Biophysical Chemistry 142 (2009) 1–6

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Thermodynamic approach to oxygen delivery *in vivo* by natural and artificial oxygen carriers

Enrico Bucci *

Department of Biochemistry and Molecular Biology, University of Maryland Medical School, 108 North Greene St., Baltimore MD 21201, United States

ARTICLE INFO

Article history:
Received 30 September 2008
Received in revised form 19 December 2008
Accepted 19 December 2008
Available online 31 December 2008

Keywords:
Blood flow
Oxygen delivery
Oxygen gradients
Facilitated diffusion
Oxygen carriers
Blood substitutes

ABSTRACT

Oxygen is a toxic gas, still indispensable to aerobic life. This paper explores how normal physiology uses the physico-chemical and thermodynamic characteristics of oxygen for transforming a toxic gas into a non toxic indispensable metabolite. Plasma oxygen concentration is in the range of 10^{-5} M, insufficient to sustain metabolism. Oxygen carriers, present in blood, release oxygen into plasma, thereby replacing consumed oxygen and buffering PO_2 near their P_{50}. They are the natural cell-bound carriers, like hemoglobin inside red cells, myoglobin inside myocytes, and artificial cell-free hemoglobin-based oxygen carriers (HBOC) dissolved in plasma. Metabolic oxygen replacement can be defined as cell-bound and cell-free delivery. Cell-bound delivery is retarded by the slow diffusion of oxygen in plasma and interstitial fluids. The 40% hematocrit of normal blood compensates for the delay, coping with the fast oxygen consumption by mitochondria. Facilitated oxygen diffusion by HBOCs corrects for the slow diffusion, making cell-free delivery relatively independent from P_{50}. At all oxygen affinities, HBOCs produce hyperoxygenations that are compensated by vasoconstrictions. There is a strict direct correlation between the rate of oxygen replacement and hemoglobin content of blood. The free energy loss of the gradient adds a relevant regulation of tissues oxygenation. Oxygen is retained intravascularly by the limited permeability to gases of vessel walls.

1. Introduction

Oxygen is a most toxic substance, 20% of breathing air. Oxygen toxicity is related to its activity, i.e. its tension in the environment. A few mm under the surface of skin (as reported in several paper originating from the laboratory of Intaglietta and reported in Ref. [1]) the oxygen tension in air, 155 Torr, decreases to near 30 Torr inside the capillaries and about 1 Torr or less in the tissues [1]. This indicates a low permeability of skin to oxygen, which protects the internal organs into non toxic level of oxygen tension. The only internal region of the body exposed to oxygen tensions higher the 100 Torr is the pulmonary alveoli. From these, a continuous mass flow through plasma in the vasculature feeds oxygen to tissues. However, the free oxygen concentration in plasma is only near the low end of 10^{-5} M range. This low concentration, is insufficient to provide the amount of oxygen necessary for, and to cope with, the rate of mitochondrial metabolism, which requires a transport of oxygen close to 1 L min^{-1} [2]. This problem of insufficiency is solved by the presence of oxygen carriers, which release oxygen into the flow, thereby replacing consumed oxygen and adding the necessary amounts of oxygen at the proper rate. Two kinds of carriers are available: the natural cell-bound carriers, such as hemoglobin inside red cells and myoglobin inside myocytes, and cell-free artificial hemoglobin-based oxygen carriers (HBOC) in plasma.

This paper explores the thermodynamics underneath the physiologic processes that drive the oxygen diffusion *in vivo*, from lungs to tissues.

2. Characteristics of the flow

2.1. The Fick law

The Henry factor, *K*, defines the oxygen solubility, as in:

$$PO_2 = \frac{[FO]}{K} \tag{1}$$

where [FO] is the molar concentration of free oxygen in solution corresponding to a partial pressure of free oxygen (PO_2). Eq. (1) allows measurements of PO_2 for monitoring variations of [FO]. Therefore,

Abbreviations: BC, buffer capacity of the carrier; BO, bound oxygen on the carrier; BO_{carr}, hemoglobin carried oxygen; *D*, diffusion coefficient; D_K, Diffusion coefficient inclusive of oxygen salvation; DS_t, time dependent desaturation; RF, empirical retarding factor; [FO], molar concentration of free oxygen in solution; FO, free oxygen in solution; HBOC, hemoglobin based oxygen carrier; *K*, Henry factor, oxygen solubility in water; $O_{2rep,t}$, rate of formation of replacing oxygen; PO_2, partial pressure of free oxygen in solution; Sat, fractional oxygen saturation of the carrier.

* Tel.: +1 443 451 5038; fax: +1 410 510 1457.
E-mail address: ebucci@umaryland.edu.

0301-4622/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2008.12.009

throughout this paper, PO_2 will be used rather than [FO] in the following discussions. The gradient of PO_2 from about 100 Torr at the lungs to 2 Torr in the muscles interstial space produces (starting at the lungs) a longitudinal negative gradient of oxygen concentration in plasma throughout the vasculature, which follows the Fick law as described by:

$$J_s = -D_K \frac{\partial(PO_2)}{\partial(s)} \tag{2}$$

where J_s is the flow rate at position s .and D is the coefficient of diffusion of oxygen in the medium. The K subscript indicates the oxygen solvation. Thus, as written, D_K becomes a coefficient of oxygen transport, where D defines the rate of diffusion, and K the amount of transported material. The profile of Eq. (2) is shown in Fig. 1. The transport is not linear with distance and begins with a sharp decrease, followed by a shallow, slowly decreasing gradient.

2.2. Energy of the flow

The free energy loss (DG), which drives the flow can be estimated using:

$$DG = -RT \ln\left[(PO_2)_1 / (PO_2)_2\right] \tag{3}$$

where subscripts 1 and 2 refer to the initial and final PO_2 of the gradient, A free energy loss that starts at near 120 Torr in the alveoli and is near 2 Torr in the interstitial space of the muscles for decreasing to near 1 Torr inside the cells can be estimated to be near a negative pressure of 2–3 Torr. Notably, the energy of the negative pressure does NOT modify the P_{50} of the oxygen carriers present in blood and it is evenly distributed at all levels of the PO_2 gradient.

3. Oxygen carriers and their buffer capacity

Oxygen carriers are those substances, like hemoglobin and myoglobin, which bind and release molecular oxygen when exposed to a variable partial pressure of free oxygen (PO_2) compatible with their oxygen affinity. The carriers are not oxygen pumps; they passively equilibrate with the prevailing PO_2 as empirically described by the Hill equation:

$$Sat = \frac{(PO_2)^n}{(P_{50})^n + (PO_2)^n} \tag{4}$$

where Sat is the fractional oxygen saturation of the carrier and n is the oxygen binding cooperativity index. P_{50} is the value of PO_2 at which Sat = 0.5. For tetrameric hemoglobin $n = 1.0$ in the absence of cooperativity, and $1 < n < 4$, when cooperativity is present. The values of P_{50} and n in Eq. (4) define the PO_2 range within which the carrier binds and releases oxygen. For example, for a carrier with $P_{50} = 30$-Torr and $n = 3.0$, Sat varies from 0.1 to 0.9 when PO_2 goes from 15 to 60 Torr. In myoglobin with $P_{50} = 2.0$ and $n = 1.0$ for the same Sat variation PO_2 goes from 0.20 to 20 Torr. Outside of the binding ranges, variations of PO_2 fail to modify Sat, and the carrier remains either fully oxygenated for PO_2s above the upper limit of the range or fully deoxygenated below the lower limit. For all values of cooperativity (n), the carriers are half saturated when $PO_2 = P_{50}$.

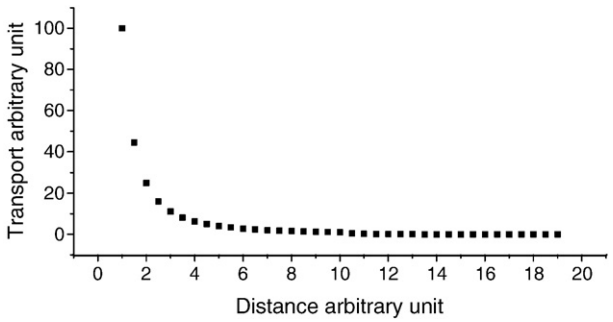

Fig. 1. Profile describing the dependence on distance of diffusion transport in arbitrary units of concentrations, according to the Fick Law.

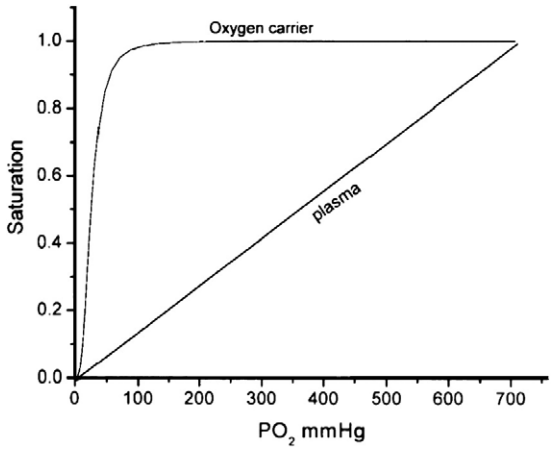

Fig. 2. Schematic of the longitudinal gradient of free oxygen through plasma compared to the oxygen binding and release by an oxygen carrier.

As shown in Fig. 2, the oxygen carrier binds oxygen at the origin of the gradient in the lungs but does not release its bound oxygen (BO) to the gradient until the decreasing plasma PO_2 becomes compatible with the exchange range of the carrier. As mentioned, the carrier is not an oxygen pump and only equilibrates with the prevailing PO_2, replacing consumed oxygen. Thus, the gradient is buffered at values of PO_2, determined by the sensitivity of the carrier to changes of PO_2 (i.e., to its buffer capacity). The buffer capacity of the carrier (BC) is defined by:

$$BC = \frac{\partial(Sat)}{\partial(PO_2)} \tag{5}$$

Fig. 3 shows the semi-logarithmic profile of the buffer capacities of red cells and myoglobin. The BC ranges of PO_2 correspond to the ranges of oxygen binding. The high oxygen-binding cooperativity makes the buffer capacity of red cells very strong and sharp. In myoglobin, the absence of cooperativity makes its buffer capacity shallower and much broader. They overlap extensively, thereby

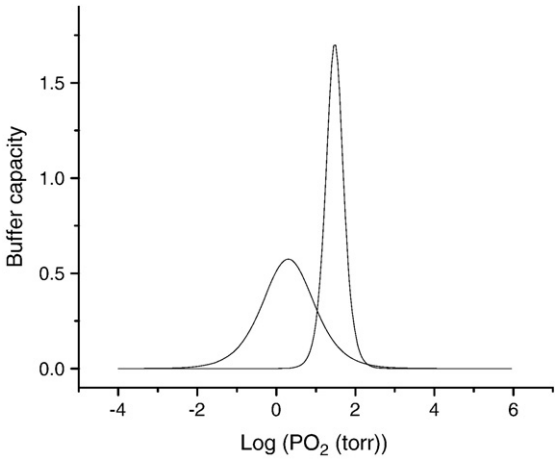

Fig. 3. Semilogarithmic profile (dSat/dLog(PO_2) of the buffer capacity of myoglobin (lower curve, $P_{50} = 2.0$ torr, $n = 1.0$) and hemoglobin in red cells (upper curve, $P_{50} = 30.0$ Torr, $n = 3.0$). The maximum strength is at the respective P_{50} values, 2.0 and 30.0 Torr, respectively.

covering the entire range of the oxygen gradient from lungs to tissues. Red-cell and myoglobin buffers have maximum value at their P_{50}—30 Torr and 2 Torr, respectively.

3.1. Buffer capacity of artificial HBOCs

The buffer capacities of artificial HBOCs follow the same rules as outlined above. Their maximum capacity will be at $PO_2 = P_{50}$, and their range will be defined by their oxygen affinity and oxygen-binding cooperativity.

4. Cell-bound oxygen delivery in vivo

4.1. Plasma longitudinal gradient and red cell buffering

The presence of a negative longitudinal PO_2 gradient *in vivo* was confirmed by several independent laboratories, as reported by Tsai et al. [1] and shown in Fig. 4. The gradient ends into a plateau at the capillary level, where the strong buffer capacity of red cells (P_{50} = 30-Torr) keeps PO_2 near 30 Torr and blood is found to be close to half saturation with oxygen. The variable data around an oxygen tension of 30 Torr are all within the buffer capacity range of red cells and reflect a different supply/consumption oxygen balance in different animal models and different tissues (see Fig. 4).

In interstitial fluid, myoglobin absorbs the oxygen diffusing from the capillaries and buffers the oxygen tension near its maximum buffer capacity: $PO_2 = P_{50} = 2$ Torr. Also in this case, the buffer will balance consumption and supply. Wittenberg and Wittenberg [3,4] report that in cardiac muscle myoglobin is 70% saturated, while in skeletal muscles, it is 50% saturated, which implies that in the heart, PO_2 is stabilized near 5 Torr, and in the muscles, near 2 Torr, both within the buffer range of myoglobin. Apparently, oxygen supply prevails over consumption in the heart, while it is in perfect equilibrium with consumption in the muscles.

5. Thermodynamics of the physiology of oxygen replacement

5.1. Thermodynamics

The quantity and rate of formation of the replacing oxygen is the difference between the amount of oxygen carried by blood hemoglobin and what is left there after replacing-oxygen is released to consumption at the capillaries:

$$O2_{repl,t} = BO_{carr} - \partial Sat / \partial t \tag{6}$$

where BO_{carr} is the amount of oxygen carried by hemoglobin and $O2_{rep,t}$, is the time dependent (rate of) formation of replacing oxygen.

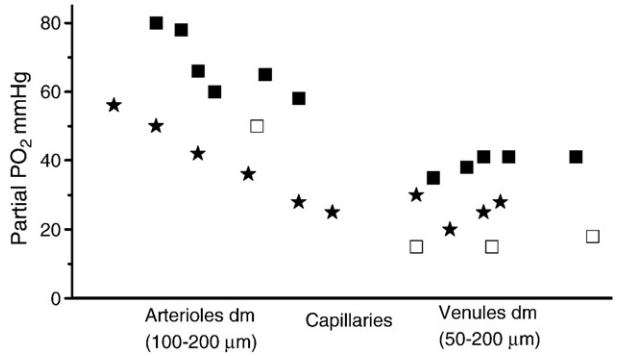

Fig. 4. Distribution of oxygen tension in the microcirculation of different animal models. □, dog gracilis ms; ★, hamster skinfold; ■, rat cerebral brain cortex. Adapted from Tsai AG, Johson PC, Itaglietta M (2003) oxygen gradients in the microcirculation, Physiol Rev 83:933–963.

The continuous resupply of BO_{carr} implies that the SAT changes on the right side of Eq. (6) includes both the release of oxygen at the capillaries and the rebinding of oxygen at the alveoli. It can be described by:

$$DS_t = \underbrace{\{(Sat)_t BO_{carr}(\exp(-k_{off})\}}_{\text{desaturation}} - \underbrace{\{(1-Sat)_t BO_{carr}\exp(k_{on})([FO])_{alv}\}}_{\text{resaturation}} \tag{7}$$

where DS_t is the decreasing saturation of BO_{carr}, at time t, k_{off} is the off-kinetic constant of hemoglobin, k_{on} is the on-kinetic constant and $[FO]_{alv}$ is the oxygen concentration at the alveoli. Because of continuous replacement, alveolar $\{FO\}_{alv}$ is constant, therefore "resaturation" is a pseudo first order kinetics. The balance between desaturation and resaturation, i.e. between consumption and supply of oxygen, regulates the PO_2 prevailing at the capillaries where oxygen delivery occurs. Eq. (6) can be rewritten as:

$$O2_{repl,t} = BO_{carr} - DS_t \tag{8}$$

Moreover, an imbalance is present between the speed of oxygen consumption and speed of replacement. The rate, *in vivo*, of the reaction of cytochrome oxydase with oxygen at the mitochondria is probably as fast as the *V*max measured *in vitro*. Instead the supply of oxygen, released by red cells into the flow, must migrate through various watery compartments (plasma, interstitial fluid, red cells cytoplasm) and their associated membranes before reaching mitochondria. As anticipated by Federspiel and Popel [5], the low oxygen solubility in water, and consequently the low diffusion coefficient D_k, slow its migration through plasma. Thus, an imbalance is create between a slow speed of supply and a fast speed of consumption. This phenomenon can be described by adding to Eq. (8) an empirical parameter, the retarding factor, $0 < RF < 1$, inclusive of all barriers crossed by the replacing-oxygen:

$$O2_{repl,t} = (BO_{carr} - Ds_t)RF \tag{9}$$

The speed is lowest when RF approaches 0 (zero), maximal when $RF = 1$.

5.2. Physiology

It is interesting to observe how the parameters in Eqs. (6)–(9) interact in order to assure to metabolism adequate amounts and rate of replacing oxygen. When all other parameter in Eq. (7) are "normal", the low RF is compensated by the amount of BO_{carr} supplied by a 40% hematocrit (i.e. about 14 g dL^{-1} of hemoglobin with about 200 ml L^{-1} of bound oxygen). When the off-kinetics of red cells hemoglobin is lowered by mutations (high affinity blood) it lowers the speed of desaturation in Eq. (7), in turn lowering the amount and rate of formation of $O2_{rep,t}$. The speed of formation of replacing oxygen is restored by an increase of BO_{carr} produced by polycytemia [6]. Conversely, an increased k_{off} lowers the oxygen affinity of blood and increases the speed of desaturation. The potential hyperoxygenation requires a decrease of BO_{carr}, resulting in anemia [6]. When the tension of oxygen in alveolar air decreases because of altitude, the lower Hb saturation at the lungs (lower BO_{carr}) is compensated by an increasing hematocrit (50–55%). Acclimatization takes a few days. Upon returning to sea level, where alveolar oxygen tension decreases, in a few days the hematocrit decreases to "normal" values [7]. It appears that changes of hematocrit, buffer variations of the parameters in Eq. (7).

Also, there is a correlation between BO_{carr} and blood flow. An acute decrease of hematocrit, consequent to a hemorrhagic event or an exchange transfusion, implies a decrease of BO_{carr}. The response is an increased blood flow [8], produced by a vasodilation, which increases the timely amount of BO_{carr} restoring a normal time formation of

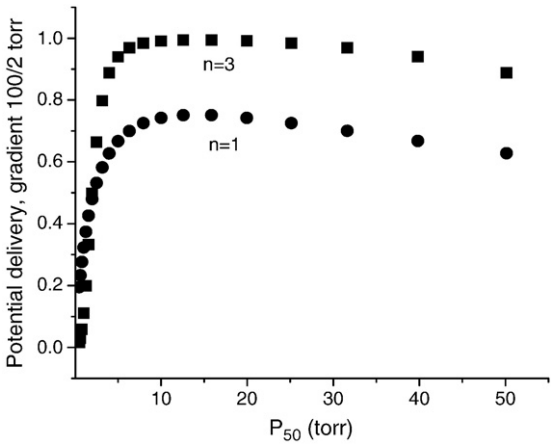

Fig. 5. Dependence on P_{50} of the potential oxygen delivery by oxygen carriers exposed to a gradient of oxygen tension between 100 and 2 Torr.

replacing-oxygen. To the contrary when hyperoxygenation is produced by acute infusions of extra red cells or cell free oxygen carriers [8], a lower blood flow, produced by a vasoconstriction, reduces the speed of circulating blood and available BOcarr [8].

5.3. A useful paradox

It seems as if the oxygen carried by blood hemoglobin contributes to control hematocrit, blood flow and vasoactivity. Oxygen bound to hemoglobin does not have any physico-chemical activity and from the thermodynamic point of view it does not exist. Therefore the sensitivity of the regulatory system to quantities of "non existent" oxygen is a thermodynamic paradox. The paradox is solved when Eqs. (6)–(9) show that BO_{carr} responds to the quantity and rate of formation of replacing oxygen, i.e. the quantity and rate of oxygen consumption. The amount of circulating BO_{carr} can be estimated experimentally measuring the blood concentration of hemoglobin, and its variations. Thus, a thermodynamic paradox offers to physiologists means to follow the rate of oxygen consumption, the balancing of the parameters in Eq. (7) and interpret the meaning of blood flow changes.

5.4. Notably

There must be a sensory system capable of monitoring not only the quantity but *also the rate* of oxygen consumption. In fact it is an imbalance between the speeds of supply and consumption that triggers the cascade of regulatory events discussed above.

6. Cell-free oxygen delivery

Cell-free plasma-based HBOCs add two new parameters to the regulation of free oxygen flow: 1) they facilitate oxygen diffusion through plasma, and as a consequence, 2) they increase the rate of formation of replacing oxygen.

As described by Wittenberg et al. [4], the flow rate of oxygen across a liquid filled membrane increases when the liquid contains cell-free oxygen carriers, such as hemoglobin or myoglobin. This phenomenon results from the translational component of the Brownian motions of the cell-free carrier molecules in solution. Thus, the rate of translational diffusion of the carriers through the liquid is critical to the facilitation, which is obviously inversely proportional to the viscosity of the medium and the size of the carrier [4]. The bulky and viscous red cells cannot contribute to the phenomenon.

Thus, plasma-based HBOCs produce an exaggerated D_K for the diffusion of free oxygen through plasma, and the RF in Eq. (9) increases toward unity, making the speed of oxygen replacement by HBOCs much faster than that by red cells. In fact, it was shown that under reduced oxygen tension (30% oxygen, rather than the classic 95%), a Ringer solution of 3 g dL^{-1} cell-free DECA (i.e., HbA crosslinked with sebacic acid [9]) was able to keep alive and functional intestinal rabbit membranes superfused in Ussing chambers, whereas a hemoglobin-equivalent suspension of bovine red cells, with similar oxygen affinity and binding cooperativity, failed to do so [10]. Because of the facilitated diffusion through the solution, the speed of oxygen replacement was much faster with cell-free DECA, coping with the rate of oxygen consumption by the tissue.

The question arises whether, with a value of the RF approaching unity, cell-free oxygen delivery is still dependent on the oxygen affinity and binding cooperativity of HBOCs. Numerical computations, using the Hill Eq. (4), show in Fig. 5 that, for a gradient between 100 and 2 Torr, there is a very small dependence of fractional oxygen release with P_{50}s between 3 and 50 Torr. Notably, affinities with P_{50} as low as 3 Torr would allow total release of oxygen in the presence of high binding cooperativity and even a substantial delivery in the absence of cooperativity.

A recent mini-review by Koehler et al. [8] confirmed that HBOCs with P_{50} varying from 3 to 30 Torr all delivered oxygen to tissue. The evidence was their interference with blood flow in brain microcirculation. They stabilized cerebral blood flow to normoxia in animals made anemic by 40%–50% exchange transfusions (Fig. 6). Notably, HBOCs with P_{50} near 3 Torr and no binding cooperativity reduced the infarct size in mouse brains produced by occlusion of the middle cerebral artery. In all cases normoxia was concomitant with a vasoconstriction not dependent on scavenging of nitric oxide. The vasoconstriction was necessary to avoid the hyperoxygenation produced by the excessive amount of replacing oxygen resulting from facilitated diffusion [8]. Also, when blood viscosity was increased by infusion of polyvinylpyrrolidone, vasoconstriction reverted into a potent vasodilation. Whether this implies a return to normoxia is still under investigation.

Notably, HBOCs also facilitate the diffusion of the oxygen delivered by red cells still present in blood after exchange transfusion. In fact, the amount of hemoglobin added to blood with HBOCs produced normoxia even when it was near half that removed with the red cells by exchange transfusions [8]. This hypothesis is supported by data obtained by Page et al. [11] using a silicone artificial capillary. The rate of oxygen diffusion from a capillary fed with a fluid containing equivalent hemoglobin amounts of red cells and HBOCs was very near that of a pure solution of HBOCs.

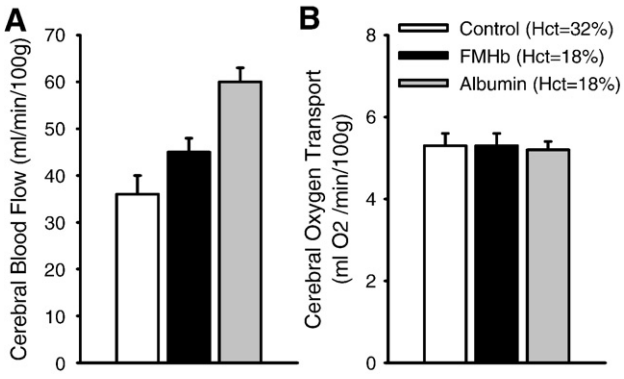

Fig. 6. Normoxia produced by fumaryl crosslinked hemoglobin (FMHb) ($P_{50} = 17$, $n = 1.2$, MW = 64 kDA) in cats after exchange transfusion with either 6% albumin or 6% FMHb. Note the lower blood flow increase in (A) and the resulting normal cerebral blood flow in the presence of FMHb in (B). Adapted from Koehler RC, Fronticelli C, Bucci E (2008) Bucci Insensitivity of cerebral oxygen transport to oxygen affinity of haemoglobin-based oxygen carriers. (2008) Biochem Biophys Acta 1784:1387–1394.

7. The difference between cell-bound and cell-free oxygen delivery

Using cell-bound oxygen carriers, normal physiology succeeds in lowering the partial pressure of oxygen in breathed air, near 150 mmHg, to the vanishing pressures present in body fluids, while delivering large amounts of oxygen to tissues.

Cell-free HBOCs add to this scenario their Brownian motions. The resulting facilitated diffusion in practice decreases the distance of watery itineraries, bypasses normal physiology, resulting in overwhelming diffusion of oxygen [8].

8. Corollaries

8.1. A neglected oxygen delivery regulation

The free energy loss of the oxygen gradient, as discussed above in Eq. (3), implies that the amount of oxygen in solution adds 2–3 Torr of energy to the solvation of oxygen in plasma and interstitial fluids. The excess solvation is negligible until the flow reaches the interstitial fluid, where at vanishing pressures below 2 Torr, oxygen metabolism approaches stochastic characteristics. The extra dissolved oxygen may duplicate the number of oxygen molecules available to consumption.

Moreover, even small changes of PO_2 at the end of the gradient would modify in a significant way the energy loss, in Eq. (3), modulating oxygen solvation according to consumption needs.

8.2. Hyperoxygenation

The inevitable result of hyperoxygenation is the formation of oxygen radicals, which are not reabsorbed and neutralized by rebinding to either cell-bound or cell-free hemoglobins. They are strong oxidative agents, that damage the endothelial layers of the vessels. Probably they are responsible *in vitro* for the increased permeability of endothelial layers exposed to hemoglobins [12] and *in vivo* for leakages through endothelial gaps opened in the vessels of rat intestine by infusion of Peg-Hb (hemoglobin coupled on the surface with polyoxiethyleneglycol) [13]. This hypothesis is supported by the observation that selenites reduce leakages [14]. It may be proposed that hyperoxygenation contributes to the adverse effects and myocardial infarctions observed in patients infused with HBOCs [15].

Notably, facilitated oxygen diffusion still produces low levels of PO_2, as dictated by the buffer capacity of the HBOCs, especially by the high-affinity carriers. This observation encourages the view that by controlling hyperoxygenation and related oxygen radicals, HBOCs could be used pharmacologically for administering measured amounts of necessary extra oxygen in clinical practice.

8.3. Oxygen vascular retention

Dispersion of oxygen at the capillaries requires retention during the long journey from lungs to tissues. Two factors limit oxygen transit losses. The buffer capacity of the carriers will retain oxygen until the oxygen tension of the gradient at the periphery becomes compatible with their oxygen affinities. The best retention is provided by high affinity HBOCs. Also, a low diffusion constant of the vascular walls (lower than that of plasma and interstitial fluid) provides a septum with proven low permeability to oxygen. In fact, as discussed above, Eq. (2) anticipates an initial sharp drop of PO_2 when oxygen diffuses into a region with lower D_K values (i.e., lower diffusion rate). This phenomenon would result in PO_2 levels lower in the abluminal side than in the luminal side of the vessels. That is why Tsai et al. [1] report that PO_2 is approximately 10% lower in the abluminal than in the luminal side of the arterioles, more so for larger arteries. Thus, to a first approximation, the vascular system before the capillaries can be considered a long tube impermeable to oxygen, as originally proposed by Krogh [16] and supported by data from Golub et al. [17].

The limited permeability of the vascular walls to gases is probably the reason why hemoglobin scavenging of nitric oxide inside the luminal side of the vessels does not affect the nitric oxide diffused from the endothelium toward the abluminal side [18] into the muscle layers of the vessels. It justifies the observation that when HBOCs do not extravasate, systemic vasoconstrictions are not produced [19].

8.4. Tissues without myoglobin

The muscles and endothelial layers of the vasculature do not have capillary beds and feed only on oxygen diffusing through the endothelial walls. These layers do not have the buffering regulatory means of myoglobin at $PO_2 = 2$ Torr. Therefore, they are exposed to higher levels of PO_2, especially in large arteries and heart coronaries, where PO_2 is highest. The higher PO_2 makes these organs vulnerable to oxygen toxicity, which in the long run may contribute to vascular pathology.

Skin does not have myoglobin. Fortunately, skin has a very low permeability to air oxygen, which otherwise would freely diffuse inside the body, burning tissues and membranes. As shown by Eqs. (1) and (2), the very low cutaneous diffusion coefficient decreases the oxygen tension in skin parenchyma, compensating for the absence of myoglobin. Myoglobin is present only in myocytes, therefore, a similar regulation may modulate PO_2 in other organs ad tissues without myocytes.

Transgenic mice totally deprived of myoglobin show normal vital signs [20]. It may be anticipated that, in the absence of buffering myoglobin, their muscle systems are exposed to a relatively high PO_2, still buffered to nearly 30 Torr by red cells. Survival is allowed. The long-term effect of this situation is difficult to assess because of the short life span of mice, further shortened by experimental protocols.

8.5. A special case is brain

In the absence of myoglobin, the neurons are exposed to oxygen tensions higher than in other tissues. The large amount of brain lipids (brain may be considered a blob of lipids) allows a much higher solvation of oxygen than plasma. As shown by Eq. (1), the consequent increase of the Henry factor decreases the ratio $[PO_2]/[FO]$ reducing the oxygen tension produced by free oxygen in the brain. The higher solvation of oxygen also results in a higher D_K, which, in turn, allows a very fast diffusion of oxygen through the parenchyma, suggesting a functional role of the lipid environment. Still, brain is another vital organ potentially exposed to high oxygen tension, which may contribute in the long run to clinical problems.

9. A disclaimer

The physiology of oxygen transport and distribution in vivo is a very complex process. This presentation focused only on a special aspect of this phenomenon—the thermodynamic aspects of the flow of free oxygen from lungs to tissues. Approximations in this discussion were used only for stressing the relationship between thermodynamics and physiology. Arbitrary parameters were used to simulate the profile of the Fick law and stressing the physiologic meaning of the initial drop.

The author hopes that these considerations and models will help to clarify physiologic events presently under investigation, help the design of new artificial oxygen carriers, and update the guidelines of the regulators for allowing clinical trials.

Acknowledgements

This paper is dedicated to the 75th birthday of Dr. Clara Fronticelli long time and most esteemed collaborator.

The manuscript was read by Dr. Clara Fronticelli and Dr. Raymond C. Koehler (Johns Hopkins Medical Institutions) who provided very

constructive critiques and was supported in part by the Dept. of Biochemistry and Molecular Biology of the University of Maryland Medical School.

Editing by Ms. Tzipora Sofare is gratefully acknowledged.

References

[1] A.G. Tsai, P.C. Johnson, M. Intaglietta, Oxygen gradients in the microcirculation, Physiol. Rev. 83 (2003) 933–963.
[2] J. Emsley, The 13th Element, Wiley, NY, 2001, p. 201.
[3] J.W. Wittenberg, B.A. Wittenberg, Myoglobin function reassessed, J. Exp. Biol. 206 (2003) 2011–2020.
[4] J.W. Wittenberg, The molecular mechanism of hemoglobin-facilitated oxygen diffusion, J. Biol. Chem. 241 (1966) 104–114.
[5] W.J. Federspiel, A.S. Popel, Theoretical analysis of the effect of the particulate nature of blood on oxygen release in capillaries, Microvasc. Res. 32 (1986) 164–189.
[6] H. Wajcman, F. Galactéros, Hemoglobins with high oxygen affinity leading to erythrocytosis. New variants and new concepts, Hemoglobin 29 (2) (2005) 91–106.
[7] G.R. Zubieta-Calleja, P.-E. Paulev, L. Zubieta-Calleja, G. Zubieta-Castillo, Altitude adaptation through hematocrit changes, J. Physiol. Phamacol. 58 (Suppl 5) (2007) 811–818.
[8] R.C. Koehler, C. Fronticelli, E. Bucci, Insensitivity of cerebral oxygen transport to oxygen affinity of haemoglobin-based oxygen carriers, Biochem. Biophys. Acta 1784 (2008) 1387–1394.
[9] E. Bucci, A. Razynska, H.E. Kwansa, B. Matheson-Urbaitis, M.O.' Hearne, J.A. Ulatowski, R.C. Koehler, Production and characteristics of an infusible oxygen-carrying fluid based on hemoglobin intramolecularly cross-linked with sebacic acid, J. Lab. Clin. Med. 128 (1996) 146–153.
[10] E. Bucci, T.L. Watts, H.E. Kwansa, A. Fasano, A Different efficacy in vitro of hemoglobin-based carriers and red cells, Adv. Exp. Med. Biol. 510 (2003) 89–92.
[11] T.C. Page, W.R. Light, C.B. McKay, J.D. Hellums, Oxygen transport by erythrocyte/hemoglobin solution mixtures in an in vitro capillary as a model of hemoglobin-based oxygen carrier performance, Microvasc. Res. 55 (1998) 54–64.
[12] R.D. Dull, B.J. DeWitt, R. Dinavahi, L. Schwartz, C. Hubert, N. Pace, C. Fronticelli, Quantitative assessment of hemoglobin-induced endothelial barrier dysfunction, J. Appl. Physiol. 97 (5) (2004) 1930–1937.
[13] A.L. Baldwin, Modified hemoglobins produce venular interendothelial gaps and albumin leakage in the rat mesentery, Am. J. Physiol. 277 (2 Pt 2) (1999) H650–H659.
[14] A.L. Baldwin, E.B. Wiley, A.G. Summers, A.I. Alayash, Sodium selenite reduces hemoglobin-induced venular leakage in the rat, Am. J. Physiol, Heart Circ. Physiol. 284 (2003) H81–H91.
[15] C. Natanson, S.J. Kern, P. Lurie, S.M. Banks, S.M. Wolfe, Cell-free hemoglobin based blood substitutes and risk of myocardial infarction and death, JAMA 299 (19) (2008) 2304–2312.
[16] A. Krogh, The number and distribution of capillaries in muscles with the calculation of the oxygen pressure necessary for supplying the tissue, J. Physiol. 52 (1918) 409–515.
[17] A.S. Golub, M.C. Barker, R. Pittman, Microvascular oxygen tension in rat mesentery, Am. J. Physiol, Heart Circ. Physiol. 294 (2008) 21–28.
[18] Y. Asano, R.C. Koehler, J.A. Ulatowski, R.J. Traystman, E. Bucci, Effect of cross-linked hemoglobin transfusion on endothelial-dependent dilation in cat pial arterioles, Am. J. Physiol. 275 (4 Pt 2) (1998) H1313–H1321.
[19] B. Matheson, H.E. Kwansa, A. Rebel, T. Mito, R.C. Koehler, E. Bucci, Vascular response to infusions of a nonextravasating hemoglobin polymer, J. Appl. Physiol. 93 (2002) 1479–1486.
[20] D.J. Garry, J.A. Ordway, J.N. Lorentz, N.B. Radford, E. Chin, R.W. Grange, R. Bassel-Duby, R.S. William, Mice without myoglobin, Nature 395 (1998) 905–908.