Control and coordination of gas transfer in fishes

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Recent developments pertaining to the control and coordination of gas transfer in fishes have been reviewed. Gill ventilatory water flow can markedly affect blood respiratory and blood acid–base status. Although arterial oxygen content traditionally has been considered the predominant factor controlling ventilation, we present evidence for additional involvement of both blood acid–base status and circulating catecholamines. An analysis of the independent effects of blood oxygen content, acid–base status, and catecholamines in controlling ventilation is confounded by the interrelationships among these variables. It is likely, however, that each factor is involved to some extent in ventilatory control in fishes. Blood oxygen transport is affected by the carrying capacity of the blood and red blood cell chemical status. Blood oxygen-carrying capacity is increased during periods of stress by adrenergic release of red blood cells from the spleen. Concurrently, adrenergic stimulation of red blood cell Na^+-H^+ exchange, reduction of intracellular nucleoside triphosphates, swelling of red blood cells, and respiratory alkalosis all tend to increase oxygen affinity and capacity of hemoglobin. Results of recent in vivo studies indicate that adrenergic inhibition of plasma bicarbonate dehydration may contribute to the respiratory acidosis after exhaustive exercise in fishes. Evidence is presented to show that hypoxemia, rather than blood acidosis per se, is the proximate stimulus for catecholamine mobilization during periods of stress in fishes.

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Les découvertes récentes reliées au contrôle et à la coordination des échanges gazeux chez les poissons ont fait l'objet d'une révision. Le courant d'eau de ventilation des branchies peut affecter considérablement les conditions respiratoires et l'équilibre acide–base du sang. Bien que la concentration d'oxygène dans le sang artériel ait toujours été considérée comme le principal facteur en cause dans la ventilation, nous apportons ici des preuves du rôle additionnel de l'équilibre acide–base du sang et des catécholamines en circulation. Les rôles respectifs de la concentration d'oxygène dans le sang, de l'équilibre acide–base et des catécholamines dans le contrôle de la ventilation sont difficiles à déterminer à cause de l'interrelation entre ces variables. Il est cependant probable que chacun de ces facteurs joue son propre rôle dans le contrôle de la ventilation chez les poissons. Le transport de l'oxygène dans le sang est affecté par la capacité limite du sang et par la condition chimique des érythrocytes. La capacité en oxygène du sang augmente durant les périodes de stress grâce à la libération adrénergique d'érythrocytes par la rate. En même temps, la stimulation adrénergique des échanges Na^+-H^+ des érythrocytes, la réduction des triphosphates dans les nucléosides intracellulaires, le renflement des érythrocytes et l'alcalose respiratoire sont des phénomènes qui tendent tous à augmenter l'affinité de l'hémoglobine pour l'oxygène et sa capacité limite. Les résultats d'études récentes in vivo indiquent que l'inhibition adrénergique de la déshydratation des bicarbonates plasmatiques peut contribuer à l'acidose respiratoire après un exercice épuisant chez les poissons. Nous démontrons ici que c'est une hypoxémie, plutôt qu'une acidose du sang per se, qui est le stimulus immédiat de la mobilisation des catécholamines durant les périodes de stress chez les poissons.

[Traduit par la revue]

Introduction

Internal respiratory status in fishes is determined by the combined processes of branchial gas transfer and blood gas transport. Branchial gas transfer ultimately reflects the two convective components, ventilatory water flow and lamellar blood perfusion, as well as the diffusive properties of the gill epithelium. Blood gas transport is affected by numerous factors, including blood oxygen-carrying capacity, red blood cell status, cardiac output, and regional blood flow distribution. These various determinants of branchial gas transfer and blood gas transport are modulated by fish to match gas transfer/transport with metabolic requirements or to correct environmentally induced respiratory disturbances.

Various aspects of this broad topic have been reviewed extensively in the past few years (e.g., Randall 1982; Randall and Daxboeck 1984; Wood and Perry 1985; Perry 1986; Nikinmaa 1986; Shelton *et al.* 1986). In the present paper, we focus on recent developments in three particular areas: ventilatory control, blood oxygen transport, and carbon dioxide excretion. Particular emphasis is placed on the roles of the gases themselves, and of circulating catecholamines, in regulating these processes.

Ventilatory control

Ventilation volume ($\dot{V}w$) may vary up to 30-fold in response to changes in environmental gas tensions and internal metabolic demands. In general, these variations are effected by large changes in ventilatory stroke volume and only small changes in breathing frequency, a strategy with obvious energetic advantages when pumping a medium of high density and viscosity, such as water. Interest has centred on three factors as possible ventilatory regulators.

Oxygen

There is abundant evidence that ventilation in fish is primarily keyed to O_2 rather than to CO_2 or pH, in contrast to ventila-

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tion in most air-breathing animals. This undoubtedly reflects the fact that the capacitance of water for O_2 is only about 1/30th of its capacitance for CO_2 (i.e., O_2 is difficult to obtain whereas CO_2 is easy to excrete). The original theoretical prediction of Rahn (1966) that the rates of $\dot{V}$w needed to achieve normal O_2 uptake would lower arterial blood CO_2 tension (Pa_{CO_2}) to very low levels (a few torr), has been confirmed by numerous investigators. However, the fact that the fish gill is hyperventilated with respect to CO_2 excretion does not mean that CO_2 excretion, Pa_{CO_2}, and arterial pH (pH_a) are unaffected by ventilation. Indeed, the fact that this is *not* the case provides some of the strongest evidence in favour of the dominance of the O_2 drive over any CO_2 or pH effects. Thus, during environmental hypoxia, ventilation is stimulated greatly despite resulting decreases in Pa_{CO_2} and elevation of pH_a ("respiratory alkalosis," Dejours 1973; Randall and Jones 1973; Eddy 1974; Soivio *et al.* 1981; Thomas and Hughes 1982*a*, 1982*b*; Thomas 1983; Thomas *et al.* 1986; Fievet *et al.* 1987; Tetens and Christensen 1987; Boutilier *et al.* 1988). Conversely, during environmental hyperoxia, ventilation is inhibited despite resulting increases in Pa_{CO_2} and depression of pH_a ("respiratory acidosis", Randall and Jones 1973; Dejours 1973; Wood and Jackson 1980; Truchot *et al.* 1980; Wilkes *et al.* 1981; Thomas *et al.* 1983; Heisler *et al.* 1988). Even during normoxia, decreases in $\dot{V}$w are associated with increases in Pa_{CO_2} and decreased CO_2 output (Iwama *et al.* 1987). Therefore, ventilatory adjustment of acid–base status, which often has been discounted (e.g., Shelton and Croghan 1988), does occur in fish. Furthermore, it is both rapid (as revealed by the extracorporeal blood loop of Thomas and Hughes 1982*a*, 1982*b*) and powerful, because at the low levels of Pco_2 in fish blood, small changes have large effects on pH_a. This may also be of direct adaptive value, for example in regulating O_2 uptake by increasing blood O_2 affinity/capacity during hypoxia (Lykkeboe and Weber 1978; Tetens and Lykkeboe 1985; Tetens and Christensen 1987) and decreasing it during hyperoxia (Wilkes *et al.* 1981). These immediate effects on red blood cell intracellular pH (RBC pH_i), induced by ventilatory adjustments of Pa_{CO_2}, would be additional to those mediated through catecholamines and intracellular nucleoside triphosphate (NTP) levels (see later).

The identity and location of the receptors for the primary O_2 drive on ventilation remain unknown. However, circumstantial evidence strongly points to blood-based receptors on the arterial side downstream from the gills, possibly in the brain (Jones and Milsom 1982). Eclancher (1972) and Bamford (1974) documented a 5-s delay between hypoxic water first contacting the gills and the start of hyperventilation, and a 7- to 12-s delay after injection of hypoxemic blood into the ventral aorta. This presumably reflects the circulation time for hypoxemic blood to reach the arterial–central receptors. The peripheral O_2 receptors in contact with the external water (Daxboeck and Holeton 1978; Smith and Jones 1978; Milsom and Brill 1986) on the first gill arch appear to be involved in cardiac rather than ventilatory control. Selective hypoxic stimulation of these peripheral receptors does not elicit hyperventilation under general normoxic conditions. Saunders and Sutterlin (1971) demonstrated that hyperventilation still occurred in response to perfusion of hypoxemic blood into the dorsal aorta when the gills were bypassed entirely. However, hyperventilation did occur in response to arterial injections of cyanide (Eclancher and Dejours 1975; Smatresk 1986) or arterial hypoxemia induced by anemia or carbon monoxide under general normoxic conditions (Holeton 1977; Smith and Jones 1982). This suggests that the receptors respond to arterial blood O_2 content (Ca_{O_2}) or delivery rate, rather than to Pa_{O_2} itself. Indeed, Smith and Jones (1982) showed that $\dot{V}$w was essentially a linear function of Ca_{O_2} under a variety of experimental conditions in trout.

Carbon dioxide and (or) pH

Ventilation clearly affects Pa_{CO_2} and internal acid–base status (see above), but is the reverse true? Early studies, which often employed unrealistically high Pco_2 exposures, produced no obvious conclusions (reviewed by Dejours 1973). More recent studies have generally reported increases in $\dot{V}$w associated with Pco_2 elevations within the physiological range (i.e., <10 torr, Dejours 1973; Randall and Jones 1973; Janssen and Randall 1975; Randall *et al.* 1976; Neville 1979; Truchot *et al.* 1980; Thomas and Le Ruz 1982; Smith and Jones 1982; Thomas *et al.* 1983). Several of these have also conclusively eliminated environmental pH changes as the cause of the hyperventilation. The explanation generally offered, however, is that hypercapnic hyperventilation is an indirect effect of hypoxemia mediated through the O_2 receptor system, i.e., Ca_{O_2} is lowered by the Bohr and Root effects associated with respiratory acidosis. The strongest evidence in favour of this conclusion is the demonstration of Smith and Jones (1982) that $\dot{V}$w was directly related to Ca_{O_2} during hypercapnia, hypoxia, and hyperoxic hypercapnia in trout. Thus, a level of environmental hyperoxia sufficient to maintain arterial O_2 content eliminated the hyperventilation caused by hypercapnia. We certainly do not dispute that part of the response to CO_2 must result from hypoxemia. We believe, however, that there now exists sufficient evidence to indicate that CO_2 and (or) pH also can stimulate $\dot{V}$w though mechanisms independent of O_2.

First, in disagreement with Dejours (1973) and Smith and Jones (1982), both Thomas *et al.* (1983) and S.F. Perry and R. Kinkead (unpublished data) have shown that exposure of trout to moderate Pco_2 levels (1–7 torr) (1 torr = 133.3 Pa) during intense hyperoxia resulted in large increases in $\dot{V}$w. Indeed, Smith and Jones (1982) reported that slightly higher levels of Pco_2 (7–15 torr) induced increases in $\dot{V}$w in their trout, which were not completely eliminated by hyperoxia. Truchot *et al.* (1980) have obtained similar results in dogfish, though the extent of hyperventilation was smaller. Heisler *et al.* (1988) have also presented evidence that during hyperoxia, dogfish make fine adjustments in their depressed $\dot{V}$w in response to arterial acid–base status. Second, in the starry flounder, $\dot{V}$w appeared insensitive to large reductions in Ca_{O_2} induced by experimental anemia, but increased as soon as Pa_{CO_2} began to rise and pH_a fell (Wood *et al.* 1979; Wood *et al.* 1982; C. M. Wood, unpublished data; Fig. 1). Third, the Atlantic skate exhibited a long-lasting hyperventilation in response to environmental hypercapnia, even though Pa_{O_2} and Ca_{O_2} remained unchanged (C. M. Wood, M. S. Graham, and J. D. Turner, unpublished data; Fig. 2). Finally, after exhaustive exercise in trout, when Ca_{O_2} was close to normal, the extent of hyperventilation was related to the extent of Pa_{CO_2} elevation and (or) pH_a depression (C. M. Wood and R. S. Munger, unpublished data).

Though all these observations point to a stimulatory role for CO_2 on $\dot{V}$w, none localize the receptors, or determine whether the proximate stimulus is Pco_2 itself or an associated change in pH. In mammals, the primary CO_2 drive on ventilation is mediated mainly through the central chemoreceptive area in

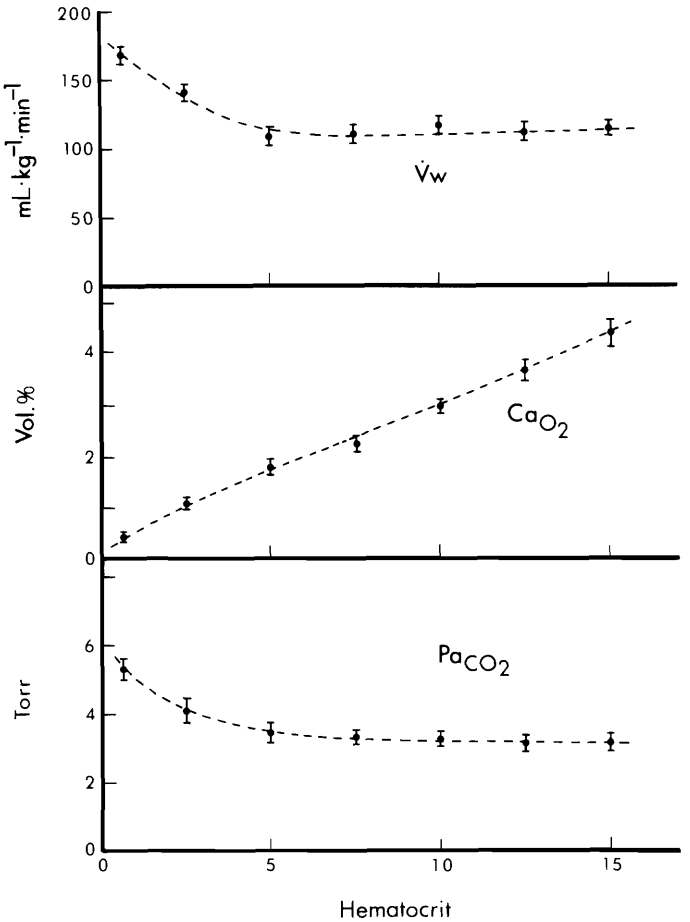

FIG. 1. Changes in ventilation volume ($\dot{V}$w), directly measured, arterial O_2 content (Ca_{O_2}), and arterial CO_2 tension (Pa_{CO_2}) in starry flounder (*Platichthys stellatus*) rendered progressively anemic by removal of red blood cells. Data are shown as mean values ± 1 SEM for each fish ($n = 6-9$) over hematocrit intervals of 2.5%. Note the constancy of $\dot{V}$w over a wide range of Ca_{O_2}, and the simultaneous increase of $\dot{V}$w and Pa_{CO_2} at very low hematocrits. Data from Wood *et al.* (1979, 1982) and C. M. Wood (unpublished).

the medulla, though peripheral chemoreceptors in major arteries are also responsive to changes in both Pa_{CO_2} and pH_a (O'Regan and Majcherczyk 1982; Schlaefke *et al.* 1983). For the central chemoreceptors, physically dissolved CO_2, rather than pH_a, appears to be the important variable, but exerts its effect by crossing the blood–brain barrier and lowering pH in medullary fluids. The proximate stimulus is thought to be the decrease in cerebrospinal fluid (CSF) pH, medullary interstitial pH, medullary intracellular pH (pH_i), or some combination thereof.

In the skate during hypercapnia, CO_2 immediately crossed the blood–brain barrier, driving down both brain pH_i and CSF pH (Figs. 2B, 2C). CSF pH was rapidly and completely regulated, but hyperventilation persisted long after CSF pH returned to control levels (Fig. 2B). Brain pH_i also was rapidly regulated, but not to the same extent (Fig. 2C). $\dot{V}$w appeared best correlated with pH_a, which was adjusted more slowly (Fig. 2A). Pa_{CO_2} itself (or CSF PCO_2 which was in equilibrium) did not appear to be the proximate stimulus because Pa_{CO_2} continued to rise gradually whereas $\dot{V}$w declined (Fig. 2D). N. Heisler and co-workers (personal communication) and C. M. Wood and R. S. Munger (unpublished data) also noted stronger correlations of $\dot{V}$w with pH_a than with Pa_{CO_2} in hyperoxic dogfish and postexercise trout, respectively. However, it must be appreciated that Pa_{CO_2} changes were a major cause of pH_a changes in these studies, and in any event, correlation does not demonstrate causation. By way of contrast, Janssen and Randall (1975) demonstrated that hyperventilation in trout could be provoked by arterial injections of either HCl or $NaHCO_3$; both treatments caused increases in Pa_{CO_2}, but only the former lowered pH_a. A further confounding factor is that many of these investigations showing PCO_2/pH effects on ventilation may have been complicated by the release of catecholamines into the bloodstream, another factor thought to stimulate $\dot{V}$w (see later). We believe that no definite conclusions can be drawn until experiments have been performed manipulating each variable separately, while simultaneously preventing changes in Ca_{O_2} and adrenergic effects. Such experiments will not be easy.

Having argued that there is a CO_2/pH effect on ventilation, we are obligated to answer the pertinent question raised first by Dejours (1973): What is its physiological meaning in a water breather, where hyperventilation cannot protect against external hypercapnia? One answer has been provided by Dejours himself, specifically that hypoxia and hypercapnia often occur simultaneously in natural waters, so the two may simply act together. However, we propose two additional explanations. First, the CO_2/pH control may constrain the extent of hyper- or hypo-ventilation mediated by the primary O_2 control during normocapnic hypoxia and hyperoxia, respectively, so as to avoid unacceptable changes in internal acid–base status. In support of this idea, Heisler *et al.* (1988) have shown that the degree of hypoventilation in hyperoxic dogfish appears to be limited by the extent of acid–base disturbance. Second, we propose that CO_2/pH control plays an important role in driving ventilation after exhaustive exercise, to correct the O_2 debt in the tissues.

Postexhaustion, Ca_{O_2} is almost normal (Primmett *et al.* 1986; Milligan and Wood 1987) and the fish are generally motionless, so neither hypoxemia nor proprioceptive stimulation can be a major influence. Pa_{CO_2} levels, however, are elevated by 1–6 torr in all species that have been examined (see summary Fig. 5 in Wood and Perry 1985), despite the many factors that should favour CO_2 excretion at this time (Perry 1986). We attribute this "CO_2 retention" to a functional inhibition of HCO_3^- dehydration through the RBC, caused by catecholamine mobilization (Fig. 3A; discussed more fully below). The effect is similar to that occurring during treatments that reduce blood carbonic anhydrase activity, such as anemia (Fig. 1) or pharmacological blockade (Haswell and Randall 1978; Swenson and Maren 1987; Henry *et al.* 1988). We hypothesize that CO_2 output is transiently inhibited until the PCO_2 diffusion gradient across the branchial epithelium has risen sufficiently to compensate. Addition of bovine carbonic anhydrase to the circulating blood plasma relieved this limitation so that the postexercise elevation of Pa_{CO_2} was reduced by half (Fig. 3B; C. M. Wood and R. S. Munger, unpublished data). Postexercise $\dot{V}$w similarly was reduced by 50%, supporting the idea that this Pa_{CO_2} elevation and (or) the accompanying acidosis is an important stimulus for hyperventilation at this time.

Catecholamines

Catecholamines are mobilized into the blood plasma in many of the circumstances in which $\dot{V}$w is stimulated, specifically

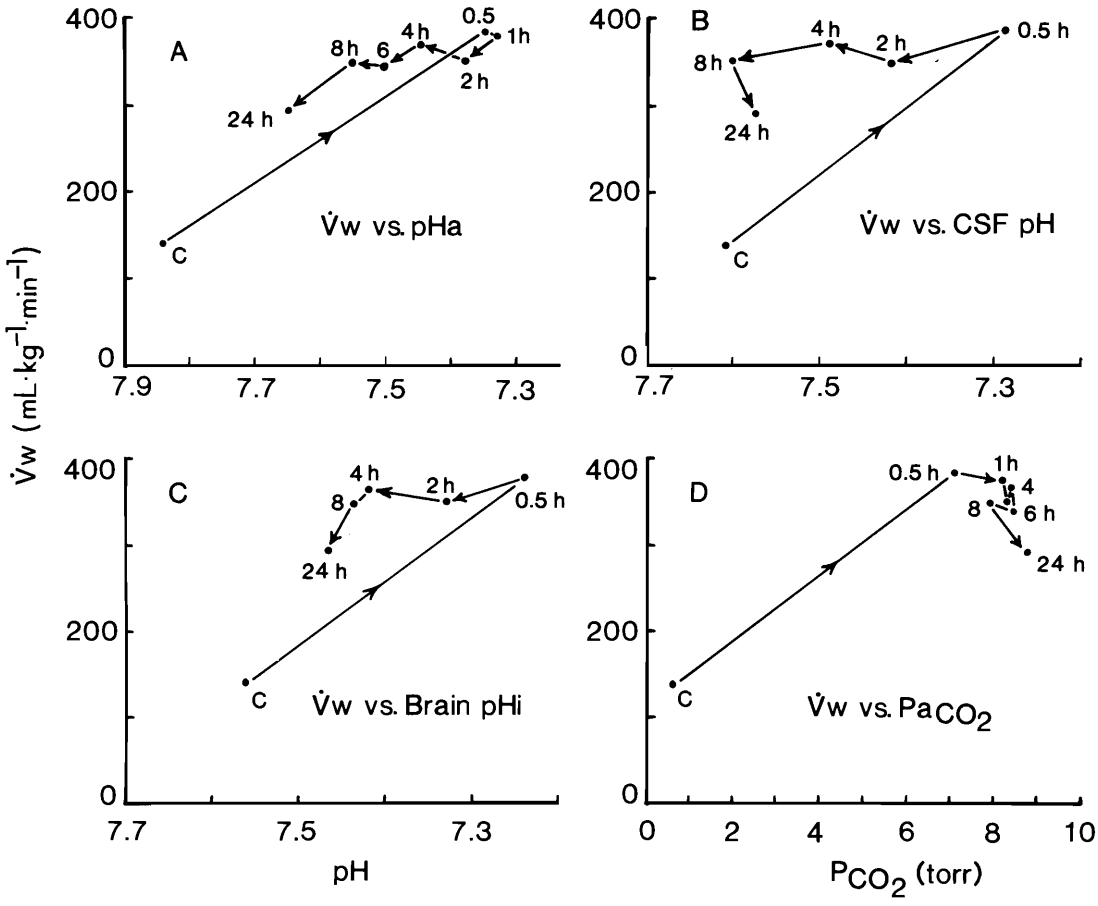

FIG. 2. Relationships between $\dot{V}w$ (directly measured) and (A) arterial pH (pH_a), (B) cerebrospinal fluid pH (CSF pH), (C) brain intracellular pH (pH_i), and (D) arterial CO_2 tension (Pa_{CO_2}) during 24 h of environmental hypercapnia (inspired P_{CO_2} = 7.5 torr) in the Atlantic big skate (*Raja ocellata*). Means (n = 11 for $\dot{V}w$, 5–11 for other parameters) are plotted at each time. (C. M. Wood, M. S. Graham, and J. D. Turner, unpublished data.)

hypoxia (Butler *et al.* 1979; Tetens and Christensen 1987; Fievet *et al.* 1987; Tetens *et al.* 1988; Boutilier *et al.* 1988), anemia (Iwama *et al.* 1987), hypercapnia (Perry 1986; Perry *et al.* 1987), acid infusions (Boutilier *et al.* 1986), and strenuous exercise (Ristori and Laurent 1985; Butler *et al.* 1986; Primmett *et al.* 1986; Milligan and Wood 1987). Exogenously administered catecholamines appear to directly stimulate $\dot{V}w$ via β-adrenoreceptors (the "summer effect"), or inhibit $\dot{V}w$ via α-adrenoreceptors (the "winter effect"), the net response depending on seasonal factors (Peyraud-Waitzenegger *et al.* 1980) in a similar manner to cardiovascular and RBC effects in fish (Part *et al.* 1982; Nikinmaa and Jensen 1986). Though the location of these adrenoreceptors is unknown, it is notable that catecholamines traverse the blood–brain barrier in fish (Peyraud-Waitzenegger *et al.* 1979; Nekvasil and Olson 1986) and so could exert direct effects on respiratory neurones in the medulla. In higher vertebrates, catecholamines also modify the sensitivity of the peripheral chemoreceptors (O'Regan and Majcherczyk 1982).

It is possible, therefore, that many of the responses discussed earlier, and attributed to O_2, CO_2, or pH stimuli, resulted at least partially from mobilized catecholamines. Though there have been few tests of this hypothesis, there are also few data to refute it. It has been demonstrated recently that simultaneous hyperoxia prevented the mobilization of catecholamines which occurred during hypercapnia in trout (S. F. Perry, D. J. Randall, P. Fletcher, and R. Kinkead, unpublished data; see Fig. 5) but did not eliminate the hyperventilation during hypercapnia (S.F. Perry and R. Kinkead, unpublished data). In contrast, D. J. Randall (personal communication) has found that β-adrenoreceptor blockade with propranolol eliminated the hyperventilatory response to acid infusion or hypoxia in the same species. Thus, no clear conclusions can be drawn as yet, and there is an obvious need for further research. Nevertheless, it is difficult to imagine that ventilatory responses to so important an environmental parameter as O_2 could be mediated solely by a humoral mechanism, with its attendant lack of speed. Hyperventilation or hypoventilation occurs about 5 s after the altered inspired P_{O_2} contacts the gills, which is a small fraction of the complete blood circulation time (Eclancher 1972; Bamford 1974). We predict that if adrenergic mechanisms are involved, they are neural and (or) have a modifying rather than a primary influence.

Blood oxygen transport

At any particular partial pressure of oxygen in plasma, the total concentration of O_2 in blood is primarily dependent upon (*i*) intracellular RBC status and (*ii*) blood O_2-carrying capacity.

Red blood cell chemical status

Acidification of the teleost RBC not only decreases the affinity of hemoglobin for O_2, thereby impairing O_2 uptake at the

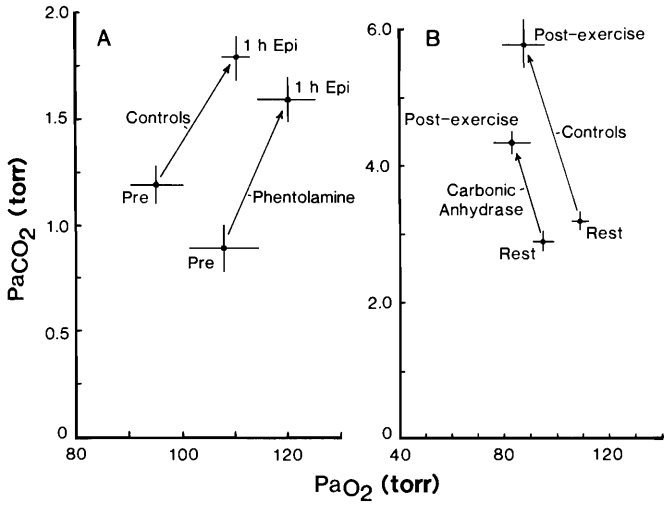

FIG. 3. (A) Changes in simultaneously measured arterial CO_2 (Pa_{CO_2}) and O_2 (Pa_{O_2}) tensions in rainbow trout caused by intravascular infusion of L-epinephrine (Epi) for 1 h (final blood epinephrine concentration = 5×10^{-8} M) under control conditions or after pretreatment with the α-adrenoreceptor antagonist phentolamine (2 mg · kg body weight^{-1}). Note that both Pa_{CO_2} *and* Pa_{O_2} increased in each instance, indicating the the rise in Pa_{CO_2} was not related to branchial diffusive or convective limitations. Values are shown as means ± 1 SEM; $n = 6$ for each group. Data are replotted from Vermette and Perry (1988*a*). (B) Changes in simultaneously measured Pa_{CO_2} and Pa_{O_2} caused by 6 min of exhaustive exercise in rainbow trout. The carbonic anhydrase group ($n = 11$) received an intravascular injection of 10 mg · kg^{-1} bovine carbonic anhydrase (approximately 25 000 Wilbur–Anderson units per kilogram) just before the resting sample. Note the reduction in postexercise Pa_{CO_2} compared with that of controls ($n = 8$), without effect on Pa_{O_2}. (C. M. Wood and R. S. Munger, unpublished data.)

gill (see earlier), but also can depress Ca_{O_2} via the Root effect (Root 1931). Thus, environmental disturbances or metabolic adjustments that promote blood acidosis are normally accompanied by hypoxemia. It is apparent, however, that several fish species are capable of preferentially regulating RBC pH_i during extracellular acidosis (i.e., at any given value of extracellular pH, RBC pH_i is elevated above that predicted from the in vitro relationship between RBC pH_i and extracellular pH), thereby reducing the extent of the blood hypoxemia. Preferential regulation of RBC pH_i has been observed during or after a variety of experimentally induced stresses, including exhaustive exercise in striped bass (Nikinmaa *et al.* 1984) and rainbow trout (Primmett *et al.* 1986; Milligan and Wood 1987), environmental hypercapnia in trout (Perry *et al.* 1987; Vermette and Perry 1988*b*), combined hypercapnia–hypoxia in tench (Jensen and Weber 1985*b*), hypoxia in trout (Fievet *et al.* 1987; Tetens and Christensen 1987), and intravascular acid infusion in trout (Boutilier *et al.* 1986). On the other hand, more sluggish species such as the starry flounder (Milligan and Wood 1987; Wood and Milligan 1987) and American eel (Hyde *et al.* 1987) display diminished capacity for RBC pH_i regulation during periods of extracellular acidosis. The results of in vivo studies that have incorporated catecholamine measurements and (or) employed selective adrenoreceptor blockade during extracellular acidosis (e.g., Nikinmaa *et al.* 1984; Primmett *et al.* 1986; Boutilier *et al.* 1986; Perry and Vermette 1987; Vermette and Perry 1988*a*, 1988*b*; Fievet *et al.* 1987; Milligan and Wood 1987; Wood and Milligan 1987) demonstrate that regulation of RBC pH_i is a β-adrenergic event. An analysis of in vitro studies (Nikinmaa 1982; Cossins and Richardson 1985; Baroin *et al.* 1984; Perry and Vermette 1987) reveals that the mechanism of adrenergic RBC pH_i regulation involves β-receptor mediated stimulation of an amiloride-sensitive Na^+-H^+ antiporter (Borgese *et al.* 1987; Nikinmaa *et al.* 1987) on the RBC membrane (see review by Nikinmaa 1986). Attempts to characterize the RBC β-receptor by using pharmacological techniques have produced conflicting results. Bennett and Rankin (1985) provided evidence that the receptor in trout RBCs is not of the β_1 subtype, based on the greater potency of epinephrine than norepinephrine. Conversely, Tetens *et al.* (1988) demonstrated that norepinephrine was more potent than epinephrine in trout RBCs, and therefore concluded that most red cell β-adrenergic responses in vivo are mediated exclusively by norepinephrine (i.e., the β_1 subtype).

Adrenergic regulation of RBC pH_i apparently is affected by seasonality. The capacity for in vivo RBC pH_i regulation is drastically reduced or abolished completely in trout during winter (Nikinmaa and Jensen 1986; van Dijk and Wood 1988). This phenomenon, though puzzling, is consistent with the seasonal changes in the ventilatory and cardiovascular responses to catecholamines (see earlier). The reduced ability to regulate adrenergic RBC pH_i during winter may be restricted to intact animals; Tetens *et al.* (1988) were unable to demonstrate seasonal differences in RBCs of trout, assessed in vitro.

Factors other than adrenergic stimulation of RBC Na^+-H^+ exchange can contribute to elevation of intracellular pH during periods of extracellular acidosis or hypoxemia. Jensen (1986) demonstrated a pronounced inverse relationship between hemoglobin oxygen ($Hb-O_2$) saturation and RBC pH_i in tench blood over the normal physiological range of blood O_2 saturation. The large effect of $Hb-O_2$ saturation on RBC pH_i in tench compared with mammalian blood presumably reflects the pronounced Haldane effect in teleost fish blood in conjunction with lower intracellular buffering capacity. $Hb-O_2$ saturation not only affects RBC pH_i, but also affects the adrenergic responsiveness of RBCs. Motais *et al.* (1987) have shown that the sensitivity of the Na^+-H^+ antiporter to β-adrenergic stimulation is inversely proportional to $Hb-O_2$ saturation. Although the mechanism is unclear (Motais *et al.* 1987), this phenomenon would allow a greater ability to raise RBC pH_i during hypoxic conditions, when there is an urgent need to increase $Hb-O_2$ affinity/capacity. Although the Haldane effect likely is the major contributing factor raising RBC pH_i when $Hb-O_2$ is depressed, reductions in intracellular NTP levels may also increase RBC pH_i. NTP levels decrease in fish blood upon deoxygenation in vivo (Wood *et al.* 1975; Lykkeboe and Weber 1978; Soivio *et al.* 1980; Jensen and Weber 1982, 1985*a*; Tetens and Lykkeboe 1985; Boutilier *et al.* 1988) or in vitro (Tetens and Lykkeboe 1981; Milligan and Wood 1987). In accordance with the passive Donnan distribution of H^+ ions across the fish RBC membrane (Albers and Goetz 1985; Heming *et al.* 1986), reduced intracellular levels of the negatively charged impermeable organic phosphates will elevate RBC pH_i. Similarly, RBC swelling, induced actively by adrenergic stimulation of Na^+-H^+ exchange (see Nikinmaa 1986) or passively by acidification (Nikinmaa *et al.* 1987), can further raise RBC pH_i as fixed negative charges on Hb and organic phosphates are diluted, causing a shift in the Donnan ration for H^+ ions. The combined effects of Hb deoxygenation, reduced NTP levels, and swelling may explain the regulation of RBC pH in tench exposed to hypoxia–hypercapnia

(Jensen and Weber 1985*b*) because RBCs of this species apparently are insensitive to catecholamines (Jensen 1987).

Nucleoside triphosphates are negative allosteric modifiers of hemoglobin O_2 affinity (Wood *et al.* 1975). Therefore, the RBC intracellular concentration of NTPs (more specifically the NTP:Hb ratio) can markedly affect blood O_2 content. NTP/Hb levels are reduced during periods of hypoxia (see above), after exhaustive exercise (Milligan and Wood 1987), and during chronic metabolic acidosis (Walker *et al.* 1989). Elevations in plasma catecholamines occur in all three conditions, so the decrease in NTP/Hb may result from both diminished oxidative metabolism (due to lack of O_2) and direct adrenergic effects. Milligan and Wood (1987) reported that application of stress levels of catecholamines (total [epinephrine + norepinephrine] = 92 nmol) to red cells in vitro abolished the CO_2-induced Bohr and Root effects in trout but not in flounder. In these experiments, catecholamines significantly depressed NTP levels in trout blood only, reaffirming the insensitivity of flounder RBCs to adrenergic stimulation. Similar effects on the in vitro O_2 dissociation curve of trout blood, using much higher levels of epinephrine (5×10^{-6} M), were reported earlier by Nikinmaa (1983).

The decline in NTP/Hb caused by catecholamines is abolished by the β-adrenoreceptor antagonist propranolol, and is thought to involve metabolic degradation of intracellular NTP stores (Nikinmaa 1986). In white sucker subjected to metabolic acidosis due to saline exposure, this decrease in NTP/Hb levels was associated with an increase in O_2 affinity which was independent of RBC pH_i (Walker *et al.* 1989). Conversely, during the first few minutes of acute hypoxia exposure in trout, Tetens and Christensen (1987) observed an increased Hb-O_2 affinity associated with β-adrenergic pH_i regulation, but in the absence of any changes in RBC organic phosphate levels. Thus the catecholamine-induced reduction in NTP/Hb levels and the catecholamine-induced stimulation of Na^+-H^+ exchange, separately or in combination, are two important mechanisms that can increase the affinity of Hb for O_2 during periods of hypoxia or extracellular acidosis. These mechanisms are complemented by the nearly instantaneous respiratory alkalosis accompanying hyperventilation (see earlier). The theoretical analysis of Malte and Weber (1987) demonstrates the energetic importance of all these factors, for it shows that increased blood O_2 affinity plays a major role in reducing the ventilatory requirement for branchial O_2 uptake.

Blood oxygen-carrying capacity

The circulating levels of hemoglobin in blood can be increased during exercise or environmental disturbance by several strategies, including hemoconcentration (Wood and Randall 1973; Milligan and Wood 1986; see review by Wood and Perry 1985) and recruitment of RBCs from the spleen (Milligan and Wood 1982; Yamamoto *et al.* 1980, 1985; Yamamoto 1987, 1988). Vermette and Perry (1988*a*) demonstrated that intravascular epinephrine infusion caused an α-receptor mediated rise in blood hemoglobin levels. These results are consistent with the study of Nilsson and Grove (1974), showing α-adrenergic contraction of the spleen, and a more recent study (S. F. Perry, D. J. Randall, P. Fletcher and R. Kinkead, unpublished data) demonstrating significant decreases in spleen weight and hemoglobin content after injection of epinephrine in trout. Predictably, the adrenergic elevation of blood hemoglobin was abolished in splenectomized fish.

The α-adrenergic release of RBCs from the spleen can occur at low levels of epinephrine and appears to be dose dependent, as illustrated in Fig. 4. The adrenergic recruitment of RBCs and the concomitant rise in blood O_2-carrying capacity during periods of hypoxemia (induced by exercise, acidosis, hypoxia) is a dominant mechanism regulating Ca_{O_2}, especially in fish species lacking adrenergic control of RBC pH_i (tench, flounder, eel) or during winter, when fish lose the capacity for β-adrenergic RBC pH_i regulation. Vermette and Perry (1988*b*) have suggested that an α-adrenoreceptor mediated increase in blood O_2-carrying capacity may be the most significant response contributing to regulation of Ca_{O_2} during external hypercapnia in trout, because α-receptor blockade produced a significantly greater reduction in Ca_{O_2} than did β-receptor blockade.

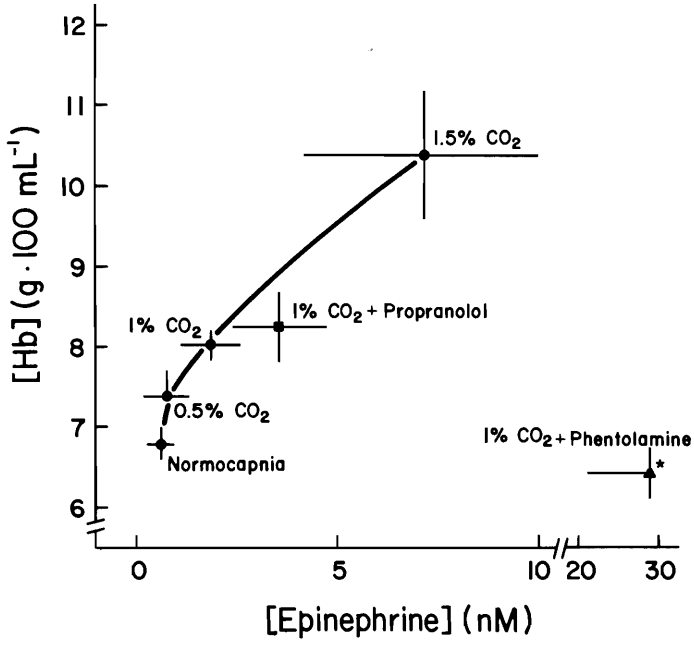

FIG. 4. Relationship between blood epinephrine levels and hemoglobin concentration in rainbow trout during progressive increases in inspired CO_2 tension (normocapnia to 1.5% CO_2). Each value represents data (± 1 SEM) from a separate group of fish ($n = 5-12$). Note that pretreatment of fish with the α-adrenoreceptor antagonist phentolamine abolished the increase in [Hb], despite the fact that epinephrine levels were elevated to the greatest extent. (S. F. Perry, D. J. Randall, P. Fletcher, and R. Kinkead, unpublished data.)

Carbon dioxide excretion

The transport of CO_2 in fish blood has been recently reviewed (Perry 1986). The prevailing model for CO_2 transfer involves the catalyzed dehydration of plasma bicarbonate (HCO_3^-) within the RBC to form physically dissolved CO_2, and the subsequent diffusion of physically dissolved CO_2 across the gill epithelium. It is likely that the process of plasma HCO_3^- conversion to dissolved CO_2, rather than branchial CO_2 diffusion or blood/water convection, is the rate-limiting step in CO_2 excretion. It has been suggested (Wood and Perry 1985; Perry 1986), on the basis of in vitro studies (T. Heming and S. F. Perry, unpublished data), that elevated epinephrine levels may impair CO_2 excretion by reducing the rate of plasma HCO_3^- dehydration. As proposed by Wood and Perry (1985), adrenergic inhibition of RBC HCO_3^- dehydration may be the basis for the consistently observed respiratory acidosis after

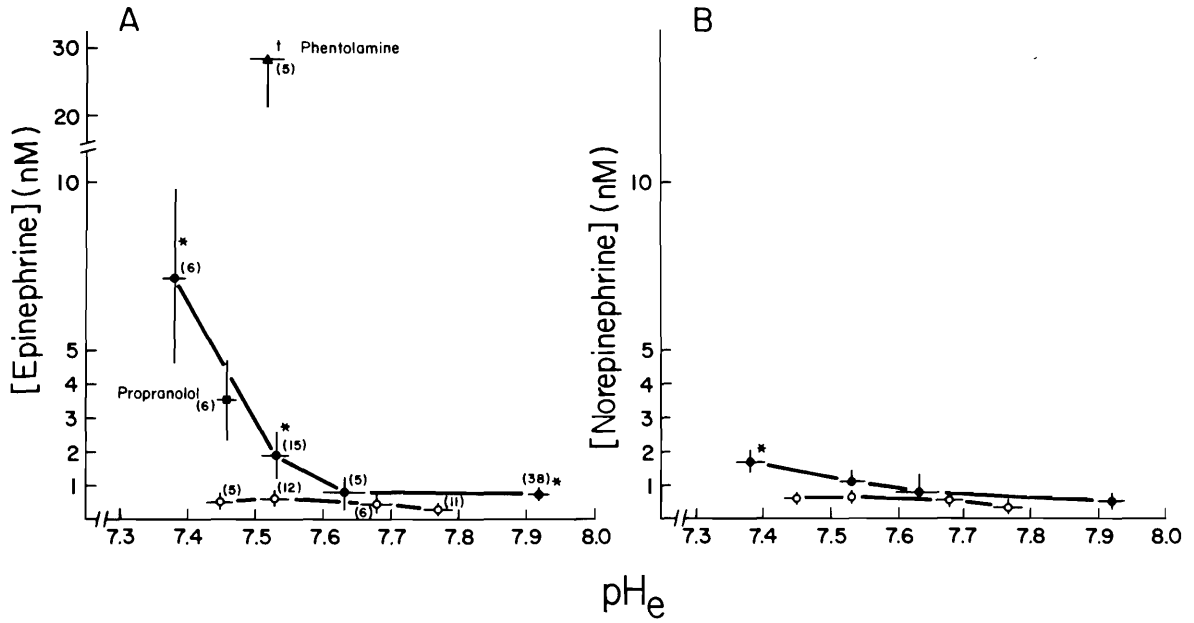

FIG. 5. Relationship between whole blood pH (pH_e) and circulating levels of (A) epinephrine and (B) norepinephrine in rainbow trout during progressive increases in inspired CO_2 tension (P_{CO_2}, see Fig. 4) under normoxic (●—●) or hyperoxic (○—○) (inspired P_{O_2} = 500–600 torr) conditions. Values are shown as means ± 1 SEM; numbers in parentheses are sample sizes. *, significantly different ($P < 0.05$, unpaired t-test) from corresponding value of inspired P_{CO_2} in the hyperoxic group; †, significantly different from the corresponding value of inspired P_{CO_2} in the normoxic group. (S. F. Perry, D. J. Randall, P. Fletcher, and R. Kinkead, unpublished data.)

exhaustive exercise in fish, a period when catecholamines are elevated (Ristori and Laurent 1985; Primmett *et al.* 1986; Butler *et al.* 1986; Milligan and Wood 1987). The transient postexercise increase in $P_{a_{CO_2}}$ cannot be explained by branchial diffusive or convective limitations because $P_{a_{O_2}}$ is either unchanged or actually elevated (see Wood and Perry 1985). Indeed, the higher catecholamine levels after exercise probably enhance branchial gas diffusive conductance (Pettersson 1983; Perry *et al.* 1985). Recently, C. M. Wood and R. S. Munger (unpublished data) have demonstrated that the typical postexercise respiratory acidosis in trout is significantly reduced by pretreatment of fish with carbonic anhydrase; $P_{a_{O_2}}$ is unaffected by this treatment (Fig. 3B) These results strongly suggest that RBC carbonic anhydrase is inaccessible to dehydrate plasma HCO_3^- after exercise. Moreover, intravascular infusion of epinephrine or the β-adrenoreceptor agonist isoproterenol induces a similar state of respiratory acidosis in trout (Perry and Vermette 1987; Vermette and Perry 1988*a*) even while raising $P_{a_{O_2}}$ (Fig. 3A). The epinephrine-induced hypercapnia is not abolished by pretreating fish with the α-adrenoreceptor antagonist phentolamine, (Fig. 3A), although gill gas diffusive and ventilatory conductances clearly are enhanced. These results strongly support the hypothesis that β-adrenergic inhibition of plasma HCO_3^- dehydration induces a transient condition of respiratory acidosis. The physiological significance of the adrenergic respiratory acidosis may lie in the stimulation of ventilation (see earlier). Because of simultaneous RBC pH_i and NTP/Hb regulation (see earlier), there is no adverse effect on blood O_2 transport.

Actual measurements of CO_2 excretion after burst exercise or following epinephrine injection into resting fish have failed to demonstrate a reduction of CO_2 excretion under these conditions (Steffensen *et al.* 1987). It is likely, however, that inhibition of plasma HCO_3^- dehydration would cause only a transient reduction of CO_2 excretion as P_{CO_2} levels rise in the blood until a new steady state is achieved. The methodology employed by Steffensen *et al.* (1987) may not have allowed detection of the putative transient inhibition of CO_2 excretion. Clearly, the idea of adrenergic control of CO_2 excretion in fishes remains controversial and warrants further research.

The involvement of catecholamines in the control of gas transfer

Throughout this paper we have emphasized the involvement of O_2, CO_2, and circulating catecholamines in regulating branchial gas transfer and blood gas transport. Summaries of resting and stress-related levels of catecholamines in fish plasma have been presented by several groups (Milligan and Wood 1987; Vermette and Perry 1988*a*; Tetens *et al.* 1988). An analysis of the physiological state of the fish in these instances reveals a common feature, namely blood acidosis/hypoxemia at times of catecholamine elevation. The hypoxemia can be primary in origin (e.g., during exposure to external hypoxia) or secondary to Bohr and Root effects. Thus, both acidosis and hypoxemia are possible stimuli for the mobilization of catecholamines from chromaffin tissue. Blood catecholamine levels have been monitored during normoxic or hyperoxic hypercapnia in an attempt to elucidate the stimulus for catecholamine mobilization (S. F. Perry, D. J. Randall, P. Fletcher, and R. Kinkead, unpublished data). The results of this study (Fig. 5) demonstrate that hypoxemia, not blood acidosis per se, is the factor promoting the release of catecholamines. It is noteworthy that significant adrenergic effects, including RBC pH_i regulation and elevation of blood [Hb] were observed at epinephrine levels below 5 nmol (see Fig. 4). It is apparent, therefore, that even slight elevations of circulating catecholamine levels in vivo can profoundly affect $C_{a_{O_2}}$. In Fig. 6 we summarize the various factors under adrenergic control that serve to regulate blood O_2 content after hypoxemia-mediated

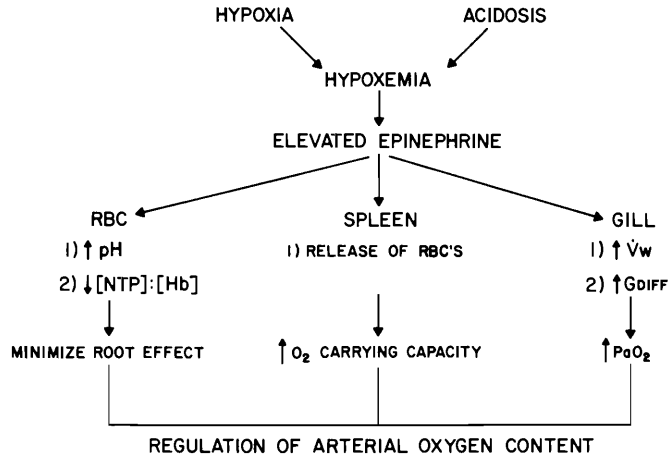

FIG. 6. Model summarizing the series of physiological events regulating arterial oxygen content after mobilization of epinephrine into the bloodstream. At the gill, ventilation volume ($\dot{V}w$) and diffusive conductance (G_{diff}) are increased, causing a rise in arterial O_2 tension (Pa_{O_2}). At the spleen, red blood cells are released, causing an increase in O_2-carrying capacity. At the RBC, intracellular pH is elevated and the nucleoside triphosphate to hemoglobin ratio (NTP:Hb) is reduced, increasing Hb-O_2 affinity/capacity and minimizing Bohr and Root effects induced by extracellular acidosis.

release of catecholamines. At the gill, ventilation and diffusive conductance are increased, thereby elevating Pa_{O_2}. Concurrently, RBCs are released from the spleen as a result of α-adrenergic contraction and cause an increase in blood O_2-carrying capacity. At the red blood cell, stimulation of β-adrenoreceptors causes an elevation of intracellular pH and a reduction in NTP/Hb levels. These changes are important to enhance O_2 loading at the gills and minimize Root effects. Thus, the catecholamine mobilization plays a critical role in regulating arterial O_2 content in many fishes during periods of hypoxemic stress. A different situation may exist in elasmobranch fishes (Metcalfe and Butler 1988) which lack a Root effect.

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