THE JOURNAL OF EXPERIMENTAL ZOOLOGY 263:143–159 (1992)

Mechanisms of Ion and Acid-Base Regulation at the Gills of Freshwater Fish

G.G. GOSS, S.F. PERRY, C.M. WOOD, AND P. LAURENT
Department of Biology, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5 (G.G.G., S.F.P.); Department of Biology, McMaster University, Hamilton, Ontario, Canada L8S 4K1 (C.M.W.); CNRS, Laboratoire de Morphologie Fonctionelle et Ultrastructurale des Adaptations, F-67037 Strasbourg Cedex, France (P.L.L.)

ABSTRACT This review examines the branchial mechanisms utilized by freshwater fish to regulate internal acid-base status and presents a model to explain the underlying basis of the compensatory processes. Rainbow trout, *Oncorhynchus mykiss*, and brown bullhead, *Ictalurus nebulosus*, were examined under a variety of experimental treatments which induced respiratory and metabolic acid-base disturbances. Acid-base regulation was achieved by appropriate adjustments of Na^+ and Cl^- net fluxes across the gills which, in turn, were accomplished by variable contributions of three different branchial mechanisms: 1) differential changes in Na^+ and Cl^- diffusive effluxes, 2) changes in internal substrate (H^+, HCO_3^-) availability, and 3) morphological adjustments to the gill epithelium. *Differential* diffusive efflux of Na^+ over Cl^- was involved only during periods of metabolic alkalosis. The importance of internal substrate availability was demonstrated using a two-substrate model. According to the model, ionic flux rates (J_{in}^{Cl-}, J_{in}^{Na+}) are determined not only by the concentration of the external ion (Na^+, Cl^-) but also by the concentration of the internal counterion (H^+, HCO_3^-). This system provides for an "automatic negative feedback" to aid in the compensation of metabolic acid-base disturbances. Morphological alteration of the gill epithelia and the associated regulation of chloride cell (CC) fractional area is an essential third mechanism which is especially important during respiratory acid-base disturbances. Specifically, fish vary the availability of the CC associated Cl^-/HCO_3^- exchange mechanism by physical covering/uncovering of CCs by adjacent pavement cells.

The ability to regulate internal acid-base status is of fundamental importance. In freshwater fish, this is achieved by a combination of branchial, renal, and metabolic processes to regulate the production, uptake, and excretion of acidic and basic equivalents (Heisler, '86; Truchot, '87; McDonald et al., '89b). The primary site for acid-base regulation is the gills (Cameron, '80; McDonald and Wood, '81; Evans, '82; Wood, '88), although changes in renal acid excretion (Wood and Caldwell, '78; Wheatly et al., '84; Perry et al., '87b; Goss and Wood, '90b) and intracellular buffering (Cameron, '80; Heisler and Neumann, '80) may also contribute significantly. In this paper we specifically focus on the branchial mechanisms involved in the regulation of acid-base homeostasis. Classically, changes in internal pH are thought to reflect alterations in either or both of the variable components of the Henderson-Hasselbalch equation, P_{CO_2} or $[HCO_3^-]$ where alterations in P_{CO_2} predominantly reflect respiratory changes while change in HCO_3^- is caused largely by metabolic adjustments. An alternate view (Stewart, '78, '83) attributes changes in pH to alterations in one or more of three independent variables, P_{CO_2}, total weak acids (A_{tot}), or strong ion difference (SID). SID is defined as the summated activity of all strongly dissociated cations (Na^+, K^+, Mg^{2+}, Ca^{2+}) minus that of all strongly dissociated anions (Cl^-, lactate$^-$, HPO_4^{2-}, SO_4^{2-}) in solution. Therefore, in any system in which P_{CO_2} and A_{tot} are constant (i.e., no respiratory component to the acid-base disturbance), changes in the acid-base status in one solution must result from differential movements of strong ions between solutions to cause a change in the SID. These two approaches should be viewed as complementary rather than contradictory. The classical view provides a practical approach to the measurement and diagnosis of acid-base status. P_{CO_2}, pH, and total CO_2 can all be measured directly with great accuracy and $[HCO_3^-]$ can be easily derived. The SID approach is much more difficult to apply

Received April 29, 1991; revision accepted October 31, 1991.

experimentally because of the cumulative error involved in multiple ion measurements, as well as the uncertainties in the quantification of A_{tot} (Cameron, '89; Cameron and Iwama, '89). However, it is extremely valuable in providing a clear theoretical basis for the linkage between ionic and acid-base regulation.

Freshwater fish exchange strong ions and acid-base equivalents across the gills between the extracellular fluid and the external environment. During a metabolic acid or base load (when P_{CO_2} does not change), the net flux of acid-base equivalents from the extracellular and intracellular compartments of the fish to the external environment might therefore be estimated by knowing the difference between the net fluxes of anions and cations from the fish to the water (equation 1). In general, this will be close to the difference between Na^+ and Cl^- net fluxes owing to the fact that Na^+ and Cl^- are the major ions in extracellular physiological systems.

$$J_{net}^{H+} = J_{net}^{anions} - J_{net}^{cations} \quad (1)$$

In addition, the net flux of an ion between the fish and the water can be resolved into two individual components, the influx and the efflux (Maetz, '56). Adjustments of either of these two components of ion balance will alter the net flux of that ion and, therefore, affect net acid-base equivalent transfer. The present paper provides evidence for independent manipulation of both the influx and efflux components of ion balance and acid-base regulation, and attempts to provide insight into some of the controlling mechanisms.

EXPERIMENTAL PROCEDURES

Six different experimental treatments were employed to alter internal acid-base status in rainbow trout (*Oncorhynchus mykiss*, range 180–400 g), and brown bullhead (*Ictalurus nebulosus*, range 150–350 g). The fish were chronically catheterized in the dorsal aorta to permit infusion and/or repetitive blood sampling, and in the urinary bladder to eliminate the contribution of the kidney to measured fluxes in the water. These treatments were as follows: I) control; II) sham (NaCl) infusion (19 h, 140 mequiv/L NaCl, infusion rate = 400 μequiv/kg/h); III) metabolic acidosis induced by HCl infusion (19 h, 70 mequiv/L HCl – 70 mequiv/l NaCL, infusion rate = 210 μequiv/kg/h). This treatment was used instead of 140 mequiv/L HCl because preliminary experiments indicated that the fish could not tolerate infusion of the latter; IV) metabolic alkalosis induced by $NaHCO_3$ infusion (19 h, 140 mequiv/L $NaHCO_3$, infusion rate = 410 μequiv/kg/h); V) respiratory acidosis induced by exposure to environmental hyperoxia (water P_{O_2} (P_{wO_2})>500 torr) for 72 h, followed by metabolic alkalosis induced by acute removal of the prolonged (72 h) hyperoxic stimulus; and VI) respiratory acidosis induced by exposure to environmental hypercapnia (2% CO_2 in air, P_{wCO_2} = 15 torr) for 48 h, followed by metabolic alkalosis induced by acute removal of the prolonged (48 h) hypercapnic stimulus. Exposure to either hypercapnia or hyperoxia has been shown to induce a severe respiratory acidosis which is compensated for over 48–72 h by increasing $[HCO_3^-]_a$ (Wood and Jackson, '80; Perry et al., '87a). Subsequent removal of the hypercapnia or hyperoxia rapidly alleviates the respiratory acidosis yet $[HCO_3^-]_a$ remains elevated, thereby producing a metabolic alkalosis (Cameron, '76; Perry et al., '87a; Wood et al., '84). Most experiments were performed using rainbow trout, while treatment VI (exposure to environmental hypercapnia and removal) was performed using brown bullhead.

EFFLUX REGULATION

A mode of acid-base regulation which only recently has received attention is manipulation of the efflux component to ion balance (Evans and Cameron, '86; Wood, '88; McDonald et al., '89b; Goss and Wood, '90a,b). Changing the SID in the extracellular compartment through the differential loss of strong ions across the gills must constrain a change in the acid-base status of that compartment. For example, a greater efflux of anions than cations from the extracellular compartment to the water will increase the extracellular SID (given equivalent rates of influx), thereby constraining an increase in extracellular pH and HCO_3^- according to physico-chemical laws (Gamble, '22; Stewart, '78,'83) of electroneutrality. This response would be appropriate for an acidotic animal. Conversely, if cation efflux exceeds anion efflux, this will decrease the SID, constraining a decrease in pH and HCO_3^- levels, an appropriate response for an alkalotic animal. Since Na^+ and Cl^- are the major strong ions diffusing into the water, the contribution of differential diffusive efflux to acid-base regulation can be approximated by monitoring the *differential* efflux of these two ions.

The method commonly used to measure rates of ionic outflux ($J_{out}^{Na+,Cl-}$) from freshwater fish is to calculate outflux as the difference between the net flux ($J_{net}^{Na+,Cl-}$) and the influx ($J_{in}^{Na+,Cl-}$), the latter measured using radioisotopes. The results of several previous studies using this method

(Cameron, '76; Wood et al., '84; Perry et al., '87a) suggest that differential diffusive efflux plays a role in acid-base regulation. However, this method is complicated by the presence of exchange diffusion components (Na^+/Na^+ and Cl^-/Cl^- self-exchanges; Maetz, '72; De Renzis, '75; Wood et al., '84) which are included in the outflux measurements. Possible differential changes in the rates of these self-exchanges confound interpretation.

The method originally employed to measure the diffusive efflux of ions from a freshwater animal was to transfer the animal to distilled water and then monitor the appearance of Na^+ and Cl^- over a short period (Shaw, '59; McDonald and Rogano, '86). The influx component to ion balance (including exchange diffusion) would be absent owing to the unavailability of external ions. However, we (Goss and Wood, '90a) have shown that when fish are placed in distilled water, there is an immediate large decrease in the branchial transepithelial potential (TEP: blood relative to water as zero), probably owing to the reduction in water Ca^{2+} and Mg^{2+} (Eddy, '75). This would change the net driving forces for the effluxes of anions and cations, and thereby produce artifactual values for differential diffusive efflux. Recently, we (Goss and Wood, '90a,b) have developed a modification of Shaw's ('59) original technique whereby fish are placed in water containing normal levels of Ca^{2+}, Mg^{2+}, alkalinity, and pH, but lacking Na^+ and Cl^-. Under these conditions, TEP does not change and both the influx and exchange diffusion components are eliminated. Using this technique, the true diffusive effluxes of Na^+ and Cl^- can be measured to determine if there is a *differential* loss of one over the other as a means of compensating an acid-base disturbance.

Figure 1 shows the effluxes of Na^+, Cl^-, and the sum $[Na^+ - Cl^-]$ efflux or *differential* diffusive efflux (predicted net H^+ flux) in trout under a number of different acid-base disturbances (procedures I–V). Diffusive efflux measurements in each of the NaCl-infused, HCl-infused, and $NaHCO_3$-infused groups were made 9 h after the start of infusion while in the post-hyperoxia group, measurements were taken 1 h after return to normoxia. Although NaCl infusion resulted in greater Na^+ (Fig. 1A) and Cl^- (Fig. 1B) effluxes compared to control values, there was no *differential* diffusive efflux of one ion with respect to the other (Fig. 1C); $[Na^+ - Cl^-]$efflux was not significantly different from zero. Metabolic acidosis induced by HCl infusion resulted in lower Na^+ and Cl^- effluxes compared to NaCl-infused fish, but again the differential diffusive efflux was unchanged (Fig. 1C), indicating no role for efflux

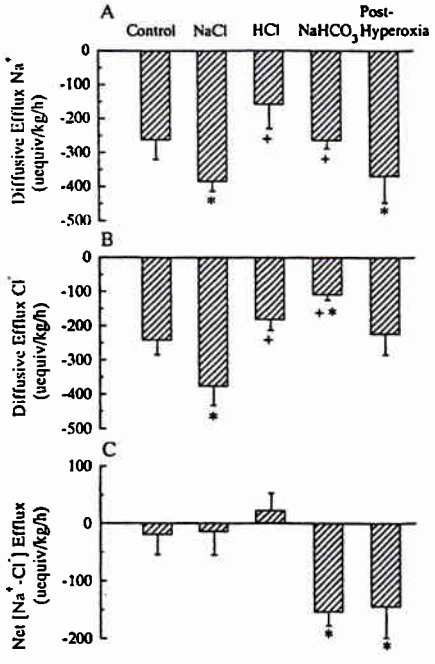

Fig. 1. Diffusive efflux of (A) Na^+, (B) Cl^-, and (C) *differential* diffusive efflux $[Na^+\text{-}Cl^-]$ under five different experimental conditions: I) control (n = 12); II) sham (NaCl) infusion (9 h, 140 mequiv/l NaCl, infusion rate = 400 μequiv/kg/h, n = 12); III) metabolic acidosis induced by HCl infusion (9 h, 70 mequiv/L HCl + 70 mequiv/L NaCl, infusion rate = 210 μequiv/kg/h, n = 6); IV) metabolic alkalosis induced by $NaHCO_3$ infusion (9 h, 140 mequiv/l $NaHCO_3$, infusion rate = 410 μequiv/kg/h, n = 12); and V) metabolic alkalosis induced by acute removal of a prolonged hyperoxic (measured 1 h post-hyperoxia after 72 h hyperoxic stimulus: P_{O_2} > 500 torr, n = 10). * indicates significantly different from control. + indicates significantly different from NaCl (sham) infused fish. $P < .05$. Means ± 1 SEM. (Data from Goss and Wood, '90a,b, '91.)

modulation in compensating this particular metabolic acidosis. On the other hand, metabolic alkalosis caused by either $NaHCO_3$ infusion or post-hyperoxic recovery produced a significant *differential* loss of Na^+ over Cl^- of up to ~150 μequiv/kg/h. At these times, net H^+ influx [measured as the sum of the ammonia efflux and the change in the titratable acidity of the water (as described by McDonald and Wood, '81)] was 300–500 μequiv/kg/h (Goss and Wood, '90a,b). Therefore, *differential* diffusive efflux may account for up to 35–50% of the total acid-base compensation during metabolic alkalosis.

The mechanisms controlling the rates of diffusive efflux in fish remain unclear. Diffusive efflux probably occurs via paracellular rather than transcellular routes (Evans, '82; McDonald et al., '89b). Therefore, regulation of tight junction selectivity may play a role in determining the magnitude of

diffusive efflux for each of the ions. Although there is no direct evidence for the regulation of tight junction selectivity in the fish gill, there is evidence for the active regulation of tight junction selectivity in "leaky" or low resistance epithelia (e.g., amphibian gallbladder epithelium, mammalian intestinal brush border; Madera, '88) in which the transepithelial resistance is altered to regulate the flow of water and solutes around the cell. An alternative possibility is that the regulation could be passive. For example, the rise in extracellular pH during metabolic alkalosis might alter the net charge on the paracellular channels, thereby promoting the cation (Na^+) efflux over anion (Cl^-) efflux. Clearly, much more work is needed to clarify the controlling factors. Nevertheless, whatever the proximate mechanisms involved, it is clear that the regulation of diffusive efflux and more importantly, *differential* diffusive efflux ($[Na^+ - Cl^-]$), plays an important role in compensating alkalotic disturbances of acid-base balance.

INFLUX REGULATION

Freshwater fish maintain a large ion concentration gradient between their extracellular fluids and the dilute external environment. Krogh ('38,'39) first suggested that the uptake of Na^+ and Cl^- by the gills of freshwater fish was accomplished by independent electroneutral exchanges. More specifically, he suggested that Na^+ was exchanged for endogenous H^+ or NH_4^+ (both of which are acidic equivalents) and that Cl^- was exchanged for endogenous HCO_3^- (a basic equivalent). Independent manipulation of these Na^+/acid and Cl^-/base exchange mechanisms would therefore also provide a direct means of regulating the internal acid-base status. These ideas were strongly reinforced by the work of Maetz's group in the 1960s and 1970s (e.g., Maetz and Garcia-Romeu, '64; Garcia-Romeu and Maetz, '64; Maetz, '72, '73; De Renzis and Maetz, '73; De Renzis, '75). More recently, it was shown quite conclusively that when freshwater fish are challenged experimentally with an acid-base disturbance, they respond by *dynamically* manipulating their branchial Na^+ and Cl^- uptake rates in a manner which is consistent with acid-base regulation (Cameron, '76; McDonald et al., '83; Wood et al., '84; Perry et al., '87a; McDonald and Prior, '88; Goss and Wood, '90a,b). Catecholamines (Girard and Payan, '77; Perry et al., '84; Vermette and Perry, '88; McDonald et al., '89a) and cortisol (Laurent and Perry, '90; Goss et al., '92) have both been examined as possible controlling agents, but no clear conclusions have yet emerged.

There is general agreement that the Na^+/acid and Cl^-/base exchange mechanisms are located on the apical (water-facing) membranes of gill transport cells. However, the energetics of transport have not yet been worked out because measurements of intracellular Na^+ and Cl^- activities and potentials have not been made in the transporting cells. Na^+/K^+ ATPase pumps on the basolateral (extracellular fluid-facing) membranes undoubtedly play a role, but it is not clear how this system alone could power both Na^+ and Cl^- uptake. Recently, Avella and Bornancin ('89, '90) have questioned the traditional model for Na^+ uptake (Na^+/acid exchange) and suggested an alternate mechanism whereby the excretion of protons (H^+) and uptake of Na^+ are accomplished by an H^+ pump powered by an H^+ ATPase on the apical membrane. The H^+ pump would be coupled electrochemically to an Na^+ channel by the apical membrane potential. It is important to point out that direct evidence for either mechanism is still lacking. Therefore, this review does not distinguish between either of the two proposed mechanisms for the excretion of H^+ and uptake of Na^+. However, both mechanisms result in the outward translocation of H^+ (NH_4^+) and inward movement of Na^+.

A common tool for the examination of ion transport is to examine the effect of substrate concentration on the rate of transport, an approach borrowed from enzyme biochemistry. In brief, this method involves measuring the unidirectional influx rate ($J_{in}^{Na+,Cl-}$) over increasing external ion or "substrate" concentrations (Shaw, '59; Maetz, '72; De Renzis and Maetz, '73; Wood and Randall, '73; Perry and Wood, '85). The relationships generated are described by Michaelis-Menten first-order kinetics, for enzymes with one substrate, and yield estimates of the affinity of the enzyme ($1/K_m$) and the maximal transport rate (J_{max}) at saturating substrate concentrations. Recently, we (Goss and Wood, '90a,b; Goss and Wood, '91) have shown that in freshwater trout, alterations in the acid-base status by a variety of experimental procedures cause changes in these kinetic variables (K_m,J_{max}).

Figure 2 shows a series of typical first-order kinetic curves generated by sequentially increasing the external Cl^- concentration ($[Cl^-]_e$: Fig. 2A) and external Na^+ concentration ($[Na^+]_e$: Fig. 2B) in fish under control conditions and during $NaHCO_3$ infusion (treatment IV). At each concentration, J_{in}^{Cl-} and J_{in}^{Na+} were measured with radiotracers over 0.5 h intervals. Notice that at every $[Cl^-]_e$, J_{in}^{Cl-} was higher in the $NaHCO_3$-infused group compared to the control group while J_{in}^{Na+} was

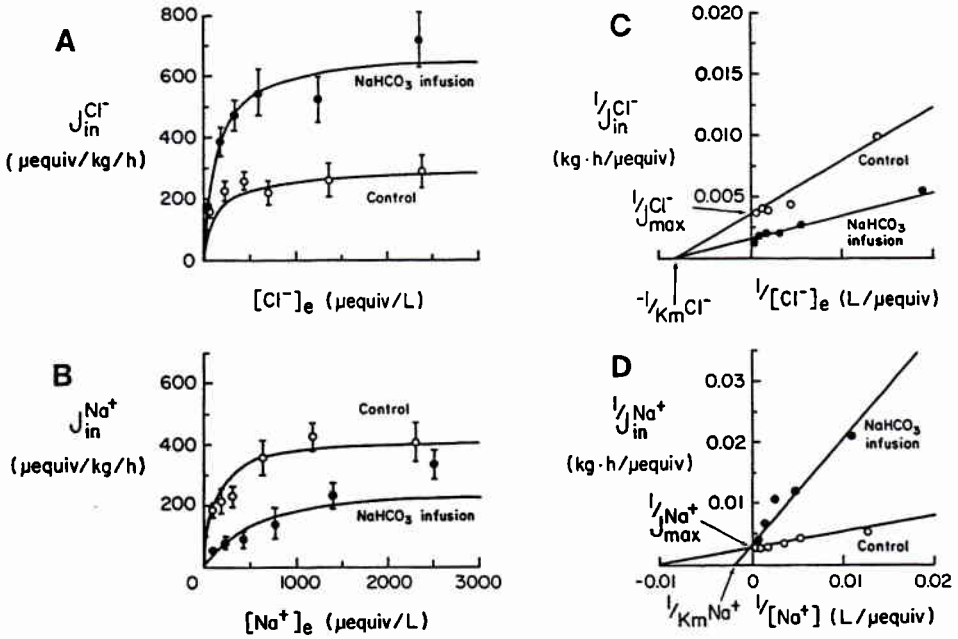

Fig. 2. Classical one-substrate kinetics of chloride influx ($J_{in}^{Cl^-}$:A) and sodium influx ($J_{in}^{Na^+}$:B) as a function of external ion concentration ([ion]$_e$) during control conditions (n = 6) and during $NaHCO_3$ infusion (10–14 h, 140 mequiv/L $NaHCO_3$, infusion rate = 410 μequiv/kg/h, n = 6). Double reciprocal Lineweaver-Burke plots (C,D) linearize the data allowing for estimations of the kinetic parameters (K_m, J_{max}). Means ± 1 SEM. (Data from Goss and Wood, 90a,b.) See text for further details.

lower at each $[Na^+]_e$ in the $NaHCO_3$-infused group. These data were then analyzed using Lineweaver-Burke plots to yield estimates of K_m and J_{max} for each transporter (K_m, J_{max}). Figure 2C shows that the metabolic alkalosis resulting from $NaHCO_3$ infusion caused an increase in $J_{max}^{Cl^-}$ (decrease in $1/J_{max}^{Cl^-}$; y-intercept) while in Figure 2D, $J_{max}^{Na^+}$ was reduced (a small but significant increase in $1/J_{max}^{Na^+}$) by $NaHCO_3$ infusion. In addition, $NaHCO_3$ infusion resulted in an increase in $K_m^{Na^+}$ (Fig. 2D) while $K_m^{Cl^-}$ was unaffected (Fig. 2C). Treatments I–V were examined in a similar manner. It was concluded that the alterations in ionic uptake rates during acid-base disturbances are achieved either through increases or decreases in J_{max} while K_m can only increase (i.e., decreased affinity) relative to control values (Goss and Wood, '90a,b, '91; Wood and Goss, '90).

This review focuses on two possible mechanisms underlying the alterations in $J_{max}^{Na^+}$ and $J_{max}^{Cl^-}$: 1) alteration of internal substrate (H^+, HCO_3^-) availability and 2) morphological alteration of the gill epithelium. The conclusion reached is that the observed changes reflect alterations in both the *apparent* J_{max}s caused by changes in internal substrate availability, and in *true* J_{max}s caused by morphological adjustments of the transporting epithelium.

SUBSTRATE AVAILABILITY

Classically, alterations in K_m are interpreted as changes in the active site of the enzyme while alterations in J_{max} are thought to occur by changes in the total number of active sites available. However, the traditional one-substrate type kinetic analysis assumes that the system is operating for an enzyme with only one active site. Each of the transport mechanisms involved in acid-base regulation (Na^+/H^+, H^+ pump/Na^+ channel, Cl^-/HCO_3^- exchanges) also have an internal active site for the counterion. In order for the one-substrate model to be valid under these circumstances, the internal active site must be completely saturated with the internal counterion at all times so as to not limit the rate of functioning of the transporter (i.e., ratio: [counterion]/$K_m >> 1$). Kirschner ('88), showed that *apparent* Na^+ saturation kinetics in frog skin is based not only on the availability of Na^+ on the mucosal surface but also on the availability of the counterion (H^+). The idea that the rate of the transporter is also set by the concentration of the internal counterion is appealing as a mechanism for acid-base regulation. During metabolic acidosis, the concentration of H^+ increases and the concentration of HCO_3^- decreases in the extracellular fluid. If similar changes occurred in the intracellular compartment of the gill epithelia cells, these

would act to stimulate Na^+ uptake and reduce Cl^- uptake thereby increasing "acid" excretion and decreasing "base" excretion. Conversely, during an uncompensated metabolic alkalosis, the concentration of H^+ decreases and the concentration of HCO_3^- increases which would reduce Na^+ uptake and increase Cl^- uptake, thereby reducing acid excretion and increasing base excretion. These mechanisms would constitute an "automatic negative feedback" system for the control of acid-base balance.

A number of workers have speculated that such a system might operate in the gills of freshwater fish (e.g., Maetz and Garcia-Romeu, '64; De Renzis, '75; Perry et al., '81; Wood et al., '84; McDonald and Prior, '88) but there has been no evidence to support this idea. However, recently we have applied two-substrate kinetics analysis, which is well described in enzymology (Florini and Vestling, '57; Michal, '85), to the problem (Wood and Goss, '90; Goss and Wood, '91). This model incorporates the substrate availability of *both* the external ion and the internal counterion as factors affecting the rate of ion transport. From Figure 2C,D, one sees that *apparent* J_{max}^{Cl-} is altered by the infusion of $NaHCO_3$. However, in the two-substrate model, the rate of the transporter is determined not only by the number of transport sites available, but also by the availability of the internal substrate (H^+ or HCO_3^-).

Figure 3 (from Goss and Wood, '91) shows the application of the two-substrate model to measurements of *apparent* J_{max}^{Cl-} under a variety of acid-base disturbances. The analysis is complex and need not be discussed fully in this paper; the reader is referred to Goss and Wood ('91) for a complete description of the theory, methods, and implications of the model. In general, the experimental data fit to the model extremely well using arterial plasma $[HCO_3^-]$ and $[H^+]$ to represent the internal substrate concentrations. Figure 3A is a standard Lineweaver-Burke or double reciprocal plot for the determination of *apparent* J_{max}^{Cl-} and *apparent* K_m^{Cl-}. According to the two-substrate model, the slope of the plots and hence the *apparent* J_{max}^{Cl-} values are determined by the concentration of the internal counterion, $[HCO_3^-]_a$. Further analysis (Fig. 3B–D) based on a modified Lineweaver-Burke approach yields the *true* J_{max}^{Cl-*} (indicated with an asterisk), *true* K_m^{Cl-*}, and *true* K_m and *true* J_{max}* for the counterion ($K_m^{HCO_3-*}$, $J_{max}^{HCO_3-*}$). The J_{max}* values are those which would apply if both the internal and the external substrate were at saturating concentrations. The results from

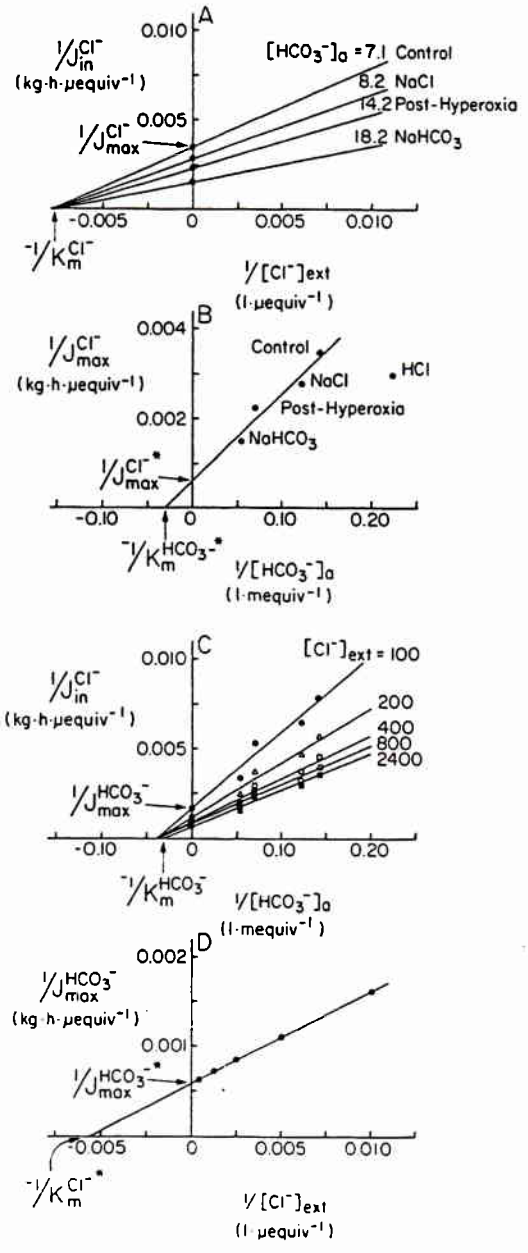

Fig. 3. Two-substrate kinetics analysis for the branchial Cl^-/HCO_3^- exchange system in rainbow trout. Analysis was performed using the experimental conditions I, II, IV, and V. HCl infusion (procedure III) was omitted from the analysis. $[HCO_3^-]_a$ values represent concentrations (mequiv/L) of bicarbonate in arterial blood plasma of each treatment group. (Figure reprinted with permission from Goss and Wood, '91.) See text for further details.

a two-substrate type of analysis showing the *true* K_m*s and *true* J_{max}*s in comparison to one-substrate control values are shown in Table 1 (from Goss and Wood, '91).

One important contribution of the two-substrate analysis is the demonstration that *true*

TABLE 1. A comparison of the results from classical Michaelis-Menten one-substrate analysis for the NA^+ and Cl^- transporters with the results from a two-substrate type of analysis[1]

	One-substrate analysis	Two-substrate analysis
J_{max}^{Na+} (μequiv/kg/h)	420	1,642
J_{max}^{Cl-} (μequiv/kg/h)	286	1,696
$J_{max}^{HCO_3-}$ (μequiv/kg/h)	—	1,702
K_m^{Na+} (μequiv/L)	99	—
K_m^{H+} (μequiv/L)	—	0.036 (pH = 7.444)
K_m^{Cl-} (μequiv/L)	127	135
$K_m^{HCO_3-}$ (mequiv/L)	—	33.3

[1]See text for further details.

J_{max}^{Cl-,HCO_3-}* (1,700 μequiv/kg/h; Table 1) is 4–5 times higher than *apparent* J_{max}^{Cl-} (286 μequiv/kg/h) measured during control conditions. Similarly, *true* J_{max}^{Na+}* (1,642 μequiv/kg/h) was 4 times higher than *apparent* J_{max}^{Na+} (420 μequiv/kg/h) measured under control conditions. The high *true* values of J_{max}* indicate that the capacity of the fish to absorb Na^+ and Cl^- from the external water is far higher than the measured influx rates under control conditions. The rate of transport of the external ion in each case is limited by the availabilities of both the external ion and the internal counterion. This model does not account for changes in K_m of the transporter for the external substrate (Fig. 2D) which likely occur through changes in the active site of the enzyme. However, it does provide estimates for *true* K_m^{H+}* and *true* $K_m^{HCO_3-}$* and explains why the transporters are not saturated by the internal counterions. Table 1 shows that under control conditions, the *true* K_m^{H+}* is 0.036 μequiv/L (pH 7.44) while $[H^+]_a$ is normally 0.012 μequiv/L (pH 7.90) and *true* $K_m^{HCO_3-}$* is 33.3 mequiv/L while $[HCO_3^-]_a$ is 5–8 mequiv/L. Therefore, the ratio of [counterion]/K_m << 1 for both H^+ and HCO_3^- meaning that the internal counterions are not at saturating concentrations and thus must limit the rates of the transporters.

One problem with this analysis is that it was performed using measured values for the extracellular acid-base status. We have assumed that changes in intracellular acid-base status are at least qualitatively, if not quantitatively, linked to changes in the extracellular acid-base status. In support of this assumption, a separate series of experiments (Goss and Wood, '91) using the ^{14}-C-DMO technique (Milligan and Wood, '86) has demonstrated that global gill epithelial intracellular acid-base status qualitatively follows extracellular acid-base status. Therefore, the results presented here, although only qualitative estimates, show that internal availability of H^+ and HCO_3^- provides an "automatic negative feedback" mechanism for internal acid-base regulation.

According to this model, however, there are several instances where changes in extracellular acid-base status would inappropriately affect branchial Cl^-/HCO_3^- exchange. For example, extracellular HCO_3^- levels are elevated during uncompensated respiratory acidosis, and further elevated during and after metabolic compensation. At these times, the model predicts a stimulation of Cl^-/HCO_3^- exchange which would exacerbate rather than correct the acidosis, yet clearly J_{in}^{Cl-} remains the same or is reduced in almost all cases (Cameron, '76; Wood et al., '84; Cameron and Iwama, '87; Perry et al., '87a; Goss and Wood, '90a,b; Goss and Wood, '91; Goss et al., '92). This apparent paradox (a lower J_{in}^{Cl-} at a time when $[HCO_3^-]_a$ is elevated) suggests that there are complementary mechanisms altering ion fluxes at the gill during acid-base disturbances.

MORPHOLOGICAL REGULATION

The second mechanism by which J_{max} could be regulated is by adjustments of the number of functional transporters present on the gill epithelia surfaces. HCl infusion was omitted from the analysis because it clearly deviated from the predicted relationship. According to the two-substrate model, this indicates that during HCl infusion, *true* J_{max}^{Cl-*,HCO_3-} increased even though internal substrate (HCO_3^-) decreased. A likely cause would be an increase in the number of transport sites. In other epithelia (e.g., turtle bladder: Steinmetz, '87; cortical collecting tubules: Schwartz et al., '85), the number of transport sites is controlled by the rapid insertion/removal of the transport sites to/from the external surface. This alteration in the epithelium is accompanied by morphological adjustment of both the cell surface and ultrastructure (Schwartz et al., '85). Cameron and Iwama ('87) first suggested that morphological alteration of the fish gill epithelia might be involved in the regulation of acid-base status. Furthermore, Cameron ('89) has suggested that future studies on the mechanisms of acid-base status should focus on the relationships between morphological alteration of the gill and the physiological adjustments during acid-base disturbances.

The location of the Na^+ and Cl^- transporters with respect to cell type has been a topic of controversy in the past few years. It has been postulated that the chloride cells (CC) are the sites of active uptake of both Na^+ and Cl^- in freshwater fish (Laurent and Dunel, '80; Laurent et al., '85; Avella et al., '87; Avella and Bornancin, '89; Perry and Laurent, '89; Laurent and Perry, '90). The ultrastructural features of the CC, including large numbers of mitochondria and an extensive tubular network, make it a likely candidate as an ion transporting cell. By contrast, the pavement cells (PVC), which constitute as much as 95% of the total gill epithelial surface area, are generally thought to have little role in the uptake of Na^+ and Cl^- and, hence, acidic and basic equivalent excretion. Although Girard and Payan ('80) proposed a model in which lamellar PVCs were the sites of Na^+ uptake in FW trout, their suggestion was based on the premise that the CC did not occur on the lamellar epithelium, a situation later shown to be untrue (Laurent et al., '85; Perry and Wood, '85; Avella et al., '87; Avella and Bornancin, '90). The PVCs display a complex system of microridges and microvilli on their external surfaces and have well-developed golgi (Hughes, '79; Laurent and Dunel, '80). However, they contain few mitochondria. The lack of mitochondria has led to the belief that PVCs do not or cannot function in ionic or acid-base regulation. However, until recently, no studies have directly addressed the impact of acid-base disturbances on the morphological or ultrastructural characteristics of the PVCs.

We have examined the physiological and morphological responses of 1) brown bullhead to 48 h environmental hypercapnia (P_{wCO_2} = 15 torr) and its subsequent removal (Goss et al., '92) and 2) rainbow trout to environmental hyperoxia (P_{wO_2} > 500 torr) and its subsequent removal (Goss and Wood, '90a; G.G. Goss, C.M. Wood, P.L. Laurent, and S.F. Perry, in preparation). Figure 4 shows the physiological (Fig. 4A,B) and morphological (Fig. 4C) responses to these acid-base disturbances. In the bullhead hypercapnia series, ion fluxes and morphological measurements (CC fractional area) were determined in the same fish. At various times during the hypercapnia regime, ion fluxes were measured over 3 h using radiotracers ($^{22}Na^+$, $^{36}Cl^-$). At the end of the flux period, the fish were killed and their gills fixed for morphometric and ultrastructural analysis (for a complete description of the methods, see Goss and Wood, '90a; and Goss et al., '92). In the trout hyperoxia regime, ion fluxes and morphometric measurements were determined in different fish subjected to identical exposure regimes.

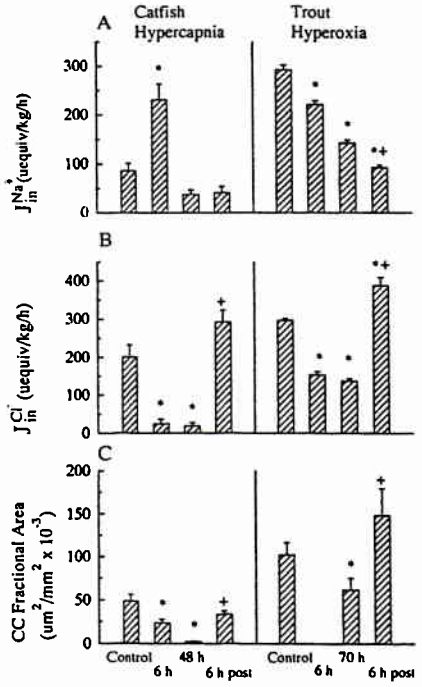

Fig. 4. The effect of hypercapnia (P_{wCO_2} = 15 torr: bullheads, *Ictalurus nebulosus*, n = 6) and its subsequent removal or hyperoxia (70 h, P_{wO_2} > 500 torr: trout, *Oncorhynchus mykiss*, n = 12, **A,B**; n = 7, **C**) and its subsequent removal on **(A)** whole body influx of sodium (J_{in}^{Na+}), **(B)** whole body influx of chloride (J_{in}^{Cl-}), and **(C)** chloride cell fractional area. * indicates significantly different from control values. $P < .05$. Means ± 1 SEM. (Data from Goss et al., '92.)

Respiratory acidosis induced by either environmental hypercapnia or hyperoxia resulted in complex changes in ion fluxes in a manner consistent with acid-base regulation. In bullheads, J_{in}^{Na+} was initially greatly stimulated (150% increase) while J_{in}^{Cl-} was rapidly reduced to 15% of the initial control value. By 48 h, the acidosis was compensated and J_{in}^{Na+} was reduced to a level near that of J_{in}^{Cl-}. These alterations in ion fluxes were accompanied by a large reduction in CC fractional area (CCFA). CCFA was reduced to 50% of the initial value after 6 h and only 10% of control after 48 h hypercapnia. A metabolic alkalosis resulting from removal of the hypercapnic stimulus (6 h post) resulted in a large stimulation of J_{in}^{Cl-} while J_{in}^{Na+} remained unchanged; CCFA was greatly increased at this time to a level near control values.

In trout, initial exposure to hyperoxia resulted in a slight decrease in J_{in}^{Na+} while J_{in}^{Cl-} was reduced rapidly to 50% of initial control values. Although a reduction in J_{in}^{Na+} by itself is not

an appropriate response to an acidosis, the combination of a large fall in J_{in}^{Cl-} and a small fall in J_{in}^{Na+} resulted in a net Cl^- loss greater than that of Na^+ (not shown) which is an appropriate response. The initial reduction in J_{in}^{Na+} during hyperoxia in trout is likely due to a reduction in perfusion and ventilation of the gill (Wood and Jackson, '80) while the persistent reduction in J_{in}^{Cl-} is likely due to a combination of reduced perfusion/ventilation and a reduction in the number of transporters through morphological alterations. By 72 h, the acidosis was fully compensated and J_{in}^{Na+} is reduced further to a level equal to that of J_{in}^{Cl-}. Exposure to hyperoxia also resulted in a 50% decrease in CCFA by 70 h (Fig. 4C). Unfortunately, morphometric measurements were not made during early hyperoxic acidosis. Removal of the hyperoxic stimulus (metabolic alkalosis) resulted in a further decrease in J_{in}^{Na+} while J_{in}^{Cl-} was greatly stimulated. Concurrently, CCFA was greatly increased in the 6 h post-hyperoxic period.

Figure 5 shows representative scanning electron microscope (SEM) photographs from bullheads exposed to hypercapnia and recovery and trout exposed to hyperoxia, respectively. For both species, exposure to a respiratory acidosis resulted in a reduction in the area of CCs on the filamental epithelium (Fig. 5C,D) compared to control conditions (Fig. 5A,B), while a metabolic alkalosis resulted in an increase in the area of CCs on the filamental epithelium (Fig. 5E,F). These changes are reflected in the morphometric data (Fig. 4C).

Representative transmission electron microscope (TEM) photographs corresponding to the SEM photographs of Figure 5 are shown in Figure 6. CCs during control periods possess a very typical ultrastructure, with large numbers of mitochondria and an extensive tubular network near the apical membrane (Fig. 6A,D). After prolonged periods of exposure to an acidotic stimulus, the apical exposure of the CC is reduced (Fig. 6B,E; corresponding to the decrease in CCFA, Fig. 4) as adjacent PVCs appear to cover over the CCs. In addition, there appear to be fewer organelles and a general disorganization of the CC ultrastructure after prolonged exposure to an acidosis, probably associated with a reduced CC functioning (and reduced J_{in}^{Cl-}; Fig. 4B) at this time. Return to normocapnia (Fig. 6C) or normoxia (Fig. 6F) results in increases in the apical exposure of CCs (increased CCFA: Fig. 4C) and a general return of the ultrastructural organization seen under control conditions, indicating a return in functioning of the CC.

An obvious and important feature of these data is the correspondence between the changes in J_{in}^{Cl-} and CCFA and the lack of correspondence between J_{in}^{Na+} and CCFA (Fig. 4). When CCFA is reduced, J_{in}^{Cl-} is reduced while when CCFA is increased, J_{in}^{Cl-} is increased. The degree of reduction of J_{in}^{Cl-} roughly corresponds to the degree of reduction in CCFA during respiratory acidosis (approximately 90% during hypercapnia in bullheads, 50% during hyperoxia in trout). However, during the metabolic alkalosis, the increase in J_{in}^{Cl-} is usually greater than the increase in CCFA, probably related to increased substrate availability ($[HCO_3^-]_a$ is elevated) at this time. The correspondence between J_{in}^{Cl-} and CCFA suggests that the Cl^-/HCO_3^- exchange mechanism is located on the CC. Long lasting reductions of J_{in}^{Cl-} to compensate acidosis are accomplished by physical covering of the CCs (and hence Cl^-/HCO_3^- exchange sites) by adjacent PVCs. This method of lowering *true* J_{max}^{Cl-*} can be viewed as essential to counteract the opposing influence of elevated internal $[HCO_3^-]$ which would tend to stimulate J_{in}^{Cl-}.

The lack of correspondence between J_{in}^{Na+} and CCFA (Fig. 4) suggests that the Na^+ uptake mechanism (either Na^+/H^+ exchange or an H^+ pump/Na^+ channel) is not associated with the CC. We have recently presented evidence that during hypercapnic acidosis in bullheads, PVCs undergo dramatic morphological changes, both externally and ultrastructurally. Microvillar density increased (measured using the SEM) and the numbers of mitochondria located in PVCs increased (Goss et al., '92). These changes suggest an increase in the metabolism of the PVC, perhaps associated with H^+ excretion and Na^+ uptake.

The mechanisms controlling these alterations in gill morphology remain unclear though cortisol may be involved. Experimentally elevated cortisol titres are known to cause increases in CCFA (Perry and Wood, '85; Laurent and Perry, '90) but the role it plays in the regulation of the morphological responses to acid-base disturbances remains to be investigated.

SUMMARY

This review presents evidence for three mechanisms which are variably utilized to regulate acid-base status. A model incorporating the proposed mechanisms and their locations is shown in Figure 7. The concentration of the external ion is assumed to be constant in this model while the relative size of the lettering for the counterion (H^+, HCO_3^-) represents the relative changes in the concentration of that particular counterion during the

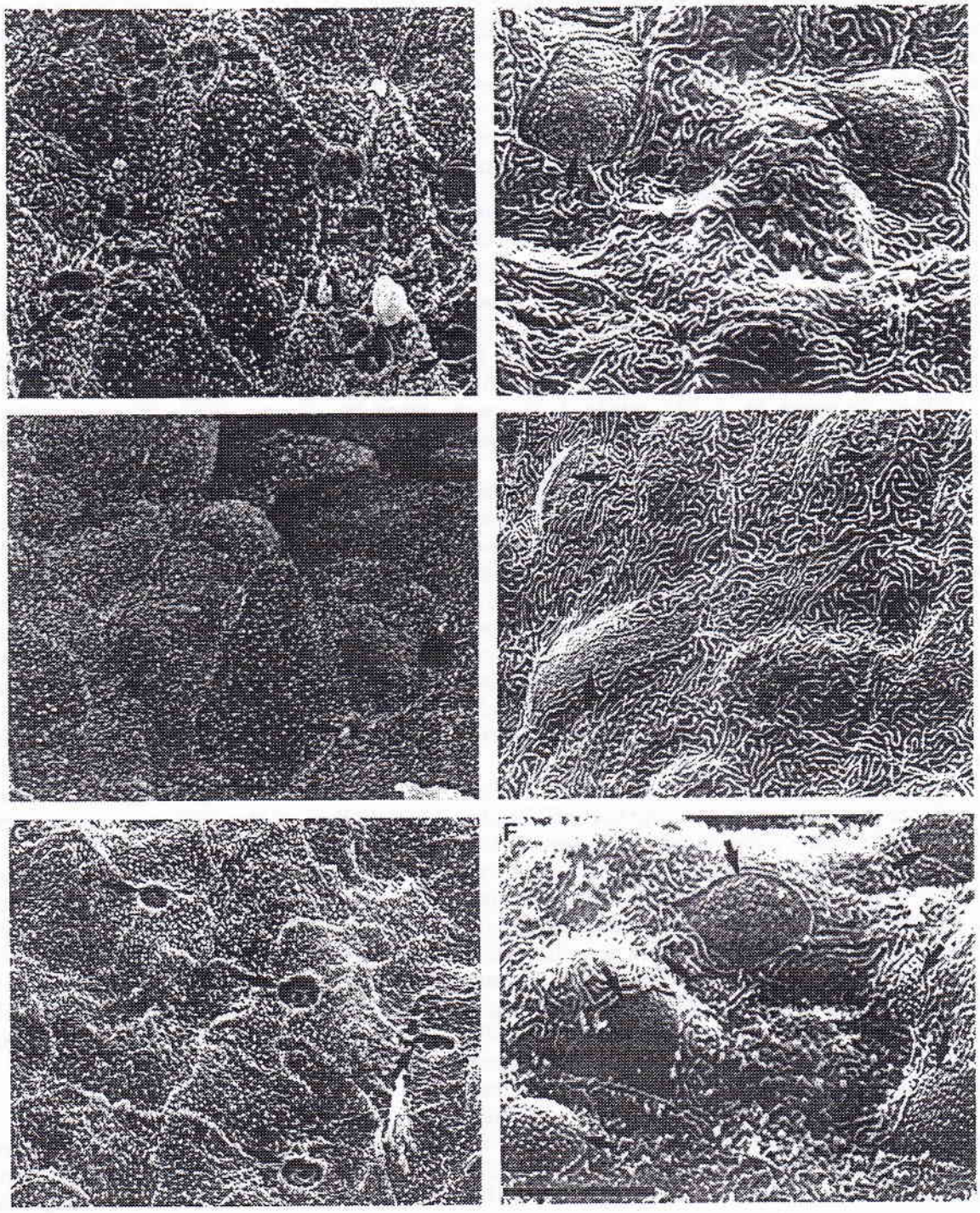

Fig. 5. Representative scanning electron microscopy (SEM) photographs of the filamental epithelium for bullhead (**A–C**) and trout (**D–F**) under control conditions, after 48 h exposure to hypercapnia or 70 h exposure to hyperoxia, and 6 h after removal of the hypercapnic or hyperoxic stimulus. Note the reduction in the CC apical exposure and change in the appearance of the CCs after exposure to hypercapnia/hyperoxia, while 6 h post-hypercapnia/6 h post-hyperoxia CC apical exposure is increased. Arrows indicate chloride cells. Bar = 10 μm. See text for further details.

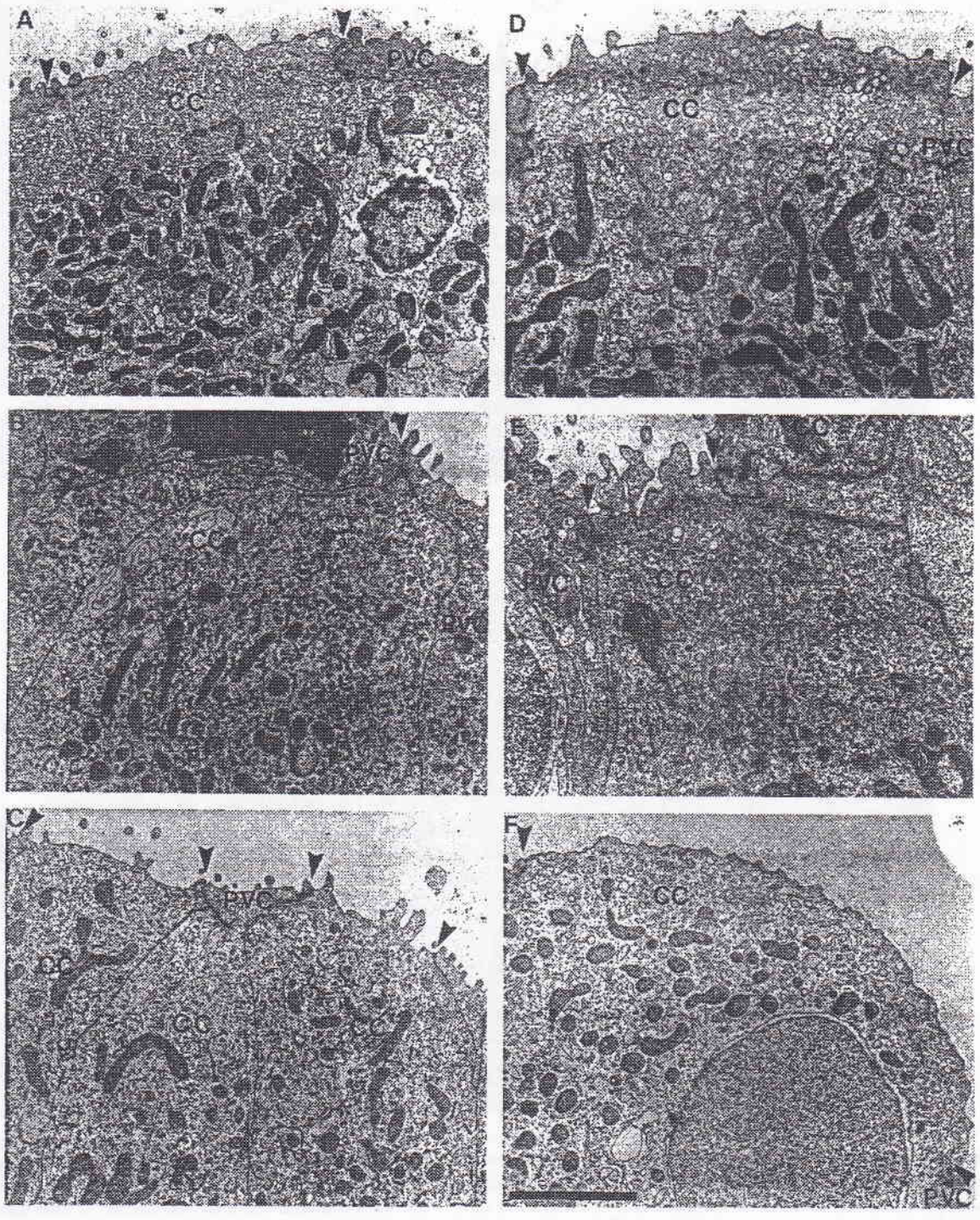

Fig. 6. Representative transmission electron microscopy (TEM) photographs of the filamental epithelium for bullhead (A–C) and trout (D–F) under control conditions, after 48 h exposure to hypercapnia or 70 h exposure to hyperoxia, and 6 h after removal of the hypercapnic or hyperoxic stimulus. Note the reduction in the CC apical exposure and change in the ultrastructure of the CC (reduced mitochondria) after exposure to hypercapnia/hyperoxia. After 24 h post-hypercapnia/6 h post-hyperoxia, CC apical surface exposure is increased. CC = chloride cell, PVC = pavement cell, arrowheads indicate CC-PVC junctions. Bar = 5 μm. See text for further details.

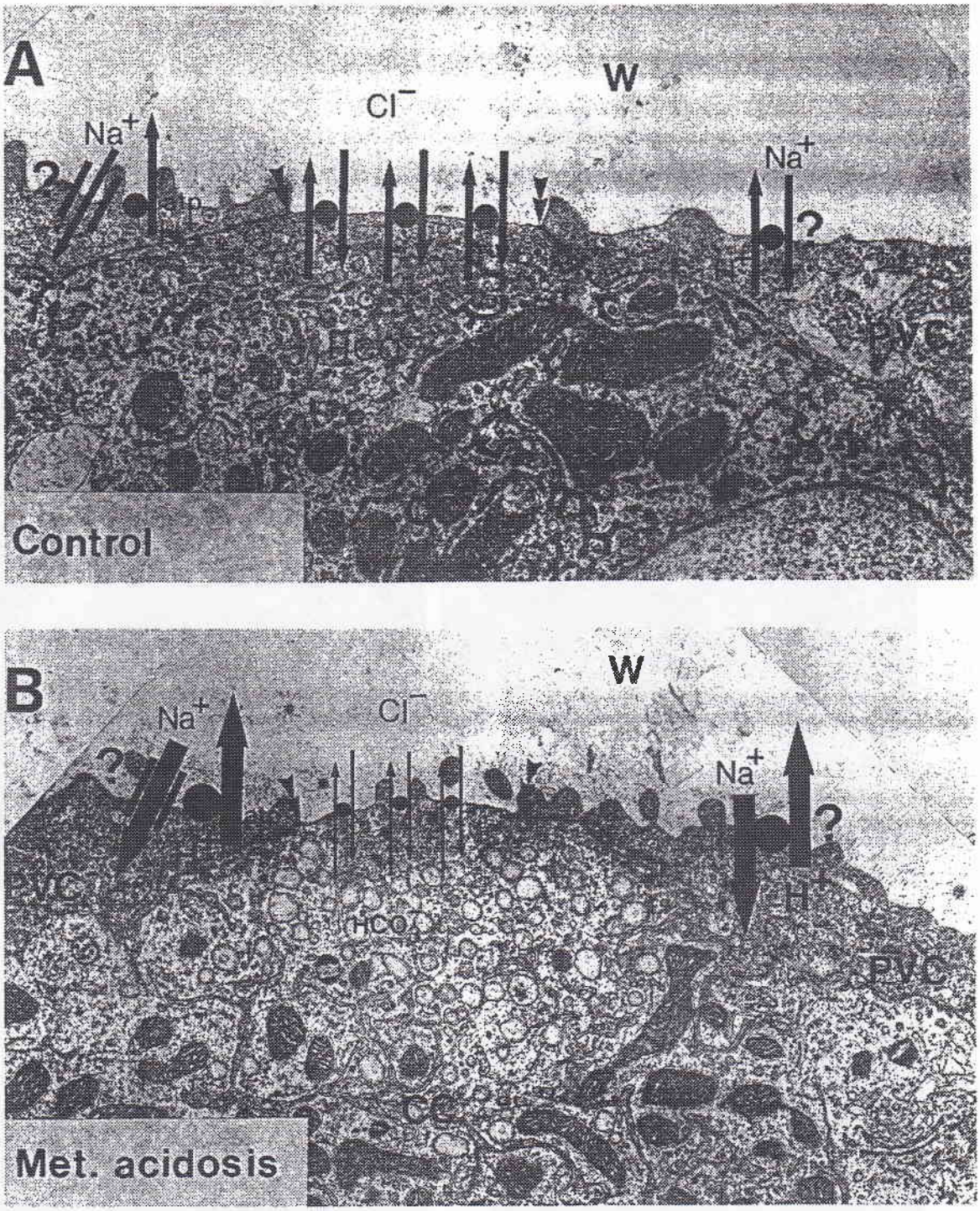

Fig. 7 Diagrammatic model illustrating the various mechanisms employed for acid-base regulation under control conditions (A), during a metabolic acidosis (B), a respiratory acidosis (C), and a metabolic alkalosis (D). The size of the arrows connected to the ion transport mechanisms indicates the relative rates according to the two-substrate model while the size of the lettering relative to control indicates the relative concentrations during each acid-base disturbance. Note that during a metabolic acidosis, $[HCO_3^-]$ is reduced and so is the rate for Cl^-/HCO_3^- exchange while $[H^+]$ is increased resulting in an increase in the rate of Na^+/H^+ exchange (H^+ pump/Na^+ channel) activity. During a respiratory acidosis, note the reduction in CC apical surface area and reduction in the number of Cl^-/HCO_3^- exchangers present. During a metabolic alkalosis, there is a combination of an increase in CC area (hence increased Cl^-/HCO_3^- exchangers), increased rate for the Cl^-/HCO_3^- exchangers, and decreased rate for the Na^+/H^+ (H^+ pump/Na^+ channel) according to the two-substrate model and a contribution from the diffusive efflux (broken arrows) mechanism to compensate for the acid-base disturbance. CC = chloride cell; PVC = pavement cell; W = water. See text for further details.

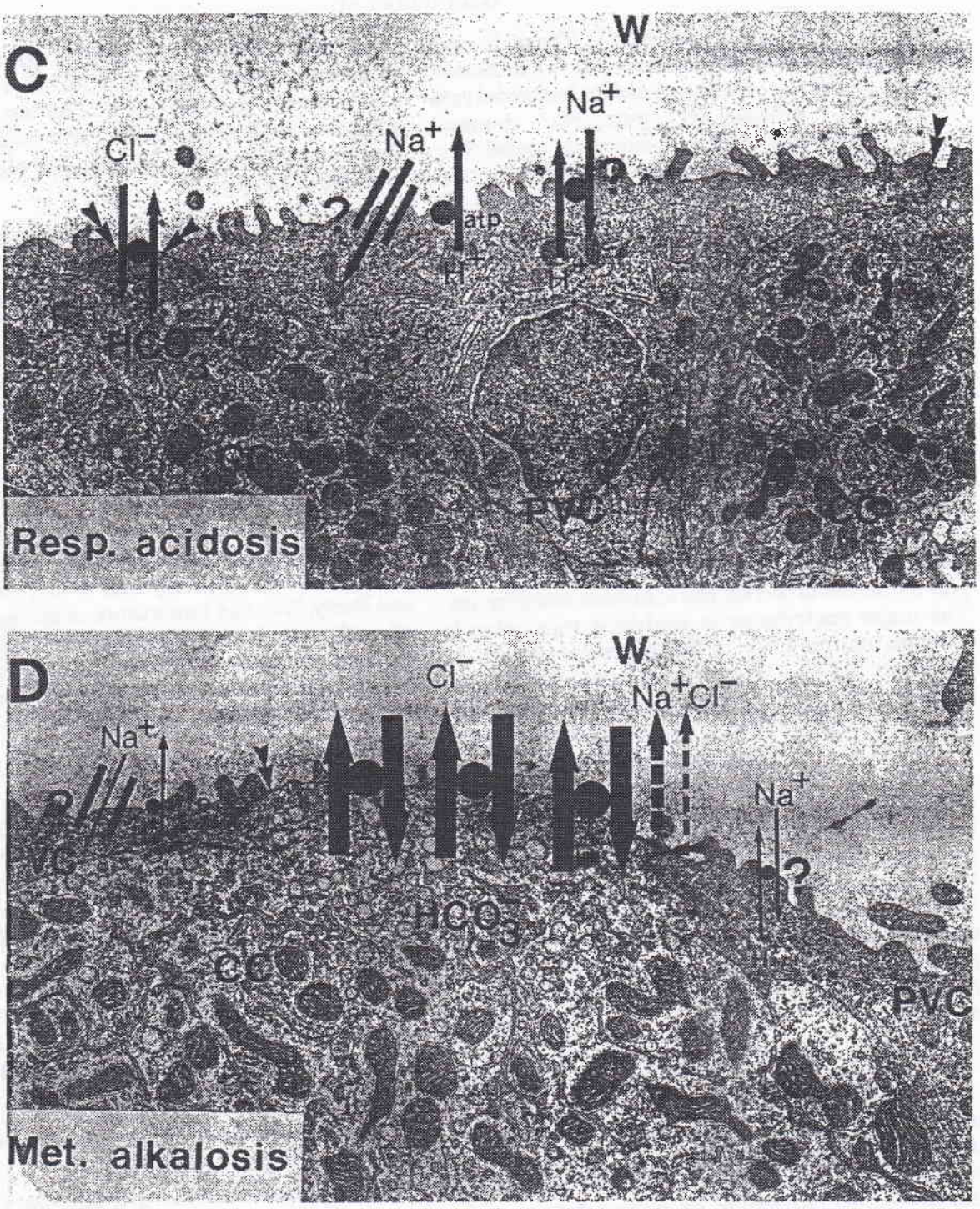

Figure 7. C, D.

various acid-base disturbances. In addition, the relative size of the arrows represents the relative "rate" for that transporter as determined by the substrate availability. The mechanism(s) involved in Na^+ uptake (Na^+/H^+ exchange, H^+ pump/Na^+ channel) remain controversial and therefore both have been indicated in each panel accompanied by a question mark (?). Cell junctions between CCs and PVCs are indicated by double arrowheads.

Under control conditions, the Cl^-/HCO_3^- exchange system located on the CC and the Na^+/H^+ exchange system (H^+ pump/Na^+ channel) located on the PVC function at a "normal" rate (Fig. 7A). During a metabolic acidosis, there is a reduction

in $[HCO_3^-]$ and an increase in $[H^+]$. These changes in counterion availability would result in a decreased rate of Cl^-/HCO_3^- exchange and an increased rate of Na^+/H^+ exchange (H^+ pump/Na^+ channel) activity according to the two-substrate model (Fig. 7B). During and after compensation of a respiratory acidosis, $[HCO_3^-]$ is greatly elevated yet J_{in}^{Cl-} is reduced (Fig. 4). This is accomplished by a reduction in the apical exposure of the CC, thereby reducing the number of Cl^-/HCO_3^- exchangers which are exposed to the water (Fig. 7C). This may be accomplished either by covering the CC by adjacent PVCs or actual removal of the Cl^-/HCO_3^- exchanges from the plasma membrane. In addition, many PVCs undergo changes in their ultrastructure including a more apical polarization and an increase in the number of mitochondria present. We suggest that this accounts for increased H^+ excretion through the Na^+/H^+ exchange (H^+ pump/Na^+ channel) mechanism during an acidosis. During a metabolic alkalosis, there are a number of strategies employed to correct the acid-base disturbance. The major contributor to acid-base regulation is through manipulation of the influx component. At this time, $[HCO_3^-]$ is greatly elevated and $[H^+]$ is reduced which are the appropriate changes in substrate availability to account for the increased "rate" of Cl^-/HCO_3^- exchange and reduced "rate" of Na^+/H^+ exchange (H^+ pump/Na^+ channel). Second, CCFA is increased rapidly to increase the number of Cl^-/HCO_3^- exchange sites. Third, during periods of metabolic alkalosis, there is also a contribution from *differential* diffusive efflux. The combination of all three mechanisms probably accounts for the relatively rapid (24 h) compensation from a metabolic alkalosis as opposed to the slower (48–72 h) compensation from a respiratory or metabolic acidosis.

Future directions

The freshwater fish gill continues to serve as a useful model for the examination of acid-base regulating epithelia, yet clearly there are several aspects of the model that remain controversial or unresolved. In particular, we believe future studies should focus on the areas described below.

Localization and quantification of the Cl^-/HCO_3^- and Na^+/H^+ (H^+ pump/Na^+ channel) mechanisms

Although we have presented evidence for the placement of these transport mechanisms on the CC and PVC, respectively, the evidence is circumstantial and correlational. Precise localization of the transporters through the use of immunocytochemical techniques is required. The effects of alterations in acid-base status on the numbers of transporters should also be examined to confirm that changes in transport numbers correlate with changes in *true* J_{max+}.

The mechanism(s) linking Na^+ uptake and H^+ excretion

Elucidation of the mechanism(s) involved in Na^+ uptake and H^+ excretion is essential. The two current models (Na^+/H^+ and H^+ pump/Na^+ channel mechanisms) should be tested using morphological, immunocytochemical, pharmacological, and/or electrophysiological techniques.

The mechanism(s) responsible for morphological adjustments in gill epithelia

Although it is known that elevated cortisol causes morphological alterations of the gill epithelia under control conditions (Perry and Wood, '85; Laurent and Perry, '90), the importance of cortisol in mediating the morphological responses to acid-base disturbance remains to be resolved. In addition, the possible involvement of other humoral agents (e.g., prolactin, atriopeptin) should also be investigated.

The contribution of neural control in the regulation of acid-base status

It is known that both adrenergic and cholinergic neurons are present in the gill (Bailly et al., '89) and the former have been implicated in the control of branchial Ca^{2+} uptake (Donald, '89). However, to our knowledge, no studies have been conducted to determine if there is a neuronal component in the regulation of acid-base status. Direct neuronal control of the activities of the Cl^-/HCO_3^- and Na^+/H^+ exchange (H^+ pump/Na^+ channel) systems remains a possibility which should be investigated.

The importance of the gill boundary layer in ionic and acid-base regulation

The importance of the gill boundary layer in ionoregulation, CO_2 excretion, and NH_3 excretion in fish living in freshwater has recently received a great deal of attention (Wright et al., '86; Wright et al., '89; Playle and Wood, '89). Water chemistry in the boundary layer may be very different from that in the bulk medium. Differences in the pH, ion concentrations, and/or acidic/basic equivalent concentrations in the gill microenvironment will affect substrate availability and competition for the external substrate site and, hence, acidic/basic

equivalent transfers to or from the fish. The importance of changes in this microenvironment and its effect on acid-base regulation should be investigated.

Importance of catecholamines in control of acid-base transfers

Pharmacological doses of catecholamines have been shown to variably alter the rates of influx for both Na^+ and Cl^- in FW fish. Initially, β-adrenergic stimulation was found to stimulate J_{in}^{Na+} (Girard and Payan, '77; Payan and Girard, '78; McDonald et al., '89a) and inhibit J_{in}^{Cl-} (Perry et al., '84). Catecholamines are released during some acid-base disturbances (e.g., acid infusion: Boutilier et al., '86; exercise: Tang and Boutilier, '88; hypercapnia: Perry et al., '87a). However, these surges in catecholamines are transient in nature and do not necessarily correspond directly to the alterations in ion fluxes. Furthermore, it has recently been shown that during hyperoxic acidosis, catecholamines are not released (Perry et al., '89) despite the alteration of ionic fluxes which occur (Wood et al., '84; Goss and Wood, '90a). Therefore, the role of catecholamines in the compensation of acid-base remains unclear. Future research should focus on the role of catecholamines in controlling ionic and acidic/basic equivalent fluxes at the gill.

Mechanisms and control of diffusive efflux of ions

Although it has been shown that there is a role for *differential* diffusive efflux in the regulation of acid-base status during metabolic alkalosis, the route of transport (presumably paracellular) and the control of this mechanism has not been investigated. Future studies should focus on the mechanisms for ion loss and examination of the controlling mechanisms.

ACKNOWLEDGMENTS

We thank many people for excellent help in the completion of the projects on which this review is based. At McMaster University in Hamilton, Canada, Steve Munger, Rod Rhem, and Michael Kovacevic and at CNRS in Strasbourg, France, Dr. Suzanne Dunel-Erb, Claudine Chevalier, Guy Bombarde, and Francois Scheer are all gratefully acknowledged. Funding for these projects was provided by NSERC grants to Dr. S.F. Perry and Dr. C.M. Wood and a CNRS grant to Dr. P.L. Laurent. G.G. Goss is supported by an NSERC postgraduate scholarship.

LITERATURE CITED

Avella, M., and M. Bornancin (1989) A new analysis of ammonia and sodium transport through the gills of the freshwater rainbow trout (*Salmo gairdneri*). J. Exp. Biol., *142*:155–175.

Avella, M., and M. Bornancin (1990) Ion fluxes in the gills of freshwater and seawater salmonid fish. In: Animal Nutrition and Transport Processes. 2. Transport, Respiration and Excretion: Comparative and Environmental Aspects. J.P. Truchot and B. Lahlou, eds. Karger, Basel, Comp. Physiol. Vol. 6, pp. 1–13.

Avella, M., A. Masoni, M. Bornancin, and N. Mayer-Gostan (1987) Gill morphology and sodium influx in the rainbow trout (*Salmo gairdneri*) acclimated to artificial freshwater environments. J. Exp. Zool., *241*:159–169.

Boutilier, R.G., G.K. Iwama, and D.J. Randall (1986) The promotion of catecholamine release in rainbow trout, *Salmo gairdneri*, by acute acidosis: Interactions between red cell pH and hemoglobin oxygen-carrying capacity. J. Exp. Biol., *123*:145–157.

Cameron, J.N. (1976) Branchial ion uptake in Arctic grayling: Resting values and the effects of acid-base disturbance. J. Exp. Biol., *64*:711–725.

Cameron, J.N. (1980) Body fluid pools, kidney function, and acid-base regulation in the freshwater catfish *Ictalurus punctatus*. J. Exp. Biol., *86*:171–185.

Cameron, J.N. (1989) Acid-base homeostasis: Past and present perspectives. Physiol. Zool. *62*:845–865.

Cameron, J.N., and G.K. Iwama (1987) Compensation of progressive hypercapnia in channel catfish and blue crabs. J. Exp. Biol., *133*:183–197.

Cameron, J.N., and G.K. Iwama (1989) Compromises between ion regulation and acid-base regulation in aquatic animals. Can. J. Zool., *67*:3078–3084.

De Renzis, G. (1975) The branchial chloride pump in the goldfish *Carassius auratus*: Relationships between Cl^-/HCO_3^- and Cl^-/Cl^- exchanges and the effect of thiocyanate. J. Exp. Biol., *63*:587–602.

De Renzis, G., and J. Maetz (1973) Studies on the mechanism of chloride absorption by the goldfish gill: Relation with acid-base regulation. J. Exp. Biol., *59*:339–358.

Donald, J.A. (1989) Adrenaline and branchial nerve stimulation inhibit $^{45}Ca^{++}$ influx into the gills of rainbow trout, *Salmo gairdneri*. J. Exp. Biol., *141*:441–445.

Eddy, F.B. (1975) The effect of calcium on gill potentials and on sodium and chloride fluxes in the goldfish, *Carassius auratus*. J. Comp. Physiol., *96*:131–142.

Evans, D.H. (1982) Salt and water exchange across vertebrate gills. In: Gills. Society for Experimental Biology Seminar Series, Vol. 16. D.F. Houlihan, J.C. Rankin, and T.J. Shuttleworth, eds. Cambridge University Press, Cambridge, pp. 149–172.

Evans, D.H., and J.N. Cameron (1986) Gill ammonia transport. J. Exp. Zool., *239*:17–23.

Florini, J.R., and C.S. Vestling (1957) Graphical determination of the dissociation constants for two-substrate enzyme systems. Biochim. Biophys. Acta, *25*:575–578.

Gamble, J.L. (1922) Carbonic acid and bicarbonate in urine. J. Biol. Chem., *51*:295–310.

Garcia-Romeu, F., and J. Maetz (1964) The mechanisms of Na^+ and Cl^- uptake by the gills of a freshwater fish, *Carassius auratus*. I. Evidence for an independent uptake of Na^+ and Cl^- ions. J. Gen. Physiol., *47*:1195–1207.

Girard, J.P., and P. Payan (1977) Kinetic analysis of sodium

and chloride influxes across the gills of trout in freshwater. J. Physiol. (Lond.), *273*:195–209.

Girard, J.P., and P. Payan (1980) Ion exchanges through respiratory and chloride cells in freshwater- and seawater-adapted teleosteans. Am. J. Physiol., *7*:R260–R268.

Goss, G.G., and C.M. Wood (1990a) Na^+ and Cl^- uptake kinetics, diffusive effluxes, and acidic equivalent fluxes across the gills of rainbow trout. II. Responses to environmental hyperoxia. J. Exp. Biol., *152*:521–547.

Goss, G.G., and C.M. Wood (1990b) Na^+ and Cl^- uptake kinetics, diffusive effluxes, and acidic equivalent fluxes across the gills of rainbow trout. II. Responses to bicarbonate loading. J. Exp. Biol., *152*:549–571.

Goss, G.G., and C.M. Wood (1991) Two-substrate kinetics analysis: A novel approach linking ionic and acid-base transport in freshwater trout. J. Comp. Physiol., *161*:635–646.

Goss, G.G., P.L. Laurent, and S.F. Perry (1992) Evidence for a morphological component in acid-base regulation during environmental hypercapnia in the brown bullhead (*Ictalurus nebulosus*). Cell Tissue Res., in press.

Heisler, N. (1986) Acid-base regulation in fishes. In: Acid-Base Regulation in Animals. N. Heisler, ed. Elsevier, Amsterdam, pp. 309–356.

Heisler, N., and P. Neumann (1980) The role of physico-chemical buffering and of bicarbonate transfer processes in intracellular pH regulation in response to changes of temperature in the larger spotted dogfish (*Scyliorhinus stellaris*). J. Exp. Biol., *85*:99–110.

Hughes, G. (1979) Scanning electron microscopy of the respiratory surfaces of trout gills. J. Zool., *188*:443–453.

Kirschner, L.B. (1988) Basis for apparent saturation kinetics of Na^+ influx in freshwater hyperregulators. Am. J. Physiol., *254*:R984–988.

Krogh, A. (1938) The active absorption of ions in some freshwater animals. Z. Verg. Physiol., *25*:335–350.

Krogh, A. (1939) Osmotic Regulation in Aquatic Animals. Cambridge University Press, Cambridge.

Laurent, P., and S. Dunel (1980) Morphology of gill epithelia in fish. Am. J. Physiol., *238*:R147–R159.

Laurent, P.L., and S.F. Perry (1990) Effects of cortisol on gill chloride cell morphology and ionic uptake in the freshwater trout, *Salmo gairdneri*. Cell Tissue Res., *259*:429–442.

Laurent, P., H. Höbe, and S. Dunel-Erb (1985) The role of environmental sodium chloride relative to calcium in gill morphology of freshwater salmonid fish. Cell Tissue Res., *240*:675–692.

Madera, J.L. (1988) Tight junction dynamics: Is paracellular transport regulated? Cell, *53*:497–498.

Maetz, J. (1956) Les échanges de sodium chez le poisson *Carrassius auratus* L. Action d'un inhibiteur de l'anhydrase carbonique. J. Physiol. (Paris), *48*:1085–1099.

Maetz, J. (1972) Branchial sodium exchange and ammonia excretion in the goldfish *Carassius auratus*. J. Exp. Biol., *58*:255–275.

Maetz, J. (1973) Na^+/NH_4^+, Na^+/H^+ exchanges and NH_3 movement across the gills of *Carassius auratus*. J. Exp. Biol., *58*:255–275.

Maetz, J., and F. Garcia-Romeu (1964) The mechanisms of Na^+ and Cl^- uptake by the gills of a freshwater fish, *Carassius auratus*. II. Evidence for NH_4^+/Na^+ and HCO_3^-/Cl^- exchanges. J. Gen. Physiol., *47*:1209–1227.

McDonald, D.G., and C.M. Wood (1981) Branchial and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri*. J. Exp. Biol., *98*:273–287.

McDonald, D.G., and M.S. Rogano (1986) Ion regulation by the rainbow trout in ion-poor water. Physiol. Zool., *59*:318–331.

McDonald, D.G., and E.T. Prior (1988) Branchial mechanisms of ion and acid-base regulation in the freshwater rainbow trout, *Salmo gairdneri*. Can. J. Zool., *66*:2699–2708.

McDonald, D.G., Walker, R.L., and Wilkes, P.R.H. (1983) The interaction of environmental calcium and low pH on the physiology of the rainbow trout, *Salmo gairdneri*. 2. Branchial ionoregulatory mechanisms. J. Exp. Biol., *102*:141–155.

McDonald, D.G., Y. Tang, and R.G. Boutilier (1989a) The role of β-adrenoreceptors in the recovery from exhaustive exercise in freshwater adapted rainbow trout. J. Exp. Biol. *147*:471–491.

McDonald, D.G., Y. Tang, and R.G. Boutilier (1989b) Acid and ion transfer across the gills of fish: Mechanisms and regulation. Can. J. Zool., *67*:3046–3054.

Michal, G. (1985) Determination of Michaelis constants and inhibitor constants. In: Methods of Enzymatic Analysis, Vol. 1. H.U. Bergmeyer, ed. Verlag Chemie, Weinheim pp. 86–104.

Milligan, C.L., and C.M. Wood (1986) Tissue intracellular acid-base status and the fate of lactate after exhaustive exercise in the rainbow trout. J. Exp. Biol., *123*:93–121.

Payan, J.P., and J.P. Girard (1978) Mise en évidence d'un échange Na^+/NH_4^+ dans la branchie de la truite adaptée à l'eau de mer: Contrôle adrénergique. C.R. Acad. Sci. Paris Ser. D., *286*:335–338.

Perry, S.F., and C.M. Wood (1985) Kinetics of branchial calcium uptake in the rainbow trout: Effects of acclimation to various external calcium levels. J. Exp. Biol., *116*:411–433.

Perry, S.F., and P.L. Laurent (1989) Adaptational responses of rainbow trout to lowered external NaCl concentration: Contribution of the branchial chloride cell. J. Exp. Biol., *147*: 147–168.

Perry, S.F., M.S. Haswell, D.J. Randall, and A.P. Farrell (1981) Branchial ion uptake and acid-base regulation in the rainbow trout *Salmo gairdneri*. J. Exp. Biol., *92*:289–303.

Perry, S.F., P. Payan, and J.P. Girard (1984) Adrenergic control of branchial chloride transport in the perfused head of the freshwater rainbow trout (*Salmo gairdneri*). J. Comp. Physiol., *154*:269–274.

Perry, S.F., S. Malone, and D. Ewing (1987a) Hypercapnic acidosis in the rainbow trout (*Salmo gairdneri*). I. Branchial ion fluxes and blood acid-base status. Can. J. Zool., *65*:888–895.

Perry, S.F., S. Malone, and D. Ewing (1987b) Hypercapnic acidosis in the rainbow trout (*Salmo gairdneri*). II. Renal ionic fluxes. Can. J. Zool., *65*:896–902.

Perry, S.F., R. Kinkead, P. Gallaugher, and D.J. Randall (1989) Evidence that hypoxemia promotes catecholamine release during hypercapnic acidosis in rainbow trout (*Salmo gairdneri*). Respir. Physiol., *77*:351–364.

Playle, R.C., and C.M. Wood (1989) Water chemistry changes in the gill microenvironment of rainbow trout: Experimental observations and theory. J. Comp. Physiol. B, *159*:527–537.

Schwartz, G.J., J. Barasch, and Q. Al-Awqati (1985) Plasticity of functional epithelial polarity. Nature, *318*:368–371.

Shaw, J. (1959) The absorption of sodium ions by the crayfish *Astacus pallipes* (Lereboullet). I. The effect of external and internal sodium concentration. J. Exp. Biol., *36*:126–144.

Steinmetz, P.R. (1987) Electrogenic proton transport by intercalated cells of tight urinary epithelia. In: Proton Passage Across Cell Membranes. G. Bock and J. Marsh, eds. Wiley, Chichester, pp. 122–138.

Stewart, P.A. (1978) Independent and dependant variables of acid-base control. Respir. Physiol., *33*:9–26.

Stewart, P.A. (1983) Modern quantitative acid-base chemistry. Can. J. Physiol. Pharmacol., *61*:1444–1461.

Tang, Y., and R.G. Boutilier (1988) Correlation between release of catecholamines and disturbance of blood pH in rainbow

trout (*Salmo gairdneri*) following exhaustive exercise. Am. J. Physiol., *255:*R395–R399.

Truchot, J.P. (1987) Comparative Aspects of Extracellular Acid-Base Balance. Springer Verlag, Berlin.

Vermette, M.G., and S.F. Perry (1988) The effects of prolonged epinephrine infusion on the physiology of the rainbow trout, *Salmo gairdneri*. III: Branchial solute fluxes. J. Exp. Biol., *128:*255–267.

Wheatly, M.G., H. Hōbe, and C.M. Wood (1984) The mechanisms of acid-base and ionoregulation in the freshwater rainbow trout during environmental hyperoxia and subsequent normoxia. II. The role of the kidney. Respir. Physiol., *55:* 155–173.

Wood, C.M. (1988) Acid-base and ionic exchanges at gills and kidney after exhaustive exercise in the rainbow trout. J. Exp. Biol., *136:*461–481.

Wood, C.M., and D.J. Randall (1973) Sodium balance in the rainbow trout (*Salmo gairdneri*) during extended exercise. J. Comp. Physiol., *82:*235–256.

Wood, C.M., and F.H. Caldwell (1978) Renal regulation of acid-base balance in a freshwater fish. J. Exp. Zool., *205:*301–307.

Wood, C.M., and E.B. Jackson (1980) Blood acid-base regulation during environmental hyperoxia in the rainbow trout (*Salmo gairdneri*). Respir. Physiol., *42:*351–372.

Wood, C.M., and G.G. Goss (1990) Kinetic analysis of the relationships between ion exchange and acid-base regulation at the gills of freshwater fish. In: Animal Nutrition and Transport Processes. 2. Transport, Respiration and Excretion: Comparative and Environmental Aspects. J.P. Truchot and B. Lahloue, eds. Karger, Basel, Comp. Physiol., Vol. 6, pp. 119–136.

Wood, C.M., M. Wheatly, and H. Hōbe (1984) The mechanisms of acid-base and ionoregulation in the freshwater rainbow trout during environmental hyperoxia and subsequent normoxia. III. Branchial exchanges. Respir. Physiol., *55:* 175–192.

Wright, P., T. Heming, and D.J. Randall (1986) Downstream pH changes in water flowing over the gills of rainbow trout. J. Exp. Biol., *126:*499–481.

Wright, P.A., D.J. Randall, and S.F. Perry (1989) Fish gill water boundary layer: A site of linkage between CO_2 and ammonia excretion. J. Comp. Physiol., *158:*627–635.