

**FACTORS LIMITING CARBON DIOXIDE EXCRETION
ACROSS THE FISH GILL**

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In 1994 Dave Randall wrote "...plasma bicarbonate is excreted by erythrocytic dehydration catalyzed by carbonic anhydrase and the CO₂ so formed then diffuses into the water across the gill epithelium" (Randall and Daxboeck, 1984). By inference, this statement implicated erythrocyte Cl⁻/HCO₃⁻ exchange as the rate-limiting step in overall CO₂ excretion in fish. In this paper, following nearly twenty years of gathering data, we arrive at exactly the same conclusion but extend Randall's original model to explain the origin and consequences of the apparent diffusion limited nature of CO₂ excretion.

Recently, Desforges *et al* (2001) demonstrated that an intra-arterial injection of 5 mg kg⁻¹ carbonic anhydrase (CA) reduced the arterial partial pressure of CO₂ (PaCO₂) in trout by 0.23 ± 0.05 mm Hg; saline injection was without effect. Because breathing and venous blood gases were unaffected by CA, the effect of extracellular CA in lowering PaCO₂ was presumably caused solely by a specific enhancement of CO₂ excretion owing to acceleration of HCO₃⁻ dehydration within the plasma. These results provided the first *in vivo* evidence that the accessibility of plasma HCO₃⁻ to red blood cell (RBC) CA constrains CO₂ excretion under resting conditions. Because the velocity of RBC Cl⁻/HCO₃⁻ exchange governs HCO₃⁻ accessibility to RBC CA, these results also provided evidence that CO₂ excretion at rest is limited by the relatively slow rate of Cl⁻

/HCO₃⁻ exchange. More recently, we have been able to successfully treat trout with DIDS *in vivo* to block RBC Cl⁻/HCO₃⁻ exchange. The blockade of RBC Cl⁻/HCO₃⁻ exchange led to a rapid and specific elevation of PaCO₂, decreased CO₂ excretion and caused a marked fall in the respiratory exchange ratio (MCO₂/MO₂; Figure 1). The effects of blocking RBC Cl⁻/HCO₃⁻ exchange with DIDS were reversed by the addition of exogenous CA to the plasma (Figure 1). Thus, these data clearly show that RBC Cl⁻/HCO₃⁻ exchange is the rate-limiting step in CO₂ excretion in trout and presumably other teleost fish.

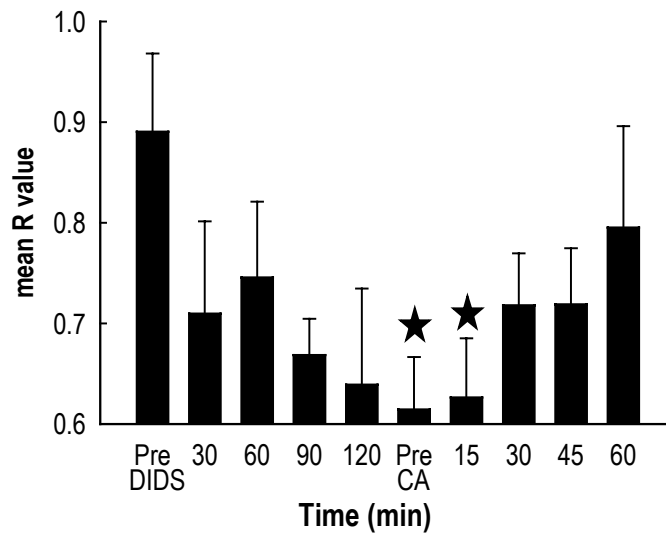


Figure 1. The effects of DIDS treatment and subsequent intra-arterial injection of carbonic anhydrase (CA) on the respiratory exchange ratio (R) in rainbow trout (*Oncorhynchus mykiss*). Data are shown as mean \pm 1 SEM; significant differences ($P < 0.05$) are indicated by asterisks. From K.M. Gilmour, P.R. Desforges and S.F. Perry; unpublished data.

The conversion of HCO₃⁻ to CO₂ as blood flows through the gill is constrained by the slow velocity of RBC Cl⁻/HCO₃⁻ exchange. We have been testing the idea that this chemical equilibrium limitation may be the underlying explanation for CO₂ transfer behaving as a diffusion-limited system. Evidence for diffusion limitations was obtained by examining the effects of altered cardiac output (and hence gill transit time) on PaCO₂ (used as an index of CO₂ transfer efficiency). As shown in Figure 2, PaCO₂ was markedly influenced by small changes in

cardiac output (V_b). These results provide compelling evidence that CO_2 excretion in trout, unlike O_2 uptake, behaves as a diffusion-limited system. Because the impact of altered V_b on PaCO_2 was eliminated by prior treatment of fish with exogenous CA (Desforges *et al* 2002), the origin of the diffusion-limitation is related to the slow velocity of RBC $\text{Cl}^-/\text{HCO}_3^-$ exchange rather than to the diffusion across the gill of CO_2 , itself.

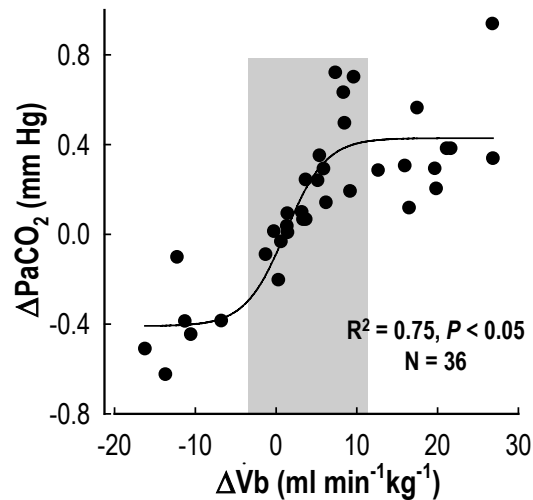


Figure 2. The relationship between the changes in cardiac output (ΔV_b) and the changes in arterial PCO_2 (ΔPaCO_2 ; $n = 36$) in rainbow trout (*Oncorhynchus mykiss*). The changes in V_b were elicited by vascular volume loading or by plasma removal. Note the significant sigmoidal correlation between V_b and PaCO_2 ($r^2 = 0.75$; $P < 0.05$). The shaded area represents the linear portion of the curve ($r^2 = 0.72$; $n = 20$). From Desforges *et al* (2002).

Because CO_2 transfer behaves as a diffusion-limited system whereas O_2 transfer is believed to be perfusion-limited (Malte and Weber, 1985), any changes in the diffusive gas conductance of the gill would be expected to cause changes in PaCO_2 with considerably less impact on PaO_2 . Indeed, two recent studies demonstrated that experimental reduction of gill surface area by gill ligation (Julio *et al* 2000) or re-routing of blood through the lamellar vascular sheet after injection of endothelin 1 (Perry *et al* 2001) caused marked increases in PaCO_2 that were reversed or prevented by pre-treatment of fish with CA.

Acknowledgements

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