What is the oxygen tension \textit{in vivo}?

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Circulatory system: delivery of nutrients and oxygen ($O_2$), removal of waste, transport between organs, endocrine pathway, heat exchange, immunological and fluid balance

$O_2$ is required by mammalian cells to support metabolism. It cannot be obtained directly from the environment in sufficient quantity (diffusion)

It has been resolved by two convective driven processes: air pump (the lungs) and a fluid pump (the heart)

As blood passes through the lung, $O_2$ diffuses down into the bloodstream, where it binds to the hemoglobin in the red blood cells (RBCs) and is carried by convective transport through the heart and large and small arteries to the microcirculatory vessels where the partial pressure gradient favors diffusion from the RBC to the tissue
Outline

• Is there a consensus for tissue pO$_2$?
• Methods to measure tissue O$_2$ tension \textit{in vivo}
• How is O$_2$ delivered?
• Importance of intravascular - tissue O$_2$ gradient
• How do \textit{in vivo} O$_2$ tensions compare to \textit{in vitro} experiments?
Consensus for tissue pO$_2$?

Until recently, it was assumed that offloading of O$_2$ from the blood to the tissue occurred mostly in the capillaries.

“Capillaries are the sole suppliers of O$_2$ to the tissue” is a cornerstone of physiology--Krogh and Erlangen in 1918, who developed the “Krogh cylinder model”

all oxygen exchange takes place at the capillary, with the entrance pO$_2$ being the large artery and the exit pO$_2$ being the large vein under reduced blood flow or low arterial oxygen level, sites at the greatest radial distance from the venous end of the capillary would lack the most O$_2$

This model ignores heterogeneity of capillary network and hemodynamics, and assumes O$_2$ exchange only at the capillary level.
Consensus for tissue \( \text{pO}_2 \)? (1)

\( \text{pO}_2 \)s, different tissues and techniques

<table>
<thead>
<tr>
<th>Tissue (species, reference)</th>
<th>Technique</th>
<th>( \text{pO}_2 ) range, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cheek Pouch</strong> (hamster, Duling BR Circ Res 31: 481–489, 1972)</td>
<td>Microelectrode</td>
<td>18 - 12</td>
</tr>
</tbody>
</table>

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Consensus for tissue pO$_2$? (2)

pO$_2$s, different tissues and techniques

<table>
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<th>Tissue (species, reference)</th>
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<th>pO$_2$ range, mmHg</th>
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</thead>
<tbody>
<tr>
<td>Intestine Villus and Submucosa (rabbit, Bohlen HG et al Am J Physiol Heart Circ Physiol 269: H1342–H1348, 1995)</td>
<td>Spectrophotometric</td>
<td>64 - 38</td>
</tr>
</tbody>
</table>
Measuring in vivo tissue \(pO_2\)

Polarographic electrode

Fluorescence quenching

Phosphorescence quenching

EPR oximetry

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Polarographic electrode

O₂ molecules diffuse to the cathode and are immediately reduced by applying polarization tension.

pO₂ on the surface of the electrode (platinum cathode) is zero.

Reduction current is determined by O₂ diffusion.

Assuming constant diffusion, tissue pO₂ is only determined by reduction current.

Polarographic system consists of a tension generator and a current meter.
# Polarography electrode

<table>
<thead>
<tr>
<th>Electrodes</th>
<th>Cons</th>
<th>Pros</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clark</strong></td>
<td>Consume O\textsubscript{2}, requires a stable boundary layer, noisy, slow time response, <em>perturbs tissue environment</em></td>
<td>Simple, easy to use, economic</td>
</tr>
<tr>
<td><strong>Whalen</strong></td>
<td>Fragile, <em>perturbs tissue environment</em></td>
<td>Low O\textsubscript{2} consumption, Low drift, noise and variability, Fast time response</td>
</tr>
<tr>
<td><strong>Surface</strong></td>
<td>Slow time response, price, <em>perturbs tissue environment</em></td>
<td>Low noise and variability, No motion artifacts</td>
</tr>
</tbody>
</table>

Both anode and cathode sealed with a lipophilic membrane.

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Hemoglobin Spectrophotometric

Blood microvessels pO$_2$ can be determined by evaluating O$_2$ saturation of hemoglobin (Hb), through measurements of Hb light absorption at different wavelengths. It has been implemented initially utilizing two and three wavelengths, and even full spectrum. The technique utilizes optical means that are easily implemented at the microscope. However, it depends on the Hb absorption spectrum at local conditions (pCO$_2$, pH, temp, ...), the tissue optical properties and light scattering. Does not provide information about tissue PO$_2$. PO$_2$ obtained with spectrophotometric technique agree with periarteriolar microelectrode measurements.


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Cryoscopic Hb and Myoglobin

Estimates O$_2$ in the vascular lumen and parenchymal cells Hb and myoglobin (Mb) saturations

Copper plate cooled with liquid nitrogen is rapidly applied to the surface of the tissue (cooling 500 µm below the surface in 50 ms)

Isosbestic wavelengths for Hb and Mb are used to determine O$_2$ saturation

Measurements made for a variety of vascular and tissue sites at a fixed time point

Rate of cooling does not prevent water crystallization, limiting optical resolution and measurements accuracy

EPR oximetry

Electron paramagnetic resonance (EPR) is the resonant absorption of microwave radiation by paramagnetic systems in the presence of an applied magnetic field.

EPR is based on the fact that the spectra of paramagnetic species can reflect interactions with other unpaired spins.

Dissolved $\text{O}_2$ cannot be observed directly by EPR, but its presence can be quantified by measuring the effects it produces in the spectra of the appropriate radical.

Soluble and Solid probes
Fluorescence quenching

$O_2$ will quench fluorescence by colliding with the fluorescent molecule when the latter is in the excited state.

Number of collisions will be proportional to the amount of $O_2$ present per unit volume.

Advantages: low $O_2$ consumption and spatial resolution.

Disadvantages: obtains a 2-D projection of 3-D events, affected by fluorophore concentration.
Phosphorescence quenching

Based on the rate of decay of excited phosphorescence from Pd-porphyrin bound to albumin and the local \( pO_2 \) (Stern-Volmer equation)

Phosphorescence emission results from transition into a triplet state by absorbing light (short flash) and then passing from this state to a singlet ground state

Pd-porphyrin releases the absorbed energy as light or transferred this energy to \( O_2 \), which prevents light emission

Light emission is quenched, fewer photons are emitted, translates into a shorter time constant

\[ EV, \text{ extravascular} \]
\[ PV, \text{ perivascularch} \]
\[ IV, \text{ intravascular} \]

\[ \text{Rate of phosphorescence decay depends on } O_2 \text{ amount (dye concentration independent)} \]
O$_2$ consumption by phosphorescence quenching

Phosphorescence consumes O$_2$ depending on the concentration of the dye and the total energy delivered by the light source.

Emission and the phosphorescence decay obtained may be the summation of signals from adjoining areas, particularly in the neighborhood of a microvessel (no uniform where the oxygen field).

Problems can be circumvented by using (i) repeated light excitation of low intensity over a period that allows diffusion to replenish the consumed oxygen and (ii) averaging the signals.


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Microcirculatory preparations

*Surgically Exposed Tissue Preparations (most common)*
- Acute
- Anesthesia varies among laboratories (type and regimes)
- Surgical preparation involves exposing and/or excising the tissue by removal out of the body cavity (cremaster and mesenteric)
- Suffusing solution used to mimic *in vivo* conditions influences blood flow and $O_2$
- For optical techniques, tissue may be covered with polyvinyl film or enclosed

*Environment Isolated Preparations*
- Allows tissue to recover from the acute effects of surgery and can be studied in the unanesthetized state

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How is O$_2$ delivered?

Convective transport = Diffusion flux out of the vessel = O$_2$ consumed

\[ QC_{\text{blood}} \Delta S = -2 \pi R_0 \Delta L D \alpha \frac{dP_{O_2}}{dr_{r=R_0}} - M_{\text{avg}} \pi (R_i^2 - R_0^2) \Delta L \]

Convective transport, difference between O$_2$ entering and exiting a segment

Diffusion flux out of the vessel, diffusion constant (D), O$_2$ solubility (\( \alpha \)), and pO$_2$ radial gradient

O$_2$ consumed, is defined by average consumption rate (\( M_{\text{avg}} \))


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Intravascular - $O_2$ gradient

Radial gradient is steepest in the arteriolar network and diminishes in the capillary and venular regions.

Steepest radial gradients are in the immediate vicinity of the vasculature, arteriolar vessels and can not be explained on the basis of diffusion alone.


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How does critical $pO_2$ in vivo compare to in vitro? (1)

Critical $pO_2$: $pO_2$ required to support oxidative metabolism

Skeletal muscle, in vitro

$PO_2$ at NADH breakpoint = 0.96 mmHg

How does critical pO$_2$ \textit{in vivo} compare to \textit{in vitro} ? (2)

Critical pO$_2$: pO$_2$ required to support oxidative metabolism

Skeletal muscle, \textit{in vivo}

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Effects of mismatching *in vivo* and *in vitro* O$_2$ tensions (1)

Effects of pO$_2$ during shear exposure on BAEC respiration

Exposing BAEC to steady laminar shear stress at higher oxygen tensions than physiological pO$_2$s results in peroxynitrite formation and inactivation of the electron transport chain.

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Summary

• *In vivo*, the interstitial pO$_2$ is not uniform

• Heterogeneity occurs on many levels: morphological, hemodynamics and metabolic

• Arterioles are as important as capillaries in oxygenating the tissue

• O$_2$ exiting the circulation, implies the existence of large blood/tissue oxygen gradients

• Capillary/tissue O$_2$ gradients are maximal in the lung (50 mmHg) and minimal in the resting tissues (0.5 mmHg)

• The fundamental understating of how O$_2$ is managed *in vivo* influences the translation of *in vitro* studies into physiological and pathophysiological mechanisms
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Effects of pO$_2$ during shear exposure on EC respiration

BAEC exposed to steady laminar shear stress results in peroxynitrite formation and inactivation of the electron transport chain

Polarography electrode

Clark electrode consumes oxygen, generating a current proportional to the \( O_2 \) concentration. Requires stable boundary/diffusion layer.

Whalen electrode has a recess (metal surface from the glass micropipette tip), eliminates motion free layer. They have low drift and \( O_2 \) consumption (10-6 \( \mu l/min \)) and fast time constant (1s). They are fragile and their presence introduces perturbations of the tissue, noisy when used in flowing blood.

Surface electrodes have both cathode and anode sealed with a lipophilic membrane to prevents impurities and eliminates motion artifacts. Their dimension (10–20 \( \mu m \)) increases catchment volume and the time to form a stable boundary layer. Often configured into an array and provided a histogram of \( O_2 \) tensions.
How is O₂ delivered?
How is $O_2$ delivered?

In vascular beds with low metabolic tissue demand (resting skeletal muscle), there are significant longitudinal gradients of $pO_2$ in the arteriolar circulation.

Tissue with higher metabolic demand (brain and intestine) had lower gradients.

Longitudinal arteriolar $pO_2$ gradient reflects the ratio of blood flow to metabolic $O_2$ demand.

$O_2$ delivery by capillaries varies, among vascular beds. Low, resting skeletal muscle and high, brain and myocardium.

Higher venular $pO_2$ relative to capillary and tissue $pO_2$ are explained by arterio-venous shunts, anatomic distribution and the Bohr effect.
EPR oximetry

Electron paramagnetic resonance (EPR) is the resonant absorption of microwave radiation by paramagnetic systems in the presence of an applied magnetic field.

EPR is based on the fact that the spectra of paramagnetic species can reflect interactions with other unpaired spins.

Dissolved O$_2$ cannot be observed directly by EPR, but its presence can be quantified by measuring the effects it produces in the spectra of the appropriate radical.

Spatial information can be obtained using EPR imaging (EPRI).
EPR oximetry, probes

Particulate (Solid) probes
- Lithium phthalocyanine (LiPc)
- Sugar chars
- Fusinite
- Coal
- India ink

Soluble probes
- Nitroxides
- Trityl radicals

The collision frequency $w$, according to the hard sphere theory of Smoluchowski is

$$w = 4\pi R^2 (D_{SL} + D_{O_2}) [O_2]$$

which translates to EPR line-broadening as

$$Dw = k D_{O_2} [O_2]$$