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EDITORIAL

# The Physics of Oxygen Delivery: Facts and Controversies

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**Abstract**

At the microvascular level, the radial oxygen gradient is greater in arterioles than in any other vascular segment and thus drives the oxygen from the blood (high concentration, source) into the perivascular tissue (low concentration, sink). Thus, arterioles appear to be the main suppliers of oxygen to the tissue, in contrast to the capillaries, where the oxygen gradient is only a few millimeters of mercury. However, longitudinal oxygen loss from arteriolar blood is higher than can be solely accounted for by diffusion. This discrepancy becomes evident when determining how oxygen is distributed in the microvascular network, an approach that requires confirmation of the data in terms of mass balance and thermodynamic considerations. A fundamental difficulty is that measuring tissue $Po_2$ is complicated by methods, exposure of tissue, interpretation, and resolution. The literature reports mean tissue $Po_2$ as low as 5 and up to 50 mm Hg. This large variability is due to the differences in techniques, species, tissue, handling, and interpretation of signals used to resolve $Po_2$ levels. Improving measurement accuracy and physiological interpretation of the emerging $Po_2$ data is ongoing. We present an analysis of our current understanding of how tissue is supplied by oxygen at the microscopic level in terms of present results from laboratories using differing methods. *Antioxid. Redox Signal.* 12, 683–691.

Our understanding of how oxygen is transported from blood into the tissues is based on the measurement of the oxygen partial pressure in different tissue structures. This is a multiscale analysis that ranges from overall systemic mass balances to details of how oxygen is distributed at the microscopic level, at the scale of blood capillaries and the mitochondria. Whereas oxygen management at the systemic level is fairly well understood, oxygen regulation on the microscopic scale continues to undergo conceptual challenges as new techniques and measurement procedures become available. Older and newer techniques remain technically complex, to the extent that they are seldom available at a single laboratory, thus making it difficult to compare methods or even to perform ancillary experiments such as mass-balance analyses that would give confidence to the accuracy and relevance of results.

## Biophysics of Oxygen Distribution in the Tissues

Determining the optimal steady-state oxygen environment for a given cell type remains a principal question. Before actual measurements of tissue oxygen distribution, tissue cells were thought to be exposed and adapted to significantly different $Po_2$ environments, depending on their location. This is because the microcirculation has longitudinal oxygen-concentration gradients that convert arterial blood into venular and venous blood. In this scenario, cells near arterioles would have greater availability of oxygen in comparison to cells in the proximity of venules.

A significant variability in tissue $Po_2$ could be related to the presence of cells with differing capacities and adaptabilities to extended exposure to different oxygen tensions. For example, the endothelium lining the microvasculature could have a different function in arterioles, capillaries, and venules and therefore require different levels of oxygen exposure. Alternatively, tissues during normal conditions could present a relatively uniform and narrowly regulated profile, regardless of their proximity to specific microvascular structures. In this context, an important function of oxygen transport to tissues at rest by capillaries may be that of equilibrating tissue $Po_2$ differences by transporting oxygen between regions with high and low $Po_2$ values. These considerations lead to the basic question of what is the variability of tissue $Po_2$ under normal conditions.

### *Measurement of tissue $Po_2$*

Polarographic Clark needle electrodes were the first method used to measure tissue $Po_2$. Whalen *et al*. (72) used this technique, providing early information on the oxygen distribution in the tissue. A multielectrode version of the polarographic electrode subjects the tissue to less trauma, but is

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able to assess only surface $Po_2$. It has been used successfully to map surface tissue $Po_2$ distributions (38). The next significant technical advancement in $Po_2$ methodology was made by the group directed by Prof. David F. Wilson, who pioneered the oxygen phosphorescence quenching technique (67). This method requires the injection of a metallo-organic protein (palladium porphyrin) dye and is based on the quenching of excited phosphorescence by oxygen, providing $Po_2$ measurements wherever the dye is present, independent of its concentration. Initially $Po_2$ measurements were made in tissue microregions where the dye was injected intravenously (54, 59). Later, perivascular $Po_2$ measurements were found to be possible because of the natural extravasation of the protein–dye complex (30). Then Wilson and co-workers (73, 74) developed a method by which the dye–protein complex is excited with light modulated at different frequencies, a technique that yields $Po_2$ distribution in terms of continuous histograms of intra- and extravascular $Po_2$, showing the existence of substantial portions of the tissue (skeletal muscle) with $Po_2$ in the range of 100 to 140 mm Hg. The physiological implications of the resulting histograms are discussed by Tsai *et al*. (65).

### *Tissue exposure*

The variability of tissue $Po_2$ emerging from the use of different or the same measurement techniques can be also a function of the method used to isolate the tissue from the environment. The cremaster and cheek-pouch preparations of the rat or hamster are often used for microvascular studies of skeletal muscle and require the tissue to be exposed and irrigated with a salt solution equilibrated with 100% $N_2$ or with 95% $N_2$/5% $O_2$ to maintain a pseudo-physiologic condition. Measurements with oxygen microelectrodes require continuous bathing of the tissue surface with the suffusing solution, which acts as an infinite sink or source of oxygen. This creates an artificial oxygen-boundary condition at the surface of the tissue, which will distort the tissue oxygen distribution. Studies with this type of tissue isolation have also been conducted on the brain surface (28).

Optical oxygen measurements can be made by covering the exposed tissue with a transparent barrier (*e.g.*, polyvinyl film or glass), which adequately provides isolation from environmental influences. Polyvinyl film has been used to isolate the tissue under study in acute conditions associated with the hamster cheek pouch (3, 58), cremaster (29), and the mesentery (24) preparations. Window-chamber techniques, although limited to tissue folds, are probably optimal in preserving the "milieu interieur" because they are designed to be contiguous to the intact skin, and thus sealed and independent from the environment. This method establishes an impermeable boundary condition, allows the tissue adjacent to the barrier to evolve its own $Po_2$, and provides a period of observation in the order of weeks. Window preparation was originally developed for the rabbit ear (46) and then implemented in rodents (1); it initially depended on the formation of scar tissue to fill the chamber. Reinhold (44) developed a dorsal skin-fold window chamber in the rat that allowed visualizing the microcirculation of a thin layer of preexisting tissue in the dorsal region, which includes a skin retractor muscle and subcutaneous tissue. This model was refined (39) and then adapted to hamsters (16), rats (55), and mice (34). A cranial window was developed to allow direct observation of the pial vasculature (35).

### *Internal consistency of the data*

The uncertainties introduced by the method for exposing the tissue, the variability between tissue, differences in methods, and the intrinsic potential variability due to the location of the measurement site along the microvasculature are complicating factors, and this method should be calibrated against known or accepted standards. When this is not possible, it may still be possible to obtain independent checks on the data by carrying out additional measurements that test the internal consistency of the data, such as performing a mass-balance analysis in which the amount of oxygen arriving by convection must equal the amount of oxygen exiting by convection, diffusion, and consumption.

An example of the internal inconsistency of available information for skeletal muscle can easily be found with a quick perusal of the literature. Oxygen microelectrodes are the basis for a well-established method and source of information on tissue $Po_2$ data; however, they are not exempt from contradictions. Whalen and co-workers (70, 71) reported that 75% of tissue $Po_2$ values were between 0 and 5 mm Hg in guinea pig gracilis (70) and cat heart muscle (71), whereas measurements in the cat soleus muscle averaged $18.9 \pm 1.8$ mm Hg. Lash and Bohlen (33) reported values on the order of 30 mm Hg for rat spinotrapezius muscle. Whalen's low values may be due to anesthesia. In contrast, Wilson *et al*. (73) " . . . conclude that the currently available data are consistent with mean oxygen pressures in normal skeletal muscle interstitium of 35–45 Torr and with there being negligible volumes with oxygen pressures <15 Torr." The deconvolution method used by these investigators yielded a significant number of measurements beyond 100 mm Hg, above arterial blood $Po_2$, and some even beyond atmospheric $Po_2$.

The internal consistency of the data was analyzed by using information on intravascular measurements of the longitudinal arteriolar $Po_2$ gradient to match the rate of oxygen exit with the diffusion flux from microvessels (43). This analysis showed that oxygen exit was greater than the diffusional flux by a factor of 10. Several studies were carried out to attempt to eliminate this factor of 10 discrepancy, which was summarized by Vadapalli *et al.* (66), but no conclusions were reached.

A mass-balance analysis between the rate of oxygen exit from arterioles and the diffusional rate of exit from these vessels used measurement of the perivascular tissue $Po_2$ gradient in the rat mesentery preparation (62). The phosphorescence quenching technique was used to measure $Po_2$, and blood flow was measured optically. It also showed that the cause for the large rate of outward diffusion was the steep gradients at the microvascular walls, an effect compatible with the hypothesis that the microvascular wall consumes a large quantity of oxygen. Similar conclusions were reached independently by Shibata and co-workers (49–51) by using a different preparation. This study supports the results of Popel *et al*. (43) (*i.e.*, oxygen exits the arterioles at a rate that is greater than that solely due to passive diffusion).

Microelectrode- or phosphorescence-based methods consume oxygen; therefore, it is critical to estimate the oxygen loss by the measuring technique. As of this writing, the only attempt to compare two techniques simultaneously

was performed by Buerk *et al*. (6), who compared the phosphorescence-quenching and microelectrode methods in the tissue of the hamster dorsal-window chamber. Results show no difference between the two methods. This finding indicates that both techniques could be subject to the same errors due to oxygen consumption, and tissue $Po_2$ in the unanesthetized condition is on the order of 22 mm Hg. This value also was obtained by using multi-wire surface electrodes in the same tissue (38). Other techniques have not been subjected to this kind of direct correlation.

Cabrales *et al*. (9) used mass balance to validate the dynamics of intra- and extravascular $Po_2$ changes due to oxygen exit from mechanically occluded arterioles. The rate at which oxygen concentration decreased in the occluded arteriolar segments was compared with the diffusional flux determined from data on the $Po_2$ gradient in the tissue with satisfactory results, within the limits of accuracy inherent to the techniques used.

Internal consistency of the data also was found in analyzing tissue and intracapillary oxygen transients from passage of single red blood cells (RBCs) in capillaries. The studies by Golub and Pittman (23) showed significant intracapillary $Po_2$ transients as a result of the passage of RBCs in capillaries of the mesentery (23), to the extent that capillary blood $Po_2$ was as high as 35 mm Hg when an RBC was present, and decreased to 10 mm Hg in the intra-RBC space (*i.e.*, in the plasma between RBCs). Because plasma does not consume oxygen, if its $Po_2$ decreases to 10 mm Hg, then this is because it equilibrates with the oxygen $Po_2$ in the pericapillary tissue, following the plasma-to-tissue oxygen concentration gradient. This process requires that the tissue $Po_2$ be lower than the plasma $Po_2$ (*i.e.*, 10 mm Hg). However, a substantial discrepancy appears in the value of mesenteric tissue $Po_2$, because the same authors reported tissue $Po_2$ in avascular areas of the mesentery to be consistently greater than 50 mm Hg (22). A recent study in the capillaries of the rat spinotrapezius muscle (2) yielded similar results, although the magnitude of the differences in $Po_2$ due to RBC passage was not so pronounced.

Cabrales and Intaglietta (8) carried out a similar study in the capillaries of the hamster window-chamber model, exploring the possibility that the significant reduction of intra-RBC plasma was due to oxygen consumption of the phosphorescence-quenching technique. They reduced the excitation intensity and found that the $Po_2$ difference between RBCs and intra-RBCs plasma became progressively lower and was eliminated if results were extrapolated to zero-intensity excitation light. Here the internal consistency of the data was explored by a simple and direct variation of the technique.

Data on human tissue $Po_2$ is scarce because most clinical information is derived from photometric measurements that determine the oxygen saturation of hemoglobin. Oxygen partial pressure in the anterior tibial muscle was measured in sedentary healthy subjects with a microelectrode at the end of a 0.45-mm catheter, yielding a $Po_2$ of $27.3 \pm 12.1$ mm Hg (57). A recent study using Clark electrodes found that $Po_2$ in the abdominal subcutaneous tissue of lean healthy subjects was $55 \pm 9$ mm Hg (40).

### *Systematic exploration of tissue $Po_2$ variability*

Tissue $Po_2$ during normal conditions was thought until recently to be in the range of 20 to 25 mm Hg. Recent clinical studies propose that this value may be as high as 50 mm Hg (73), a result supported by the measurements in human adipose tissue (40). However, these measurements in humans were made with Clark electrodes, which tend to yield higher values because they introduce a significant perturbation of the tissue microenvironment and sample an oxygen field that includes the contribution of microvessels in addition to the interstitium.

The lower limit for tissue $Po_2$ is about 2 mm Hg, at which point the tissue metabolism switches from aerobic to anaerobic metabolism (45). Therefore, under normal resting conditions, an ample margin of safety exists to protect the tissue from anoxia; however, this is limited by the variability of tissue $Po_2$. As an example, the $Po_2$ variability measured with the phosphorescence-quenching technique in the tissue of the hamster dorsal-window-chamber preparation, in which the tissue is isolated from the environment with glass, is reported to have a standard deviation ($\sigma$) of about $\pm 4$ mm Hg. Thus, in normal resting conditions, in which this tissue is supposed to average 22 mm Hg, cellular anoxia is at least $3\sigma$ distant from the mean, ensuring that fewer than 0.1% of the cells are at risk. At present, it not evident why tissue $Po_2$ should be as high as 220 mm Hg in terms of the risk for tissue anoxia, and the rationale for tissue $Po_2$ being in the range of 50 mm Hg is not apparent.

Extravascular $Po_2$ heterogeneity could be an intrinsic consequence of the intravascular longitudinal $Po_2$ gradient from arterioles to capillaries and venules. However, this source of variability is not demonstrated by results obtained from *in vivo* studies (64). This is partially due to the longitudinal microvascular $Po_2$ gradient tendency to reverse in venules because of anatomic pairing with countercurrent flowing arterioles, allowing oxygen diffusion from arterioles to venules, and convective shunts that transport oxygen from arterioles directly to venules, thus bypassing transit through capillaries (14, 56). Comparatively large oxygen gradients at the arteriolar and venular walls may be a mechanism whereby the higher blood $Po_2$ is reduced to a perivascular $Po_2$ level in proximity of the average interstitial $Po_2$. Therefore, the presence of a "U"-shaped intravascular longitudinal oxygen gradient (spanning arterioles and venules) and the presence of perivascular gradients could be the reason for the $Po_2$ being uniformly distributed in the tissue and not significantly influenced by the proximity to arterioles and venules.

## The Distribution of Tissue $Po_2$

A systematic survey was made of the variability of tissue $Po_2$ in the hamster dorsal-window-chamber model by using the phosphorescence-quenching technique (65). Tissue $Po_2$ measurements were performed according to two methods: (a) a "random" protocol, in which measurements were made along five vertical and three horizontal axes, the microscopic field being advanced in approximately 150-$\mu$m steps along each axis to a location where the $Po_2$ was measured; and (b) a "focused" protocol in which tissue $Po_2$ measurements were made in areas void of large feeding vessels and between capillaries. The random protocol includes intravascular, perivascular, or interstitial tissue measurements and combinations of each, whereas the focused protocol interrogates intercapillary tissue.

Results for the random protocol were that average tissue $Po_2$ was $25.1 \pm 8.9$ mm Hg, ranging from 63.1 to 5.5 mm Hg.

As expected, the focused protocol yielded a lower and narrower tissue $Po_2$ that averaged $20.2 \pm 7.7$ mm Hg, with 36.8 and 14.8 mm Hg maxima and minima, respectively. The low values in the random protocol corresponded to the oxygen levels measured in terminal lymphatics (24).

Nolte *et al*. (38) used the multiwire polarographic electrode to survey tissue $Po_2$ distribution in the open hamster dorsal window chamber. These data are presented in Fig. 1, together with the data from Tsai *et al*., for the random protocol previously discussed. The same figure shows the data reported by Wilson *et al*. (74) for skeletal muscle.

The data of Wilson *et al*. (74) presented histograms of tissue fractions with $Po_2$ showing a continuous variability from near-zero values to arterial values as high as 140 mm Hg, with 20% of the tissue volume being in the range of 100 to 140 mm Hg. It should be noted that the maximum $Po_2$ achieved by arterial blood in the lung is about 100 mm Hg. The existence of a longitudinal gradient of $Po_2$ is a well-established feature of $Po_2$ distribution in the vasculature (64), and it is impossible for blood $Po_2$ to exceed the $Po_2$ of blood in the lungs. Boegehold and Johnson (4) recorded, by using Whalen microelectrodes in 55-$\mu$m diameter arterioles of the cat sartorius muscle, a periarteriolar $Po_2$ of $52.1 \pm 3.1$ mm Hg [standard error of the mean (SEM)] with tissue $Po_2$ of $22.8 \pm 3.3$ mm Hg. Smith *et al.* (53, 54), by using the oxygen phosphorescence-quenching technique, measured a $Po_2$ of $37 \pm 2$ mm Hg in similar-sized arterioles in rat spinotrapezius muscle and $31 \pm 3$ mm Hg in venules. Therefore, periarteriolar tissue $Po_2$ has a very a narrow range that prevents over oxygenation of tissue.

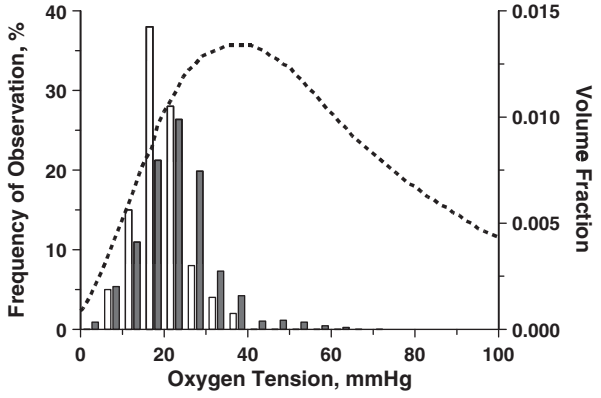


**FIG. 1. Tissue $Po_2$ obtained from three different techniques illustrates the variability of findings and how difficult it is to compare available data.** The histograms are obtained from the hamster dorsal-window-chamber model. Nolte *et al*. (38) obtained the $Po_2$ distribution by using a multiwire polarographic electrode array placed on top of the tissue suffused with a heated buffer solution (*white bars*). Tsai *et al*. (56), by using the sealed window chamber and a random discrete sampling of the tissue, constructed a histogram (*black bars*) with a slightly higher peak with the shift of the histogram to the left, as compared with one obtained with the electrode array. The technique used by Wilson *et al*. (74) resolves the emission signal and produces a curve (*dotted line*) representing the volume fraction of tissue (right y-axis) as a function of $Po_2$. In this representation, the high values, which reach 140 mm Hg, and lower values ($-10$ mm Hg) are not shown. However, an interpretation of this curve is to equate the peak ($\sim 50$ mm Hg) as the average tissue $Po_2$ within the hamster check pouch. A study that provides data in the form of tissue $Po_2$ histograms is that of Nolte *et al*., who used the multiwire polarographic electrode to survey tissue $Po_2$ distribution in the open hamster dorsal-window chamber (38).

Wilson *et al*. (73) used the signal-processing technique, Maximal Entropy Analysis, to resolve their measurements of light emission. Their interpretation of their results is that the broadening of the $Po_2$ histogram leading to the inclusion of a significant population of extreme nonphysiologic values is "intrinsic to the technique" and that " . . . the uncertainty in the determination of phosphorescence life time increases with decreasing S/N (signal to noise ratio)." They truncate the data at 140 mm Hg; however, it is possible and likely that the cause for the appearance of very high $Po_2$ values as a consequence of their method to resolve the $Po_2$ from the emission by using variable frequencies may affect the $Po_2$ distribution as a whole, including the low values of the distribution. They recently reported similar results for tumors, tissues that tend to be underoxygenated (73).

In conclusion, little agreement exists between results on tissue $Po_2$ distribution, and historically, a trend appears continuously to increase the estimate of the magnitude of tissue $Po_2$, as we compare results by Whalen *et al*. (70–72) with our data and that generated from studies in Professors Pittman's and Wilson's laboratories.

## Noninvasive Indirect Methods Used to Assess Tissue Oxygenation

No direct methods are used to measure tissue oxygen levels. In the clinic, assessment of oxygenation is made by monitoring intravascular hemoglobin oxygen saturation ($HbO_2Sat$) based on measurements of light absorption, a method specific to intravascular oxygenation that is only a surrogate measurement for perivascular and interstitial tissue $Po_2$ distribution. The technique determines percentage hemoglobin oxygen saturation by measuring the light absorbed at a reference wavelength and the isobestic wavelength of oxygen and deoxyhemoglobin (15). Optical monitoring of oxy- and deoxyhemoglobin by near-infrared spectroscopy (NIRS) was original developed as a noninvasive optical technique for bedside monitoring of oxygenation (52), and in combination analytical methods resolves both blood flow and $HbO_2Sat$ in vascular beds (68). It is most accurate when light absorption by the tissue is minimized, as with transillumination of thin tissues. The problem with these techniques is the transmission of light through tissue, which depends on absorption and scattering effects. Scattering due to flowing RBCs and tissues requires multiwavelength analysis to resolve RBC density to measure saturation accurately. Markers in combination with red-light optical tomography (18) are used to tag hypoxic zones within the tissue in tumors treated with photodynamic therapy (7); however, they differentiate only anoxia from normoxia. Raman spectroscopy, implemented to measure microvascular $HbO_2Sat$ (60), does not provide an absolute definition for the zero $Po_2$ baseline.

## Tissue $Po_2$ and How Oxygen Exits the Circulation

The classic concept explaining oxygen exit from the circulation is that it is driven by the oxygen-concentration gradient between blood and tissue, moving from the source to the sink

(31). The only modification to this concept has been that the major oxygen-concentration gradient has been found at the blood–tissue interface in the arterioles but not in capillaries (12). Thus the mirror-image analogy whereby oxygen is absorbed in lung capillaries and delivered in the tissue capillaries does not appear to hold for many tissues because lung capillaries present well-defined large $Po_2$ gradients, whereas tissue capillaries could be in oxygen near-equilibrium.

The literature in general supports capillaries being the principal suppliers of oxygen to the tissues; however, the balance between arterioles and capillaries being the suppliers of oxygen to the tissues varies from organ to organ, and with the activity level of the tissue involved. Functional studies of skeletal muscle also show that muscle capillarity and aerobic capacity are related (42). An approach to determine the relative contribution of arterioles *versus* capillaries is that of comparing their change of oxygen saturation between the precapillary and the capillary compartments. Tsai *et al*. (64) made a summary of the available data and showed that the reduction of oxygen saturation is greater in the capillaries of the myocardium, the exercising gracilis muscle, and the rat cortex. Most other tissues show a trend for a greater decrease in oxygen saturation in the precapillary circulation. One of the tissues studied in greatest detail is the hamster check pouch (under anesthesia) (10) and the unanesthetized hamster window chamber (26). In these tissues, the precapillary oxygen exit is 3.0 and 2.5 times the capillary change in oxygen saturation, respectively.

Studies into the mechanisms of oxygen transportation from blood into the tissue show that a significant amount of oxygen is delivered by the arteriolar circulation, an effect that determines the presence of longitudinal microvascular oxygen gradients. This is documented in a variety of tissues (64). This phenomenon requires the existence of a corresponding radial oxygen intraluminal/extraluminal oxygen gradient (19, 61, 64), which is the driving force that determines oxygen exit from blood vessels. It is usually assumed that these oxygen-concentration gradients are determinants of the process of diffusion; however, other mechanisms not fully dependent on the random, thermally driven molecular/atomic momentum exchange that causes diffusion are conceptually possible, provided that at steady state and in the absence of external energy sources, the transfer of matter does not violate thermodynamic principles.

Mass-balance considerations indicate that the longitudinal gradient of blood $Po_2$ in the microcirculation must be matched by a corresponding rate of oxygen exit through the vessel wall, because blood is not a significant oxygen-consuming compartment. Most attempts to establish this mass balance show that the oxygen exit rate is, in general, significantly higher than accounted for by estimating the oxygen diffusion rate across the vessel walls (43).

Tsai *et al*. (62), using the oxygen phosphorescence-quenching technique, found that longitudinal and radial fluxes did indeed balance, because steep oxygen-concentration gradients exist across the vessel wall. To recapitulate, if no oxygen-concentration gradients exist at the vessel wall, in the presence of a finite oxygen flux, a virtually infinite diffusional conductivity through the vessel wall must exist. Such a possibility is not readily apparent. A significant amount of oxygen is delivered to the tissue *via* the arterioles. The arteriolar wall is composed of endothelium and smooth muscle, which also require oxygen to sustain their metabolism. The literature and many experimental investigations show that these cells have a resistance to oxygen diffusion that is not different from that of other tissues. These rudimentary factors require the presence of commensurate oxygen-concentration gradients at the arteriolar walls.

Carvalho and Pittman (10) reported that the transmural $Po_2$ difference (*i.e.*, across the arteriolar vessel wall) in $45.8 \pm 5.4$-$\mu$m diameter arterioles in the hamster check pouch, is $1.0 \pm 2.7$ (SEM, $n = 5$) mm Hg, or $1.0 \pm 6.7$ (SD) mm Hg, and a significant oxygen loss is found from arterioles, similar to that found by other investigators. These data probably are uninterpretable in terms of conventional physical principles, because the small transmural $Po_2$ gradient and large standard deviation suggest that either (a) measurement errors introduce excessive noise, and the arterioles are in oxygen equilibrium with the tissue, precluding the existence of a longitudinal oxygen gradient; or b) the technique is accurate and shows that a substantial number of arterioles are oxygenated by the tissue (*i.e.*, have a negative $Po_2$ gradient). A combination of the two possibilities is likely, and no conclusion can be drawn from this result unless a local mass balance of oxygen fluxes is made.

### Is Nondiffusion-Transported Oxygen Possible?

The results obtained by the studies of Carvalho and Pittman and other studies suggest that mechanisms other than diffusion transport oxygen across the vessel walls. The endothelium of the microvascular wall may present a tunneling-like process, which, even in the presence of weak oxygen concentration gradients, may transfer large amounts of oxygen. It is generally assumed that the diffusion resistance across the lipid bilayer and cell cytosol does not exceed the diffusion resistance of a water layer of the same thickness (17, 36). However, small uncharged molecules homologous to oxygen, such as $H_2O$, $CO_2$, CO, and $NH_4$, penetrate the cell membranes through pores (channels) made of membrane proteins. These molecules are transported through membranes with the aid of aquaporin and, possibly, the band 3 protein (69). Moreover, it was demonstrated experimentally that oxygen can also penetrate through these channels. Mechanistically, this is due to the ability of bulky organic radicals to form microcavities in the lipid environment, in which oxygen mobility and concentration can be significantly greater than in the unmodified lipid bilayer (27).

Therefore, mechanisms exist that could explain a high diffusivity of oxygen across the cell membrane. A problem arises with this concept because, after oxygen transverses the cell membrane, it has to diffuse through the cytosol. This is a fluid environment that presumably lacks the high-diffusivity channels for gas molecules. Furthermore, this medium has a viscosity greater than that of water, with direct consequences on the magnitude of the diffusion constant, which is inversely proportional to the viscosity of the medium in which it takes place. Therefore, it can be expected that oxygen mobilities in the cytosol media limit oxygen diffusion across the vessel wall as well.

A fundamental question to be resolved is to determine the oxygen demand of arterioles *in vivo* to be able to choose between a simple diffusion-process oxygen exit from arterioles and one combined with high oxygen consumption. At

present, the basic approach to resolve this is improving the spatial-resolution measurements of oxygen concentration.

## Oxygen Consumption by Blood Vessels, an Alternate Hypothesis

The endothelium is one of the most active biochemical cell systems of the mammalian organism, producing metabolites that regulate many critical homeostatic mechanisms. The activity of the endothelium addresses the major regulatory system, such as the control of inflammation, maintenance of anticoagulatory function, control of fluid exchange, sensing of mechanical events at its surfaces (mechanotransduction), and production of metabolites in response to varying oxygen supply and local demand.

### *Estimates of oxygen consumption of the vasculature*

The respiration of vascular tissue, with data mostly derived from isolated vessel segments, slices, or rings, and focused on the respiration rate of smooth muscle, with no particular attention to the status of the endothelium. The majority of reported values in a review by Paul (41) were between 1.1 and $4.5\times10^{-3}$ ml $O_2$/min/g or three orders of magnitude smaller than measurements related to the respiration of arterioles in whole organs with intact endothelium.

### *Organ studies*

Curtis *et al*. (11) used a detergent to strip the endothelium of the blood vessels of the dog hindlimb and found that the oxygen consumption of the preparation decreased by 35% (11). These authors explored alternatives to explain the decreased oxygen consumption, including interference with fluid balance, edema formation, and plugging of capillaries, reducing functional capillary density. Assuming that the endothelium is of the order of 0.1% of the tissue mass, we may conclude that the oxygen consumption of this tissue *in situ* is about 350 times that of the remainder of the tissue.

Ye *et al.* (75) perfused the rat hindlimb, kidney, intestine, and the mesentery at constant flow and found that vasoconstriction induced by the administration of noradrenaline or vasopressin significantly increases oxygen consumption by 75% on constriction. Vasoconstriction *per se* reduces functional capillary density and tissue perfusion (20); therefore, it is not likely that the increased oxygen consumption was due to increased tissue perfusion, as was subsequently proposed (9). Marshall and Davies (37) also found that hindlimb oxygen consumption of the animals breathing normal air, after being adapted to chronic hypoxia, increased by 1.53 ml $O_2$/min/g arteriole.

## Microscopic Methods to Determine Oxygen Consumption of the Endothelium

With the exception of the studies by Bruttig and Joyner (5) and Kuehl *et al*. (32), the literature reports that oxygen consumption of the endothelial cells is about two orders of magnitude lower than that shown by the *in vivo* studies.

The consumption of oxygen of the vessel wall (endothelium), deduced from the work of Tsai *et al*. (62), is 4.5 ml $O_2$/min/cm tissue. From the data of Duling *et al*. (13), arterioles of similar dimension (22 $\mu$m diameter) have an oxygen consumption of 0.1 ml $O_2$/min/cm. The results of Santilli *et al*. (47) for the rabbit aorta yield the value of 1.6 ml $O_2$/min/g tissue.

Sharan *et al*. (48), by using oxygen electrodes on pial arterioles, reported oxygen gradients of $1.17\pm0.06$ mm Hg/$\mu$m, which was reduced to $0.68\pm0.04$ mm Hg/$\mu$m when the vessels were dilated. This finding is in accordance with those of several of our studies using the phosphorescence-quenching technique, which shows that vasodilatation reduces the oxygen gradient (25), and *vice versa* (20). Shibata *et al*. (50) also found similar correlations between changes in oxygen-consumption rate as a function of vasodilation and vasoconstriction of arterioles in the rat cremaster muscles by using the phosphorescence-quenching technique. The studies of Sharan *et al*. (48) and Tsai *et al*. (62) appear to yield similar results. Wall thickness is not reported, and all oxygen measurements used to construct a profile are carried out at 10-$\mu$m intervals. Thus, the uncertainty due to the catchment volume of the electrode may lead to gradients at the vessel wall that may be somewhat greater than reported. However, it is unfortunately not possible to check the internal consistency of the data by an independent calculation or measurement.

## Lymphatic and Tissue Oxygen Distributions

Terminal lymphatic fluid is the compartment farthest removed from the oxygen supply, and therefore would present the lowest $Po_2$ in the tissue due to oxygen consumption by the tissue and the lymphatic vessel wall. Lymphatic $Po_2$ distribution was measured in the hamster dorsal-window-chamber model (24). Tissue $Po_2$ averaged $24.6\pm2.7$ mm Hg. Lymphatic fluid $Po_2$ in small and collecting vessels was $18.4\pm2.6$ and $18.0\pm2.4$ mm Hg, respectively. The significant difference between tissue and intralymphatic $Po_2$ was due in part to the presence of an oxygen gradient across the lymphatic wall, which was $3.7\pm1.3$ and $6.0\pm1.2$ mm Hg for terminal and collecting lymphatics, respectively. This oxygen gradient was assumed to be due to the oxygen consumption by cellular components of the lymphatic wall. Thus, the increased vessel-wall gradient found in collecting lymphatics was reconciled with the findings that these microlymphatic vessels tend to be contiguous to arterioles, whereas the terminal lymphatics are dispersed in the tissue. These findings indicate that terminal lymphatics have the lowest $Po_2$ in the tissue and differ from the concept that venules have the lowest oxygen tension.

## Clinical Implications of Tissue Oxygen Distribution

The conceptualization that tissue is permeated by oxygen at an approximately constant $Po_2$ level leads to the conclusion that in normal conditions, the tissue is not exposed to anoxia. However, the existence of this intrinsic variability associated with decreased oxygen delivery, and therefore tissue $Po_2$, increases the probability of the incidence of ischemic regions. What can be done to reduce this risk and the related damage? When does this risk start? How does it progress? When does it become lethal? These questions cannot be fully answered without understanding how tissue $Po_2$ is managed locally, and how these effects are related to clinically measured tissue $Po_2$. Clinical methods for measuring "tissue $Po_2$" measure average microvascular blood-oxygen saturation. This global parameter, as previously discussed, is indirectly related to tissue $Po_2$ and may be incorrect in conditions of resuscitation involving therapeutic approaches aimed at restoring blood

pressure by vasoconstriction (19). At the other end of the spectrum, tissue $Po_2$ is used in clinical scenarios as one of the indicators for deciding the level of amputation in patients with severe limb ischemia.

The most critical aspect of oxygen distribution in clinical conditions is when it becomes a relevant factor in deciding therapy, particularly the intensive therapy environment. Two parameters require critical evaluation: (a) the absolute level of tissue oxygen; and (b) the intrinsic variability of tissue oxygen at the prevalent level. Present clinical methods approximate the first, because they derive information only from intravascular conditions, but do not address the second feature. This is particularly relevant because evidence suggests that tissue-perfusion inhomogeneity can be addressed clinically with newly formulated plasma expanders (63).

## Summary

Accurate determination of tissue $Po_2$ is technically difficult because of problems in assessing the measurement site. Additionally, incongruent results from different methods are difficult to compare, leading to widely differing interpretations of how the $Po_2$ is distributed in tissue. The question remains as to the actual value of tissue $Po_2$. Laboratories analyzing oxygen transport, having made significant contributions beyond the analysis based on the Krogh cylinder, are uncovering different, nonexclusive possibilities of how oxygen is transported from blood through the vessel wall into the tissue. This review highlights the limitations to our understanding of oxygen transport to tissue. Many questions that remain to be explored, particularly in terms of instrumentation, and mathematical modeling of oxygen transport (21). An understanding of the meaning of the tissue $Po_2$ measurement is needed to improve diagnosis and treatment in the clinical setting.

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