Review

Microvascular and tissue oxygen distribution

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Abstract

Understanding of oxygen delivery by the microcirculation has been dominated by the unitary component analysis of Krogh and Erlangen focussed on oxygen transport mediated by single capillaries, oxygenation of tissue as a whole being extrapolated from findings on oxygen exchange in these vessels. This analysis is under revision since capillaries are not sole sources of oxygen. It is increasingly apparent that arterioles are a significant equivalent source, while venules may serve as sinks for capillary and arteriolar oxygen. As a consequence detailed descriptions of the architecture of the microcirculation based on the tissue cylinder conceptualization does not yield new information given the non-exclusive role of capillaries as purveyors of oxygen to tissue. In the present study we investigate how tissue is oxygenated directly from the arteriolar supply on the basis of current results with newly developed optical techniques for the measurement of local intra- and extravascular pO2 by phosphorescence decay. This methodology shows that tissue regions between arterioles and venules have essentially uniform tissue pO2. The only experimentally detectable gradients in pO2 are those present in the immediate vicinity of arterioles. Findings on vascular longitudinal gradients are used to devise a model that links convective and diffusive processes, showing how blood viscosity, blood oxygen-carrying capacity and the slope of the oxygen dissociation curve are linked in determining intravascular and tissue pO2. The integrated approach provides a numerical basis for interpreting consequences of alterations in transport properties of blood applicable to the field of blood substitutes.

Keywords: Oxygen transport; Capillaries; Oxygen uptake

1. Introduction

Survival of mammalian cells is based on the availability of an aqueous medium as a source of nutrients and oxygen to support metabolism. In multicellular organisms these needs cannot be met by direct contact with the environment, inducing the evolution of the circulatory systems to meet this demand. Changes in fluid environment are relatively slow while metabolic requirements are variable and may occur at a much faster rate; however, since aqueous medium management and metabolic supply depend on the same system of delivery, it follows that oxygen supply and fluid exchange are, in principle, linked.

Transport phenomena involved in fluid exchange and balance are better understood than those of oxygen delivery even though conceptually neither of these systems conserves material in terms of mass balance involving tissue and microcirculation. Fluid exchange in the tissue/microcirculation systems loses fluid into the lymphatics, while in the context of metabolic processes the system loses oxygen through consumption. This superficial commonality does not necessarily reflect a detailed similarity between these two transport systems; however, it suggests that these systems may in part be analyzed by models based on similar principles, or at least related. For instance, in fluid exchange we recognize the presence of regions of high hydraulic pressure within the capillary network that filter fluid into the tissue, and regions of low pressure that absorb fluid while the lymphatic system serves as an escape mechanism that stabilizes tissue fluid volume. In this analysis usually no attempt is made to assign an exclusive tissue region to a capillary. In fact, early modelling in this area recognized that the region

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influenced by a capillary is determined by the transport from surrounding capillaries [28].

By contrast, thinking about oxygen delivery has been dominated by the elegant analysis of Krogh and Erlangen [41] who focused on how oxygen transport from blood to tissue is mediated in single capillaries and a surrounding tissue cylinder isolated from the surrounding cylinders (the so-called Krogh model). Tissue oxygenation as a whole was, and in many circumstances still is, extrapolated from the behavior of these unitary components. While fluid exchange is also considered at the unitary level, analysis and experience indicate that effects involve primarily macroscopic tissue regions. By contrast, analysis of oxygen transport has led to the concept of the microscopic ‘lethal corner’, the tissue site most distant from the inflow end of the capillary and therefore most vulnerable to impairment of capillary blood flow.

A problem associated with the Krogh model arises from the observation that capillaries are not the sole source of oxygen in the microcirculation, and may not be the most important supply for a significant fraction of the tissue in direct contact with arterioles. This phenomenon was demonstrated by Duling and Berne [10] who found that significant amounts of oxygen exit the arteriolar network. The longitudinal oxygen gradient (direction of blood vessel axis) when related to the vessel blood flow provides a quantitative measure of the rate of oxygen loss from arterioles. Kuo and Pittman [42] found that this rate of exit was of the order of 10 times that which could be accounted for by periarteriolar oxygen consumption, on the assumption that the oxygen gradients across the vessel wall are negligible and predicted by the level of oxygen consumption of parenchymal tissue. This finding led to the suggestion that the diffusion constant of the wall tissue may be an order of magnitude higher than previously thought [55]. This supposition is in contrast with extensive work indicating that there may be significant barriers to oxygen transfer to tissue on the luminal side of the blood/tissue interface in vessels smaller than about 15 μm diameter [2,24]. Furthermore, Torres Filho et al. [63], utilizing the phosphorescence decay technique in the skinfold of SCID mice obtained evidence for the presence of large gradients at the vessel wall, and when the corresponding diffusion gradients are used to carry out the mass balance, there is no need to invoke abnormally large diffusion constants.

The premise that organs present uniform arrays of equally-spaced capillaries with concurrent flow underlies the Krogh cylinder model. In recent years considerable effort has been directed toward detailed description of the architecture of the capillary networks, in part to obtain a better understanding of the applicability of this model to the in vivo situation. In the process it has become evident that overlying and adjacent capillary networks may have countercurrent or concurrent flow, in part as a consequence of the presence of cross-capillaries [73]. Furthermore, arterioles and venules can serve as sources or sinks for oxygen carried in the surrounding capillaries [71], and venules may serve as sinks for oxygen in the capillary network [55]. These phenomena lead to an elevated level of oxygen tension in some regions of the tissue, thus allowing for diffusion on a larger scale than that restricted to single non-interacting capillaries, a situation that supports the alluded-to similarity between fluid and oxygen transport. Given these considerations, it is unrealistic to expect that detailed descriptions of capillary network architecture should yield insight on oxygen delivery to tissue, a statement supported by the lack of specific in vivo experimental evidence showing the existence of the so-called ‘lethal corner’.

In most analyses of oxygen delivery by blood vessels it is assumed that it is due to oxygen flux from a spatially continuous source. Tsai and Intaglietta [66] studied the problem of tissue oxygenation by discrete oxygen sources by computer simulation and found that longitudinal penetration of oxygen (direction of the capillary axis) and radial penetration transport of oxygen into the tissue vary depending on the relationship of oxygen-carrying capacity or hematocrit and blood flow velocity. Thus at constant flux but increased flow velocity and lower hematocrit a larger tissue volume is oxygenated at a lower average PO2 and vice versa—a finding that is not contemplated by the tenets of Krogh’s model, and which may explain results in hemodilution showing the increase of tissue oxygenation at lower hematocrits and higher flow velocities [36,78].

Understanding of microvascular oxygen transport has become a key issue in the treatment of acute and chronic ischemic disease, where the answer to problems is not forthcoming from the understanding of events in single capillaries, or detailed analysis of microvascular architecture. This suggests that the problem may be analyzed from a broader perspective, including the interaction of convection and diffusion in the transport process, regulation, and the architectural anatomy of the microcirculation in the context of biophysical properties of blood.

2. Microvascular oxygen transport: the analytical problem

Oxygen transport from the lungs to the tissue is the resultant of a balance between the rate at which it leaks out of the blood vessels and the velocity at which it is carried by the moving blood. Outward oxygen diffusion from arterial vessels is present throughout the circulation since there is no specific barrier to its passage across the vessel wall other than the exit rate limitation imposed by diffusion and geometry of the vessels expressed by the permeability surface area product [12,46]. Oxygen transport in the circulation is different from the transport of fluids through a system of impermeable conduits since in permeable tubes the mass balance between input and output is a function of flow rate.
Experimental findings support these premises. Direct measurements of arteriolar microvessel intravascular pO$_2$ correlated to locally measured blood flow velocity show that blood pO$_2$,blood and flow velocity v are empirically related by the expression:

\[ pO_{2,\text{blood}} = 30 + 2.5 \nu \text{ mmHg}; \]

(\(\nu = \text{mm/s}; P < 0.001\))

This expression has a correlation coefficient of 0.503 which is relatively weak; however, it has a high level of significance because it is derived from 265 independent measurements [35]. The significance of this relationship is that changes in cardiac output affect proportionately the oxygen content of all segments of the vascular tree.

The relationship between blood flow velocity, oxygen-carrying capacity and rate of oxygen leakage is given by a mass balance equation for a segment of an arterial or arteriolar blood vessel with branching order \(i\). For any axial length \(dx\) the rate of oxygen entering the upstream cross-section at \(x\) is \(m_i\) given by:

\[ m_i = Q_i [S_{0,i} + n_i pO_{2,i}] F(\text{Htc}) \]

where \(Q_i\) is the entering volumetric flow rate and \(F\) is a function representing the intrinsic oxygen-carrying capacity of blood related to the hematocrit (Htc). \(S_{0,i} + n_i pO_{2,i}\) is the linearized functional relationship that determines blood oxygen as a function of the prevailing partial pressure of oxygen pO$_2$,i and which approximates the hemoglobin oxygen dissociation relationship in the given branch \(i\) of length \(L_i\) (\(S_{0,i}\) is the saturation at zero pO$_2$ for each vessel segment \(i\)). The oxygen content of blood decreases along the blood vessel in proportion to the rate of oxygen exit across the vessel wall \(m_{\text{diffusion}}\). This rate of oxygen exit is determined by the oxygen permeability coefficient \(P\), the surface area of the vessel \(\pi d_i dx\) (\(d_i\): inside vessel diameter) and the difference between blood and tissue pO$_2$,i - pO$_2$,s,i = pO$_2$,i; therefore the rate of oxygen exit per unit \(dx\) is:

\[ dm_{\text{diffusion}} = P \sigma d_i c pO_{2,i} dx \]

where \(c\) is the oxygen solubility in tissue. The assumption of a fixed tissue pO$_2$ cannot be generalized for all tissue types, but may be valid for tissues where this parameter has been measured in vivo, such as subcutaneous connective tissue and skeletal muscle, according to our results. In this equation pO$_2$,i is the variable rather than oxygen content since oxygen diffusion is driven by the gradient of oxygen partial pressure, which in a gas is proportional to oxygen concentration. In blood, oxygen concentration is related to pO$_2$ through the oxygen saturation curve which describes the oxygen held in chemical binding. In this system it is practical to express mass balance as a function of partial pressures rather than concentrations (molecule per unit volume) because of the changes present at the blood/vessel wall interface. Within an infinitesimal distance in either direction from the interface pO$_2$ is identical; however, oxygen concentration changes abruptly and in tissue oxygen diffusion is driven by tissue pO$_2$ gradient which is not related to the amount of oxygen in blood.

Combining Eqs. 2 and 3 to describe oxygen mass balance for a blood vessel cylinder of length \(dx\) we obtain the differential equation:

\[ \frac{dpO_{2,i}^*}{dx} = \frac{\sigma d_i c p}{Htc Q_i n_i} \]

where \(F(\text{Htc}) = \text{Htc}\).

Integration of Eq. 4 over the extent of the vascular segment \(L_i\) yields:

\[ pO_{2,i}^* = pO_{2,0}^* e^{-k_i} \]

where \(k_i = \frac{\pi d_i c p L_i}{\text{Htc} n_i Q_i}\)

which indicates that if we know pO$_2$, at the beginning of the vascular segment (i.e., the end of the previous segment \(i-1\)), we can calculate pO$_2$ for the end of the \(i\)th segment. Knowing pO$_2$ at \(i = 0\) (i.e., central oxygen tension) allows us to predict pO$_2$ at any subsequent order of vascular branching according to:

\[ pO_{2,i}^* = pO_{2,0}^* e^{-\sum_{i=1}^{\infty} k_i} = pO_{2,0}^* e^{-k_i} \]

Blood flow in any segment is given by Poiseuille's equation:

\[ Q_i = \frac{\pi D_i^4 \Delta P_i}{128 \mu L_i} \]

where \(\Delta P_i\) is the pressure drop in the segment length \(L_i\), and \(\mu\) is the viscosity of blood. Introducing this expression into Eq. 6 and solving for \(K_n\) we obtain:

\[ K_n = \frac{\mu}{\text{Htc} \sum_{i=1}^{n} n_i d_i^2 \Delta P_i} \]

The significance of this result is that it shows how microvascular pO$_2$ is determined by the geometry of the blood vessels, the vessel oxygen wall permeability, rheological properties of blood, oxygen content of blood and the shape of the oxygen dissociation curve.

This development is a simplification of previous solutions of this problem particularly in what regards the assumption of uniform tissue oxygen. The problem of diffusion in an inhomogeneous tissue has been addressed by Popel and Gross [54], Klitzman et al. [38], and Hsu and Secomb [26]. The theoretical study by Wieringa et al. [74] consider the implications of intercapillary oxygen exchange coupled with heterogeneous flow distribution, reaching the conclusion that this interaction is determinant in creating a tissue oxygen environment that is significantly less inhomogeneous than that resulting from the non-interacting capillary cylinder hypothesis. The potential presence of additional large-scale fluxes of oxygen be-

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tween arterioles and venules [12,13] further supports the assumption made in this development that tissue oxygen is prevalently uniform.

There are several factors that are superposed on the phenomena described by the present analysis. In particular, exchange of oxygen in the microcirculation is critically dependent on the relative contributions of red blood cells and plasma and the chemical processes associated with interactions of hemoglobin with oxygen and carbon dioxide, plasma proteins and the effects in red blood cells [27]. Furthermore, in skeletal muscle transport of oxygen is facilitated by the presence of myoglobin which acts as a tissue oxygen carrier [22].

Our development reflects the critical role played by the velocity of blood and therefore blood viscosity in partitioning in arterioles and capillaries. It is noteworthy that changes in intrinsic oxygen-carrying capacity of blood (i.e., variations in hematocrit and blood viscosity) influence blood oxygenation depending on how the ratio $\mu_v/\text{Htc}$ is affected. In this scenario any decrease of intrinsic blood oxygen-carrying capacity not accompanied by lowered blood viscosity, and therefore increased flow velocities, lowers intravascular $pO_2$ prior to blood arriving at the capillaries. Conversely, at constant intrinsic oxygen-carrying capacity, the circulation can deliver proportionally more oxygen to the capillaries at higher flow rates, since blood with a higher $pO_2$ arrives at the capillaries. Furthermore, any increase in intrinsic oxygen-carrying capacity of blood, or availability of blood to the arterioles that may be caused by the presence of hemoglobin in solution (rather than within the red blood cell) [9] requires that blood viscosity should be increased if the normal distribution of intravascular $pO_2$ is to be maintained, as shown by the studies of Waschke et al. [70].

3. The technology for $pO_2$ measurements in the microcirculation

The earliest methodology for measurements of $pO_2$ at the cellular level is based on the so-called Clark electrode [8] which was miniaturized and constructed to fit within the tip of a glass micropipette [72]. These electrodes can be placed in contact with the tissue area of interest or inserted into the tissue. There are a few reports of their successful use in measuring blood $pO_2$, and the paucity of data suggests that direct contact of the microelectrodes with blood poses substantial technical difficulties. Duling and Berne [10] argued that perivascular measurements of tissue $pO_2$ constitute a representative measurement of intravascular $pO_2$; however, there appear to be significant $pO_2$ gradients in the immediate vicinity of the vessel wall as shown by our own results (see below), by Duling et al. [11], who found differences as large as 27 mmHg across the microvascular wall, and as indicated by Groebe and Thews [23].

Polarographic electrodes have been developed in a multiwire configuration where as many as 8 microelectrodes are packaged in a bundle where the exposed microelectrode area is placed in contact with the tissue through a membrane [37]. This technique is reported to be useful for obtaining tissue surface histograms; however, the size of the electrode package precludes knowledge of location of electrodes relative to the underlying blood vessels, consequently it cannot be used to obtain specific understanding of the dynamics of oxygen transport because measurements are dissociated from considerations of blood vessel distribution. Furthermore, the size of electrodes (10–20 μm diameter wires) and the effective distance of the electrode from the tissue, probably at least of the order of thickness of the membrane, suggest that the spatial resolution for each electrode is not adequate to obtain measurements from the tissue only, and that each measurement is always a mix of data from several sources including tissue and blood vessels [19].

Early studies with single electrodes noted that perivascular $pO_2$ is not always a good estimate of intravascular $pO_2$. This problem was resolved by the introduction of microvessel blood spectrophotometry. This technique evaluates oxygen saturation of hemoglobin through measurements of light absorption at different wavelengths of the hemoglobin absorption spectrum. This indirect technique is attractive because it relays only optical means which are easily implemented at the microscope [52]. However, it depends on a precise knowledge of the hemoglobin absorption spectrum and the relationship between the oxygen dissociation curve for hemoglobin and $pO_2$, a relation that is strongly influenced by local carbon dioxide concentration and pH, parameters that are not easily determined in blood in the microcirculation. A further limitation of this technique is that it cannot be used to measure tissue $pO_2$.

Microcirculatory $pO_2$ in both blood and tissue can presently be measured by the Pd-cuproporphyrin phosphorescence decay technique [62] based on the oxygen-dependent quenching of phosphorescence emitted by metalloporphyrins bound to albumin [68,75]. Animals receive a slow intravenous injection of albumin/Pd-meso-tetra(4-carboxyphenyl)porphyrin 15 mg/ml at 0.05 ml/min (Porphyrin Products, Logan, UT) which is allowed to circulate for 10 min prior to measurements. After pulsed light excitation to the triplet state of oxygen, the energy causing phosphorescence is transferred to free oxygen molecules present in the measuring site. In this process phosphorescence lifetime decreases in proportion to the local oxygen tension. The relationship between phosphorescence lifetime $\tau$ and $pO_2$ is described by the Stern-Volmer equation:

$$\tau_0/\tau = 1 + k_q \cdot \tau_0 \cdot pO_2$$

(9)

where $\tau_0$ and $\tau$ are the phosphorescence lifetimes in the absence of oxygen and at a given $pO_2$ respectively and $k_q$ is the quenching constant. Both $\tau_0$ and $k_q$ are substance-
specific, are pH- and temperature-dependent, and have been previously determined. Binding of the porphyrins to albumin provides protection from self-quenching and reduces oxygen-quenching, which is advantageous when measuring high oxygen concentration. The porphyrin compound remains mostly intravascular for several hours after intravenous or intra-arterial application and allows non-invasive determination of intravascular PO2 over that period. There is a small and steady leakage of the albumin-bound dye that is related to the normal exchange of albumin between blood and tissue. This process causes the porphyrin compound to be detectable by this technique in the tissue about 30 min after injection, thus allowing measurement of tissue PO2. The technique is independent of absolute light-intensities and blood porphyrin concentrations.

Decay curves from rectangular measurement areas about 7 × 100 μm located parallel to the vessel under study are analyzed using a standard single exponential least-squares numerical fitting technique. Resultant time constants are used in the Stern-Volmer equation to calculate PO2. When light emission is measured at a specific point along the optical path, consideration must be given to contribution of sources overlying and underlying the measurement plane. With the phosphorescence decay technique signals from other areas along the same optical axis have a different constant. Since quenching at a specific PO2 results in a single exponential [68], in some applications there would be a need for multiple exponential fitting algorithms [1,49,69] and a means for assigning a specific location to each exponential found. Current use of the technique has not revealed the need for this type of analysis [34,35,59,60].

The level of tissue oxygen partial pressure that is not adequate for supporting oxidative metabolism can be determined by measuring the rise of tissue fluorescence 405 nm as mitochondria become reduced when oxygen supply falls below a preset limit. The PO2 at which the shift in metabolism occurs is not known precisely, being estimated to be in the range of 0.5–2 mmHg in the mitochondria. As cells become progressively anoxic, fluorescence increases, reaching a plateau at a value of PO2 that is near zero mmHg. This technique is useful to detect oxygen availability in the tissue, a parameter that is measured by stopping oxygen supply and measuring the time necessary for the rise in fluorescence [65]; however, it cannot be used as a quantitative measurement of tissue PO2 at the higher ranges of this parameter [7].

4. Experimental findings on PO2 distribution in the microcirculation

The studies of Duling and Berne [10] reported periarteriolar PO2 values of about 30 mmHg when measured with microelectrodes. Relating this to the O2 dissociation curve of hemoglobin shows that one-third of the arterial oxygen exits the circulation prior to arrival at the capillaries. Similar magnitudes of O2 loss from arterioles were also found in the pial microcirculation [11,30]. Possible recipients for the oxygen exiting prior to the capillaries have been studied in efforts to understand the mechanism of tissue oxygenation. Shunting of O2 away from the arterioles to parallel venules was found to be negligible under normal conditions [58], while Ellsworth and Pittman [12] showed diffusive shunting from arterioles to capillaries. Theoretical evaluation of the extent of shunting has been found to be about 40–50% of the arteriolar loss [57]. Another proposed oxygen sink is consumption by the tissue surrounding the arterioles; Kuo and Pittman [42] concluded from experimental and theoretical analysis that only 10–15% of the arteriolar oxygen loss can be accounted for by periarteriolar tissue consumption. With a multitude of experimental evidence of oxygen loss by the arterioles, there is still no concrete explanation for the large arteriolar losses.

Our own measurements in blood and tissue of the awake hamster window preparation using the phosphorescence technique [64] in the awake, unanesthetized ham-
ster skinfold preparation reported in Fig. 1 show that intraluminal \( p_{O_2} \) in 64.3 \( \mu \)m A1 arterioles is 57.5 \( \pm \) 7.5 mmHg while A4 terminal arterioles have \( p_{O_2} \) of 34.8 \( \pm \) 6.9 mmHg. Small venules do not exhibit any correlation between \( p_{O_2} \) and order or branching or diameter and have an average \( p_{O_2} \) of 29.7 \( \pm \) 9.3. It should be noted that tissue of the hamster skin fold chamber includes both of muscle and connective tissue. These results show that in this intact tissue, without anesthesia, according to the oxygen dissociation curve for hamster blood. 20% of the oxygen in blood exits the microcirculation in arterioles, while a very small fraction difficult to estimate without directly measuring tissue consumption, is delivered by the capillaries. In this preparation, which consists mostly of subcutaneous connective tissue and muscle, under normal conditions tissue \( p_{O_2} \) is 23.5 \( \pm \) 5.3 mmHg, with no evidence for the presence of areas with lower \( p_{O_2} \), a value in agreement with that of Boegehold and Johnson [3] who reported 22.8 mmHg in tissue sites at the venous end of the capillary network for anesthetized cat sartorius muscle utilizing microelectrodes.

5. Oxygen gradients at the arteriolar blood/tissue interface

There is substantial controversy regarding the issue of how oxygen is unloaded from the microvessels. The work of Hellums et al. [25] summarized in a recent review supports the concept of significant gradients in the fluid layer in contact with the vessel wall and therefore the existence of a marked resistance to outward diffusion of oxygen. This conclusion is reached from analytical considerations [47] and in vitro experimental modeling [4]. This resistance arises from theoretical considerations regarding the gradient in oxygen tension between the wall and the central blood core, showing that it should be of the order of 10 mmHg in arterioles of 30–50 \( \mu \)m diameter. Furthermore, the existence of this resistance would significantly overestimate the rate at which oxygen is unloaded from the microcirculation.

These results are difficult to interpret when related to in vivo findings because all direct measurements of the change in arteriolar oxygen content along the length of the vessels show that oxygen unloading occurs at such a rate that the diffusion coefficient for oxygen in the tissue would have to be about 10 times that which it is normally assumed to be, even if there is no resistance to oxygen unloading on the blood side of the blood/vessel wall interface. The same result was reached by Hsu and Secomb [26] who treated the diffusion problem between arterioles and tissue by Green’s function method. In their work they found that they could predict the oxygen distribution found in capillaries by Ellsworth et al. [13]; however, the calculated outward oxygen flux was an order of magnitude smaller than that measured in capillaries.

The phosphorescence decay technology provides means for evaluating simultaneously intra- and extravascular \( p_{O_2} \), and therefore it is a direct method of evaluating both longitudinal vessel blood \( p_{O_2} \) gradients (blood oxygen saturation gradients) and the perivascular \( p_{O_2} \) tissue gradients that presumably determine the rate of oxygen exit from blood vessels. In a very large number of determinations (\( n > 200 \)) we have found that A1 arterioles in the 50–60 \( \mu \)m diameter range exhibit a perivascular oxygen gradient by which oxygen tension in the tissue, on average, falls by 17.5 mmHg over a distance estimated to be about 3.5 \( \mu \)m, since this is the center of a 7-\( \mu \)m-wide measuring area. Progressively smaller arterioles have proportionately smaller extra/intra-luminal \( p_{O_2} \) differences at the vessel wall as shown in Fig. 1. The existence of these gradients was recently demonstrated by Itoh et al. [29]. In this work \( p_{O_2} \) maps of the oxygen gradients in the mesentery were obtained with an oxygen-quenching fluorescent membrane, showing that gradients are circumscribed by the vicinity of arterioles. When these gradients are used to estimate the rate of oxygen exit by diffusion from the arteriolar microvessels, we do not find significant differences between longitudinal oxygen unloading and rate of outward diffusion.

Such large oxygen gradients in the vicinity of the arteriolar wall are in contrast with the hypothesis that the rate of exit of oxygen from arterioles is facilitated by a high diffusion constant as suggested by Pepel et al. [55], or that they are due to high oxygen solubility in tissue as proposed by Pittman [51]. It has also been proposed that a significant drop in \( p_{O_2} \) occurs as oxygen passes from being contained by hemoglobin to a region where it is dissolved in tissue water [23]; however, the corresponding oxygen gradient would be due to the increased resistance due to the transition, further hindering the release of oxygen, and therefore making it even more difficult to identify the oxygen exit flux necessary to balance the longitudinal decrease of oxygen content. Conversely, our findings could be interpreted in terms of a very high rate of oxygen utilization by the endothelium as suggested of by the study of Bruttig and Joyner [6], showing that endothelium is capable of metabolic activity which can be 100-fold that of other cell species. It should be noted that capillary endothelium has the largest surface in the circulation and therefore a maximal exposure to blood oxygen per unit amount of tissue. It is interesting to speculate whether one may extrapolate from results in arterioles to the capillaries and conclude that endothelium is a major oxygen sink.

When the intraluminal \( p_{O_2} \) is referred to the oxygen saturation curve for the corresponding animal species, we find that approximately 35% of the oxygen in blood is released to the tissue between the A1 arterioles and the capillaries, and that about 9% of this oxygen is either recovered or shunted to the venules as shown in Fig. 1. Since steep oxygen gradients are also present near venular
walls, venular perivascular tissue appears to be at about the same \( pO_2 \), as the remainder of the tissue, thus precluding a re-oxygenation of venular blood by direct transfer from the tissue, but rather due to convective or diffusive shunts that account for about 25% of the oxygen delivered by the arteriolar supply.

The origin of these gradients is not established. They are not due to increased resistance to diffusion, because this would further hinder the oxygen exit measured experimentally. The alternative is that this gradient is due to the presence of a highly metabolic tissue in the vascular wall. Our own experiments in the study of the effects of prosta-cyclin on tissue oxygenation show that these agents, in healthy tissue under un-anesthetized conditions, cause both dilatation and constriction in different vessels of the microvascular network, with little effect on tissue oxygenation. A1 vessels that constrict have wall gradients of about 26 mmHg, while those that dilate have gradients of the order of 14 mm Hg. Since the change in diameter and gradient are anti-correlated, while they are directly correlated in the normal vasculature as shown in Fig. 1, we interpreted this result as the expression of the relationship between vessel wall metabolism and vessel wall tone, in support of the concept that vessel wall metabolism is a factor determining tissue oxygenation distribution.

6. Role of capillaries in tissue oxygenation

Our findings based on simultaneous measurements of intra- and extravascular \( pO_2 \) indicate that in connective tissue and muscle at rest capillaries are not the only suppliers of oxygen, and that they may rank in second place to oxygen delivered by arterioles. Furthermore, the presence of increased blood oxygen tension in venules relative to both tissue and capillaries is evidence of the presence of convective and diffusive shunts whose physiological role is not established.

An important finding is that tissue \( pO_2 \) is essentially uniform at the microscopic level on a scale of about 7 \( \times 100 \) \( \mu \)m. A similar finding was obtained by Toth et al. [65] who determined that tissue oxygen availability at arterial and venous capillary sites was essentially the same as on a scale of round areas of 20 \( \mu \)m diameter. We also find that in tissue the only experimentally detectable gradients in \( pO_2 \) are those present in the immediate vicinity of arterioles and venules; therefore, since tissue \( pO_2 \) is found to be the same in locations near and distal from arterioles, as well as in the proximity of venules, tissue \( pO_2 \) uniformity would appear to be a feature of tissue oxygenation distribution.

As a consequence, in the tissues studied, the resulting tissue oxygenation appears to be determined by the arteriolar diffusional oxygen source supply, oxygen diffusion and consumption (metabolism) of the related tissue mass, and the venous exit, with the configuration of the intervening capillary network mostly providing a sufficient functional capillary density for smoothing out tissue \( pO_2 \) irregularities. In this context it is interesting to compare normal tissue and tumor microvascular networks. In normal tissue such as skeletal muscle, kidney and liver, the different parts of the network are well differentiated and capillaries are arranged in a specific pattern, while in tumors there is a random system of interconnecting vessels [32]. Both ordered and disordered configurations achieve the same result in terms of oxygen supply to the tissue; consequently, it may be that anatomical arrangement of either normal or tumor systems is neither determined by nor critical to oxygen delivery. The tissue fluid transport process is similarly independent of capillary network configuration since net tissue fluid pressure is determined by the relationship between capillary surface area exposed to forces favoring filtration versus that exposed to absorption (see Ref. [21] for a detailed analysis of this process).

The possible independence of tissue oxygenation from capillary network configuration suggests that conclusions based on Krogh cylinder modelling may not realistically portray the role of capillaries in tissue oxygenation. The relevance of Krogh cylinder modelling to tissue oxygenation has been repeatedly analyzed (see Refs. [39,51,53] for a detailed discussion of assumptions and consequences); however, only recently has there been an increased emphasis in exploring the limitations of the critical non-interaction assumption [26,74] which leads to a level of tissue inhomogeneity not found in our studies.

There are additional factors not contemplated by the Krogh-type analysis that tend to even out tissue oxygenation if tissue cylinders are allowed to interact. One deals with the assumption that blood is a homogenous medium, and therefore a continuum source of oxygen, which is valid for the larger microvessels. Capillary hematocrit is usually significantly lower than central, being of the order of half of the systemic value in most tissues. This discrepancy between central and peripheral hematocrit is due to plasma skimming [48] and the separation of red blood cells occurring at bifurcations with different flow rates, whereby the stream with the highest flow rate collects the greater number of red blood cells [17]. Mass balance considerations indicate that the presence of low-hematocrit capillaries must be compensated by red blood cell and leukocyte shunts as shown by Ley et al. [43].

Federspiel and Sarelius [15] dealt with the theoretical limit at which capillary blood transits from homogeneous to a particulate system, and concluded that it is at a hematocrit of about 20%, which is the average value for this parameter in most tissues. However, the intrinsic variability of capillary perfusion causes large temporal dependent departures from this value and either selective or induced hemodilution leads to considerably lowers levels. Furthermore, when blood oxygenation is analyzed by assuming red blood cells to be discrete oxygen sources [5,66], the shape and volume of tissue oxygenated is...
simultaneously dependent on the flow velocity and the hematocrit, such that high velocity flows yield greater axial extension of the oxygenated zone, with shallower and smaller radial penetration.

Capillary flow is modulated continuously by the process of vasomotion, leading to an interrelated change of blood flow velocity and hematocrit [14] whereby the net red blood cell flux is approximately constant since lower hematocrits are usually concomitant with higher flow velocities due to lowered blood viscosity. The non-linearity of the oxygenation process evidenced by the study of Tsai and Intaglia etta [66] shows that although flux, the product of hematocrit and flow velocity, is relatively constant, underlying changes in velocity and hematocrit will not yield constant oxygenation. When the analysis of oxygen delivery by single sources is extended to non-steady conditions prevailing with vasomotion [67], results show that the volume of tissue oxygenated increases for all regimes of flow variability, but the average tissue pO₂ is smaller. Therefore, as a consequence of the non-linearity of tissue oxygen transport, temporally averaged tissue oxygenation is different from steady tissue oxygenation even though the same number of red blood cells arrive at the tissue per unit time.

These considerations show that due to the variability of hematocrit and flow velocity different capillaries deliver oxygen to different tissue configurations, which change in time as a function of the temporal variability of capillary flow and hematocrit. It is plausible to conclude that most differences in pO₂ will be smoothed out as shown in our studies if capillaries are allowed to interact on the basis of the differences in oxygen partial pressure between their surrounding regions.

7. The role of regulation in microvascular oxygenation

Oxygen delivery to the microcirculation is an actively regulated phenomenon which has been subjected to extensive studies [33]. The preceding discussion shows that as a consequence of mass balance between convection and diffusion, oxygen supply to the tissue is directly related to all factors that govern blood flow. This result suggests that blood viscosity, oxygen-carrying capacity and position of the dissociation curve for hemoglobin within the microvasculature and the vessel wall gradients become determinant and interacting factors regulating microvascular oxygen tension, while branching patterns and number and vessel dimensions become secondary. Factors such as viscosity and flow velocity determine shear stress at the blood vessel wall. Production of the vasodilator prostacyclin by endothelium is a direct function of shear stress generated by blood at the vascular wall [16,20], a phenomenon that is the basis of so-called ‘blood-flow-induced vasodilatation’ [44,61]. Consequently the relationship between the oxygen transport properties of blood (hematocrit, flow velocity and the resulting blood viscosity), the release of regulatory mediators [45] and the distribution of pO₂ in the circulation [31] indicate that structure and control of the microcirculation is linked and matched to the flow and transport properties of blood; therefore, blood and microvessels must be viewed as a unit in order to analyze oxygen distribution and delivery.

The previous considerations lead to the conceptualization of how oxygen is transported in the microcirculation that is in part similar to that developed to interpret fluid balance. In fluid exchange and balance we recognize regions of high and low hydrostatic (hydraulic) pressure that move fluid by a convective process through the tissue, resulting in virtually uniform tissue pressure. In parallel with this, in terms of oxygen there is a high O₂ partial pressure source, namely arterioles, the tissue per se and the venous exit. Steep gradients at the arteriolar wall should be the factor causing tissue pO₂ to be uniform throughout. The uniformity of tissue pO₂ is also the resultant of other factors, including the quasi-random arrangement of oxygen sources and sinks, and the random arrangements of the direction of blood flow in the tissue [13]. As a result the tissue compartment is relatively evenly oxygenated, much in the same way as it has uniform tissue fluid pressure, the arterioles being the only organic structures exposed to a wide range of pO₂ conditions. This exposure, however, is directly linked to the autoregulatory process, since these blood vessels (organs) have specific reactions to changes from the blood pO₂ that they are meant to recognize as their normal environment, in the same way that they autoregulate to maintain both flow and pressure at a preset level for each order of branching. The arteriolar vessel wall may limit the exposure of tissue to the elevated oxygen partial pressure of blood through consumption, with the effect that tissue pO₂ is essentially constant. Similarly, the vessel wall reacts through the myogenic process to changes in hydraulic pressure striving to maintain downstream pressure (i.e., the pressure that governs fluid exchange) constant.

The present analysis highlights the significance of arterioles in supplying oxygen to the tissue. The question arises as to the role of capillaries in tissue oxygenation, namely how much oxygen they contribute and what is their role in insuring uniform tissue oxygenation. Fig. 1 based on the data of Kerger et al. [24] and Torres Gilbo et al. [64] shows that in the hamster the skinfold capillaries release very little of the blood’s oxygen content. Capillary pO₂ is 28.5 ± 4.9 mm Hg while tissue pO₂ is 23.5 ± 5.3 mmHg; however, most of the oxygen delivered by the capillary would appear to be used in the vicinity of the capillary since the pericapillary oxygen gradient is about 4 mmHg. Therefore the actual amount of oxygen delivered by the capillaries may be restricted to supplying this thin pericapillary region since there is little evidence of further oxygen delivery, as indicated by the fact that small venule pO₂ is slightly higher than capillary pO₂. Unpublished
findings from our laboratory also show that although functional capillary density and tissue oxygenation are correlated, lowering functional capillary density lowers uniformly tissue oxygenation, without causing oxygenation inhomogeneity.

The microcirculation is the only system where the same cellular species (endothelium, smooth muscle) are exposed to a significant range of oxygen tensions at steady state as a consequence of the large longitudinal vascular oxygen gradient. Therefore the vascular wall of each component of the microcirculation appears to be adapted to operate within a specific range of pO₂’s. Given that oxygen exit from the blood vessels prior to arrival at the microcirculation is physically fixed at about 20% of the available oxygen supply, and that the vascular regulation of pO₂ is directly related to the pO₂ of blood in contact with each vascular compartment, it would appear that oxygen regulation and the nature of the oxygen dissociation curve of hemoglobin are intimately related. Therefore an oxygen dissociation curve with a high affinity for oxygen up to the knee in the curve at 50 mmHg (p50 at 27 mmHg) is specifically designed to lower blood pO₂ to 50 mmHg with the exit of 20% of oxygen which is used to support the vessel wall oxygen of arteries and large arterioles, while the low affinity present beyond 50 mmHg may be designed to supply oxygen to the tissue. It should be noted that 2nd/3rd-order arterioles contribute more than any other arteriolar order to vascular resistance and flow regulation [50] and have the highest density of adrenergic innervation [56], suggesting that both sensors and mechanical effectors are present at the location in the microcirculation where the O₂ dissociation curve changes slope. This suggests that flow regulation and the nature of the oxygen dissociation curve of hemoglobin may be interrelated as shown in Fig. 2.

Historically, capillaries were first perceived as the connection between the arteriolar and venous system. Their role as suppliers of oxygen to the tissue is a direct consequence of observation of the lung, where it was readily determined that these conduits were the structures in charge of delivering atmospheric oxygen to blood. Since capillaries were soon found to be also present in tissue, it was automatically assumed that their function in tissue would be a mirror image or a reversal of that in the lung, namely that of unloading the blood oxygen to the tissue [40]. It could be that this conclusion is only partially correct. The lung is the only organ whose tissue is mostly capillaries and in lung the capillaries may not be capillaries in the sense of tubes as they appear in the tissue, but primarily surfaces held together by tethers [18]. Therefore, lung and tissue capillaries may be functionally different entities as regards the transmission of oxygen to and from the tissue.

8. Summary and conclusions

The microcirculation is a system that develops in higher forms of life to provide the necessary fluid and gas environment to the cellular components of three-dimensional tissues. This function avails itself of the transport processes of diffusion and convection, where the transport of both oxygen and water, particularly in the tissues, appears to have similar features. Analysis of unitary single capillary processes for either process was a key to understanding the mechanisms operational in the management and control of both fluids. Understanding of both systems is evolving from a phase of analysis of the mechanism to one of integration focused on the behavior of the tissue system as whole. The underlying connection between some of the physical principles and mechanisms appears to lead to similar results for the distribution of oxygen and water in tissue, namely that it is quite uniform, and that transport
within the tissue is primarily determined by the large-scale gradients, and secondarily by the distribution of capillaries, a conclusion that parallels that reached by Ellsworth et al. [13].

Data obtained with the phosphorescence decay technique show that in some tissues arterioles play a key role in oxygenating tissue, and that capillaries are secondary to this process. This finding is valid for muscle at rest and during exercise. The steep oxygen gradients in the vicinity of the microvascular wall indicate that not all of the oxygen that exits the arterioles is delivered to the tissue, since a portion may be consumed by the vessel wall itself. It is likely that this situation is quite different in working muscle where the dilatation of arterioles may lower the arteriolar wall metabolism, causing more oxygen to become available to the tissue, while also delivering more oxygen to the capillaries.

The relationship between microvascular and tissue oxygen distribution and local blood flow velocity is important because blood flow velocity is an integral factor in setting the rate of oxygen exit from the microvessels. Blood flow velocity changes depending on tissue oxygen demand, and is a critical variable when physical properties of blood such as oxygen-carrying capacity and viscosity are altered by the introduction of plasma expanders. These considerations are particularly applicable to the emerging field of blood substitutes [76,77] where efficacy and clinical outcome are dependent on how the microcirculation responds to the alteration of blood transport properties described by the present analysis. Furthermore, it is quite likely that intravascular oxygen gradients become significantly altered when red blood cells are substituted by oxygen carriers of markedly different characteristics such as liposomes and molecular solutions, as viscosity, wall metabolism and wall shear stress change.

In conclusion, our analysis and experimental findings show that tissue oxygenation is the combined result of both arteriolar and capillary transport processes, and that mass transport analysis applied to individual capillaries does not yield results applicable to the tissue that we study because our experimental findings do not support the existence of a significant heterogeneity in tissue oxygenation. Of the many factors that affect tissue oxygenation we have focused on blood flow velocity and the vessel wall gradients as necessary features to achieve a complete understanding of the process. However, we are not yet able to link blood transport and vessel wall metabolism, which would appear to be the next necessary step in understanding tissue oxygenation in terms of both the physical and metabolic barriers that oxygen must negotiate in its passage from blood to tissue.

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