Branchial Ion and Acid-Base Transfer in Freshwater Teleost Fish: Environmental Hyperoxia as a Probe

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Abstract

In freshwater fish, linked ion and acid-base fluxes across the gills are dynamically manipulated to regulate internal acid-base status in the face of environmental challenges. Relevant theory, methodology, and a synthesis of current ideas are presented, based on experiments with hypercapnia, low pH, acid and base infusion, temperature change, enforced exercise, toxicants, and other challenges. Hyperoxia may be the most useful of these treatments, for it is devoid of confounding factors caused by other probes—high water $[H^+]$*, catecholamine mobilization, and metabolic rate changes. In rainbow trout, hyperoxia (inspired* P_{O_2} $[P_{IO_2}] > 500$ *Torr) induces a pure respiratory acidosis that is fully compensated over 72 h by net acid excretion, more than 90% of which occurs via the gills. Return to normoxia induces a pure metabolic alkalosis that is more rapidly compensated by branchial acid uptake (= base excretion). Use of this protocol has demonstrated that (1) respiratory acidosis is largely compensated by adjustments of* Cl^- *versus base exchange, while both* Cl^- *versus base and* Na^+ *versus acid exchanges contribute during metabolic alkalosis; (2) these dynamic adjustments in* Na^+ *and* Cl^- *influx are achieved by complex changes in both substrate affinity and maximum transport capacity* (J_{max})*;* Na^+ *and* Cl^- *affinities appear to be maximal under control conditions and can only be decreased, but* J_{max} *values can be either increased or decreased depending on the internal acid-base status; (3) differential diffusive efflux of* Na^+ *relative to* Cl^- *provides an additional mechanism of net acid flux during alkalosis; (4) large decreases in branchial intracellular fluid volume,* Na^+*, and* Cl^- *levels occur during respiratory acidosis and are corrected during normoxic recovery; (5) mean gill intracellular pH remains extremely stable. The importance of the internal acid-base status of the transport cells, and a possible regulatory role for extracellular pH, are discussed.*

Introduction

The mechanisms of ion and acid-base transfer across the gills of freshwater fish have been studied intensively in recent years. It is now clear that changes

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in the chemical and physical characteristics of the external environment can have profound effects on these processes, and furthermore that such disturbances can be very useful experimental probes of normal gill function. The first objective of the present article is to summarize the experimental background to our current understanding and to synthesize major points of consensus or dispute, employing data from a variety of freshwater species. The second goal is to outline recent progress using environmental hyperoxia as a probe for analyzing the branchial mechanisms of ion and acid-base regulation in the rainbow trout. Morphological aspects of these processes are considered in detail by Laurent and Perry (1991).

Historical Background

The high capacitance of water for CO_2 relative to O_2 restricts the ability of aquatic animals to regulate internal acid-base status through ventilatory adjustments of blood P_{CO_2} (Rahn 1966; Randall and Cameron 1973; Cameron 1978, 1979). Instead, the major portion of pH control is achieved by the exchange of acidic or basic equivalents between the extracellular fluid and the external environment, resulting in changes in plasma HCO_3^- levels. The presence of independent, electroneutral Na^+-versus-"acid" (H^+, NH_4^+) and Cl^--versus-"base" (HCO_3^-, OH^-) exchanges on the gills, involved simultaneously in ion uptake and acid-base regulation (fig. 1), was first suggested by August Krogh in the 1930s (summarized in Krogh 1939). However, it was not until almost three decades later that concrete evidence for the existence of these exchanges began to accumulate (Shaw 1959; Garcia-Romeu and Maetz 1964; Maetz and Garcia-Romeu 1964; Kerstetter, Kirschner, and Rafuse 1970; Kerstetter and Kirschner 1972; Maetz 1972, 1973; de Renzis and Maetz 1973; Kirschner, Greenwald, and Kerstetter 1973; Ehrenfeld 1974; de Renzis 1975). This progress was made possible both by the application of radiotracer techniques for the measurement of unidirectional fluxes of Na^+ and Cl^- between an animal and its aquatic environment and by the development of theory and practice for measuring the associated acid-base fluxes. (Note: the acids and bases are alternatively termed "acidic or basic equivalents," "fixed acid or base," "nonvolatile acid or base," "metabolic acid or base," "delta bicarbonate," "bicarbonate equivalents," and "net H^+" by various authors. For the sake of convenience, the simpler if less rigorous terms "acid" or "base" will be used here, with the recognition that acid excretion is equivalent to base uptake, and vice versa, in terms of the acid-base status of the animal. In practical terms net acid flux can be measured as the sum, signs considered, of the total ammonia flux [i.e., $NH_3 + NH_4^+$]

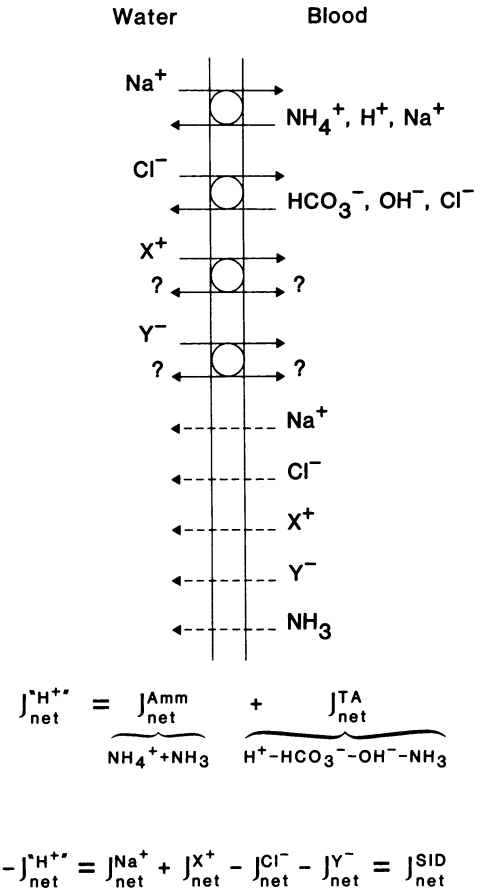

Fig. 1. A simple model of ion and acid-base exchanges across the gills of a freshwater fish. X^+ and Y^- represent other strongly dissociated cations and anions, respectively. Solid lines represent carrier-mediated fluxes; broken lines represent simple diffusive fluxes. The first equation illustrates the principle behind the measurement of net acid flux ($J^{H^+}_{net}$) as the sum of the total ammonia flux (J^{Amm}_{net}) and the titratable acidity flux (J^{TA}_{net}), with signs considered. The second equation illustrates the relationship dictated by strong ion difference (SID) theory between net acid flux and the difference between net strong cation and net strong anion flux (J^{SID}_{net}).

and the flux of the component that changes water pH ["titratable acidity"], as illustrated in fig. 1. The latter is usually quantified by titration to a fixed pH, or by measuring the change in water pH or HCO_3^- concentration at constant P_{CO_2} [for a detailed description of theory and methods, see Maetz 1973; Cameron 1980; McDonald and Wood 1981; Holeton, Neumann, and Heisler 1983*b*].)

Notably, Maetz and Garcia-Romeu (1964) demonstrated that injection of internal NH_4^+ and HCO_3^- loads stimulated Na^+ and Cl^- uptake, respectively, in goldfish, while addition of NH_4^+ and HCO_3^- to the external environment inhibited Na^+ and Cl^- uptake, respectively. Kerstetter and Kirschner (1972) were the first to directly relate blood pH and ion exchange, showing that HCO_3^- injections simultaneously increased blood pH and Cl^- influx in anaesthetized rainbow trout. Maetz (1973) and de Renzis and Maetz (1973) provided the first direct correlations between net Na^+ flux and net acid flux, and between net Cl^- flux and net base flux (fig. 2). These were obtained by pretreating goldfish in deionized water to stimulate their Na^+ and Cl^- uptake rates and then measuring the Na^+-versus-acid relationship in an Na_2SO_4 solution (to eliminate Cl^--versus-base exchange) and the Cl^--versus-base relationship in a choline chloride solution (to eliminate Na^+-versus-acid

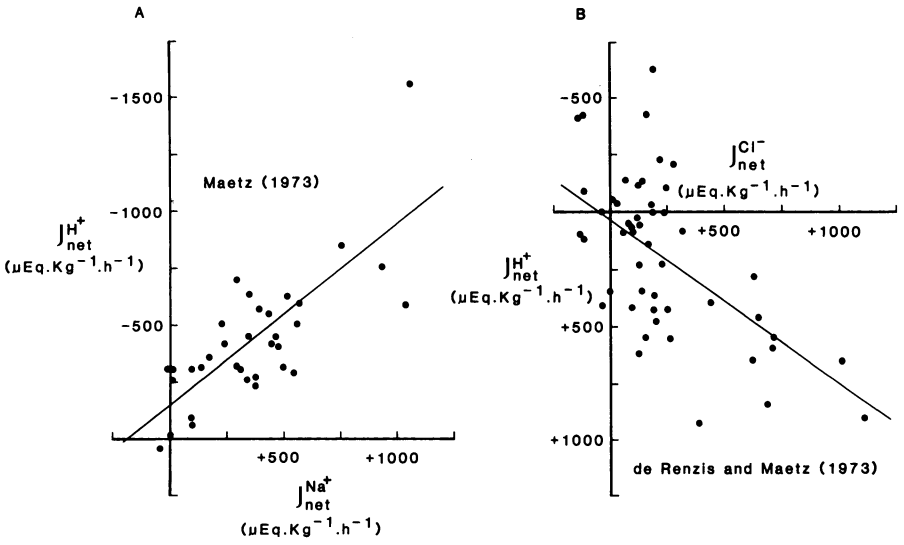

Fig. 2. Relationships (A) between net Na^+ flux ($J^{Na^+}_{net}$) and net acid flux ($J^{H^+}_{net}$) and (B) between net Cl^- flux ($J^{Cl^-}_{net}$) and net base flux (negative $J^{H^+}_{net}$) across the gills of goldfish (Carassius auratus), as first demonstrated by Maetz (1973) and de Renzis and Maetz (1973), respectively. Units are µeq/kg/h. $J^{H^+}_{net}$ was measured as the sum of the total ammonia flux (J^{Amm}_{net}) and the titratable acidity flux (J^{TA}_{net}), with signs considered. The fish were preadapted to deionized water for 3 wk to stimulate uptake rates, fitted with bladder catheters to eliminate renal fluxes, and then tested in either (A) Na_2SO_4 media, to eliminate Cl^--vs.-base exchange, or (B) choline chloride media, to eliminate Na^+-vs.-acid exchange. The figure is redrawn and recalculated from Maetz (1973) and de Renzis and Maetz (1973).

exchange). These workers also supplied the first evidence that branchial ion exchange could affect internal acid-base status, and vice versa. Preadaptation of goldfish to Na^+-free media induced internal acidosis, followed by a preferential stimulation of Na^+ uptake upon return to normal media. Preadaptation to Cl^--free media induced alkalosis, and a subsequent preferential Cl^- uptake. However, interpretation of all these experiments was complicated by the fact that internal ion levels as well as acid-base status were disturbed by loading with NH_4^+ and HCO_3^- salts and by adaptation to artificial media. Furthermore, the blood acid-base data, obtained by caudal puncture, were questionable.

The modern era of understanding was ushered in by the work of Cameron (1976), who was the first to demonstrate that branchial exchanges could be dynamically adjusted by the animal in a manner that would help correct an internal acid-base disturbance, even though there was no initial ionic disturbance. Specifically, using the Arctic grayling, Cameron (1976) employed environmental hypercapnia to induce an internal respiratory acidosis and found that the fish responded with decreased branchial Cl^- influx and increased Na^+ influx, interpreted as achieving acid excretion and/or base uptake. Using cannulation methodology to obtain reliable acid-base data for blood, Cameron (1976) also showed that an acute rise in temperature induced an internal alkalosis, the compensation of which was associated with a relatively greater stimulation of Cl^- than Na^+ influx.

Cameron (1979, 1980) was also the first to explicitly recognize the importance of the SID approach elaborated by Stewart (1978, 1983) in understanding this type of branchial response, though it is clear that Maetz's group (Maetz 1972, 1973; de Renzis and Maetz 1973; de Renzis 1975) also adopted the same theoretical orientation. The SID is the difference in concentration between all fully dissociated cations and anions in solution, and is viewed as one of the three "independent" variables that determine acid-base status (the other two are P_{CO_2} and total weak acid present, mainly protein in blood). Previously, acid-base regulation and ionoregulation had been considered separate or only partially related phenomena, a viewpoint that is still unfortunately prevalent today in some quarters. In contrast, SID theory demonstrates that the two are fundamentally linked via physicochemical principles, most importantly the constraints of electrical neutrality. The SID approach is complex in theoretical detail (Stewart 1978, 1983) and often clumsy in practice (see below), but conceptually illuminating. The basic principle is illustrated in figure 1. Simply stated, the net difference between strong cation (mainly Na^+) and strong anion (mainly Cl^-) fluxes across the gills will dictate the net acid flux in the opposite direction, no matter how these fluxes occur (fig. 1). Viewed in this light, an acid uptake

can result equally from a loss of Na^+ or a gain of Cl^-. While the mechanism may well occur via direct carrier-mediated coupling (e.g., Na^+/acid, Cl^-/base exchangers), this is not the only possibility; simple diffusive fluxes of strong cations and anions may also contribute (fig. 1). Thus an acid "flux" does not necessarily involve the physical movement of H^+ et cetera across the gills, for an aqueous medium is an infinite source or sink of H^+ and OH^- ions. If the net effect of the strong electrolyte flux is to change the internal SID, there has occurred an addition of acid or base to the blood, and thus effectively an acid flux.

Modern Studies of Branchial Ion and Acid-Base Transfer

Over the past 15 yr, a large number of studies have further examined the relationship(s) between branchial ion and acid-base exchange in freshwater fish, as summarized in the Appendix. In general, these investigations have applied an experimental disturbance to either the external or internal environment of intact, nonanesthetized fish and then followed (1) the net acid-base flux at the gills, (2) the unidirectional and net Na^+ and/or Cl^- fluxes at the gills, and (3) the internal acid-base and ionic status, as assessed from blood samples drawn from chronic, indwelling cannulae. Only studies that fulfill at least two of these three criteria have been included in the Appendix. Two conclusions can be drawn immediately from this summary. First, as is usually the case in fish physiology, our knowledge is based on only a very few species, most notably the rainbow trout, the channel catfish, and the European carp. The goldfish, the standard subject of earlier workers, appears to have fallen out of favor, probably because it is too small to reliably cannulate. Second, the most commonly used experimental treatments have been environmental hypercapnia, perhaps because of Cameron's (1976) original success with this approach, and environmental acid exposure, the latter reflecting widespread concern about acidic precipitation.

Current Synthesis

A detailed analysis of these papers (and related studies) yields a number of specific conclusions.

1. There is general consensus that fish dynamically manipulate their branchial ionic exchange mechanisms in order to compensate imposed acid-base disturbances, unless the severity of the treatment (e.g., acute acid exposure) prevents this. The few studies yielding negative results on this

point can be explained by the ion-poor nature of the ambient water (e.g., Cameron and Wood 1978; Perry et al. 1981; Heisler 1982) or by reinterpretation of the original data—see, for example, the discussion of Wood, Wheatly, and Hobe (1984) on Bornancin, de Renzis, and Maetz (1977).

2. In those studies where the appropriate measurements have been made the relationship predicted by SID theory appears to hold (fig. 1). That is, the net acid flux is approximately equal but opposite to the difference between net strong cation and anion flux. On a quantitative basis, Na^+ and Cl^- are by far the most important electrolytes involved, though, in some investigations, overall agreement has been improved by also taking into account the much smaller fluxes of other strong electrolytes (e.g., K^+, Ca^{2+}, Mg^{2+}, $SO_4^=$).

3. It is clear that both Na^+-versus-acid and Cl^--versus-base exchanges can be dynamically adjusted. An important exception is the eel, where Cl^--versus-base transfer is virtually absent (see, e.g., Hyde and Perry 1987, 1989). In general, adjustment of Cl^--versus-base exchange seems to be quantitatively more important than modulation of Na^+-versus-acid transfer. However, this conclusion has been challenged by two recent studies of McDonald, Tang, and Boutilier (1989*a*, 1989*b*) on trout. There is no consensus on the circumstances that determine whether one, the other, or both are altered.

4. The morphological localization of Na^+-versus-acid and Cl^--versus-base exchanges on the gill epithelium of freshwater teleosts has been the subject of lively controversy. One view, based largely on work with the isolated perfused trout head preparation, favors the pavement or "respiratory" cells (e.g., Girard and Payan 1980; Payan, Girard, and Mayer-Gostan 1984). The other, based largely on descriptive morphology, favors the mitochondrial-rich or "chloride" cells (e.g., Laurent, Hobe, and Dunet-Erb 1985). A consensus now appears to be building in favor of the latter as the major, though not necessarily sole, site of ion exchange. This conclusion is based on correlations between ion transport activity and chloride-cell structure and distribution (Laurent et al. 1985; Perry and Wood 1985; Avella et al. 1987; Cameron and Iwama 1987; Perry and Laurent 1989; Laurent and Perry 1991) and on a methodological reassessment of the perfused trout head preparation and the circulatory pathways therein relative to chloride-cell distribution (Gardaire et al. 1985).

5. There is general agreement that the exchange mechanisms are located on the apical (water-facing) membranes of the transporting cells, with the major point of energy input being Na^+/K^+ ATPase pumps on the basolateral membranes. Kirschner (1980) and McDonald and Prior (1988) have provided useful model diagrams. While such a system is probably sufficient to drive Na^+ influx, it is unclear whether the electrochemical gradients are

large enough to also support Cl^- influx. In this regard, it is interesting that the presence of a Cl^- and/or HCO_3^--dependent ATPase has been reported in the gills of freshwater fish (Kerstetter and Kirschner 1974; Bornancin et al. 1977; de Renzis and Bornancin 1977). Measurements of intracellular ion levels and membrane potentials in the transporting cells, both of which are currently lacking, are required before any definitive conclusions can be drawn.

6. Both Na^+ and Cl^- influxes have been characterized as saturable processes whose rates vary with external substrate concentrations (e.g., $[Na^+]_{ext}$) in an approximately hyperbolic manner. As such, these relationships can be described by the constants J_{max} (maximum influx rate) and K_m (affinity = external substrate concentration at ½ J_{max}) according to classical one-substrate Michaelis-Menten analysis:

$$J_{in} = \frac{J_{max}^{Na^+} \times [Na^+]_{ext}}{K_m^{Na^+} + [Na^+]_{ext}} \quad (1)$$

These parameters vary considerably between species; within a species, adaptation to more dilute media (i.e., lower external substrate concentration) is achieved by substantial increases in J_{max}, sometimes accompanied by small decreases in K_m. Changes in J_{max} are thought to represent alterations in the number of transport sites available, and have recently been correlated with changes in the number of chloride cells on the lamellar epithelium (Avella et al. 1987). To date, there is no information whether these kinetic parameters can be dynamically manipulated in response to internal acid-base status.

7. There appears to be little dispute that HCO_3^-, and not OH^-, is the base anion exchanged against Cl^- (although there is no experimental evidence on this point!). Conversely, it remains highly controversial whether H^+ or NH_4^+ is the acid cation exchanged against Na^+ (despite a massive amount of experimental work directed at this question). Indeed, it remains unsettled whether ammonia moves across the gills as NH_3, NH_4^+, or both. Analysis of this controversy is beyond the scope of this article; the reader is referred to Maetz (1973), Kormanik and Cameron (1981), Cameron and Heisler (1983), Holeton et al. (1983*b*), Wright and Wood (1985), Cameron (1986), Evans and Cameron (1986), McDonald and Prior (1988), Wood (1988, 1989), McDonald et al. (1989*a*), and Randall, Lin, and Wright (1991) for the detailed arguments. Given this uncertainty, at present there appears to be no reason to abandon the "flexible" model (Maetz 1973; Wright and Wood 1985)—that is, that Na^+ can be exchanged against either or both of NH_4^+ and H^+, and ammonia flux can occur either via NH_3 diffusion or Na^+-versus-NH_4^+ exchange. The relative rates of these processes will depend on the relevant

electrochemical gradients, the acid-base status of the animal, the relative availability of the substrates, and the chemistry of the boundary layer at the gills (discussed in detail by Randall et al. 1991). However, it should be emphasized that, as long as net acid flux is measured correctly (as the sum of the total ammonia and titratable components; see fig. 1 and discussion in Historical Background), the resolution of this controversy is immaterial to the quantification of Na^+-versus-acid exchange.

8. Until very recently, attention has been focused exclusively on modulation of Na^+ and Cl^- influxes as mechanisms for acid-base adjustment. A priori, there is no reason why modulation of Na^+ and Cl^- effluxes could not also play a role in freshwater fish. (Note: for the sake of clarity, the term "outflux" is used to refer to the total outflux determined by radiotracer methods, which may contain a component of exchange diffusion, while the term "efflux" is reserved for that portion which does not comprise exchange diffusion.) This could occur either by reversal of the exchange mechanisms (e.g., Na^+ efflux vs. H^+ influx) or by differential diffusive efflux of Na^+ and Cl^- leading to SID changes (fig. 1). These possibilities were suggested by Wood et al. (1984) and Wood (1988) on the basis of unidirectional flux measurements, while McDonald and Prior (1988) and McDonald et al. (1989*a*) presented detailed quantitative arguments for differential diffusive efflux regulation based on similar radiotracer methods. A complication of all these studies is that exchange diffusion (fig. 1) accounts for a significant fraction of the unidirectional ion fluxes in freshwater fish (see, e.g., Maetz 1972; Wood and Randall 1973; de Renzis 1975). With standard radiotracer techniques, the possibility exists that differential Na^+-versus-Cl^- outflux could be simply a difference in the extent of Na^+/Na^+-versus-Cl^-/Cl^- exchange diffusion, rather than a true differential efflux.

9. Little is known of the neuronal or hormonal mechanisms for the control of branchial fluxes of ions versus acids and bases. The involvement of circulating catecholamines has often been suggested, on the basis of observations that Na^+ influx is increased via β-adrenoreceptor stimulation and Cl^- influx via α-receptor stimulation in the perfused trout head preparation (Payan and Girard 1978; Perry, Payan, and Girard 1984). Preliminary in vivo experiments with epinephrine infusion supported this idea (Wood and Perry 1985), but more thorough pharmacological studies have yielded equivocal results (Vermette and Perry 1987; van Dijk and Wood 1988; Vermette and Perry 1988*a*, 1988*b*; McDonald et al. 1989*b*). At present, it would appear that exogenously administered catecholamines (generally very high doses) promote net branchial acid excretion, but there is little evidence that endogenously mobilized catecholamines do the same. The possibility exists that at least some ion and acid-base effects of exogenous catecholamines

and adrenergic antagonists are indirect, resulting from changes in total perfusion or flow distribution within the secondary lamellae, gill filaments, and recurrent circulation of the gills. Adrenergic nerves in the gills (Donald 1984) have recently been implicated in the control of branchial Ca^{2+} uptake (Donald 1989) and could be involved instead of, or in addition to, plasma catecholamines but have not yet been investigated in relation to acid-base balance. Cortisol mobilization is another possibility, though the only evidence to date is of very long term rather than dynamic effects on branchial ion exchanges (Perry and Wood 1985; Perry and Laurent 1989; Laurent and Perry 1991).

10. An alternative regulatory mechanism suggested by several studies (de Renzis 1975; Wood et al. 1984; McDonald and Prior 1988) is that the rates of Na^+-versus-acid and Cl^--versus-base exchanges may be linked directly to the internal acid-base status of the fish. Such a system would act as an automatic servomechanism to control acid-base balance. Internal alkalosis would increase the availability of base anions (i.e., HCO_3^-) and decrease the availability of acid cations (i.e., H^+ and NH_4^+), thereby driving Cl^- influx and base excretion and inhibiting (or even reversing) Na^+ influx and acid excretion. Internal acidosis would have directly opposite effects. Thus, the transport systems might respond to the internal substrate concentrations (acid or base) in a similar manner to their well-documented responses to external substrate concentrations (Na^+ or Cl^-; see 6 above). As the exchange mechanisms are thought to be located on the apical membranes, the relevant internal acid-base status would be presumably that present inside the transporting cells. To date, there have been no measurements of gill intracellular pH or kinetic analyses of transport in relation to internal acid-base status, so there is no direct evidence to support this idea.

11. Branchial ionoregulatory mechanisms are perturbed by a wide variety of environmental toxicants; such effects are often the proximate cause of lethality. Evans (1987) has provided a detailed review of the recent literature, and additional information on the uptake of xenobiotics is presented by McKim and Erickson (1991). In general, toxicants tend to inhibit Na^+ and Cl^- influxes and stimulate Na^+ and Cl^- outfluxes, with accompanying disturbances in internal acid-base status if the net ion fluxes result in SID changes. The latter situation has been worked out in detail for acute environmental acid exposure (Wood 1989). Elevations in Na^+ and Cl^- outfluxes result from increases in gill diffusive permeability associated with damage or inflammation of the epithelium caused by the toxicants. The mechanisms of influx inhibition range from direct competition (e.g., blockade of Na^+ influx by high environmental H^+ and NH_4^+) through mixed competitive and

noncompetitive inhibition (e.g., Cu effects on Na^+ influx) to physical damage of the chloride cells (e.g., Al effects).

In summary, it is clear from the preceding synthesis that our understanding of branchial mechanisms of ion and base transfer in freshwater fish has increased dramatically over the past 15 yr. However, it is equally clear that this progress has uncovered new areas of uncertainty that were not even considered 15 yr ago. In the following experimental section on the freshwater rainbow trout, environmental hyperoxia has been employed as an experimental probe for some of these controversial areas.

Environmental Hyperoxia as an Experimental Probe

In the past, hyperoxia has been used only sparingly as a tool for analyzing the branchial mechanisms of ion and acid-base regulation (see the Appendix, table A1); however, it may well be the most useful environmental disturbance for probing normal gill function. In particular, hyperoxia and its removal induces internal acid-base disturbances while avoiding the increased water $[H^+]$ associated with environmental hypercapnia or acid exposure; external H^+ may directly inhibit branchial exchanges in addition to the intended effect on internal acid-base status (see, e.g., Perry, Malone, and Ewing 1987; Wood 1989). Furthermore, hyperoxia does not elevate circulating catecholamine levels (Perry et al. 1989), in contrast with hypercapnia (Perry et al. 1987), acid exposure (Wood 1989), acid infusions (Boutilier, Iwama, and Randall 1986), strenuous exercise (Milligan and Wood 1987), and epinephrine infusions (Perry and Vermette 1987). Hyperoxia also avoids the large changes in metabolic rate associated with environmental temperature change and exercise. The one complicating feature of hyperoxia is an accompanying alteration of gill water and blood flow, but this is common to all the treatments listed in the Appendix, table A1. The present synthesis of hyperoxic responses in the freshwater rainbow trout integrates new, previously unpublished information with earlier data obtained under identical exposure conditions (Wood and Jackson 1980; Hobe, Wood, and Wheatly 1984*b;* Wheatly, Hobe, and Wood 1984; Wood et al. 1984).

Material and Methods

A standard protocol, consisting of a normoxic control period, then 72 h of hyperoxia (inspired $P_{O_2} > 500$ Torr), and finally a 24-h period of normoxic recovery, has been used throughout. The exposure regime, water-quality

characteristics, cannulation of the fish, blood sampling, flux-measurement techniques, most other analytical methods, and statistical analyses have been described by Hobe et al. (1984*b*), Wheatly et al. (1984), and Wood et al. (1984). Therefore, only previously undescribed methods are given here.

Ion and Acid Fluxes and Ion Uptake Kinetics

Fluxes were measured under control normoxic conditions, during the first 8 h and final 7 h of hyperoxic exposure, and during the first 8 h of normoxic recovery. In order to increase temporal resolution in flux measurements, a specially designed low-volume chamber (~1.4 L for a 300-g fish) that did not stress the fish, as judged by normal acid-base status, ion balance, and cortisol levels, was employed. This system allowed measurements of unidirectional Na^+ and Cl^- and net acid fluxes over 0.5-h periods. Kinetic measurements of Na^+ and Cl^- uptake took approximately 4 h to complete. Therefore, these were performed during segments of the hyperoxia protocol where the 0.5-h measurement series had determined that steady-state fluxes were relatively stable. Before and after the kinetic measurements, fish were held in normal tap water. The actual kinetic determinations were performed in a synthetic medium that lacked NaCl but had the same pH, Ca^{2+}, Mg^{2+}, and titratable alkalinity as normal tap water. For each measurement, the water NaCl level was sequentially increased at 0.5-h intervals (nominally 50, 150, 300, 600, 1,200, and 2,400 μeq/L) by addition from a radioactively labeled stock solution. Thus, specific activity in the water was kept relatively constant. A brief radioisotopic mixing period after each sequential addition of the stock solution (prior to the start of the flux period) served to minimize error associated with simple adsorption of label to mucus on the fish's surface. The uptake rate in each period was determined from the disappearance of ^{22}Na or ^{36}Cl from the water (into the fish). Values of K_m and J_{max} in the Michaelis-Menten relationship (eq. [1]) were determined separately for each fish by standard Eadie-Hofstee regression analyses (Michal 1985).

Diffusive Effluxes, Transepithelial Potential (TEP), and Cortisol

Diffusive effluxes were measured according to an approach pioneered by Shaw (1959). This method has been neglected in favor of radiotracer techniques in recent years, but has the great advantage of avoiding the complicating effects of exchange diffusion discussed earlier. In brief, at various times during the hyperoxic regime, the normal tap water in the low-volume fish chamber was rapidly replaced with the synthetic NaCl-free medium. Disturbance to the fish was minimal. The rates of appearance of Na^+ and

Cl^- in the medium over the first 10 min provided a direct measure of their diffusive efflux rates; exchange diffusion was eliminated because influx was prevented by the absence of external substrate.

Branchial TEP (blood relative to the external normal tap water as zero) was measured at various times during the hyperoxic regime by a high-impedance voltmeter (10^{-12} Ω), as described by Perry and Wood (1985). The dorsal aortic catheter was used to make electrical contact with blood without disturbance to the fish. Plasma cortisol was measured with a ^{125}I-radioimmunoassay kit (Corning).

Intracellular pHi, Fluid Volumes, and Intracellular Ions

Fish were killed at various times during the hyperoxic exposure regime for the measurement of these parameters in the gills. Intracellular pH (pHi) was determined from the plasma-to-tissue distribution (12 h after injection) of ^{14}C-DMO (5,5-dimethyl-2,4-oxazolidinedione; 7 μCi/kg), with ^{3}H-PEG-4000 (polyethylene glycol, mol wt = 4,000; 28 μCi/kg) as the extracellular fluid (ECF) space marker. Of several ECF markers tested (mannitol, inulin, PEG), PEG was the only one to yield reliable, reproducible estimates of extracellular space in gill tissue. Surficial gill tissue (i.e., mainly lamellae and filament surface) was obtained by rapidly removing gill arches 1, 3, and 4, blotting dry, and then using a microscope slide to gently scrape the surface of each arch into a homogeneous slurry on a glass plate. Subsequent analytical methods and equations for calculating intra- and extracellular fluid volumes (ICFVs and ECFVs) and pHi were identical to those described by Milligan and Wood (1986*b*) and Wright, Randall, and Wood (1988). Red-blood-cell pHi was measured by the freeze-thaw method (Zeidler and Kim 1977). Gill ICFVs and pHi values were corrected for the presence of trapped red cells on the basis of the measured hemoglobin content of the gill homogenate. For measurement of intracellular ion levels, gill samples, obtained as described above, were dried to a constant weight and then ground to a fine powder with a mortar and pestle. The powder was incubated for 2 wk in 10–100 vol 8% perchloric acid and the acid extract then analyzed for Na^+ and K^+ by atomic absorption (Varian 1275-AA) and Cl^- by coulometric titration (Radiometer CMT10). Tissue ions were initially expressed on a wet-weight basis and then corrected to an intracellular-water basis using the measured ECFVs and ICFVs and plasma ion levels in the same samples.

The Basic Response to the Normoxia-Hyperoxia-Normoxia Regime

Because ventilation is primarily controlled by an O_2 drive in fish (Dejours 1973), environmental hyperoxia causes a pronounced hypoventilation. A

pure respiratory acidosis of endogenous origin results (fig. 3); the trout retains CO_2 because of reductions in both ventilation and gill perfusion (Wood and Jackson 1980). This acidosis is then compensated by acid excretion, more than 90% of which occurs via the gills (fig. 4). Despite the persistence of high arterial P_{CO_2} (Pa_{CO_2}), extracellular pH (pHa) is returned to normal by the buildup of HCO_3^- in the blood plasma (fig. 3). The response is essentially complete by 72 h. Subsequent return to normoxia causes a pure metabolic alkalosis of endogenous origin; pHa rises well above normal because the retained CO_2 is quickly washed out, whereas the accumulated HCO_3^- persists for some time (fig. 3). The rate of net base excretion during

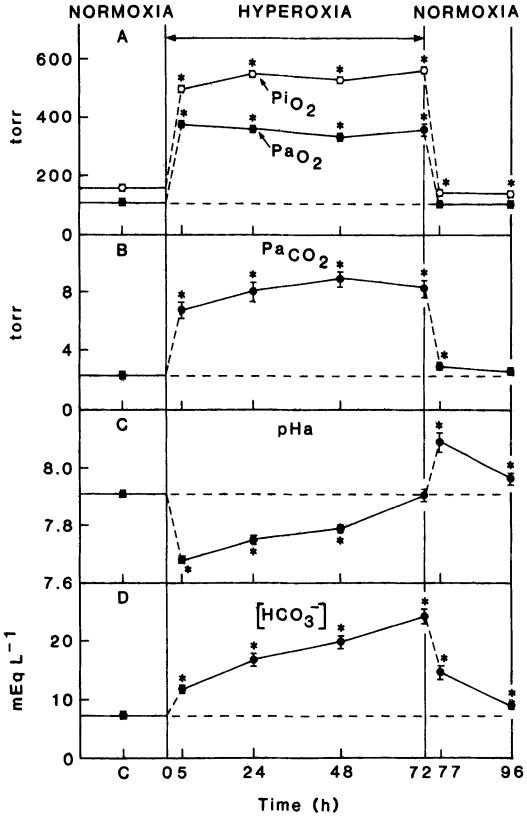

Fig. 3. Changes in arterial blood gases and plasma acid-base status of rainbow trout prior to hyperoxic exposure (normoxic control, indicated by C*), during 72 h of hyperoxic exposure, and during 24 h of subsequent normoxic recovery. Values are means, and each error bar represents plus or minus one standard error of the mean (SEM) (*n = 7–12*). An asterisk (*) indicates* $P < 0.05$ *relative to the normoxic control value. The figure is redrawn from Hobe et al. (1984*b*).*

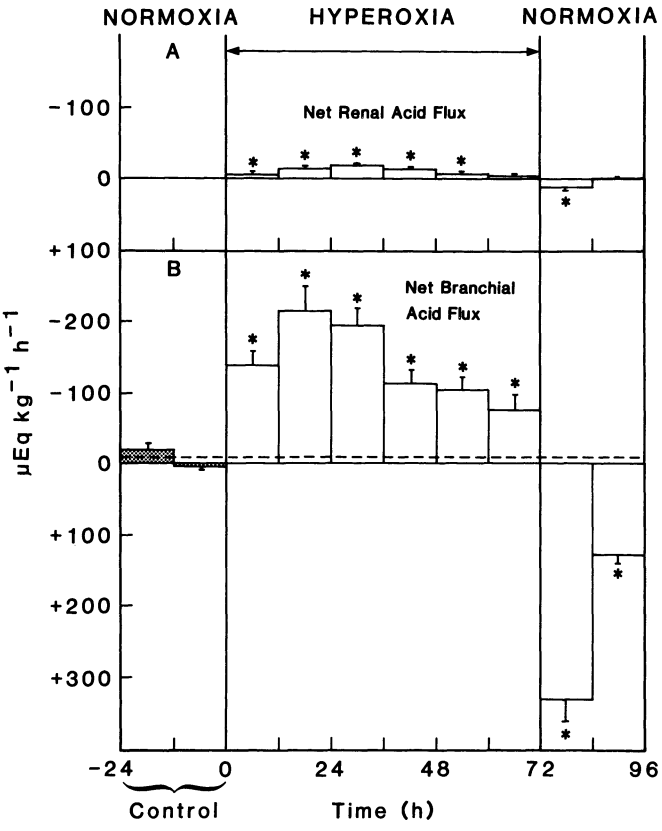

*Fig. 4. Changes in net renal and branchial acid fluxes of rainbow trout over successive 12-h periods prior to hyperoxic exposure (normoxic control), during 72 h of hyperoxic exposure, and during 24 h of subsequent normoxic recovery. Values are means; each error bar represents ±1 SEM (*n = 12*). An asterisk (*) indicates* $P < 0.05$ *relative to the normoxic control value. The figure is redrawn from Wood et al. (1984).*

alkalosis is considerably higher than the rate of net acid excretion during acidosis (fig. 4).

The Relative Roles of Na^+-versus-Acid and Cl^--versus-Base Exchange

Throughout the hyperoxia regime, the relationship predicted by SID theory (Stewart 1978, 1983) holds, with an approximately −1:1 relation between net acid flux and net $[Na^+ - Cl^-]$ flux. This is illustrated specifically by figure 6 in Wood et al. (1984). The net $[Na^+ - Cl^-]$ flux is positive during the net acid excretion of hyperoxia and negative during the net base excretion of

hyperoxic recovery. Overall, changes in Cl^- flux play a larger role than changes in Na^+ flux during the net acid excretion of hyperoxia, while the two contribute about equally during the net base excretion of normoxic recovery. Thus net branchial Na^+ balance is approximately zero or slightly positive during hyperoxic acidosis, and negative during the alkalosis of normoxic recovery. In contrast, net Cl^- balance is highly negative during hyperoxic acidosis and positive during recovery alkalosis. The involvement of both exchanges during recovery, in contrast to mainly just the Cl^- exchange during hyperoxia, is one reason for the faster correction of alkalosis than acidosis.

Unidirectional flux measurements over 0.5-h intervals demonstrated complex, time-dependent changes in the influx and outflux components of both Na^+ and Cl^- exchange. Figure 5 summarizes mean values over four 4-h periods of relative stability during the regime. Influx of Cl^- is inhibited throughout hyperoxic acidosis and stimulated during recovery alkalosis. Influx of Na^+ is initially unchanged during hyperoxic acidosis but inhibited during recovery alkalosis; outflux of Na^+ is stimulated during recovery. These changes are all in accord with the directions of net acid flux at these times. However, superimposed are paradoxical decreases in Na^+ influx and Cl^- outflux during final hyperoxia and increases in Cl^- outflux during normoxic recovery. These may reflect exchange diffusion or nonspecific effects caused by decreases in gill ventilation and perfusion during hyperoxia and increases in these factors upon return to normoxia.

Changes in plasma Na^+ and Cl^- concentrations generally reflect the pattern of net fluxes at the gills, though they are somewhat damped by exactly opposite changes in renal Na^+ and Cl^- fluxes. Notably, the plasma HCO_3^- buildup (fig. 6*D*) that restores pHa to control levels during hyperoxia is accompanied by an almost equimolar drop in plasma Cl^- (fig. 6*B*); there is negligible rise in plasma Na^+ (fig. 6*A*). Other strong electrolytes in plasma (K^+, Ca^{2+}, $HPO_4^=$) change only slightly, so the increase in plasma SID (fig. 6*C*) is almost equal to the decrease in plasma Cl^-. The SID change is also very similar to the change in plasma HCO_3^-, though with much greater variability (cf. fig. 6*D* and 6*C*). This nicely illustrates a point made earlier. The SID approach is often clumsy and imprecise in practice because of the need for multiple measurements and the compound error that may result. Measurements of HCO_3^- levels and net acid fluxes are far more accurate than measurements of SID levels and fluxes, yet the latter are required to elucidate the actual mechanisms involved.

In summary, the hyperoxia responses are in accord with the majority of previous studies (see Current Synthesis, point 3). While acid-base correction may involve changes in both Na^+ and Cl^- balance, the latter plays an overall

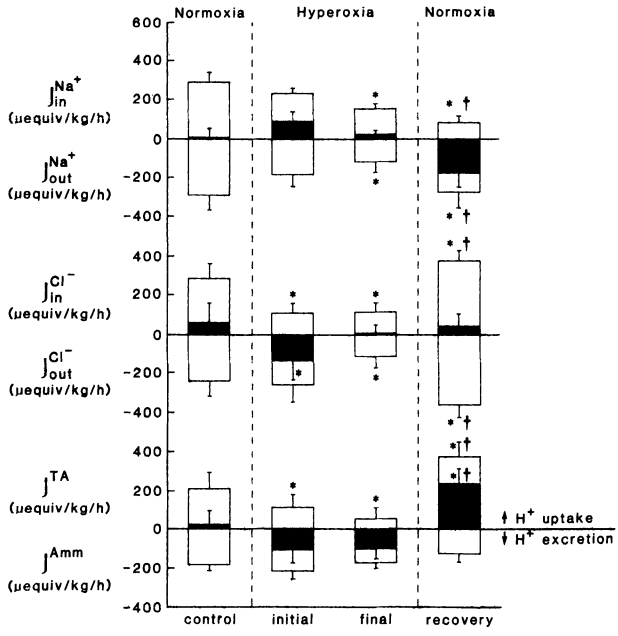

*Fig. 5. Unidirectional influxes (upward bars), outfluxes (downward bars), and net fluxes (stippled bars) of (top) Na^+ and (center) Cl^- across the gills of rainbow trout at four periods during the normoxia-hyperoxia-normoxia regime. The bottom panel provides a comparable display for acid flux; the upward bar represents titratable acidity flux, the downward bar total ammonia flux, and the stippled bar their arithmetic sum (= net acid flux). The four periods are normoxic control ("control"), 4–8 h of hyperoxia ("initial"), 68–72 h of hyperoxia ("final") and 4–8 h after return to normoxia ("recovery"). The fluxes are average values for eight individual 0.5-h determinations over each 4-h period for each fish. Values are means; each error bar represents ±1 SEM (*n* = 8–16). An asterisk (*) indicates $P < 0.05$ relative to the normoxic control value; a plus sign (+) indicates $P < 0.05$ relative to the hyperoxic "final" value. The figure is taken from unpublished data of G. G. Goss and C. M. Wood.*

greater role. The apparently opposite findings of McDonald et al. (1989*a*, 1989*b*) were based on influx data only; their net flux data do not conflict with this conclusion. A possible explanation for the dominance of Cl^- is the lower concentration of Cl^- than Na^+ in blood plasma. The Cl^- balance has the freedom to increase or decrease over a wide range without altering plasma osmotic pressure, which is stabilized by the reciprocal change in plasma HCO_3^-. In contrast, Na^+ balance may be limited to decreases only,

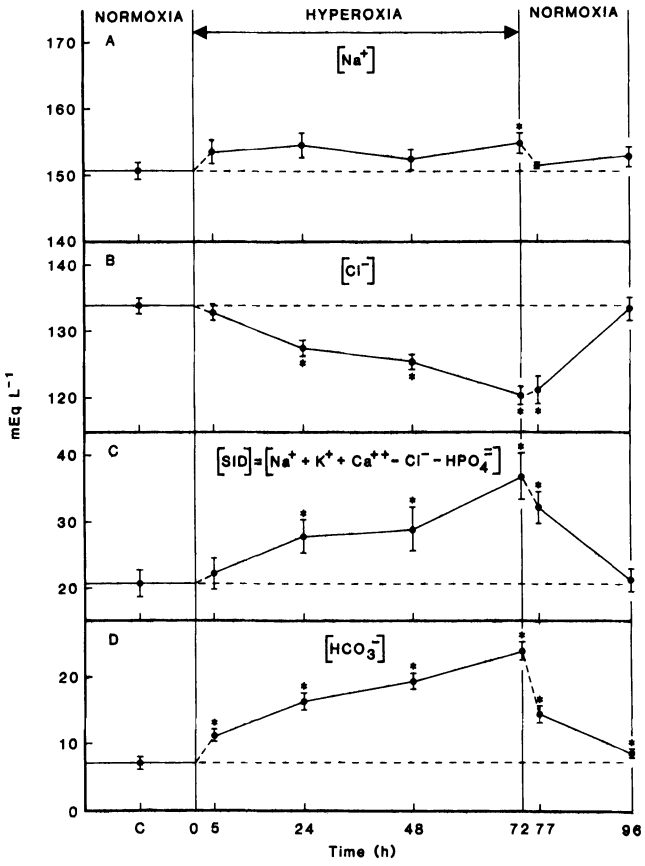

Fig. 6. Changes in plasma electrolytes of rainbow trout during the normoxia-hyperoxia-normoxia regime. Other details are as in fig. 3. The figure is redrawn from Wheatly et al. (1984) and Hobe, Wood, and McMahon (1984b); panel C is recalculated from raw data.

because an Na^+ balance more positive than control values would force an increase in plasma osmotic pressure.

Na^+ and Cl^- Uptake Kinetics in Relation to Internal Acid-Base Status

During the four chosen periods of relative stability (fig. 5), the uptake kinetics of both Na^+ and Cl^- can be described by simple Michaelis-Menten relationships (e.g., fig. 7). The kinetic parameters K_m and J_{max} exhibit significant variation among the periods (table 1), providing the first reported evidence that these kinetic parameters can be dynamically manipulated in response to internal acid-base status (see Current Synthesis, points 6 and

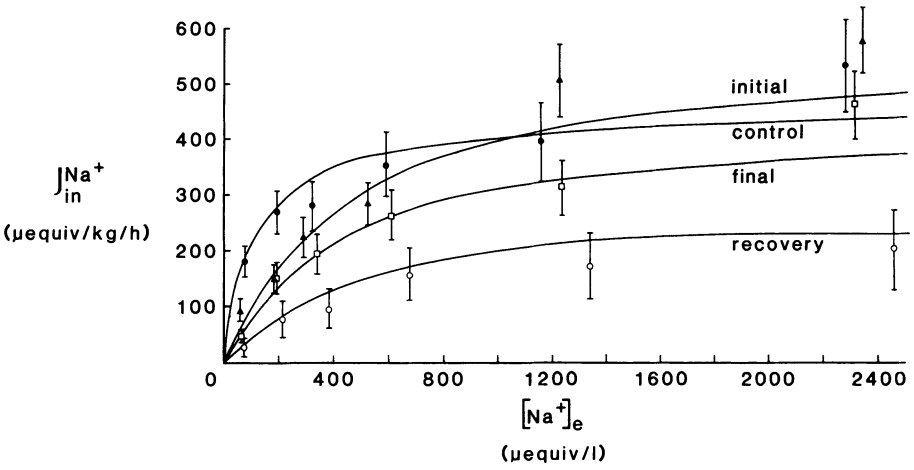

Fig. 7. The kinetic relationships between branchial Na^+ influx and external Na^+ concentration in rainbow trout at various times during the normoxia-hyperoxia-normoxia regime. Periods correspond with those of fig. 5. Curves were fitted via the Michaelis-Menten relationship with mean estimates of K_m *and* J_{max} *for each period, as determined by Eadie-Hofstee regression analyses for all individual fish. Closed circles, control; closed triangles, initial; open squares, final; open circles, recovery. Values are means; each error bar represents ±1 SEM (*n = *12). The figure is taken from unpublished data of G. G. Goss and C. M. Wood.*

10). Note in particular that decreases in Na^+ and Cl^- influx during hyperoxia are associated with increases in K_m (i.e., decreased affinity), without alternations of J_{max}. However, during the alkalosis of normoxic recovery, increased Cl^- influx is achieved by a decrease in $K_m^{Cl^-}$ to control levels and a large increase in $J_{max}^{Cl^-}$, while the decrease in Na^+ influx at this time reflects a further increase in $K_m^{Na^+}$ and a large decrease in $J_{max}^{Na^+}$. Based on these and other experiments with acid and base loading (G. G. Goss and C. M. Wood, unpublished results), our tentative conclusion is that K_m values are normally at a minimum (i.e., maximum affinity) under control conditions and can only be increased, but J_{max} values can be either increased or decreased depending on the internal acid-base status.

As yet, we know little about the mechanisms involved in altering K_m and J_{max}. The possibility of nonspecific effects of O_2 or ventilation/perfusion changes, especially with respect to K_m, cannot be eliminated. However, the J_{max} changes are consistent with the results of the acid- and base-loading experiments. Avella et al. (1987) have correlated long-term changes in $J_{max}^{Na^+}$ with changes in chloride-cell numbers and surface area on the lamellar epithelium in trout. Laurent and Perry (1991) have reported increases in

TABLE 1

Kinetics constants for Na^+ and Cl^- influx in rainbow trout at various times during the normoxia-hyperoxia-normoxia regime

	Normoxia (Control)	Hyperoxia		Normoxia (Recovery)
		Initial	Final	
$K_m^{Na^+}$ (μeq/L)	114 ± 25	445 ± 73[a]	401 ± 94[a]	559 ± 53[a]
$K_m^{Cl^-}$ (μeq/L)	165 ± 26	174 ± 35	248 ± 41[a]	137 ± 13[b]
$J_{max}^{Na^+}$ (μeq/kg/h) ...	456 ± 81	579 ± 68	433 ± 87	310 ± 65[a,b]
$J_{max}^{Cl^-}$ (μeq/kg/h) ...	292 ± 35	341 ± 33	272 ± 49	445 ± 54[a,b]

Source. G. G. Goss and C. M. Wood, unpublished data.
Note. Periods correspond with those of fig. 5. Values are means ± 1 SEM. $N = 11–12$.
[a] $P < 0.05$ relative to normoxic control value.
[b] $P < 0.05$ relative to final hyperoxic value.

the apical surface area and internal vesicular activity of individual chloride cells of trout after 2–4 d of hyperoxic exposure. It remains problematic whether these phenomena could be responsible for the very rapid changes (<4 h) seen in the present study. It is also difficult to see how alterations in the apical exposure of chloride cells could change $J_{max}^{Na^+}$ and $J_{max}^{Cl^-}$ in opposite directions. On the basis of work with isolated frog skins, Kirschner (1988) has raised the interesting suggestion that Na^+ influx could be limited by the availability of the internal counterion (i.e., H^+ or NH_4^+). In light of our findings on the internal acid-base status of the gill epithelium (below), this possibility is now being actively investigated as a general explanation for changes in $J_{max}^{Na^+}$ and $J_{max}^{Cl^-}$ during acid-base disturbance.

The Role of Differential Diffusive Efflux of Na^+ versus Cl^-

Unidirectional flux measurements with radiotracers (fig. 5) do not suggest any differential Na^+-versus-Cl^- outflux of a polarity that would aid acid-base regulation by SID effects. However, as noted earlier, such measurements may not provide a true measure of efflux rates because of the confounding effects of exchange diffusion. When true diffusive efflux is measured by rapidly exposing the fish to NaCl-free media, reductions in both Na^+ and Cl^- efflux are seen during hyperoxia, and increases in both Na^+ and Cl^-

efflux during normoxic recovery. There is no evidence of a differential diffusive efflux of Na^+ versus Cl^- during hyperoxia, though the data are rather variable. However, throughout the first 8 h of normoxic recovery, Na^+ efflux significantly exceeded Cl^- efflux by 90–150 μeq/kg/h. The SID effect of this differential flux (fig. 1) could account for up to 50% of the net acid uptake or base excretion at this time. This involvement of a differential efflux mechanism (in addition to the dual effects on Na^+ and Cl^- uptake; fig. 5) is a second reason for the faster correction of acidosis than alkalosis.

Branchial TEP has been measured as an alternative approach to the question of differential Na^+-versus-Cl^- efflux. It increases significantly from its normal slightly negative value of about −1 mV during normoxia to about +1 mV during hyperoxia, and then returns to negative values during normoxic recovery (fig. 8). These changes are small, and the possibility of a conductance change in the epithelium cannot be overlooked. However, if this is not the explanation, these data suggest that a differential efflux of Cl^- over Na^+, which is too small to measure with the NaCl-free-media technique, may occur during hyperoxia.

The mechanism(s) controlling differential efflux are unknown, although evidence is mounting that the paracellular passage of ions across many ep-

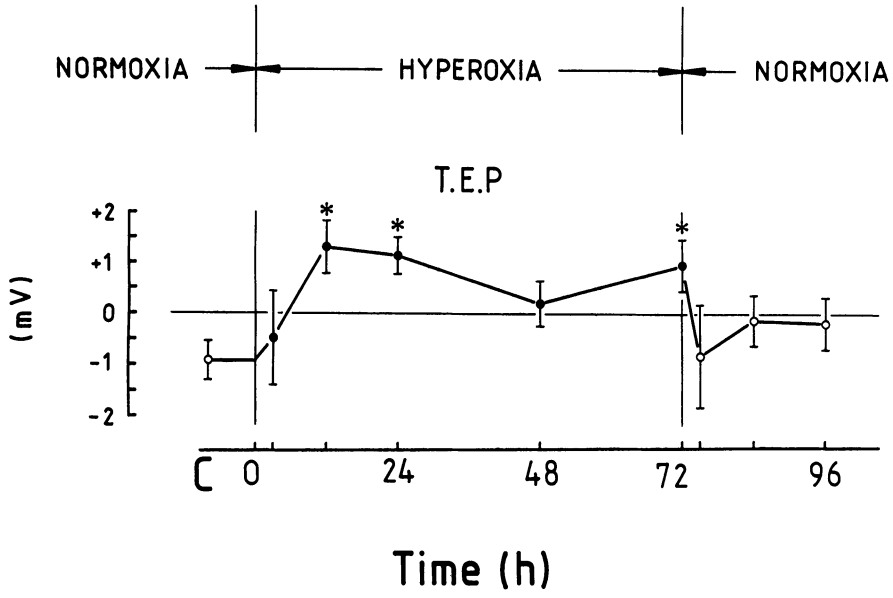

*Fig. 8. Changes in branchial TEP of rainbow trout during the normoxia-hyperoxia-normoxia regime. Values are means; each error bar represents ±1 SEM (*n = *12). An asterisk indicates* $P < 0.05$ *relative to normoxic control value. The figure is taken from unpublished data of C. M. Wood and J. LeMoigne.*

ithelia is an actively regulated process (Madera 1988). A simple explanation would be that the permselectivity of the paracellular diffusion channels in the gills to Na^+ and Cl^- may be responsive to internal acid-base status, in much the same way as it is sensitive to external water pH and calcium levels (Wood 1989). Another possibility is that shrinkage of the branchial cells during hyperoxia, and subsequent increases in cell volume during normoxic recovery, as outlined below, may alter the permeability characteristics of the tight junctions.

Intracellular Fluid Volume and Ion Levels in the Gills

Changes in ICFV and ion concentrations in epithelial cells are often associated with alterations in Na^+ and Cl^- transport, either as causes or consequences of the latter (e.g., Schultz and Hudson 1986). However, to date there is no information on this topic in the gills of freshwater fish.

Hyperoxia induces a marked fall in gill ICFV, reduction in intracellular Na^+ and Cl^- concentrations, and increase in intracellular K^+ (fig. 9). On the basis of these three ions alone, the intracellular SID would be greatly increased. The changes in these parameters do not all occur simultaneously. The Na^+ and Cl^- reductions are stable by 12 h of hyperoxia and do not change thereafter; however, the more rapid K^+ increase (3 h) returns to control levels by 48 h. The ICFV continues to decrease until 48 h, by which time the reduction is almost 20%. During normoxic recovery, Na^+, Cl^-, and ICFV return toward control levels only slowly. The decrease in ICFV during hyperoxia reflects both a reduction in total gill water content and a relative shift of fluid from ICFV to ECFV (fig. 9). These changes appear to be unique to gill tissue; other tissues examined (white muscle, brain) show either an increase or no change in ICFV, and only small changes in intracellular ion levels. The gill effects may be related to the alterations in branchial chloride-cell and pavement-cell morphology during hyperoxic exposure that have recently been described by Laurent and Perry (1991).

Hyperoxia has been reported to cause a shrinkage of trout red blood cells in vitro (Soivio, Westman, and Nyholm 1974). To ensure that the reduction in gill ICFV was not a direct effect of O_2 itself, a similar respiratory acidosis has been induced by exposing trout to normoxic hypercapnia (inspired P_{CO_2} [P_{ICO_2}] = 7.5 Torr). Hypercapnia causes a pattern of ICFV reduction and intracellular ion changes very similar to that seen during hyperoxia (fig. 9). Thus, these effects are specifically associated with the acid-base disturbance and/or the branchial ion fluxes involved in correcting the disturbance. However, none of these intracellular changes (ICFV, Na^+, K^+, or Cl^-) is

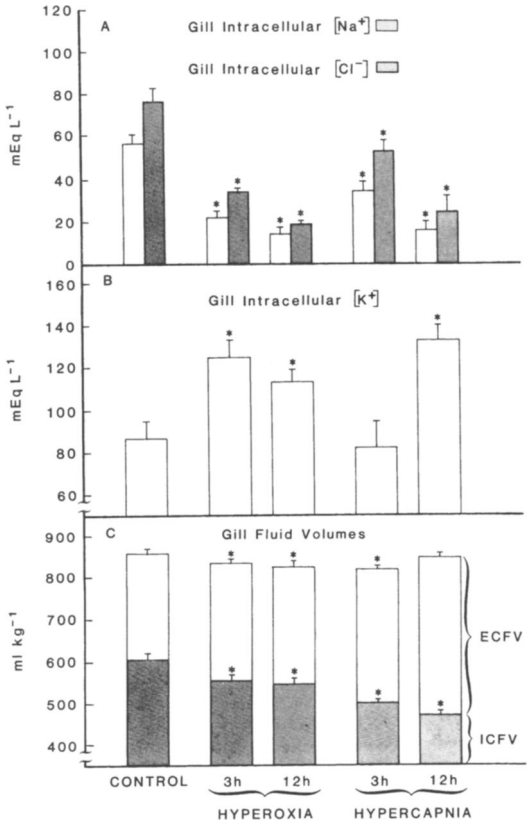

Fig. 9. The influence of 3 or 12 h of exposure to either hyperoxia or normoxic hypercapnia (P_{ICO_2} = 7.5 Torr) on mean gill intracellular Na^+, Cl^-, and K^+ concentrations, and gill ICFVs and ECFVs in rainbow trout. Values are means; each error bar represents ±1 SEM (n = 6–15). *An asterisk (*) indicates* $P < 0.05$ *relative to normoxic control value. The figure is taken from unpublished data of C. M. Wood and J. LeMoigne.*

well correlated with the rate of net acid flux across the gills (see below). A great deal more work will be required to understand the functional significance of the intracellular responses.

Intracellular pH in the Gills

While there has been considerable speculation about branchial cell pHi and its role in the control of ion and acid fluxes (e.g., Maetz and Garcia-Romeu 1964; de Renzis 1975; Haswell, Randall, and Perry 1980; Perry et al.

1981; Wood et al. 1984; McDonald and Prior 1988), the present data are the first such measurements. An important caveat is that the DMO technique measures a mean pHi for the entire surficial gill tissue, not just the transporting cells. Under control normoxic conditions, mean branchial pHi is about 7.4, rather similar to red-blood-cell pHi (fig. 4). This value is somewhat higher than white-muscle pHi and considerably lower than the pHa or the pHi in brain tissue (Milligan and Wood 1986*b*).

The most remarkable feature of gill pHi is its constancy, varying by less than 0.1 pH unit over the entire normoxia-hyperoxia-normoxia regime (fig. 10*B*) despite the large changes in pHa (fig. 10*A*) and in acid and base flux through the epithelium (fig. 4). A similar set of measurements during environmental hypercapnia (C. M. Wood and J. LeMoigne, unpublished results) confirms this minimal variation of gill pHi in the face of acid-base disturbance. The calculated increase in intracellular SID in the gills noted earlier may be a manifestation of the pHi-regulatory mechanism, for this is the appropriate response required to compensate a P_{CO_2} elevation. The regulatory ability of the gill tissue is even more remarkable in comparison to the responses of other tissues in trout. Much larger, significant changes in

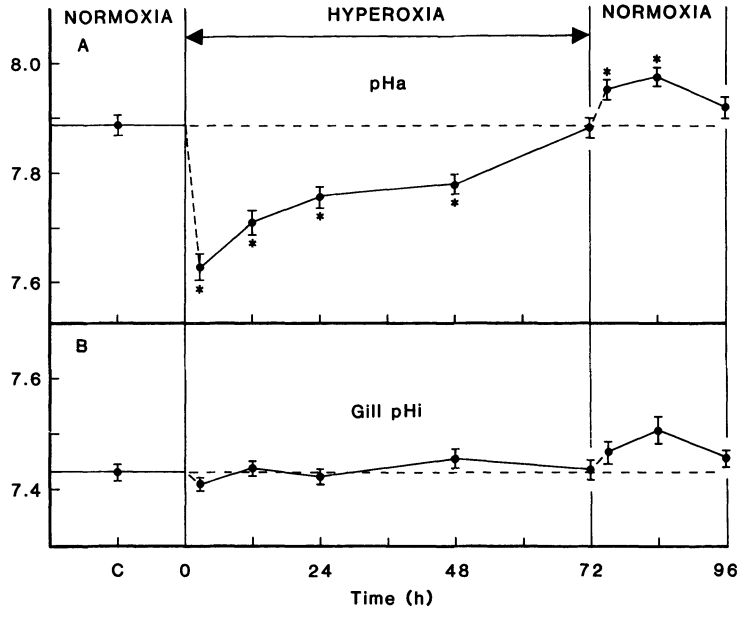

Fig. 10. Simultaneously measured changes in pHa and gill pHi during the normoxia-hyperoxia-normoxia regime. Values are means; each error bar represents ± 1 SEM (n = *8–15). An asterisk (*) indicates* $P < 0.05$ *relative to normoxic control value. The figure is taken from unpublished data of C. M. Wood and J. LeMoigne.*

pHi occur during the hyperoxic regime in brain, red cells, and white muscle (C. M. Wood and J. LeMoigne, unpublished results), though pHi changes are generally not as large as pHa changes because of the higher intracellular buffer capacity.

The relative stability of mean gill pHi does not oppose the earlier suggestion that the rates of Na^+-versus-acid and Cl^--versus-base exchange may be set by the internal availability of acid cations and base anions respectively. First, variations in pHi are generally much smaller than those in pHa, and only small changes in pHi may be needed to cause large changes in transport or metabolism (Roos and Boron 1981). Such changes may be close to the resolution of the DMO technique. Second, the very small changes that were observed correlated well with alterations in net acid flux (see fig. 11). Finally, it must be remembered that the transport cells probably constitute only a

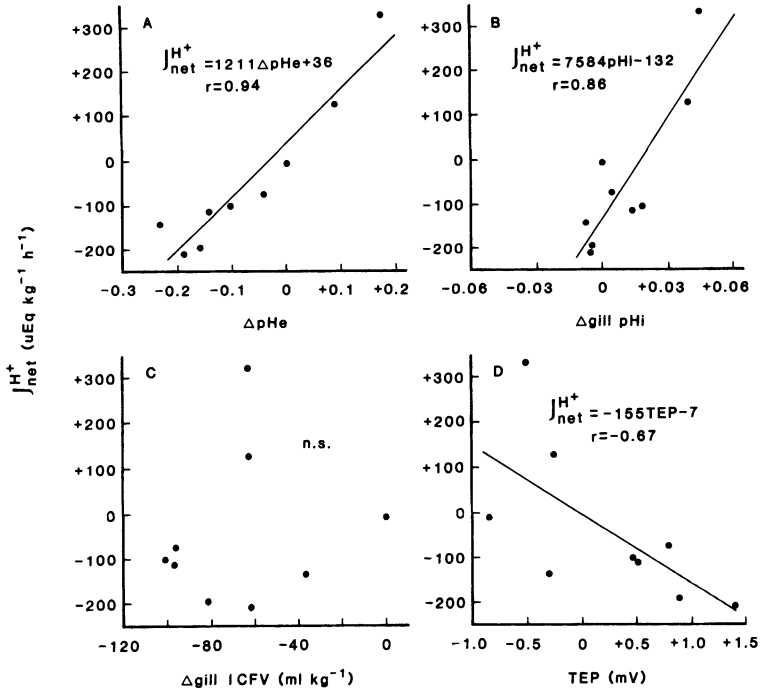

Fig. 11. Correlations in rainbow trout between branchial net acid flux and the deviations from normoxic control values in (A) arterial pHa = extracellular pHe, (B) mean gill pHi, (C) gill ICFV, and (D) TEP. The equations of the regression lines and correlation coefficients are given for the significant relationships ($P < 0.05$). The figure is based on data from Wood et al. (1984) and unpublished data of G. G. Goss, J. LeMoigne, and C. M. Wood.

small volume fraction of the total epithelium; changes in their pHi may be much greater than changes in the mean pHi value for the whole tissue.

Summary: The Control of Acid-Base Fluxes across the Gills

The present study has shown that at least three different mechanisms are involved in net branchial acid flux: Na^+-versus-acid exchange, Cl^--versus-base exchange, and differential diffusive efflux of Na^+ versus Cl^-. It may be naive to seek a single controlling factor for all three, but some possibilities will be examined. As discussed earlier, it is unlikely that plasma catecholamines were mobilized in the present experiments (Perry et al. 1989), although a role for branchial adrenergic nerves remains possible (Donald 1984). Plasma cortisol, which was monitored, showed only one small variation, a slight increase upon the return to normoxia. Figure 11 displays relationships between some of the other potential controlling factors examined in the present study and the net acid flux across the gills. The data sets are from different experiments, so only mean values taken at the same times during the standard-normoxia-hyperoxia-normoxia regime have been plotted against one another.

By far the strongest correlation is with ΔpHe, the deviation of blood pH from control levels (fig. 11*A*). Other blood acid-base parameters such as $Paco_2$, HCO_3^-, and plasma SID concentration (also individual plasma ions) show no relationship with branchial acid flux. There is a strong relationship with Δgill pHi (fig. 11*B*). However, this must be viewed cautiously because, while some pHi values at various times during the regime were significantly different from one another, none were significantly different from the normoxic control value (fig. 10*B*). The lack of relationship with Δgill ICFV (fig. 11*C*) is representative of a similar absence of correlation with other intracellular parameters—Na^+, K^+, Cl^-—in contrast with the strong relationships with pHe and pHi. The significant though rather variable relationship with branchial TEP (fig. 11*D*) probably reflects the contribution of the diffusive efflux component.

While correlation cannot prove causation, the relationship between ΔpHe and net acid flux is intuitively attractive. It is the blood pHe, and not plasma ions, HCO_3^-, or SID concentration that is ultimately returned to normal by adjustment of ion transfers at the gills. From a feedback point of view, it makes sense that deviation in the regulated parameter (pHe) drives the compensatory response. This is not to negate the earlier argument about the possible role of pHi in the transporting cells, but to suggest that the latter should be keyed in some way to pHe.

Acknowledgments

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Appendix

TABLE A1

Experimental investigations over the past 15 yr of the relationship(s) between ion and acid-base exchange in the gills of intact, unanesthetized freshwater fish (only studies that examined at least two of the following are included: [1] net acid-base flux; [2] unidirectional and net Na^+ and/or Cl^- fluxes; [3] internal acid-base and/or ionic status via catheterization)

Experimental Treatment and Reference	Species
Environmental hypercapnia:	
Cameron 1976	*Thymallus arcticus*
Cameron and Wood 1978	*Hoplias malabaricus, Hoplerythrinus unitaeniatus*
Cameron 1980	*Ictalurus punctatus*
Perry et al. 1981	*Oncorhynchus mykiss*
Claiborne and Heisler 1984	*Cyprinus carpio*
Cameron 1985	*Ictalurus punctatus*
Claiborne and Heisler 1986	*C. carpio*
Perry et al. 1987	*O. mykiss*
Environmental hyperoxia:	
Bornancin et al. 1977	*Anguilla anguilla*
Wood et al. 1984	*O. mykiss*
Wheatly et al. 1984	*O. mykiss*
Hobe et al. 1984*b*	*O. mykiss*
Environmental temperature change:	
Cameron 1976	*Thymallus arcticus*
Cameron and Kormanik 1982*a*	*I. punctatus*
Smatresk and Cameron 1982	*Lepisosteus oculatus*
Forced-air exposure/breathing:	
Heisler 1982	*Symbranchus marmoratus*
Hyde and Perry 1987	*Anguilla rostrata*

Experimental Treatment and Reference	Species
Environmental toxicant exposure:	
Zinc:	
Spry and Wood 1985	*O. mykiss*
Copper:	
Lauren and McDonald 1985, 1987	*O. mykiss*
Saline:	
Wilkes and McMahon 1986	*Catostomus commersoni*
Ammonia:	
Cameron 1986	*I. punctatus*
Aluminum:	
Booth et al. 1988	*Salvelinus fontinalis*
Wood et al. 1988	*S. fontinalis*
McDonald and Milligan 1988	*S. fontinalis*
Environmental acid exposure:	
McDonald and Wood 1981	*O. mykiss*
Ultsch, Ott, and Heisler 1981	*C. carpio*
Booth, Jancsz, and Holeton 1982	*O. mykiss*
Holeton, Booth, and Jancsz 1983*a*	*O. mykiss*
McDonald 1983	*O. mykiss*
McDonald, Walker, and Wilkes 1983	*O. mykiss*
Hobe et al. 1984*a*	*C. commersoni*
Wright and Wood 1985	*O. mykiss*
Hobe 1987	*O. mykiss, C. commersoni*
Hobe and McMahon 1988	*C. commersoni*
Audet, Munger, and Wood 1988	*O. mykiss*
Audet and Wood 1988	*O. mykiss*
Wood 1989	*O. mykiss*
Acid or base infusion:	
Cameron 1980	*I. punctatus*
Cameron and Kormanik 1982*b*	*I. punctatus*
Claiborne and Heisler 1986	*C. carpio*
McDonald and Prior 1988	*O. mykiss*
McDonald et al. 1989*a*	*O. mykiss*
Catecholamine infusion:	
Perry and Vermette 1987	*O. mykiss*
Vermette and Perry 1987	*O. mykiss*
McDonald et al. 1989*b*	*O. mykiss*
Strenuous exercise:	
Holeton et al. 1983*b*	*O. mykiss*
Milligan and Wood 1986*a*, 1986*b*	*O. mykiss*
Wood 1988	*O. mykiss*
Tang, McDonald, and Boutilier 1989	*O. mykiss*
McDonald et al. 1989*b*	*O. mykiss*

Literature Cited

AUDET, C. E., R. S. MUNGER, and C. M. WOOD. 1988. Long term sublethal acid exposure in rainbow trout (*Salmo gairdneri*) in softwater: effects on ion exchanges and blood chemistry. Can. J. Fisheries Aquatic Sci. 45:1387–1398.

AUDET, C. E., and C. M. WOOD. 1988. Do rainbow trout (*Salmo gairdneri*) acclimate to low pH? Can. J. Fisheries Aquatic Sci. 45:1399–1405.

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.

BOOTH, J. H., G. F. JANCSZ, and G. F. HOLETON. 1982. Cl^-, K^+, and acid-base balance in rainbow trout during exposure to, and recovery from, sublethal environmental acid. Can. J. Zool. 60:1123–1130.

BOOTH, C. E., D. G. MCDONALD, B. P. SIMONS, and C. M. WOOD. 1988. The effects of aluminum and low pH on net ion fluxes and ion balance in the brook trout, *Salvelinus fontinalis*. Can. J. Fisheries Aquatic Sci. 45:1563–1574.

BORNANCIN, M., G. DE RENZIS, and J. MAETZ. 1977. Branchial Cl^- transport, anion stimulated ATPase, and acid-base balance in *Anguilla anguilla* adapted to freshwater: effects of hyperoxia. J. Comp. Physiol. 117:313–322.

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 pHi 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.

———. 1978. Regulation of blood pH in teleost fish. Respir. Physiol. 33:129–144.

———. 1979. Excretion of CO_2 in water-breathing animals: a short review. Mar. Biol. Lett. 1:3–13.

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

———. 1985. The bone compartment in a teleost fish, *Ictalurus punctatus:* size, composition, and acid-base response to hypercapnia. J. Exp. Biol. 117:307–318.

———. 1986. Responses to reversed NH_3 and NH_4^+ gradients in a teleost (*Ictalurus punctatus*), an elasmobranch (*Raja erinacea*) and a crustacean (*Callinectes sapidus*): evidence for NH_4^+/H^+ exchange in the teleost and the elasmobranch. J. Exp. Zool. 239:183–195.

CAMERON, J. N., and N. HEISLER. 1983. Studies of ammonia in the rainbow trout: physico-chemical parameters, acid-base behavior and respiratory clearance. J. Exp. Biol. 105:107–125.

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. A. KORMANIK. 1982*a*. Intracellular and extracellular acid-base status as a function of temperature in the channel catfish, *Ictalarus punctatus*. J. Exp. Biol. 99:127–142.

———. 1982*b*. The acid-base responses of gills and kidney to infused acid and base loads in the channel catfish, *Ictalurus punctatus*. J. Exp. Biol. 99:143–160.

CAMERON, J. N., and C. M. WOOD. 1978. Renal function and acid-base regulation in two Amazonian erythrinid fishes: *Hoplias malabaricus,* a water-breather,

and *Hoplerythrinus unitaeniatus,* a facultative air-breather. Can. J. Zool. 56: 917–930.

CLAIBORNE, J. B., and N. HEISLER. 1984. Acid-base regulation and ion transfers in the carp (*Cyprinus carpio*) during and after exposure to environmental hypercapnia. J. Exp. Biol. 108:25–43.

———. 1986. Acid-base regulation and ion transfers in the carp (*Cyprinus carpio*): pH compensation during graded long- and short-term environmental hypercapnia, and the effect of bicarbonate infusion. J. Exp. Biol. 126:41–62.

DEJOURS, P. 1973. Problems of control of breathing in fishes. Pages 117–133 in L. BOLIS, K. SCHMIDT-NIELSON, and S. H. P. MADDRELL, eds. Comparative physiology: locomotion, respiration, transport, and blood. American Elsevier, New York.

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

DE RENZIS, G., and M. BORNANCIN. 1977. A Cl^-/HCO_3^- ATPase in the gills of *Carassius auratus:* its inhibition by thiocynate. Biochim. Biophys. Acta. 467:192–207.

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. 1984. Adrenergic innervation of the gill of brown and rainbow trout *Salmo trutta* and *S. gairdneri.* J. Morphol. 182:307–316.

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

EHRENFELD, J. 1974. Aspects of ionic transport mechanisms in crayfish *Astacus leptodactylus.* J. Exp. Biol. 61:57–70.

EVANS, D. H. 1987. The fish gill: site of action and model for toxic effects of environmental pollutants. Environ. Health Perspect. 71:47–58.

EVANS, D. H., and J. N. CAMERON. 1986. Gill ammonia transport. J. Exp. Zool. 239: 17–23.

GARCIA-ROMEU, F., and J. MAETZ. 1964. The mechanism of sodium and chloride uptake by the gills of a fresh-water fish, *Carassius auratus.* I. Evidence for an independent uptake of sodium and chloride ions. J. Gen. Physiol. 47:1195–1207.

GARDAIRE, E., M. AVELLA, J. ISAIA, M. BORNANCIN, and N. MAYER-GOSTAN. 1985. Estimation of sodium uptake through the gill of rainbow trout *Salmo gairdneri.* Exp. Biol. 44:181–189.

GIRARD, J. P., and P. PAYAN. 1980. Ion exchanges through respiratory and chloride cells in freshwater- and seawater-adapted teleosteans. Am. J. Physiol. 238:R260–R268.

HASWELL, M. S., D. J. RANDALL, and S. F. PERRY. 1980. Fish gill carbonic anhydrase: acid-base regulation or salt transport? Am. J. Physiol. 238:R240–R245.

HEISLER, N. 1982. Intracellular and extracellular acid-base regulation in the tropical freshwater teleost fish *Symbranchus marmoratus* in response to the transition from waterbreathing to airbreathing. J. Exp. Biol. 99:9–28.

HOBE, H. 1987. Sulfate entry into soft water fish (*Salmo gairdneri; Catostomus commersoni*) during low ambient pH exposure. J. Exp. Biol. 133:87–109.

HOBE, H., and B. R. MCMAHON. 1988. Mechanisms of acid-base and ionoregulation in white suckers (*Catostomus commersoni*) in natural soft water. II. Exposure to a fluctuating pH regime. J. Comp. Physiol. 158B:67–79.

HOBE, H., C. M. WOOD, and B. R. MCMAHON. 1984*a*. Mechanisms of acid-base and ionoregulation in white suckers (*Catostomus commersoni*) in natural soft water. I. Acute exposure to low ambient pH. J. Comp. Physiol. 154B:149–158.

HOBE, H., C. M. WOOD, and M. G. WHEATLY. 1984*b*. The mechanisms of acid-base and ionoregulation in the freshwater rainbow trout during environmental hyperoxia and subsequent normoxia. I. Extra- and intracellular acid-base status. Respir. Physiol. 55:139–154.

HOLETON, G. F., J. H. BOOTH, and G. F. JANCSZ. 1983*a*. Acid-base balance and Na^+ regulation in rainbow trout during exposure to, and recovery from, low environmental pH. J. Exp. Zool. 228:21–32.

HOLETON, G. F., P. NEUMANN, and N. HEISLER. 1983*b*. Branchial ion exchange and acid-base regulation after strenuous exercise in the rainbow trout (*Salmo gairdneri*). Respir. Physiol. 51:303–318.

HYDE, D. A., and S. F. PERRY. 1987. Acid-base and ionic regulation in the American eel (*Anguilla rostrata*) during and after prolonged aerial exposure: branchial and renal adjustments. J. Exp. Biol. 133:429–447.

———. 1989. Differential approaches to blood acid-base regulation during exposure to prolonged hypercapnia in two freshwater teleosts: the rainbow trout (*Salmo gairdneri*) and the american eel (*Anguilla rostrata*). Physiol. Zool. 62:1164–1186.

KERSTETTER, T. H., and L. B. KIRSCHNER. 1972. Active chloride transport by the gills of rainbow trout (*Salmo gairdneri*). J. Exp. Biol. 56:263–272.

———. 1974. HCO_3^- dependent ATPase activity in the gills of rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 488:581–589.

KERSTETTER, T. H., L. B. KIRSCHNER, and D. RAFUSE. 1970. On the mechanisms of sodium ion transport by the irrigated gills of rainbow trout (*Salmo gairdneri*). J. Gen. Physiol. 56:342–359.

KIRSCHNER, L. B. 1980. Control mechanisms in crustaceans and fishes. Pages 157–222 in R. GILLES, ed. Mechanisms of osmoregulation in animals. Wiley, New York.

———. 1988. Basis for apparent saturation kinetics of Na^+ influx in freshwater hyperregulators. Am. J. Physiol. 254:R984–R988.

KIRSCHNER, L. B., L. GREENWALD, and T. H. KERSTETTER. 1973. Effect of amiloride on sodium transport across body surfaces of freshwater animals. Am. J. Physiol. 224: 832–837.

KORMANIK, G. A., and J. N. CAMERON. 1981. Ammonia excretion in animals that breathe water: a review. Mar. Biol. Lett. 2:11–23.

KROGH, A. 1939. Osmotic regulation in aquatic animals. Cambridge University Press, Cambridge.

LAUREN, D. J., and D. G. MCDONALD. 1985. Effects of copper on branchial ionoregulation in the rainbow trout, *Salmo gairdneri* Richardson: modulation by water hardness and pH. J. Comp. Physiol. 155B:635–644.

———. 1987. Acclimation to copper by rainbow trout, *Salmo gairdneri*: physiology. Can. J. Fisheries Aquatic Sci. 43:1488–1496.

LAURENT, P., H. HOBE, 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.

LAURENT, P., and S. F. PERRY. 1991. Environmental effects on fish gill morphology. Physiol. Zool. 64:4–25.

MCDONALD, D. G. 1983. The interaction of environmental calcium and low pH on the physiology of the rainbow trout, *Salmo gairdneri.* I. Branchial and renal net ion and H^+ fluxes. J. Exp. Biol. 102:123–140.

MCDONALD, D. G., and C. L. MILLIGAN. 1988. Sodium transport in the brook trout *Salvelinus fontinalis:* effects of prolonged low pH exposure in the presence and absence of aluminum. Can. J. Fisheries Aquatic Sci. 45:1606–1613.

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., Y. TANG, and R. G. BOUTILIER. 1989*a*. Acid and ion transfer across the gills of fish: mechanisms and regulation. Can. I. Zool. 67:3046–3054.

———. 1989*b*. 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., R. L. WALKER, and P. R. H. WILKES. 1983. The interaction of environmental calcium and low pH on the physiology of the rainbow trout, *Salmo gairdneri.* II. Branchial ionoregulatory mechanisms. J. Exp. Biol. 102: 141–155.

MCDONALD, D. G., and C. M. WOOD. 1981. Branchial and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri,* at low environmental pH. J. Exp. Biol. 93: 101–118.

MCKIM, J. M., and R. J. ERICKSON. 1991. Environmental impacts on the physiological mechanisms controlling xenobiotic transfer across fish gills. Physiol. Zool. 64:39–67.

MADERA, J. L. 1988. Tight junction dynamics: is paracellular transport regulated? Cell 53:497–498.

MAETZ, J. 1972. Branchial sodium exchange and ammonia excretion in the goldfish *Carassius auratus.* Effects of ammonia loading and temperature changes. J. Exp. Biol. 56:601–620.

———. 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 mechanism of sodium and chloride 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.

MICHAL, G. 1985. Determination of Michaelis constants and inhibitor constants. In H. U. BERGMEYER, ed. Methods of enzymatic analysis. Vol. 1. Verlag Chemie, Weinheim.

MILLIGAN, C. L., and C. M. WOOD. 1986*a*. Intracellular and extracellular acid-base status and H^+ exchange with the environment after exhaustive exercise in the rainbow trout. J. Exp. Biol. 123:93–121.

———. 1986*b*. Tissue extracellular acid-base status and the fate of lactate after exhaustive exercise in the rainbow trout. J. Exp. Biol. 123:123–144.

———. 1987. Regulation of blood oxygen transport and red cell pHi after exhaustive exercise in rainbow trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). J. Exp. Biol. 133:263–282.

PAYAN, P., and J. P. GIRARD. 1978. Mise en évidence d'un échange Na^+/NH_4^+ dans la

branchie de la truite adaptée a l'eau de mer: controle adrénèrgique. C. R. Acad. Sci. 286D:335–338.

PAYAN, P., J. P. GIRARD, and N. MAYER-GOSTAN. 1984. Branchial ion movements in teleosts: the roles of respiratory and chloride cells. Pages 39–63 in W. S. HOAR and D. J. RANDALL, eds. Fish physiology. Vol. 10B. Academic Press, New York.

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., 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.

PERRY, S. F., and P. 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., S. MALONE, and D. EWING. 1987. 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., P. PAYAN, and J. P. GIRARD. 1984. Adrenergic control of branchial chloride transport in the isolated perfused head of the freshwater trout (*Salmo gairdneri*). J. Comp. Physiol. 154:269–274.

PERRY, S. F., and M. G. VERMETTE. 1987. The effects of continuous epinephrine infusion on the physiology of rainbow trout, *Salmo gairdneri.* I. Blood respiratory, ionic and acid-base status. J. Exp. Biol. 128:235–253.

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.

RAHN, H. 1966. Aquatic gas exchange: theory. Respir. Physiol. 1:1–12.

RANDALL, D. J., and J. N. CAMERON. 1973. Respiratory control of arterial pH as temperature changes in rainbow trout *Salmo gairdneri.* Am. J. Physiol. 225: 997–1002.

RANDALL, D., H. LIN, and P. A. WRIGHT. 1991. Gill water flow and the chemistry of the boundary layer. Physiol. Zool. 64:26–38.

ROOS, A., and W. F. BORON. 1981. Intracellular pH. Physiol. Rev. 61:296–434.

SCHULTZ, S. G., and R. L. HUDSON. 1986. How do sodium-absorbing cells do their job and survive? News Physiol. Sci. 1:185–189.

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.

SMATRESK, N. J., and J. N. CAMERON. 1982. Respiration and acid-base physiology of the spotted gar, a bimodal breather. II. Responses to temperature change and hypercapnia. J. Exp. Biol. 96:281–293.

SOIVIO, A., K. WESTMAN, and K. NYHOLM. 1974. Changes in hematocrit values in blood samples treated with and without oxygen: a comparative study with four salmonid species. J. Fish Biol. 6:763–767.

SPRY, D. J., and C. M. WOOD. 1985. Ion flux rates, acid-base status, and blood gases in rainbow trout, *Salmo gairdneri,* exposed to toxic zinc in natural softwater. Can. J. Fisheries Aquatic Sci. 42:1332–1341.

STEWART, P. A. 1978. Independent and dependent variables of acid-base control. Respir. Physiol. 33:9–26.

———. 1983. Modern quantitative acid-base chemistry. Can. J. Physiol. Pharmacol. 61:1444–1461.

TANG, Y., D. G. MCDONALD, and R. G. BOUTILIER. 1989. Acid-base regulation following exhaustive exercise: a comparison between freshwater- and seawater-adapted rainbow trout (*Salmo gairdneri*). J. Exp. Biol. 141:407–418.

ULTSCH, G. R., M. E. OTT, and N. HEISLER. 1981. Acid-base and electrolyte status in carp (*Cyprinus carpio*) exposed to low environmental pH. J. Exp. Biol. 93: 65–80.

VAN DIJK, P. L. M., and C. M. WOOD. 1988. The effects of beta-adrenergic blockade on the recovery process after strenuous exercise in the rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol. 32:555–570.

VERMETTE, M. G., and S. F. PERRY. 1987. The effects of prolonged epinephrine infusion on the physiology of the rainbow trout, *Salmo gairdneri*. II. Branchial solute fluxes. J. Exp. Biol. 128:255–267.

———. 1988*a*. Adrenergic involvement in blood oxygen transport and acid-base balance during hypercapnic acidosis in the rainbow trout, *Salmo gairdneri*. J. Comp. Physiol. 158B:107–115.

———. 1988*b*. Effects of prolonged epinephrine infusion on blood respiratory and acid-base status in rainbow trout: alpha and beta effects. Fish Physiol. Biochem. 4:189–202.

WHEATLY, M. G., H. HOBE, 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.

WILKES, P. R. H., and B. R. MCMAHON. 1986. Responses of a teleost (*Catastomus commersoni*) to hypersaline exposure. II. Transepithelial flux of sodium, chloride and "acidic equivalents." J. Exp. Biol. 121:95–113.

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.

———. 1989. The physiological problems of fish in acid water. Pages 125–152 in R. MORRIS, E. W. TAYLOR, D. J. A. BROWN, and J. A. BROWN, eds. Acid toxicity and aquatic animals. Cambridge University Press, Cambridge.

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 S. F. PERRY. 1985. Respiratory, circulatory and metabolic adjustments to exercise in fish. Pages 1–22 in R. GILLES, ed. Circulation, respiration and metabolism. Springer, Heidelberg.

WOOD, C. M., R. C. PLAYLE, B. P. SIMONS, G. G. GOSS, and D. G. MCDONALD. 1988. Blood gases, acid-base status, plasma ions, and hematology in adult brook trout (*Salvelinus fontinalis*) under acid/aluminum exposure. Can. J. Fisheries Aquatic Sci. 45:1575–1586.

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., M. WHEATLY, and H. HOBE. 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. A., D. J. RANDALL, and C. M. WOOD. 1988. The distribution of ammonia and H^+ between tissue compartments in lemon sole (*Parophrys vetulus*) at rest, during hypercapnia, and following exercise. J. Exp. Biol. 136:149–175.

WRIGHT, P. A., and C. M. WOOD. 1985. An analysis of branchial ammonia excretion in the freshwater rainbow trout: effects of environmental pH change and sodium uptake blockade. J. Exp. Biol. 114:329–353.

ZEIDLER, R., and D. H. KIM. 1977. Preferential hemolysis of postnatal calf red cells induced by internal alkalinization. J. Gen. Physiol. 70:385–401.