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Original Research Communication

Time-Dependant Oxygen Partial Pressure in Capillaries and Tissue in the Hamster Window Chamber Model

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ABSTRACT

The possibility of a plasma oxygen diffusion barrier implies a significant resistance to oxygen diffusion and the existence of capillary erythrocyte-associated transients of oxygen. This effect was analyzed by measuring intracapillary blood and tissue pO_2 in the hamster chamber window model using a noninvasive intravital microscopy palladium porphyrin phosphorescence decay technique for two set light excitations (high and low). Using high light excitation, intracapillary blood pO_2 was 13.7 ± 6.1 mm Hg, and increased to 18.0 ± 4.5 mm Hg for low light excitation. For high light excitation, intracapillary blood pO_2 peaks were in the range of 25–30 mm Hg, and the lowest values were in the range of 5–10 mm Hg. Reducing the excitation provided a more uniform pO_2 ranging 15–25 mm Hg. With temporal reduction in blood capillary pO_2, levels were correlated to the increase in phosphorescent amplitude that corresponded to plasma gaps. Tissue pO_2 measured at low light excitation in the proximity of capillaries was 23.1 ± 1.8 mm Hg. In conclusion, low intracapillary blood pO_2 measurements at full hematocrit are an artifact, only observed when oxygen consumption by the measurement technique was excessive and/or absorption of the excitation light was increased by the absence of RBCs. These findings suggest that resistance to oxygen diffusion in plasma is a minor factor in tissue oxygenation by capillaries in the hamster model. *Antioxid. Redox Signal.* 9, 845–853.

INTRODUCTION

Our understanding of the tissue oxygen supply mechanism is influenced by the theoretical work of Krogh (24), who assumed that oxygen is released by capillaries, described as parallel cylinders whose diameter is compatible with the transit of red blood cells (RBCs). In this analysis, oxygen delivery was proposed to occur from a uniform column of blood at a higher oxygen concentration than the surrounding tissues. The exchange properties of Krogh's configuration have been exhaustively analyzed by means of mathematical models, and recently reviewed by Golub and Pittman (15). A feature of these models first proposed by Hellums (19), it is the assumption of a significant plasma oxygen transfer resistance from the RBC to the tissue due to the plasma oxygen diffusion properties. The presence of a oxygen diffusion barrier from RBC to plasma, and eventually to the parenchyma, has also been shown mathematically (44).

Tissue oxygen delivery occurs when oxygen bound to hemoglobin in RBCs diffuses to the surrounding plasma and from there to the tissues. Analytical descriptions of oxygen diffusion from RBCs to plasma in general show little evidence that the presence of oxygen diffusion resistance significantly hinders oxygen transfer from RBCs to the tissue. Furthermore, when blood pO_2 is <40 mm Hg, the steepness of the hemoglobin oxygen saturation acts as an additional diffusion driving force, with the sigmoidal hemoglobin oxygen equilibrium curve being a major determinant for the oxygen flux direction dependence (9, 18).

The existence of a plasma oxygen diffusion barrier in the capillaries implies the existence of erythrocyte-associated transients of oxygen concentration as RBCs pass through the

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capillaries, as reported by Pittman (30) and Golub and Pittman (15) in the rat mesentery. This work shows that plasma pO_2 in between RBCs can attain values as low as 5 mm Hg, which are significantly lower than the tissue oxygen tension reported for the same preparation (16, 40). This finding indicates that there may be a pO_2 difference between RBCs and the inter RBC plasma on the order of 15 mm Hg.

Studies of capillary and tissue pO_2 distribution in the mesentery and other tissues indicate that average tissue pO_2 is in the range of 20–25 mm Hg (42). An exception was reported by Hangai–Hoger *et al.* (17), who found that areas of exposed mesentery, distant from the microvascular networks by several hundred microns, had a tissue oxygen tension that averaged 3.7 ± 3.3 mm Hg. These authors, however, noted that " . . . distribution of tissue pO_2 may be in part a consequence of how the tissue is exposed for analysis. *In situ* the thin mesenteric membrane void of adipose tissue and blood vessels is folded and in close proximity to the remainder of the tissue, in such a fashion that the connective tissue avascular areas are in direct contact with the surface of highly vascularized adipose tissue, leading to more uniform tissue pO_2."

Since cell-free plasma oxygen consumption should be extremely low, it is unlikely that intercapillary plasma has a pO_2 levels lower than tissue pO_2. If tissue pO_2 is significantly lower than RBC pO_2, then plasma oxygen would tend to equilibrate with the surrounding tissue. The analysis of the erythrocyte-associated transients of oxygen requires measurements of tissue pO_2 in the neighborhood of the capillaries where plasma pO_2 is measured. This information shows the direction of oxygen fluxes between RBCs, plasma, and tissue, and indicates the conditions in which RBC-related oxygen transients are a significant phenomenon in the tissue oxygen delivery.

This study documented oxygen tension variations in flowing capillaries of the hamster window preparation using the a similar technique to the one used by Pittman and Golub (15) and Pittman (30), and related these findings to the tissue pO_2 measured in the vicinity of the capillaries studied. This preparation was used because tissue is intact and isolated from ambient air. Microvascular oxygen measurements were made using the phosphorescence quenching methodology (15, 22, 38). Since laboratories implement this technique using different components, where the intensity of light excitation is a variable that determines oxygen consumption by the measuring technique, we performed this study at two different levels of light excitation, where the low level was set at the limit of signal detection.

METHODS

Animal preparation

Investigations were performed in 55–65 g golden Syrian hamsters (Charles River Laboratories; Boston, MA). Hamsters were fitted with a dorsal chamber window (10, 13). This model has been extensively used for investigations of the intact microvasculature of adipose and subcutaneous tissue and skeletal muscle in conscious animals (10). Animals were anesthetized for window implantation, and 2 days later for carotid artery and jugular vein catheterization. Four days after the initial surgery, the microvasculature was examined; only animals passing an established systemic and microcirculatory inclusion criteria, which includes having tissue void of low perfusion, inflammation, and edema, were entered into the study (41). Animal handling and care followed the "NIH Guide for the Care and Use of Laboratory Animals". Experimental protocol was approved by the local animal care committee.

Inclusion criteria

Animals were suitable for the experiments if: (a) systemic parameters were within normal range, namely, heart rate (HR) >300 beats/min, mean arterial pressure (MAP) >90 mm Hg, systemic hematocrit (Hct) >45%, and arterial oxygen partial pressure (paO_2) >55 mm Hg; and (b) no signs of edema or bleeding were present during microscopic examination of the tissue.

Systemic parameters

MAP and HR were recorded continuously (MP 150, Biopac System; Santa Barbara, CA). Hct was measured from centrifuged arterial blood samples taken in heparinized capillary tubes (Readacrit Centrifuge; Clay Adams, Division of Becton–Dickinson, Parsippany, NJ). Hemoglobin content was determined spectrophotometrically from a single drop of blood (B-Hemoglobin, Hemocue, Stockholm, Sweden).

Blood chemistry

Arterial blood was collected in heparinized glass capillaries (0.1 ml) and immediately analyzed for paO_2, $paCO_2$, base excess (BE), and pH (Blood Chemistry Analyzer 248, Bayer, Norwood, MA).

Intravascular and tissue oxygen tension (pO_2)

High resolution noninvasive intracapillary and tissue pO_2 measurements were made using a modified phosphorescence quenching microscopy system (38, 39). The pO_2 technique is based on the oxygen-dependent quenching of phosphorescence emitted by albumin-bound palladium-meso-tetra(4-carboxyphenyl) porphyrin (Pd-TCPP; Porphyrin Products, Inc., Logan, UT) complex after pulsed light excitation. Phosphorescence quenching microscopy is not dependent on dye concentration, and the decay time is inversely proportional to the pO_2 level. The technique is well suited for detecting hypoxia, because its decay time is inversely proportional to the pO_2 level, causing the method to be more precise at low pO_2s. The phosphorescent technique allows for precise localization of the pO_2 measurements and is used to measure both intravascular and extravascular pO_2, since the albumin–dye complex continuously extravasates from the circulation into the interstitial tissue. Pd-porphyrin complex is excited by a flash lamp (MVS-2601; PerkinElmer Salem, MA.) using pulsed light (10 μsec duration) at a rate necessary for the measurements (depending on time resolution needed, 30 Hz in tissue and 80 Hz in the intracapillary blood). The flash lamp was set for high mode (1,000 V) with capacitors C1–C4 removed. Pulsed light passed through a 420 nm band pass

filter (Oriel Corp, Franklin, MA; Transmittance of 48%); this was used for high light excitation measurements. A second 420 nm band pass filter (T: 48%) was used to reduce the excitation light pulse by ~50%; this was used for the low light excitation measurements. Problems related to the microscopy of thick tissues were circumvented by using a partial confocal effect obtained by placing a 100 μm pin-hole in the excitation light path (20). Phosphorescence emission is passed through an adjustable rectangular optical slit and light filter (630 nm cutoff) and is captured by a high sensitivity photomultiplier (R1477-06, Hamamatsu, Japan). Signals were visualized on an oscilloscope (TDS 2002, Tektronix; Beaverton, OR) and transferred to an analog input (BNC-2110, National Instruments; Austin, TX) connected to a high-performance data-acquisition board (PCI-6070E, 1.25 MSamples/sec, National Instruments, Austin, TX). Phosphorescence decay curves were analyzed online, using a standard single exponential least squares numerical fitting technique, and the resultant time constant was applied to the Stern–Volmer equation to calculate oxygen tension (pO_2), using predetermined parameters of the lifetime in the absence of oxygen and the quenching constant corrected for this animal model.

Animals received a slow intravenous injection of 15 mg/kg body weight at a concentration of 10.1 mg/ml of a Pd-TCPP. This was allowed to circulate for 10 min prior to pO_2 measurements. The measurement site was microscopically selected by an adjustable optical window. Intravascular pO_2 measurements were made by placing the rectangular optical window over a region of ~4 × 4 μm within the capillary. These measurements were made using a high (100X) magnification objective (Olympus LUMPL-FL 100X, N.A. 1.0; New Hyde Park, NY). Perivascular pO_2 measurements were made next to the capillaries studied using a low magnification (40X) objective (Olympus LUMPL-FL 40X, N.A. 0.8). Tissue pO_2 was measured in regions void of vessels within intercapillary spaces with an optical window size of about 10 × 10 μm.

The high magnification objective was used for blood capillary measurements since it provided sufficient spatial resolution at 80 pulses/sec with high and low light excitation to excite the phosphorescent. Measurements in the tissue were made using a low magnification objective rather than a high magnification objective at a rate of 30 pulses/sec at high light excitation, because when tissue pO_2 measured with high magnification objective produced a significant decrease in pO_2 (Fig. 1), a phenomenon likely due to oxygen consumption in the stationary medium.

To distinguish between RBCs and plasma, we simultaneously recorded the phosphorescence emission amplitude at the beginning of a decay curve after the end of the excitation pulse (5 μsec average phosphorescence signal, 10 μsec after pulse excitation). The phosphorescence probe is uniformly dissolved in plasma and does not enter the RBCs; therefore the maximum amplitude of the phosphorescence signal is proportional to the amount of plasma in the detection volume (15). The records obtained from individual measurements for both blood capillary pO_2 levels and emission phosphorescence amplitude signals were filtered using a 12 Hz low pass cut-off frequency digital filter to reduce signal variability.

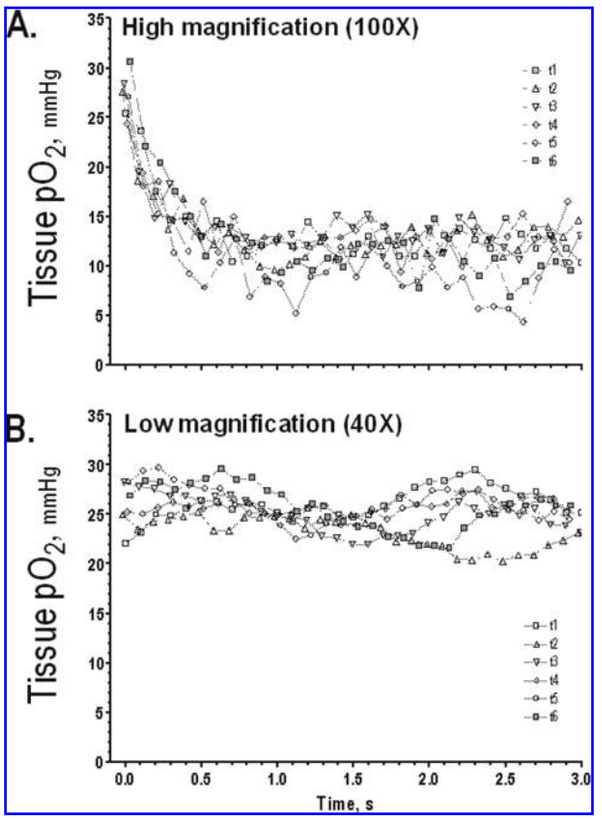

FIG. 1. Tissue pO_2 as a function of time. Measurements carried out with two optical magnifications (**A**) high magnification and (**B**) low magnification, at six different tissue locations: t1, t2, t3, t4, t5, and t6; high light excitation (*black symbols*) and low light excitation (*empty symbols*). Each measurement was made using 30 excitation flashes (10 flashes/sec), and measurements were continued at the shown intervals for 3 sec. High magnification (100X objective) concentrated the excitation light in an area of 10 μm diameter, while the low magnification (40X objective) spread the light over an area of 70 μm diameter. Tissue pO_2 level was not affected by the measuring technique when using the low magnification at high or low light phosphorescent excitations.

Animal experimental setup

The unanesthetized animal was given 20 min to adjust to the tube environment before the control systemic parameters (MAP, HR, blood gases, and Hct) were measured. The conscious animal in the tube was then fixed to the microscopic stage of a transillumination intravital microscope (IMT-2 Olympus). The tissue image was projected onto a charge-coupled device camera (COHU 4815) connected to a videocassette recorder and viewed on a monitor.

Phosphorescent oxygen consumption

Blood samples from the animals who received an intravenous injection of Pd-TCPP were collected in heparinized glass capillaries (0.1 ml), 15 min after injection. Blood samples were sealed, preserved at 4ºC, and wrapped in aluminum

foil until the animal experiment was completed. The Pd-TCPP plasma solutions were obtained after spinning down blood at 1,000 *g* for 5 min. A Pd-TCPP plasma solution (36 μl) was placed in a UV quartz cuvette (NSG Precision Cells Inc., Farmingdale, NY). Sample irradiation was performed with the identical conditions as the animal experiments. Flash light excitation was delivered to the surface of the cuvette through the microscope 100X objective (80 Hz, 5 sec). The concentration of the metalloporphyrin was 0.5 mg/dl, estimating a plasma volume in these animals to be 2–2.5 ml. Plasma samples were equilibrated with 10% oxygen before experiments to reproduce *in vivo* conditions.

Data analysis

Results are presented as mean ± standard deviation unless otherwise denoted. Data within each group were analyzed using nonparametric *t*-test (Mann Whitney test), one way nonparametric analysis of variance ANOVA (Kruskal–Wallis test), and when appropriate, post hoc analyses performed with the Dunn's Multiple Comparison Test. Pearson correlation (product moment correlation) was used to test linear correlation between phosphorescence emission amplitude and intracapillary pO_2 levels. All statistics were calculated using GraphPad Prism 4.01 (GraphPad Software, Inc., San Diego, CA). Changes were considered statistically significant if $p < 0.05$.

RESULTS

A total of 8 animals (58 ± 4 g) were studied.

Systemic parameters

Hematocrit and hemoglobin were 48 ± 1% and 14.7 ± 0.5 g/dl, respectively. MAP and HR were 102 ± 8 mm Hg and 432 ± 27 bpm, respectively.

Laboratory parameters

Systemic blood gas parameters pH, PaO_2, and $PaCO_2$ were 7.36 ± 0.02, 62.5 ± 6.3 mm Hg, and 48.6 ± 4.8 mm Hg, respectively. There were not significant changes in systemic or laboratory parameters before and after the measurements.

Capillary oxygen tension

A total of 28 capillaries were studied, 16 for high light excitation (single optical filter) measurements, and 12 for low light excitation (double optical filter) measurements. Each field of observation was chosen for study at locations in the tissue where a single capillary was observed in sharp focus.

Perivascular pO_2s were measured in all cases before and after the determination of erythrocyte-associated transients of oxygen to determine any possible disturbance of the conditions by the

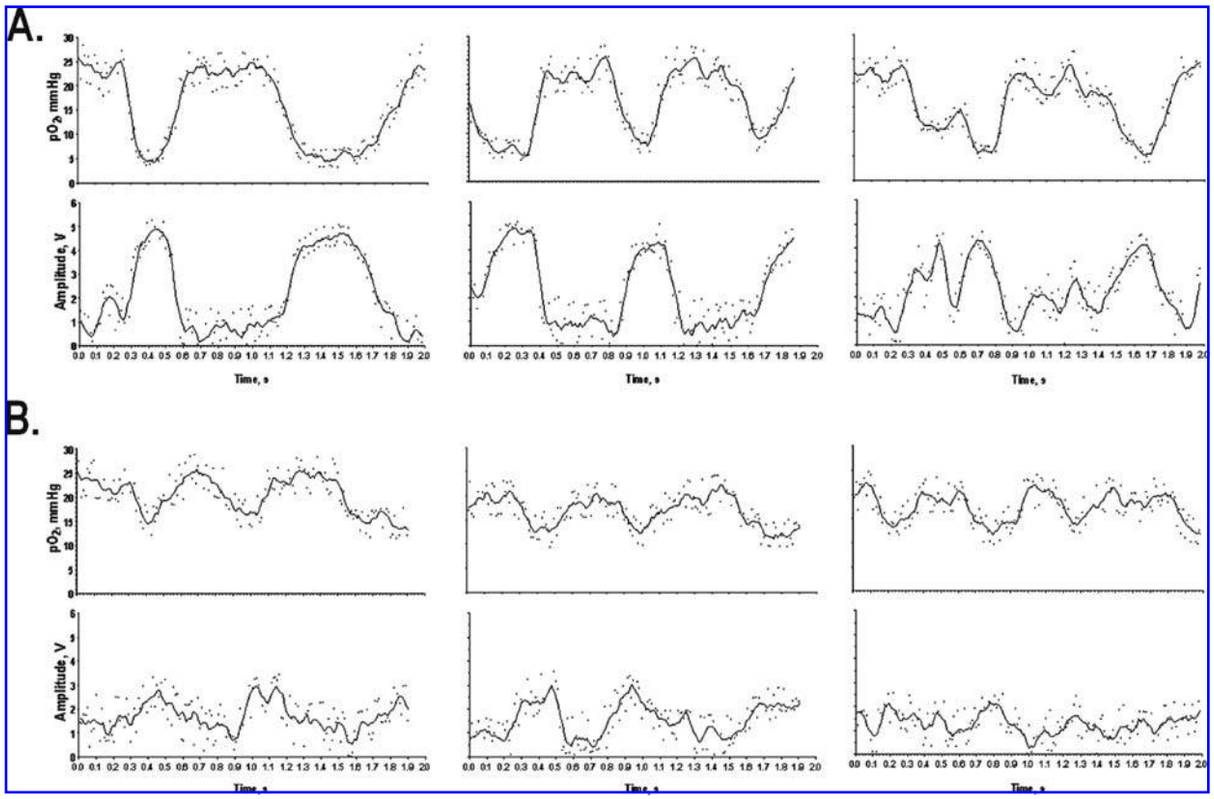

FIG. 2. Capillary pO_2 variability as a function of excitation intensity. This figure presents six series of intracapillary blood pO_2 levels and the phosphorescent emission amplitude in different capillaries; high light excitation (**A**) and low light excitation (**B**). *Dots* present each pO_2 reading and the *solid line* the filtered trend (12 Hz low pass filter). Both series of data were used for statistical analysis. The data of each series was used to obtain a histogram of intracapillary blood pO_2.

measurements. Perivascular tissue pO_2 measured with high intensity light excitation pulses (single optical filter) before erythrocyte-associated transients of oxygen measurements was 22.4 ± 1.3 mm Hg (n = 14 locations, before) and 21.9 ± 1.5 mm Hg (n = 14 locations, after). Using low light intensity excitation pulses (two optical filters), the respective measurements were 23.1 ± 1.8 mm Hg (n = 12 locations, before) and 22.8 ± 1.9 mm Hg (n = 12 locations, after). Each pO_2 measurement was obtained, averaging 1 sec measurements (30 flashes/sec). The differences in tissue pO_2 were not statistically significant.

Fluctuations of capillary blood pO_2 levels were measured using high light flash excitation with high magnification (100X objective) at 80 flashes/sec. A reduction in flash excitation intensity to low light intensity excitation mitigated blood capillary pO_2 levels fluctuation with high magnification at 80 flashes/sec reading rate. Simultaneous records containing 160 consecutive points of pO_2 and phosphorescent amplitude measurements made in a capillary for 2 sec are shown in Fig. 2 (2A, high light excitation; and 2B, low light excitation).

The peak pO_2 values obtained using a high light excitation were in the range of 25–30 mm Hg, and the lowest values in the record were in the range of 5–10 mm Hg for all the capillaries studied (mean pO_2 was 13.7 ± 6.1 mm Hg). Filtered and unfiltered records obtained of blood capillary pO_2 levels and emission phosphorescence amplitude signals were correlated to establish potential relations. In these conditions, temporal variations of capillary blood pO_2 appear to be directly related to the increases in emission phosphorescent amplitude as evidenced by the relationship between signal amplitude and capillary pO_2 (Fig. 3A, unfiltered; and Fig. 3B, filtered data). The regression coefficient was $R^2 = 0.68$ for the whole data set with high light excitation. This regression coefficient increased to 0.93 when the data was filtered. Both Spearman's correlations for filtered and unfiltered data were statistically significant ($p < 0.05$, filter = 212; unfiltered = 2,560).

When the intensity of the excitation was reduced to low light excitation intensity, the pO_2 distribution was more homogenous, ranging from ~15–25 mm Hg (Fig. 2B, low light excitation). Visual inspection of the records suggests a relationship between the decrease of capillary blood pO_2 levels and the phosphorescent emission amplitude. The linear fit between capillary blood pO_2 and phosphorescence intensity had regression coefficients of $R^2 = 0.36$ for the unfiltered data and 0.41 for the filtered data (Fig. 3A, unfiltered; and Fig. 3B, filtered data), both Spearman's correlations statistically significant ($p < 0.05$, filter = 160 measurements; unfiltered = 1,920 measurements).

Data on tissue pO_2 and capillary blood pO_2 was pooled to construct the distribution of oxygen in the tissue (n = 1560 measurements) and intracapillary pO_2 (n = 2560 measurements for high light excitation; and n = 1920 measurements for low light excitation). Histograms of the intracapillary pO_2 distribution for capillaries measured is shown in Fig. 4A, and shows a significant differences in the trend of data obtained with high and low light excitation intensity ($p < 0.05$). The high light excitation yields a bimodal distribution for capillary pO_2, low pO_2 levels were centered at about 6–8 mm Hg and the higher pO_2 levels were centered between 20 and 22 mm Hg.

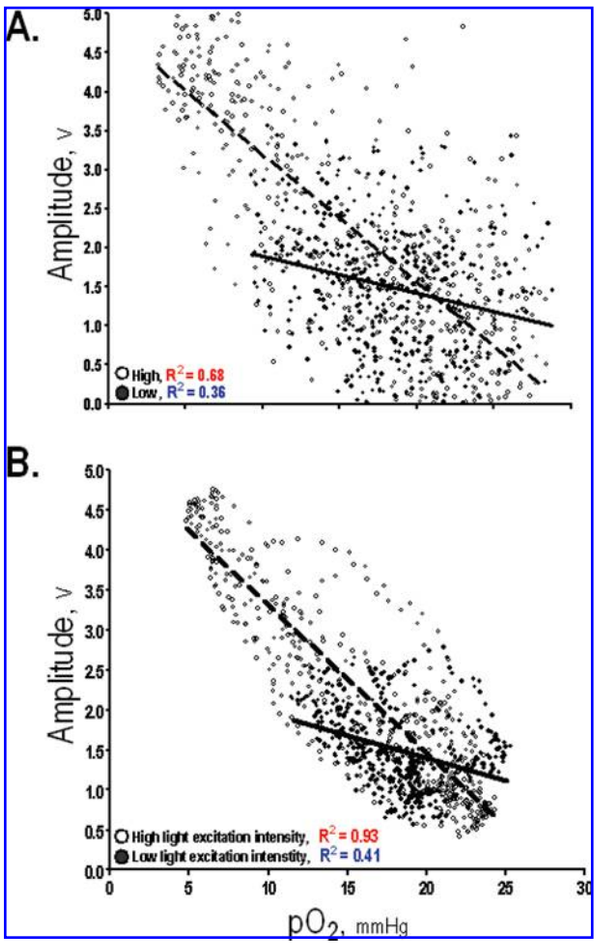

FIG. 3. Relation between phosphorescent emission amplitude and intracapillary pO2 levels for high and low light excitation. *Upper panel*, (**A**): all data. *Lower panel*, (**B**): data filtered with the 12 Hz low pass filter. A high number of measurements, filtered and unfiltered data for high and low level of light excitation intensities (oxygen consumption by the method) and pO_2 measured were statistically significantly correlated (regression coefficients: high, R^2 = 0.68/0.93; low R^2 = 0.36/0.41).

The histograms for tissue oxygen measurements obtained before and after erythrocyte-associated transients of oxygen measurements presented in Fig. 4B show an average tissue pO_2 of 22.9 ± 2.1 mm Hg, which is higher than the average capillary pO_2 (13.7 ± 6.1 mm Hg, high light excitation; 18.0 ± 4.5 mm Hg, low light excitation).

The distribution of tissue pO_2 shows no values below 10–15 mm Hg; however, when high light excitation was used, almost half of the measurements of capillary blood pO_2 were significantly lower than the surrounding pericapillary tissue pO_2 measured.

Ex vivo measurements of plasma pO_2 show different rates of decay due to oxygen consumption, depending on the intensity of the light flash excitation (Fig. 5), the rate of oxygen consumption being approximately proportional to the intensity of the excitation.

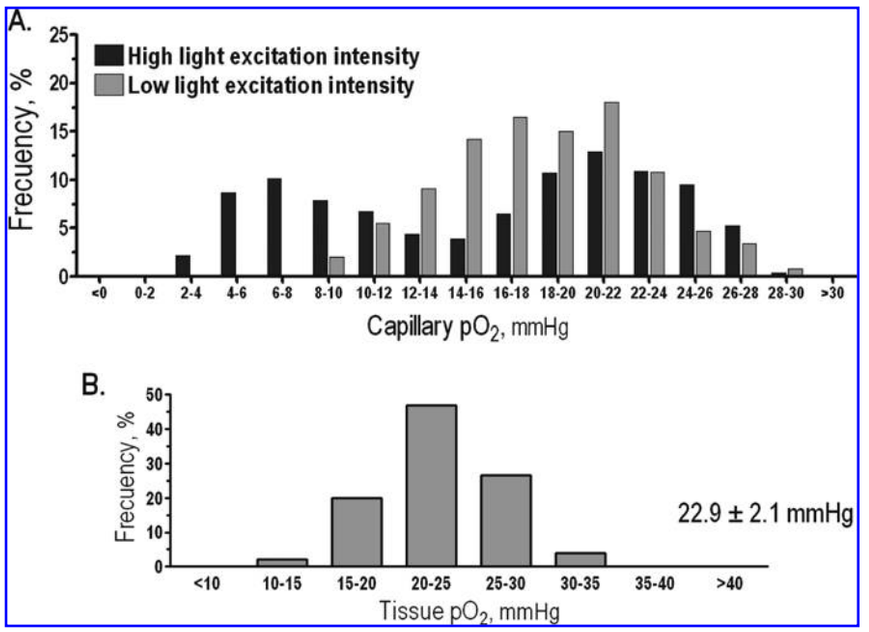

FIG. 4. Capillary pO_2 histograms for high and low light excitation. Capillaries measured are divided into high (*dark gray*) and low (*light gray*) excitation light intensity (**A**). The high light intensity phosphorescence excitation produced a bimodal histogram, while the reduction of the phosphorescence excitation eliminated the bimodal distribution. (**B**) presents the distribution of tissue oxygen tension. Tissue pO_2 has a mean greater than capillary pO_2, a phenomenon attributed to the prevalent oxygen delivery to this tissue by arterioles.

DISCUSSION

The principal finding of this study is that the magnitude of the capillary blood pO_2 transients measured using the phosphorescence quenching technique are partially dependent on the intensity of excitation. Therefore, the oxygen consumption of the measuring technique at high light excitation intensity counts for the reduction in pO_2 values measured.

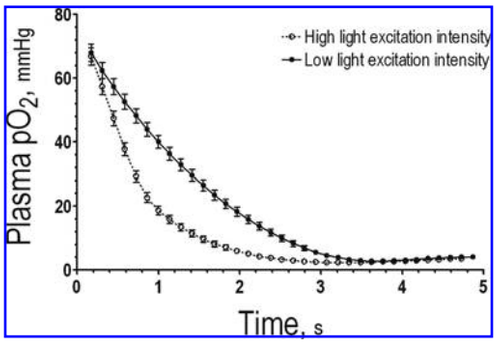

FIG. 5. Oxygen concentrations recorded in a cuvette containing an aqueous plasma solution of Pd-TCPP. Experiments were performed at similar rate of excitation for the capillary experiments (80 flashes/sec). After 4–5 sec, all the oxygen in the 36 μl was consumed. The difference in oxygen consumption as a function of the intensity of excitation is confirmed by this simple experiment. When the identical experiment was performed using an albumin solution and identical concentration of Pd-TCPP, the rate of oxygen consumption was significantly decreased (0.84 μ*M*/flash high light excitation and 0.39 μ*M*/flash for low light excitation). Consequently, calibration of the phosphorescent system for *in vivo* measurements must reproduce the experimental conditions in which the technique is used and a calibration using blood and large volumes does not reproduce microvascular conditions.

When the light to excite the phosphorescent is concentrated into a small volume within the capillaries, it caused large RBC/plasma pO_2 gradients (15). Decreasing the phosphorescent excitation intensity significantly reduced the RBC plasma oxygen gradients. Extrapolation to zero excitation intensity suggests RBC/plasma gradients are not present unless there is oxygen consumption in the plasma.

A similar result was obtained measuring tissue pO_2 with different optical magnifications. The use of a high magnification objective (100X), concentrates the excitation light to the extent that oxygen consumption by the technique is a major factor in the measurement in a stationary medium, such as interstitial fluid (Fig. 1A). The problem is of lesser significance in moving fluid such as capillaries, where oxygen is replenished by convection.

The record of blood capillary pO_2 obtained at low light excitations shows a variability of ~5 mm Hg. This variability may be due to the alternating passage of RBCs and plasma gaps, noise in the measuring system, changes in the tissue, movement of the preparation, and variability in the excitation light. The strongest component of this variability may be due to the residual oxygen consumption by the technique, which cannot be completely eliminated since it is intrinsic to the method. The changes in capillary blood pO_2 levels at high light excitation were on the order of 20 mm Hg, as commented before, low light excitation produced a max change of 5 mm Hg. This suggests that a further reduction of light excitation by 25% would completely eliminate the effects due to oxygen consumption in plasma by the measuring technique. However, at these low levels of light excitation, phosphorescent emission can be also significantly reduced, and the signal to noise ratio decreases to the extent where curve fitting to the exponential function becomes unstable.

The finding that low blood capillary pO_2 levels measured by phosphorescence are due to high light excitation levels over a small area is further supported by a high negative

regression between the measured excitation intensity and the resulting plasma pO_2 for high and low excitation intensity ($R^2 = 0.93$; $p < 0.05$, for the12 Hz digitally filtered data). This regression coefficient decreased at lower excitation levels ($R^2 = 0.41$), and the regression coefficient could vanish if the technique did not consume oxygen (Fig. 3).

Oxygen distribution in the hamster chamber window has been studied in a variety of conditions, showing that in most circumstances capillaries are the blood vessel with the lowest pO_2, since collecting venules and venules have progressively higher pO_2 levels (21, 42). pO_2 in the tissue surrounding the capillaries tends to be uniform, even in the proximity of arterioles due to the high oxygen gradients present in the arteriolar vessels walls (21, 36), presumably arising due to oxygen consumption by the arteriolar vessel wall (36). In the venular circulation, pO_2 is significantly higher than in capillaries; however, since vessel wall gradients are also present in the venular vessel wall, the net effect is that tissue pO_2 tends to be uniform.

The uniformity of tissue pO_2, which in the case of the hamster window tissue used in these experiments was 22.9 ± 2.1 mm Hg, demonstrates that the erythrocyte associated transients of oxygen recorded in capillaries (at high light intensity excitation) are not representative of physiological conditions, since there is no mechanism that accounts for the existence of blood pO_2 values below the average tissue pO_2 value.

Our results show that capillary plasma pO_2 is in the range of 5–10 mm Hg for high light excitation levels in the absence of RBCs. The histogram of tissue pO_2 (Fig. 4B) shows no tissue region <10 mm Hg, only 1% of the tissue being in the range of 10–15 mm Hg, and 99% of the tissue pO_2 is >15 mm Hg, causing tissue pO_2 to be consistently higher than plasma pO_2. Therefore, it is physically impossible in this preparation for plasma to reach the values obtained with high light intensity excitation unless there is a significant consumption of oxygen in the plasma, a process that occurs at high light excitation levels of palladium-porphyrin phosphoresce.

In conditions of extreme hemodilution (11% hematocrit), with large plasma gaps between RBCs, erythrocyte-associated transients of oxygen may be present since oxygen is released from the plasma into the tissue through a greater surface area available for its release from RBCs. However, theoretical studies of the circulation of plasma in the space between RBCs show the existence of an annular vortex coaxial with the capillary, with portions of the fluid traveling at a higher velocity than the RBCs, which introduces a significant amount of mixing in between capillary RBCs (5, 14, 32–34). Therefore, our results and the possibility of significant plasma mixing indicate that the diffusion resistance to oxygen passage from RBCs to plasma and tissue in the hamster window chamber model is not significant. In the hamster chamber window model at extreme hemodilution conditions, which may have facilitated the development of erythrocyte-associated transients of oxygen and therefore magnified the effect of the related plasma resistance to oxygen diffusion, oxygen extraction is identical to that found at a normal Hct when functional capillary density and flow are maintained (6–8, 43). Other animal models presented similar results during hemodilution (3, 11, 23, 26–28).

The concept of diffusion resistance was proposed on the basis of the theoretical analysis in tubes with 27 μm diameter (2, 25, 37). These studies were carried out at very low flow rates to obtain a longitudinal oxygen concentration drop along the tubes, which may be a factor in decreasing the rate in which plasma is mixed between RBCs. A similar analysis using a 10 μm diameter tubes showed a faster oxygen uptake in hemoglobin solutions (facilitated diffusion) than in RBC suspensions (29). However, oxygen diffusion resistance in plasma was not clearly established as a limiting factor. Whether the theoretical and *in vitro* results describe conditions in larger microvessels is still an open question, since *in vivo* experimental data consistently show that more oxygen exits the microvessels than can be accounted for by conventional diffusion in the surrounding tissue (1, 31, 35).

Our results show that, when phosphorescence quenching is used to measure pO_2 in superficial structures *in vivo* and in applications involving intravital microscopy, photochemical oxygen consumption is of minimal importance when excitation light intensity is maintained at a low level. However, if the average excitation intensity is increased by reducing the excitation volume and/or increasing the rate of measurements, perturbation of the local oxygen concentration becomes significant. Conventional phosphorescence quenching microscopy used to measure pO_2 levels in large tissues regions uses low excitation frequencies (10 Hz, flashes/sec) over a short time (1–3 sec) and low optical magnification (4, 22, 38, 40).

The rate of photochemical oxygen consumption decreases as oxygen concentration is reduced, because the probability of a porphyrin triplet undergoing a direct decay to the ground state increases. Thus, relatively fewer triplets are quenched by oxygen, leading to a reduced rate of singlet oxygen formation. A practical and perhaps somewhat counterintuitive consequence of this aspect of the photochemistry of phosphorescence quenching is more likely to perturb tissue (by singlet oxygen formation) at relatively higher tissue oxygen tensions.

Buerk *et al.* (4) compared the tissue oxygen tensions measured with a 5 μm tip recessed electrode and the phosphorescence quenching microscopy technique (10 × 10 μm) in the hamster window chamber model. This work used moderate levels of light excitation, and distributed the light to an area of ~140 μm in diameter and showed oxygen consumption was minimal, even when the excitation lasted as long as 1 min, which is consistent with our results.

The present study shows that the tissue of the hamster window chamber at rest has a temporal and spatial distribution of pO_2 that is relatively uniform and primarily influenced by the oxygen exiting from arterioles and venules, a model proposed by Ellsworth and Pittman (12). The spatial and temporal uniformity of the tissue is also reflected in an approximately uniform distribution of capillary blood pO_2, since the capillaries appear to be mostly in oxygen equilibrium with the tissue, and only contribute minimally to the oxygen supply. The histograms of tissue and capillary pO_2 show regions of the tissue whose pO_2 exceeds the maximal pO_2 measured in capillaries. The histograms of capillary pO_2 shows an average of 18.0 ± 4.5 mm Hg, while the average tissue pO_2 is significantly higher being 22.9 ± 2.1 mm Hg, therefore capillaries in this model do not deliver oxygen to the tissue. This result is

compatible with an oxygen distribution for this tissue where most of the oxygen is delivered by the comparatively high pO_2 arterioles and venules.

In summary, our results show that the existence of substantial pO_2 gradients in the plasma between adjacent RBCs is mostly due to photochemical consumption of oxygen in plasma during the measurement. Measurements of pericapillary tissue pO_2 in the same region where erythrocyte-associated transients of oxygen were detected show that tissue pO_2 level is always significantly higher than the low transient pO_2 measured with high intensity light excitation, a configuration only possible if plasma consumes a significant amount of oxygen. The presence of erythrocyte-associated transients of oxygen support the concept that there is a substantial plasma oxygen diffusion resistance (15), also postulated in model studies of oxygen transfer from blood to tissue. However, the lack of significant erythrocyte-associated transients of oxygen measurements, when oxygen consumption by the technique is minimal, suggests that resistance to oxygen diffusion in plasma is not a major component in the capillary circulation of our hamster window model. In our model, erythrocyte-associated transients of oxygen can only be demonstrated when an external factor consumes plasma oxygen, a nonphysiological condition created by the measurement technique. This study shows that the interpretation of capillary pO_2 measurements requires information on the pO_2 in the surrounding tissue. Finally, present results show that the interstitial tissue of the experimental model has a pO_2 predominantly equal or higher than intra capillary pO_2, supporting the concept that for some tissues at rest, arterioles may be the principal suppliers of oxygen (12).

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ABBREVIATIONS

Hct, systemic hematocrit; HR, heart rate; MAP, mean arterial pressure; Pd-TCPP, palladium-meso-tetra(4-carboxyphenyl) porphyrin; pO_2, partial pressure of oxygen; RBC, red blood cell.

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