2s provided the possibility to make statistical comparisons of the PO2 data, obtained in two different sets of experiments under identical physiological conditions.

Intravascular PO2 in mesenteric arterioles was found to be 65.0 ± 1.4 mmHg (mean ± SE), arteriolar diameter was 18.8 ± 0.7 μm and number of measured vessels was 78 (See Fig. 6). Intersitial PO2 measured above arterioles was 62.7 ± 2.0 mmHg and PO2 at a distance 5 μm from the blood-wall interface was 62.2 ± 2.1 mmHg (diameter was 20.2 ± 0.9 μm and number of measured profiles was 84). Thus no significant transmural PO2 drop was found in the PO2 measurements made separately in mesenteric arterioles and outside of arterioles of the same size (the statistically insignificant difference was 2.3–2.8 mmHg).

Extrapolation of the parabolic PO2 profile obtained in mesenteric tissue to the location of the blood-wall interface (Fig. 6) showed that the intravascular PO2 value was consistent with this curve, indicating that wall oxygen consumption is not substantially different from that in the tissue (60–68 nl O2·cm−3·s−1) (21). This result is in a good agreement with previously reported data on oxygen consumption rates in the arteriolar and arterial wall obtained with the Cartesian diver technique and microelectrodes [expressed in nl O2·cm−3·s−1: 60 (28), 22–63 (41), 80 and 155 (4)]. The recent report of simultaneous PO2 measurements in blood and tissue made with two different phosphorescence probes administered to intra- and extravascular compartments showed no large PO2 difference (73), providing support for our results.

Conclusions

PQM opened the opportunity for noninvasive measurements of PO2 in microvessels in organs isolated from atmospheric oxygen. However, the application of this technique to microscopic PO2 measurements in stationary fluids has not been validated. In PQM the energy of excitation and concentration of the phosphorescent probe are selected to keep the oxygen consumption by the method well below 1% of the measured PO2 per single excitation flash. Multiple excitations of the same sampled volume can significantly increase the error of measurements because of the accumulated photoconsumption of oxygen. We analyzed the measuring performance of two types of PQM instruments with different combinations of the sizes of excitation and detection areas and flash rates in connection to oxygen depletion by the method in the sampled microscopic volume. The combination of a large excitation area and rapid flash rate yields a situation that maximizes the accumulated photoconsumption artifact. It was found that instruments of the LEA type have a high critical velocity that makes the PO2 measurement unreliable in most microvessels. This type of instrument produces a larger PO2 depletion in stationary tissue than in flowing blood, thus creating the apparent transmural PO2 drop in arterioles. The proposed model of accumulated photoconsumption also predicts artifacts contributing to the reported longitudinal PO2 gradient in arterioles and the PO2 rise in venules, high vascular wall and mesenteric tissue respiration rates, low and uniform tissue PO2, and low PO2 in lymphatic microvessels. These results have an alternative explanation because of the accumulated photoconsumption artifact intrinsic for LEA instruments with a high flash rate. This development obviates the need for a radical revision of Krogh’s concept of a dominating role for capillaries in oxygen transport to tissue, as suggested by PO2 data obtained with LEA instruments.

It is critically important to minimize the artifact of accumulated oxygen photoconsumption by using PQM to perform accurate PO2 measurements in microvessels and tissue. The SEA type of PQM technique has been designed to prevent the accumulation of photoconsumption of oxygen in flowing blood and lymph. The development of the scanning SEA instrument eliminated the accumulated oxygen depletion in PO2 measurements in stationary tissue and fluids. Thus the further improve-
Innovative Methodology

H2916

P02: MEASUREMENT IN MICROVESSELS AND TISSUE


