2008 Sunrise Free Radical School
Presentation by:

Pedro Cabrales, Ph.D.

What is the oxygen tension *in vivo*?

Pedro Cabrales

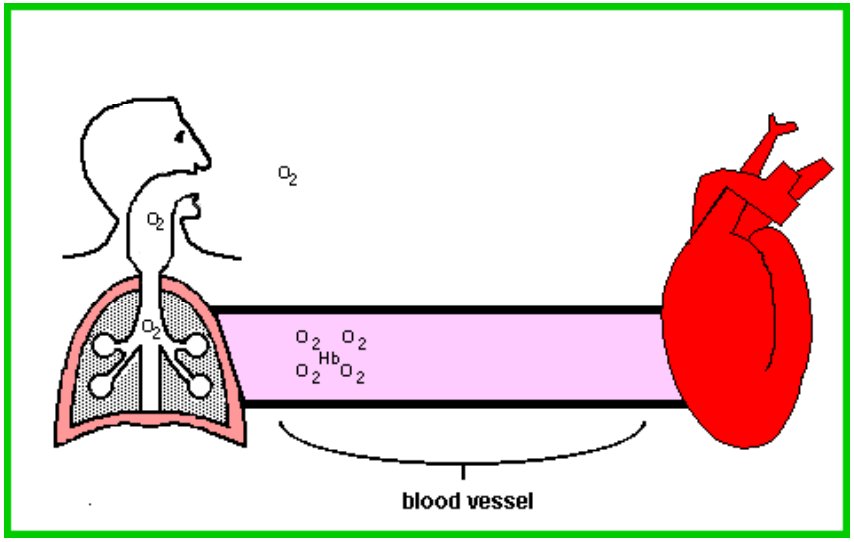
La Jolla Bioengineering Institute
Microhemodynamics Laboratory
University of California, San Diego

15th Annual Meeting of the SFRBM, Nov. 19-23, 2008

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 *in vivo*
- How is O_2 delivered?
- Importance of intravascular - tissue O_2 gradient
- How do *in vivo* O_2 tensions compare to *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 pO_2? (1)

pO_2s, different tissues and techniques

Tissue (species, reference)	Technique	pO_2 range, mmHg
Cheek Pouch (hamster, Duling BR Circ Res 31: 481–489, 1972)	Microelectrode	18 - 12
Spinotrapezius Ms (rat, Boland EJ et al J Appl Physiol 62: 791–797, 1987)	Microelectrode	26 - 13
Sartorius Ms (cat, Boegehold MA et al Am J Physiol Heart Circ Physiol 254: H929–H936, 1988)	Microelectrode	40 - 22
Sartorius Ms (cat - low flow, Boegehold MA et al Am J Physiol Heart Circ Physiol 254: H929–H936, 1988)	Spectrophotometric	14 - 9
Skinfold (hamster, Intaglietta M et al Cardiovasc Res 32: 632–643, 1996)	Phosphorescence	34 - 29
Skinfold (hamster - perivascular, Intaglietta M et al Cardiovasc Res 32: 632–643, 1996)	Phosphorescence	30 - 21
Spinotrapezius Ms (rat, Shonat RD Am J Physiol Heart Circ Physiol 272: H2233–H2240, 1997)	Phosphorescence	32 - 22
Brain (rat - cortex, Vovenko EP Pflügers Arch 437: 617–623, 1999)	Microelectrode	57 - 31

Consensus for tissue pO_2? (2)

pO_2s, different tissues and techniques

Tissue (species, reference)	Technique	pO_2 range, mmHg
Retractor Ms (hamster, Ellsworth ML et al Am J Physiol Heart Circ Physiol 252: H1031–H1040, 1987)	Spectrophotometric	30 - 21
Retractor Ms (hamster, Kuo I Am J Physiol Heart Circ Physiol 254: H331–H338, 1988)	Spectrophotometric	30 - 22
Myocardium (dog, Honig CR et al Adv Exp Med Biol 248: 591–599, 1989)	Cryoscopic	64 - 25
Retractor Ms (hamster, Swain DP et al Am J Physiol Heart Circ Physiol 256: H247–H255, 1989)	Spectrophotometric	24 - 23
Gracillis Ms (dog - 4 Hz stimulation, Honig CR et al Am J Physiol Heart Circ Physiol 261: H2031–H2043, 1991)	Cryoscopic	31 - 20
Intestinal Villus and Submucosa (rat, Bohlen HG et al Am J Physiol Heart Circ Physiol 269: H1342–H1348, 1995)	Spectrophotometric	48 - 30
Intestine Villus and Submucosa (rabbit, Bohlen HG et al Am J Physiol Heart Circ Physiol 269: H1342–H1348, 1995)	Spectrophotometric	64 - 38

Measuring *in vivo* tissue pO_2

Polarographic electrode

Davies PW and Brink F, *Rev. Sci. Instrum*. 1942

Fluorescence quenching

Longmuir IS and Knopp JA, *J Appl Physiol*. 1976

Phosphorescence quenching

Vanderkooi JM et al, J Biol Chem. 1987

EPR oximetry

Swartz HM et al, Biochemistry. 1989

Polarographic electrode

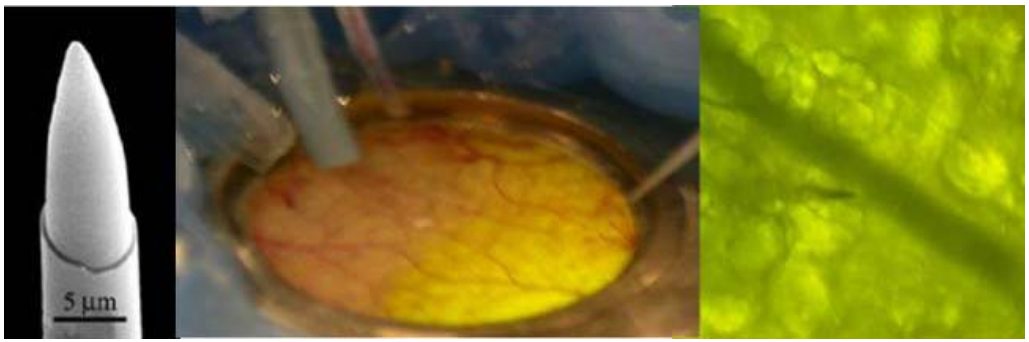
O_2 molecules diffuse to the cathode and are immediately reduced by applying polarization tension

pO_2 on the surface of the electrode (platinum cathode) is zero

Reduction current is determined by O_2 diffusion

Assuming constant diffusion, tissue pO_2 is only determined by reduction current

Polarographic system consists of a tension generator and a current meter



Polarography electrode

Electrodes	Cons	Pros
Clark	Consume O_2, requires a stable boundary layer, noisy, slow time response, *perturbs tissue environment*	Simple, easy to use, economic
Whalen Metal surface from the glass micropipette tip	Fragile, *perturbs tissue environment*	Low O_2 consumption Low drift, noise and variability Fast time response
Surface Both anode and cathode sealed with a lipophilic membrane	Slow time response, price, *perturbs tissue environment*	Low noise and variability No motion artifacts

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

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

Pittman RN And Duling BR. Measurement of percent hemoglobin in the microvasculature. *J Appl Physiol* 38: 321–327, 1975

Steenbergen JM, Lash JM, And Bohlen HG. Role of lymphatic system in glucose absorption and the accompanying microvascular hyperemia. Am J Physiol Gastrointest Liver Physiol 267: G529–G535, 1994.

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

Gayeski TEJ and Honig CR. Oxygen gradients from sarcolema to cell interior in a red muscle at maximal oxygen consumption. Am J Physiol Heart Circ Physiol 251: H789–H799, 1986

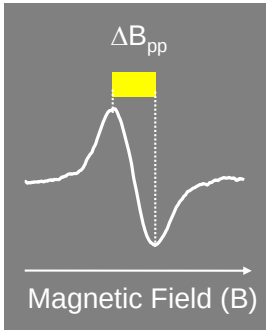
EPR oximetry

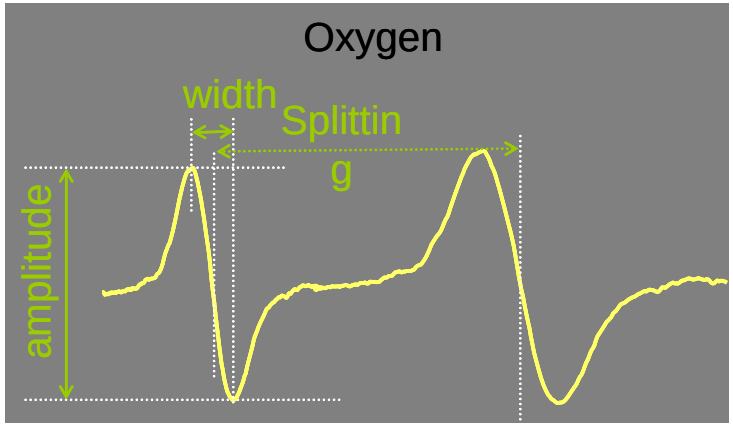
Electron paramagnetic resonance (EPR) is the resonant absorption of microwave radiation by paramagnetic systems in the presence of an applied magnetic field

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

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

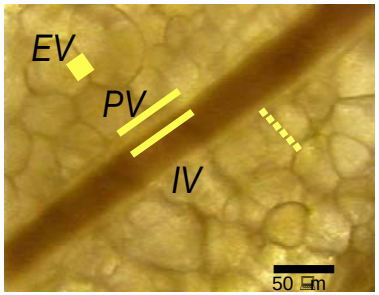
Phosphorescence quenching

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

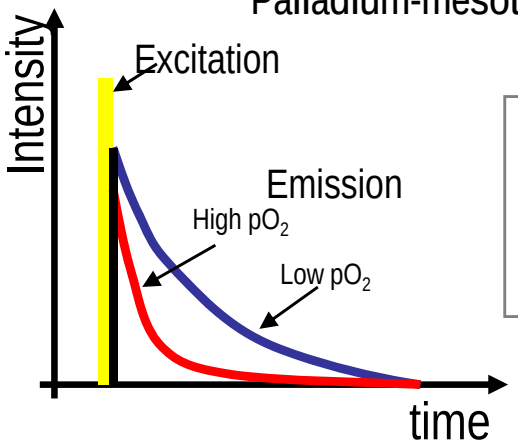
Pd-porphyrin releases the absorbed energy as light or transferred this energy to O_2, which prevents light emission

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



***EV*, extravascular**
***PV*, perivascular**
***IV*, intravascular**

Palladium-mesotetra-(4-carboxyphenyl)porphyrin

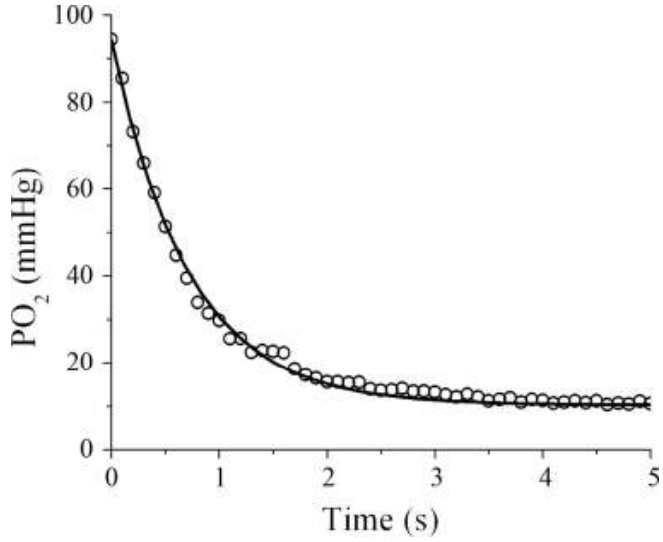


Rate of phosphorescence decay depends on O_2 amount (dye concentration independent)

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)



Golub AS et al. Am J Physiol Heart Circ Physiol 294: H2905-H2916 2008

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

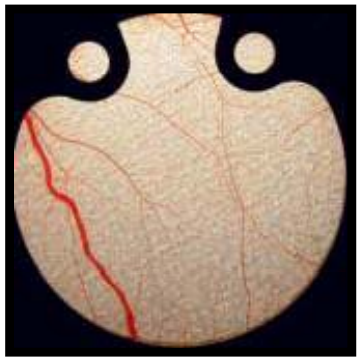
Microcirculatory preparations

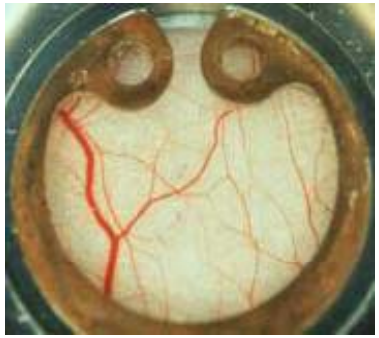
Surgically Exposed Tissue Preparations (most common)

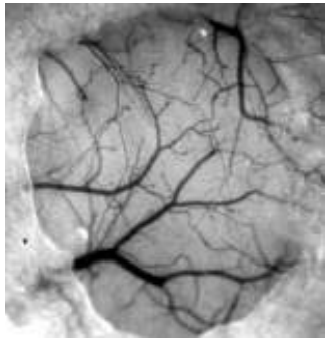
- Acute
- Anesthesia varies among laboratories (type and regimes)
- Surgical preparation involves exposing and/or excising the tissue by removal out of the body cavity (cremaster and mesenteric)
- Suffusing solution used to mimic *in vivo* conditions influences blood flow and O_2
- For optical techniques,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

Environment Isolated Preparations

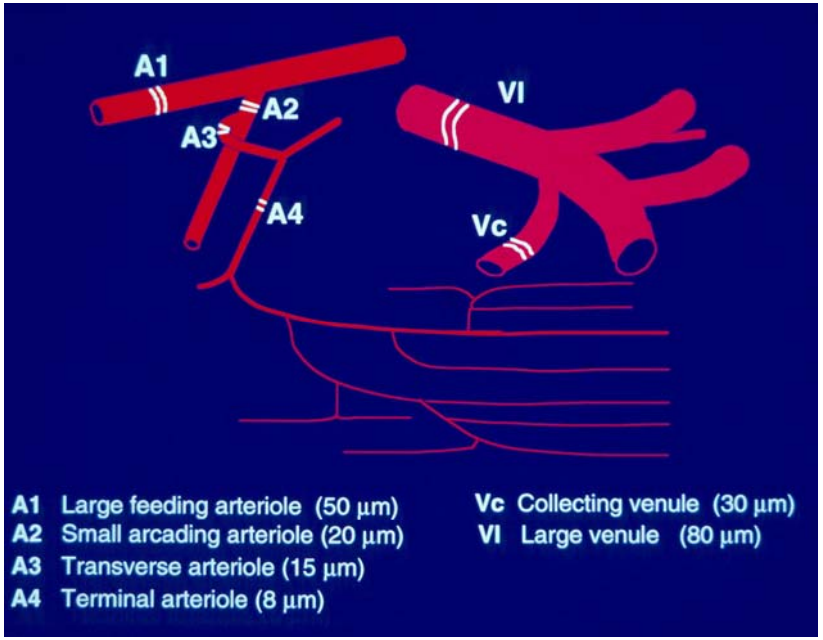
- Allows tissue to recover from the acute effects of surgery and can be studied in the unanesthetized state

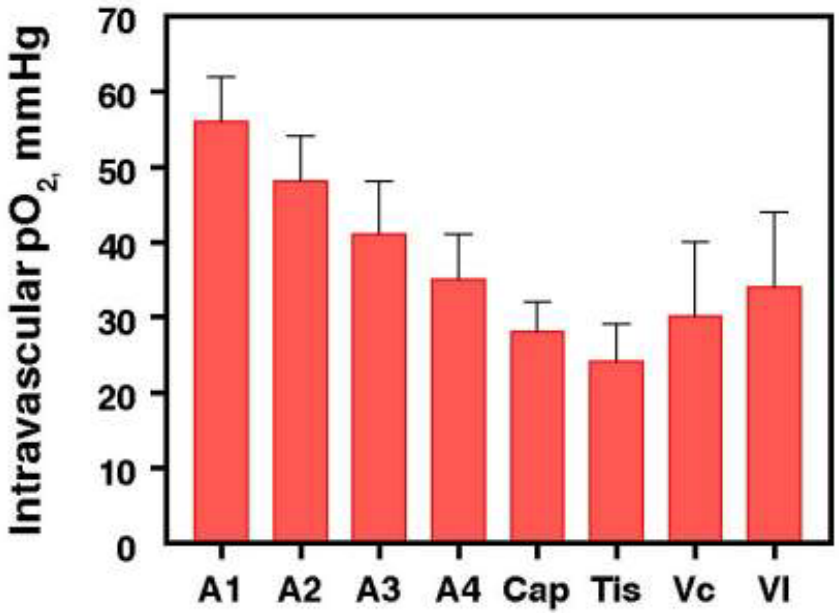






How is O_2 delivered?





Kerger *et al*., Systemic, subcutaneous microvascular oxygen tension in conscious Syrian golden hamsters. *Am J Physiol* 1995;268:H802-810.

Convective transport = Diffusion flux out of the vessel = O_2 consumed

$$\mathrm{QC_{blood}\Delta S} = \; -2\pi R_0 \Delta L D \alpha \frac{\mathrm{dP_{O_2}}}{\mathrm{d}r_{r=R_0}} = M_{\mathrm{avg}} \pi (R_{\mathrm{t}}^2 - R_0^2) \Delta L$$

Convective transport, difference between O_2 entering and exiting a segment

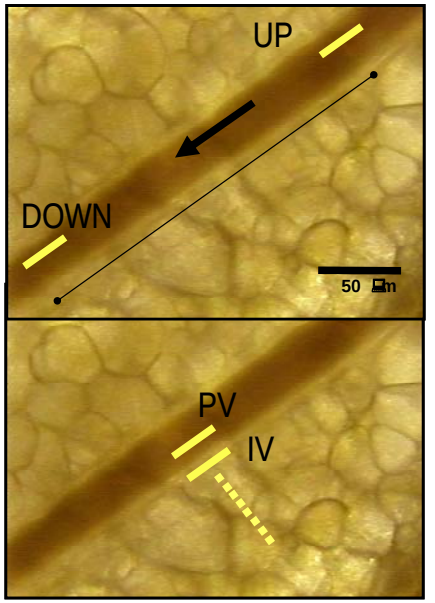
Diffusion flux out of the vessel, diffusion constant (D), O_2 solubility (α), and pO_2 radial gradient

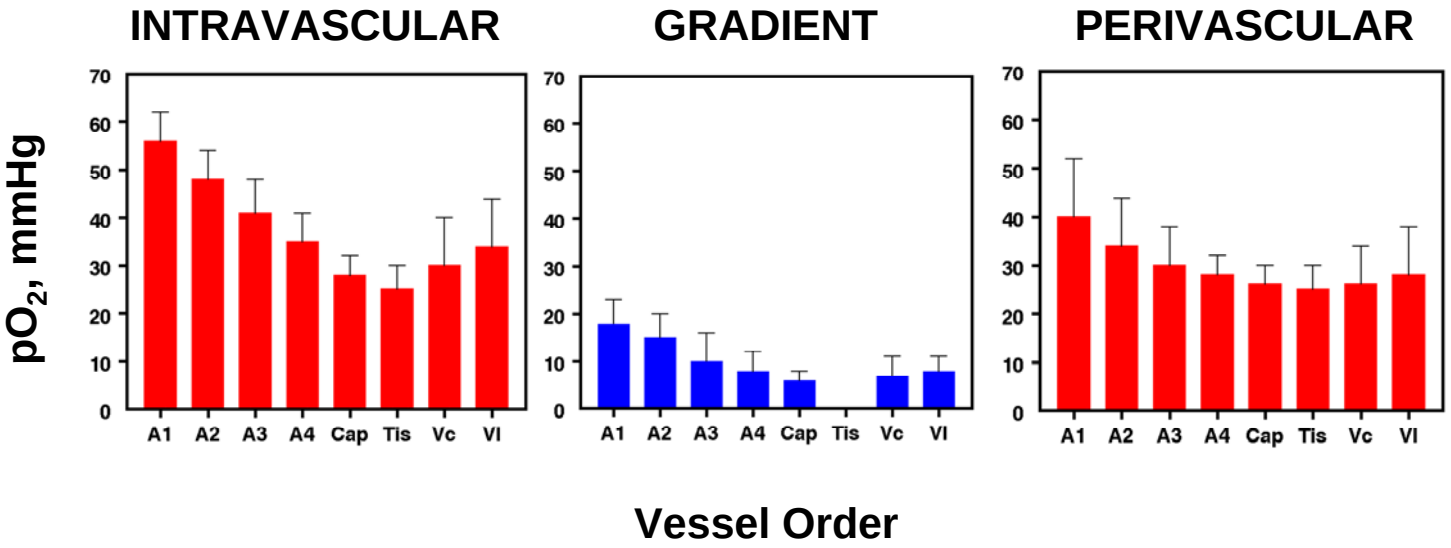
O_2 consumed, is defined by average consumption rate (M_{avg})

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





O_2 loss during convection

is equal to

Diffusive O_2 loss + O_2 consumption

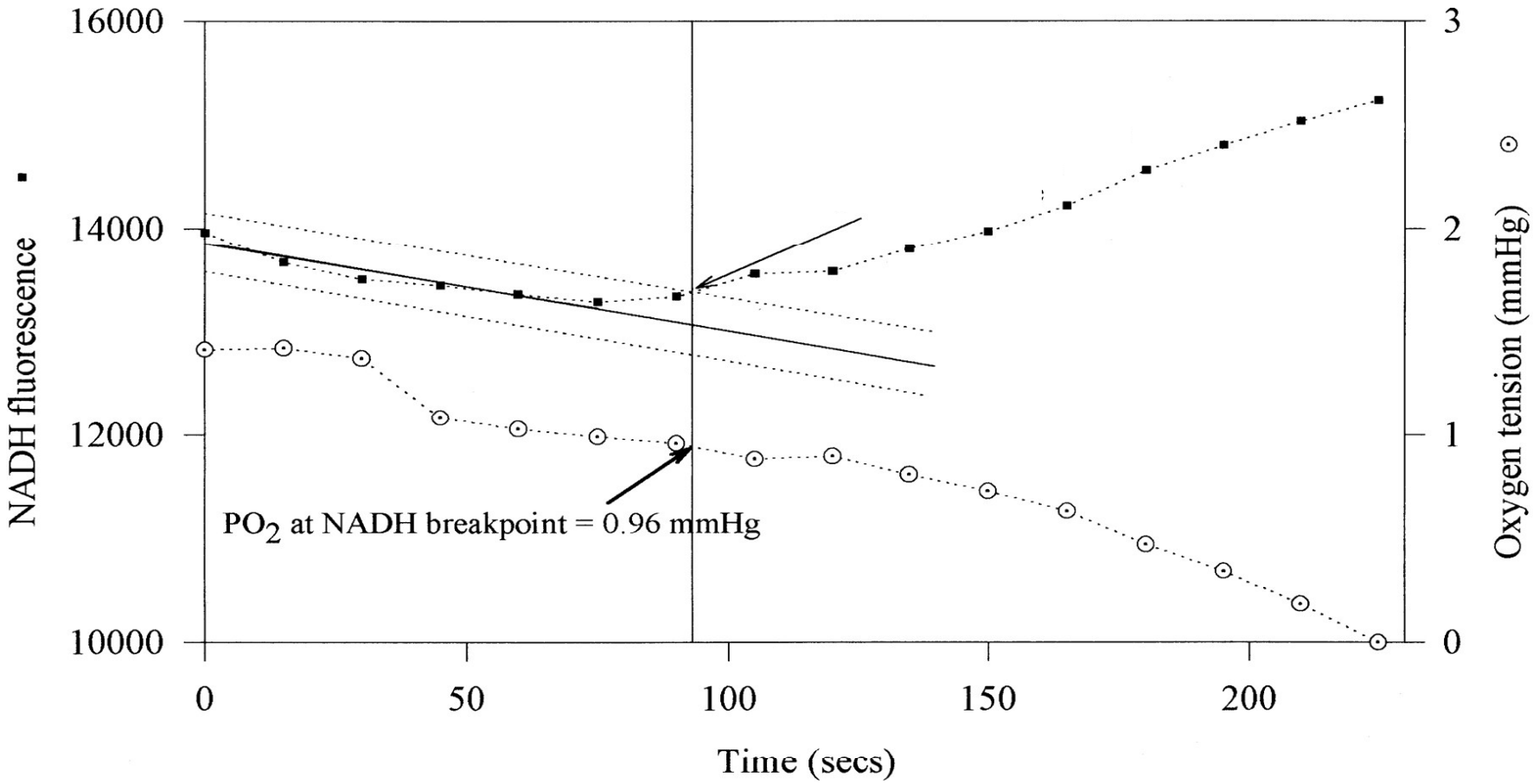
EV, extravascular
PV, perivascular
IV, intravascular

Kerger *et al*., Systemic, subcutaneous microvascular oxygen tension in conscious Syrian golden hamsters. *Am J Physiol* 1995;268:H802-810.

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***

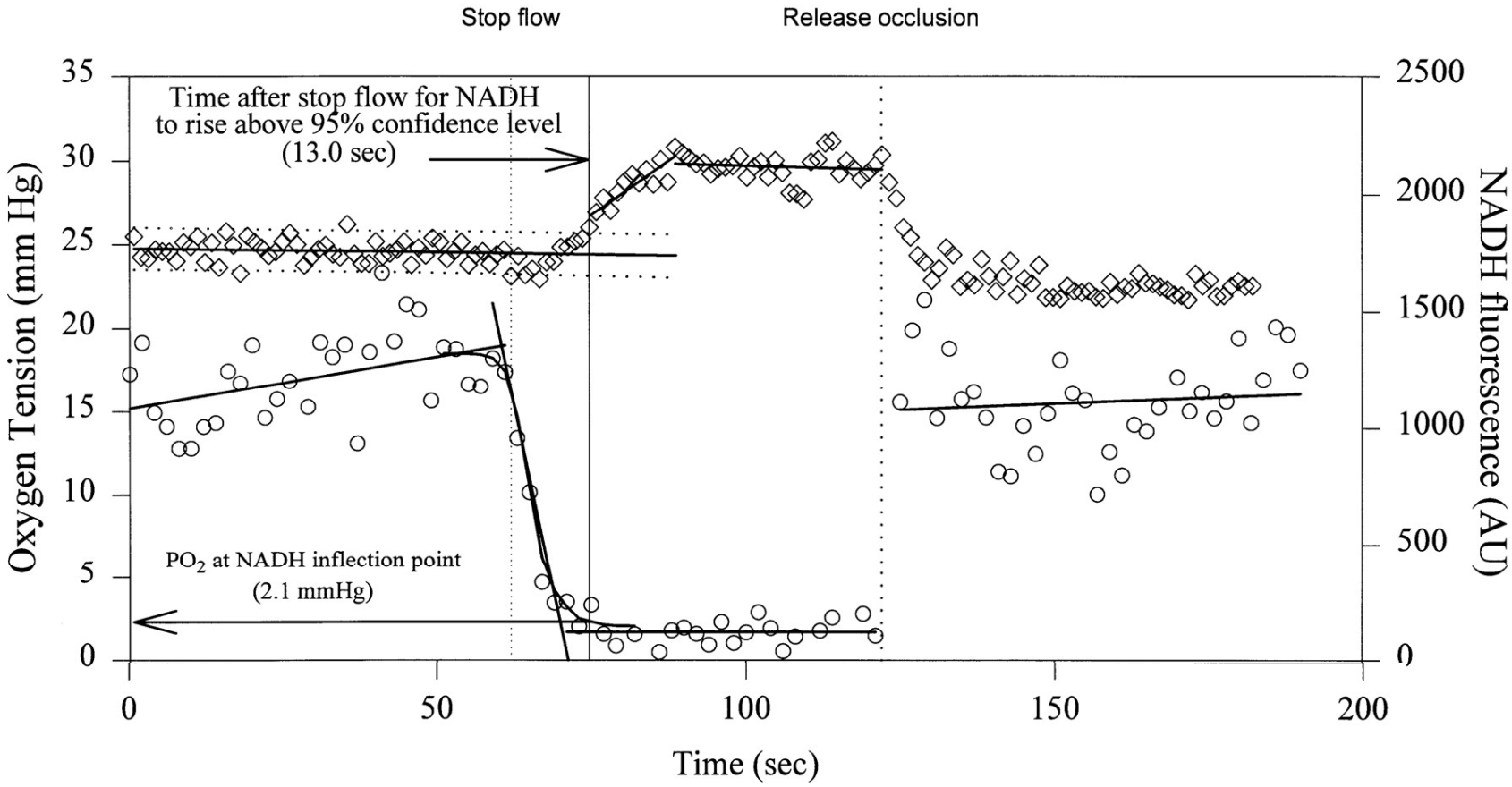


Richmond KN et a. Am J Physiol Cell Physiol 273: C1613-C1622 1997

How does critical pO_2 *in vivo* compare to *in vitro* ? (2)

Critical pO_2: *pO_2 required to support oxidative metabolism*

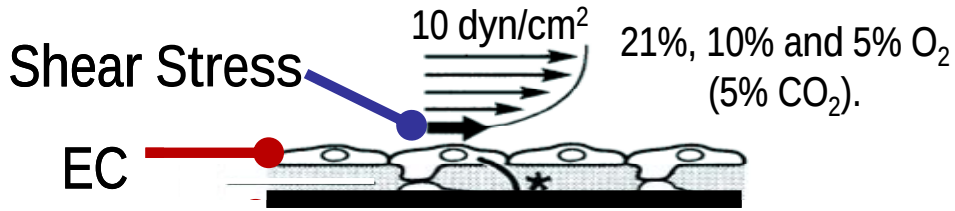
Skeletal muscle , ***in vivo***

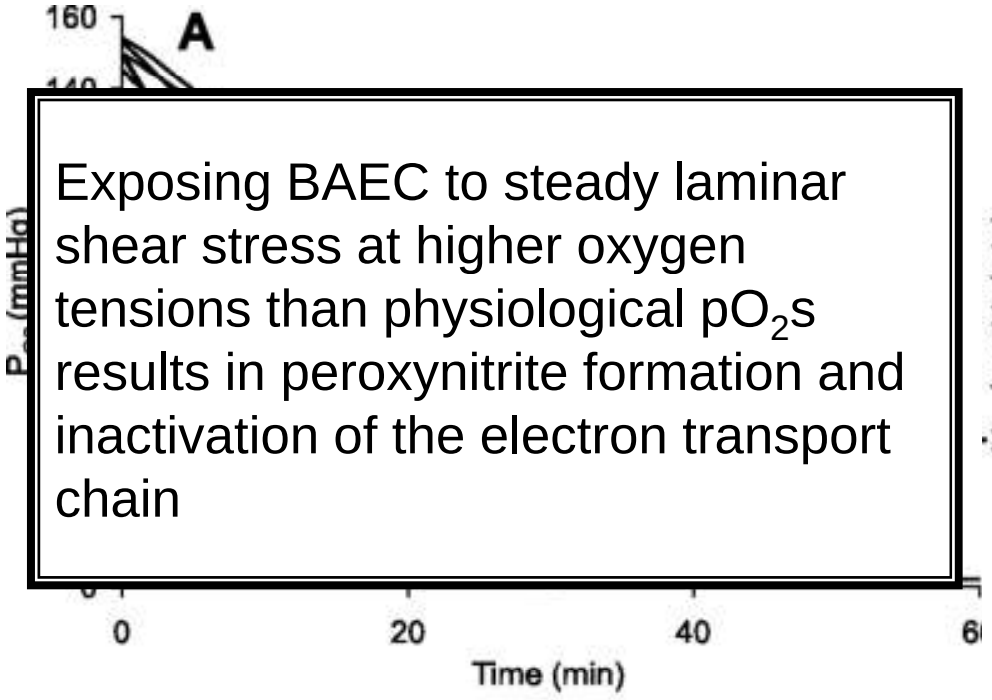


Richmond KN et al Am J Physiol Heart Circ Physiol 277: H1831-H1840 1999

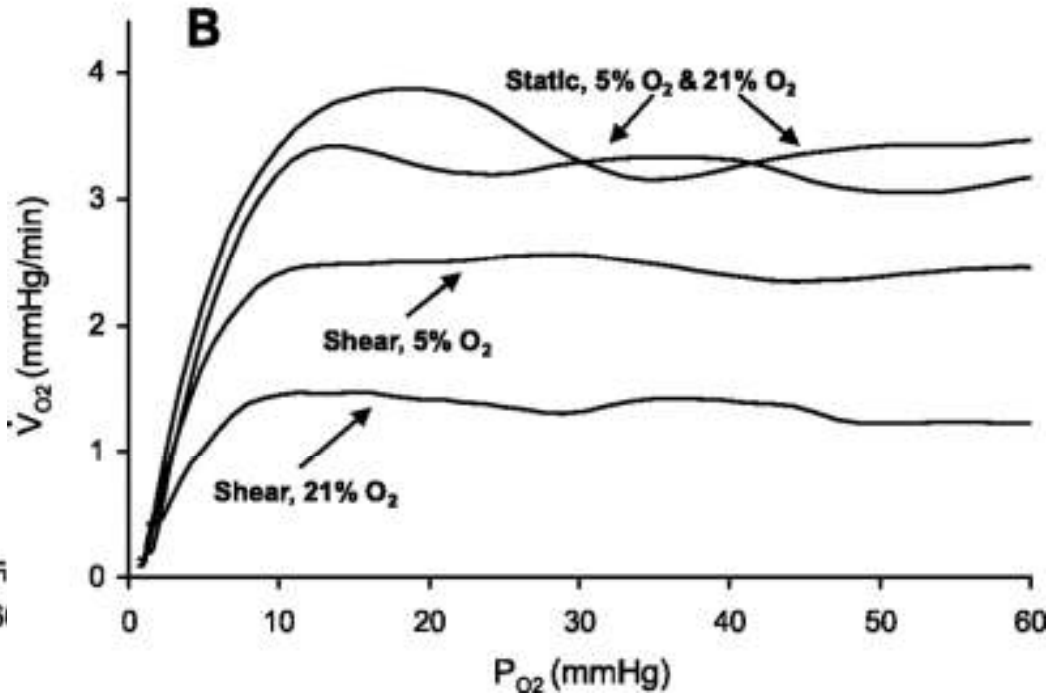
Effects of mismatching *in vivo* and *in vitro* O_2 tensions (1)

Effects of pO_2 during shear exposure on BAEC respiration





Exposing BAEC to steady laminar shear stress at higher oxygen tensions than physiological pO_2s results in peroxynitrite formation and inactivation of the electron transport chain



Jones CI et al Am J Physiol Cell Physiol 295: C180-C191 2008

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

Acknowledgements

UCSD team:

Marcos Intaglietta, Ph.D.

Paul C. Johnson, Ph.D.

Amy G. Tsai, Ph.D.

Funding sources : NIH Heart and Lung Institute R24 HL64395, R01 HL62354, R01 HL62318, R01 HL76182, P01 HL71064 and US Army PR023085.

Acknowledgements

UCSD team:

Marcos Intaglietta, Ph.D.

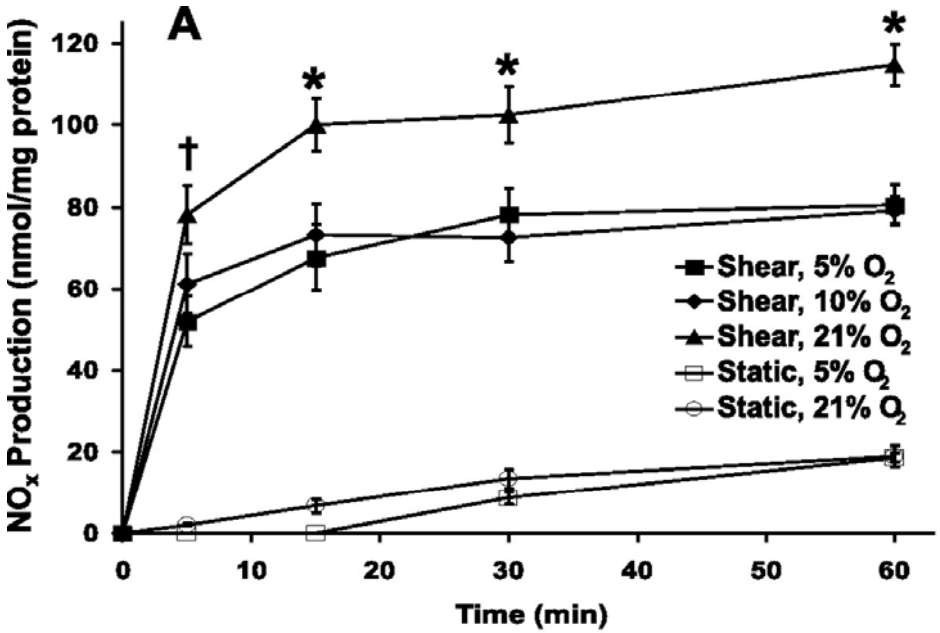
Paul C. Johnson, Ph.D.

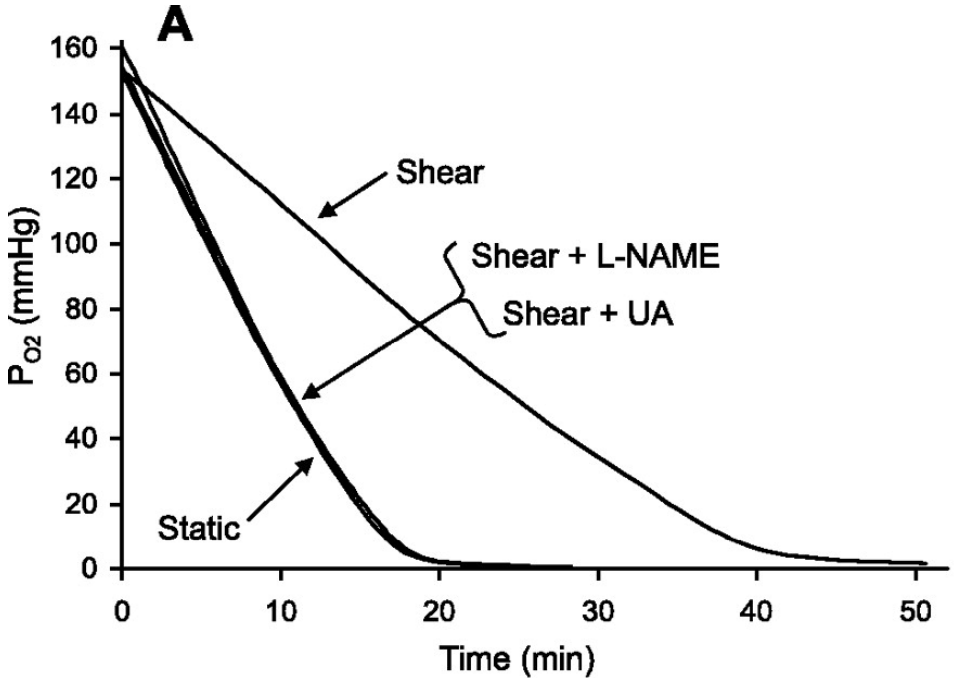
Amy G. Tsai, Ph.D.

Funding sources : NIH Heart and Lung Institute R24 HL64395, R01 HL62354, R01 HL62318, R01HL76182, P01 HL71064 and US Army PR023085.

Effects of mismatching *in vivo* and *in vitro* O_2 tensions (2)

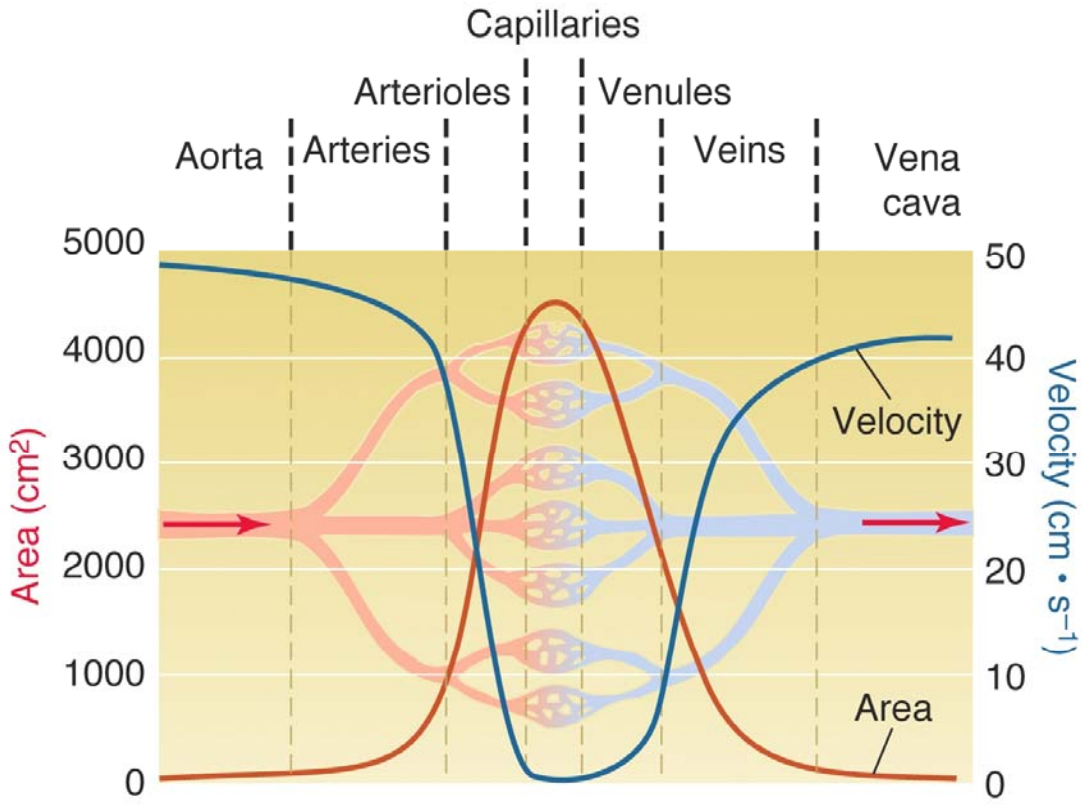
Effects of pO_2 during shear exposure on EC respiration





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

Jones CI et al Am J Physiol Cell Physiol 295: C180-C191 2008


Capillaries
Arterioles
Venules
Aorta
Arteries
Veins
Vena cava
5000
4000
3000
2000
1000
0
Area (cm²)
50
40
30
20
10
0
Velocity (cm • s⁻¹)
Velocity
Area

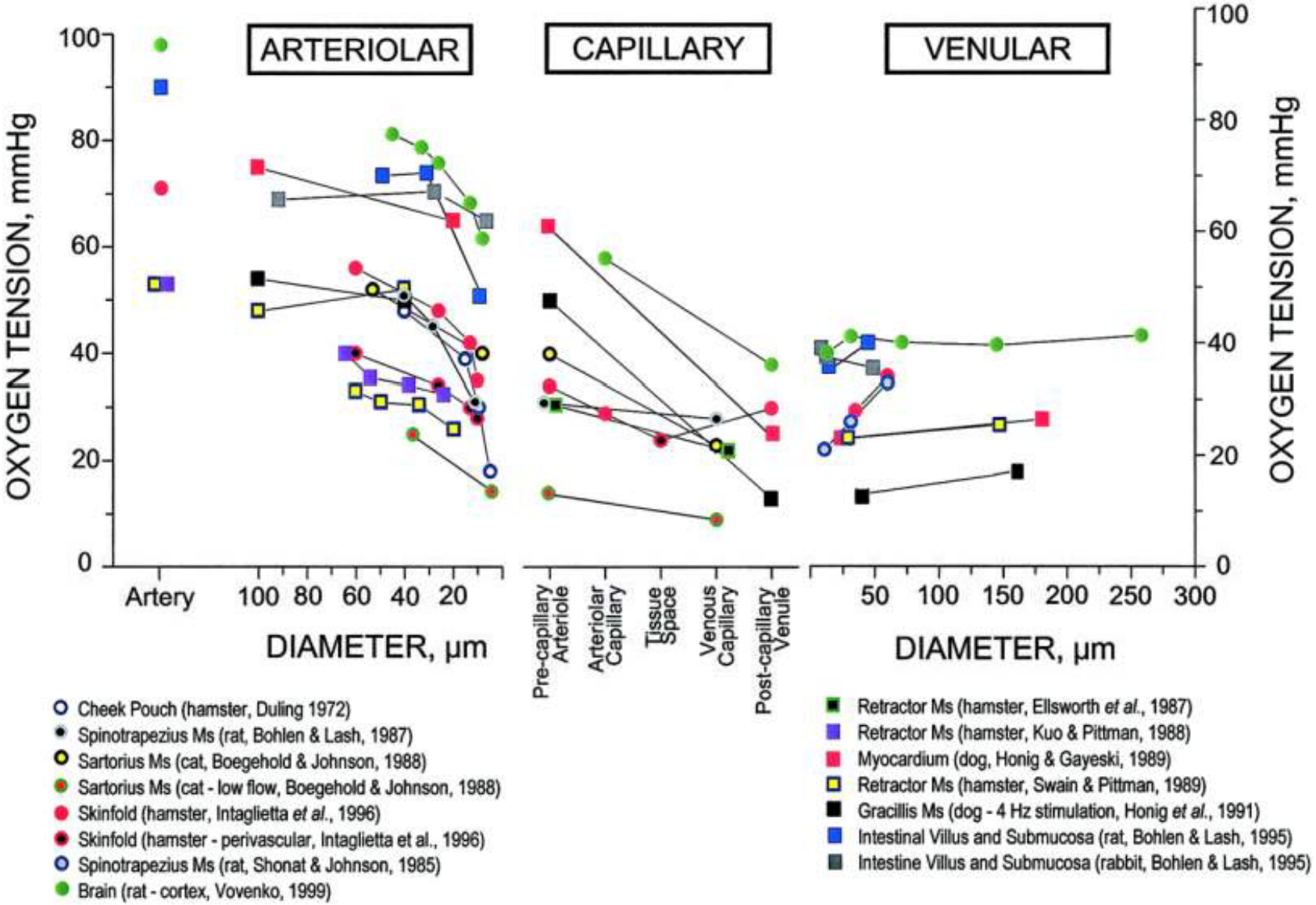
Polarography electrode

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 µl/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 µm) 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 O_2 delivered?



TSAI, A. G. et al. Physiol. Rev. 83: 933-963 2003

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

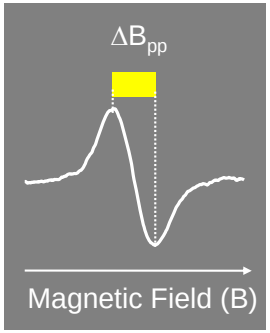
EPR oximetry

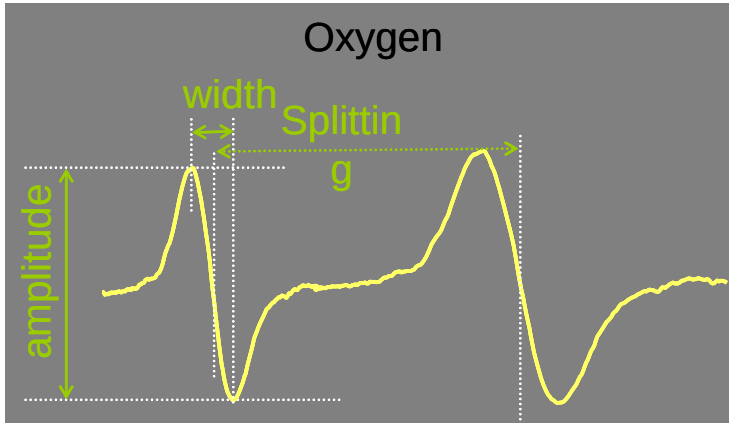
Electron paramagnetic resonance (EPR) is the resonant absorption of microwave radiation by paramagnetic systems in the presence of an applied magnetic field

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

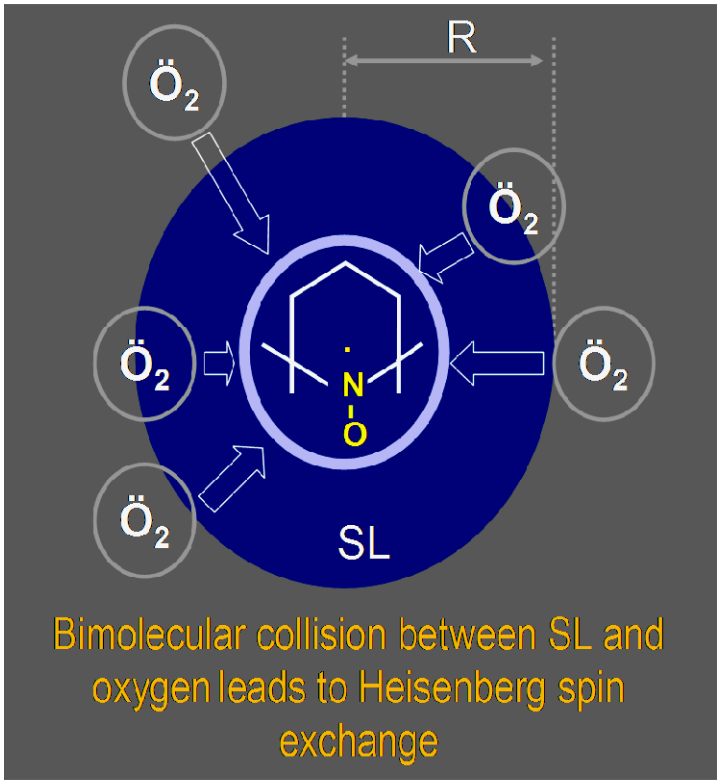
Spatial information can be obtained using EPR imaging (EPRI)





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

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

which translates to EPR line-broadening as

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