J. exp. Biol. **152**, 549–571 (1990)
Printed in Great Britain © *The Company of Biologists Limited 1990*

Na^+ AND Cl^- UPTAKE KINETICS, DIFFUSIVE EFFLUXES AND ACIDIC EQUIVALENT FLUXES ACROSS THE GILLS OF RAINBOW TROUT

II. RESPONSES TO BICARBONATE INFUSION

BY GREG G. GOSS* AND CHRIS M. WOOD

Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada L8S 4K1

Accepted 15 May 1990

Summary

Adult rainbow trout fitted with arterial and bladder catheters were chronically infused with either bicarbonate (as $NaHCO_3$) or NaCl for 19 h at approximately 410 μequiv $kg^{-1} h^{-1}$. $NaHCO_3$ infusion produced a pure exogenous metabolic alkalosis of approximately 0.35 pH units accompanied by a decrease in plasma [Cl^-] but no change in plasma [Na^+]. Alkalosis stimulated Cl^- influx and inhibited Na^+ influx (measured at 10–16 h infusion), resulting in a negative Na^+ balance, a positive Cl^- balance and a large net basic equivalent excretion (=acidic equivalent uptake) across the gills. The latter was approximately equal to the rate of HCO_3^- loading. The kidney accounted for approximately 13 % of the acid–base compensation.

Kinetic analysis revealed that reductions in J_{in}^{Na} were accomplished by increases in K_m^{Na} (463 μequiv l^{-1}; $NaHCO_3$-infused *vs* 276 μequiv l^{-1}; NaCl-infused) and large decreases in J_{max}^{Na} (262 μequiv $kg^{-1} h^{-1}$ *vs* 689 μequiv $kg^{-1} h^{-1}$) while stimulation of J_{in}^{Cl} was accomplished by large increases in J_{max}^{Cl} only (674 μequiv $kg^{-1} h^{-1}$ *vs* 360 μequiv $kg^{-1} h^{-1}$). Thus, J_{max} can be increased or decreased in response to acid–base disturbance, but K_m can only be increased; the Na^+ and Cl^- carriers operate close to maximum affinity under control conditions. Basic equivalent excretion was described by a virtually identical kinetic curve to that of the Cl^- uptake. $NaHCO_3$ infusion also induced a differential diffusive efflux of Na^+ over Cl^- which could account for up to 35 % of the acid–base compensation during alkalosis.

Introduction

Metabolic alkalosis during recovery from hyperoxia was associated with a greatly increased Cl^- influx, a reduced Na^+ influx and a large uptake of acidic equivalents across the gills, indicative of dynamic modulation of Na^+/acidic

*Present address: Department of Biology, University of Ottawa, 30 George Glinski Pr., Ottawa, Ontario, Canada K1N 6N5.

Key words: hyperoxia, uptake kinetics, K_m, J_{max}, efflux, Michaelis–Menten.

equivalent and Cl^-/basic equivalent exchanges (Goss and Wood, 1990). Alterations in Na^+ and Cl^- influx were achieved by changes in both the K_m (inverse of affinty) and J_{max} (maximal transport rate) of the respective transporters. However, this period is also characterized by marked changes in the perfusion and ventilation characteristics of the gills (Dejours, 1972, 1973; Wood and Jackson, 1980), raising the possibility that these 'non-specific' effects either contributed to, or detracted from, the observed responses. The primary goal of the present study was, therefore, to examine the same phenomena during a comparable metabolic alkalosis, but without the possible complicating effects of perfusion and ventilation changes.

Infusion of base in the form of $NaHCO_3$ results in an exogenously produced metabolic alkalosis which is, at least qualitatively, similar to that found during recovery from hyperoxia (Claiborne and Heisler, 1986; Heisler *et al.* 1988; McDonald and Prior, 1988; cf. Hõbe *et al.* 1984). However, all previous studies on this topic have used a bolus infusion method to induce the alkalosis. The present study, instead, has employed chronic infusion to create a relatively steady state of metabolic alkalosis within the fish, a balance between HCO_3^- loading and excretion. The treatment was designed to produce an alkalosis of similar magnitude to that occurring during recovery from hyperoxia while avoiding the time course effects which complicated the hyperoxia study.

Differential diffusive efflux of Na^+ and Cl^- across the gills is also thought to play a significant role in acid–base correction in some circumstances (McDonald and Prior, 1988; McDonald *et al.* 1989). Some evidence for the involvement of this mechanism during post-hyperoxia alkalosis was obtained by Goss and Wood (1990) using a new method (efflux to NaCl-free water). Therefore, a second objective of the present study was to employ this technique to examine the contribution of differential diffusive efflux in detail at various times during the infusion period.

Blood acid–base status was examined during $NaHCO_3$ infusion to ensure that this treatment did, in fact, induce a similar alkalosis to that found during recovery from hyperoxia. The renal contribution to acid–base regulation during chronic infusion of $NaHCO_3$ was also assessed. Control infusions of neutral NaCl at the same concentration and rate were performed in all experiments to detect possible non-specific responses due to volume or salt loading.

Materials and methods

Experimental animals

Rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792); 250–400 g) were obtained and acclimated to 15±0.5°C in Hamilton tapwater as described in Goss and Wood (1990). To allow for repetitive blood sampling, infusion and urine collection, trout were anaesthetized (MS-222 1:10000; Sigma) and fitted with a dorsal aortic cannula filled with heparinized Cortland saline (Wolf, 1963;

50 i.u. ml^{-1} sodium heparin; Sigma), according to the method of Soivio *et al.* (1972), and a urinary catheter, according to the method of Wood and Randall (1973). The fish were then allowed to recover for 72 h before experimentation in the boxes described by Goss and Wood (1990).

Experimental water

Acclimation and experimental tapwater and the artificial NaCl-free water used in series II and III had the same composition as described in Goss and Wood (1990).

Experimental series

The term outflux (unidirectional outflux) refers to outflux in series I and III obtained by radioisotopic measurement of influx (J^X_{in}) and net flux (J^X_{net}). The term efflux (diffusive efflux) refers to effluxes measured in series II by a method that did not require radioisotopes.

Series I

Series I was designed to characterize the net and unidirectional ion and acid–base fluxes across the gills and kidney, and plasma ion and acid–base status associated with chronic infusion of either 140 mmol l^{-1} NaCl (control, N=5) or 140 mmol l^{-1} $NaHCO_3$ (experimental, N=6). This concentration (140 mmol l^{-1}) was chosen so as to minimize any changes in the osmolarity of the plasma. A further objective was to determine 4 h periods during which these fluxes might be relatively stable, thereby allowing the planned kinetic uptake measurements of series III.

Repetitive blood samples (300 μl) were withdrawn *via* the cannula at the following times: 0 (initial), 10, 15 and 19 h after the start of infusion. Blood samples were analyzed for arterial pH (pHa) and plasma total [CO_2], [Na^+] and [Cl^-]. After the initial blood sample, the fish were then infused *via* the arterial catheter with either 140 mmol l^{-1} NaCl or 140 mmol l^{-1} $NaHCO_3$ at a rate of 2.92±0.11 ml kg^{-1} h^{-1} (N=22) using a Gilson Minipuls peristaltic pump. The bicarbonate loading rate was therefore 410 μequiv kg^{-1} h^{-1}. The goal was to induce an alkalosis of similar proportion to that found in recovery from hyperoxia by Hõbe *et al.* (1984), while minimizing the volume load. This rate of infusion and HCO_3^- concentration were chosen on the basis of an initial trial experiment at a range of rates (not shown). After 10 h of infusion, the boxes were closed and both $^{24}Na^+$ (4.0 μCi) and $^{36}Cl^-$ (1.0 μCi) were added. An initial period of 10 min was allowed for complete mixing. Thereafter, water samples (40 ml) were taken at 0.5 h intervals for 6 h and analyzed for $[Na^+]_e$, $[Cl^-]_e$, total ammonia (Amm), titratable alkalinity (TAlk), and $^{24}Na^+$ and $^{36}Cl^-$ cts min^{-1}. The boxes were flushed after 3 h of closure to ensure that the ambient ammonia (Amm) levels did not exceed 200 μequiv l^{-1}. Following the flushing, the appropriate amount of isotope was re-added to the box.

Series II

Series II was designed to measure the simple diffusive efflux of Na^+ and Cl^- from rainbow trout chronically infused with either 140 mmol l^{-1} NaCl (N=11) or 140 mmol l^{-1} $NaHCO_3$ (N=12). This was performed concurrently with each of series I and III and therefore involved the same fish. The results from the two sets were not significantly different from each other and were therefore combined as series II. Control diffusive efflux measurements were taken immediately before control blood sampling; separate experimental diffusive efflux measurements were taken immediately prior to the 10 h and 19 h blood samples in each infusion group. At each measurement time the boxes were flushed with NaCl-free water of normal pH, Ca^{2+} and Mg^{2+} content and TAlk, and diffusive effluxes were measured by monitoring the appearance of Na^+ and Cl^- in the external water over 0–10 min, as described by Goss and Wood (1990).

Urine samples were collected in this series from an 8 h pre-sampling control period, the first 10 h of infusion, 10–15 h of infusion and 15–19 h of infusion. These samples were analyzed for urine flow rate (UFR), total Amm and titratable acidity minus bicarbonate [$TA-HCO_3^-$]. In a separate, identical series of experiments on different fish, urinary [Na^+] and [Cl^-] and UFR were measured to determine the ion excretion rates from the kidney during NaCl infusion (N=5) and $NaHCO_3$ infusion (N=5).

Series III

Series III was designed to measure the uptake kinetics of Na^+ and Cl^- in fish chronically infused with either 140 mmol l^{-1} NaCl (N=5) or 140 mmol l^{-1} $NaHCO_3$ (N=6). The protocol for sampling of blood and infusion of salts was performed as outlined in series I. After 10 h of infusion, the flow of tapwater to the box was stopped and uptake kinetics determined over the following 4 h. Methods were identical to those described in Goss and Wood (1990), except that Na^+ and Cl^- kinetics were determined simultaneously in the same fish through the use of $^{24}Na^+$ and $^{36}Cl^-$. A common stock solution containing approximately 4.0 μCi $^{24}Na^+$ and 1.0 μCi $^{36}Cl^-$ was used for all additions to ensure the maintenance of a relatively constant specific activity over the entire range of $[NaCl]_e$.

Analytical techniques and calculations

Analytical methods were identical to those described by Goss and Wood (1990), except for the following. Plasma and urinary [Na^+] were appropriately diluted in 0.2 % HNO_3 for atomic absorption (Varian AA1275). Plasma and urinary [Cl^-] were determined by coulometric titration (Radiometer CMT-10). Duplicate 5 ml water samples were counted for the sum of $^{36}Cl^-$ and $^{24}Na^+$ cts min^{-1} by scintillation counting (LKB Rackbeta, model 1217) immediately following the end of the experiment. The $^{24}Na^+$ isotope was allowed to decay for at least 50 half-lives (half-life=14.96 h) and the sample was recounted assuming that only

$^{36}Cl^-$ cts min^{-1} (half-life=30 000 years) remained. The $^{24}Na^+$ counts were obtained by subtraction and corrected for decay during the flux period in question by:

$$A_0 = \frac{A}{e^{-\ln 2 \cdot t/T_{1/2}}}, \quad (1)$$

(Wang *et al.* 1975), where A_0 is the value corrected to time t_0, A is the value obtained by subtraction of the original counts, t is the time elapsed in hours and $T_{1/2}$ is the half-life of the $^{24}Na^+$ (14.96 h).

Blood samples were drawn from the dorsal aortic cannula. Arterial pHa and plasma total CO_2 were immediately determined by standard Radiometer techniques (Wood and Jackson, 1980). The remainder of the sample was centrifuged and the plasma frozen for later ionic analysis. Pa_{CO_2} and plasma HCO_3^- levels were calculated by the rearrangement of the Henderson–Hasselbalch equation using the values of pK′ and αCO_2 tabulated in Boutilier *et al.* (1984).

As in mammalian physiology, urinary acid output can be calculated as:

$$([Amm] + [TA - HCO_3^-])UFR. \quad (2)$$

Urine [Amm] was determined colorimetrically (Verdouw *et al.* 1978). $[TA-HCO_3^-]$ was measured as a single value in the double-titration procedure recommended by Hills (1973).

Calculations of ion and acidic equivalent fluxes and transformation of the data by Michaelis–Menten kinetics and Eadie–Hofstee regression analysis were performed as outlined in Goss and Wood (1990), as were the statistical analyses. A significance level of $P<0.05$ was employed throughout.

Results

Internal responses

Plasma $[Na^+]$ did not change in the $NaHCO_3$-infused group but in NaCl-infused fish there was a significant increase after 15 h of infusion (Fig. 1A). Plasma $[Cl^-]$ remained unchanged in the NaCl-infused group, despite the Cl^- load, but $NaHCO_3$ infusion resulted in a large loss of Cl^- from the plasma (133 to 108 mmol l^{-1}, Fig. 1B). As in fish recovering from exposure to hyperoxia, a pure metabolic alkalosis was present in fish infused with $NaHCO_3$, characterized by a large increase in arterial plasma $[HCO_3^-]$ (from 9.2 to 19.3 mmol l^{-1}, Fig. 1C) with no elevation in Pa_{CO_2} (Fig. 1D). As a result, pHa was greatly elevated from 7.89 to 8.25 (Fig. 1E). NaCl infusion did not cause any change in pHa, Pa_{CO_2} or $[HCO_3^-]$.

Renal responses

With a constant infusion rate of 2.92 ml kg^{-1} h^{-1}, urine flow rate (UFR) increased by 86 % (2.85±0.17 to 5.12±0.60 ml kg^{-1} h^{-1}) and 80 % (2.60±0.19 to 4.70±0.82 ml kg^{-1} h^{-1}) in the NaCl- and $NaHCO_3$-infused groups, respectively, at 10–16 h. These amounted to about 80 % of the infusion rate. Volume loading of

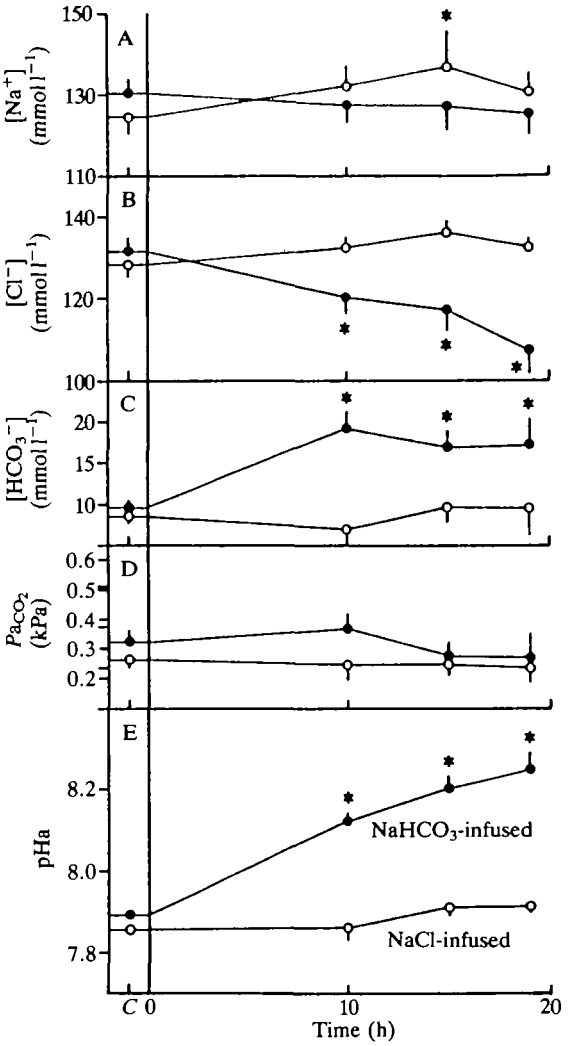

Fig. 1. Changes in (A) plasma $[Na^+]$, (B) plasma $[Cl^-]$, (C) plasma $[HCO_3^-]$, (D) plasma P_{CO_2} and (E) whole-blood pH in the arterial blood of rainbow trout (pHa) during 19 h of chronic infusion with either 140 mmol l^{-1} NaCl (○; N=10) or 140 mmol l^{-1} $NaHCO_3$ (●; N=12) (series I and III). C is the pre-infusion control measurement. Significant differences from control value (P<0.05) are indicated by an asterisk (*). Values are means±1 S.E.M.

the fish was therefore relatively small, minimizing any complications that might be caused by changes in the cardiovascular system. Net basic equivalent excretion (=net acidic equivalent uptake) *via* the kidney increased dramatically in $NaHCO_3$-infused fish from a control value of 2 μequiv kg^{-1} h^{-1} to 85 μequiv kg^{-1} h^{-1}, solely as a result of a large increase in the $[TA-HCO_3^-]$ component of renal acid–base flux (Fig. 2B). Net acidic equivalent uptake did not change in the NaCl-infused

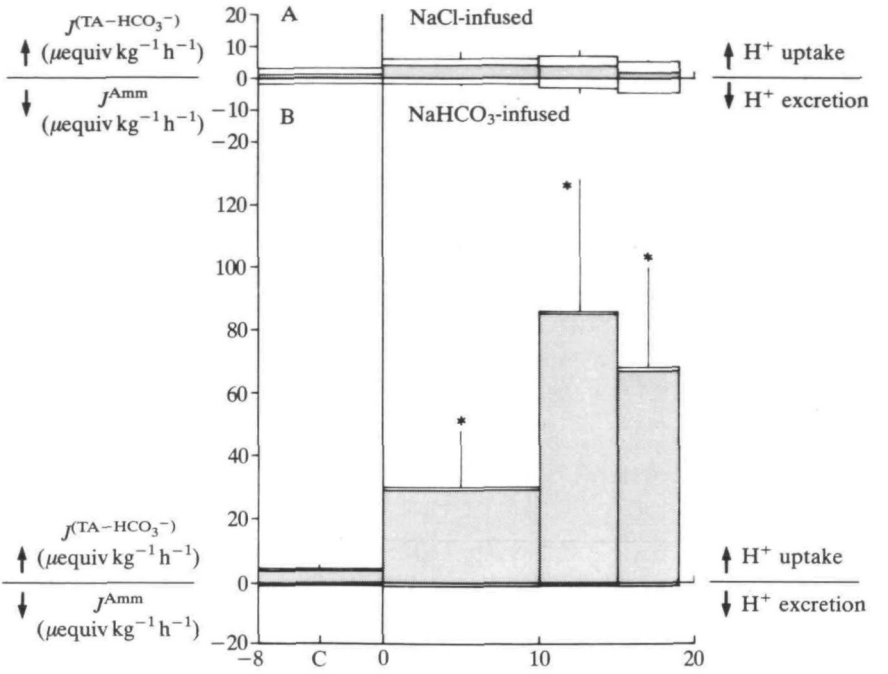

Fig. 2. The net flux of acidic equivalents (J^{H}_{net}) *via* the urine of rainbow trout during 19 h of chronic infusion with either (A) 140 mmol l^{-1} NaCl (N=5) or (B) 140 mmol l^{-1} $NaHCO_3$ (N=6) (series I and III). Positive values ($J^{TA-HCO_3^-}$) indicate net acidic equivalent uptake (=basic equivalent excretion), negative values (J^{Amm}) indicate net acidic equivalent excretion. J^{H}_{net} (shaded) is the sum of these two variables. Standard error bars have been omitted from J^{H}_{net} values for clarity. See legend of Fig. 1 for other details.

fish (Fig. 2A). In a separate group of fish, urinary Na^+ and Cl^- excretion rate (data not shown) during NaCl infusion rose from control values of 12.5±1.4 and 10.2±1.5 μequiv kg^{-1} h^{-1}, respectively, to 49.9±9.9 and 58±11.8 μequiv kg^{-1} h^{-1} over the 19 h infusion period, whereas during $NaHCO_3$ infusion, urinary Na^+ and Cl^- excretion rates rose from 16.9±3.0 and 15.2±5.3 μequiv kg^{-1} h^{-1} to 32.0±7.3 and 27.4±3.1 μequiv kg^{-1} h^{-1}, respectively, over the same time period. However, in each treatment, there was no differential loss of one ion over the other.

Unidirectional and net branchial fluxes – series I

Infusion of NaCl or $NaHCO_3$ resulted in a highly negative J^{Na}_{net} (Fig. 3) of about −200 μequiv kg^{-1} h^{-1} in both cases, but there were large differences in the magnitude of the influx and efflux components of net ion balance. In particular, J^{Na}_{in} was reduced by about 50 % from 400 to 200 μequiv kg^{-1} h^{-1} in the $NaHCO_3$-infused group relative to the NaCl-infused group. J^{Na}_{out} was more variable in both groups, but overall was lower by about the same amount as J^{Na}_{in} as a result of

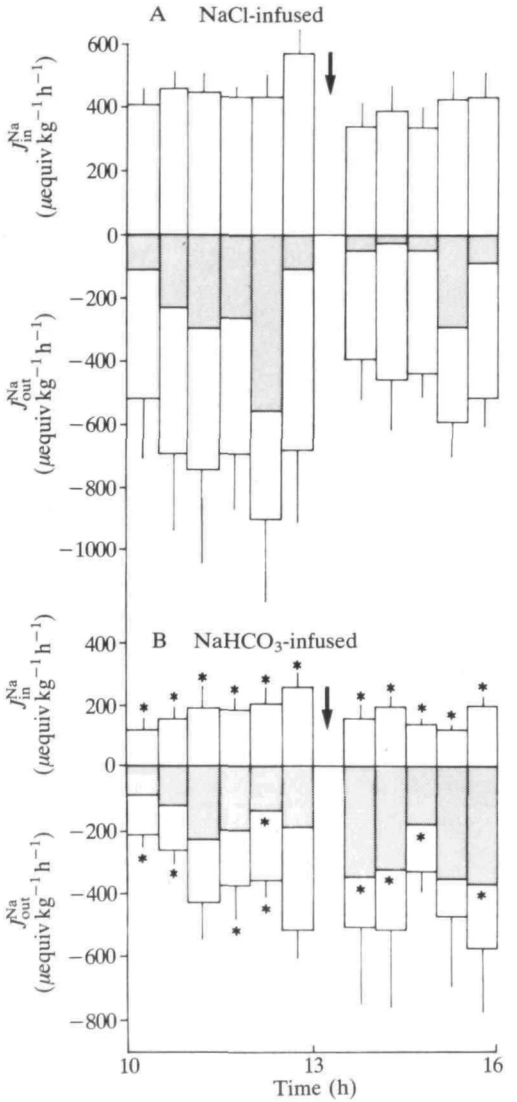

Fig. 3. Unidirectional and net flux rates for Na^+ across the gills of rainbow trout after 10–16 h of chronic infusion with either (A) 140 mmol l^{-1} NaCl (N=5) or (B) 140 mmol l^{-1} $NaHCO_3$ (N=6) (series I). Fluxes were measured over 0.5 h intervals. Positive values represent movement into the fish (J_{in}^{Na}), negative values represent movement out of the fish (J_{out}^{Na}). Shaded areas indicate the net movement of Na^+ (J_{net}^{Na}) between the fish and the water; standard errors have been omitted for the sake of clarity. Large arrows indicate the period when the boxes were flushed to prevent ammonia build-up. Significant differences between $NaHCO_3$-infused and NaCl-infused groups (P<0.05) for each flux period are indicated with an asterisk (*). Overall, mean J_{in}^{Na} was significantly smaller in $NaHCO_3$-infused fish compared to the NaCl-infused fish. Mean J_{out}^{Na} was also significantly smaller in $NaHCO_3$-infused fish, while J_{net}^{Na} was not significantly different between groups (P<0.05). Values are means±1 S.E.M.

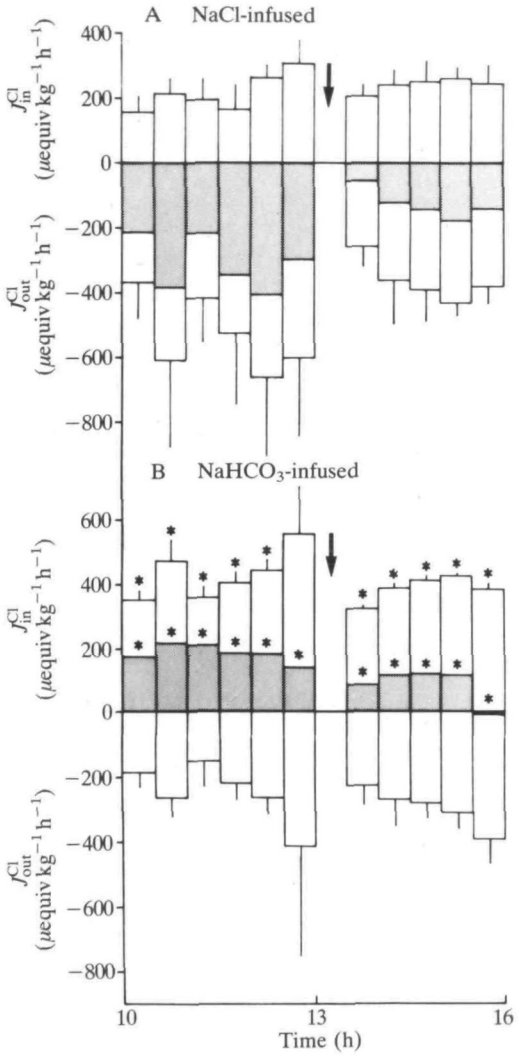

Fig. 4. Unidirectional and net flux rates for Cl^- across the gills of rainbow trout after 10–16 h of chronic infusion with either (A) 140 mmol l^{-1} NaCl (N=5) or (B) 140 mmol l^{-1} $NaHCO_3$ (N=6) (series I). Overall, mean J_{in}^{Cl} was significantly greater in $NaHCO_3$-infused fish compared to NaCl-infused fish. J_{net}^{Cl} was also significantly different in the $NaHCO_3$-infused fish, while J_{out}^{Cl} was not significantly different (P<0.05). See legend of Fig. 3 for other details.

$NaHCO_3$ infusion. Responses of Cl^- fluxes were very different. In the NaCl-infused group, J_{net}^{Cl} was again highly negative (−200 μequiv kg^{-1} h^{-1}; Fig. 4A) and approximately equal to J_{net}^{Na} (Fig. 3A). However, infusion of $NaHCO_3$ resulted in a positive J_{net}^{Cl} of 150 μequiv kg^{-1} h^{-1}, caused by a greatly stimulated J_{in}^{Cl} (Fig. 4B), which was approximately doubled from 200 to 400 μequiv kg^{-1} h^{-1} compared to the NaCl-infused group. The differences in J_{in}^{Cl} and J_{net}^{Cl} were significant at almost

every individual flux period and significant overall compared with the NaCl-infused group. J_{out}^{Cl} tended to be smaller at most times in the $NaHCO_3$-infused fish, but none of these differences was significant.

There were profound differences in acidic equivalent fluxes between the two groups. While the NaCl-infused control group were in approximate H^+ balance (Fig. 5A), the $NaHCO_3$-infused group exhibited a highly positive J_{net}^{H} of about 400–500 μequiv $kg^{-1} h^{-1}$ (Fig. 5B), which was essentially equal to the rate of $NaHCO_3^-$ infusion (mean of 410 μequiv $kg^{-1} h^{-1}$). This difference was entirely due to an approximately threefold elevation of J^{TA} from 200 to 600 μequiv $kg^{-1} h^{-1}$ as a consequence of $NaHCO_3$ infusion. J^{Amm} remained unchanged at about -200 μequiv $kg^{-1} h^{-1}$ in both infusion groups (Fig. 5A,B). The differences in J_{net}^{H} and J^{TA} between the two groups were significant at every flux period and significant overall, while for J^{Amm} there were no significant differences between the two groups.

The results of these experiments (Figs 3,4,5) indicated that the Na^+, Cl^- and acidic equivalent fluxes in the two groups were in approximate steady state from 10 to 14 h after the start of infusion. This period was therefore chosen as the experimental period for the planned uptake kinetic measurements in series III.

J_{net}^{H} vs $[J_{net}^{Na} - J_{net}^{Cl}]$ – *series I*

The relationship between J_{net}^{H} and $[J_{net}^{Na} - J_{net}^{Cl}]$ in these experiments based on all individual simultaneous flux measurements from the two groups is shown in Fig. 6. The overall relationship was highly significant ($r = -0.692$, $N = 120$, $P < 0.001$), as described by the linear regression equation:

$$[J_{net}^{Na} - J_{net}^{Cl}] = -0.68 J_{net}^{H} - 74 . \quad (4)$$

This slope was significantly different from 1.0, that of the line of equality, suggesting that differential movement of the two strong ions, Na^+ and Cl^-, may not explain all of the net movement of acidic equivalents across the gills in this experiment. The y-intercept (-74 ± 25) was not significantly different from 0 ($P > 0.05$).

Diffusive efflux – series II

Control diffusive efflux of Na^+ in trout measured before the beginning of infusion was about -160 and -250 μequiv $kg^{-1} h^{-1}$ in the NaCl- and $NaHCO_3$-infused groups, respectively (Fig. 7). These values were not significantly different from each other. In the NaCl-infused group, Na^+ efflux increased significantly by 140 % to -384 μequiv $kg^{-1} h^{-1}$ at 19 h while there was no change in the efflux of Na^+ in the $NaHCO_3$-infused group. Control diffusive Cl^- efflux in the fish to be infused with NaCl was -166 μequiv $kg^{-1} h^{-1}$ while the mean diffusive efflux for the group to be infused with $NaHCO_3$ was -306 μequiv $kg^{-1} h^{-1}$. The reason for this significant difference is unknown, for the two groups were treated identically up to this time. It may be a random effect of the small sample size. Infusion of NaCl resulted in an increase in diffusive efflux of Cl^- by 125 % to

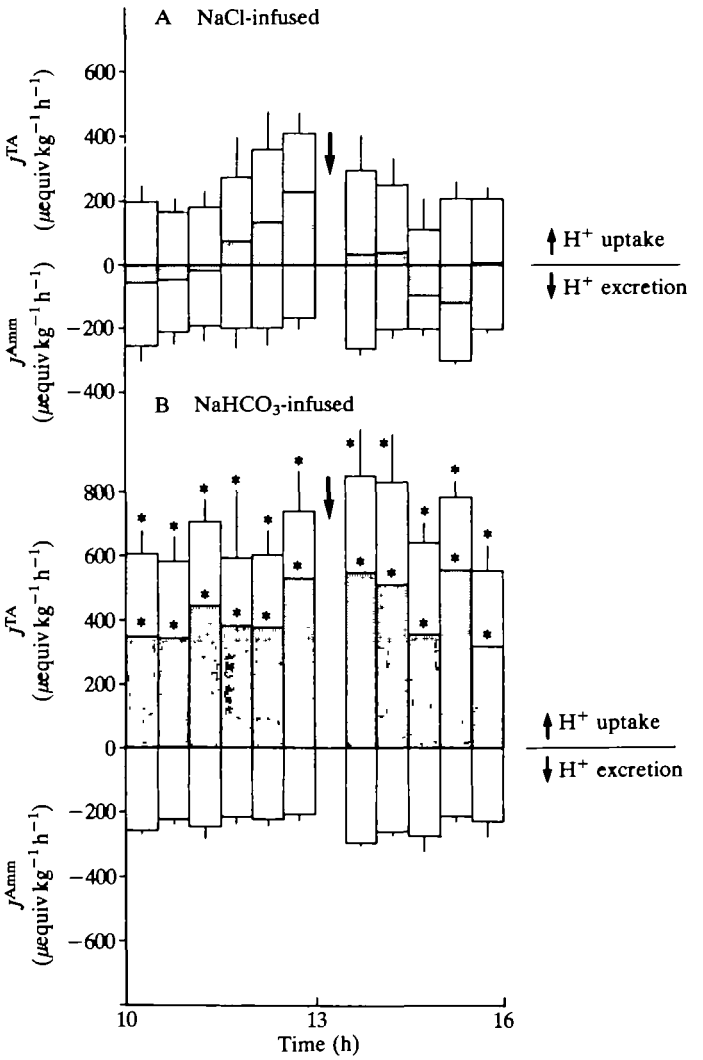

Fig. 5. Branchial flux rates of titratable acidity (J^{TA}), total ammonia (J^{Amm}) and net acidic equivalents (J^{H}_{net}) after 10–16 h of chronic infusion with either (A) 140 mmol l^{-1} NaCl (N=5) or (B) 140 mmol l^{-1} $NaHCO_3$ (N=6) (series I). Positive values indicate acidic equivalent uptake, negative values indicate acidic equivalent excretion. Shaded areas indicate net acidic equivalent (J^{H}_{net}) flux as the sum of the two components: J^{TA} and J^{Amm}, signs considered; standard errors have been omitted for the sake of clarity. Overall, mean J^{TA} and J^{H}_{net} were significantly greater in $NaHCO_3$-infused fish compared to NaCl-infused fish (P<0.05) while there were no significant differences in J^{Amm} between groups. See legend of Fig. 3 for other details.

$-376\,\mu equiv\,kg^{-1}\,h^{-1}$ at 19 h, while infusion of $NaHCO_3$ resulted in a decrease in the diffusive efflux of Cl^- by 65 % to $-108\,\mu equiv\,kg^{-1}\,h^{-1}$. The net difference (i.e. $Na^+ - Cl^-$) in the efflux rates of these ions was not significantly different from

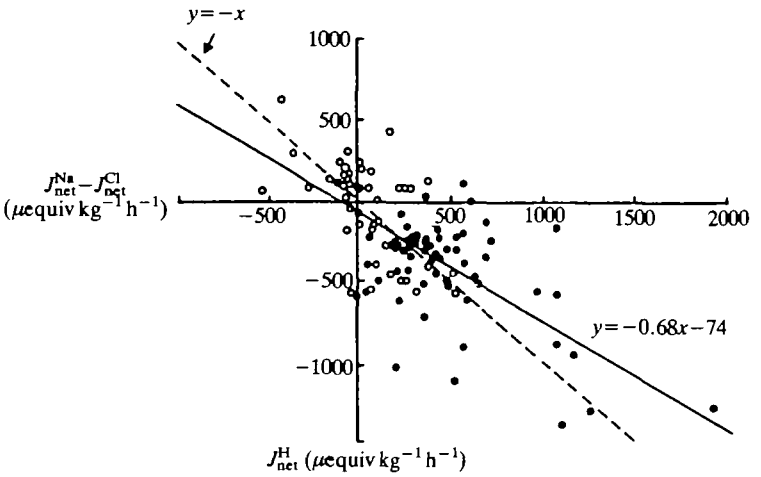

Fig. 6. The relationship between branchial net acidic equivalent flux rate (J^{H}_{net}) and the simultaneously measured differential net flux rate of Na^+ (J^{Na}_{net}) and Cl^- (J^{Cl}_{net}) after 10–16 h of chronic infusion with either 140 mmol l^{-1} NaCl or 140 mmol l^{-1} $NaHCO_3$ (series I). Open circles (○) represent individual values for NaCl-infused fish while closed circles (●) represent individual values for $NaHCO_3$-infused fish. The dashed line represents the line of equivalence (1:1 ratio) while the solid line was fitted by least squares regression analysis: $[J^{Na}_{net}-J^{Cl}_{net}]=-0.68(\pm0.07)J^{H}_{net}-74(\pm25)$, ($r$=0.692, N=120, P<0.001).

zero in either group at the control measurement or in the NaCl-infused group at any time during the experimental regime. However, infusion of $NaHCO_3$ resulted in significant changes in the diffusive efflux of Na^+ and Cl^- such that Na^+ efflux exceeded Cl^- efflux by −60 μequiv kg^{-1} h^{-1} at 10 h and by −150 μequiv kg^{-1} h^{-1} at 19 h (Fig. 7).

Uptake kinetics – series III

The uptake kinetic curves obtained by observation of J^{Na}_{in} and J^{Cl}_{in} over increasing $[NaCl]_e$ in both NaCl- and $NaHCO_3$-infused groups are shown in Fig. 8. The lines fitted to the data are based on the Michaelis–Menten model using the means of the K_m and J_{max} values obtained for individual fish by Eadie–Hofstee regression analysis, as described by Goss and Wood (1990). J^{Na}_{in} in $NaHCO_3$-infused fish was lowered by 50–75 % compared to that of the NaCl-infused fish over the entire concentration range (Fig. 8A). In contrast, J^{Cl}_{in} was approximately doubled in $NaHCO_3$-infused fish compared with NaCl-infused fish at every $[Cl^-]_e$ (Fig. 8B).

Estimates of the J_{max} and K_m of the transport system in each treatment group, based on the Eadie–Hofstee regression analyses are summarized in Fig. 9. During $NaHCO_3$ infusion, the affinity of the Na^+ transporter decreased markedly, i.e. K^{Na}_m increased significantly by 70 % from 276 to 463 μequiv l^{-1} (Fig. 9A). Concurrently, J^{Na}_{max} decreased by 60 % (689 to 262 μequiv kg^{-1} h^{-1}) in $NaHCO_3$-infused fish compared to NaCl-infused fish (Fig. 9B). The Cl^- transporter manifested no

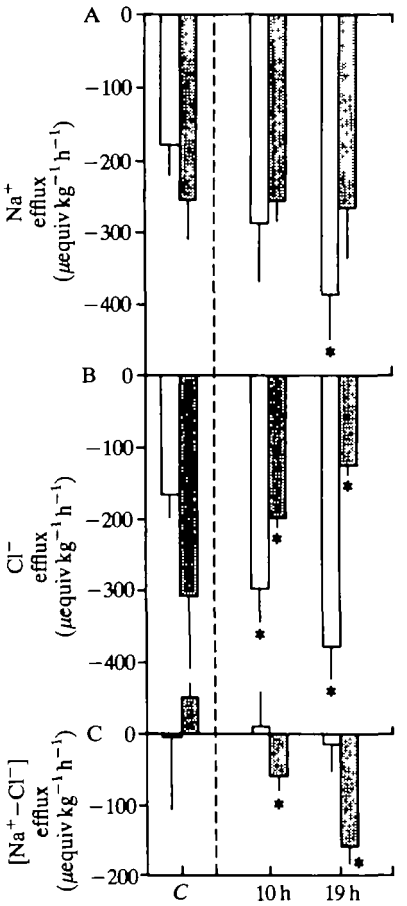

Fig. 7. Diffusive efflux of (A) Na^+, (B) Cl^- and (C) $[Na^+-Cl^-]$ across the gills of rainbow trout during chronic infusion with either 140 mmol l^{-1} NaCl (N=10) (open columns) or 140 mmol l^{-1} $NaHCO_3$ (N=12) (shaded columns) (series II). Significant differences from control value are indicated by an asterisk (*). Values are means ±1 S.E.M. *C*, control value.

change in K_m^{Cl} as a result of $NaHCO_3$ infusion (Fig. 9A) when compared with NaCl-infused fish, but J_{max}^{Cl} was almost doubled (360 to 674 μequiv kg^{-1} h^{-1}, Fig. 9B) in $NaHCO_3$-infused fish.

The effects of increasing $[NaCl]_e$ on the unidirectional outfluxes of Na^+ and Cl^- as well as on J_{net}^{H} and its components were also examined in the kinetic experiments. At almost every level of $[NaCl]_e$, both J_{out}^{Na} and J_{out}^{Cl} were significantly lower in the HCO_3^--loaded fish compared to the NaCl-infused controls (data not shown), in agreement with the steady-state fluxes of series I (cf. Figs 3 and 4). This difference was much more marked for J_{out}^{Cl} (two- to fourfold difference) than for J_{out}^{Na} (1.5-fold to twofold difference). As $[NaCl]_e$ was increased, both J_{out}^{Na} and J_{out}^{Cl} tended to increase in each group, though the data were very variable, reflecting

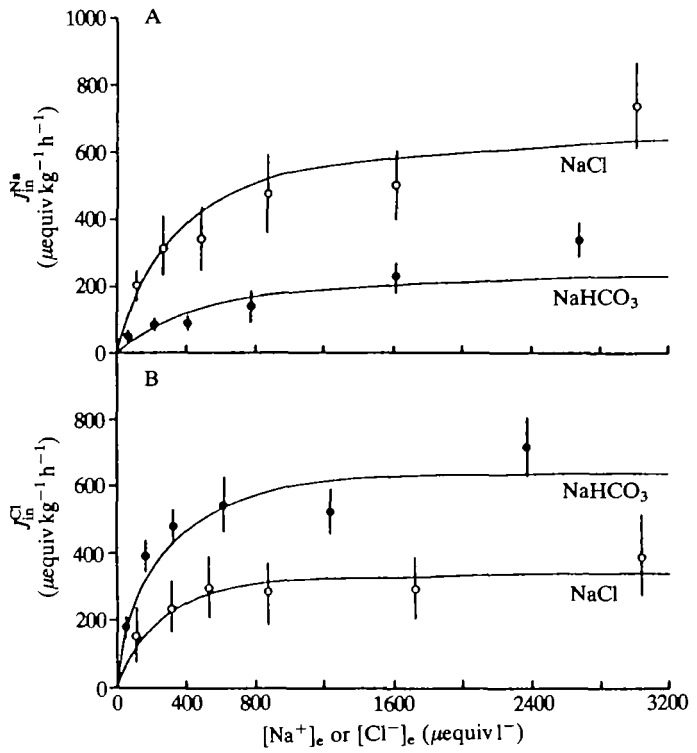

Fig. 8. The kinetics of (A) sodium influx (J_{in}^{Na}) and (B) chloride influx (J_{in}^{Cl}) as a function of the $[Na^+]_e$ and $[Cl^-]_e$, respectively, during chronic infusion with either 140 mmol l^{-1} NaCl (○; N=5) or 140 mmol l^{-1} $NaHCO_3$ (●; N=6) (series III). Curves were drawn by Michaelis–Menten analysis from mean estimates of K_m and J_{max} obtained by Eadie–Hofstee regression analysis for all individual fish in the group. Mean J_{in} values have been plotted at the mean $[Na^+]_e$ or $[Cl^-]_e$ for each point. Values are means±1 S.E.M.

difficulties in making accurate J_{out} measurements at high substrate concentration over short flux periods, as discussed by Goss and Wood (1990). The only significant effect was an increase in J_{out}^{Na} at the two highest $[NaCl]_e$ levels in $NaHCO_3$-loaded fish.

The flux of titratable acidity (J^{TA}) and the net flux of acidic equivalents (J_{net}^{H}) were significantly higher in the $NaHCO_3$-infused group compared to the NaCl-infused group at every $[NaCl]_e$ (Fig. 10). In contrast, J^{Amm} was not significantly different between the two groups at any $[NaCl]_e$. In NaCl-infused fish, J_{net}^{H}, J^{TA} and J^{Amm} were not significantly altered as $[NaCl]_e$ increased (Fig. 10A). In marked contrast, there were significant elevations in both J^{TA} and J_{net}^{H} as $[NaCl]_e$ increased in the $NaHCO_3$-infused fish; J^{Amm} was unaffected. When plotted, these increases also seemed to follow typical Michaelis–Menten uptake kinetic curves. Thus, one could estimate the K_m and J_{max} of the acidic equivalent uptake (=basic equivalent excretion) in terms of $[NaCl]_e$. This was accomplished by plotting J_{net}^{H} *versus*

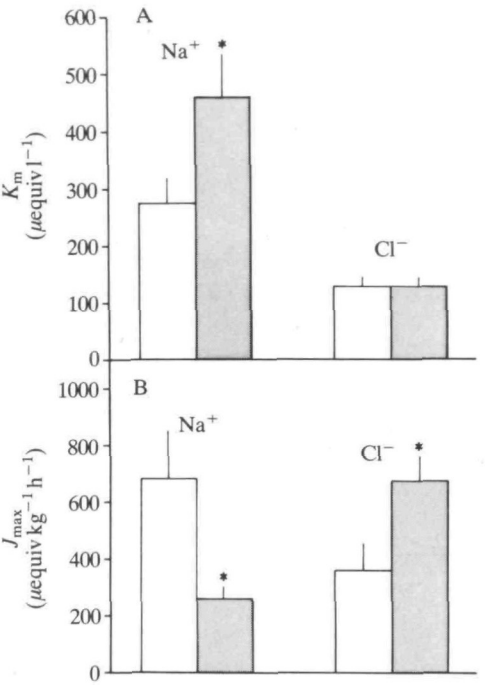

Fig. 9. Mean estimates of (A) the affinity (K_m) and (B) the maximum transport rate (J_{max}) of the Na^+ and Cl^- transporters during chronic infusion with either 140 mmol l^{-1} NaCl (open bars; N=5) or 140 mmol l^{-1} $NaHCO_3$ (shaded bars; N=6) (series III). Values significantly different (P<0.05) from NaCl-infused fish are indicated with an asterisk (*). Values are means±1 S.E.M.

$J^H_{net}/[NaCl]_e$ in an Eadie–Hofstee regression analysis (Michal, 1985) for each individual fish. 'J^H_{max}' was obtained from the y-intercept and 'K^H_m' from the slope. From this analysis it was found that the K^H_m (*expressed in terms of* $[NaCl]_e$) of the transporter was 133±57 μequiv l^{-1} and the J^H_{max} was 753±150 μequiv kg^{-1} h^{-1}. These may be compared with the very similar values for the Cl^- transporter (K^{Cl}_m=135±9 μequiv l^{-1}; J^{Cl}_{max}=674±89 μequiv kg^{-1} h^{-1}) and the very different values for the Na^+ transporter (K^{Na}_m=463±82 μequiv l^{-1}; J^{Na}_{max}=262±43 μequiv kg^{-1} h^{-1}). The affinity and maximal transport rates of the Cl^- transporter were not significantly different from those of the acidic/basic equivalent transporter, while the Na^+ transporter was significantly different in both terms. This similarity of kinetic parameters provides strong evidence for the linkage of Cl^- influx and net acidic equivalent uptake (=basic equivalent excretion) in this particular situation.

Discussion

Blood acid–base status during $NaHCO_3$ infusion

Chronic infusion of $NaHCO_3$ produced a number of significant changes in the

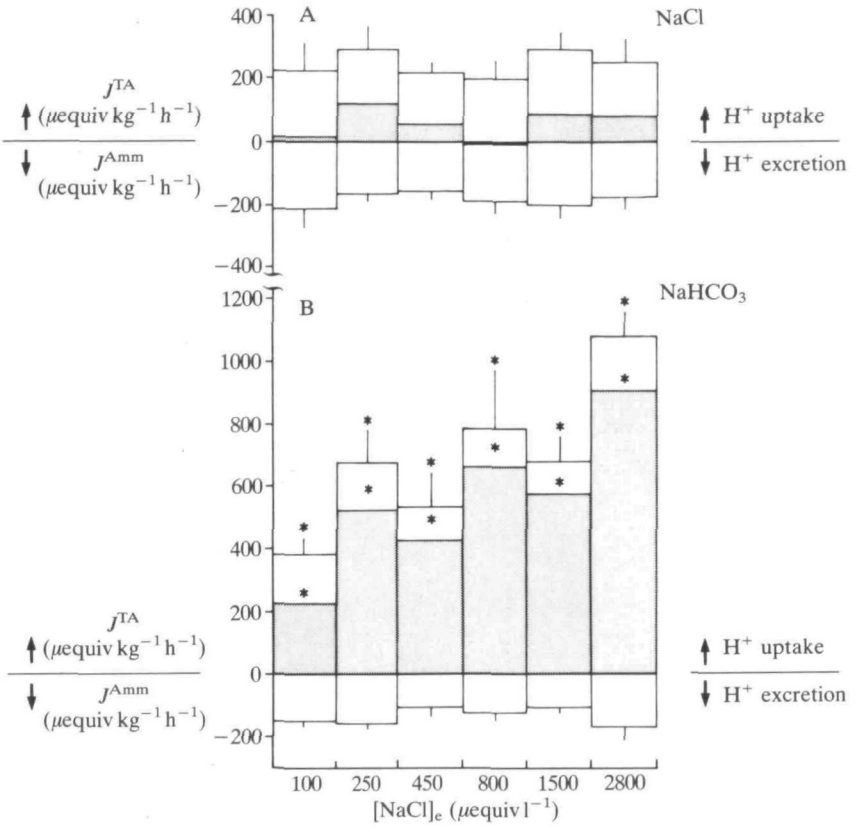

Fig. 10. Titratable acidity (J^{TA}), total ammonia (J^{Amm}) and net acidic equivalent (J^{H}_{net}) flux rates across the gills of rainbow trout as a function of the $[NaCl]_e$ during chronic infusion with either (A) 140 mmol l^{-1} NaCl (N=5) or (B) 140 mmol l^{-1} $NaHCO_3$ (N=6) (series III). Positive values indicate acidic equivalent uptake, negative values indicate acidic equivalent excretion. Shaded areas indicate net acidic equivalent flux (J^{H}_{net}) as the sum of the two components: J^{TA} and J^{Amm}, signs considered. Standard errors have been omitted for the sake of clarity. Significant differences between groups (P<0.05) for each flux period are indicated with an asterisk (*). Values are means±1 S.E.M. Significant differences for both J^{TA} and J^{H}_{net} within the $NaHCO_3$-infused group are indicated below. There were no significant differences for J^{Amm}. Lines underscore treatments which are not significantly different from one another (P<0.05).

100 450 250 1500 800 2800

blood and plasma composition of the rainbow trout, compared to few changes in fish infused with NaCl (Fig. 1). The increases in plasma $[HCO_3^-]$ and pHa were similar to those levels found during the first few hours of normoxic recovery from 72 h of environmental hyperoxia (Wood and Jackson, 1980; Hõbe *et al.* 1984). The elevation in pHa in response to $NaHCO_3$ infusion occurred without any significant

changes in Pa_{CO_2} (i.e. a pure metabolic alkalosis; Fig. 1D). Despite the Na^+ load, plasma [Na^+] remained unchanged but plasma [Cl^-] was partially replaced by HCO_3^- (Fig. 1A,B) in a similar manner to that found by Wheatly *et al.* (1984) in hyperoxic trout. This presumably reflects the constraint of electroneutrality, with Na^+, Cl^- and HCO_3^- being the major ions involved in the present situation.

Continuous infusion of $NaHCO_3$ at a rate of 410 μequiv kg^{-1} h^{-1} did not result in a constantly increasing plasma [HCO_3^-]. Instead, the fish reached a point where excretion rate matched infusion rate (Figs 1C and 5). Plasma [HCO_3^-] in the present study was not elevated beyond the proposed plasma HCO_3^- threshold of 30 mmol l^{-1} (Claiborne and Heisler, 1984), although this concept remains the subject of controversy (Cameron and Iwama, 1987).

Renal response to chronic infusion

In both the NaCl-infused and the $NaHCO_3$-infused fish, urine flow rate (UFR) increased by 80 % of the infusion rate. If the remaining 20 % of the infusion rate stayed in the extracellular fluid (ECF), this would have acted to increase ECF volume in a 300 g fish by an average of 3.3 ml over the 19 h infusion period. Assuming an ECF volume of 27 % body weight (Milligan and Wood, 1982), this volume would amount to a 4 % increase in ECF volume and even less if some penetrated into the intracellular compartment. Therefore, it is likely that there was a minimal change in the blood volume over the entire experimental period. The implication of this result is that the chronic infusion of either NaCl or $NaHCO_3$ did not greatly alter the perfusion characteristics of the gills. While ventilation was not measured, it also seems likely that these treatments would not cause any substantial changes, based on current knowledge of ventilatory control in fish (Perry and Wood, 1989). Thus, any observed changes in ion and acidic equivalent fluxes should be much less subject to the confounding 'non-specific' effects (i.e. perfusion and/or ventilation changes) that are known to occur during recovery from exposure to hyperoxia.

The kidney is known to play a much smaller role than the gills in compensation of acid–base disturbances with the relative renal contribution varying from 7 to 32 % (McDonald and Wood, 1981; Cameron and Kormanick, 1982; Holeton *et al.* 1983; Wheatly *et al.* 1984; Perry *et al.* 1987*b*; Vermette and Perry, 1987*b*; Wood, 1988). This appears to have been the case in the present study, where the kidney contributed a maximum of 13 % to the total net base excretion in $NaHCO_3$-infused fish (Fig. 2B). This excretion rate was probably due to the increased UFR, and thus probably glomerular filtration rate, coupled with a large increase in the filtered HCO_3^- load. Infusion of $NaHCO_3$ may have surpassed the renal threshold for the reabsorption of HCO_3^-, as discussed by Wheatly *et al.* (1984). Urine ion measurements obtained in the accompanying study do not indicate any differential efflux of Na^+ over Cl^-; however, it must be assumed that this excretion of HCO_3^- was accompanied by equimolar amounts of strong cations in order to maintain electroneutrality. Ca^{2+} and K^+ excretion rates *via* the urine have been reported to change during acid–base disturbances (McDonald and Wood, 1981; Wheatly *et al.*

1984; Perry *et al.* 1987*b*) but, unfortunately, they were not measured in the present study. Therefore, it appears that the kidney played a significant but much smaller role in comparison to the gills in ion and acid–base regulation in $NaHCO_3$-infused trout, in agreement with previous studies.

Branchial ion and acidic equivalent fluxes

Infusion of $NaHCO_3$ resulted in a greater J_{in}^{Cl} compared to NaCl infusion (Fig. 4) while J_{in}^{Na} was greatly reduced (Fig. 3). In both groups there was a large branchial net loss of Na^+ (approx. $-200\,\mu equiv\,kg^{-1}\,h^{-1}$) due to infusion of Na^+ salts. However, in $NaHCO_3$-infused fish, there was a net branchial Cl^- gain (approx. $200\,\mu equiv\,kg^{-1}\,h^{-1}$) over the exposure period while in NaCl-infused fish there was a net Cl^- loss (approx. $-200\,\mu equiv\,kg^{-1}\,h^{-1}$). These results confirm those of previous studies showing that the fish adjust their branchial Na^+/acidic equivalent and Cl^-/basic equivalent transporters in a manner consistent with the correction of acid–base status (Cameron, 1976; McDonald *et al.* 1983, 1989; Wood *et al.* 1984; Perry *et al.* 1987*a*,*b*; Wood, 1988; Goss and Wood, 1990). Furthermore, the fish were in approximate acid–base equilibrium, with the rate of J_{net}^{H} (approx. $+400\,\mu equiv\,kg^{-1}\,h^{-1}$) corresponding to the rate of HCO_3^- infusion (approx. $410\,\mu equiv\,kg^{-1}\,h^{-1}$), which in turn corresponded to the difference between J_{net}^{Na} and J_{net}^{Cl} (approx. $-400\,\mu equiv\,kg^{-1}\,h^{-1}$).

However, these fish were clearly not in Na^+ and Cl^- equilibrium – i.e. acid–base homeostasis was achieved at the expense of ionic homeostasis. In the face of a loading rate of $410\,\mu equiv\,kg^{-1}\,h^{-1}$ for both Na^+ and Cl^- in the NaCl-infused trout, and of $410\,\mu equiv\,kg^{-1}\,h^{-1}$ for Na^+ and $0\,\mu equiv\,kg^{-1}\,h^{-1}$ for Cl^- in the $NaHCO_3$-infused trout, the gill net fluxes (see above) were clearly not in balance. The urinary fluxes (Fig. 2) at best could account for only a small part of this difference. The plasma ion data (Fig. 1) were also clearly not in accord with the net balance situation of the fish. For example, plasma [Cl^-] declined and plasma [Na^+] was stable in the $NaHCO_3$-infused trout (which were in overall positive balance for both Na^+ and Cl^-). From this we conclude that the 'missing' Na^+ and Cl^- entered the intracellular compartment or some other sink within the fish. The homeostatic role of this compartment is a topic of interest in future research.

In both the $NaHCO_3$-loaded fish and fish undergoing the normoxia–hyperoxia–normoxia regime (Goss and Wood, 1990), acidic equivalents were exchanged in approximately equimolar amounts for the difference in the net flux of Na^+ and Cl^-. In both studies, changes in J_{net}^{Cl} were more important than changes in J_{net}^{Na} in contributing to the dynamic response. In turn, changes in J_{in}^{Cl} were more important than those in J_{out}^{Cl}. Alterations in the net flux of acidic equivalents in these experiments were accomplished as a result of increases in the J^{TA} component, with no significant changes in J^{Amm} (Fig. 5). This provides additional evidence for the linkage of Cl^- influx and the flux of basic equivalents. In terms of influx manipulations, compensation from an alkalosis occurs mainly though stimulation of the Cl^-/basic equivalent exchange with a minor, but significant, role for the

inhibition of Na^+/acidic equivalent exchange (Claiborne and Heisler, 1984; Wood *et al.* 1984). Modulation of differential diffusive efflux also plays a significant role (see below). The continued excretion of ammonia during alkalosis when Na^+/acidic equivalent exchange is inhibited indicates that ammonia excretion probably follows the flexible model of Wright and Wood (1985), i.e. *via* either or both Na^+/NH_4^+ exchange and NH_3 diffusion across the gill epithelium. Cameron and Heisler (1983) found that the latter alone was sufficient to explain ammonia excretion under control conditions.

In the present study there was not a large stimulation of J_{out}^{Cl} in $NaHCO_3$-infused fish (Fig. 4). This suggests that the simultaneous increases in both J_{out}^{Na} and J_{out}^{Cl} occurring immediately after return to normoxia in the previous hyperoxic studies (Wood *et al.* 1984; Goss and Wood, 1990) were probably the result of changes in the perfusive and ventilatory characteristics noticed during this time (Dejours, 1972, 1973; Wood and Jackson, 1980). Thus, they would constitute a non-specific response, rather than a response to acid–base status.

Relationship between net branchial ion flux and net acidic equivalent flux

While the relationship between the flux of acidic equivalents and the difference between the net fluxes of Na^+ and Cl^- was linear and highly significant (Fig. 6), it did not yield a $-1:1$ stoichiometry, in contrast to the results during exposure to hyperoxia (Goss and Wood, 1990). The greater and more prolonged excursions of pHa may have been a factor here. Although the net fluxes of Na^+ and Cl^- made up a large part of the charge balance (approx. 70%), other ions must certainly have been involved. Increased fluxes of other ions across the gills have been reported under certain conditions (e.g. K^+, McDonald and Wood, 1981; Eddy, 1985; Ca^{2+}, Perry and Wood, 1985; SO_4^{2-}, Hõbe, 1987) and a similar lack of $-1:1$ stoichiometry has been found in several other studies (Claiborne and Heisler, 1984; Perry *et al.* 1987*a*; Vermette and Perry, 1987*a*; Wood, 1988).

Diffusive efflux and acid–base regulation

Infusion of $NaHCO_3$ resulted in a differential diffusive efflux of Na^+ over Cl^- (Fig. 7), confirming the result found during recovery from exposure to hyperoxia (Goss and Wood, 1990). This acts to decrease the strong ion difference (SID) and constrain a necessary net gain of acidic equivalents inside the fish, thereby aiding in reducing the alkalosis. Differential diffusive efflux (Fig. 7), when compared with J_{net}^{H} (Fig. 5) at comparable times, may have accounted for as much as 35% of the total branchial net acidic equivalent uptake (=basic equivalent excretion). Goss and Wood (1990) estimated a figure up to 50% during post-hyperoxic alkalosis. Thus, differential diffusive efflux constitutes a third mechanism of acid–base regulation during metabolic alkalosis (in addition to stimulations of J_{in}^{Cl} and inhibition of J_{in}^{Na}).

An important contribution of the present study was the near simultaneous measurement of the differential diffusive efflux (to NaCl-free water) and the measured outflux of Na^+ and Cl^- (using radio-tracer analysis) in the same fish

Table 1. *Comparison of the measured diffusive efflux values (N=12) for Na^+, Cl^- and †$Na^+ - Cl^-$ (10 h; series II) with unidirectional outflux measurements (N=6) of J^{Na}_{out}, J^{Cl}_{out} and $[J^{Na}_{out} - J^{Cl}_{out}]$ (10–12 h; series I) during chronic infusion with either NaCl or $NaHCO_3$*

Treatment		Diffusive efflux (10 h; series II)	Unidirectional outflux (10–12 h; series I)
NaCl infusion	Na^+	−283±88	−535±96*
	Cl^-	−299±46	−479±93*
	$Na^+ - Cl^-$†	16±50	−55±67
$NaHCO_3$ infusion	Na^+	−255±39	−354±50*
	Cl^-	−200±29	−216±26
	$Na^+ - Cl^-$†	−55±22	−138±67*

All measurements in μequiv kg^{-1} h^{-1}. Values are mean±1 S.E.M.

* Significantly different ($P<0.05$) from diffusive efflux.

† Difference between Na^+ and Cl^- diffusive efflux or difference between Na^+ and Cl^- unidirectional outflux.

during acid–base disturbance. Previous studies addressing the role of differential outflux/efflux in acid–base regulation have employed only the radioisotopic method (Wood *et al.* 1984; Wood, 1988; McDonald and Prior, 1988; McDonald *et al.* 1989). Comparison of the estimates in Table 1 indicates that there were significant differences in the estimated size of the differential outflux/efflux component of acid–base balance, depending on the technique applied. Radioisotopic outflux measurements were probably higher because of the presence of exchange diffusion (Cl^-/Cl^-, Na^+/Na^+; see Goss and Wood, 1990). The exchange diffusion component of radioisotopically measured outflux, by definition, can play no role in acid–base correction. Therefore, the diffusive efflux method (to NaCl-free water) provides a more accurate technique for assessing the role of differential diffusive efflux in acid–base regulation.

Na^+ and Cl^- uptake kinetics – changes during $NaHCO_3$ infusion

Infusion of $NaHCO_3$ resulted in complex alterations in both K_m^{Na} and J_{max}^{Na} of the Na^+ transporter, while for the Cl^- transporter there was no significant change in K_m^{Cl} but a large increase in J_{max}^{Cl} (Figs 8, 9). The observed inhibition of Na^+ influx in $NaHCO_3$-infused fish (Fig. 3) was accomplished by decreasing both the affinity (increased K_m^{Na}) and the maximum transport rate (J_{max}^{Na}) of the Na^+ transporter (Fig. 9). This is directly comparable with the response in Na^+ kinetics noted during recovery from hyperoxia by Goss and Wood (1990). Changes in Cl^- influx during recovery from hyperoxia occurred as a result of both decreases in K_m^{Cl} (but only relative to the elevated value of final hyperoxic acidosis) and increases in J_{max}^{Cl}, whereas, in the present study, only an increased J_{max} occurred in response to metabolic alkalosis (Fig. 9). The values of K_m^{Cl} were the same during HCO_3^-

infusion (132 μequiv l^{-1}); NaCl-infusion (135 μequiv l^{-1}; Fig. 9) and recovery from hyperoxia (135 μequiv l^{-1}; Goss and Wood, 1990).

Therefore, it appears that K_m of both the Na^+ and Cl^- transporters under control normoxic conditions operate *at or near the maximum affinity* (low K_m). Reducing the influx can then be accomplished by increasing K_m or by decreasing J_{max}. However, increases in influx at this time can be accomplished only by increasing J_{max}, because K_m under control conditions is already close to the minimum possible value. It is not surprising that there is a limit to which affinity can be increased, and the transporters normally operate close to this maximal affinity. No such restriction is apparent for J_{max}, which could be either increased or decreased with respect to control levels for the purpose of acid–base correction. Interestingly, the K_m of the Na^+ transporter was increased (Fig. 9) during infusion with NaCl compared to the control normoxia value (Fig. 9). This might be the result of the Na^+ load, even in the absence of an acid–base disturbance. Infusion of $NaHCO_3$ resulted in a further reduction in the affinity of the Na^+ transporter.

The possible mechanisms for changes in K_m and J_{max} were dealt with at length in Goss and Wood (1990). However, the present experiment has indicated that changes in K_m and J_{max} observed during post-hyperoxic alkalosis were *not* the result of the 'non-specific' mechanisms (perfusive and convective changes, altered O_2 levels) that complicated interpretation of the post-hyperoxia responses.

The increased excretion of acidic equivalents as $[NaCl]_e$ was increased in the kinetics experiment (Fig. 10) was similar to, but much more pronounced, than the corresponding relationship during post-hyperoxic recovery. This occurred as a direct result of alterations in the J^{TA} component while J^{Amm} remained unchanged. These data indicated that the external availability of NaCl was directly limiting on the rate of acidic equivalent excretion and support the hypothesis that recovery from an acid disturbance should occur faster in water with a higher $[NaCl]_e$ (Perry, 1982; Tang *et al.* 1988; McDonald *et al.* 1989).

The uptake of acidic equivalents (=basic equivalent excretion) seemed to follow typical Michaelis–Menten kinetic curves (Fig. 10). The observation that kinetic parameters of basic equivalent excretion, when expressed in terms of $[NaCl]_e$, were very similar to those of Cl^-, provides perhaps the strongest evidence to date that the major uptake mechanism involved in the excretion of basic equivalents across the gills is the Cl^- transporter.

This research was supported through an NSERC operating grant to CMW. Many thanks are extended to M. Kovacevic and R. Rhem for excellent technical support throughout the project.

References

Boutilier, R. G., Heming, T. A. and Iwama, G. K. (1984). Physico-chemical parameters for use in fish respiratory physiology. In *Fish Physiology*, vol. XA (ed. W. S. Hoar and D. J. Randall), pp. 403–430. New York: Academic Press.

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

Cameron, J. N. and Heisler, N. (1983). Studies of ammonia in the rainbow trout: physico-chemical parameters, acid–base behaviour and respiratory clearance. *J. exp. Biol.* **105**, 107–126.

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

Cameron, J. N. and Kormanick, G. A. (1982). Intracellular and extracellular acid–base status as a function of temperature in the freshwater channel catfish, *Ictalurus punctatus*. *J. exp. Biol.* **99**, 127–142.

Claiborne, J. B. and Heisler, N. (1984). Acid–base regulation and ion transfers in the carp (*Cyprinus carpio*) during and after exposure to environmental hypercapnia. *J. exp. Biol.* **108**, 25–43.

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

Dejours, P. (1972). Action des changements de P_{O_2} et de P_{CO_2} de l'eau sur la ventilation et l'equilibre acide–base du sang chez quelques poissons teleosteens. *J. Physiol., Paris* **65**, 386A.

Dejours, P. (1973). Problems of control of breathing in fishes. In *Comparative Physiology: Locomotion, Respiration, Transport, and Blood* (ed. L. Bolis, K. Schmidt-Nielsen and S. H. P. Maddrell), pp. 117–133. New York: American Elsevier.

Eddy, F. B. (1985). Uptake and loss of potassium by rainbow trout (*Salmo gairdneri*) in freshwater and dilute sea water. *J. exp. Biol.* **118**, 277–286.

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

Heisler, N., Toews, D. P. and Holeton, G. F. (1988). Regulation of ventilation and acid–base status in the elasmobranch *Scyliorhinus stellaris* during hyperoxia-induced hypercapnia. *Respir. Physiol.* **71**, 227–246.

Hills, A. G. (1973). *Acid–Base Balance. Chemistry, Physiology, Pathophysiology*. Baltimore: The Williams and Wilkens Co.

Hõbe, H. (1987). Sulphate entry into soft-water fish (*Salmo gairdneri; Catostomus commersoni*) during low ambient pH exposure. *J. exp. Biol.* **133**, 87–109.

Hõbe, H., Wood, C. M. and Wheatly, M. G. (1984). The mechanisms of acid–base and ionoregulation in the freshwater rainbow trout during environmental hyperoxia and subsequent normoxia. I. Extra- and intracellular acid–base status. *Respir. Physiol.* **55**, 139–154.

Holeton, G. F., Neumann, P. and Heisler, N. (1983). Branchial ion exchange and acid–base regulation after strenuous exercise in the rainbow trout (*Salmo gairdneri*). *Respir. Physiol.* **51**, 303–318.

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

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

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

McDonald, D. G. and Wood, C. M. (1981). Branchial, and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri*, at low environmental pH. *J. exp. Biol.* **93**, 101–118.

Michal, G. (1985). Determinations of Michaelis constants and inhibitior constants. In *Methods of Enzymatic Analysis*, vol. 1 (ed. H. U. Bergmeyer), pp. 86–103. Weinheim: Verlag Chemie.

Milligan, C. L. and Wood, C. M. (1982). Disturbances in haematology, fluid volume distribution and circulatory function associated with low environmental pH in the rainbow trout, *Salmo gairdneri*. *J. exp. Biol.* **99**, 397–415.

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

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

PERRY, S. F., MALONE, S. AND EWING, D. (1987*b*). Hypercapnic acidosis in the rainbow trout (*Salmo gairdneri*). II. Renal ionic fluxes. *Can. J. Zool.* **65**, 896–902.

PERRY, S. F. AND WOOD, C. M. (1985). Kinetics of branchial calcium uptake in the rainbow trout: effects of acclimation to various external calcium levels. *J. exp. Biol.* **116**, 411–433.

PERRY, S. F. AND WOOD, C. M. (1989). Control and coordination of gas transfer in fishes. *Can. J. Zool.* **67**, 2961–2970.

SOIVIO, A., WESTMAN, K. AND NYHOLM, K. (1972). Improved method of dorsal aorta catheterization: haematological effects followed for three weeks in rainbow trout (*Salmo gairdneri*) *Finn. Fish. Res.* **1**, 11–21.

TANG, Y., NOLAN, S. AND BOUTILIER, R. G. (1988). Acid–base regulation following acute acidosis in seawater-adapted rainbow trout, *Salmo gairdneri*: a possible role for catecholamines. *J. exp. Biol.* **134**, 297–312.

VERDOUW, H., VAN ECHTELD, C. J. Å. AND DEKKERS, E. M. J. (1978). Ammonia determinations based on indophenol formation with sodium salicylate. *Water Res.* **12**, 399–402.

VERMETTE, M. G. AND PERRY, S. F. (1987*a*). The effects of prolonged epinephrine infusion on the physiology of the rainbow trout, *Salmo gairdneri*. III. Branchial solute fluxes. *J. exp. Biol.* **128**, 255–267.

VERMETTE, M. G. AND PERRY, S. F. (1987*b*). The effects of prolonged epinephrine infusion on the physiology of the rainbow trout, *Salmo gairdneri*. III. Renal ionic fluxes. *J. exp. Biol.* **128**, 268–285.

WANG, C. H., WILLIS, D. L. AND LOVELAND, W. D. (1975). *Radiotracer Methodology in the Biological, Environmental, and Physical Sciences*. New Jersey: Prentice Hall.

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

WOLF, K. (1963). Physiological salines for freshwater teleosts. *Prog. Fish Cult.* **25**, 135–140.

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

WOOD, C. M. AND JACKSON, E. B. (1980). Blood acid–base regulation during environmental hyperoxia in the rainbow trout (*Salmo gairdneri*). *Respir. Physiol.* **42**, 351–372.

WOOD, C. M. AND RANDALL, D. J. (1973). The influence of swimming activity on water balance in the rainbow trout (*Salmo gairdneri*). *J. comp. Physiol.* **82**, 257–276.

WOOD, C. M., WHEATLY, M. G. AND HØBE, H. (1984). The mechanisms of acid–base and ionoregulation in the freshwater rainbow trout during environmental hyperoxia and subsequent normoxia. III. Branchial exchanges. *Respir. Physiol.* **55**, 175–192.

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