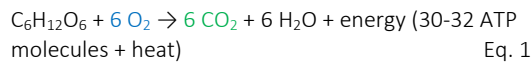


## BOX 1

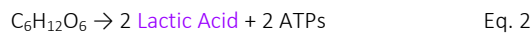
# Role of metabolic gases (O<sub>2</sub> and CO<sub>2</sub>) in mammalian fluids and cell culture media

*Homeostasis* describes the maintenance of a steady-state in an organism. In mammalian bodies, this includes maintaining the equilibrium of temperature, water (H<sub>2</sub>O), physiological processes involving oxygen (O<sub>2</sub>), carbon dioxide (CO<sub>2</sub>), and pH of extracellular fluids, but also blood sugar levels, and the concentrations of sodium-, potassium- and calcium ions. Acid-base chemistry and levels of dissolved gases within mammalian fluids are driven by aerobic and anaerobic metabolic processes (Eq. 1 – 2), and regulated by the respiratory and cardiovascular systems.

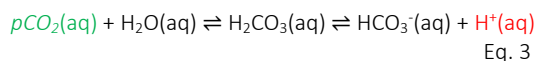
### Aerobic metabolism



### Anaerobic metabolism



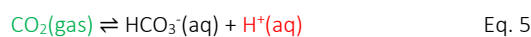
The critical role of dissolved gases in moderating physiological processes has long-been recognized<sup>1</sup>, but gained newfound attraction upon the discovery that cells can sense and adapt to varying levels of O<sub>2</sub>, revealing oxygen as a primary regulator of fundamental physiological processes (2019 Nobel Prize in Physiology or Medicine). O<sub>2</sub> and CO<sub>2</sub> are important signalling molecules but fundamentally differ in their behaviour upon dissolution in metazoan fluids and culture media. CO<sub>2</sub> dissociates to produce carbonic acid and spontaneously forms H<sup>+</sup> ions and reacts with HCO<sub>3</sub><sup>-</sup> to stabilize pH in the body (Eq. 3-4).



### Anaerobic metabolism influences media pH



In cell culture medium, the bicarbonate buffering system operates through the Le Chatelier's principle (Eq. 5). Commercial incubators with a CO<sub>2</sub>-rich atmosphere (usually set to 5%) enable the CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> buffering. Free carbonic ions in the medium react with the extra H<sup>+</sup> ions to form carbonic acid, and stabilize pH.



### Ideal Gas Law

$$PV = nRT \quad \text{Eq. 6}$$

where P = pressure (mmHg), V = volume (L), n = number of molecules of gas (mol), R = ideal gas constant (62.364 L mmHg K<sup>-1</sup> mol<sup>-1</sup>), and T = temperature (K).<sup>3</sup>

Henry's Law states that dissolved oxygen (μM) in the liquid medium is proportional to the partial pressure of oxygen in air. In solution, oxygen solubility decreases with increasing temperature and ionic strength and increases with atmospheric pressure.<sup>4</sup>

### Henry's Law

$$P \cdot \gamma_{\text{O}_2} = H \cdot x^* \quad \text{Eq. 7}$$

where P is pressure of the gas mixture above the medium,  $\gamma_{\text{O}_2}$  is the mole fraction of O<sub>2</sub> within this mixture, H is Henry's constant, and x\* is the equilibrium mole fraction of O<sub>2</sub> in the medium immediately adjacent to the interface.<sup>5</sup>

### Henderson-Hasselbalch equation

$$\text{pH} = \text{pK}_a + \log\left(\frac{[\text{B}]}{[\text{HB}]}\right) \quad \text{Eq. 8}$$

where pH is related to the acid dissociation constant (pK<sub>a</sub>) and concentration of the buffer's unprotonated [B] and protonated [HB] forms.<sup>2</sup>

Within the human body, finite limits of O<sub>2</sub>, CO<sub>2</sub>, and pH differ between organs and fluids. O<sub>2</sub> and pH follow a reverse trend than the finite limits of CO<sub>2</sub>.<sup>6</sup>

### *In vivo* homeostasis and finite limits (e.g., Arterial blood gas<sup>7,8</sup>) versus *In vitro* variability<sup>9</sup>

Variable	<i>In vivo</i>	<i>In vitro</i>
pH	7.35 to 7.45	6.65 to 7.4
pCO <sub>2</sub>	35 to 45 mmHg	23 to 137 mmHg
pO <sub>2</sub>	75 to 100 mmHg	118 to 167 mmHg
HCO <sub>3</sub> <sup>-</sup>	22 to 26 mEq	-

As shown in Human erythroleukemia cells (K562), *in vitro* values can drift as much as to a pH 6.7, CO<sub>2</sub> 11% and O<sub>2</sub> 16%, which is outside *in vivo* finite limits<sup>9</sup>.

In the human body, respiratory and cardiovascular systems (among other systems and mechanisms; e.g., kidneys) ensure that cells *in vivo* receive sufficient O<sub>2</sub> and acid-base stability. These systems are in charge of pH homeostasis, and would be difficult to maintain without acid urinary excretion, and the ability to generate new bicarbonate. Failure to regulate dissolved gases and acid-base chemistry within the finite limits that maintain homeostasis<sup>10</sup> can be hallmarks of numerous hormone and metabolic disorders, as well as carcinogenesis<sup>11</sup>.

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