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STRATEGIES FOR ACID-BASE REGULATION
IN THE AMERICAN EEL (Anquilla rostrata)

BY

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A THESIS SUBMITTED TO THE UNIVERSITY OF OTTAWA IN PARTIAL
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ABSTRACT

A role for branchial ion exchange mechanisms in compensation of extracellular acidoses has been noted in teleosts and is related, in part, to the selective modulation of branchial Cl⁻/HCO₃⁻/(OH⁻) or Na⁺/NH₄⁺(H⁺) exchange. In an attempt to characterize compensatory mechanisms utilized by the American eel (Anguilla rostrata), observations of blood acid-base and ionic variables were made in eels subjected to either prolonged air-exposure or external hypercapnia.

It was revealed that the American eel (Anguilla rostrata), though not particularly well-adapted for prolonged aerial respiration, is able to withstand the hypoxemia and acidosis associated with extended air-exposure (36 h). An incapacity for aerial gas transfer caused an accumulation of metabolic H⁺ ions and respiratory CO₂ which contributed to a severe mixed acidosis, compensated, albeit slowly, upon return to water. The changes observed in the flux of strong ions (strong ion difference flux, SIDF) during recovery from air-exposure were attributed primarily to modification of branchial Na⁺/acid exchange. Moreover, a significant correlation existed between the rate of SIDF and the rate of branchial acid excretion. Net renal acid efflux was low (approximately 6.5% of total acid clearance) both during and after prolonged air-exposure. Due to the slow rate of acid-base recovery after exposure to air, it was suggested that the eel
possesses a limited capacity for acid-base regulation, a result of uniquely low rates of branchial Cl⁻/HCO₃⁻ exchange.

However, the eel was capable of regulating the acidosis associated with hypercapnia equally well as the trout in spite of limited Cl⁻/HCO₃⁻ exchange and an enormous stimulation of branchial Na⁺ uptake during hypercapnia was detected in eels. Furthermore, there was no stimulation of branchial Na⁺ uptake in eels acclimated to low Na⁺ water, which demonstrate an inability to regulate hypercapnic acidosis. Interestingly, a relationship arose between whole blood pH and the external concentration of Na⁺ in eels during steady state conditions. It is concluded that the eel relies on modulation of branchial Na⁺/NH₄⁺/H⁺ exchange rather than Cl⁻/HCO₃⁻/(OH⁻) exchange to compensate extracellular acidoses. However, as the eel appears to react only slowly to changes in blood acid-base status, it is speculated that hormonal adjustments such as plasma catecholamine surges may play a limited role in the manipulation of these processes.
RESUME

Un rôle compensatoire des acidoses extracellulaires a été noté pour les mécanismes d'échanges ioniques branchiaux chez les télesostes, et est associé, en partie, à la modulation sélective des échanges branchiaux de Cl⁻/HCO₃⁻/(OH⁻) ou de Na⁺/NH₄⁺(H⁺). Pour essayer de caractériser les mécanismes compensatoires qui sont utilisés par l'anguille américaine (Anguilla rostrata), des observations des variables acido-basiques et ioniques sanguines ont été fait avec des anguilles asujetties à une exposition à l'air prolongée ou à l'hypercapnie externe.

Il a été révélé que l'anguille américaine (Anguilla rostrata), bien que n'étant pas particulièrement bien adaptée pour la respiration aérienne prolongée, est capable de résister à l'hypoxémie et à l'acidose associées à une exposition prolongée à l'air (36 h). Une incapacité de faire un transfert de gaz aérien causa une accumulation d'ions métaboliques H⁺ et de CO₂ respiratoire, qui ont contribués à une acidose mixte sévère, qui fut compensée lentement au retour à l'eau. Les changements observés dans le flux d'ions forts (flux différentiel d'ions forts, SIDF) durant le rétablissement suite à l'exposition à l'air ont été attribués à la modification d'échanges Na⁺/acide branchiale. En plus, une corrélation significante existe entre le taux de la SIDF et le taux de l'excrétion branchiale d'acide. Le flux rénal net d'acide était bas (environ 6.5% du dégagement total d'acide)
durant et après l'exposition à l'air. Due au faible taux de récupération acido-basique après l'exposition à l'air, il a été suggéré que l'anguille possède une capacité limitée pour la régulation acido-basique résultant des taux faibles d'échanges branchiaux de Cl⁻/HCO₃⁻.

Néanmoins, l'anguille est capable de réguler l'acidose associée à l'hypercapnie aussi bien que la truite en dépit de l'échange limitée de Cl⁻/HCO₃⁻; une stimulation énorme de l'influx branchiale de Na⁺ a été détectée. Il n'y eu pas de stimulation de l'influx branchiale de Na⁺ dans les anguilles acclimatées à une eau à teneur faible de Na⁺, ce qui démontra une inabilité de réguler l'acidose hypercapnique. Curieusement, une relation fut démontrée entre le pH du sang complexé et la concentration du milieu en Na⁺ durant des conditions stables.

Il est conclu que l'anguille se fie sur la modulation de l'échange branchiale de Na⁺/NH₄⁺(H⁺) au lieu de l'échange de Cl⁻/HCO₃⁻(OH⁻) pour compenser les acidoses extracellulaires. Comme l'anguille réagit lentement aux changements dans son status acido-basique sanguin, il est spéculé que des ajustements hormonaux tel que des vagues de catécholamines pourrait jouer un rôle limité dans la manipulation de ces procédés.
ABBREVIATIONS

\[ \text{C}_{\text{CO}_2} \] \ldots total carbon dioxide
\[ \text{C}_{\text{O}_2} \] \ldots total oxygen
\[ \text{CO}_2 \] \ldots carbon dioxide
\[ \text{Hct} \] \ldots hematocrit
\[ \text{RBC} \] \ldots red blood cell
\[ \text{P}_{\text{O}_2} \] \ldots partial pressure of oxygen
\[ \text{P}_{\text{CO}_2} \] \ldots partial pressure of carbon dioxide
\[ \text{pHe} \] \ldots whole blood pH
\[ \text{RBC pH} \] \ldots red blood cell pH
\[ \text{SID} \] \ldots strong ion difference
\[ \text{UFR} \] \ldots urine flow rate
\[ \text{J}_{\text{NET}} \] \ldots net flux
\[ \text{J}_{\text{IN}} \] \ldots influx
\[ \text{J}_{\text{OUT}} \] \ldots efflux
\[ \text{SIDF} \] \ldots strong ion difference flux
\[ \text{J}_{\text{TA}} \] \ldots net flux of titratable alkalinity
\[ \text{J}_{\text{AMM}} \] \ldots net flux of ammonia
\[ \text{M}_{\text{CO}_2} \] \ldots excretion of carbon dioxide
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CHAPTER 1

GENERAL INTRODUCTION
The relationship between an organism and its immediate environment is extremely dynamic and, inevitably, the environment imposes stress upon an animal which challenges its regulatory mechanisms. One important physiological indicator of this relationship in an animal is pH because the maintenance of pH is critical to the integrity of both structural and enzymatic proteins.

Acid-base Disturbances

Perturbations of blood acid-base status in fishes, which often reflect changes in the homeostatic relationship with the environment, are categorized generally as being of either metabolic or respiratory origin.

Metabolic acidosis is most commonly associated with exercise and is characterized by a reduction in whole blood pH and an elevation of plasma lactate concentrations (Wood and Perry, 1985; Perry, Daxboeck, Emmett, Hochachka and Brill, 1985; Heisler, 1986; Milligan and Wood, 1986a; Butler, 1986). However, when used to study acid-base regulation, it has been shown that a component of the recovery observed in trout is a result of metabolic adjustments and is related to the clearance of lactate (Milligan and Wood, 1986b). Furthermore, the acidosis encountered by fish following exhaustive exercise is not purely of metabolic origin as elevations in arterial PCO2 have been reported (Perry et al., 1985; Wood and Perry, 1985; Heisler, 1986; Butler, 1986). Although the respiratory component of this acidosis is relatively minor, it is termed
nevertheless a mixed acidosis. In a similar fashion, exposure to air for prolonged periods of time in "air-breathing" fishes can result in a mixed acidosis (Berg and Steen, 1965; Heisler, 1982; see also reviews by Johansen, 1970; Randall, Burggren, Farrell and Haswell, 1981). Both lactic acid and mineral acid loads have been infused intravascularly into fish in an attempt to mimic the changes encountered following exhaustive exercise (Wood and Caldwell, 1978; Kobayashi and Wood, 1980; Cameron and Kormanik, 1982; McDonald, Walker, Wilkes and Wood, 1982). The infusion of either lactic acid or mineral acid (i.e. HCl) omits the respiratory component of the acidosis. Moreover, acid-base disturbances of external origin, which occur naturally as a result of changes in environmental pH (i.e. acid rain), superficially cannot be distinguished from an acidosis of purely metabolic origin (Neville, 1979; Packer, 1979; McDonald, Hobe and Wood, 1980). Indeed, artificial acidification of the environment has been used to study mechanisms of acid-base regulation (McWilliams and Potts, 1978; McDonald and Wood, 1981).

Acid-base disturbances of respiratory origin are compensated by air-breathers using ventilation and, although seldom encountered naturally, respiratory acid-base disturbances in fishes are similarly related to ventilation. In fishes, evidence suggests that ventilation is strongly oriented to the extraction of O₂ from the water rather than the elimination of CO₂ (Saunders, 1962; Holeton and Randall, 1967; Davis and
Cameron, 1971; Randall and Jones, 1973; Dejours, Toulmond and Truchot, 1977; Dejours, 1978; Wood and Jackson, 1980; Smith and Johnes, 1982) and, because the capacitance of water for CO₂ is greater than that for O₂ (Piiper, Dejours, Haab and Rahn, 1971), fish hyperventilate with respect to CO₂ (Dejours, 1978). Therefore, the ability to modulate arterial PₐCO₂ is limited by the requirement for O₂ in fishes. This characteristic has been used as a tool in an attempt to understand mechanisms of acid-base regulation. Respiratory acid-base disturbances have been imposed on fish simply 1) by elevating environmental PₐCO₂, which results in elevated arterial PₐCO₂ (hypercapnia) and hence a respiratory acidosis (exogenous hypercapnia; Cameron, 1976; Perry, Haswell, Randall and Farrell, 1981; Perry, 1982; Thomas, 1983; Toews, Holeton and Heisler, 1983; Claiborne and Heisler, 1984; Claiborne and Heisler, 1986; Perry, Malone and Ewing, 1987a,b; Cameron and Iwama, 1987) or 2) by elevating environmental PₐO₂ (hyperoxia), which results in hypoventilation and hence elevated PₐCO₂ (endogenous hypercapnia; Bornancin, De Renzis and Maetz, 1977; Wood and Jackson, 1980; Thomas, 1983; Hobe, Wood and Wheatly, 1984; Wheatly, Hobe and Wood, 1984; Wood, Wheatly and Hobe, 1984).

**Mechanisms of Acid-base Regulation**

In fish, pH regulation is accomplished initially using buffers which help to maintain a steady state pH and reduce the effects of acid-base disturbances (see Heisler, 1986a,b).
There are two distinct buffer systems in fish: the \( \text{CO}_2 \)-bicarbonate buffer system and the non-bicarbonate buffer system. Briefly, the \( \text{CO}_2 \)-bicarbonate buffer system is capable only of buffering \( \text{H}^+ \) ions of non-respiratory origin and its effects can be predicted by the Henderson-Hasselbalch equation. The non-bicarbonate buffer system, which consists mainly of protein residues with \( pK \) values close to physiological \( \text{pH} \) (e.g. histidine), can buffer both respiratory and non-respiratory acid-base disturbances. Non-bicarbonate buffering in the extracellular fluid is considerably lower than that of the intracellular fluid, although the intracellular buffering capacity varies in the different tissues of the body (Heisler, 1986a). Neither the bicarbonate or non-bicarbonate buffer system is capable of restoring homeostatic conditions following an acid-base disturbance and both act transiently to prevent potentially lethal changes in \( \text{pH} \) until homeostatic conditions are restored by some other means. Indeed, true regulation of \( \text{pH} \) disturbances occurs in an electroneutral manner by the active transfer of acidic/basic equivalents between the animal and the environment. In fish, the kidney and the gill have been identified as tissues involved in the active transfer of acidic or basic equivalents during the process of acid-base regulation, which is inextricably linked to ionic regulation.

The Kidney
The role of the kidney in restoring blood acid-base status during periods of internal acidosis/alkalosis can be estimated i) by the concentration of buffers in the urine (e.g. phosphate, ammonia) as there is an absolute amount by which the kidney can reduce pH, ii) by urine flow rate, as the clearance of acid is fundamentally related to the volume of urine cleared, iii) by characterization of the acid-base disturbance and iv) by examining a possible species-dependent response. During metabolic acidosis caused by the infusion of mineral acid in trout (Wood and Caldwell, 1978) and catfish (Cameron and Kormanik, 1982) or by external acidification in trout (McDonald and Wood, 1981), renal acid excreting mechanisms can account for clearance of between 33 and 100% of the accumulated acid load. However, the injection of lactic acid into trout, which mimics the elevation of ECF metabolic acid load following exhaustive exercise, can account for only 6% of total acid clearance (Kobayashi and Wood, 1980). Indeed, following exhaustive exercise, renal acid excretion accounts for only 8% of the net $H^+$ efflux (Wood, 1988) and during purely respiratory acid-base disturbances (e.g. hypercapnic acidosis, hyperoxic acidosis), there is similarly little or no reliance on adjustments of renal acid excretion (Cameron, 1980; Wood et al., 1984, Wheatley et al., 1984; Perry et al., 1987a,b). Indeed, the predominant role of the kidney during acid-base disturbances appears to be the retention of plasma $[HCO_3^-]$ (Pitts, 1974; Wood and Jackson, 1980; Wheatly
et al., 1984; Perry et al., 1987b) rather than the excretion of acidic equivalents.

The Gill

The gill, therefore, is pre-eminent in the compensation of internal acidoses in fish. Indeed, the modulation of branchial ionic uptake mechanisms, known to be important in ionic regulation (Maetz, 1970; Evans, 1982; 1984; Payan, Girard and Mayer-Gostan, 1984), has been linked to changes in blood acid-base status (deRenzis and Maetz, 1975; Perry et al., 1981; Perry and Vermette, 1987). Moreover, evidence suggests that dynamic manipulation of branchial ionic exchange mechanisms occurs in fishes during internal acid-base disturbances (Cameron, 1976; Wood et al., 1984; Claiborne and Heisler, 1984; Perry et al., 1987a). At the gill, the movement of Na\(^+\) and Cl\(^-\) is determined i) by the electroneutral exchange of Na\(^+\) for H\(^+\)(NH\(_4\))\(^+\) and Cl\(^-\) for HCO\(_3\)\(^-\)(OH\(^-\)) at the apical surface of the gill epithelium, ii) by the passive efflux which is determined by both the electrochemical gradient and gill permeability and iii) by exchange diffusion (see Evans, 1984; Wood, 1988). Strong ion theory (Stewart, 1980) dictates that it is the net flux of all strong ions (predominantly Na\(^+\) and Cl\(^-\)) that determines the net movement of acidic equivalents and hence, modulation of either the uptake or efflux of an ion can result in changes of strong ion flux. During periods of internal acidosis, stimulation of branchial Na\(^+\) uptake and/or inhibition of branchial Cl\(^-\) uptake
can cause differential changes in the net flux of these ions. As a result, there can be an excretion of acidic equivalents to the external environment or an accumulation of bicarbonate within the fish, hence regulation of the acidosis. With the notable exception of the Arctic grayling (Thymallus arcticus; Cameron, 1975), fish appear to rely predominantly on inhibition of branchial Cl⁻/HCO₃⁻(OH⁻) exchange to regulate hypercapnic acidosis (Wood et al., 1984; Claiborne—and Heisler, 1984; 1986; Perry et al., 1987a).

The American Eel (Anquilla rostrata)

The eel (Anquilla sp.) is unusual among euryhaline teleosts because it lacks significant Cl⁻/HCO₃⁻ exchange, as indicated by almost undetectable branchial Cl⁻ influx (Kirsch, 1972; Bornancin et al., 1977). Thus, an examination of the American eel (Anquilla rostrata) presents a unique opportunity to study the relative contributions of the Cl⁻/HCO₃⁻ and Na⁺/H⁺ exchange mechanisms to acid-base balance. Although dynamic manipulation of the Cl⁻/HCO₃⁻ exchange has been reported in the eel (Bornancin et al., 1977), extremely low activity would preclude a role in acid-base regulation.

As fish rely, at least partially, on modulation of branchial Cl⁻/HCO₃⁻ exchange to compensate internal acid-base disturbances, the objective of this thesis was to determine the relative importance of branchial ionic exchange mechanisms in acid-base regulation in the eel. This was accomplished by i) quantifying the changes in blood acid-base status following
exposure to an acidosis and ii) examining the mechanisms used to compensate this acidosis. Two model acidotic stress systems were used to accomplish this; prolonged exposure to air and exposure to external hypercapnia.
CHAPTER 2

GENERAL METHODS
Experimental Animals

American eels (*Anquilla rostrata*) were obtained from an eel ladder associated with the Saunders Hydroelectric Dam in Cornwall, Ontario and were transported on ice to the University of Ottawa. Rainbow trout (*Salmo gairdneri*) of both sexes were obtained from Thistle Springs Trout Farm (Ashton, Ontario) and were transported to the University of Ottawa in hyperoxic water to reduce the stress associated with transport.

All fish were held on a 12 h light: 12 h dark photoperiod in large fiberglass aquaria (Living Stream; Toledo, Ohio) supplied with flowing, aerated and dechlorinated City of Ottawa tap water ([Na$^+$] = 0.10 mM, [Cl$^-$] = 0.15 mM, [Ca$^{2+}$] = 0.35 - 0.40 mM, [K$^+$] = 0.03 mM, pH = 7.5 - 8.0) for at least two weeks prior to experimentation. Water temperature varied between 5 and 15°C seasonally. Trout were maintained on a diet of dried commercial trout pellets (Purina Trout Chow) withheld 48 h prior to experimentation. Eels were kept unfed at all times.

Surgical Techniques

To permit periodic blood and urine sampling during experiments, indwelling cannulae were implanted chronically into fish using several techniques. In eels, indwelling cannulae were implanted into either the caudal artery, the pneumogastric artery or the urinary bladder. In trout, cannulae were implanted into the dorsal aorta.
For cannulation of the caudal artery, eels were anaesthetized in a solution of ethyl-m-aminobenzoate (2 g L\(^{-1}\); MS 222, Sigma) adjusted to pH 7.5 - 8.0 with Tris buffer (Trizma Base, Sigma) and an incision was made approximately 10 cm caudal to the anus and slightly below the lateral line. The haemal arch was then exposed and polyethylene tubing (Clay Adams PE 50; ID = 0.58 mm; OD = 0.96 mm) was inserted into the caudal artery after puncturing the vessel wall with a 26 gauge hypodermic needle. The wound was then sutured, the cannula was secured to the dorsal fin and the fish was placed in the experimental holding box where it was allowed to recover from the effects of surgery.

In a similar fashion, indwelling cannulae were implanted into the pneumogastric artery. Following anaesthesia, an incision was made 3 cm caudal to the heart and right latero-ventrally. The pneumogastric artery was isolated caudal to the gall bladder and dorsal to the gas bladder. The artery was ligated shut caudally and clamped rostrally. A small incision was made in the vessel wall with microscissors and polyethylene tubing (Clay Adams PE 50; ID = 0.58 mm; OD = 0.97 mm) was inserted. A purse-string suture (000 suture silk) was used to ligate the vessel wall to the cannula. To ensure patency of the cannula, it was passed through the body wall and sutured to the lateral body wall using 00 suture silk. The wound was then sutured, and the fish was transferred to an opaque Perspex box (vol 3 L) where it was allowed to recover.
from the effects of anaesthesia and surgery for at least 48 h prior to experimentation.

To allow continuous urine collection, urinary bladder catheters were also implanted into eels lacking other cannulae. A polyethylene tube (Clay Adams PE 60; ID = 0.76 mm; OD = 1.22 mm) was inserted through the urinary papilla and into the urinary bladder a distance of approximately 1 cm. To prevent the occurrence of kinks, a more flexible length of tubing (Tygon) was attached to the catheter and sutured to the anal fin. Following surgery, eels were transferred to experimental holding boxes where they were allowed to recover for at least 48 h before experimentation commenced.

To permit serial blood sampling from the dorsal aorta, trout were anaesthetized in a solution of ethyl-m-aminobenzoate (0.1 g L⁻¹; MS 222, Sigma) buffered to pH 7.0 with NaHCO₃ then placed onto an operating table (modified from Smith and Bell, 1967) which allowed continuous irrigation of anaesthetic. Indwelling cannulae were implanted into the dorsal aorta (Smith and Bell, 1964) using flexible polyethylene tubing (Clay Adams PE 50). The trout were revived on the operating table, by irrigation of the gills with aerated water, then transferred to opaque Perspex holding boxes (vol 3 L) where they were allowed to further recover for at least 48 h before experimentation commenced.

Branchial Solute Fluxes
All branchial solute fluxes in eels were determined between the months of May and October in an attempt to control for seasonal effects. Studies on trout were performed either November or December of 1987. Branchial unidirectional Na\(^+\) and Cl\(^-\) fluxes were determined by monitoring the disappearance of \(^{22}\)Na (as NaCl; Amersham) or \(^{36}\)Cl (as HCl; ICN) from the experimental holding box. Approximately 1.0 uCi of \(^{22}\)Na or 1.5 uCi of \(^{36}\)Cl was added to each box and allowed to mix for 15 min. An initial water sample (20 ml) was removed following the mixing period and another after 6 h. Activity of \(^{22}\)Na or \(^{36}\)Cl was determined immediately on 5.0 ml samples while the remaining water was stored (\(-20^\circ\) C) for subsequent ionic analysis (Na\(^+\), Cl\(^-\), K\(^+\), NH\(_4^+\)) and determination of branchial net ionic fluxes. Branchial net acid fluxes were determined according to McDonald and Wood (1981) from measurements of titratable alkalinity and ammonia concentrations (see below) on initial and final water samples. The methodology does not discern between the excretion of acid and uptake of base but this is inconsequential with respect to acid-base regulation.

**Analytical Procedures**

Blood pH was determined with a micro-capillary pH electrode (Radiometer G299A) maintained at ambient water temperatures in conjunction with a Radiometer PHM-71 acid-base analyzer and BMS3-MK2 blood micro-system. \(C_{CO2}\) of the blood was determined on 100 ul samples using a carbon dioxide analyzer (Corning Model 905). Blood \(P_{CO2}\) and bicarbonate
concentration ([HCO$_3^-$]) were calculated from the measured $C_{CO_2}$ and pH values according to the Henderson-Hasselbalch equation. Values for the appropriate dissociation constants of carbonic acid and the solubility coefficients of CO$_2$ at various temperatures and pH's were obtained from Boutilier, Heming and Iwama (1984). Metabolic acid load (Davenport, 1974), in mM, was calculated according to the following equation:

$$\text{Metabolic acid load} = [\text{HCO}_3^-]_1 - [\text{HCO}_3^-]_2 - (\text{pH}_1 - \text{pH}_2)$$ (1)

where is the in vitro non-carbonic acid buffer value of eel blood at a hematocrit of 20% (-10.12 mmol L$^{-1}$ whole blood; see Chapter 3). Plasma and urine osmolalities were determined using a Wescor 5100C vapor pressure osmometer recalibrated with 100 mmol kg$^{-1}$ standard to compensate for low urine osmolality. Plasma, urine and water concentrations of Na$^+$, K$^+$ and Ca$^{2+}$ were determined by flame emission spectrophotometry (Varian model Spectra AA-10). Cl$^-$ levels were determined by amperometric titration (Buchler-Cotlove Chloridometer). [Pi] was determined spectrophotometrically using a commercial assay kit (Sigma) and total ammonia was measured by a micro-modification of the salicylate-hypochlorite reaction (Verdouw, vanEchteld and Dekkers, 1968). Pi and ammonia determinations were performed on plasma samples deproteinized with 12% trichloracetic acid. $^{22}$Na and $^{36}$Cl activities were determined on 5 ml water samples by liquid scintillation counting (LKB 1215 Rackbeta). Branchial net fluxes ($J_{NET}$) for these electrolytes and unidirectional fluxes (e.g. $J_{IN}$, $J_{OUT}$) for
\( \text{Na}^+ \) and \( \text{Cl}^- \) were determined according to Maetz (1956). Accordingly, influxes \( (J_{IN}) \) and net gains by the animal have positive signs while effluxes \( (J_{OUT}) \) and net losses have negative signs.
CHAPTER 3

PHYSIOLOGICAL CONSEQUENCES OF PROLONGED AIR-EXPOSURE IN
THE AMERICAN EEL, ANGUILLA ROSTRATA: BLOOD
RESPIRATORY AND ACID-BASE STATUS
Introduction

The American eel, *Anquilla rostrata*, although lacking specialized air-breathing organs, is known to make routine excursions onto land and may even migrate short distances between ponds across damp meadows. The physiological mechanisms for aerial respiration in the eel have been studied previously (Berg and Steen, 1965; 1966). These studies have revealed that air-exposed eels gulp and retain air in the buccal cavity from which they are able to extract oxygen via transfer across the gills. Based on their measurements of oxygen uptake and blood oxygen content, Berg and Steen (1965) suggested that branchial \( O_2 \) uptake, in conjunction with cutaneous \( O_2 \) transfer and \( O_2 \) reabsorption from the swim bladder, was sufficient to meet the metabolic requirements of air-exposed eels at 7° C but not at 15° C. However, it was noted that eels encounter a severe acidosis during exposure to air, presumably due to the inability to excrete \( CO_2 \).

Unfortunately, the blood sampling methodology employed (animals were not catheterized to permit serial blood sampling) by Berg and Steen (1965) and the absence of critical measurements and calculations (e.g. blood \( P_{O_2} \), \( P_{CO_2} \) and bicarbonate) did not allow a valid or complete assessment of the respiratory and acid-base properties during prolonged aerial exposure. More recent studies (Kirsch and Nonnotte, 1977; Smith, Duiker and Cooke, 1983) have demonstrated that eel skin is poorly vascularized and relatively impermeable compared to
respiratory epithelia, hence unimportant in transcutaneous gas transfer. These studies have revealed that cutaneous oxygen uptake, although a significant component of total oxygen uptake, is largely a reflection of oxygen consumption by the skin itself. The majority of gas transfer into the circulation, therefore, may occur across the gills when eels are in air even though cutaneous O₂ uptake may be high (Berg and Steen, 1965). Given the absence of special gill modifications permitting effective aerial gas transfer, arterial blood gas tensions and acid-base status may be affected adversely.

In this chapter, the consequences of prolonged air-exposure on blood respiratory and acid-base properties in the eel have been evaluated in an attempt to determine the effectiveness of aerial gas transfer. Of particular interest is a quantification of the acidosis associated with air-exposure which arises presumably due to the inability of the eel to excrete CO₂. The results will be compared with those obtained from true amphibious fishes (e.g. Synbranchus marmoratus) which also use gills and/or buccal epithelia for gas transfer while in air.
Materials and Methods

Experimental Animals

Immature American eels (Anguilla rostrata) weighing between 97 and 298 g (mean weight = 184.9 ± 17.0 (SE) g; N = 42) were utilized in this study. Water temperature varied between 4 and 9°C during the course of the experiments.

IN VITRO PROTOCOL

Buffering Capacity:

Approximately 3 - 5 ml of blood was withdrawn slowly (1 ml min⁻¹) via caudal artery cannulae from eels that had recovered from surgery for at least 48 h. Blood from 7 eels was pooled, centrifuged and the plasma removed. The plasma and red blood cells (RBC's) were re-mixed in round-bottom tonometer flasks (each containing 25 units ammonium heparin) to yield predetermined haematocrits (Hcts) of 10, 15, 20 and 30%. The tonometer flasks were placed in a constant temperature (10°C) water bath, shaken continuously, and gassed initially with 0.25% CO₂ in air for 45 min before samples were withdrawn. The flasks were gassed subsequently for 30 min with 0.5, 1.0 and 2.0% CO₂ in air. At each P_CO₂, 0.7 ml of blood was removed and analyzed for Hct, whole blood pH (pHₑ) and total carbon dioxide content (C_CO₂). Gas mixtures were obtained using a Worsthoff gas mixing pump. Non-bicarbonate buffer values (Ω), used in calculations of base deficit (see below), were determined for each Hct according to the relationship:
\[ \xi = \frac{C_{CO_2}}{pH}. \]  

(2)

Finally, a regression relating Hct and buffer value was established.

**Oxygen Dissociation Curves, Root Effect, pHe versus RBC pH Relationship:**

Whole blood oxygen dissociation curves were constructed and \( P_{50} \)'s determined using the mixing technique described by Haab, Piiper and Rahn (1960). Briefly, blood from 7 eels was pooled, divided into two equal portions and placed in tonometer flasks kept at 10\(^\circ\) C (see above). One flask was gassed with 100\% \( O_2 \) and the other with 100\% \( N_2 \). In a separate experiment (\( N = 7 \)), flasks were gassed with 0.25\% \( CO_2 \) in \( O_2 \) and 0.25\% \( CO_2 \) in \( N_2 \). All gases were supplied from pre-analyzed precision gas mixtures (Air-Products Inc.; Ottawa, Ontario). Blood was withdrawn anaerobically from each flask in varying proportions, mixed thoroughly and analyzed for total oxygen content (\( C_{O_2} \)), oxygen partial pressure (\( P_{O_2} \)), Hct and pHe. Log \( P_{50} \) was expressed as a function of whole blood pH. The magnitude of the Root effect and the relationship between pHe and RBC pH were quantified on pooled blood (\( N = 7 \)) by measuring \( C_{O_2} \), pHe and RBC pH as carbon dioxide tension was varied between 0 and 2 \% (0, 0.25, 0.5, 1.0, 1.5, 2.0 \%) \( CO_2 \) in oxygen.

**In Vivo Protocol**

For these experiments, the holding apparatus consisted of an opaque plastic tube (4 cm internal diameter) perforated
with small holes (0.75 cm diameter; density = 15/dm²) that permitted adequate water or air convection but prevented excessive movement of the eels. The tube was stoppered at both ends and suspended in an opaque acrylic chamber (volume = 6 L; Perspex) that was supplied with flowing water. The holding chamber was aerated vigorously with compressed air to enhance air/water convection. The caudal artery cannula (see Chapter 2 for details of cannulation) was secured outside of the holding box to allow easy access and was flushed at least once daily with freshwater teleost saline (Wolf, 1963) containing 10 units ml⁻¹ heparin (ammonium salt; Sigma).

Eels were exposed to air for a period of 36 h and then subsequently returned to water for an 18 h period of recovery. Exposure to air (10°C) was achieved by draining the water from the eel holding box to a level below that of the suspended tube. The remaining water served to humidify the air surrounding the eel and ensured that the skin remained moist. Blood samples (0.7 ml) were withdrawn from the caudal artery cannulae immediately before air-exposure, at periodic intervals during air-exposure (1, 2, 3, 6, 12, 24 and 36 h), and after return to water (1 and 18 h). Control eels were kept in water for 54 h. A decline in sample size resulted due to problems with cannula patency in both control and air-exposed eels during the latter stages of experiments. To compensate for this problem, an additional group of eels were subjected to a blood sampling regimen that commenced after 3 h of
air-exposure. This is the reason for the variable N numbers. Additionally, serial blood sampling and resultant anaemia caused arterial CO₂ to decline even in control animals. For this reason, both absolute and corrected CO₂ values are presented. The corrected CO₂ values were obtained by relating the absolute CO₂ to the initial Hct:

\[
\text{Corr. CO}_2 = \text{CO}_2 \times \frac{\text{initial Hct}}{\text{Hct}}
\]  

(3)

Hct, pHe, RBC pH, PO₂, CO₂ and CCO₂ were determined immediately on each sample as described in Chapter 2 (Analytical Procedures). The remaining blood was centrifuged and the plasma was stored (-70°C) for subsequent analysis of lactate concentrations, determined using conventional enzymatic methods described in Bergmeyer (1974). Approximately 0.7 ml of saline was reinjected slowly into the caudal artery cannula following each sample to partially restore blood volume.

**Statistical Analysis**

In Figures, variability of the data is indicated by ± 1 SE. Results have been statistically analyzed using two-tailed, paired or unpaired Student's t-tests between sample means with a 5% level of significance.
Results

In Vitro Experiments

The relationship between non-bicarbonate buffering capacity ($\bar{\gamma}$) and Hct in eel blood was determined to be:

$$\bar{\gamma} = 2.73 + 0.377(\text{Hct}) \quad (r = 0.988).$$  \hspace{1cm} (4)

Mean initial Hct in vivo was 19.6\% yielding an average initial blood buffering capacity of \(-10.12\) mM L\(^{-1}\) whole blood. Eel blood, in vitro, displayed significant Bohr and Root effects (Fig. 1A,B). The Bohr coefficient was calculated to be \(-0.38\) based on a comparison of O\(_2\) dissociation curves at P\(_{CO2}\) zero (nominal P\(_{CO2}\)) and 3.75 torr. Total oxygen content varied with whole blood pH according to the relationship:

$$C_{O2} = -14.47 + 2.55 \text{ pHe} \quad (\text{Fig. 1B}).$$  \hspace{1cm} (5)

A decrease in pHe from 8.1 to 7.5 (the pHe reduction observed during air-exposure) caused C\(_{O2}\) to decrease by 25\%, in vitro.

In Vivo Experiments

The effect of prolonged air-exposure on selected blood respiratory variables is illustrated in Fig. 2. Arterial C\(_{O2}\) declined rapidly during the initial 3 h of air-exposure and then more gradually for the remainder of the air-exposure period. Arterial C\(_{O2}\) returned to control levels immediately upon returning the eels to water (Fig. 2A,B). Arterial P\(_{O2}\) decreased from about 75 torr to 25 torr during the initial 3 h of air-exposure and thereafter remained stable. Re-immersion in water caused an immediate rise in P\(_{O2}\) to approximately 125
Figure 1. In vitro respiratory properties of eel blood showing
A) oxygen dissociation curves at P_{CO2} = 0 torr (●●, P_{50} = 10 torr, N = 7) and 3.75 torr (○○, P_{50} = 21 torr, N = 7), and B) the magnitude of the CO2-induced Root
effect (CO2 = 2.55 pHe - 14.47, r = 0.988). All
determinations were performed on pooled blood. See text
for further details.
Figure 2. The effects of prolonged air-exposure (●●) on selected arterial blood respiratory variables in the American eel including A) total O₂ content, B) total O₂ content corrected for serial sampling dilution, C) oxygen tensions (P₀₂), D) hematocrit (Hct) and E) lactate concentration ([lactate]). Control eels (○○) were left in water for the entire 54 h period. N numbers are indicated in parentheses above the data points in Fig. 2A. * represents significant difference from control value at corresponding time; + represents significant difference from pre-exposure value (pre).
torr (Fig. 2C). Both the control and air-exposed eels displayed a similar pattern of Hct adjustment during the first 12 h of air/water exposure. However, between 12 h and 36 h, the air-exposed eels tended to have higher Hcts (Fig. 2D). Blood lactate levels increased after 6 h in air and continued to increase until the eels were returned to water, at which time, blood lactate levels declined toward control levels (Fig. 2E).

Eels in air developed severe extracellular and RBC acidosis (Fig. 3A). A comparison of the relationship between pH and RBC pH in vitro (Fig. 4A) and in vivo (Fig. 4B) demonstrates that RBC pH was not regulated during air-exposure but simply conformed to the in vitro relationship. The extracellular acidosis was at least partially respiratory in origin as indicated by the elevated PCO₂ (Fig. 3D). However, a metabolic component is also suggested by the decline in blood total CO₂ in the latter stages of air-exposure (Fig. 3C). The temporal changes in whole blood acid-base status during air-exposure are shown on a pH-HCO₃⁻ diagram (Fig. 5). This makes it clear that the acidosis was composed of respiratory and metabolic components throughout the air-exposure period as evidenced by the persistent hypercapnia and base deficit. In the first hour after return to water, the change in whole blood acid-base status followed the in vitro buffer line, indicating purely respiratory compensation. The elevation of pH in the subsequent 17 h primarily reflected the clearance of metabolic acid.
Figure 3. The effects of prolonged air-exposure (---) on selected arterial blood acid-base variables in the American eel including A) whole blood pH (pHe), B) red blood cell pH (RBC pH), C) total CO₂ content (C_CO₂) and D) CO₂ tension (P_CO₂). All other details as in Fig. 2.
Figure 4. A) The relationship between whole blood pH (pHe) and red blood cell pH (RBC pH) in vitro (stippled area represents ± 1 SE about the linear regression). The pHe versus RBC pH relationship was determined by equilibrating pooled blood from catheterized eels (N = 7) with a range of CO₂ tensions (0.25, 0.5, 1.0 and 2.0 %). B) Superimposition of in vivo pH values (± 1 SE) on the in vitro line. The numbers associated with each point indicate the duration of air-exposure, in hours. P represents pre-exposure values; R1 and R18 represent 1 and 18 hours after return to water, respectively.
A

RBC pH

7.6
7.4
7.2

B

RBC pH

7.7
7.5
7.3
7.1

pHe

7.5
7.7
7.9
8.1

In vitro
Figure 5. A pH-HCO₃⁻ diagram showing the temporal changes in blood acid-base status in the American eel during and after 36 h of continuous air-exposure. The dashed lines represent the in vitro whole blood non-HCO₃⁻ buffer line ($\beta = -10.1 \text{ mM L}^{-1}$). The numbers associated with each point indicate the duration of air-exposure, in hours. P represents pre-exposure acid-base values; R1 and R18 represent 1 and 18 hours after return to water, respectively.
Discussion

During prolonged aerial exposure, the American eel displays a limited capacity for gas transfer as indicated by the decreases and increases in arterial blood oxygen and carbon dioxide tensions, respectively. While in air, the eel is known to gulp and retain air in the buccal cavity for extended periods of time (Steen and Kruysse, 1964; Berg and Steen, 1965, 1966). According to Berg and Steen (1965), the gradual removal of O₂ from the gulped air accounts for approximately one third of total oxygen uptake while the remaining two thirds is accomplished by O₂ transfer across the skin. The results of more recent studies, however, indicate that eel skin is poorly suited for transcutaneous gas transfer and may simply extract sufficient O₂ from the ambient environment (air or water) to meet cutaneous metabolic requirements. Indeed, the skin of the closely related European eel (*Anguilla anguilla*), is poorly vascularized, displays a low value of Krogh's constant for O₂ diffusion (3.1 - 4.3 X 10⁻¹⁵ mol sec⁻¹ cm⁻¹ torr⁻¹) and consumes O₂ at a greater rate than cutaneous O₂ uptake (Kirsch and Nonnotte, 1977). Smith et al. (1983) also reported poor vascularization in the skin of the Australian short-finned eel (*Anguilla australis*). Although these authors reported a high percentage of total oxygen uptake by the skin (47 %), they were unable to assess the actual transcutaneous transfer (e.g. water-to-blood). In light of these results and because of the difficulty in
distinguishing between cutaneous O$_2$ uptake and transcutaneous O$_2$ transfer, it is possible that Berg and Steen (1965) overestimated the importance of cutaneous O$_2$ uptake in the oxygenation of arterial blood. There are no direct measurements of transcutaneous CO$_2$ movements in fishes inhabiting air. The results of the present study, however, suggest that eel skin does not contribute significantly to CO$_2$ excretion while in air, based on the steady rise in arterial P$_{CO2}$ during the period of prolonged air-exposure. The pattern of gulping and retaining air in the buccal cavity employed by the eel during aerial exposure, is similar to the pattern of aerial respiration utilized by other fishes that use gills, modified gills or buccopharyngeal tissue to extract oxygen while breathing air (e.g. Synbranchus marmoratus; Johansen, 1966; Heisler, 1982; Graham and Baird, 1984; Amphipnous cuchia; Lomholt and Johansen, 1976; see also reviews by Johansen, 1970; Randall et al., 1981). However, there are differences apparent in the efficiency of aerial gill oxygen transfer between those air-breathing fishes that commonly rely on aerial gas exchange (e.g. Synbranchus) and the predominantly water-ventilating eel. Assuming negligible transcutaneous oxygen uptake in air and water, the decrease in arterial P$_{O2}$ from 75 to 25 torr must reflect a major reduction in the efficiency of branchial O$_2$ transfer. Presumably, this reduced efficiency is due to the absence of counter-current convective processes, a stagnation of the ventilatory medium

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which reduces the blood-to-air $P_{O_2}$ gradient as $O_2$ is extracted continuously from the gill cavity (the duration of breath-holding can be as long as 5 min; Berg and Steen, 1965), and a decrease in diffusive conductance with the collapse of gill lamellae. In contrast, *Synbranchus* can maintain arterial $P_{O_2}$ while breathing air (Johansen, 1966; Heisler, 1982) perhaps as a consequence of buccal epithelial vascularization and structural modifications to the gill that prevent the lamellae from collapsing. The reduction in arterial $P_{O_2}$ during air-exposure, combined with a pronounced reduction in RBC pH and concomitant Bohr and Root effects, contributed to a marked lowering of arterial oxygen content. The magnitude of the Root effect reported here (-2.55 vol%/$P_{H_2}O$ unit) is similar to that reported previously for *Anguilla vulgaris* (Steen, 1963) and *Anguilla anguilla* (Bridges, Hlastala, Riepl and Scheid, 1983). As the presence of a pronounced Root effect is disadvantageous during prolonged air-exposure, it is not surprising that air-breathers such as *Synbranchus* (Johansen, 1966; 1970), *Amphipnous* (Lomholt and Johansen, 1976), *Electrophorus* (Johansen, Lenfant, Schmidt-Neilsen and Peterssen, 1968) and *Neochanna* (Wells, Forster and Meredith, 1984) do not exhibit significant Root effects. Thus, these fish are capable of maintaining haemoglobin $O_2$ saturation despite the severe extracellular acidosis that often accompanies air-exposure (see below). The low oxygen affinity ($P_{50} = 21$ torr at $P_{CO_2} = 3.75$ torr; $P_{H_2}O = 7.76$) measured in
vitro and presumably even lower O\textsubscript{2} affinity in vivo during air-exposure (P\textsubscript{CO\textsubscript{2}} = 2–9 torr) cannot be considered advantageous for air-breathing when arterial P\textsubscript{O\textsubscript{2}} is 24 torr (Fig. 2). The high P\textsubscript{50} values in amphibious air-breathing fishes, however, that are capable of maintaining arterial P\textsubscript{O\textsubscript{2}}, clearly are of adaptive significance for aerial respiration. It must be emphasized that the prevailing view that air-breathing fishes display lower blood O\textsubscript{2} affinity than aquatic forms (e.g. Lenfant and Johansen, 1972) has been challenged (Powers, 1980). Although differences in P\textsubscript{50} between air-breathing and water-breathing fishes may not always be obvious in vitro, they might exist in vivo because of elevated CO\textsubscript{2} levels in air-breathers (Randall et al., 1981). The acidosis reported in this study was attributed to retention of respiratory CO\textsubscript{2} as well as addition of metabolic acid to the blood. Carbon dioxide retention also has been observed in the blood of Synbranchus during air breathing (Johansen, 1966; Heisler, 1982). Evidence for an inability to excrete CO\textsubscript{2} by fishes utilizing gills for aerial gas exchange is supported further by low gas exchange ratios (Lomholt and Johansen, 1976; see also Randall et al., 1981) and low CO\textsubscript{2} tensions in expired air (Graham and Baird, 1984). It is likely that the P\textsubscript{CO\textsubscript{2}} of the air that is retained within the buccal cavity rapidly equilibrates with venous blood, preventing further CO\textsubscript{2} excretion during the latter stages of the breath-hold. Indeed, Lomholt and Johansen (1976) have
demonstrated that the $P_{CO_2}$ within the air sacs in *Amphipnous* reaches equilibrium with blood perfusing the air sacs after only 2 min of an average 8-10 min breath-hold. In the absence of $CO_2$ excretion ($MCO_2$) measurements in the present study, it is speculated that $MCO_2$ declined as each breath-hold progressed thereby causing a persistent elevation of arterial $P_{CO_2}$. Increasing the frequency of ventilation presumably would increase $CO_2$ excretion but this strategy is not utilized by the eel perhaps to limit convective water loss. The observation of base deficit during air-exposure (Fig. 5) indicates that a metabolic acid was added to the blood. It is assumed that the metabolic $H^+$ ions originated from the hydrolysis of internal ATP stores and/or hydrolysis of ATP combined with anaerobic glycolysis (Hochachka and Mommsen, 1983) as indicated by the gradual elevation of plasma lactate levels (Fig. 2E). Thus, it would appear that the eel may cope with prolonged air-exposure by increasing anaerobic metabolism which is not surprising, given the low arterial $O_2$ contents that have been measured. The base deficit, representative of metabolic acid added to the blood, occurred shortly after air-exposure was initiated (Fig. 5) whereas excess lactate only appeared in the blood following 12 h. The discrepancy between the appearance of metabolic acid and lactate in the blood may reflect degradation of internal (presumably muscular) energy stores (Creatine-Pi and ATP) which sustain metabolism during the initial stages of air-exposure, followed
by a switch to anaerobic glycolysis and lactate production in
the latter stages of air-exposure. Alternatively, the delayed
appearance of lactate in the blood may have resulted from
preferential retention of lactate in the tissue of origin
compared to rapid release of $\text{H}^+$ ions. The precise source of
this lactate is not known. Temporal discrepancies between
lactate and metabolic acid appearance in the blood have
frequently been observed after exhaustive exercise in fish and
invariably lactate levels peak at a later time (Wood and
Perry, 1985). The observation that RBC pH during air-exposure
conformed to the in vitro relationship between pH and RBC pH
is in sharp contrast to the preferential regulation of RBC pH
that has been observed in other fishes during extracellular
acidosis (Primett, Randall, Mazeaud and Boutilier, 1986;
Boutilier, Iwama and Randall, 1986; Perry et al., 1987a). The
regulation of RBC pH during acid-base disturbances in fishes
is due to adrenergic stimulation of erythrocytic $\text{Na}^+/\text{H}^+$
exchange (see review by Nikinmaa, 1986). Thus, it would
appear that the eel does not elevate plasma catecholamines
during air-exposure or alternatively eel RBC's are insensitive
to catecholamines. The latter hypothesis is favored as
epinephrine levels are expected to be high during air-exposure
to mobilize tissue glycogen stores for anaerobic glycolysis.
Certainly, this is an area that warrants further
investigation.
In conclusion, this study has revealed that the eel is able to withstand the hypoxemia and acidosis associated with extended air-exposure (36 h). Effective loss of the gill as a gas exchange structure results in an oxygen debt which eventually necessitates a switch to anaerobic metabolism. Accumulation of metabolic $H^+$ ions and respiratory $CO_2$ contribute to a severe mixed acidosis which, mediated by Root and Bohr effects, further exacerbates the $O_2$ debt. Hence, in the absence of an aerial gas exchange organ, the eel is not particularly well-adapted for aerial respiration but its unusual tolerance to hypoxemia and acidosis does permit short-term terrestrial excursions.
CHAPTER 4

ACID-BASE AND IONIC REGULATION IN THE AMERICAN EEL (ANGUILLA ROSTRATA) DURING AND AFTER PROLONGED AERIAL EXPOSURE: BRANCHIAL AND RENAL ADJUSTMENTS
Introduction

An interest in the physiological consequences of aerial exposure in eels has developed because of their habit of making routine excursions onto land to migrate between ponds. Unlike true amphibious air-breathing fishes (e.g. Synbranchus marmoratus), however, the eel is not particularly well-adapted for aerial gas transfer (see Chapter 3). Consequently, during prolonged air-exposure, the eel sustains a severe extracellular acidosis of respiratory and metabolic origin (Berg and Steen, 1965; see also Chapter 3) that is compensated gradually upon return to water (Chapter 3). The two major sites of acid excretion in fishes are the gill and kidney (see review by Heisler, 1984). During air-exposure, the gill is eliminated as a potential site of acid-base regulation and the importance of the kidney is questionable as there is the possibility of reduced urine flow to counteract dehydration. Upon return to water, both locations are available for transfer of excess acidic equivalents into the external medium. There is reason to suspect, however, that the eel has a limited capacity to modify branchial acid excretion compared to other fishes because of the extremely low rates of Cl⁻/HCO₃⁻ exchange (Kirsch, 1972; Bornancin et al., Maetz, 1977). There is ample evidence to suggest that modulation of this pathway is the prevalent mechanism for regulating internal acidosis in fish (Wood et al., 1984; Wood and Perry,
1985; Claiborne and Heisler, 1986; Perry et al., 1987a; Wood, 1988).

Thus, prolonged air-exposure followed by immediate return to water presents an opportunity to study the partitioning of acid-base regulation between the gill and kidney as a function of water availability and to examine the effectiveness of the gill in acid-excretion in the apparent absence of significant Cl⁻/HCO₃⁻ exchange. In this chapter, the branchial and renal adjustments have been assessed in the American eel (Anguilla rostrata) during and after 36 h of continuous air-exposure.
Materials and Methods

Experimental Animals

Immature American eels (Anguilla rostrata) weighing between 50.5 and 156.5 g (mean weight = 84.3 ± 13.8 (SE) g; N = 90) were utilized in these experiments (see Chapter 2). Water temperature ranged between 4 and 9° C during the course of the experiments and both control and experimental fish were subjected to the same temperature variations.

Protocol

Eels were exposed to air for a period of 36 h and then subsequently returned to water for an 18 h period of recovery. Blood samples (0.7 ml) were withdrawn from the caudal artery (see Chapter 2) before air-exposure, at periodic intervals during air-exposure and after return to water. Control eels were kept in water for 54 h. Blood samples were immediately centrifuged and the plasma stored (-70° C) for subsequent analysis of plasma solutes (Na⁺, K⁺, Ca²⁺, Cl⁻, total ammonium and inorganic phosphate (Pi)), and osmolality.

Branchial Solute Fluxes

Time periods chosen for the branchial flux determinations were 0-6 h before air-exposure and 0-6 h, 6-12 h following 36 h of continuous air-exposure. All other details have been outlined in Chapter 2 (General Methods).

Renal Solute Fluxes

Urine was collected continuously in plastic vials for 6
or 12-h periods before air-exposure (0-6, 6-12 h), during air-exposure (0-12, 12-24, 24-36 h) and after air-exposure (0-6, 6-12 h) from eels that had cannulae implanted into the urinary bladder (Chapter 2). Evaporation was minimized by covering the plastic collection vials with parafilm. Urine samples were analyzed immediately after collection for pH and volume. 100 μl of urine was diluted 20 fold, acidified (1% V/V) with 1 M nitric acid and left frozen at -20°C until further assays could be performed. Samples then were thawed and total ammonia, Pi, Na⁺, Cl⁻, K⁺ and Ca²⁺ concentrations were determined. The remaining urine was stored at -70°C for later determination of osmolality. Urine from a separate group of fish (N = 16) was used immediately following collection to determine total urinary acid excretion. Total urinary acid excretion was calculated as the sum of the titratable component (TA-HCO₃⁻) and the non-titratable component (total ammonia) multiplied by the urine flow rate (UFR). Urinary [TA-HCO₃⁻] was measured by adding a known volume of 0.02 M HCl to 200 μl of urine to lower the pH below 5.0, then vigorously aerating and agitating the sample for 15 min to remove CO₂. NaOH (0.02 M) was then gradually added using a microburette (Gilmont) to restore urine pH to the blood pH representative of the particular sampling period. The difference between the quantities of acid and base added to the urine yielded the titratable component of urinary acid excretion.
Statistical Analysis

Data shown in Figures and Tables are means ± 1 SE. Where appropriate, a paired or unpaired Student's t-test was used to compare sample means and 5% was taken as the fiducial limit of significance.
Results

Plasma Solute

Plasma osmolality increased markedly throughout the air-exposure period (Fig. 6A) suggesting significant dehydration. Osmolality was restored rapidly to initial values upon re-immersion in water. Ammonia and Pi concentrations in the plasma changed in a similar manner as osmolality during and after air-exposure (Fig. 6B,C). The effects of air-exposure on the major plasma electrolytes are illustrated in Fig. 7. A gradual elevation of [K+] occurred (in the absence of hemolysis), which was reversed following air-exposure during the 12 h period of recovery in water (Fig. 7C). Notably, plasma levels of Na+, Cl− and Ca2+ did not parallel the changes in plasma osmolality although [Na+] did tend to increase during air-exposure. Although plasma [Cl−] actually decreased while eels were in air, this observation may not be physiologically relevant because [Cl−] remained low even after return to water (Fig. 7B). Plasma [Ca2+] was stable throughout the experiment.

Branchial Adjustments

Using standard radiotracer methodology, it was not possible to detect branchial influx of Cl− in either control or experimental animals over 6 h measuring periods. For this reason, net Cl− fluxes rather than unidirectional Cl− fluxes have been presented in Fig. 8. As a consequence of stimulated JINNa+ and to a lesser extent reduced JOUTNa+ (not significantly different at 0-6 h post air-exposure), JNETNa+ was

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Figure 6. The effects of prolonged air-exposure (○-○, N = 7) on selected plasma variables in the American eel including A) osmolality, B) ammonia concentrations and C) inorganic phosphate concentrations. Control eels (○, N = 7) were left in water for the entire experiment (54 h). * represents significant difference from the control value at the corresponding time; + represents significant difference from pre-exposure values.
Figure 7. The effects of prolonged air-exposure on the concentrations of selected plasma ions including A) Na\(^+\), B) Cl\(^-\), C) K\(^+\) and D) Ca\(^{2+}\). All other details as in Fig. 6.
Figure 8. The effects of prolonged air-exposure in the American eel on branchial solute fluxes. A) Na\(^+\) influx (\(J_{IN}Na^+\), positive values), Na\(^+\) efflux (\(J_{OUT}Na^+\), negative values) and Na\(^+\) net flux (\(J_{NET}Na^+\), solid bars); B) Cl\(^-\) net flux (\(J_{NET}Cl^-\)); C) K\(^+\) net flux (\(J_{NET}K^+\)); D) the net strong ion difference flux (\(J_{NET}Na^+ + J_{NET}K^+ - J_{NET}Cl^-\); SIDF). Flux studies were performed at 0-6 h before air-exposure (Pre) and at 0-6 and 6-12 h following air-exposure (Post). All fluxes are expressed as umol kg\(^{-1}\) h\(^{-1}\). N numbers are 16 for control fish (clear bars) and 15 for experimental fish (cross-hatched bars). * represents significant difference from the control value at the corresponding time; + represents significant difference from pre-exposure values.
immediately upon returning the eels to water (Fig. 8A). At 6-12 h post air-exposure, $J_{\text{NETNa}}$ was not significantly different from the pre air-exposed values although it remained significantly elevated above the control eels. In contrast, $J_{\text{NETCl}}$ was reduced (e.g. more negative) to the same extent in both control and experimental animals presumably as a result of increased $J_{\text{OUTCl}}$ since $J_{\text{INCl}}$ was undetectable. Due to the differential adjustments of branchial net fluxes of Na$^+$, K$^+$ and Cl$^-$ (Fig. 8), a significant pattern emerged with respect to the difference between cation and anion net fluxes ($J_{\text{NETNa}}+J_{\text{NETK}}-J_{\text{NETCl}}$, Fig. 8D). $J_{\text{NETNa}}+J_{\text{NETK}}-J_{\text{NETCl}}$ was elevated during the initial 6 h following air-exposure and remained elevated (although not significantly different from initial values) during the 6-12 h flux period. Based on empirical evidence (Wood et al., 1984; Wood, Boutilier and Randall, 1986; Vermette and Perry, 1987), the magnitude of $J_{\text{NETNa}}+J_{\text{NETK}}-J_{\text{NETCl}}$ is the primary factor influencing branchial net acid excretion ($J_{\text{NETh}}$). Consequently, one would predict an increase in branchial $J_{\text{NETh}}$ following air-exposure. Indeed, branchial $J_{\text{NETh}}$ did increase following air-exposure (Fig. 9) and was similar in magnitude to the increase in $J_{\text{NETNa}}+J_{\text{NETK}}-J_{\text{NETCl}}$ (approximately 100 umol kg$^{-1}$ h$^{-1}$ of additional $J_{\text{NETh}}$). The rise in $J_{\text{NETh}}$ was attributable to ammonia excretion increasing to a greater extent than titratable acid uptake (Fig. 9A).

Renal Adjustments
Figure 9. Branchial excretion of acidic equivalents in the American eel before and after prolonged air-exposure. Shown are A) the components of branchial acid excretion, titratable acidity flux ($J_{TA}$) and ammonia excretion ($J_{AMM}$) expressed as umol kg$^{-1}$ h$^{-1}$ and B) the sum of these which represents net acid excretion ($J_{NETH^+}$). Flux rates are expressed as umol kg$^{-1}$ h$^{-1}$. All other details as in Fig. 8.
Urine flow rate (UFR) decreased during air-exposure and was associated with a pronounced increase in urine osmolality (Fig. 10). Consequently, concentrations of the various urinary solutes (Na⁺, Cl⁻, K⁺, Pi, NH₄⁺), with the notable exception of Ca²⁺, increased throughout the air-exposure period (Figs. 11,12). Urinary excretion rates of the various solutes, however, remained unchanged in air due to the depressed UFR (Figs. 11,13). Again, the exception was renal Ca²⁺ excretion, which actually decreased during air-exposure (Fig. 13D). Upon returning the eels to water, UFR was stimulated tremendously and osmolality was returned to control values. Urinary solute excretion rates were increased during the recovery phase in water due to the stimulation of UFR and/or elevated urinary solute concentrations (Figs. 11,13).

The involvement of the kidney in acid-base regulation during and following air-exposure in the American eel is illustrated in Fig. 14. The reduction in UFR during air-exposure precluded any substantial contribution of the kidney to acid-base regulation although there was a statistically significant increase in urinary J_{NETH⁺} in the final 18 h of exposure. Renal J_{NETH⁺} was stimulated markedly during the first 6 h following return to water due to the combined effects of elevated UFR (Fig. 10), J_{TA-HCO₃⁻} (Fig. 14A) and J_{AMM} (Fig. 14B).
Figure 10. The changes in A) urine osmolality and B) urine flow rate (UFR) associated with prolonged air-exposure in the American eel. Collection periods were increased to 12 h during air-exposure. N numbers are 16 for control fish (clear bars) and 15 for experimental fish (cross-hatched bars). * represents significant difference from the control value at the corresponding time; + represents significant difference from pre-exposure values.
Figure 11. The effects of prolonged air-exposure in the American eel on urinary buffers, ammonia ([AMM]) and inorganic phosphate ([Pi]). Represented are A) ammonia concentrations ([AMM]), B) ammonia excretion rate (J_{AMM}), C) inorganic phosphate concentrations ([Pi]) and D) Pi excretion rate (J_{Pi}). All other details as in Fig. 10.
Figure 12. The effects of prolonged air-exposure in the American eel on urine ion concentrations including A) Na⁺, B) Cl⁻, C) K⁺ and D) Ca²⁺. N numbers are 16 for control fish (clear bars) and 15 for experimental fish (cross-hatched bars). * represents significant difference from the control value at the corresponding time; + represents significant difference from pre-exposure values.
Figure 13. The effects of prolonged air-exposure in the American eel on renal excretion rates (umol kg$^{-1}$ h$^{-1}$) of various ions including A) Na$^+$ ($J_{Na^+}$), B) Cl$^-$ ($J_{Cl^-}$), C) K$^+$ ($J_{K^+}$) and D) Ca$^{2+}$ ($J_{Ca^{2+}}$) with prolonged air-exposure. N numbers are 16 for control fish (clear bars) and 15 for experimental fish (hatched bars). * represents significant difference from the control value at the corresponding time; + represents significant difference from pre-exposure values.
Figure 14. The effects of prolonged air-exposure in the American eel on the renal efflux rates of A) titratable acid minus bicarbonate ($J_{TA-HCO_3^-}$; measured as a single component), B) ammonia ($J_{AMM}$), C) the net excretion of acidic equivalents ($J_{NETH^+}$) and D) urine pH. Fluxes expressed as umol kg$^{-1}$ h$^{-1}$. All other details as in Fig. 13.
Discussion

Blood Acid-base Status

The results of the present study demonstrate that the eel, whether in air or water, possesses a severely limited capacity for acid-base regulation. During air-exposure, blood pH is reduced because of retention of respiratory CO₂ and increased anaerobic metabolism (see Chapter 3). As such, the acid-base disturbance associated with prolonged air-exposure is similar to the mixed extracellular respiratory/metabolic acidosis frequently observed in fish after exhausting exercise (see review by Wood and Perry, 1985). Indeed, the accumulation of metabolic acid (base deficit) in the blood after 36 h in air (9.53 mM) is approximately equal to the extracellular fluid (ECF) metabolic acid load in a variety of fish species immediately post-exercise (Scyliorhinus stellaris, Piiper, Meyer and Drees, 1972; Salmo gairdneri, Milligan and Wood, 1986a; Katsuwonus pelamis, Perry et al., 1985; Raja ocellata, Wood and Perry, 1985). Although the recovery from exhausting exercise is a distinctly different physiological process than the recovery from air-exposure, a comparison of the results above with those obtained from exercising fish nonetheless does reveal the inefficiency of acid-base regulation in the eel. Faced with similar extracellular metabolic acid loads, fishes recovering from exhausting exercise are capable of restoring blood acid-base status usually within 12 h whereas the eel recovering from air-exposure manages to clear just 50%
of the ECF acid load after 18 h recovery in water. It is speculated that the inefficiency of acid-base regulation in the eel compared to other fishes is related to fundamental differences in branchial ionic exchange mechanisms although the possibility of slow metabolic H⁺ removal leading to delayed recovery cannot be excluded.

Branchial Adjustments

It is well established that branchial Cl⁻ influx in the eel is exceptionally low compared to other teleosts (1.2 umol kg⁻¹ h⁻¹; Kirsch, 1972; 3.6 umol kg⁻¹ h⁻¹; Bornancin et al., 1977). Given the low rate of $J_{\text{INCl}^-}$, it is not surprising that unidirectional $^{36}$Cl movements were undetectable over 6 h flux periods using standard radiotracer methodology (in the study of Kirsch (1972), 24 h were required to accurately quantify $J_{\text{INCl}^-}$). Because of the coupling of Cl⁻ uptake and HCO₃⁻ excretion in fishes (see reviews by Evans, 1984; Heisler, 1984), the low rate of $J_{\text{INCl}^-}$ in the eel is reflected by uniquely low plasma [Cl⁻] and relatively high [HCO₃⁻] and pH (e.g. Farrell and Lutz, 1975; Schmidt-Nielsen and Renfro, 1975; Bornancin et al., 1977; see also Table 1). Moreover, inhibition of Cl⁻/HCO₃⁻ exchange and concomitant base retention during periods of internal acidosis is eliminated as an acid-base regulatory mechanism. Of course, it is the net flux of Cl⁻ rather than the unidirectional Cl⁻ fluxes that ultimately determines the contribution of Cl⁻ movements to the net transfer of acidic equivalents across the gill. In this
regard, modulation of Cl\(^{-}\) efflux could potentially contribute to acid-base regulation. This prospect apparently is also eliminated in the eel because the passive efflux of Cl\(^{-}\) must be kept low in order to partially counteract the reduced Cl\(^{-}\) influx (Kirsch, 1972). Although the changes in Cl\(^{-}\) net flux reported here following air-exposure are consistent with the regulation of acidosis, their significance is doubted because the changes were relatively minor and control eels displayed a similar trend. The apparent inability of the eel to modulate branchial Cl\(^{-}\) movements is significant because the modification of J\(_{\text{NETCl}^{-}}\) during and/or following a variety of acid-base disturbances has been reported to be the dominant mechanism of acid-base regulation in fishes (hyperoxic acidosis, Wood et al., 1984; following exhaustive exercise, Wood and Perry, 1985; hypercapnia, Claiborne and Heisler, 1986; Perry et al., 1987a). According to strong ion difference (SID) theory (Stewart, 1980) and experimental data (Wood et al., 1984; Wood et al., 1986; Vermette and Perry, 1987), branchial acid excretion ultimately is determined by the arithmetic difference between strong cation and strong anion net fluxes (net strong ion difference flux, SIDF). In the present study, SIDF has been estimated as J\(_{\text{NETNa}^{+}}\)+J\(_{\text{NETK}^{+}}\)-J\(_{\text{NETCl}^{-}}\). The significant increase of SIDF observed after eels were returned to water is an appropriate response for compensating the acidosis accrued during air-exposure. The most significant response promoting the
elevation of SIDF was stimulation of $J_{\text{IN}}\text{Na}^+$ in the initial 6 h following re-immersion in water. As expected, there was a reasonable correlation between the rate of the SIDF and the rate of branchial acid excretion. The absolute magnitude of the branchial net acid flux during recovery from air-exposure, albeit low compared to other fishes regulating equivalent acid loads, was sufficient to account for the slow decrease in the ECF metabolic acid load (approximately 70 umol kg$^{-1}$ h$^{-1}$ over 18 h).

**Renal Adjustments**

Under normal conditions, urine resides in the bladder for variable periods of time before being excreted. However, the bladder catheterization technique only enables the collection of continuously voided tubular urine. Thus, in the present study, the contribution of the urinary bladder in acid-base or ionic regulation was not assessed. Further problems associated with analyzing urine collected continuously from gas-permeable urinary catheters include the inability to collect urine under anaerobic conditions thereby leading to CO$_2$ loss from the urine and pH fluctuations, and a considerable catheter dead space volume of approximately 0.35 ml resulting in catheter transit times which averaged 2.0 h in control fish. Prolonged air-exposure was associated with ECF dehydration as indicated by the pronounced rise in plasma osmolality and was presumably equivalent to the volume of fluid excreted by the kidney and the amount lost by evaporat-
tion across the body surfaces. The increase in plasma osmolality (about 40 mOsm kg⁻¹) cannot be accounted for by elevated levels of the measured ions (Na⁺, K⁺, Cl⁻, NH₄⁺, Ca²⁺) which suggest there were considerable changes in some unmeasured osmolyte(s). The kidney played a relatively minor role in acid-base regulation both during air-exposure and during the recovery period in water. The primary adjustment of renal function while eels were in air was a marked reduction in UFR. Clearly, this is important for minimizing dehydration but precludes a major role for the kidney in acid-base balance. Although UFR is reduced by the eel when in air, there is an obvious physiological limit in the capacity to concentrate the urine. Our results indicate that UFR in air-exposed eels approaches values reported for seawater-adapted eels (Schmidt-Nielsen and Renfro, 1975). Glomerular filtration rate (GFR) was not measured in the present study thus the cause of the reduced UFR remains unclear. The pronounced increase in urine osmotic and ionic concentrations, however, suggests that increased tubular reabsorption of water or reduced tubular fluid secretion could be involved. Schmidt-Nielsen and Renfro (1975) reported that the reduced UFR in seawater-adapted eels was not due to adjustments of GFR but rather to changes in tubular fluid reabsorption and secretion. Although renal acid excretion was significantly increased during the latter stages of air-exposure and the initial period of recovery, the magnitude
of these changes was insufficient to substantially alter the ECF acid-base status during air-exposure or assist in the recovery after air-exposure. Moreover, a significant component of the apparent increase in renal acid excretion after air-exposure must have been initiated during air-exposure but was not measured during that period due to the reduced UFR and to the catheter dead space volume. Hence, renal excretion of acidic equivalents accounted for approximately 6.5% of the metabolic acid cleared after air-exposure whereas branchial contributions accounted for 93.5% of the acid cleared. The lack of involvement of the eel kidney in acid-base regulation is perhaps best illustrated by analysis of the urine between 6 and 12 h after air-exposure. During this period, a substantial ECF metabolic acid load existed yet renal acid excretion was unaltered. The reliance of other freshwater teleosts on the kidney for acid-base regulation is variable and is probably related to species differences and the nature of the acid-base disturbance. The simulation of metabolic acidosis by infusion of mineral acid in trout (Wood and Caldwell, 1978) and catfish (Cameron and Kormanik, 1982) or external acidification in trout (McDonald and Wood, 1981) can stimulate renal acid excreting mechanisms which can account for clearance of between 33 and 100% of the accumulated acid load (33%, Cameron and Kormanik, 1982; 50%, McDonald and Wood, 1981; 100%, Wood and Caldwell, 1978). Surprisingly, the injection of lactic acid into trout, which perhaps best mimics the elevation of
ECF metabolic acid load in the eel at the conclusion of air-exposure, caused only minor changes in renal acid excretion and could account for only 6% of total acid clearance (Kobayashi and Wood, 1980). Thus, the possibility that the eel kidney can respond effectively to other types of acid-base disturbances cannot be excluded. In conclusion, the changes observed in SIDF during recovery from air-exposure were attributed primarily to modification of branchial Na⁺/H⁺ exchange. A correlation existed between the rate of SIDF and the rate of branchial acid excretion. Although the metabolic removal of acidic equivalents has been demonstrated in other fishes, the increase in the rate of branchial net acid efflux was sufficient to account for the slow rate of recovery observed (70 umol kg⁻¹ h⁻¹). Net renal acid efflux was low (approximately 6.5% of total acid clearance) both during and after prolonged air-exposure. The predominant role of the kidney during air-exposure was to minimize dehydration. It is suggested that the limited capacity for acid-base regulation displayed by the eel is, in part, a result of low rates of branchial Cl⁻/HCO₃⁻ exchange.
CHAPTER 5

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).
Introduction

Acid-base and ionic regulation are linked in fishes (see Evans, 1984; Heisler, 1984). Moreover, the relative contributions of the gills and kidneys to acid-base regulation are diverse (see Chapters 1 and 3; Cameron, 1980; Kobayashi and Wood, 1980; McDonald and Wood, 1981; Cameron and Kormanik, 1982; Wood et al., 1984; Perry et al., 1987a,b). It is apparent, however, that respiratory acid-base disturbances (e.g. hypercapnic acidosis, hyperoxic acidosis) are regulated almost exclusively by the gill with little or no reliance on renal adjustments (Cameron, 1980; Wheatly et al., 1984; Wood et al., 1984, Perry et al., 1987a,b). In the fish gill epithelium, Na\(^+\) and Cl\(^-\) uptake from the water are related to acid and base extrusions, respectively, via electroneutral Na\(^+\)/H\(^+\)(NH\(_4\)\(^+\)) and Cl\(^-\)/HCO\(_3\)^-(OH\(^-\)) exchanges on the apical (mucosal) membrane. Thus, during periods of internal acidosis, stimulation of Na\(^+\)/acid exchange and/or inhibition of Cl\(^-\)/base exchange causes excretion of acidic equivalents to the external environment. The net result is accumulation of bicarbonate within the fish and ultimately, regulation of the acidosis. With the notable exception of the Arctic grayling (Thymallus arcticus; Cameron, 1976), fish appear to rely predominantly on inhibition of branchial Cl\(^-\)/HCO\(_3\)^-(OH\(^-\)) exchange to regulate hypercapnic acidosis of endogenous (Wood et al., 1984) or exogenous (Claiborne and Heisler, 1984; 1986; Perry et al., 1987a) origin.
The eel (*Anguilla sp.*) is unusual among euryhaline teleosts because it lacks significant Cl⁻/HCO₃⁻ exchange, as indicated by almost undetectable branchial Cl⁻ influx (see Chapter 4; Kirsch, 1972; Bornancin et al., 1977). Thus, an examination of the American eel (*Anguilla rostrata*) and the rainbow trout (*Salmo gairdneri*) during hypercapnic acidosis presents an unique opportunity to study the relative contributions of the Cl⁻/HCO₃⁻(OH⁻) and Na⁺/H⁺(NH₄⁺) exchange mechanisms to acid-base balance in fishes. Although dynamic manipulation of the Cl⁻/HCO₃⁻ exchange has been reported in the eel (Bornancin et al., 1977), extremely low activity presumably will preclude a role in acid-base regulation. Indeed, in Chapter 4, it was argued that absence of significant Cl⁻/HCO₃⁻ exchange in the American eel may have been the basis for the inefficient acid-base regulation observed after prolonged air-exposure. The results of that study, however, may have been influenced by profound metabolic adjustments.

In this chapter, i) the potential of the Na⁺/H⁺(NH₄⁺) exchange pathway to modify blood pH in the eel by acclimating fish to various external Na⁺ concentrations has been assessed; ii) the ability of the trout and the eel to regulate hypercapnic acidosis has been compared, and iii) the importance of external Na⁺ levels on the ability of the eel to regulate hypercapnia has been evaluated. These experiments were performed to test the hypothesis that the eel relies
solely on adjustments of branchial Na⁺/H⁺(NH₄⁺) exchange to correct internal acidosis because it lacks the capability to modify Cl⁻/HCO₃⁻(OH⁻) exchange.
Materials and Methods

Experimental Animals

Both American eels (*Anquilla rostrata*) weighing between 108 and 233 g (mean wt = 111.2 ± 16.1 g (SE); N = 44) and rainbow trout (*Salmo gairdneri*) of both sexes and weighing between 147.3 and 398.8 g (mean wt = 267.8 ± 34.7 g (SE); N = 22) were utilized in these experiments. All fish were maintained (as outlined in Chapter 2) in large fiberglass aquaria (Living Stream; Toledo, Ohio) supplied with flowing, aerated and dechlorinated City of Ottawa tap water ([Na$^+$] = 0.10 mM, [Cl$^-$] = 0.15 mM, [Ca$^{2+}$] = 0.35 - 0.40 mM, [K$^+$] = 0.93 mM, pH = 7.5 - 8.0).

Acclimation Conditions

To increase the concentration of Na$^+$ in the water, dechlorinated, city of Ottawa tapwater (flow 1200 ml min$^{-1}$) was titrated with a solution of 6 M NaCl (0.40 ml min$^{-1}$) into holding aquaria in order to attain [Na$^+$] = 1.97 ± 0.09 mM (N = 264), [Cl$^-$] = 2.04 ± 0.11 (N = 264), [Ca$^{2+}$] = 0.35 - 0.40 mM, [K$^+$] = 0.03 mM and pH = 7.4 - 8.0. Eels were acclimated to these conditions for at least 6 weeks prior to experimentation.

Eels were acclimated for the same period of time to low Na$^+$ water. City of Ottawa tapwater was used as source water for a reverse osmosis unit (Culligan) used in conjunction with a deionizer. This system was capable of removing 95 - 98% of all ions while maintaining a water flow rate of approximately
800 ml min\(^{-1}\). The deionized water (800 ml min\(^{-1}\)) was titrated with a solution of 1.3 M CaNO\(_3\)/0.3 M CaCl\(_2\) (0.20 ml min\(^{-1}\)) into holding aquaria to attain \([\text{Na}^+] = 0.0090 \pm 0.0003 \text{ mM (N = 456), [Cl}^-] = 0.162 \pm 0.017 \text{ mM (N = 456) and [Ca}^{2+}] = 0.439 \pm 0.023 \text{ (N = 456). To maintain pH at approximately 7.8, the deionized water was further titrated with 6 M KOH (0.04 ml min}^{-1}). Eels were acclimated to these conditions for at least 6 weeks prior to experimentation.

During all experiments, eels acclimated to both high NaCl and low Na\(^+\) water were kept under these conditions.

**Branchial Solute Fluxes**

In all experiments, whole-body fluxes were determined. However, due to the relative impermeability of eel skin (Kirsch, 1972) and the negligible renal ionic fluxes (see Chapter 4), whole body fluxes essentially reflect branchial ionic fluxes. To determine branchial solute fluxes, water flow to the Perspex boxes was halted for the duration of the flux. Water temperature in the boxes was maintained by increasing the level of the water surrounding the boxes on a "wet table" and maintaining water flow to the table. The box was vigorously aerated to maintain water \(P_{O_2}\) at approximately 150 torr. Branchial Na\(^+\) and Cl\(^-\) influxes (\(J_{INNa^+}\) and \(J_{INCl^-}\)) and net fluxes (\(J_{NETNa^+}\) and \(J_{NETCl^-}\)) were determined as outlined in Chapter 2. Specific activity of the water was maintained between 3000 and 5000 DPM/\(\mu\)Mol. Water samples were removed after a 20 min mixing period and then again following the flux
period to determine Na\(^+\) or Cl\(^-\) specific activities. This flux period was 3 h for the determination of both Na\(^+\) and Cl\(^-\) influxes in trout and for determination of Na\(^+\) influx in eels. To accurately measure Cl\(^-\) influx in eels, the flux period was extended to 6 h.

**Protocol**

Following a 3 h normocapnic period, hypercapnia was induced by equilibrating water contained within the holding apparatus, which consisted of an opaque, 3 L Perspex box, with 0.8% CO\(_2\) in air. This was accomplished using a vertical, counter-current gas exchange column (see Perry et al., 1987a). The rate of water flow into the column was allowed to exceed that flowing to the boxes while an overflow was used to maintain water levels at the top of the column. Water flowing down through the column was allowed to mix with 8% CO\(_2\) in air, released at the bottom. As the gas rose, it was dispersed by marbles (diameter 1 cm), effectively increasing the surface area for gas exchange. Precise control of P\(_{CO_2}\) was attained by varying the flow of water through the column. The hypercapnic water was then passed into the experimental holding box where P\(_{CO_2}\) was monitored. Flow from the column was approximately 600 ml min\(^{-1}\), adequate to supply water to 3 fish simultaneously. A Wosthoff gas mixing pump (model M301 A-F) was used to supply the 8% CO\(_2\) in air.

**Blood Sampling Regimen**
For American eels (Anquilla rostrata), blood samples (0.6 ml) were withdrawn from cannulae implanted in the pneumogastric artery (see Chapter 2) 3 h prior to hypercapnia, at 1, 4, 12, 24, 48 and 72 h during hypercapnia, and at 1 and 24 h post-hypercapnia. In a similar fashion, blood samples were withdrawn from cannulae implanted into the dorsal aorta of trout (see Chapter 2). Due to attrition, this sampling regimen was altered slightly for trout. During the hypercapnic period, no sample was taken at 4 h and a single sample was taken at 36 h instead of at 24 h and 48 h.

Measurements were made of hematocrit (Hct), total CO₂ (CCO₂), whole blood pH (pHe) and red blood cell (RBC) pH. All analytical procedures (for determination of blood acid-base status and ionic fluxes) have been outlined in Chapter 2.

Statistical Analysis

Data shown in figures and tables are means ± 1 S.E. To test for significance between experimental groups, a 2 X 2 factorial ANOVA was performed in conjunction with a Tukey's studentized range test. To test for significant difference within experimental groups, a Student's t-test was used. The fiducial limit of significance for all tests was taken as 5%. See figure legends for details.
Results

The eel in normal water, when compared to the trout kept under similar environmental conditions displayed a metabolic alkalosis (Fig. 15). Acclimation to elevated external [Na⁺] (high NaCl water) exacerbated the metabolic alkalosis in the eel whereas acclimation to lowered external [Na⁺] (low Na⁺ water) caused a metabolic acidosis with respect to the normal water eel (Fig. 15). The acidosis developed by the eel after acclimation to low Na⁺ water did not differ significantly from the acid-base status of the trout in normal water (Fig. 15).

In the eel, branchial influx of Cl⁻ was not significantly affected by acclimation to either high NaCl or low Na⁺ water (Table 1). Branchial influx of Na⁺ was stimulated by acclimation to high NaCl water and was inhibited by acclimation to low Na⁺ water (Table 1). These results concur with the perturbations in blood acid-base status following the acclimation period. Although the rates of both $J_{IN}Na^+$ and $J_{IN}Cl^-$ were considerably lower in the eel than in the trout, the ratio of $J_{IN}Na^+$ to $J_{IN}Cl^-$ was much higher in the eel due to the extremely low values of $J_{IN}Cl^-$. An integration of the data in Table 1 and Fig. 15 revealed that blood acid-base status in acclimated animals could be estimated, at least qualitatively, by the ratio of $J_{IN}Na^+$ to $J_{IN}Cl^-$. Branchial net fluxes ($J_{NET}$) did not differ significantly between groups in which accurate measurements could be made ($J_{NET}Na^+$ or $J_{NET}Cl^-$ could not be determined with accuracy in high NaCl
Figure 15. A pH-HCO₃ diagram showing blood acid-base status of trout and eels acclimated to variable external [Na⁺] (indicated). indicates significant difference from the eel acclimated to normal water; indicates significant difference from the trout. Means were statistically compared using ANOVA in conjunction with a Tukey's Studentized range test.
Table 1: A comparison of resting branchial ionic fluxes between the rainbow trout and the American eel following acclimation to various external [Na⁺]. All values are presented as umoles kg⁻¹ hour⁻¹ ± 1 SE. * indicates significant difference from normal water eel; + indicates significant difference from the trout.

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<th>TYPE</th>
<th>J_{NET}Na⁺</th>
<th>J_{IN}Na⁺</th>
<th>J_{NET}Cl⁻</th>
<th>J_{IN}Cl⁻</th>
<th>J_{IN}Na⁺/J_{IN}Cl⁻</th>
</tr>
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<tr>
<td>TROUT (N=12)</td>
<td>-14.60</td>
<td>324.59*</td>
<td>-9.30</td>
<td>189.46*</td>
<td>1.71</td>
</tr>
<tr>
<td></td>
<td>±8.74</td>
<td>±48.83</td>
<td>±7.21</td>
<td>±28.87</td>
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<tr>
<td>EEL:</td>
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<td></td>
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<tr>
<td>NORMAL (N=12)</td>
<td>-9.70</td>
<td>33.58+</td>
<td>-4.01</td>
<td>5.92+</td>
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</tr>
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<td>±7.20</td>
<td>±3.54</td>
<td>±10.92</td>
<td>±0.93</td>
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<tr>
<td>LOW. Na⁺ (N=10)</td>
<td>3.56</td>
<td>8.16*+</td>
<td>-0.84</td>
<td>4.83+</td>
<td>1.69</td>
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<tr>
<td></td>
<td>±8.59</td>
<td>±1.29</td>
<td>±13.94</td>
<td>±1.61</td>
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<tr>
<td>HIGH NaCl (N=11)</td>
<td>---- 920.86*+</td>
<td>----</td>
<td>6.28+</td>
<td>146.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>±176.11</td>
<td></td>
<td>±0.57</td>
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</table>
water due to the high background levels of NaCl).

During hypercapnia, water PCO₂ was increased to 6.42 ± 0.23 torr (N = 77) from approximately zero torr and consequently blood PCO₂ was elevated throughout the hypercapnic period in both the normal water eel and the trout (see Fig. 16A). With the onset of hypercapnia, whole blood pH decreased in the eel and trout by approximately 0.4 and 0.35 units, respectively (Fig. 16B). The acidosis was compensated gradually during the hypercapnic period (Fig. 16B) and was associated with a rise in plasma [HCO₃⁻] (Fig. 16D). RBC pH dropped to a greater extent in the eel (0.25 units) than in the trout (0.1 unit) (Fig. 16C). A comparison of the relationship between whole blood pH and RBC pH in vitro and in vivo (during hypercapnia) revealed that RBC pH in the eel (Fig. 19), unlike the trout (Fig. 20), conformed to the in vitro relationship and therefore was not regulated preferentially over extracellular pH.

During the post-hypercapnic period, a slight alkalosis developed (Fig. 16B) due to differential rates of branchial excretion of dissolved CO₂ and acid-base relevant ions (Fig. 16A-D). The alkalosis was fully compensated by the trout after 24 h of recovery but persisted in the eel (Fig. 16B). Accordingly, plasma [HCO₃⁻] remained elevated in the eel (Fig. 16D).

The temporal changes in blood acid-base status during hypercapnia in the eel and trout are shown as pH–HCO₃⁻.
Figure 16. The effects of external hypercapnia (●—●) on selected arterial blood acid-base variables in the American eel (left) acclimated to normal water and the rainbow trout (right) including A) arterial PCO₂, B) whole blood pH, C) red blood cell (RBC) pH and D) total CO₂ (TCO₂). Control animals (○—○) were maintained in normocapnic water. N numbers are 7 for both experimental and control eels; N numbers are 6 and 5 for experimental and control trout, respectively. A Student’s t-test was used to determine (†) significant difference from control value at corresponding time, (♦) significant difference from pre-exposure value (PRE) and (‡) significant difference from both control and pre-exposure values.
Figure 17. A pH-HCO₃⁻ diagram showing the temporal changes in blood acid-base status in the American eel acclimated to normal water during and after 72 h of exposure to external hypercapnia (0.8% CO₂). The dashed line represents the in vitro whole blood non-HCO₃⁻ buffer line ($\Theta = -10.1$ mM L⁻¹ whole blood; see Chapter 3). The numbers associated with each point indicate the duration of hypercapnia, in hours. P represents pre-exposure acid-base values; Post 1 and Post 24 represent 1 and 24 h after return to normocapnia, respectively.
Figure 18. A pH-HCO₃⁻ diagram showing the temporal changes in blood acid-base status in the rainbow trout acclimated to normal water during and after 72 h of exposure to external hypercapnia (0.8% CO₂). The dashed line represents the in vitro whole blood non-HCO₃⁻ buffer line (θ = -8.2 mM L⁻¹ whole blood; Vermette and Perry, 1987). All other details as in Fig. 17.
Figure 19. The effects of external hypercapnia on the relationship between whole blood pH and RBC pH in eels acclimated to normal water. In vivo pH values (± 1 SE) have been superimposed on the in vitro relationship between whole blood pH and RBC pH (see Chapter 3). * indicates significant difference from the regression shown with 95% confidence intervals. The numbers associated with each point indicate the duration of hypercapnia, in hours. P represents pre-exposure values; Post 1 and Post 24 represent 1 and 24 h after return to normocapnia, respectively.
Figure 20. The effects of external hypercapnia on the relationship between whole blood pH and RBC pH in trout acclimated to normal water. In vivo pH values (± 1 SE) have been superimposed on the in vitro relationship between whole blood pH and RBC pH (from Vermette and Perry, 1987).
diagrams (Figs. 17, 18). After 1 h of hypercapnia, the changes in whole blood acid-base status in both the eel and the trout simply reflected non-bicarbonate buffering. During the ensuing 72 h of hypercapnia, metabolic compensation of the acidosis occurred in both the eel and the trout as indicated by movement along constant \( \text{PCO}_2 \) isopleths. After hypercapnia (Figs. 17, 18), blood acid-base status in both the eel and trout initially reflected the rapid removal of respiratory acid (\( \text{CO}_2 \)) resulting in a metabolic alkalosis. The differential abilities of the eel and trout to compensate the post-hypercapnic metabolic alkalosis were associated with differential rates of \( \text{HCO}_3^- \) removal from the blood (Figs. 17, 18).

Eels acclimated to high NaCl water exhibited responses similar to the normal water eel following the onset of hypercapnia (Fig. 21). RBC pH was elevated above the controls prior to the onset of hypercapnia and during the initial 4 h of hypercapnia, the decrease in RBC pH was less in eels acclimated to high NaCl water when compared to normal water eels during this period (Fig. 21C). Moreover, a comparison of the relationship between whole blood pH and RBC pH in vivo and in vitro (Fig. 22) revealed that RBC pH was regulated preferentially only during this time. Red blood cell pH did not rise significantly above control values until 1 h post-hypercapnia (Fig. 21C) though the values did not deviate from the in vitro relationship between whole blood pH and RBC pH at this time (Fig. 22).
Figure 21. The effects of external hypercapnia (-----) on selected arterial blood acid-base variables in the American eel acclimated to normal water (left) and acclimated to high NaCl water (right). N numbers are 5 for experimental and control eels acclimated to high NaCl water. All other details as in Fig. 16.
Figure 22. A pH-HCO$_3^-$ diagram showing the temporal changes in blood acid-base status in the American eel acclimated to high NaCl water during and after 72 h of exposure to external hypercapnia (0.8% CO$_2$). All other details as in Fig. 17.
Figure 23. The effects of external hypercapnia on the relationship between whole blood pH and RBC pH in American eels acclimated to high NaCl water. For details, see Fig. 19.
Post-hypercapnia, the changes in arterial PCO$_2$, whole blood pH and plasma C$_{CO_2}$ in eels acclimated to high NaCl water were similar to eels acclimated to normal water (Figs. 21A,B,D; 22).

The temporal changes in blood acid-base status during hypercapnia in eels acclimated to low Na$^+$ water differed significantly from eels acclimated to normal water (Fig. 24). Notably, plasma C$_{CO_2}$ (Fig. 24D) remained unchanged during 72 h of hypercapnia and whole blood pH was not even partially regulated (Fig. 24B). Post-hypercapnia, both blood PCO$_2$ and RBC pH returned to control values within 1 h (Fig. 24A,C). Whole blood pH remained significantly depressed at 1 h post-hypercapnia but returned to control levels after 24 h of recovery (Fig. 24B). Plasma C$_{CO_2}$ was not significantly elevated at any time during the experiment (Fig. 24D).

Shown as a pH-HCO$_3^-$ diagram (Fig. 25), blood acid-base status during hypercapnia in eels acclimated to low Na$^+$ water conformed to the in vitro buffer line and therefore simply reflected non-bicarbonate buffering.
Figure 24. The effects of external hypercapnia (●—●) on selected arterial blood acid-base variables in the American eel acclimated to normal water (left) and acclimated to low Na⁺ water (right). N numbers are 6 and 5 respectively for experimental and control eels acclimated to high NaCl water. All other details as in Fig. 16.
Figure 25. A pH-HCO₃⁻ diagram showing the temporal changes in blood acid-base status in the American eel acclimated to low Na⁺ water during and after 72 h of exposure to external hypercapnia (0.8% CO₂). All other details as in Fig. 17.
Discussion

Acid-base regulation in fishes during acidosis is accomplished by the transfer of $\text{HCO}_3^-$ into the body which is equivalent to the extrusion of acidic equivalents into the environment. The differential adjustments of branchial net ionic fluxes (predominantly $J_{\text{NET}Na^+}$ and $J_{\text{NET}Cl^-}$) correlate with the net movement of acidic equivalents (see Chapter 4; Wood et al., 1984; Wood et al., 1986; Claiborne and Heisler, 1986; Vermette and Perry, 1987). Adjustments of branchial net ionic fluxes during acid-base disturbances result from the modulation of either branchial efflux and/or influx. During periods of acidosis, the appropriate net flux responses are increased $J_{\text{NET}Na^+}$ or decreased $J_{\text{NET}Cl^-}$. The results of the present study indicate that the eel is entirely reliant on modulation of $J_{\text{NET}Na^+}$. We suggest that this strategy for acid-base regulation in the eel is a reflection of the exceptionally low rates of unidirectional $\text{Cl}^-$ fluxes.

Effects of Acclimation

In the eel, the branchial uptake of $\text{Cl}^-$ is not affinity-limited because elevating the external concentration of $\text{Cl}^-$ (high NaCl water) above levels speculated for Km in the eel (1mM; Bornancin et al., 1977) did not significantly affect uptake. Moreover, acclimation to low Na+ water might have been expected to cause an increase in the number of chloride cells (Laurent, Hobe and Dunel-Erb, 1985) thereby increasing the number of branchial $\text{Cl}^-/\text{HCO}_3^-$ exchange sites as occurs in
the trout (S. F. Perry and P. Laurent, unpublished). However, as no stimulation of Cl⁻ uptake was detected, it is speculated that the eel is physiologically incapable of modifying Cl⁻ uptake in freshwater and the low capacity for Cl⁻ uptake will impede dynamic modulation of net Cl⁻ flux under stressed conditions. The limited capacity for Cl⁻ uptake is reflected by decreased plasma [Cl⁻] (Chapter 4; Bornancin et al., 1977) and a significant "anion gap" (Farrell and Lutz, 1975) when compared to the trout. Unlike other fishes (Catostomus commersoni; Hobe, Wood and McMahon, 1984; Salmo gairdneri; Wood et al., 1984; Vermette and Perry, 1987; Perry et al., 1987a; Thymallus arcticus; Cameron, 1976), the magnitude of branchial Cl⁻ uptake is approximately 1/6 of branchial Na⁺ uptake in the eel and, although JİNa⁺ is much greater in the trout than the eel, plasma [Na⁺] in the eel (Chapter 4; Bornancin et al., 1977) is similar to the trout (McDonald and Wood, 1981; Perry et al., 1987a). The resulting strong ion difference (Stewart, 1980) in eel plasma correlates with elevated whole blood pH (Chapter 4; Bornancin et al., 1977). The results of the present study demonstrate a relationship between the external concentration of Na⁺ and whole blood pH in the eel due to changes in branchial Na⁺ uptake without concurrent changes in Cl⁻ uptake. During acclimation to high NaCl water, the elevation of external [NaCl] resulted in the stimulation of JİNa⁺ but did not affect JİCl⁻. Stimulation of JİNa⁺ without a concurrent increase in Na⁺ efflux would
cause a positive net flux during the initial period of acclimation until a new steady-state was achieved (e.g. \( J_{\text{NET}Na^+} \) was not significantly elevated after 6 weeks of acclimation). The observed increase in whole blood pH probably was related to a further increase in plasma SID caused by a transitory increase in Na\(^+\) net flux. Conversely, in low Na\(^+\) water, the uptake of Na\(^+\) became affinity-limited and \( J_{\text{IN}Na^+} \) was depressed. The passive efflux of Na\(^+\) would then result in a net loss of Na\(^+\) (lowered plasma [Na\(^+\)]) and hence a decrease in whole blood pH. Ultimately, however, efflux modulation restored \( J_{\text{NET}Na^+} \) to control levels (Table 1) and further adjustments of pH were prevented.

Thus, it is apparent that the changes in blood acid-base status associated with variable external [Na\(^+\)] were related to transient adjustments of \( J_{\text{NET}Na^+} \). Hence, an examination of the branchial influxes of both Na\(^+\) and Cl\(^-\) in trout and eels acclimated to high NaCl, normal and low Na\(^+\) water revealed that the ratio of \( J_{\text{IN}Na^+} \) to \( J_{\text{IN}Cl^-} \) qualitatively estimates blood acid-base status during steady-state conditions.

**Effects of Hypercapnia**

The eel is known to clear the metabolic acid load accrued during a 36 h exposure to air slowly when compared to other fishes faced with similar metabolic acid loads (Katsuwonus pelamis; Perry *et al.*, 1985; *Salmo gairdneri*; Milligan and Wood, 1986a; *Scyliorhinus stellaris*; Piiper *et al.*, 1972; *Raja ocellata*; Wood and Perry, 1985). In chapter 4, it was
postulated that the limited capacity to regulate this acid-base disturbance is due to extremely low rates of Cl⁻/HCO₃⁻ exchange in the eel. The present study shows that the eel is capable of compensating the acidosis associated with hypercapnia as well as the trout (see also Perry et al., 1987a). Indeed, the changes in blood acid-base status during hypercapnia in the eel are similar to those reported for other freshwater and marine fishes (see Cameron and Randall, 1972; Janssen and Randall, 1975; Cameron, 1976; Eddy, 1976; Randall, Heisler and Drees, 1976; Eddy, Lomholt, Weber and Johansen, 1977; Cameron, 1980; Perry et al., 1981; Perry, 1982; Thomas, 1983; Toews et al., 1983; Perry et al., 1987a). Increasing the external concentration of NaCl has no apparent effect on the ability to regulate whole blood pH during hypercapnic acidosis even though eels acclimated to high NaCl water were able to elevate plasma [HCO₃⁻] to approximately 30 mM. The inability of both the eel acclimated to high NaCl water and the eel acclimated to normal water to fully compensate whole blood pH is perhaps, in part, related to the arithmetic relationship between whole blood pH and [HCO₃⁻]. To fully compensate whole blood pH, plasma [HCO₃⁻] theoretically would have to rise to approximately 50 mM, far above the bicarbonate threshold reported for most fish (Claiborne and Heisler, 1986). Compensation of hypercapnic acidosis is inhibited by a lowered concentration of external Na⁺ revealing the dependence of the eel on the modulation of branchial Na⁺ uptake rather
than branchial Cl\textsuperscript{-} uptake. Presumably, the magnitude of any changes in Cl\textsuperscript{-} net flux are too small to result in detectable changes in blood acid-base status.

There is no apparent explanation for the relative inability displayed by the trout in this study to compensate hypercapnic acidosis when compared to previous studies (see Cameron and Randall, 1972; Eddy et al., 1977; Perry, 1982; Perry et al., 1987a) other than seasonal effects on catecholamine mediated responses; trout were exposed to hypercapnia in October and November of 1987. Catecholamines are released during hypercapnic acidosis (Perry et al., 1987a), and may modify branchial ion exchanges (see Vermette and Perry, 1987).

Changes in blood acid-base status in the eel post-hypercapnia differ from those reported in other fishes. The eel displays a persistent elevation of plasma [HCO\textsubscript{3}\textsuperscript{-}] following hypercapnia unlike the trout which rapidly returns plasma [HCO\textsubscript{3}\textsuperscript{-}] to control levels. The reduction of elevated HCO\textsubscript{3}\textsuperscript{-} in the trout post-hypercapnia is due to a net inward movement of Cl\textsuperscript{-} (Perry et al., 1987a), due to stimulation of J\textsubscript{IN}Cl\textsuperscript{-}. The stimulation of either J\textsubscript{IN}Cl\textsuperscript{-} or J\textsubscript{NET}Cl\textsuperscript{-} is not possible in the eel due to the low, constant rate of Cl\textsuperscript{-}/HCO\textsubscript{3}\textsuperscript{-} exchange.

In the study in which eels were exposed to air (Chapter 3), it was shown that RBC pH is not selectively regulated in vivo but simply conforms to the in vitro relationship between whole blood pH and RBC pH. During hypercapnia in eels
acclimated to low Na$^+$ or normal water, RBC pH also conforms to
the in vitro relationship indicating that the eel either does
not elevate plasma catecholamines or that RBC pH, unlike most
other fishes (see Nikinmaa, 1986), is unaffected by elevated
plasma catecholamines. It is unclear why eels acclimated to
high NaCl water displayed a greater propensity for RBC pH
regulation during the initial 4 h of hypercapnia. This
regulation did not persist throughout the hypercapnic period.
Regulation of RBC pH in other fishes occurs due to adrenergic
stimulation of Na$^+$/H$^+$ exchange (see Nikinmaa, 1986) and may
minimize depressions of arterial O$_2$ content during periods of
extracellular acidosis (Nikinmaa, Cech and McEnroe, 1984;
Boutilier et al., 1986; Primmett et al., 1986). Significant
Root and Bohr effects occur in vitro in eel blood. Indeed,
following the acidosis associated with hypercapnia, a 20% reduction of arterial O$_2$ content would result (Chapter 3).
This, however, does not appear to compel the eel to regulate
RBC pH.

In conclusion, it is apparent that whole blood pH in the
eel and trout during steady-state conditions is estimated by
the ratio of $J_{IN}Na^+$ to $J_{IN}Cl^-$. During hypercapnia, plasma
[HCO$_3^-$] is elevated in the eel presumably due to increases in
the activity of the branchial Na$^+$/H$^+$(NH$_4^+$) exchange, as the
elevation of plasma [HCO$_3^-$] is abolished by abrogated external
[Na$^+$]. Thus, the eel is capable of regulating the acidosis
associated with hypercapnia despite severely limited $\text{Cl}^-/\text{HCO}_3^-$ 
($\text{OH}^-$) exchange.
CHAPTER 6

DIFFERENTIAL APPROACHES TO BLOOD ACID-BASE REGULATION IN THE AMERICAN EEL (*ANGUILLA ROSTRATA*) AND THE RAINBOW TROUT (*SALMO GAIRDNERI*): BRANCHIAL ADJUSTMENTS.
Introduction

It was shown that the eel is capable of regulating the acid-base disturbance associated with external hypercapnia equally well as the trout and, moreover, a dependence upon external Na\(^+\) was demonstrated (see Chapter 5). It was tentatively concluded that the eel relies on adjustments of branchial Na\(^+\)/H\(^+\)(NH\(_4\)\(^+\)) exchange whereas the trout relies on adjustments of both branchial Cl\(^-\)/HCO\(_3\)\(^-\)(OH\(^-\)) and Na\(^+\)/H\(^+\)(NH\(_4\)\(^+\)) exchange.

In this chapter, the differential dependencies of the eel and trout on branchial ionic exchanges for acid-base regulation will be explored directly during hypercapnic acidosis. It is speculated that, unlike the trout, the eel will rely completely on modulation of branchial Na\(^+\)/H\(^+\)(NH\(_4\)\(^+\)) exchange rather than Cl\(^-\)/HCO\(_3\)\(^-\)(OH\(^-\)) exchange in an attempt to compensate this acidosis. The results will demonstrate that i) unlike the trout, the eel is unable to modify branchial Cl\(^-\)/HCO\(_3\)\(^-\)(OH\(^-\)) exchange to compensate this acidosis and ii) although a role for the modulation of branchial Na\(^+\)/NH\(_4\)\(^+\) exchange is apparent in the eel, the exact mechanisms by which branchial Na\(^+\)/NH\(_4\)\(^+\) exchange leads to compensation of this acidosis are unclear.
Materials and Methods

Experimental Animals

American eels (Anguilla rostrata) weighing between 93 and 257 g (mean wt = 123.8 ± 12.3 g; n = 76) and rainbow trout (Salmo gairdneri) of both sexes and weighing between 119.2 and 349.8 g (mean wt = 243.5 ± 23.3 g; n = 24) were utilized in these experiments (see General Methods). Eels were acclimated to low Na\(^+\) water as outlined in Chapter 5. Eels acclimated to high NaCl were not utilized in this study because i) branchial ionic fluxes could not be accurately assessed due to the high background levels of NaCl and ii) eels acclimated to high NaCl water did not differ in the ability to regulate hypercapnic acidosis.

Protocol

Hypercapnia was induced environmentally by equilibrating water contained within the holding apparatus, which consisted of an opaque, 3 L Perspex box, with 0.8% CO\(_2\) in air. This was accomplished in two ways. During the flux period, water within the Perspex box was gassed with 0.8% CO\(_2\) in air and water P\(_{\text{CO}_2}\) was maintained at approximately 6.5 torr. At all other times, this was done using a vertical, counter-current gas exchange column as outlined in chapter 5. A Wosthoff gas mixing pump (model M301 A-F) was used to supply all mixed gasses during the experiment.

Branchial Solute Fluxes
To determine branchial solute fluxes/ water flow to the Perspex boxes was halted for the duration of the flux. Water temperature in the boxes was maintained by increasing the level of the water surrounding the boxes on a "wet table" and maintaining water flow to the table. The box was vigorously aerated to maintain ambient water \( P_{O_2} \). Branchial \( Na^+ \) and \( Cl^- \) influxes (\( J^{IN}_{Na^+} \) and \( J^{IN}_{Cl^-} \)) were determined by monitoring the disappearance of either \( ^{22}Na \) (as \( NaCl \); Amersham) or \( ^{36}Cl \) (as HCl; ICN) from the external environment following the addition of isotope to each box (see Maetz, 1956). Specific activity of the water was maintained between 3000 and 5000 DPM/\( \mu \)mol and was increased gradually during the experiment to avoid backflux problems. Water samples were removed following a 20 min mixing period and then again following the flux period. Between consecutive fluxes, the Perspex boxes were flushed with appropriately gassed water for at least 15 min to avoid ammonia accumulation. This flux period was 3 h for determination of both \( Na^+ \) and \( Cl^- \) influx in trout and for determination of \( Na^+ \) influx in eels but for determination of \( Cl^- \) influx in eels, it was extended to 6 h. The activity of \( ^{22}Na \) or \( ^{36}Cl \) was then determined on 5 ml water samples while the remaining samples were stored at -70° C for later analysis of \( Na^+ \), \( K^+ \) and \( Cl^D \). Time periods chosen for the determination of branchial \( Na^+ \) influxes in eels and trout and \( Cl^D \) influxes in trout were 6-3 and 3-0 h pre-hypercapnia, 0-3, 3-6, 9-12, 21-24, 45-48
and 69-72 h during hypercapnia and 0-3, 3-6 and 21-24 h post-
hypercapnia. In eels, Cl\textsuperscript{D} influxes were determined 6-0 h pre-
hypercapnia, at 0-6, 6-12, 18-24, 42-48, 66-72 h during
hypercapnia and 0-6 and 18-24 h post-hypercapnia. The same
regimen was used for control fish. Values for net ionic
fluxes obtained from Na\textsuperscript{+} influx experiments and Cl\textsuperscript{-} influx
experiments were combined at corresponding times.

**Statistical Analysis**

Data shown in figures and tables are means ± 1 SE. To
test for significance between experimental groups, a 2 X 2
factorial ANOVA was performed in conjunction with a Tukey's
studentized range test. To test for significant difference
within experimental groups, a Student's t-test was used. The
fiducial limit of significance for all tests was taken as 5%.
See figure legends for details.
Results

With the onset of hypercapnia, water $P_{CO2}$ was elevated to $6.53 \pm 0.47$ torr ($N = 186$). In trout, both branchial $J_{INa}^+$ and $J_{INCl}^-$ were significantly reduced during the latter stages of hypercapnia (Fig. 27A,B) and remained depressed during the initial stages of recovery. However, $J_{INNa}^+$ became significantly elevated while $J_{INCl}^-$ rose to control levels at 24 h post-hypercapnia (Fig. 27A). During hypercapnia, the changes in both $J_{NETNa}^+$ and $J_{NETCl}^-$ concur with these observations (Fig. 26A,B). However, during the initial stages of recovery, $J_{NETCl}^-$ became significantly elevated without concurrent increases in $J_{INCl}^-$. At 24 h, $J_{NETNa}^+$ was not elevated significantly and the significant elevation of $J_{NETCl}^-$ was not associated with an elevation of $J_{INCl}^-$. The contributions of the net ionic fluxes to acid-base regulation appear to counteract each other as only during the initial stages of hypercapnia did SIDF (see Fig. 26C) become positive (indicating acid excretion) and only immediately post-hypercapnia did SIDF become significantly negative (indicating base excretion). $J_{NETK}^+$ did not change significantly.

In the eel acclimated to normal water, exposure to hypercapnia for 72 h resulted in a gradual stimulation of $Na^+$ influx without detectable changes in $Cl^-$ uptake (Fig. 29A,B) with a rapid return to control levels during recovery. However, $J_{NETNa}^+$ did not significantly increase until 72 h; the net flux of both $Cl^-$ and $K^+$ did not change significantly.
Figure 26. The effects of external hypercapnia (0.8% CO₂) on branchial solute fluxes in the trout acclimated to normal water. A) Na⁺ net flux ($J_{NET}Na^+$), B) the net flux of Cl⁻ ($J_{NET}Cl^-$), C) the net flux of strong ions (SIDF) and D) K⁺ net flux ($J_{NET}K^+$). All fluxes are expressed as umol kg⁻¹ h⁻¹. N numbers are 6 for both control fish (clear bars) and experimental fish (solid bars). ⭐ represents significant difference from the control value at the corresponding time; ▲ represents significant difference from pre-exposure values; ✶ represents significant difference from both pre and control values.
Figure 27. The effects of external hypercapnia (0.8% CO₂) on branchial influxes of A) sodium (J_{IN}Na⁺) and B) chloride (J_{IN}Cl⁻) in trout acclimated to normal water. N numbers are 6 for control fish (clear bars) and 6 for experimental fish (solid bars). All other details as in Fig. 26.
Figure 28. The effects of external hypercapnia (0.8% CO₂) on branchial solute fluxes in the eel acclimated to normal water. N numbers are 6 for control fish (clear bars) and 6 for experimental fish (solid bars). All other details as in Fig. 26.
Figure 29. The effects of external hypercapnia (0.8% CO₂) on branchial influxes of A) sodium (J_{IN}Na⁺) and B) chloride (J_{IN}Cl⁻) in eels acclimated to normal water. All other details as in Fig. 26.
at any point during the experiment (Fig. 28A,B,D). The arithmetic difference between the net fluxes of Na\(^+\) and Cl\(^-\) (strong ion difference flux; SIDF) followed the changes in \(J_{NETNa}^+\) and rose significantly above both control and pre-values only during the final stages of hypercapnia (Fig. 28A,C). However, during recovery, SIDF became significantly depressed when compared to controls due the significant decrease in \(J_{NETNa}^+\).

Eel acclimated to low Na\(^+\) water showed predictable changes in branchial ionic fluxes. Although significant differences were detected in both influxes (Fig. 30) and net fluxes (Fig. 31), changes were not of great magnitude and generally occurred in a manner which was counteractive to acid-base balance.
Figure 30. The effects of external hypercapnia (0.8% CO₂) on branchial solute fluxes in the eel acclimated to low Na⁺ water. N numbers are 6 for control fish (clear bars) and 6 for experimental fish (solid bars). All other details as in Fig. 26.
Figure 31. The effects of external hypercapnia (0.8% CO₂) on branchial influxes of A) sodium (J_inNa⁺) and B) chloride (J_inCl⁻) in eels acclimated to low Na⁺ water. All other details as in Fig. 26.
Discussion

The constraints of electroneutrality dictate that the difference between the net flux of strong ions (see Stewart, 1980) will exactly oppose the flux of acidic equivalents. Hence, an increase in the influx of strong ions will result in the equal efflux of acidic equivalents. The differential adjustment of branchial net ionic fluxes (predominantly \( J_{NETNa^+} \) and \( J_{NETCl^-} \)) is known to occur during acid-base disturbances in fish (Chapter 4; Wood et al., 1984; Wood et al., 1986; Claiborne and Heisler, 1986; Vermette and Perry, 1987) and is a result of the modulation of either branchial efflux or influx.

In the trout, rapid decreases in both branchial \( Na^+ \) and \( Cl^- \) uptake (\( J_{INNa^+} \) and \( J_{INCl^-} \)) resulted in the net loss of both ions and the changes in \( J_{INNa^+} \) were contrary to the process of acid-base regulation. However, the positive strong ion flux (strong ion difference flux; SIDF), which occurred initially during hypercapnia, is indicative of pH regulation and corresponded to the changes in blood acid-base status reported in Chapter 5. Similar changes in branchial ionic fluxes have been reported in trout following the intravascular infusion of epinephrine (Vermette and Perry, 1987).

Presumably, any changes in SIDF in the eel would be due to changes in \( J_{NETNa^+} \) as the eel is unable to modify \( Cl^-/HCO_3^- \) exchange. In the previous chapter, it was noted that whole blood pH in the eel was dependent on the external concentr-
ation of Na⁺. Furthermore, it was demonstrated that, while eels acclimated to normal water were capable of compensating blood acid-base status throughout the hypercapnic period, the ability to compensate hypercapnic acidosis was abolished in eels acclimated to low Na⁺ water. These results are indirect evidence that the eel relies solely on the activity of branchial Na⁺/acid exchange or the modulation of net movements of Na⁺ for compensation of hypercapnic acidosis.

However, the results of the present study do not fully concur with those of the previous chapter. Although a pronounced stimulation of branchial Na⁺ uptake was detected in eels acclimated to normal water during hypercapnia, there were no apparent effects on the net movement of Na⁺ until the termination of hypercapnia and hence no changes were detected in SIDF until this time. From this result, it can be predicted that no change in the flux of acidic equivalents would result. However, an examination of the changes in blood acid-base status (Chapter 5) reveals that regulation of whole blood pH in eels acclimated to normal water occurred throughout the hypercapnic period. Although in fishes SIDF is generally considered a function of the net movements of Na⁺ and Cl⁻, under certain circumstances, other strong electrolytes are known to make a significant contribution to branchial SIDF (K⁺-Eddy, 1985; Ca²⁺-Ferry and Wood, 1985). This could explain the apparent inability to detect changes in SIDF during hypercapnia as only the net movements of Na⁺, K⁺
and Cl\(^-\) were monitored. However, any changes in blood acid-base status which occurred independent of the movement of branchial Na\(^+\) should also have occurred in eels acclimated to low Na\(^+\) water. The inability of the eel acclimated to low Na\(^+\) water to regulate hypercapnic acidosis (Chapter 5) excludes the possibility of the involvement of other strong ions in acid-base regulation. Indeed, the only apparent difference between eels acclimated to low Na\(^+\) and eels acclimated to normal water is the ability to modify branchial Na\(^+\) uptake. Hence, although no stimulation in net flux was detected initially in eels acclimated to normal water, the ability to regulate hypercapnic acidosis must be attributed to modulation of branchial J\(_{\text{NET}Na^+}\). Indeed, the present study has shown that during recovery from hypercapnia, J\(_{\text{NET}Na^+}\) in eels acclimated to normal water drops significantly and contributes to a significant decrease in SIDF. This result concurs with compensation of an internal alkalosis, known to be present following hypercapnia (see Chapter 5) and furthermore, indicates a reliance on modulation of J\(_{\text{NET}Na^+}\).

The inability to detect changes in branchial J\(_{\text{NET}Na^+}\) during hypercapnia is perhaps, in part, related to the net efflux of sodium by the kidney. In this study, measurements were made of whole body flux because in other fish, it has been shown that the kidney has limited involvement in the excretion of acidic equivalents and functions to retain base during periods of internal hypercapnia (Cameron, 1980; Wheatly
et al., 1984; Wood et al., 1984, Perry et al., 1987a,b). However, the kidney contributes to a net efflux of Na\(^+\) (see Chapter 4; see also Cameron, 1980; Wheatly et al., 1984; Wood et al., 1984, Perry et al., 1987a,b) and hence, branchial net uptake of Na\(^+\) may have occurred and may have been stimulated during hypercapnia but was underestimated due to increased renal efflux of Na\(^+\).

Compensation of respiratory acid-base disturbances in other teleosts occurs primarily due to the modulation of branchial ion exchange sites rather than kidney function. Moreover, the response of these pumps following hypercapnic acidosis appears to be relatively rapid (approximately 12 h; Cameron, 1976; Wood et al., 1984; Claiborne and Heisler, 1984; Perry et al., 1987a), a response which can be mimicked by elevated plasma epinephrine (Vernette and Perry, 1987). Circulating catecholamines are known to be elevated in fish following a variety of stresses (acid-infusion, Boutilier et al., 1986; Tang, Nolan and Boutilier, 1988; hypercapnia, Perry et al., 1987a; exhaustive exercise, Primett et al., 1986; Milligan and Wood, 1987; hypoxia, Fievet, Motais and Thomas, 1986) and gill perfusion experiments have indicated adrenergic control of branchial ionic uptake (Payan, Matty and Maetz, 1975; Payan, 1978; Perry, Payan and Girard, 1984a,b). It seems unlikely that adrenergic stimulation of branchial Na\(^+\) uptake could account for the observed 5-fold increase in Na\(^+\) uptake which occurred in eels acclimated to normal water 72 h
after the onset of hypercapnia. Moreover, receptor desensitization is known to occur following the prolonged elevation of catecholamines within 24 h. Hence, both the role of catecholamines in the adjustment of branchial Na⁺/acid exchange and the mechanisms behind the tremendous stimulation of Na⁺ uptake during hypercapnia must be questioned. The present results suggest that the response to prolonged hypercapnic acidosis is chronic, perhaps due to increased numbers of "chloride cells" (CC), thought to be involved in the movement of both Na⁺ and Cl⁻ (see reviews by Kirschner, 1977; Karnaky, 1980; Philipott, 1980; Foskett, Bern, Machen and Conner, 1983). The nature of the stimuli which act to increase the number of CC is largely unknown (Foskett et al., 1983) although a correlation exists with elevated plasma cortisol levels. The time course required for the differentiation of CC in Tilapia (Foskett, Logsdon, Turner, Machen and Bern, 1981) closely parallels the duration of hypercapnia in this study. However, when the freshwater eel was treated with cortisol, a hormone known to elicit CC production (Perry and Wood, 1985), there were no observable effects on the net flux of Na⁺ (data not shown; see also Forrest, Cohen, Schon and Epstein, 1973).

In conclusion, this chapter has demonstrated that the trout relies on dynamic manipulation of both Na⁺/NH⁴⁺ and Cl⁻/HCO₃⁻ exchange though modulation of Cl⁻/HCO₃⁻ exchange appears to be pre-eminent in acid-base regulation. Although no
change in the net flux of Na\textsuperscript{+} was detected in the normal water eel during hypercapnia, from the observation that i) there was a stimulation of branchial Na\textsuperscript{+} uptake during hypercapnia in eel acclimated to normal water, which has demonstrated an ability to regulate hypercapnic acidosis and ii) there was no stimulation of branchial Na\textsuperscript{+} uptake in the eel acclimated to low Na\textsuperscript{+}-water, which demonstrated an inability to regulate hypercapnic acidosis, it is concluded that the eel relies on branchial Na\textsuperscript{+} uptake to compensate hypercapnic acidosis.
CHAPTER 7

GENERAL DISCUSSION
Stresses are frequently encountered by fish (exercise, temperature changes, changing environmental salinity, air-exposure, hypoxia, hypercapnia, hyperoxia) and, commonly, these stresses are associated with detrimental changes in acid-base status. Maintenance of homeostatic pH is important to the integrity of a fish and the role of branchial ion exchange processes in compensation of pH disturbances in fishes is well documented. Strong ion theory (Stewart, 1980) dictates that it is the net flux of strong ions (predominantly Na\(^+\) and Cl\(^-\) in fish) that determines the net movement of acidic equivalents. Modulation of either the uptake or passive efflux of an ion will result in changes the net flux of an ion. The compensation of acid-base disturbances can occur by the modulation of either branchial Cl\(^-\)/HCO\(_3^-\)/(OH\(^-\)) exchange or Na\(^+\)/H\(^+\)/(NH\(_4^+\)) exchange in teleosts. However, the selective inhibition of branchial Cl\(^-\)/HCO\(_3^-\)/(OH\(^-\)) exchange has been reported in numerous teleosts during acid-base disturbances (Wood et al., 1984; Claiborne and Heisler, 1984; Perry et al., 1987a). As the uptake of both Na\(^+\) and Cl\(^-\) are active processes (Evans, 1984), selective inhibition of branchial Cl\(^-\)/HCO\(_3^-\)/(OH\(^-\)) exchange during an acidosis is a more energetically efficient process than the stimulation of branchial Na\(^+\)/H\(^+\)/(NH\(_4^+\)) exchange. In the American eel (Anguilla rostrata), the ability to absorb external chloride has been greatly reduced as indicated by extremely low rates of Cl\(^-\) uptake (see Chapters 4, 5, 6; see also Kirsch, 1972; Bornancin

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et al., 1977). The objective of this thesis was to determine the importance of branchial ionic exchange sites in acid-base regulation in the eel using prolonged exposure to air and exposure to external hypercapnia as model stress systems.

Briefly, the relative incapacity for aerial gas transfer demonstrated by the eel during air-exposure led to the accumulation of metabolic H⁺ ions and respiratory CO₂ which contributed to a severe mixed acidosis. The metabolic portion of this acidosis was compensated, albeit slowly, upon return to water. The changes observed in SIF, which concurred with the net excretion of acidic equivalents, were attributed primarily to modification of branchial Na⁺/H⁺ exchange. Due to the slow rate of recovery following exposure to air and although the results of this study could have been affected by metabolic adjustments associated with hypoxemia, it was suggested that the eel displayed a limited capacity for acid-base regulation, a result of low rates of branchial Cl⁻/HCO₃⁻ exchange.

To further examine this possibility, eels were exposed to external hypercapnia. It was postulated that the eel would display a similar inefficiency of acid-base regulation during hypercapnic acidosis. However, the eel was capable of regulating the acidosis associated with hypercapnia as well as other teleosts (see Chapter 5; see also Cameron and Randall, 1972; Janssen and Randall, 1975; Cameron, 1976; Eddy, 1976; Eddy et al., 1977; Cameron, 1980; Thomas, 1983; Perry et al.,
1981; Perry, 1982; Perry et al., 1987a; Toews et al., 1983) in spite of limited \( \text{Cl}^-/\text{HCO}_3^- \) exchange. Although no change in the net flux of \( \text{Na}^+ \) was observed, a stimulation of branchial \( \text{Na}^+ \) uptake during hypercapnia in eels acclimated to normal water was detected, which correlated with the ability to regulate hypercapnic acidosis. Furthermore, there was no stimulation of branchial \( \text{Na}^+ \) uptake in eels acclimated to low \( \text{Na}^+ \) water, which were unable to regulate hypercapnic acidosis. In a similar fashion, whole blood pH was shown to vary with the external [\( \text{Na}^+ \)], further indicating dependence on branchial \( \text{Na}^+ \) movements in the eel.

Regardless, the eel is capable of regulating both the acidosis associated with air-exposure (metabolic) and hypercapnia (respiratory), albeit slowly following air-exposure. It is apparent that the eel relies on modulation of branchial \( \text{Na}^+ / \text{H}^+ (\text{NH}_4^+) \) exchange rather than branchial \( \text{Cl}^-/\text{HCO}_3^- (\text{OH}^-) \) exchange to compensate extracellular acidoses. Both exposure to air and exposure to hypercapnia resulted in the stimulation of branchial \( \text{Na}^+ / \text{H}^+ (\text{NH}_4^+) \) exchange as indicated by elevated \( \text{Na}^+ \) influx although the stimulation of branchial \( \text{Na}^+ / \text{H}^+ (\text{NH}_4^+) \) exchange occurred slowly during hypercapnia. Indeed, branchial ionic exchange mechanisms in the eel appear to react only slowly to changes in blood acid-base status. Following air-exposure, although the increase in the rate of branchial net acid efflux was sufficient to account for the slow rate of recovery observed (70 \( \text{umol kg}^{-1} \text{ h}^{-1} \)), a role has been
suggested for the metabolic removal of acidic equivalents as demonstrated in other fish (see Milligan and Wood, 1987). Assuming even partial compensation metabolically, the role attributed to branchial Na⁺/H⁺(NH₄⁺) exchange in compensation of the acidosis after air-exposure would be reduced.

The observation that RBC pH during both air-exposure and hypercapnia simply conforms to the in vitro relationship between pHe and RBC pH contrasts markedly with the observation that RBC pH is preferentially regulated in many other fishes during extracellular acidosis (Primett et al., 1986; Boutilier et al., 1986; Perry et al., 1987a) due to adrenergic stimulation of erythrocytic Na⁺/H⁺ exchange (see review by Nikinmaa, 1986). However, the starry flounder (Platichthys stellatus), which is an inactive, benthic species, like the eel, does not preferentially regulate RBC pH following exhaustive exercise. Moreover, the flounder does not elevate plasma catecholamines following exhaustive exercise (see Wood and Milligan, 1987). It has been postulated that the eel either i) does not elevate plasma catecholamines during acid-base disturbances or ii) RBCs are insensitive to catecholamines. However, when coupled with the knowledge that fish are known to elevate plasma catecholamines during stressed states (Boutilier et al., 1986; Fievet, Motais and Thomas, 1986; Primett et al., 1986; Milligan and Wood, 1987; Perry et al., 1987a; Tang, Nolan and Boutilier, 1988) and that catecholamines are known to have biological effects in eels
(ventilation, Peyraud-Waitznegger, 1979; hyperglycemia, Epple and Nibbio, 1985), erythrocytic Na+/H+ exchange appears to be insensitive to elevated catecholamines in the eel when compared to other fishes. Moreover, although the eel has demonstrated an ability to modulate branchial Na+/H+(NH₄⁺) exchange, it seems unlikely that adrenergic stimulation of branchial Na⁺ uptake could account for the observed 5-fold increase in Na⁺ uptake which occurred in eels acclimated to normal water 72 h after the onset of hypercapnia. It is speculated that i) the process of branchial Na⁺ uptake either is insensitive or becomes rapidly insensitive to elevated plasma catecholamines and ii) the eel relies on the intervention of a hormone other than catecholamines to stimulate branchial Na⁺ uptake during perturbations of blood acid-base status. This is an area which will hopefully warrant further investigation.
REFERENCES


Bridges, C.R., Hlastala, M.P., Riepl, G. and Scheid, P. (1983) Root effect induced by CO2 and by fixed acid in


extrusion mechanism during seawater adaptation of a teleost fish, the cichlid *Sarotherodon mossambicus*. J. Exp. Biol. 93, 209-224.


Johansen, K., Lenfant, C., Schmidt-Neilsen, K. and Peterssen, J. (1968). Gas exchange and control of breathing in the
electric eel *Electrophorus electricus*. Z. Vergl. Physiol. 61, 137-163.


Lomholt, J.P. and Johansen, K. (1976). Cls exchange in the


Perry, S.F. (1982). The regulation of hypercapnic acidosis in two salmonoids, the freshwater trout (Salmo gairdneri) and the seawater salmon (Onchorhynchus kisutch). Mar. Behav. Physiol. 9, 73-79.


Powers, D.A. (1980). Molecular ecology of teleost fish hemo-
globins: strategies for adapting to changing environments. Amer. Zool. 20, 139-162.


Thomas, S. (1983). Changes in blood acid-base balance in trout (Salmo gairdneri Richardson) following exposure to
combined hypoxia and hypercapnia. J. Comp. Physiol. 152, 53-57.


