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 nucleotide 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.


Les découvertes récentes ont pour objet un contrôle de la coordination des échanges gazeux chez les poissons. Le courant d’eau de ventilation des branchies peut affecter considérablement les conditions respiratoires et l’équilibre acide 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 de l’influence de l’équilibre acide du sang et des catécholamines circulantes. Les rôles respectifs de la concentration d’oxygène dans le sang, de l’équilibre acide 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 un rôle dans le contrôle de la ventilation chez les poissons. Le transport du sodium 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 contribuent tous à augmenter l’affinité de l’hémoglobine pour l’oxygène et sa capacité limite. Les résultats d’études récentes en 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.

[Intaduit 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 lamellate 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 transport 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 (V\textsubscript{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-
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 $V_w$ needed to achieve normal $O_2$ uptake would lower arterial blood $CO_2$ tension ($P_{ACO_2}$) 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, $P_{ACO_2}$, 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 $P_{ACO_2}$ and elevation of pH$_A$ (“respiratory alkalosis,” Dejours 1973; Randall and Jones 1973; Eddy 1974; Soivio et al. 1981; Thomas and Hughes 1982a, 1982b; 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 $P_{ACO_2}$ 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 $V_w$ are associated with increases in $P_{ACO_2}$ 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 1982a, 1982b) 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 $P_{ACO_2}$, 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 hypoxic blood into the ventral aorta. This presumably reflects the circulation time for hypoxic 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 hypoxic 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 ($CaO_2$) or delivery rate, rather than to $P_{AO_2}$ itself. Indeed, Smith and Jones (1982) showed that $V_w$ was essentially a linear function of $CaO_2$ under a variety of experimental conditions in trout.

Carbon dioxide and (or) pH

Ventilation clearly affects $P_{ACO_2}$ 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 $V_w$ 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., $CaO_2$ 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 $V_w$ was directly related to $CaO_2$ during hypercapnia, hypoxia, and hyperoxic hypercapnia in trout. Thus, a level of environmental hypoxia 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 $V_w$ 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 $P_{CO_2}$ levels (1–7 torr) (1 torr = 133.3 Pa) during intense hyperoxia resulted in large increases in $V_w$. Indeed, Smith and Jones (1982) reported that slightly higher levels of $P_{CO_2}$ (7–15 torr) induced increases in $V_w$ 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 $V_w$ in response to arterial acid—base status. Second, in the starry flounder, $V_w$ appeared insensitive to large reductions in $CaO_2$ induced by experimental anemia, but increased as soon as $P_{ACO_2}$ 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 $P_{AO_2}$ and $CaO_2$ remained unchanged (C. M. Wood, M. S. Graham, and J. D. Turner, unpublished data; Fig. 2). Finally, after exhaustive exercise in trout, when $CaO_2$ was close to normal, the extent of hyperventilation was related to the extent of $P_{ACO_2}$ elevation and (or) pH$_A$ depression (C. M. Wood and R. S. Mungen, unpublished data).

Though all these observations point to a stimulatory role for $CO_2$ on $V_w$, none localize the receptors, or determine whether the proximate stimulus is $P_{ACO_2}$ 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
light pH, medullary intracellular pH (pHi), or some combination thereof.

In the skate during hypercapnia, CO₂ immediately crossed the blood—brain barrier, driving down both brain pH and CSF pH (Figs. 2B, 2C). CSF pH was rapidly and completely regulated, but not to the same extent (Fig. 2C). Vw appeared best correlated with pH₂, which was adjusted more slowly (Fig. 2A). PaCO₂, itself (or CSF PCO₂ which was in equilibrium) did not appear to be the proximate stimulus because PaCO₂ continued to rise gradually whereas Vw 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 Vw with pH₂ than with PaCO₂ in hyperoxic dogfish and postexercise trout, respectively. However, it must be appreciated that PaCO₂ changes were a major cause of pH₂ 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 NaHCO₃; both treatments caused increases in PaCO₂, but only the former lowered pH₂. A further confounding factor is that many of these investigations showing PCO₂/pH effects on ventilation may have been complicated by the release of catecholamines into the bloodstream, another factor thought to stimulate Vw (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 CaO₂ and adrenergic effects. Such experiments will not be easy.

Having argued that there is a CO₂/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 CO₂/pH control may constrain the extent of hyper- or hypo-ventilation mediated by the primary O₂ 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 hyperventilation in hyperoxic dogfish appears to be limited by the extent of acid—base disturbance. Second, we propose that CO₂/pH control plays an important role in driving ventilation after exhaustive exercise, to correct the O₂ debt in the tissues.

Postexhaustion, CaO₂ is almost normal (Primmeth et al. 1986; Milligan and Wood 1987) and the fish are generally motionless, so neither hypoxia nor proprioceptive stimulation can be a major influence. PaCO₂ 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 CO₂ excretion at this time (Perry 1986). We attribute this “CO₂ retention” to a functional inhibition of HC-O⁻₂ 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 CO₂ output is transiently inhibited until the PCO₂ 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 PaCO₂ was reduced by half (Fig. 3B; C. M. Wood and R. S. Munger, unpublished data). Postexercise Vw similarly was reduced by 50%, supporting the idea that this PaCO₂ 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 Vw 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 ($P_{aCO_2}$) 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; Primmel et al. 1986; Milligan and Wood 1987). Exogenously administered catecholamines appear to directly stimulate $\dot{V}_w$ via $\beta$-adrenoreceptors (the “summer effect”), or inhibit $\dot{V}_w$ via $\alpha$-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 Majcherzyk 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 $\beta$-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 neutral 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
The ability to preferentially regulate RBC pH during periods of extracellular acidosis or hypoxemia. Jensen (1986) demonstrated a pronounced inverse relationship between hemoglobin oxygen (Hb-O2) saturation and RBC pH in tench blood over the normal physiological range of blood O2 saturation. The large effect of Hb-O2 saturation on RBC pH in tench compared with mammalian blood presumably reflects the pronounced Haldane effect in teleost fish blood in conjunction with lower intracellular buffering capacity. Hb-O2 saturation not only affects RBC pH, 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-O2 saturation. Although the mechanism is unclear (Motais et al. 1987), this phenomenon would allow a greater ability to raise RBC pH during hypoxic conditions, when there is an urgent need to increase Hb-O2 affinity/capacity. Although the Haldane effect likely is the major contributing factor raising RBC pH when Hb-O2 is depressed, reductions in intracellular NTP levels may also increase RBC pH. 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, 1985a; Tetens and Lykkeboe 1985b; 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. 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 as fixed negative charges on Hb and organic phosphates are diluted, causing a shift in the Donnan ratio 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.
Nucleoside triphosphates are negative allosteric modifiers of hemoglobin O2 affinity (Wood et al. 1975). Therefore, the RBC intracellular concentration of NTPs (more specifically the NTP:Hb ratio) can markedly affect blood O2 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 O2) 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 CO2-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 O2 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 (Nikonmaa 1986). In white sucker subjected to metabolic acidosis due to saline exposure, this decrease in NTP/Hb levels was associated with an increase in O2 affinity which was independent of RBC pH (Walker et al. 1989). Conversely, during the first few minutes of acute hypoxia exposure in trout, Tetens and Christensen (1987) observed an increased Hb-O2 affinity associated with β-adrenergic pH2 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⁺ exchange, separately or in combination, are two important mechanisms that can increase the affinity of Hb for O2 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 O2 affinity plays a major role in reducing the ventilatory requirement for branchial O2 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 (1988b) have suggested that an α-adrenoreceptor-mediated increase in blood O2-carrying capacity may be the most significant response contributing to regulation of CaO2 during external hypercapnia in trout, because α-receptor blockade produced a significantly greater reduction in CaO2 than did β-receptor blockade.

**Carbon dioxide excretion**

The transport of CO2 in fish blood has been recently reviewed (Perry 1986). The prevailing model for CO2 transfer involves the catalyzed dehydration of plasma bicarbonate (HCO3⁻) within the RBC to form physically dissolved CO2, and the subsequent diffusion of physically dissolved CO2 across the gill epithelium. It is likely that the process of plasma HCO3⁻ conversion to dissolved CO2, rather than branchial CO2 diffusion or blood/water convection, is the rate-limiting step in CO2 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 CO2 excretion by reducing the rate of plasma HCO3⁻ hydration. As proposed by Wood and Perry (1985), adrenergic inhibition of RBC HCO3⁻ dehydration may be the basis for the consistently observed respiratory acidosis after
exhaustive exercise in fish, a period when catecholamines are elevated (Ristori and Laurent 1985; Primett et al. 1986; Butler et al. 1986; Milligan and Wood 1987). The transient postexercise increase in $P_{\text{ACO}}$ cannot be explained by branchial diffusive or convective limitations because $P_{\text{AO}}$ 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 branchial pretreatment of fish with carbonic anhydrase; $P_{\text{AO}}$ is unaffected by this treatment (Fig. 3B) These results strongly suggest that RBC carbonic anhydrase is inaccessible to dehydrate plasma $\text{HCO}_3^-$ after exercise. Moreover, intravascular infusion of epinephrine or the $\beta$-adrenoceptor agonist isoprotrotenol induces a similar state of respiratory acidosis in trout (Perry and Vermette 1987; Vermette and Perry 1988a) even while raising $P_{\text{AO}}$ (Fig. 3A). The epinephrine-induced hypercapnia is not abolished by pretreating fish with the $\alpha$-adrenoceptor antagonist phentolamine, (Fig. 3A), although gill gas diffusive and ventilatory conductances clearly are enhanced. These results strongly support the hypothesis that $\beta$-adrenergic inhibition of plasma $\text{HCO}_3^-$ 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 $O_2$ excretion after burst exercise or following epinephrine injection into resting fish have failed to demonstrate a reduction of $O_2$ excretion under these conditions (Steffensen et al. 1987). It is likely, however, that inhibition of plasma $\text{HCO}_3^-$ dehydration would cause only a transient reduction of $O_2$ excretion as $P_{\text{CO}_2}$ 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 1988a; 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 $C_{\text{AO}}$. In Fig. 6 we summarize the various factors under adrenergic control that serve to regulate blood $O_2$ content after hypoxemia-mediated
release of catecholamines. At the gill, ventilation and diffusive conductance (O_{Gill}) are increased, causing a rise in arterial O_{2} tension (P_{A \text{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.

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**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 (V_{w}) and diffusive conductance (O_{Gill}) are increased, causing a rise in arterial O_{2} tension (P_{A \text{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.

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