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5 Gas Exchange

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I. INTRODUCTION

The process of gas exchange involves the transfer of oxygen (O_2), carbon dioxide (CO_2), and to a lesser extent, ammonia (NH_3), between the environment and the tissue site of use or production. In fishes, the gills are usually the primary interface for the exchange of respiratory gases between the organism and the aquatic environment, although the skin may also contribute to gas transfer in adult fishes and is generally the major site of gas exchange in embryonic and larval fishes.[171] A few fishes display adaptations for air-breathing; while the majority of such species are facultative air-breathers, meeting metabolic O_2 demands from the atmosphere because of a shortage of O_2 in the aquatic environment, a very few species are obligate air breathers which cannot survive if denied access to the atmosphere.[77] The present review, however, will focus on gas exchange across the gills of water-breathing fishes.

Fish gills are designed for the effective transfer of O_2 from water to blood, and CO_2 in the opposite direction. The movement of gases across the respiratory surface is governed by the diffusive properties of the gill epithelium, while the respiratory gases are delivered to or removed from the gill by convective processes; ventilatory water flow on the external side and lamellar blood perfusion internally. Movement of the respiratory gases between the gas exchange organ and the tissue sites of use or production is determined by the gas transport characteristics of the blood, as well as convective (blood flow) and diffusive (between blood and tissue) factors. The manipulation of convective, diffusive, and/or blood gas transport components enables gas exchange to be matched to the metabolic requirements of the fish in the face of variable physiological or environmental conditions.

Respiratory gas exchange in fishes has been the subject of a number of detailed reviews in recent years. In addition to reviews of general principles,[18,50,78,132,156,157,160] a variety of specific aspects of gas transfer have been examined including gill structure,[62,63,84,130,155] ventilation,[92,140,169] hemoglobin structure and function,[74,100,101,210] CO_2 excretion,[13,120,129] mathematical models of gas transfer,[145-147] and gas transfer during exercise or hypoxia.[79,154,218,226] The objective of the current review is to present an overview of the processes involved in the exchange of respiratory gases between the environment and the tissues. Emphasis will be placed on the manner in which the diffusive, convective, and blood gas transport properties of the gas exchange system are controlled to ensure matching between metabolic requirements and respiratory gas transfer. The present review will also focus on interactions between the movements of O_2 and CO_2, and CO_2 and NH_3. The physiological importance of such interactions has only recently become the focus of significant research effort and therefore remains largely unresolved.

II. BASIC STRUCTURE OF GILLS

Gas exchange in the fish gill occurs across the lamellae, which are flattened plate-like structures extending out from both the upper and lower surfaces of the filament [reviewed in Reference 63]. Large numbers of filaments, in turn, are arranged in rows along the paired gill arches located on either side of the pharynx so that the lamellae form a sieve through

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which the ventilatory water must pass. Water flow through the gill sieve is laminar and parallel to the lamellae. The flow of blood perfusing the lamellae is in the opposite direction to that of the water flow, creating a potentially highly efficient countercurrent system for gas exchange.[146,147] In keeping with their function as the gas exchange unit of the gill, the number of lamellae is correlated with both the size and activity of the fish, and the total area of all lamellae constitutes the total surface area of the gill available for gas transfer.[63]

Individual lamellae consist of two epithelial sheets enclosing the vascular space [reviewed in Reference 84]. The epithelial sheets are held apart by pillar cells and the lateral processes or flanges extending from the pillar cells line the vascular space. Overlying and connected to the pillar cells is a basement membrane, upon which rests the bilayered epithelium, and the bilayered epithelium, basement membrane, and pillar cell flange complex is frequently termed the functional respiratory epithelium. The inner epithelial layer consists of poorly differentiated cells and is separated from the outer layer by an extracellular space. Pavement cells, which are typically 3 to 10 μm in diameter,[84] are the predominant cell type in the outer layer of the lamellar epithelium, generally constituting more than 90% of the epithelium.[130] Chloride cells and mucous cells are also located in the lamellar epithelium. The structure of the mitochondria-rich chloride cells differs between seawater and freshwater fishes, but in both cases this cell type is usually found only at the base of the lamella. Mucous cells secrete a polyanionic glycoprotein mucous which coats the respiratory epithelium.[130] Together with the boundary layer of slow-moving water immediately adjacent to the lamellar surface, the thin mucous layer provides a gill microenvironment in which conditions may differ substantially from those present in the bulk water flow.[150,158,163]

III. TRANSPORT OF RESPIRATORY GASES IN THE BLOOD

The essential function of the gas exchange system is to meet the metabolic requirements of the cells for O_2 and to remove the CO_2 produced by cellular metabolism. This objective is achieved in the first instance by the blood, which removes CO_2 from the respiring tissues to the gas exchange surface and carries O_2 in the opposite direction. The main adaptation of blood for gas transport is the presence of the respiratory pigment hemoglobin (Hb) within the red blood cells (rbcs). Hemoglobin not only increases the O_2 carrying capacity of the blood about 20-fold in comparison to the amount which can be carried as physically dissolved O_2,[212] but through its ability to bind protons also has a profound influence on the carriage of CO_2 in the blood. The central role of hemoglobin in CO_2 transport in the blood of teleost fishes is clearly demonstrated by the observation that the CO_2 content of deoxygenated blood is greater than that of oxygenated blood.[74]

Hemoglobin is a tetrameric molecule in most fishes, although agnathans possess monomeric hemoglobin. Tetrameric hemoglobin has two α and two β chains; O_2 binds in a reversible and cooperative fashion to the four heme groups, while H^+ and CO_2 bind to specific amino acid residues in the globin chains. Carbamino CO_2 formation probably contributes little to the transport of CO_2 in fish blood, unlike in mammals,[83] because acetylation of the α-amino groups leaves only the terminal amino groups on the β-chains available to bind CO_2, where it is in direct competition with the binding of organic phosphates[74,120,168] (see below). The cooperativity of Hb-O_2 binding (conventionally characterized by the Hill coefficient, N_{Hill}) can be observed in the sigmoidal shape of the O_2 equilibrium curve ($N_{Hill} > 1$), which describes the relationship between Hb-O_2 saturation and the partial pressure of oxygen, PO_2[74] (Figure 1). Agnathan hemoglobin is exceptional in possessing a hyperbolic O_2 equilibrium curve ($N_{Hill} = 1$), as the cooperativity of O_2 binding in this case is a product of reversible association/dissociation reactions of the monomeric hemoglobin rather than of conformational changes of the tetrameric molecule, as is the situation in other piscine hemoglobins.[101] Allosteric interactions among the O_2, H^+, and CO_2 binding sites result in an interdependence

in the binding of these three ligands.[74,212] For example, the Hb-O_2 binding affinity is decreased by a reduction in pH (the Bohr effect) while an increase in the Hb-O_2 saturation is associated with the release of protons, termed Bohr protons, from hemoglobin (the Haldane effect). Thus, the acidification of the rbc interior associated with CO_2 addition to the blood in the tissues will augment O_2 unloading in the tissues via the Bohr effect, while CO_2 loss at the gills will benefit O_2 loading.[74] At the same time, CO_2 transport will be aided by the Haldane effect (Figure 2).

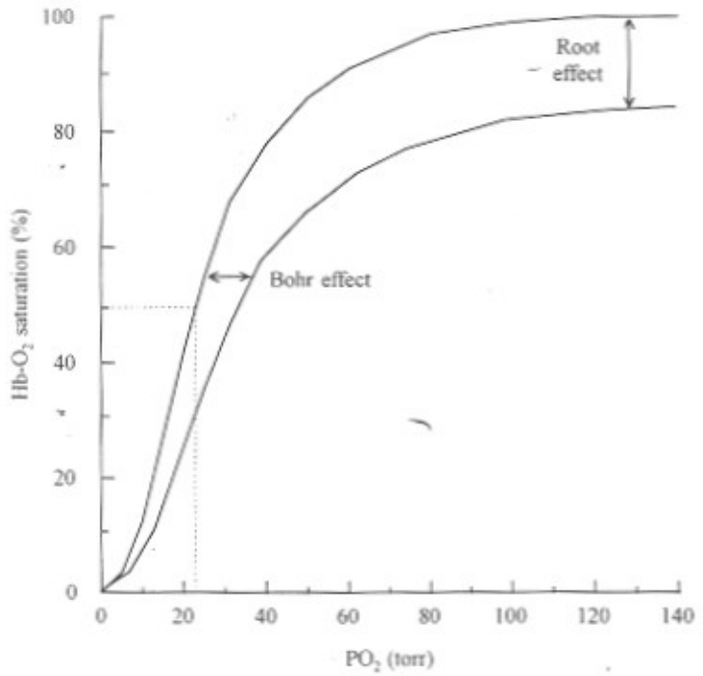

FIGURE 1. A typical sigmoidal O_2 equilibrium curve for tetrameric teleost hemoglobins, with the P_{50}, the PO_2 at which hemoglobin is 50% saturated with O_2, marked by the dotted lines. The Bohr effect describes the influence of rbc intracellular pH on the Hb-O_2 binding affinity, while the impact on Hb-O_2 binding capacity is described by the Root effect. A decrease in rbc intracellular pH increases the P_{50} (lowered affinity) and reduces the Hb-O_2 binding capacity.

The majority of carbon dioxide is transported in the blood as bicarbonate ions (HCO_3^-) in the plasma [reviewed in References 120 and 129]. Molecular CO_2 diffuses out of the tissue and into the rbc where it is hydrated to HCO_3^- and protons, $CO_2 + H_2O \Rightarrow HCO_3^- + H^+$, a reaction catalyzed by the enzyme carbonic anhydrase (CA), which is abundant within the rbc interior (Figure 2). Removal of the products ensures by the law of mass action that the reaction will continue and hence that the gradient for CO_2 diffusion from tissue to blood is maintained; HCO_3^- exits the rbc in exchange for chloride ions[21,75,116] via the band 3 anion exchanger[61,170] located in the rbc membrane (the chloride shift), while H^+ is buffered by hemoglobin.[120] Clearly, then, the increase in Hb-H^+ binding affinity associated with a reduction in Hb-O_2 saturation that is the basis of the Haldane effect will benefit CO_2 transport by augmenting CO_2 uptake by the blood in the tissues. The process is reversed at the gills (Figure 2); molecular CO_2 diffuses out of the blood to the ventilatory water along its partial pressure gradient. The loss of CO_2 drives the HCO_3^- dehydration reaction within the rbc, $HCO_3^- + H^+ \Rightarrow CO_2$, which stimulates HCO_3^- entry into the rbc via the Cl^-/HCO_3^- exchanger.[120] Protons are provided by the buffering action of hemoglobin, with the oxylabile Bohr protons of the Haldane effect contributing a significant component. In fact, rapid oxygenation of rainbow trout blood *in vitro*

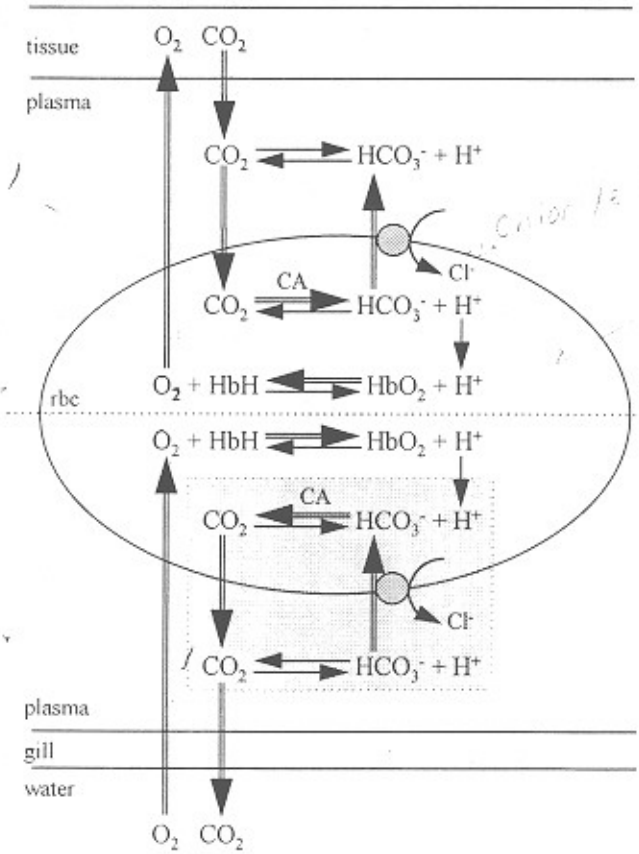

FIGURE 2. A schematic representation of the CO_2 excretion pathway in most fishes. The Jacobs-Stewart cycle is highlighted by the gray box. CA = carbonic anhydrase. See text for further details. (Modified from Perry, S. F., *Can. J. Zool.*, 64, 565, 1986.)

resulted in a 40% elevation of the CO_2 excretion rate relative to that measured at constant oxygenation status,[124] and the magnitude of the oxygenation-linked "boost" was correlated with the extent of the Haldane effect in a comparative study of four fish species.[142] Many fish hemoglobins exhibit a low buffer capacity and a large Haldane effect relative to the hemoglobins of other vertebrates, and this will enhance the importance of oxygenation-linked H^+ to CO_2 excretion.[13]

The contribution of the oxylabile Bohr protons to CO_2 excretion appears to be particularly significant in the lamprey. Unlike in other vertebrates, the permeability of the rbc membrane to HCO_3^- appears to be very low in lamprey, perhaps owing to the absence of a functional Cl^-/HCO_3^- exchanger[105,201,202] [reviewed in References 101 and 199, but see also Reference 20]. Consequently, the majority of CO_2 is transported as HCO_3^- within the erythrocyte;[201] Tufts et al.[200] have calculated that 62% of the difference in total CO_2 content between the arterial and venous blood can be accounted for by changes in the rbc total CO_2 content vs. only 8% in rainbow trout. Effective CO_2 excretion therefore requires that the CO_2 transport capacity of the rbc be maximized and in this regard it is noteworthy that the Haldane effect in lamprey blood is unusually large, enabling large oxygenation-linked changes in rbc total CO_2 content to occur.[35,104] In elasmobranch fishes, on the other hand, the Haldane effect appears to be small or absent[85,142,228] [reviewed in Reference 18], but the very high intrinsic buffering capacity of elasmobranch hemoglobins[73] may compensate for the insignificant Haldane effect.[74] Elasmobranch fishes also differ from teleosts in having CA available to

plasma reactions in the gills,[42,188,228] although the functional significance of this CA activity is not yet clear. In teleost fishes, the absence of plasma-accessible CA activity in the gills[53,54] ensures that the rbc is the sole site of significant HCO_3^- dehydration because the residence time of blood in the gills, 0.5 to 2.5 s,[18,24,64,154] is too short to permit significant HCO_3^- dehydration to occur at the uncatalyzed rate in the plasma.[120] One consequence of the nonavailability of CA activity to plasma reactions in teleost fishes is the presence of an acid-base disequilibrium, detectable as a slow increase in the plasma pH, in the arterial postbranchial blood.[44] It has been argued[86] that teleost fishes lack plasma-accessible CA activity because in its presence protons may be transferred rapidly from the plasma to the rbc interior, whereas in its absence H^+ movement across the rbc membrane is limited by the rate of the uncatalyzed plasma HCO_3^- dehydration reaction, which has a half-time of 25 to 90 s at physiological temperature and pH.[120] The advantage of this strategy to teleost fishes lies in the fact that their hemoglobin exhibits a Root effect, in which the maximal Hb-O_2 carrying capacity is reduced by acidification such that 100% saturation cannot be achieved in acidified blood even in the presence of 100 atm of pure O_2[14] (Figure 1). Thus, rapid H^+ transfer from plasma to rbc could compromise blood O_2 transport in teleost fishes.

The Root effect, which can be considered an exaggerated Bohr effect, is found only in fish hemoglobins, and among fish species its existence is correlated with the presence of a swimbladder and/or a choroid rete.[14,66,168] Thus, for example, the hemoglobin of most elasmobranch fishes, which generally lack both a swimbladder and a choroid rete, does not exhibit a strong Root effect. The generation of a local acidosis in the rete mirabile or choroid rete lowers the Hb-O_2 carrying capacity, driving off O_2 and allowing very high PO_2s to be obtained in the swimbladder or the vitreous humor of the eye.[14,119] However, because protons are normally passively distributed across the rbc membrane according to a Donnan equilibrium, the Root effect may also contribute to a reduction in the arterial blood O_2 content during periods of general internal acidosis. In a number of species, the mobilization of catecholamines into the circulation during such acid-base disturbances, by effectively uncoupling the normal dependence of rbc intracellular pH (pHi) on plasma pH, serves to avoid the Root and Bohr effect-mediated decreases in blood O_2 content which might otherwise occur[204] (see below). An alternative or additional safeguard in some species is the possession of multiple hemoglobins with different functional properties.[168,210,212] Anodic Hb components, which are found in all species, are characterized by relatively low O_2 affinities and pronounced pH sensitivities (i.e., Bohr and Root effects). In some species, including eel, trout, and catfish, cathodic Hb components having high O_2 affinities and low pH dependence are also found and may function to preserve O_2 transport during internal hypoxia and/or acidosis when the anodic Hb is unable to load sufficient O_2.[74,211,212]

In addition to the interrelationships which exist among hemoglobin O_2, CO_2, and H^+ binding, the Hb-O_2 binding affinity is also affected through allosteric interactions by the binding of organic phosphates to hemoglobin [reviewed in References 11, 74, 168, and 212]. The P_{50} (Figure 1) is lowered (i.e., Hb-O_2 binding affinity is increased) by a decrease in the concentration of nucleoside triphosphates ([NTP]) within the rbc. The increase in Hb-O_2 affinity with decreasing [NTP] occurs through direct effects, specifically a reduction in allosteric interactions, but also indirectly through modifications of the fixed charge within the rbc such that the pHi is increased, and hence, by the Bohr effect, so too is the Hb-O_2 binding affinity.[11,212] In salmonid and elasmobranch erythrocytes, adenosine triphosphate (ATP) is the predominant NTP modifier of hemoglobin function, whereas guanosine triphosphate (GTP) appears to dominate in carp, eel, goldfish, and tench.[11,212] When both ATP and GTP are present, experimental evidence indicates that GTP plays the greater modulating role because it is a more potent effector of Hb-O_2 binding affinity and undergoes larger environmentally-mediated changes in intracellular concentration.[211,212] A possible advantage of this strategy is that ATP is freed for more primary commitments related to cellular energy metabolism.[11,211]

A. Control of Blood Gas Transport

The metabolic demands of the tissues for O_2 supply and CO_2 removal are highly variable, depending on the activity level of the fish, and furthermore, large variations may occur in the environmental O_2 and CO_2 tensions encountered. For example, O_2 uptake ($\dot{M}O_2$) may be increased by 12 to 15 times above the resting rate during sustained aerobic exercise in fishes, with 93% of the increased $\dot{M}O_2$ directed towards the working muscles.[154,226] To meet the increased gas exchange requirements imposed by such physical and environmental stresses, a number of strategies are available for the control of blood gas transport. Most of these control strategies appear to be keyed to the tissue requirements for O_2, and may be divided into either quantitative modifications, i.e., adjustment of the blood O_2 carrying capacity, or qualitative modifications, involving modulation of the Hb-O_2 binding affinity or capacity.[74] It should be recognized, however, that owing to the linkage between O_2 and CO_2 transport in the blood, modification of the blood O_2 carrying capacity or Hb-O_2 binding affinity/capacity may in turn have repercussions for blood CO_2 transport.

1. Qualitative Strategies

Modifications of the Hb-O_2 binding affinity or capacity represent qualitative adaptations of the blood gas transport properties, and are generally accomplished by manipulation of the chemical microenvironment of hemoglobin within the rbc. Adjustment of rbc pHi, adjustment of rbc [NTP], or adjustment of rbc volume are the three major mechanisms utilized by fishes to modify the Hb-O_2 binding affinity or capacity.[74] In a number of teleost fishes, all three adjustments of the rbc intracellular environment are achieved through an integrated set of responses of the rbc to the mobilization of catecholamines into the circulation [reviewed in References 93,97,99,100,107,132,140,159,194, and 195].

Circulating levels of the catecholamine hormones, adrenaline and noradrenaline, increase in teleost fishes in response to a variety of physical and environmental stresses which require that O_2 transport be enhanced, such as exhaustive exercise,[17,19,91,136,151] external hypoxia,[10,37,125,133,135,190,196] and external hypercapnia.[125,128,131,193] The proximate stimulus for the release of catecholamines into the circulation from their site of synthesis and storage in the chromaffin cells of the head kidney appears to be a reduction in the blood O_2 content,[133,135] but the actual linkage between the proximate stimulus and the triggering of catecholamine release has not yet been fully elucidated.[159,195] The binding of circulating catecholamines to rbc membrane β-adrenoreceptors, which are coupled to adenylate cyclase, stimulates the formation of cyclic AMP. This second messenger, in turn, initiates a phosphorylation cascade that ultimately activates a unique, cAMP-sensitive Na^+/H^+ antiporter (β-NHE of the Na^+/H^+ exchanger or NHE gene family[8,9,49]) on the rbc membrane such that protons are extruded from the rbc in exchange for plasma Na^+, thereby elevating the intracellular pH while lowering the extracellular pH[2,26,102] (Figure 3) [reviewed in References 100 and 107]. Normally, protons are distributed passively across the rbc membrane according to a Donnan equilibrium, and changes in the extracellular H^+ concentration result in corresponding changes in the rbc pHi [reviewed in References 99, 100, 107, and 195]. The mechanism through which protons are equilibrated passively across the rbc membrane is termed the Jacobs-Stewart cycle (Figure 2): an increase in the extracellular [H^+], for example, drives the dehydration of plasma HCO_3^- to CO_2, which diffuses rapidly across the rbc membrane and is hydrated to H^+ and HCO_3^- in the presence of CA. The resultant protons are largely taken up by intracellular buffers, while HCO_3^- leaves the rbc via the band 3 Cl^-/HCO_3^- exchanger. Stimulation of rbc β-adrenoreceptors effectively uncouples the rbc pHi from its normal dependence on extracellular pH because the rate of proton extrusion from the rbc by the Na^+/H^+ antiporter is greater than the rate of re-equilibration by means of the Jacobs-Stewart cycle, which is limited by the slow speed of the uncatalyzed plasma hydration/dehydration reaction.[94,95] An increase in pHi will clearly

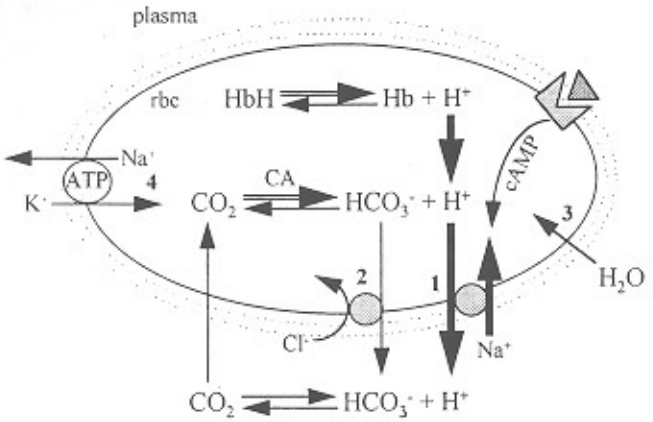

FIGURE 3. A schematic model outlining the effects of catecholamines on the teleost rbc. Stimulation of the β-adrenoreceptor by catecholamines triggers, via cAMP, activation of the rbc membrane Na^+/H^+ antiporter (1) and protons released from hemoglobin or produced by CO_2 hydration are extruded from the rbc. The rise in intracellular [HCO_3^-] resulting from CO_2 hydration stimulates Cl^-/HCO_3^- exchange (2), while the decrease in CO_2 causes a temporary reversal of the normal outwardly directed PCO_2 gradient; both factors may lead to "CO_2 retention". Osmotically obliged water enters the cell (3) following the increase in intracellular ions, leading to cell swelling. The rise in intracellular [Na^+] activates Na^+-K^+ exchange (4), which increases energy consumption and leads to a decrease in intracellular [NTP]. See text for further details. (Modified from Boutilier, R. G. and Ferguson, R. A., *Can J. Zool.*, 67, 2986, 1989 and Thomas, S. and Perry, S. F., *J. Exp. Biol.*, 263, 160, 1992.)

benefit the Hb-O_2 binding affinity and capacity through the Bohr and Root effects, respectively.

Alkalinization of the rbc shifts the equilibrium of the intracellular CO_2-HCO_3^--H^+ reactions towards CO_2 hydration, thereby increasing the intracellular HCO_3^- concentration and stimulating HCO_3^- efflux from the rbc in exchange for Cl^-.[107,195] Thus, Na^+ and Cl^- enter the rbc simultaneously and the accumulation of these osmotically active ions is accompanied by the entry of water, causing an increase in the rbc volume (Figure 3). The dilution of hemoglobin and organic phosphates at a constant Hb:NTP ratio resulting from the rbc swelling reduces Hb-NTP complexing and hence increases the Hb-O_2 binding affinity.[74,107] Further, cell swelling in itself can elevate the rbc pHi owing to the dilution of the fixed negative charges on the impermeable proteins (hemoglobin and NTPs) and a resultant shift in the Donnan distribution of protons across the rbc membrane.[74] Accumulation of Na^+ within the rbc also stimulates the Na^+/K^+ pump and its increased energetic demands may account for the decrease in the rbc intracellular [NTP] observed following adrenergic activation of the Na^+/H^+ antiporter.[11,36,206] The net result of stimulation of rbc β-adrenoreceptors by circulating catecholamines in many teleost fishes, then, is an increase in the Hb-O_2 binding affinity and capacity produced by the combined effects of rbc alkalinization, a decrease in the rbc [NTP], and swelling of the rbcs.

While most studies of rbc adrenergic responses in teleost fishes have focused on the effects of catecholamines in enhancing the Hb-O_2 binding affinity and capacity, some experimental evidence suggests that the function of the rbc in CO_2 excretion may be negatively affected by the adrenergic activation of Na^+/H^+ exchange[226] [reviewed in References 132, 140, 159, 195, and 218]. The massive extrusion of protons from the rbc and consequent shift in the rbc CO_2-HCO_3^--H^+ reactions towards H^+ and HCO_3^- formation by theory would be expected to decrease the intracellular CO_2 tension, which may produce a temporary reversal of the normal outwardly directed PCO_2 gradient between rbc and plasma (Figure 3). For example, Perry and Thomas[139] observed a transient lowering of the extracellular PCO_2 *in vivo*