Physical principles of gases relevant to hypoxic cell culture

What is "normoxia" in a cell culture incubator (gas phase)?

Air: **78% N_2**
20.9% O_2 (v/v)
1% others (nobel gases, CO_2, no H_2O)
<u>in</u>dependent of altitude!

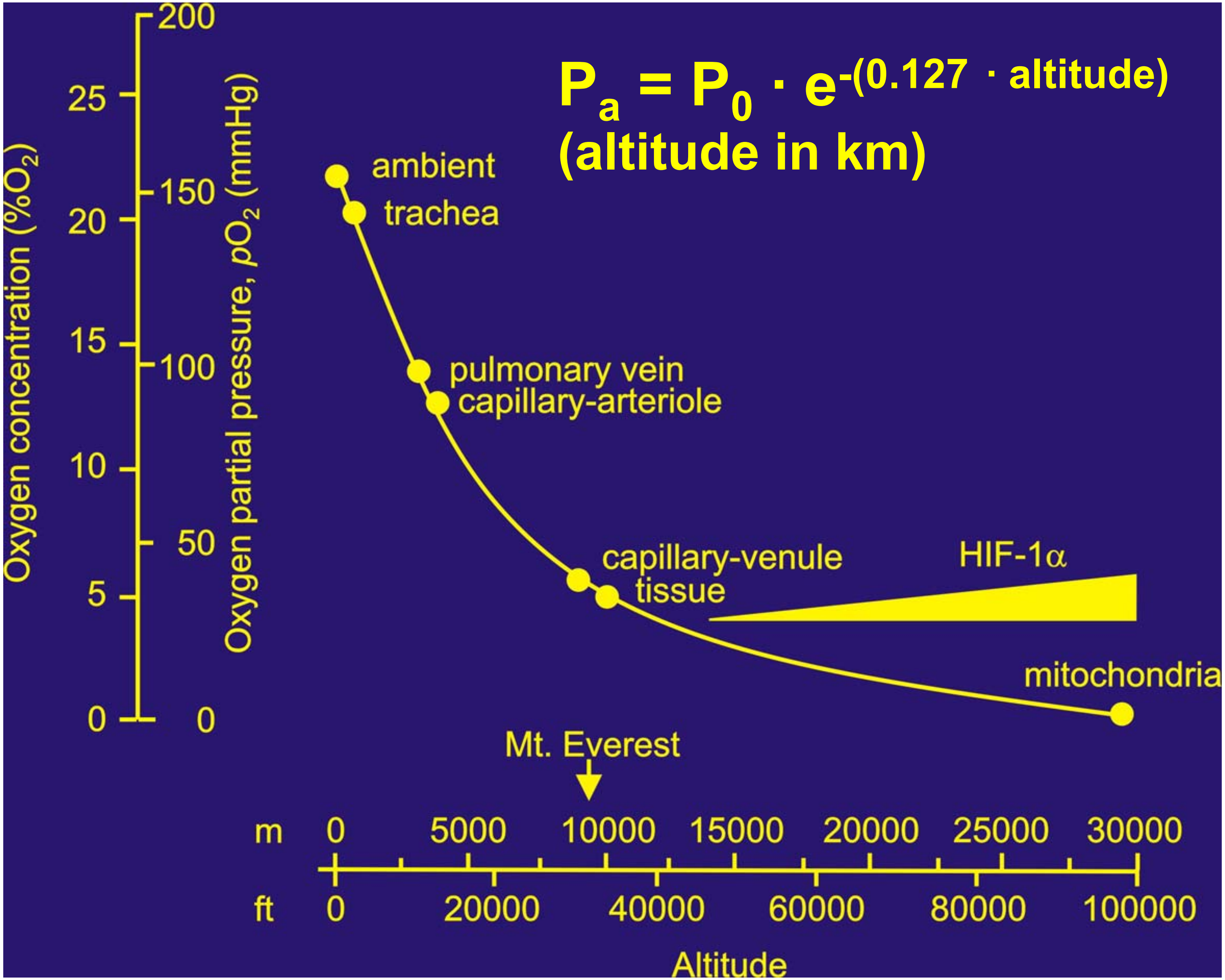
But in fact: 18.55% O_2 (in an incubator in Zürich)!
→ why?

Dalton's law: "*gas partial pressures are additive*"

pH_2O sat. = 47 mmHg (at 37°C)

Roland H. Wenger, Institute of Physiology, University of Zürich

Exponential decrease of air pressure with increasing altitude



Wenger, *AINS* 2004, 39, *S*38

Subtraction of the water vapour

$$P_a = P_0 \cdot e^{-(0.127 \cdot altitude)}$$

My lab in Zürich is at 500 m altitude:

$P_a = 760 \cdot e^{-(0.127 \cdot 0.5)} = 713.24$ mmHg
(ignoring meteorological conditions)

713.24 - 47 = 666.24 mmHg

→ the higher the altitude, the more pH_2O_g plays a role

Subtraction of the CO_2

normal CO_2 ~ 0.04%
incubator CO_2 = 5%

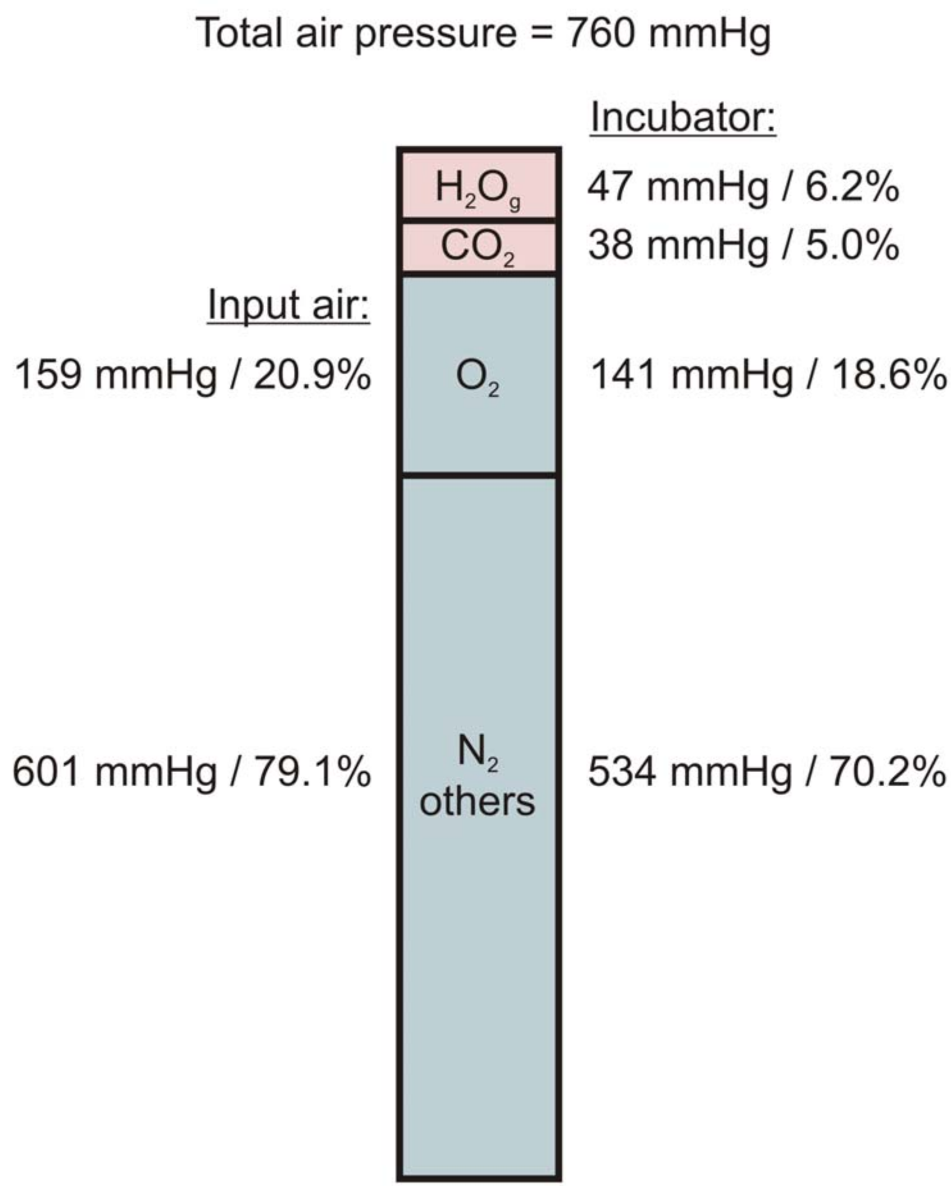
666.24 mmHg - 4.96% = 633.19 mmHg

20.9% of 633.19 mmHg = 132.33 mmHg

132.33 of 713.24 = 18.55%

→ do not set your normoxic incubator at 20% oxygen...

Summary: gas conditions in an incubator (now at sea level)



Gases in water (incubator at sea level)

Henry's law: "*p_x in gas phase = p_x in liquid phase*"

→ pO_2 in H_2O = 141 mmHg

→ pCO_2 in H_2O = 38 mmHg

"*Gas concentration c_x is proportional to partial pressure p_x*"

Bunsen's solubility constant α_x :

$$c_x = \alpha_x \cdot p_x$$

→ cO_2 in H_2O = 1.26 µM/mmHg · 141 mmHg = 177 µM

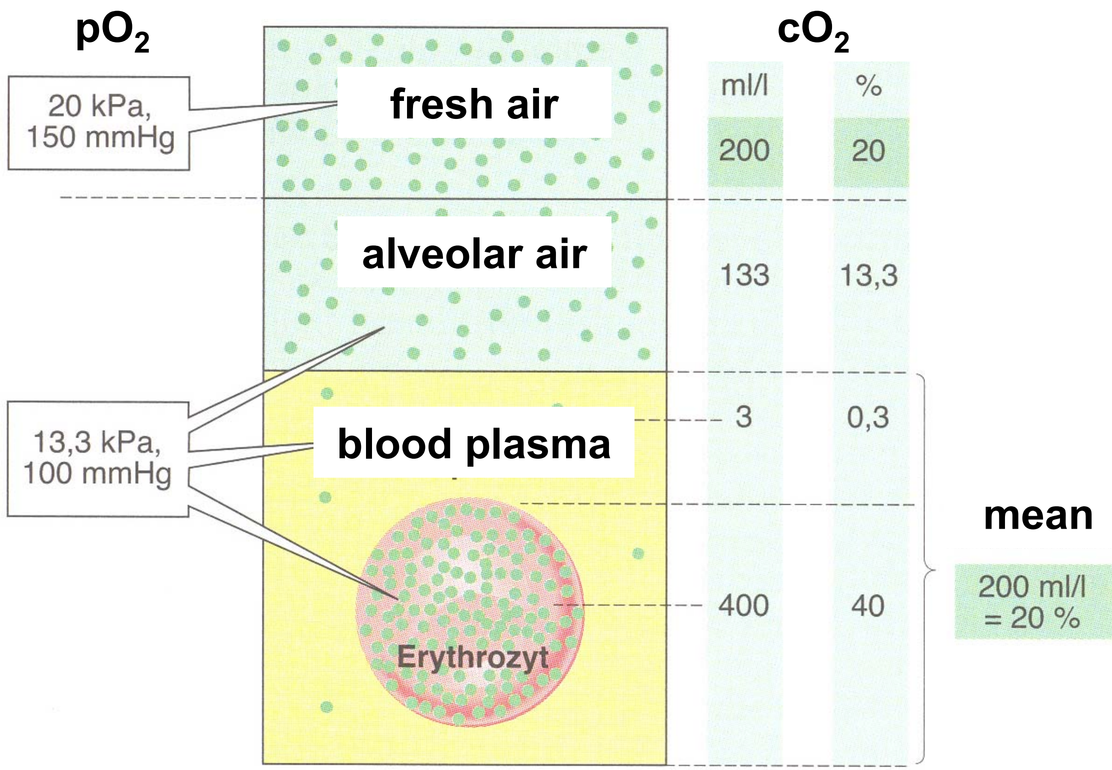
→ cCO_2 in H_2O = 30.0 µM/mmHg · 38 mmHg = 1150 µM

→ CO_2 is approx. 24-fold better "physically" soluble than O_2

(values are given for blood plasma at 37°C)

Gases in water

Attention: gas concentration in biological fluids!



O_2: oxygen-binding proteins

CO_2: chemical reaction with H_2O to HCO_3^- and H^+

→ only pO_2 but not cO_2 is "sensed"

Gases in water

Fick's diffusion capacity D_x:

"*Gas diffusion is proportional to partial pressure difference*"

$$\delta V_x / \delta t = D_x \cdot \Delta p_x$$

$$D_x = D \cdot \alpha_x \cdot A \cdot d^{-1}$$

D_x = diffusion coefficient
α_x = Bunsen solubility constant
A = contact area
d = distance

→ CO_2 easily reaches the bottom of a dish whereas O_2 shows steep gradients!

Temperature effects on gases

Ideal gas law: $P \cdot V = n \cdot R \cdot T$

$$\frac{V}{n} = R \frac{T_0}{P_0} = 22.4\ l\ mol^{-1}$$

Standard conditions:

$T_0 = 0°C = 273\ K$

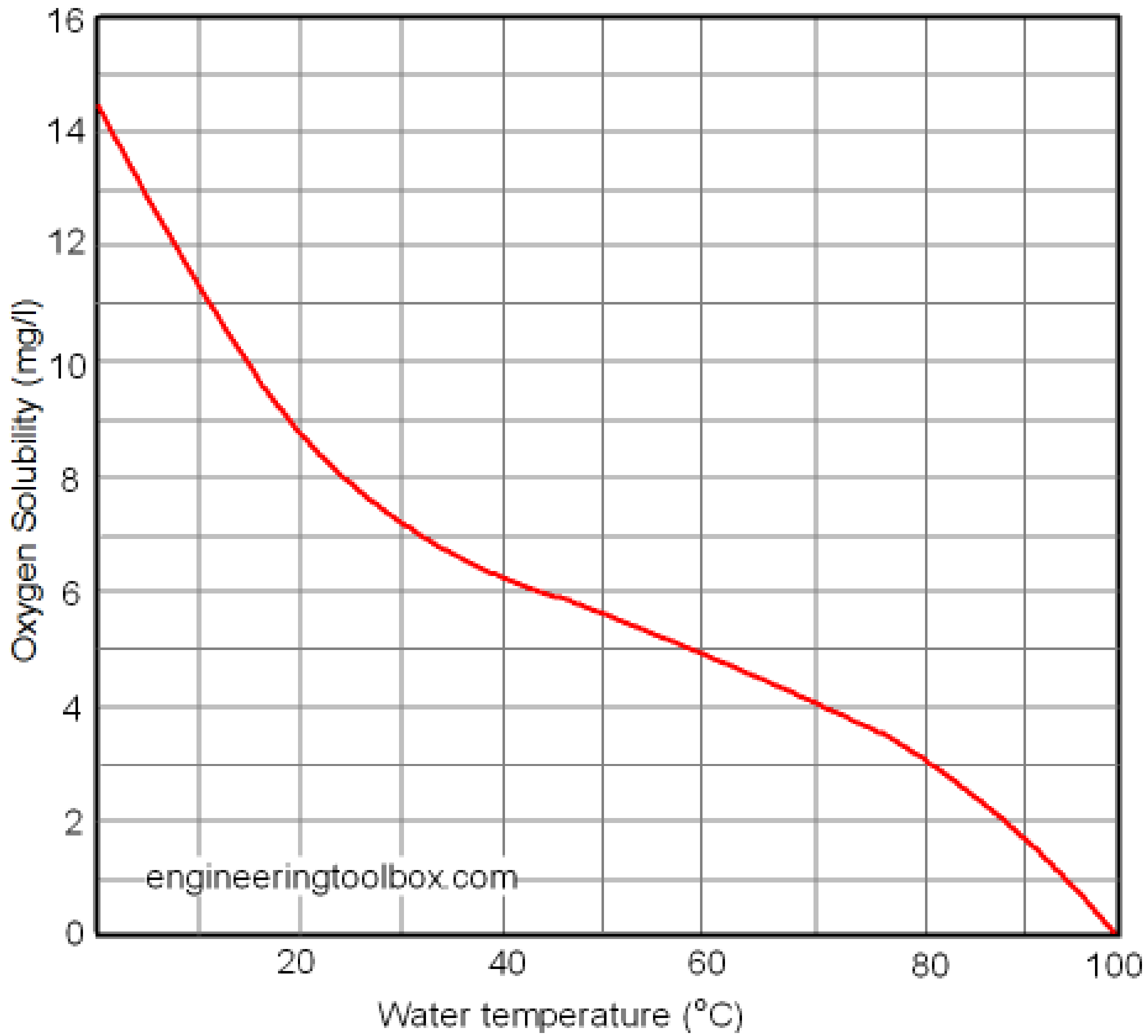
$P_0 = 760\ mmHg = 101.3\ kPa$

$R = 8.31\ l\ kPa\ mol^{-1}\ K^{-1}$

$n = 1$ mole

→ Temperature affects volume

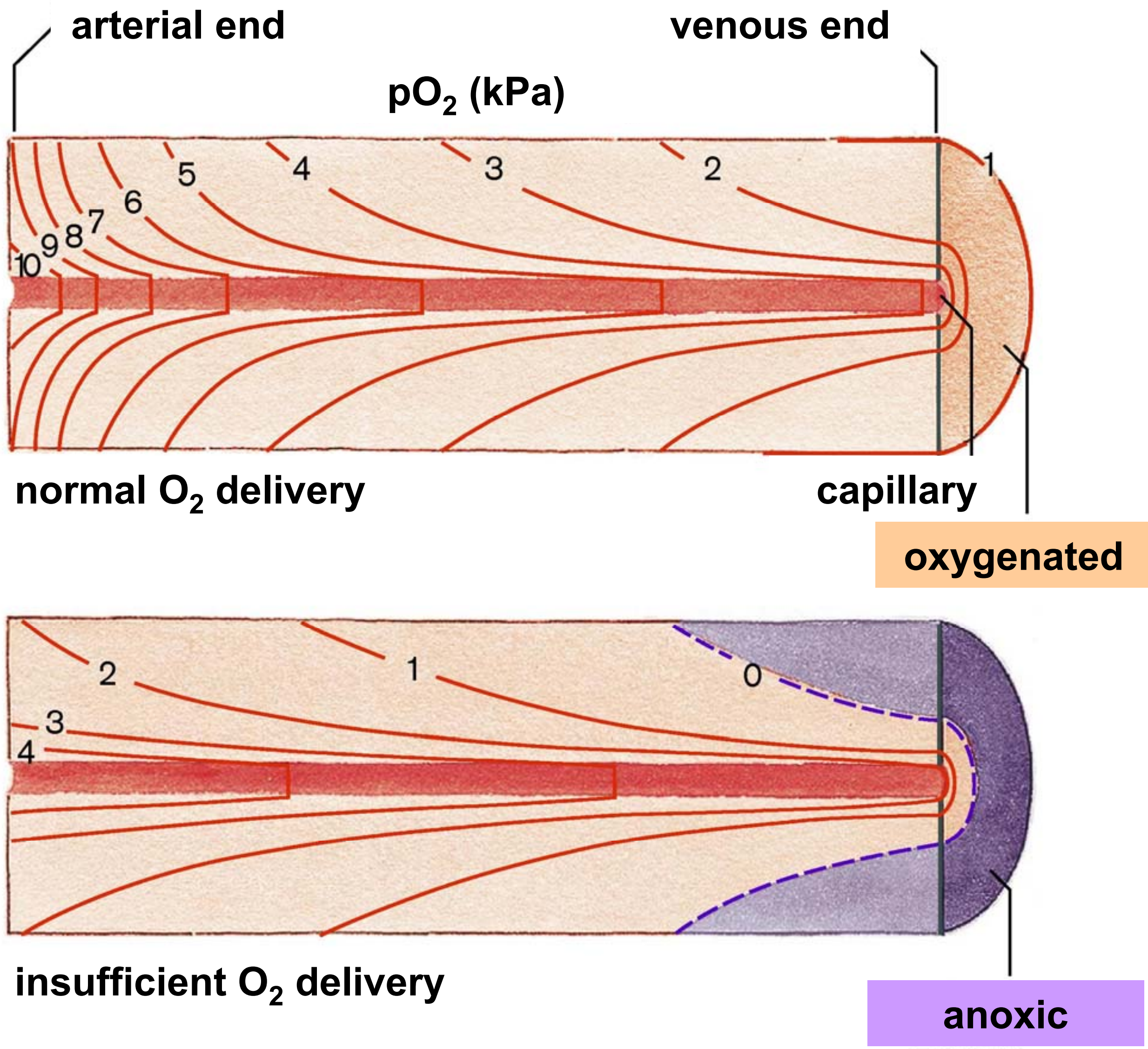
Temperature effects on gases



→ Temperature affects solubility, diffusion etc.
Only relevant for measuring devices not working at 37°C.

What is the tissue pO_2?

Krogh's tissue cylinder



→ there is no single tissue pO_2!

Pressure units
(*sorry for the use of non-standard mmHg!*)

Pressure units						
	Pascal (Pa)	Bar (bar)	Technical atmosphere (at)	Atmosphere (atm)	Torr (Torr)	Pound-force per square inch (psi)
1 Pa	≡ 1 N/m^2	10^{-5}	1.0197×10^{-5}	9.8692×10^{-6}	7.5006×10^{-3}	145.04×10^{-6}
1 bar	100,000	≡ 10^6 dyn/cm^2	1.0197	0.98692	750.06	14.5037744
1 at	98,066.5	0.980665	≡ 1 kgf/cm^2	0.96784	735.56	14.223
1 atm	101,325	1.01325	1.0332	≡ 1 atm	760	14.696
1 torr	133.322	1.3332×10^{-3}	1.3595×10^{-3}	1.3158×10^{-3}	≡ 1 Torr; ≈ 1 mmHg	19.337×10^{-3}
1 psi	6.894×10^{3}	68.948×10^{-3}	70.307×10^{-3}	68.046×10^{-3}	51.715	≡ 1 lbf/in^2