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Red Cell Physiology and Signaling Relevant to the Critical Care Setting

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Abstract

Purpose of Review—Oxygen (O_2) delivery, the maintenance of which is fundamental to supporting those with critical illness, is a function of blood O_2 content and flow. Here, we review red blood cell (RBC) physiology relevant to disordered O_2 delivery in the critically ill.

Recent Findings—Flow (rather then content) is the focus of O_2 delivery regulation: O_2 content is relatively fixed, whereas flow fluctuates by several orders of magnitude. Thus, blood flow volume and distribution vary to maintain coupling between O_2 delivery and demand. The trapping, processing and delivery of nitric oxide (NO) by RBCs has emerged as a conserved mechanism through which regional blood flow is linked to biochemical cues of perfusion sufficiency. We will review conventional RBC physiology influencing O_2 delivery (O_2 affinity & rheology) and introduce a new paradigm for O_2 delivery homeostasis based on coordinated gas transport and vascular signaling by RBCs.

Summary—By coordinating vascular signaling in a fashion that links O_2 and NO flux, RBCs couple vessel caliber (and thus blood flow) to O_2 need in tissue. Malfunction of this signaling system is implicated in a wide array of pathophysiologies and may be explanatory for the dysoxia frequently encountered in the critical care setting.

Keywords

erythrocyte; red blood cell; O_2 delivery; nitric oxide

Introduction: the Erythron

Recently, the red blood cell (RBC) series, from progenitor cells to mature erythrocytes, have been termed *the Erythron*. This convention serves to reinforce the concept of integrated tissue function as an independent organ responsible for transport of oxygen from lungs to

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tissue[1]. In adults, the total number of circulating RBCs is in steady state unless perturbed by pathologic or environmental insult. This is not so during the early stages of embryonic development. Mature RBCs have a life span of approximately 120 days, the majority of which is spent traversing capillary channels of the microcirculation. It is estimated that RBCs travel approximately 300 miles prior to senescence and clearance from the circulation, completing 170,000 circuits through the heart[2] and losing 15-20% of it's hemoglobin content[3]. RBC survival during is attributed to the unique composition of it's membrane and it's ability to rotate around the intracellular contents[4]. RBCs achieve gas transport to and from tissues, but that is not their sole function. RBCs also play an important role in regulation of regional vascular tone, vascular antioxidant systems, immune regulation and self-recognition and the physiologic response to hypoxia on both a regional and whole body level.

RBC Clearance

The estimated normal life span of a mature RBC is 110-120 days[5]. To date, clearance of normal senescent RBCs has not been clearly understood. Two mechanisms have been proposed, clustering of the band 3 (B3) membrane protein[6-9] and externalization of membrane phosphatidyl serine (PS)[10-13], both of these processes may be accelerated in the setting of critical illness, impairing oxygen transport capacity. Oxidatively modified hemoglobin (Hb) forms hemichrome aggregates, which associate with the cytoplasmic domain of the abundant membrane protein B3. Subsequent, clustering of B3 exofascial domains increases affinity of naturally occurring anti-B3 autoantibodies, which activates the complement system – leading to RBC uptake and destruction by macrophages[14]. Normally, PS is asymmetrically distributed in the plasma membrane (a process regulated by flippases). Disruption of this pattern is a well-documented mark of RBC senescence[10-13], signaling RBC removal by the reticulo-endothelial system[13]. Alternatively, RBCs may proceed through a form of ‘stimulated suicide’ similar to apoptosis (termed eryptosis), which is characterized by cell shrinkage and cell membrane scrambling, that is stimulated by Ca^{2+} entry through Ca^{2+}-permeable, PGE_2-activated cation channels, by ceramide, caspases, calpain, complement, hyperosmotic shock, energy depletion, oxidative stress, and deranged activity of several kinases (e.g. AMPK, GK, PAK_2, CK1α, JAK_3, PKC, p38-MAPK). Eryptosis has been described in the setting of ETOH intoxication, malignancy, hepatic failure, diabetes, chronic renal insufficiency, hemolytic uremic syndrome, dehydration, phosphate depletion, fever, sepsis, mycoplasma infection, malaria, iron deficiency, sickle cell anemia, thalassemia, glucose 6-phosphate dehydrogenase deficiency, and Wilson's disease[13, 15, 16].

Capture and Release of Oxygen by RBCs

Hemoglobin (Hb) is formed of 2 α and 2 β polypeptide chains each carrying a heme prosthetic group, comprised of a porphyrin ring bearing a ferrous atom that can reversibly bind an oxygen (O_2) molecule. In the deoxygenated state, the Hb tetramer is electrostatically held in a tense (T) conformation. Binding of the first O_2 molecule leads to mechanical disruption of these bonds, an increase in free energy and transition to the relaxed (R) conformation. Each successive O_2 captured by T-state Hb shifts the Hb tetramer closer to

the R state, which has an estimated 500-fold increase in O_2 affinity[17]. This concept of thermodynamically coupled "cooperativity" in O_2 binding was first described by Bohr[18] and explains the sigmoidal appearance of the O_2-Hb binding curve, also known as the oxy-Hemoglobin dissociation curve (ODC) (Figure 1)[19]. Moreover, understanding of allosteric influence of protein function by 'heterotropic effectors' (e.g. For Hb, O_2, which binds to the 'active' site (heme) is the homotropic ligand and all other molecules influencing the Hb~O_2 binding relationship are termed heterotropic effectors.) was first achieved following description of the variation in Hb~O_2 affinity[20]. In addition to the homotropic effects of ligand binding on quaternary conformational changes (e.g. cooperativity), primary ligand binding affinity (O_2) is also affected by multiple heterotropic effectors of significant physiologic relevance. The major heterotropic effectors that influence Hb O_2 affinity are hydrogen ion (H^+), chloride ion (Cl^-), carbon dioxide ($CO_{2)}$ and 2,3-diphosphoglycerate (DPG)[17].

P_{50}, the oxygen tension at which 50% of Hb binding sites are saturated, is used as a standard means to quantify change in Hb~O_2 affinity and is inversely related to the binding affinity of Hb for O_2[19]. Elevated levels of H^+, Cl^- and CO_2 reduce O_2 binding affinity (e.g. raise P_{50}). This allosteric shift in O_2 affinity, called the Bohr effect[21], arises from the interactions among the above heterotropic effectors bound to different sites on hemoglobin – all of which serve to stabilize the low energy, low affinity, T-state Hb conformation[22]. This effect is achieved by complex interactions amongst carbonic anhydrase (CA) and the B3 membrane protein (also known as anion exchange protein 1, AE1). Specifically, CA generates H^+ and HCO_3^- from CO_2 encountered in the microcirculation; HCO_3^- then exchanges for Cl^- across the RBC membrane through AE1. As a consequence, extra erythrocytic CO_2 is converted into intra-erythrocytic HCl by the CA-AE1 complex, thus acidifying RBC cytoplasm and raising p50 (lowering affinity, also termed 'right' shifting the ODC). Additionally, through the Haldane effect, CO_2 more directly lowers O_2 affinity (by binding to the N-terminus of the globin chains to form a carbamino, further stabilizing T-state Hb); carbamino formation also releases another hydrogen ion (further reinforcing the 'right shift' in ODC)[17] (Figure 2)[23]. This set of reactions is reversed in the alkaline (and low CO_2) milieu in the pulmonary circulation, leading to increased Hb~O_2 binding affinity (lower P_{50}). In sum, this physiology vastly improves O_2 transport efficiency by enhancing gas capture in the lung and release to tissue – and does so in proportion to perfusion sufficiency (in the setting of perfusion lack, acidosis and hypercpanea improve O_2 release). Of note, this tightly regulated modulation of O_2 affinity may become impaired in the setting of critical illness[24-27] and may, in part explain the dysoxia commonly observed in this setting.

Less acute modulation of P_{50} is achieved by DPG, a glycolytic intermediate that binds in an electrically charged pocket between the β chains of hemoglobin, which stabilizes the T conformation, decreasing O_2 affinity and elevating P_{50}. DPG binding also releases protons, lowering intracellular pH and further reinforcing the Bohr effect. DPG in RBCs increases whenever O_2 availability is diminished (as in hypoxia or anemia) or when glycolytic flux is stimulated[28]. Lastly, temperature significantly influences Hb~O_2 affinity. As body temperature increases, affinity lessens (P_{50} increases, ODC shifts right); the reverse happens in hypothermia. This feature is of physiological importance during heavy exercise, fever or

induced hypothermia. It should be noted that clinical co-oximetry results and blood gas values are reported at 37°C and not at true *in vivo* temperature and can lead to either under or over estimation of true $HbSO_2$% values and blood O_2 tension[29].

RBC Biophysical factors Influencing tissue perfusion

Hemorheology describes flow and deformation properties of blood and its formed elements (RBCs, WBCs and platelets). Plasma is a newtonian fluid (viscosity is independent of shear rate); its viscosity is closely related to protein content[30] and in critical illness, physiologically significant changes in viscosity may vary with concentration of acute phase reactants[31-33]. Whole blood, however, is considered a non-newtonian suspension (fluidity cannot be described by a single viscosity value)[34]; whole blood fluidity is determined by combined rheological properties of plasma and the cellular components. There is increasing evidence that pathophysiologic variation in hemorheology is a major determinant of tissue perfusion and as such, of O_2 delivery by RBCs[35].

The cellular components of blood, particularly RBCs, influence blood viscosity as a function of both number and deformability. RBC concentration in plasma (hematocrit) has an exponential relationship with viscosity and meaningfully diminishing tissue perfusion when Hct exceeds ~ 60-65[36]. RBC deformability, or behavior under shear stress, also strongly influences blood fluidity. Normal RBCs behave like fluid drops under most conditions, are highly deformable under shear and orient with flow streamlines[37]. However, during inflammatory stress, RBC tend to aggregate into linear arrays like a stack of coins (rouleaux); fibrinogen and other acute phase reactants in plasma stabilize such aggregates, significantly increasing blood viscosity – such a change in viscosity is most impactful upon O_2 delivery during low flow (e.g. low shear) states (such as in critical illness) in the microcirculation[38]. RBC biomechanics and aggregation impact blood viscosity, strongly influencing the volume and distribution of O_2 delivery (again more so, in the low-shear microcirculation, or when vessel tone is abnormal)[34]. This hemorheologic physiology is perturbed by oxidative stress (common in critical illness)[39, 40] and in sepsis[41-46]. This has been attributed to increased intracellular 2,3-DPG concentration[47], intracellular free Ca^{2+} [48] and decreased intra-erythrocytic ATP with subsequent decreased sialic acid content in RBC membranes[49]. Both increased direct contact between RBCs and WBCs and reactive oxygen species released during sepsis have also been shown to alter RBC membrane properties [50, 51].

Blood viscosity and subsequent tissue blood flow is altered in several patho-physiological states. A well-known example is catecholamine discharge, which under acute stressful conditions reduces circulating blood volume and elevates blood pressure. The resultant fluid shift leads to a higher hematocrit and increased plasma protein and overall increase in blood viscosity. Catecholamine discharge also increases the absolute circulating RBC mass secondary to reintroduction of the “reserve” RBC volume from the splanchnic circulation[52].

Under normal conditions, RBC adherence to endothelial cells (EC) is insignificant and RBC deformability permits efficient passage through the microcirculation. Again, under normal

conditions, enhanced EC adherence plays a role removal of senescent RBCs in the spleen. However, during critical illness RBC~endothelial interactions are altered by RBC injuries associated with sepsis[43, 44, 53, 54] and/or oxidative stress[40] (more so, with 'activated' endothelium, as occurs in critical illness)[54-56] and such RBC~endothelial aggregates create a physiologically significant increase in apparent blood viscosity[34]. Moreover, RBC adhesion directly damages endothelium[57-60] and augments leukocyte adhesion[61-64] further impairing apparent viscosity and microcirculatory flow. This phenomenon is commonly appreciated in the pathophysiology of vaso-occlusive crises in sickle cell disease patients [65], malaria[66], diabetic vasculopathy[67], polycythemia vera[68] and central retinal vein thrombosis[69], but may be more widespread than originally appreciated.

Influence of RBC signaling upon tissue perfusion

Normal physiologic regulation of microcirculatory blood flow instantaneously matches O_2 delivery to metabolic demand and is exquisitely responsive to change in O_2 consumption across tissue region and within regions, across time[70]. This programmed physiological response to relative tissue hypoperfusion[71] (e.g. hypoxic vasodilation; HVD) is effected in a fashion which suggests the presence of an O_2 sensor, detecting point-to-point variations in arteriolar O_2 content, and subsequently initiating a signaling mechanism capable of immediate modulation of vascular tone. Stein and Ellsworth[72] originally identified Hb as a potential circulating O_2 sensor, a fact later established *in vivo* following the discovery that $HbSO_2$, rather than plasma or tissue pO_2, directly correlated with blood flow[73]. RBCs were thus identified as vascular control elements that actively coordinate modulation of blood flow to resolve perfusion insufficiency (rather than simple transporters without a role in regulatory signaling): to date, three $HbSO_2$-dependent RBC-derived signaling mechanisms have been proposed: (1) formation and export of S-nitrosthiols, 'catalyzed' by hemoglobin (SNO-Hb hypothesis)[74, 75], (2) reduction of nitrite to NO by deoxygenated Hb (nitrite hypothesis)[76] and (3) hypoxia-responsive release of ATP (ATP hypothesis) [77]. Each (probably non-exclusive) mechanism has been described to play a role in blood flow misdistribution during various pathologic states and this newly appreciated feature of RBC physiology is centrally relevant to understanding tissue dysoxia in the critically ill.

Mechanistic appreciation of the above physiology has been achieved only after a fundamental shift in our understanding of nitric oxide (NO) biology and chemistry and this issue requires some attention here. Since the original identification of NO of as endothelium-derived relaxing factor (EDRF)[78, 79], our understanding of NO-based vascular signaling has advanced immeasurably[80, 81]. The apparent brief lifetime and fate of EDRF was originally explained by facile diffusion of NO "gas" in solution and its rapid terminal reactions (1) in vascular smooth muscle cells with the ferrous heme iron (Fe^{2+}) of soluble guanylate cyclase (sGC), and (2) in the vessel lumen with oxygenated Hb (forming MetHb and nitrate), deoxygenated Hb (forming nitrosyl hemoglobin) and superoxide (forming peroxynitrite)[82]. We have since come to appreciate much broader biological chemistry of endothelial NO[83], a large portion of which we now understand to occur through the covalent binding of NO^+ to cysteine thiols, forming S-nitrosothiols (SNO). SNO signaling follows oxidation of NOS-generated NO radicals to a NO^+ (nitrosonium) equivalent, which can then cascade across thiols in peptides and proteins to regulate protein

function in a tightly regulated fashion by enzymatic trans-S-nitrosylation reactions (akin to phosphorylation[84-86]); thereby preserving NO bioactivity[80, 81] (in fact, Hb was the first described protein to catalyze S-transnitrosylation reactions and it is this function that is essential to regulation of blood flow by RBCs)[87-91]. This far broader signaling repertoire enabled awareness that heme in sGC is not the sole (or even the principal) target of NO generated by endothelium, with a wide array of alternative sGC (cyclic guanosine monophosphate)-independent reactions following endothelial NOS (eNOS) activation[83, 84].

To recognize the central importance of vasoregulation by RBCs, it is essential to appreciate that it is now broadly accepted that (contrary to the original paradigm) endothelium-derived NO plays no direct role in the HVD response that underlies blood flow regulation[73, 92]. In fact, because of substrate (O_2) limitation, NO production by eNOS is most likely attenuated by hypoxia[93, 94]. Moreover, NOS inhibitors do not block the acute change in blood flow coupled to Hb desaturation[95]. However, NO groups captured, transported, processed and subsequently deployed by RBCs do originally arise from eNOS[74] and perhaps other NOS isoforms[96] and/or nitrite[97]. As such eNOS derived NO groups are transported by RBCs to effect HVD at a time and place remote from the original site of NO synthesis, initiating immediate modulation of vascular tone in concert with cues perfusion insufficiency, including hypoxia, hypercarbia, and acidosis[74, 75].

Processing and export of S-nitrosothiols by RBCs

The discovery that Hb could sustain S-nitrosylation (HbSNO)[74], later characterized by both mass spectrometry[98] and X-ray crystallography[99], provided an explanation as to how NO could circumvent terminal reactions with Hb. Rather than acting solely as scavengers of NO (as originally described), this chemistry enables RBCs to conserve NO bioactivity, allowing its transport throughout the circulation (Figure 3)[74, 75, 91]. The formation and export of NO groups by Hb is governed by the transition in Hb conformation that occurs in the course of O_2 loading/unloading during arteriovenous (A-V) transit[74, 75, 100, 101]. In a tightly regulated fashion, Hb captures and binds NO at its ß-hemes and subsequently converts ß-heme NO into Cys-ß93-SNO[102]. The passing of NO between heme and thiol requires heme-redox coupled activation of the NO group, which is controlled by its allosteric transition across the lung[103]. NO group export from Hb occurs when steep O_2 gradients are encountered in the periphery (HVD). This promotes NO transfer to receptor thiols, including those associated with the erythrocytic membrane protein AE-1 (band 3) [104] and extra erythrocytic thiols[90, 105] to form plasma or other cellular SNOs, which are vasoactive at low nM concentrations[74, 75]. Importantly all NO transfers in this process involve NO^+[74, 106], which protects bioactivity from Fe^{2+} heme recapture and/or inactivation (S-nitrosothiols are the only known endogenous NO compounds that retain bioactivity in the presence of Hb[74, 105, 107]). Extensive evidence supports SNO-Hb biology, whereby RBCs exert graded vasodilator and vasoconstrictor responses across the physiological microcirculatory O_2 gradient. RBCs dilate pre-constricted aortic rings at low pO_2 (1% O_2), while constricting at high pO_2 (95% O_2)[75, 100, 107, 108]. The vasodilatory response at low O_2 is enhanced following the addition of NO (or SNO) to RBCs, commensurate with SNO-Hb formation[74, 104, 107, 109]. Additionally, the vasodilatory

response is enhanced in the presence of extra cellular free thiol[107], occurs in the absence of endothelium[106, 107] (which is consistent with *in vivo* observation that HVD is endothelium independent [110]) and transpires in the time frame of normal circulatory transit (is effected over seconds), as confirmed by measurements of A-V gradients in SNO-Hb[74, 90, 100, 108]. Finally, numerous groups have demonstrated that bioactivity of inhaled NO is commensurate with SNO-Hb formation[111-116].

Metabolism of nitrite by RBCs

Nitrite, long viewed as an inactive end product of NO metabolism[117], has recently been identified as another potential store of bioactive NO in the circulation[118]. Rationale for this proposal relies on the reduction of nitrite to (and subsequent export of) NO radical by RBCs. Several RBC nitrite "reductases" have been identified, including Hb[118-120], xanthine oxidoreductase[121], and carbonic anhydrase[122]. Some have suggested that the reduction of nitrite to NO radical by deoxyHb may serve as the RBC derived signaling mechanism regulating HVD[120, 123-125]. However, this hypothesis has two major shortcomings in terms of known NO chemistry/biochemistry and HVD physiology. Firstly, to influence vascular tone, the NO radical produced from nitrite must escape RBCs at low O_2 tension in order to elicit a vasodilatory response. Established experimental evidence, however, unambiguously refutes the possibility of NO escaping RBCs as an authentic radical, especially given the proximity, high concentration, and rapid reaction kinetics ($10^7 M^{-1}s^{-1}$) of authentic NO with deoxyHb. The only plausible reconciliation of this chemistry enabling bioactivity from deoxyHb-catalyzed nitrite reduction, would be that bioactivity instead - derives from heme captured NO ($HbFe^{2+}NO$) being further converted into SNO-Hb[97, 102]. The second shortcoming relates to the fact that the nitrite reductase activity of deoxyHb is in fact symmetrical across the physiological O_2 gradient[125, 126], with maximal activity occurring at Hb p50 (the pO_2 at which Hb is 50% O_2 saturated, which for intra-erythrocytic Hb *in vivo* is ~ 27 mmHg)[124, 125]. This pO_2 does not align with peak HVD response, which also of course, increases in a steadily graded fashion as pO_2 falls in the physiological range from 100mmHg down to approximately 5mmHg ($HbSO_2$ ~ 1-2%) [71, 73]. Instead, ff RBC based vasoactivity were maximal at Hb p50, then blood flow would be diverted away from regions with pO_2 below 27 mmHg (where it would be needed most). Additionally, based upon the symmetry of Hb nitrite reductase activity at the p50, RBCs traversing vascular beds with pO_2 at p25 or p75 (or p10 and p90, etc.) would generate equal NO-based activity [123], where, in fact, gradually progressive (rather than equal) HVD responses are observed.

Adenosine Triphosphate (ATP)

ATP (but not its degradation products ADP, AMP, or adenosine [127]) has long been known to act as an endothelium dependent vasodilator in humans[77], binding to P_2Y purinergic receptors to induce local vascular tone[128, 129] and to influence conducted vasodilation[130, 131] *via* stimulation of vasoactive signals including endothelial NO, prostaglandins, and endothelial-derived hyperpolarization factors (EDHFs).

More recently, RBCs have been identified as potential sources of vascular ATP[77, 132, 133], with release being stimulated by conditions associated with diminished oxygen supply relative to demand; i.e., hypoxia, hypercapnia, and low pH[77, 132, 134]. Despite its seeming potential as a mediator of HVD, RBC derived ATP falls short on a couple of fronts. Firstly, HVD is unaltered by both endothelial denudation and eNOS deletion[106]; however, ATP vasoactivity is endothelial dependent. Secondly, blood levels of ATP rise and fall over minutes, which is not commensurate with the physiologic time scale for the RBC-based HVD response that occurs in the course of A-V transit over seconds. Despite its shortcomings in terms of acting as the primary mediator of HVD, it is more likely that Hb and ATP serve complementary vasoactive roles, in acute local and prolonged systemic hypoxia respectively[106, 135-137].

Conclusion: Blood flow disruption during critical illness by maladaptive RBC-based signaling

Evidence is mounting in support of a causal relationship between acquired RBC dysfunction and a host of perfusion-related morbidities that complicate critical illness[41, 108, 138-152]. Recently, it has been observed that levels of SNO-Hb are altered in several disease states characterized by disordered tissue oxygenation[108, 109, 153-160]. In addition, where examined, RBCs from such patients exhibit impaired vasodilatory capacity[90, 108, 109, 157, 159-161]. These data suggest that altered RBC-derived NO bioactivity may contribute to human pathophysiology. Specifically, alterations in thiol-based RBC NO metabolism have been reported in congestive heart failure[108], diabetes[109, 156], pulmonary hypertension[100, 155] and sickle cell disease[157, 162], all of which are conditions characterized by inflammation, oxidative stress and dysfunctional vascular control. Moreover, known cross-talk between SNO signaling and cellular communication via carbon monoxide[163-165], serotonin[86, 166, 167], prostanoids[168, 169], catecholamines[170-172] and endothelin[173-175] may permit broad dispersal of signals generated by dysfunctional RBCs. Precise understanding of the roles of dysregulated RBC-based NO transport in the spread of vasomotor dysfunction from stressed vascular beds may open novel therapeutic approaches to a range of pathologies.

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Key Points

- RBC dysfunction may contribute to the dysoxia commonly observed in the critically ill, by impairing delivery of RBCs to tissue (altered rheology and adhesion) or by impairing O_2 delivery from perfusing RBCs (altered p50, Bohr and Haldane shifts).
- RBCs are newly appreciated to capture, process, transport and release NO in a tightly regulated fashion that links regional blood flow to metabolic demand in support of O_2 delivery homeostasis.
- Likewise, malfunction of RBC-based control of vasoactive effectors may contribute to disordered perfusion commonly observed in the critically ill.

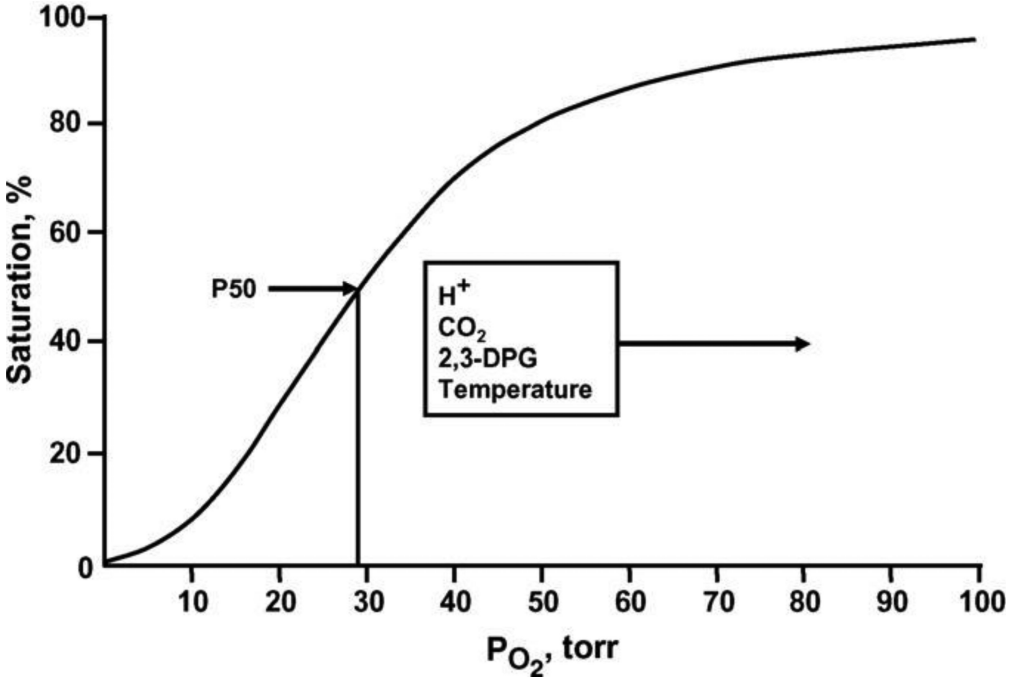

Figure 1.

The normal whole blood oxygen equilibrium curve (OEC)[19]. P_{50} is the pO_2 at which hemoglobin is half-saturated with O_2. The principle effectors that alter the position and shape of the curve under physiological conditions are indicated.

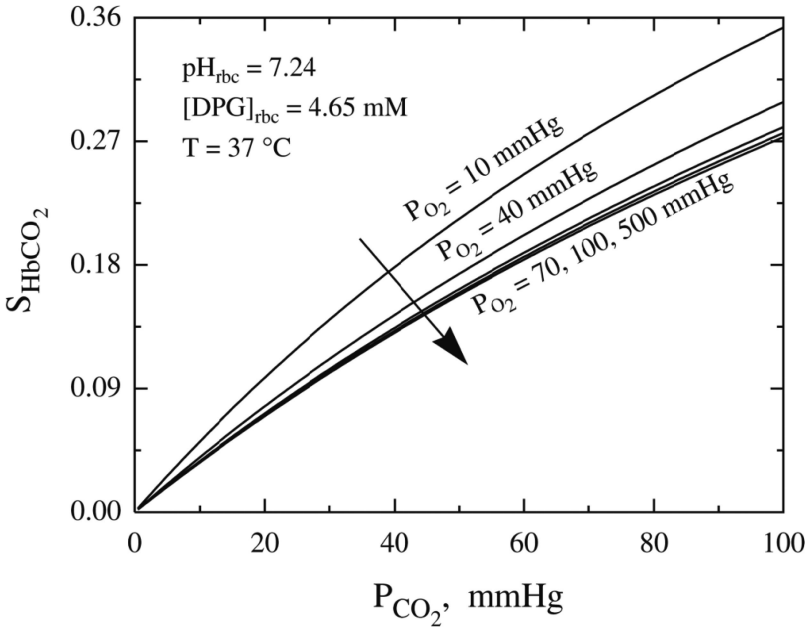

Figure 2.

The quantitative behavior of the Carbaminohemoglobin ($HbCO_2$) dissociation curves at various oxygen tension levels[23].

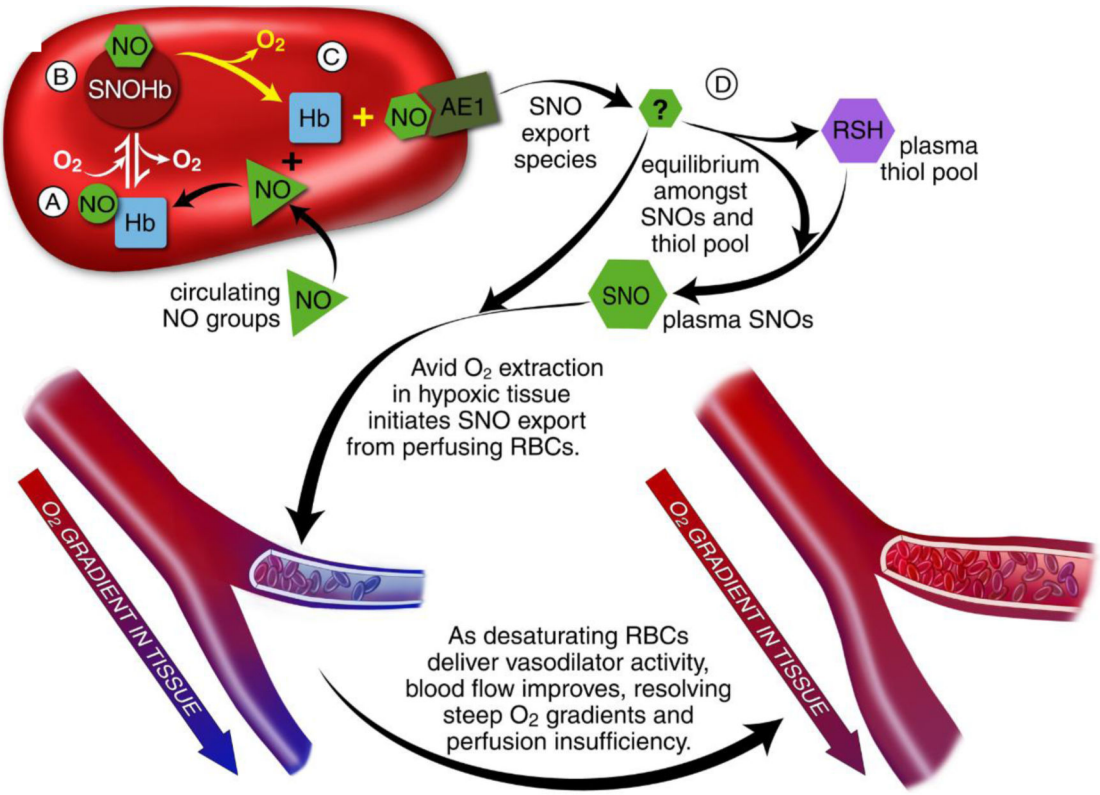

Figure 3.

RBCs transduce regional O_2 gradients in tissue to control NO bioactivity in plasma by trapping or delivering NO groups as a function of HbO_2 saturation[91]. In this fashion, circulating NO groups are processed by Hb into the highly vasoactive (thiol-based) NO congener, S-nitrosothiol (SNO). By exporting SNOs as a function of Hb deoxygenation, RBCs precisely dispense vasodilator activity in direct proportion to regional blood flow lack. Because oxy- and deoxy-Hb process NO differently, allosteric transitions in Hb conformation afford context-responsive (O_2-coupled) control of NO bioavailability, linking the sensor and effector arms of this system. Specifically, Hb conformation governs the equilibria among deoxyHbFeNO (**A**; NO sink), oxySNOHb (**B**; NO store), and acceptor thiols including the membrane protein SNO-AE1 (**C**; bioactive NO source). Direct SNO export from RBCs or S-transnitrosylation from RBCs to plasma thiols (**D**) yields vasoactive SNOs, which influence resistance vessel caliber and close this signaling loop. Thus, RBCs either trap **(A)** or export **(D)** NO groups in response to physiologic cues, linking vessel tone to tissue pO_2 in a fashion that calibrates blood flow to tissue respiration.