J. exp. Biol. **126**, 41–61 (1986)
Printed in Great Britain © *The Company of Biologists Limited 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

BY J. B. CLAIBORNE* AND N. HEISLER

Abteilung Physiologie, Max-Planck-Institut für experimentelle Medizin, D-3400 Göttingen, FRG

Accepted 5 June 1986

SUMMARY

To study both temporal and quantitative effects of hypercapnia on the extent of pH compensation in the arterial blood, specimens of carp (*Cyprinus carpio*) were exposed to a P_{CO_2} of about 7·5 mmHg (1 mmHg = 133·3 Pa) (1 % CO_2) in the environmental water for several weeks, and a second group of animals was subjected to an environmental P_{CO_2} of about 37 mmHg (5 % CO_2) for up to 96 h. A third series of experiments was designed to test the possibility that infusion of bicarbonate would increase the extent of plasma pH compensation. Dorsal aortic plasma pH, P_{CO_2} and $[HCO_3^-]$, as well as net transfer of HCO_3^--equivalent ions, NH_4^+, Cl^- and Na^+, between fish and ambient water, were monitored throughout the experiments.

Exposure to environmental P_{CO_2} of 7·5 mmHg resulted in the expected respiratory acidosis with the associated drop in plasma pH, and subsequent compensatory plasma $[HCO_3^-]$ increase. The compensatory increase of plasma bicarbonate during long-term hypercapnia continued during 19 days of exposure with plasma bicarbonate finally elevated from 13·0 mmol l^{-1} during control conditions to 25·9 mmol l^{-1} in hypercapnia, an increase equivalent to 80 % plasma pH compensation.

Exposure to 5 % hypercapnia elicited much larger acid–base effects, which were compensated to a much lesser extent. Plasma pH recovered to only about 45 % of the pH depression expected at constant bicarbonate concentration. At the end of the 96-h exposure period, plasma $[HCO_3^-]$ was elevated by a factor of 2·5 to about 28·2 mmol l^{-1}.

The observed increase in plasma bicarbonate concentration during 5 % hypercapnic exposure was attributable to net gain of bicarbonate equivalent ions from (or release of H^+-equivalent ions to) the environmental water. Quantitatively, the gain of 15·6 mmol kg^{-1} was considerably larger than the amount required for compensation of the extracellular space, suggesting that acid–base relevant ions were transferred for compensation of the intracellular body compartments. The uptake of

* Present address: Department of Biology, Georgia Southern College, Statesboro, Georgia 30460, USA.

Key words: acid–base regulation, ammonia excretion, bicarbonate exchange, bicarbonate threshold, base infusion, chloride exchange, *Cyprinus carpio*, hypercapnia, ion transfer mechanisms, sodium exchange.

bicarbonate-equivalent ions from the water was accompanied by a net release of Cl^- and, to a smaller extent, by a net uptake of Na^+, suggesting a 75 % contribution of the Cl^-/HCO_3^- exchange mechanism.

Infusion of bicarbonate after 48 h of exposure to 7·5 mmHg P_{CO_2} had only a transient effect on further pH compensation. The infused bicarbonate was lost to the ambient water, and pre-infusion levels of bicarbonate were reattained within 24 h. Repetition of the infusion did not result in a notable improvement of the acid–base status. These observations are consistent with the idea of a 'threshold' of the bicarbonate retaining and resorbing structures of the fish.

INTRODUCTION

The elevation of plasma P_{CO_2} in fish induced by exposure to ambient hypercapnia regularly results in an associated fall in plasma pH, which is, in general, at least partially compensated by elevation of plasma $[HCO_3^-]$ (see recent reviews by Heisler, 1982, 1984, 1986*b*). The time required for HCO_3^--mediated pH recovery, however, is quite variable and may range from 10–20 h (Heisler, Weitz & Weitz, 1976; Eddy, Lomholt, Weber & Johansen, 1977; Toews, Holeton & Heisler, 1983) up to several days (e.g. Janssen & Randall, 1975).

The accumulation of bicarbonate responsible for pH compensation has been attributed to Na^+/H^+, Na^+/NH_4^+ and Cl^-/HCO_3^- exchange processes in various combinations and mainly at the gills (e.g. Maetz & Garcia-Romeu, 1964; Cameron, 1976). On the basis of these mechanisms the observed variability of the time course and magnitude of compensation may be related to differences in branchial ion turnover rates, which are larger by orders of magnitude in marine than in freshwater species (Evans, 1980*b*), and/or to the availability of relevant ions in the surrounding water (Heisler, 1982*b*, 1984, 1986*d*). Indeed, in trout the time course of compensation seems to be related to the electrolyte content of the experimental water (see Heisler, 1985), and in *Scyliorhinus* the uptake rate of bicarbonate-equivalent ions has been shown to be correlated with the bicarbonate concentration of the environmental water (Heisler & Neumann, 1977; Heisler, 1985, 1986*b*).

The limited extent of pH compensation recently reported for *Cyprinus* (approx. 55 %, Claiborne & Heisler, 1984*b*) was achieved mainly by net uptake of HCO_3^- from the ambient water in exchange for Cl^-, which is in agreement with the data of DeRenzis & Maetz (1973), but in contrast to the 'standard' model of transbranchial Na^+/H^+ and Na^+/NH_4^+ exchange, which are considered to be more adaptive mechanisms for freshwater fish, voiding H^+ in exchange for Na^+ passively lost to the hypo-osmotic environment (Maetz & Garcia-Romeu, 1964; reviewed by Evans, 1980*a*, 1984, 1986; Heisler, 1980, 1984). Utilization of only one of the ion exchange pathways, as well as limited availability of relevant ions in the environmental water, may have led to a rate limitation of bicarbonate-equivalent uptake in *Cyprinus*, which in turn may be responsible for the limited pH compensation during the relatively short time of exposure (48 h, Claiborne & Heisler, 1984*b*).

Another factor may be involved. Although rate and magnitude of HCO_3^- accumulation in carp were found to be below those of other species, the plasma $[HCO_3^-]$

finally attained was close to that observed in other fishes during environmental or hyperoxia-induced hypercapnia (Heisler *et al.* 1976; Eddy *et al.* 1977; Toews *et al.* 1983; Hōbe, Wood & Wheatly, 1984). The 'maximal' [HCO_3^-] attained during hypercapnia is generally between 20 and 32 mmol l^{-1} in fishes and amphibians (Heisler, 1984, 1985, 1986*c*). Accordingly, the incomplete compensation in carp may also be related to a species-specific maximum for plasma bicarbonate concentration.

The aim of the present study was to assess the extent of pH compensation and bicarbonate accumulation in carp exposed to mild hypercapnia on a long-term basis, and to delineate the transepithelial ion transfer mechanisms exploited during exposure to high levels of environmental hypercapnia. We also studied the physiological mechanisms for bicarbonate accumulation during hypercapnia by giving infusions of bicarbonate, and tested the idea of a maximal bicarbonate concentration in the plasma of fishes, limited by a certain threshold of the ion exchange structures.

MATERIALS AND METHODS

Carp (*Cyprinus carpio*) were maintained and prepared in a manner previously described by Claiborne & Heisler (1984*b*). In brief, fish were anaesthetized with MS-222 and placed on an operating rack which allowed irrigation of the gills in order to maintain anaesthesia and oxygen supply. The dorsal aorta was cannulated, using methods modified from those described by Soivio, Westman & Nyholm (1975) and Ultsch, Ott & Heisler (1981). Fish were then placed in the appropriate experimental chamber and allowed to recover for a minimum of 24 h, and in most cases several days, before experiments were begun.

Long-term hypercapnia experiments

After implantation of the cannulae, carp (mass 1·98 ± 0·19 kg, $\bar{x} \pm$ S.E., $N = 5$) were returned to a large volume aquarium (800 l), which was one-third of the size of the aquaria used for acclimation, and held there for at least 1 month before the experiment. The aquaria were supplied with dechlorinated, aerated tap water (>100 l fish^{-1} day^{-1}), and thermostatted at 15 ± 0·5 °C. The fish were exposed to a 12 h light/dark cycle and fed regularly. To prevent tangling and unnecessary disturbance of the fish, relatively short (approx. 20 cm) cannulae were left trailing from the carp between sampling periods. Before blood samples were taken, the cannula on each fish was carefully caught and an additional extension (approx. 80 cm) of PE 60 tubing was connected to the implanted tubing. Fish were then left undisturbed for an additional 30–60 min. After this period, a blood sample was drawn from the cannula and immediately analysed. The cannula was then flushed with heparinized Ringer solution, the extension removed, and the cannula plugged with a stainless steel stopper.

The large aquarium used for CO_2 exposure was fitted with a 'P_{CO_2}-stat' system to allow modification and maintenance of elevated P_{CO_2} in the environmental water. This consisted of a water-jacketed P_{CO_2} electrode (Radiometer, Copenhagen; maintained at 15 ± 0·5 °C) connected to a pH meter, chart recorder, and a pH-stat system

operating a water flow solenoid valve. Water from the aquarium was supplied to the P_{CO_2} electrode by a roller pump. For initiation of hypercapnia, the water was bubbled with pure CO_2 (approx. 0·3 l min^{-1}), and P_{CO_2} in the water was adjusted by addition of almost CO_2-free aerated water by means of the P_{CO_2}-stat system. This system allowed the P_{CO_2} of the environmental water to be kept within ±0·5 mmHg of the predetermined level. The P_{CO_2} electrode was calibrated with water equilibrated with known CO_2 concentrations (supplied by a gas mixing pump; Wösthoff, Bochum, FRG).

After a 4-day postoperative reacclimation period, control blood samples were drawn from each fish. In order to minimize stress, the P_{CO_2} of the water was then gradually increased by adjusting the setpoint-value of the P_{CO_2}-stat system to about 1 % CO_2 over the next 5 days. P_{CO_2} was then maintained at a constant level for an additional 14 days. Experimental blood samples were collected on days 3, 6, 15 and 19 of exposure to hypercapnia. Samples of about 0·8 ml each were analysed for P_{CO_2}, pH and P_{O_2} as described by Heisler (1978). Plasma total CO_2 (T_{CO_2}) was measured conductometrically (Claiborne & Heisler, 1984*b*).

High-level hypercapnia experiments (5 % CO_2)

After surgery, carp (mass 1·40 ± 0·30 kg, $\bar{x}$ ± S.E., $N = 8$) were placed in a closed recirculation system identical to that described by Claiborne & Heisler (1984*b*). In this system, water (typical ion concentrations in mmol l^{-1}): $[Na^+]$, 0·3; $[Cl^-]$, 0·2–0·5; $[K^+]$, 0·02–0·07; $[Ca^{2+}]$, 0·8) was continuously cycled through a large aeration column, a filter system and the fish box. Air/CO_2 gas mixtures supplied to the aeration column were controlled by two gas flowmeters. Roller pumps delivered water from the fish system to ammonia electrodes and equilibration columns followed by pH electrodes, connected to appropriate amplifiers and recording equipment, thus allowing the measurement of total [ammonia] and $[HCO_3^-]$ in the ambient water throughout the experiment (see Claiborne & Heisler, 1984*b* for details).

After 24–48 h of acclimation to the experimental apparatus and a 12–15 h control period, the experiment was started by switching the gas aerating the water in the fish system to a mixture of 5 % CO_2 in air. During both the control and experimental periods, changes in ambient [ammonia] and $[HCO_3^-]$ were continuously monitored, and water samples were saved for later Na^+ and Cl^- analysis. Blood samples (approx. 0·5 ml) were collected from the dorsal aorta 0, 1, 2, 4, 8, 24, 48, 72 and 96 h following the start of the hypercapnic period, and analysed for P_{CO_2}, pH, P_{O_2} and T_{CO_2}.

Bicarbonate infusion experiments

Carp (mass 1·63 ± 0·13 kg, $\bar{x}$ ± S.E., $N = 4$) were allowed to recover from surgery and subjected to control periods and to hypercapnia in the same way and in the same apparatus as described for high-level hypercapnia experiments. The level of P_{CO_2} of approx. 7·5 mmHg was maintained for 120 h. After 48 h and again after 96 h of hypercapnia, 5 mmol kg^{-1} $NaHCO_3$ in Ringer solution was infused by means of a

roller pump into the dorsal aorta, over a 30- to 45-min period, depending on the weight of the fish. Blood and water samples were collected throughout control and hypercapnic periods, and analysed for the parameters mentioned above.

Calculations

Plasma [HCO_3^-] was calculated for each sample as the difference between T_{CO_2} and dissolved CO_2 ($\alpha_{CO_2} \times P_{CO_2}$; applying values for α obtained from the formula of Heisler, 1984, 1986*a*; note the first sign of the last line term in Heisler (1984) is misprinted and should be '+').

The amounts of each ionic species transferred between fish and environmental water during control and experimental periods – specifically, $\Delta HCO_3^-{}_w$, $\Delta NH_4^+{}_w$, $\Delta Na^+{}_w$ and $\Delta Cl^-{}_w$ – were determined from measurements of the concentration changes of these ions in the ambient water after adjustment for the total volume of the recirculation system and the mass of the animal. For details refer to Heisler (1984). It should be noted that $\Delta NH_4^+{}_w$ actually describes the total ammonia ($NH_4^+ + NH_3$) released from the fish. More than 99% of any NH_3 eliminated into the hypercapnic water will immediately be converted to (and measured as) NH_4^+ as a result of the high pK′ for the ammonia/ammonium buffer system (9·6, Cameron & Heisler, 1983). $\Delta H^+{}_{e \to w}$ is the difference between $\Delta NH_4^+{}_w$ and $\Delta HCO_3^-{}_w$ for each period. 'Net Δ' values are the differences between control and experimental averages for each period. The 'expected' plasma pH at constant [HCO_3^-] was calculated on the basis of the control plasma [HCO_3^-] and the individual plasma P_{CO_2}.

Statistical analysis using Student's *t*-test for paired or unpaired data was performed where appropriate. All calculations were accomplished on a mini- or microcomputer (Digital, PDP 11/03-L, or Kontron PSI-80) using specially designed BASIC and FORTRAN routines (Claiborne & Heisler, 1984*a*; and J. B. Claiborne, N. A. Andersen & N. Heisler, unpublished).

RESULTS

Long-term hypercapnia

The changes in plasma acid–base status during long-term exposure to hypercapnia are shown in Fig. 1. As the ambient P_{CO_2} was gradually increased to 7·5 mmHg over the first 6 days, plasma P_{CO_2} was significantly elevated from the control value of 4·4 mmHg to 10·2 mmHg ($P < 0{\cdot}001$, $N = 5$). From day 6 to day 19, plasma P_{CO_2} rose more slowly to the final value of 11·4 mmHg; an overall increase of 160%. Concurrent with the elevation of ambient P_{CO_2}, plasma pH fell from a control value of 7·91 ± 0·03 ($\bar{x} \pm$ S.E., $N = 5$) to a minimum measured at day 6 of 7·69 ± 0·03. By day 19, the pH had recovered to 7·83 ± 0·02, a value which was not significantly different from the original control pH ($P < 0{\cdot}1$, $N = 4$). During this hypercapnic exposure, plasma [HCO_3^-] increased by nearly 100% from 13·0 ± 0·7 to 25·9 ± 0·9 mmol l^{-1}.

High-level hypercapnia (5 % CO_2)

During exposure to an ambient P_{CO_2} of approx. 35 mmHg, carp exhibited much larger changes in blood acid–base status (Fig. 2) than observed at 1 % ambient CO_2. One hour after initiation of 5 % hypercapnia, plasma P_{CO_2} had increased from 4·4 to 33·3 mmHg and was elevated by nearly ninefold (from 4·36 to 37·21 mmHg) after 96 h. Plasma pH fell from 7·86 to 7·09 ($P < 0·001$, $N = 5$) during the first hour of hypercapnia. Over the following 3 days, the pH recovered to 7·41, and then did not change significantly for the remaining 24 h of the experiment. Plasma [HCO_3^-] rose from the control value of $11·4 \pm 0·7$ to $27·2 \pm 1·1$ mmol l^{-1} ($P < 0·001$, $N = 5$) after 72 h of hypercapnia. During the next 24 h plasma [HCO_3^-] increased only insignificantly to $28·2 \pm 1·4$ mmol l^{-1} ($P < 0·1$, $N = 4$).

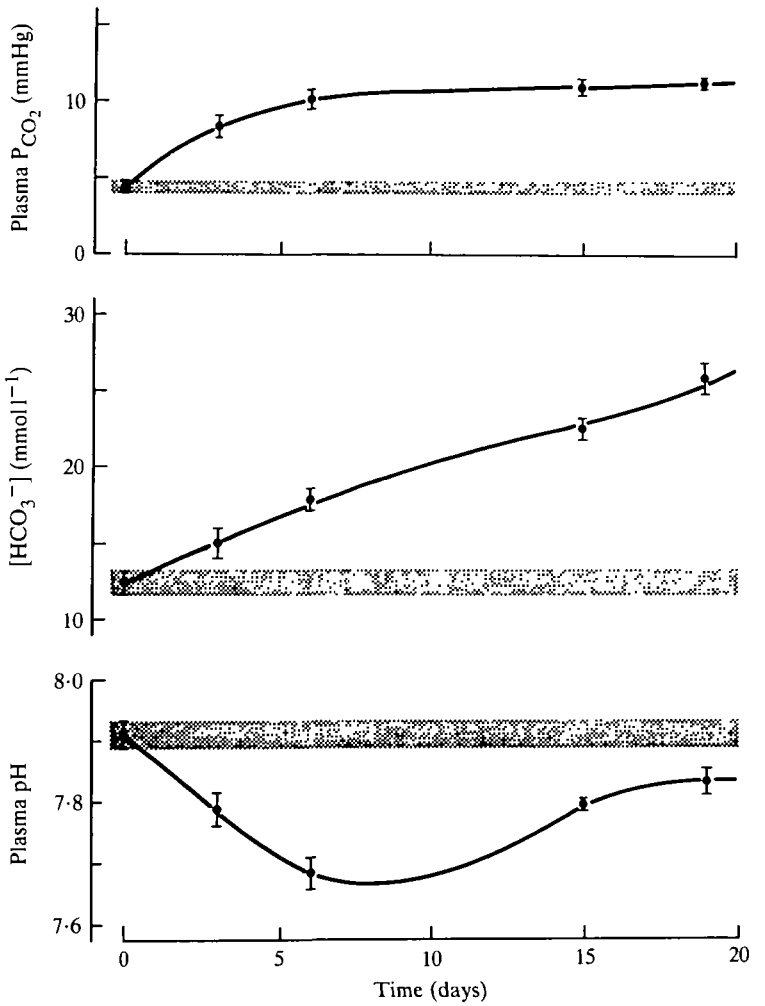

Fig. 1. Plasma P_{CO_2}, [HCO_3^-] and pH in dorsal aortic blood following the onset of long-term environmental hypercapnia of about 1 % CO_2 ($\bar{x} \pm$ S.E., $N = 5$; day 19: $N = 4$). Shaded areas represent $\bar{x} \pm$ S.E. of the pre-hypercapnic control values.

The increase in plasma [HCO_3^-] during hypercapnia is a result of net acid–base relevant ion transfer between the fish and the ambient water. During the first 72 h of exposure to 35 mmHg P_{CO_2} the ammonia release increased by about 2·5-fold (from $0{\cdot}18 \pm 0{\cdot}05$ to $0{\cdot}44 \pm 0{\cdot}4$ mmol h^{-1} kg^{-1}, $P < 0{\cdot}001$, $N = 8$), which was the main contributor to the large net H^+ transfer from the fish to the water (Fig. 3). ΔH^+ increased from 0·08 to 0·29 mmol h^{-1} kg^{-1} after 72 h, equivalent to a 350 % increase in cumulative H^+ transfer (a net H^+ extrusion of about 15·1 mmol kg^{-1}). In the four fish maintained at hypercapnia for 96 h, the rates of ammonia and H^+ excretion averaged 0·40 and 0·247 mmol h^{-1} kg^{-1}, respectively. From hours 72 to 96 of

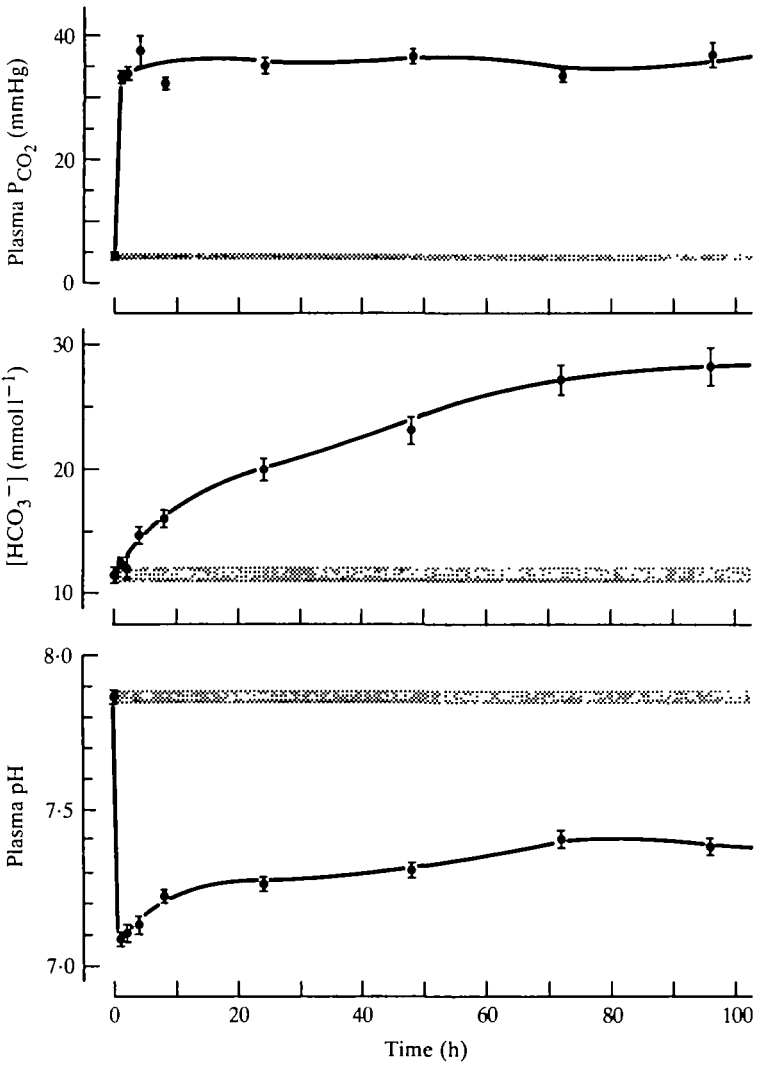

Fig. 2. Plasma P_{CO_2}, [HCO_3^-] and pH in dorsal aortic blood following the onset of 5 % hypercapnia ($\bar{x} \pm$ S.E., $N = 5$; hour 96: $N = 4$). Shaded areas represent $\bar{x} \pm$ S.E. of the pre-hypercapnic control values.

hypercapnia, the rates of ammonia and H^+ transfers in these animals were not significantly different from pre-hypercapnia control values. The release of HCO_3^- to the water increased slightly during hypercapnia, resulting in a small but insignificant increase in the rate of net HCO_3^- efflux (from $0{\cdot}10 \pm 0{\cdot}06$ during control conditions to $0{\cdot}15 \pm 0{\cdot}02$ mmol h^{-1} kg^{-1}, $N = 8$).

This transfer of acid–base relevant ions was accompanied by net movements of Cl^- and Na^+ between fish and ambient water (Fig. 4). Hypercapnia induced an alteration in net Cl^- transfer from a slight net uptake to a much larger net efflux. Over 72 h, the control uptake rate of $0{\cdot}02 \pm 0{\cdot}02$ mmol h^{-1} kg^{-1} was reversed to a net

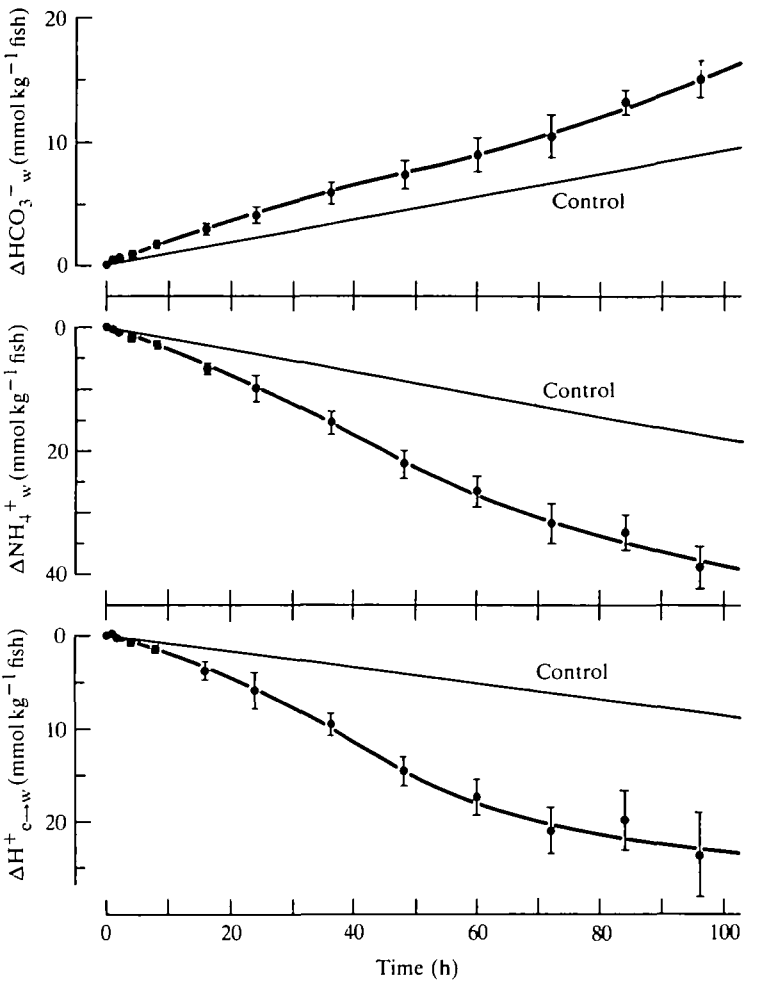

Fig. 3. Changes in the amount of water HCO_3^- and NH_4^+ during exposure to 5% ambient CO_2. The control line was determined over a 12-h period before the start of hypercapnia. In this and following ion transfer figures, the control line has been extended as a reference over the subsequent experimental periods. Cumulative H^+ efflux ($\Delta H^+_{c\rightarrow w}$) is calculated as the difference between the release of NH_4^+ and HCO_3^- ($\bar{x} \pm$ S.E., $N = 8$ for control and experimental points up to 72 h; at 84 and 96 h: $N = 4$).

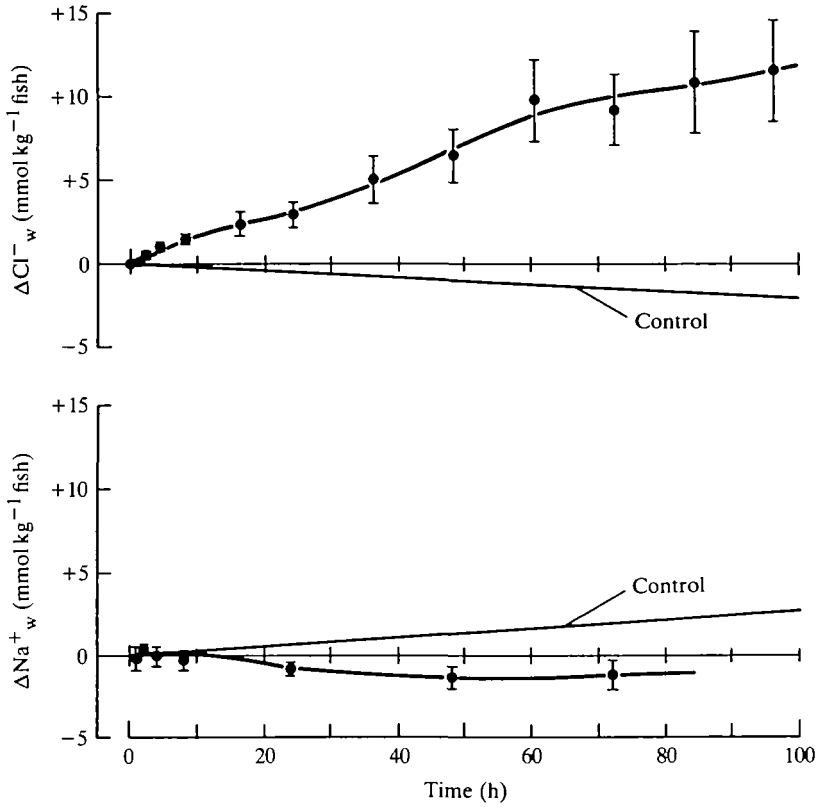

Fig. 4. Changes in water Cl^- and Na^+ concentrations during the 5 % hypercapnic period ($\bar{x} \pm$ S.E.). For ΔCl^-, $N = 8$ for control and experimental points up to hour 72; hours 84 and 96: $N = 4$. For ΔNa^+, $N = 7$ for all points.

loss of $0{\cdot}13 \pm 0{\cdot}03$ mmol h^{-1} kg^{-1} ($P < 0{\cdot}02$, $N = 8$). During this same period, net Na^+ exchange was reduced only slightly from a control efflux of $0{\cdot}03 \pm 0{\cdot}04$ to a net uptake of $-0{\cdot}01 \pm 0{\cdot}01$ mmol h^{-1} kg^{-1}.

Bicarbonate infusion

Exposure to an environmental P_{CO_2} of 7·5 mmHg in this series resulted in similar effects on the plasma acid–base status (Fig. 5), as reported before (Claiborne & Heisler, 1984*b*). After 48 h of hypercapnia, plasma pH was still significantly reduced from the controls ($7{\cdot}88 \pm 0{\cdot}04$) to $7{\cdot}64 \pm 0{\cdot}03$, although plasma [$HCO_3^-$] was increased from $12{\cdot}3 \pm 0{\cdot}7$ to $19{\cdot}6 \pm 0{\cdot}9$ mmol l^{-1} ($P < 0{\cdot}001$). Bicarbonate infusions after 48 and 96 h of hypercapnia induced a rapid increase in plasma pH initially exceeding normocapnic control values. Plasma pH was elevated maximally immediately after infusion (infusion no. 1: $7{\cdot}94 \pm 0{\cdot}06$, infusion no. 2: $7{\cdot}97 \pm 0{\cdot}09$), but returned rapidly within 2 h to sub-control levels. Plasma [HCO_3^-] was transiently elevated to 38·5 and 38·2 mmol l^{-1} immediately following bicarbonate administration (after 48 and 96 h, respectively). Within 24 h of each infusion, however,

plasma [HCO_3^-] had returned to near pre-infusion levels, stabilizing at approximately 23 mmol l^{-1}.

The pattern of transepithelial ion transfer was considerably affected by the infusions (Fig. 6). During the first 2 days of hypercapnia, a slight reduction in net HCO_3^- loss and an increase in net NH_4^+ excretion resulted in a net H^+ transfer from fish to water of $0{\cdot}22 \pm 0{\cdot}05$ mmol h^{-1} kg^{-1}. This was approximately equivalent to a threefold increase compared to the control rate of $0{\cdot}07 \pm 0{\cdot}05$ mmol h^{-1} kg^{-1}. The first infusion caused a rapid net HCO_3^- loss, which averaged $0{\cdot}20 \pm 0{\cdot}06$ mmol h^{-1} kg^{-1} over the following 24 h. The second infusion elicited an even larger

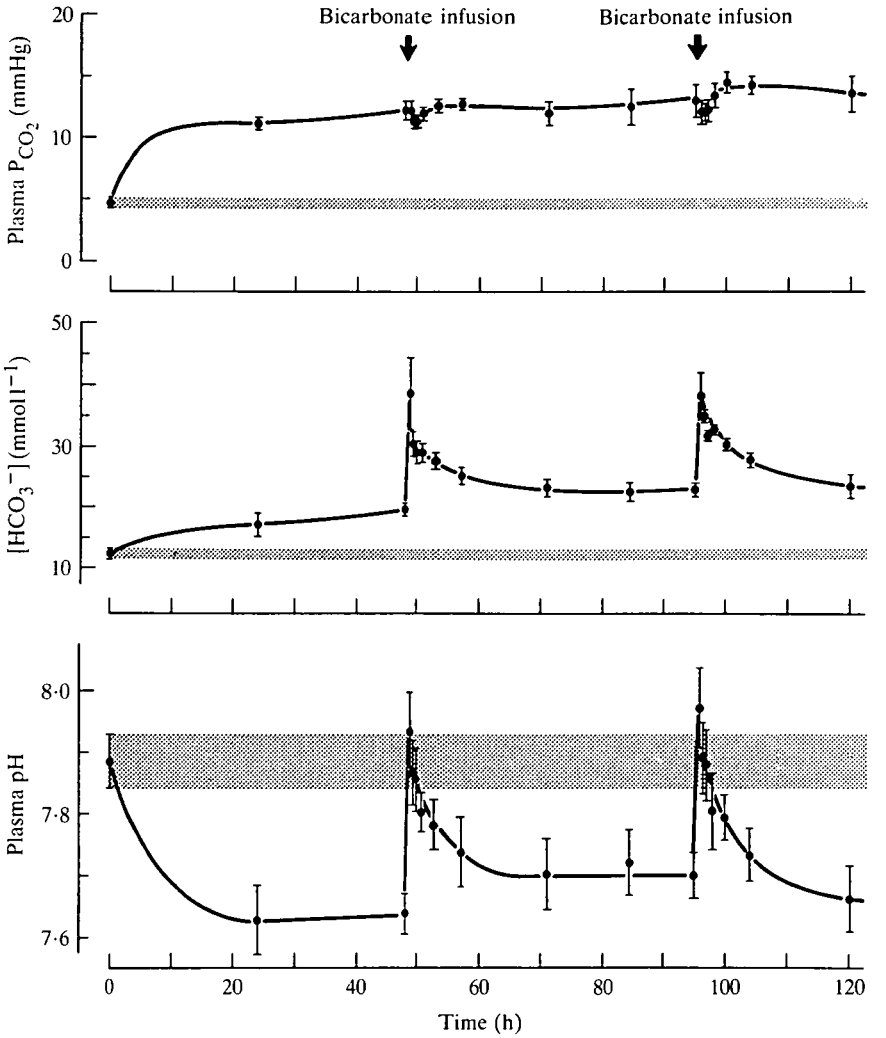

Fig. 5. Plasma P_{CO_2}, [HCO_3^-] and pH in dorsal aortic blood during exposure to 1% hypercapnia and subsequent $NaHCO_3$ infusions at 48 and 96 h ($\bar{x} \pm$ S.E., $N = 4$). Shaded areas represent $\bar{x} \pm$ S.E. of the pre-hypercapnic control values.

elimination rate of HCO_3^- (averaging $0{\cdot}31 \pm 0{\cdot}05$ mmol h^{-1} kg^{-1} – a 3·5-fold increase in relation to the control net HCO_3^- efflux). When compared to the negligible changes in $\Delta NH_4^+{}_w$ efflux, net HCO_3^- loss was responsible for a virtual shutdown in calculated net H^+ transfer ($-0{\cdot}05 \pm 0{\cdot}05$ mmol h^{-1} kg^{-1}, $P < 0{\cdot}002$) for a minimum of 24 h after infusion. Indeed, following the first infusion, 36 h were required for the $\Delta H^+{}_{c \to w}$ efflux to approach pre-infusion rates.

The changes in the transfer pattern of acid–base relevant ions were reflected in alterations of Na^+ and Cl^- movements during this experimental series (Fig. 7). As noted in the 5 % CO_2 experiment, 1 % CO_2 also caused a highly significant reversal in

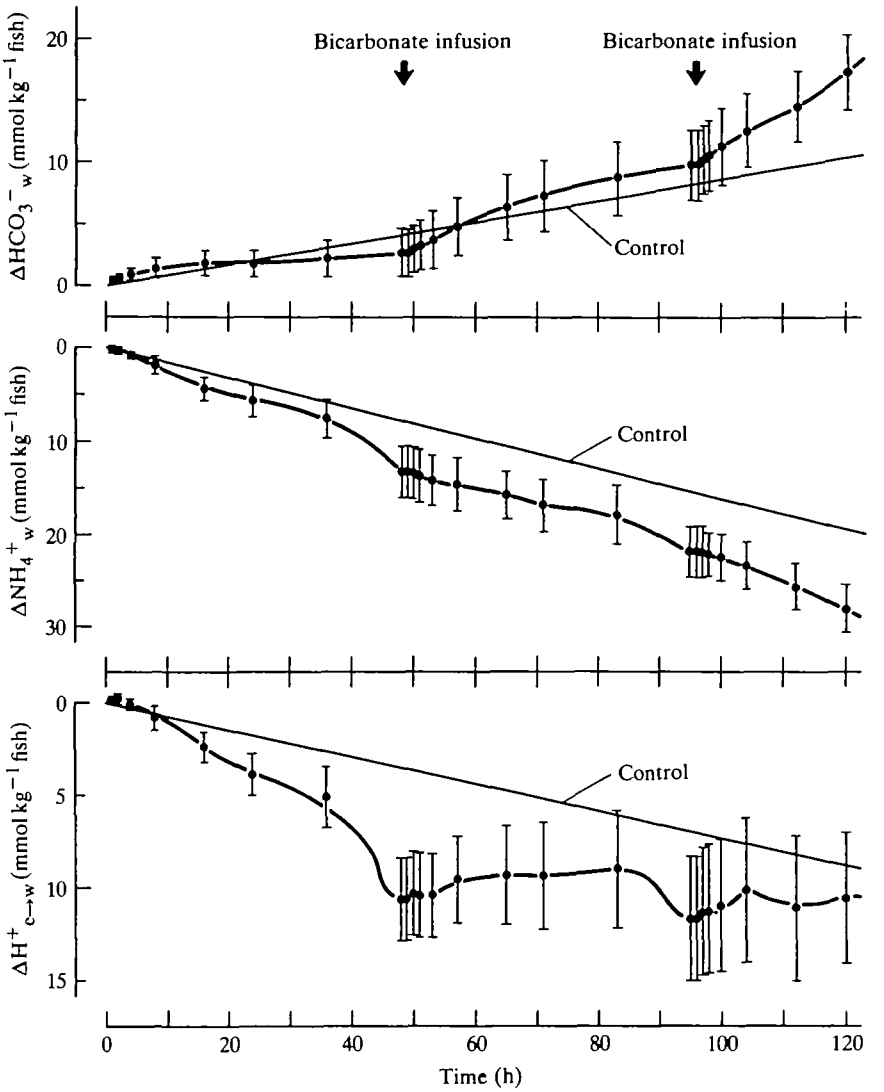

Fig. 6. Changes in water $[HCO_3^-]$, $[NH_4^+]$ and $\Delta H^+{}_{c \to w}$ during exposure to 1 % hypercapnia and subsequent $NaHCO_3$ infusions at 48 and 96 h ($\bar{x} \pm$ S.E., $N = 4$). For further explanations see legend to Fig. 3.

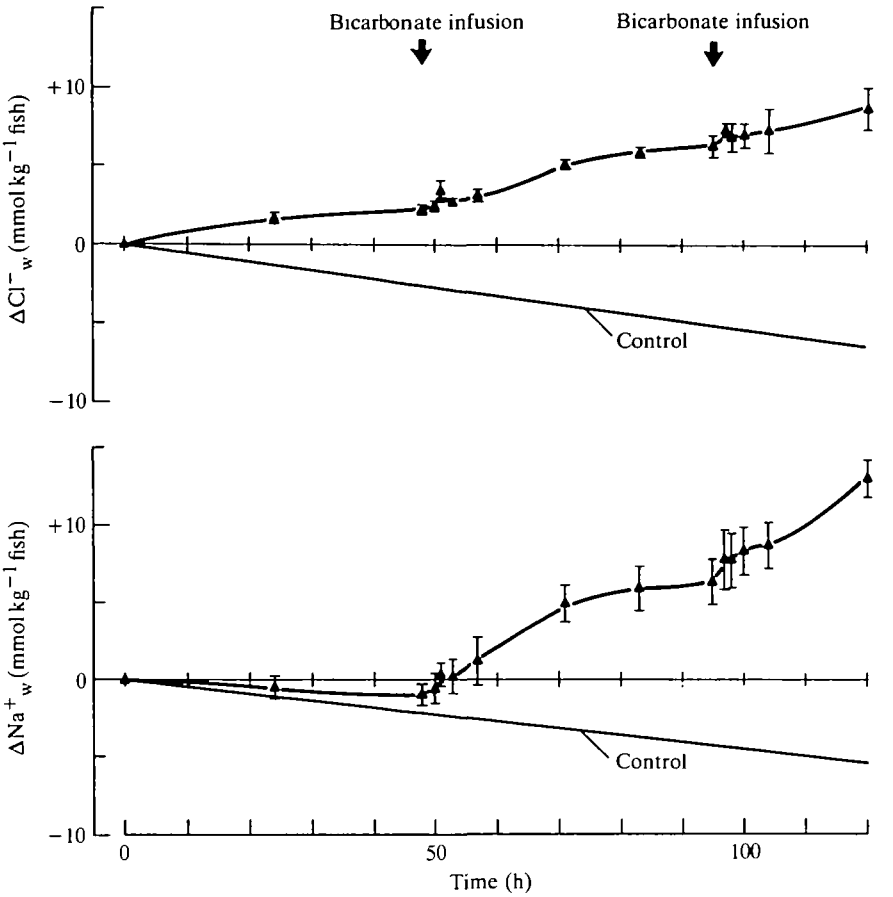

Fig. 7. Changes in water [Cl^-] and [Na^+] during exposure to 1% hypercapnia and subsequent $NaHCO_3$ infusions at 48 and 96 h ($\bar{x} \pm$ S.E., $N = 4$).

net Cl^- transfer from a net uptake ($0{\cdot}05 \pm 0{\cdot}06$ mmol h^{-1}kg^{-1}) to a net efflux ($0{\cdot}05 \pm 0{\cdot}01$ mmol h^{-1} kg^{-1}, $P < 0{\cdot}001$, $N = 4$). During the same period, ΔNa^+_w was not observed to change appreciably: the control uptake rate of $0{\cdot}05 \pm 0{\cdot}05$ mmol h^{-1} kg^{-1} was reduced slightly to $0{\cdot}02 \pm 0{\cdot}02$ mmol h^{-1} kg^{-1}. Following each $NaHCO_3$ infusion, ΔCl^-_w efflux increased by 140%, averaging $0{\cdot}13 \pm 0{\cdot}02$ mmol h^{-1} kg^{-1} ($P < 0{\cdot}05$) for the first 24 h after infusion. At the same time, net Na^+ transfer was reversed from the slight uptake rate noted above to a net efflux of 0·25 and 0·28 mmol h^{-1} kg^{-1} ($P < 0{\cdot}01$) during the 24 h period following each respective infusion.

DISCUSSION

Exposure of fish to elevated ambient P_{CO_2} regularly results in a reduction of plasma pH induced by an increase in plasma P_{CO_2} as a result of the close diffusive contact of these fluids *via* the branchial epithelium. Plasma pH is usually (with some exceptions) at least partially compensated and often recovers to control values (for

review see Heisler, 1984, 1986*b*). However, the time course and magnitude of the compensatory pH adjustment may vary considerably. Various studies on freshwater trout (*Salmo gairdneri*) have shown this species to require from about 20 to 72 h for limited to complete pH compensation during environmental hypercapnia (e.g. Cameron & Randall, 1972; Janssen & Randall, 1975; Eddy *et al.* 1977; Thomas & Le Ruz, 1982; Thomas, Fievet, Barthelemy & Peyraud, 1983) or hyperoxia-induced hypercapnia (Wood & Jackson, 1980; Hōbe *et al.* 1984). This variability reported for the trout may in part be due to the diverse magnitude of prevailing P_{CO_2} values. Another fraction of the variability, however, may be attributed to dissimilar ion concentrations in the different types of water used as the experimental environment (Heisler, 1982*b*, 1985). Indeed, while the freshwater catfish (*Ictalurus punctatus*) requires 24 h to offset the reductions in extra- and intracellular pH induced by hypercapnia (Cameron, 1980, 1985), the marine elasmobranch *Scyliorhinus stellaris* (Heisler *et al.* 1976) and the marine teleost *Conger conger* (Toews *et al.* 1983) achieve close to complete compensation within 8–10 h .

Long-term hypercapnia

When carp are exposed to a slow increase of ambient CO_2 over several days to about 7·5 mmHg P_{CO_2}, and then maintained at this level for another 14 days, it becomes evident that this species is also capable of almost complete recovery of plasma pH towards control values by elevation of plasma [HCO_3^-] (80 % of the hypercapnia-induced pH shift calculated for the hypothetical case that plasma [HCO_3^-] had remained constant at control levels). Comparison of the parameter 'control-[HCO_3^-]-standardized' pH (pH_{st}) with the experimental plasma pH (Fig. 8), indicates that the recovery process starts immediately after onset of hypercapnia, but that the main fraction of the recovery is performed after day 3 and continues for the duration of the experiment. These data conform very well with the earlier observation that carp exposed to the same level of hypercapnia for 48 h compensate only about one-half of the induced pH depression (Claiborne & Heisler, 1984*b*). Accordingly, carp seem to require even more time for net bicarbonate resorption during hypercapnia than other freshwater fishes. Further experiments are required to decide whether the low rate of net bicarbonate resorption is due to the lack of sufficient external HCO_3^- or counter ion concentrations (as mentioned above), or to a general rate-limitation of transbranchial acid–base relevant ion exchange mechanisms in this species.

High-level hypercapnia experiments

Upon exposure to approx. 35 mmHg P_{CO_2}, plasma pH recovered by about 0·15 units during the first 4 h. This fast initial recovery by bicarbonate accumulation (>3 mmol l^{-1}) is certainly not the result of the more effective ion transfer at low plasma pH. In contrast, it has to be attributed mainly to intracorporeal non-bicarbonate buffering, whereas less than one-third of this rise was due to net gain of bicarbonate from the ambient water (see Fig. 3). Buffering in the extracellular

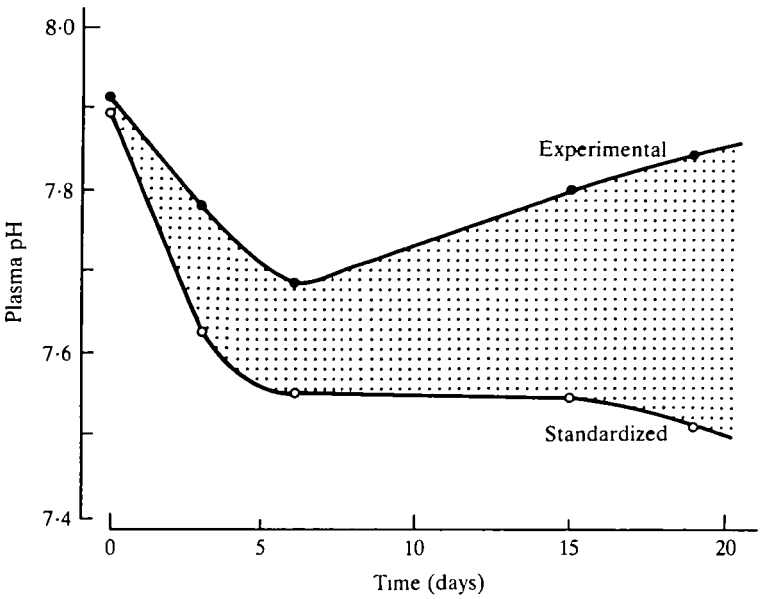

Fig. 8. Comparison of average measured plasma pH values *versus* those standardized on the basis of the control [HCO_3^-] (see Materials and Methods) during long-term exposure to mild hypercapnia ($\bar{x}$, $N = 4$). The shaded area represents the degree of extracellular pH compensation accomplished by the fish during the 19-day experiment.

compartment is poor, so that transmembrane transfer from the intracellular compartments, which occurs similarly during the initial phase of hypercapnia in *Scyliorhinus* (Heisler *et al.* 1976) and *Conger* (Toews *et al.* 1983), must be responsible for this phenomenon.

The bicarbonate-equivalent ion resorption from the water was much more effective during this series than during exposure to only 1 % CO_2. This is evident from the rise of 17 mmol l^{-1} in plasma [HCO_3^-] compared to 8 mmol l^{-1} at 1 % CO_2 (Claiborne & Heisler, 1984*b*), and from the absolute amount of about 15·6 mmol kg^{-1} of HCO_3^- gained during 96 h, which is about three times that gained during exposure to 1 % CO_2 for the same time (approx. 8 mmol l^{-1}, Claiborne & Heisler, 1984*b*). In spite of the improved efficiency of the relevant ion transfer mechanisms plasma [HCO_3^-] reached a plateau at 28 mmol l^{-1}, which accounted for only a 45 % restoration of plasma pH. Since the concentrations were not significantly different between 72 and 96 h, and no significant further transfer of acid–base relevant ion occurred (paired *t*-test), there is little likelihood that longer exposure would considerably improve compensation at this high level of hypercapnia.

The total amount of bicarbonate accumulated in the extracellular space (17 mmol $l^{-1} \times 0{\cdot}20 = 3{\cdot}4$ mmol kg^{-1}, assuming an ECS volume of 20 %; see Cameron, 1980; Heisler, 1982*b*) is much smaller than the amount of bicarbonate-equivalent ions resorbed from the ambient water (15·6 mmol kg^{-1}). Accordingly, a major proportion of the net HCO_3^- taken up was utilized to compensate the intracellular pH,

demonstrating again the high priority of intracellular pH regulation (e.g. Cameron, 1980; Heisler *et al.* 1976; Heisler, 1980, 1982*a*; Hōbe *et al.* 1984).

Mechanisms of acid–base adjustment in carp

Carp exposed to hypercapnia evidently rely heavily on transfer of acid–base relevant ions for the compensation of pH changes in their body fluids. The extent to which different transepithelial ion exchange mechanisms are exploited during this regulatory process, however, is not evident from the raw data. The regulatory response to 5 % CO_2 exposure is characterized by a large accumulation of NH_4^+ in the environmental water (>18·8 mmol kg^{-1} over 72 h), about seven times the amount released from carp at 1 % CO_2 (Claiborne & Heisler, 1984*b*). The bicarbonate release to the water was also larger than during control conditions, a phenomenon which may be considered as maladaptive. At first glance these data could be interpreted to indicate that the Na^+/NH_4^+ exchange mechanism is predominant.

In this context, however, it should be noted that the $\Delta NH_4^+{}_w$ actually describes total ammonia ($NH_4^+ + NH_3$) released from the fish (see Materials and Methods, and Heisler, 1984). Accordingly, the fraction of ammonia that is eliminated as NH_4^+ or NH_3 is not directly evident, and can be estimated only indirectly. Indirect evidence for a significant contribution of non-ionic transbranchial diffusion to the elimination of the main nitrogenous waste product of teleost fish, ammonia, has been supplied by determination of the fluxes of all relevant counter ions together with those of the acid–base relevant ions after exhausting activity in trout (Holeton, Neumann & Heisler, 1983). Measurements of the gill ammonia permeability and the effective NH_3 partial pressures on both sides of the gill epithelium in trout have suggested that ammonia is actually primarily eliminated by non-ionic diffusion as long as environmental ammonia partial presssures are low (Cameron & Heisler, 1983).

Conditions of low environmental ammonia partial pressures are met especially during environmental hypercapnia. The acidified ambient water (pH ≈ 6·5) is, according to the pK of the ammonia/ammonium buffer system, an almost infinite sink for NH_3 lost from the animal. Furthermore, preliminary data from our laboratory indicate that plasma ammonia concentration may rise from control values of 0·1–0·3 mmol l^{-1} up to 2·5 mmol l^{-1} during 48 h at 5 % hypercapnia (J. B. Claiborne, N. A. Andersen & N. Heisler, unpublished results). This rise is certainly due to increased ammonia production, as evident from the elevated overall release. The cause for the elevated protein catabolism is unclear, but may be related to unnaturally high CO_2 levels and metabolic imbalance.

It is thus not surprising that the net flux analysis of the potential counter ions for NH_4^+ (Na^+) and HCO_3^- (Cl^-) reveals that 18·8 mmol kg^{-1} net ammonium released (Fig. 3) are in imbalance with only approx. 3·2 mmol kg^{-1} of net Na^+ taken up from the water (Fig. 4). On the basis of a 1:1 electroneutral ion exchange, and the non-availability of a significant amount of monovalent ions other than Na^+ in the ambient water, less than 17 % of the ammonia can have been transferred by ionic exchange. This 17 % also includes any H^+ exchanged for Na^+, which are considered to

compete for the same carrier sites. The remainder of the ammonium ions must therefore have been eliminated by non-ionic diffusion (83 %).

Non-ionic diffusion of NH_3 to the ambient water, however, results in equivalent production of bicarbonate due to immediate ionization of ammonia to ammonium. As a result of this mechanism, an amount equivalent to 83 % of the ammonia, 15·6 mmol kg^{-1} HCO_3^- must have been produced during 72 h of 5 % hypercapnia. The difference between the amount of bicarbonate produced by ammonia ionization and the actual increase in water bicarbonate (15·6−3·7 = 11·9 mmol kg^{-1}) must then be attributed to an electroneutral 1:1 HCO_3^-/Cl^- exchange, a proposition well supported by the net Cl^- release of about 10·9 mmol kg^{-1}. These data therefore suggest that more than three-quarters of the bicarbonate-equivalent ions taken up from the environment for pH compensation during hypercapnia have been transported by the HCO_3^-/Cl^- exchange mechanism.

The above considerations can briefly be summarized by the rule that the difference in the amounts of transferred monovalent cations and anions is balanced by the amount of transferred acid–base relevant ions (Heisler, 1980, 1982*b*; Holeton & Heisler, 1983; Holeton *et al.* 1983; for details see Heisler, 1984, 1986*a*), as long as electroneutral ion exchange mechanisms are exploited and unaccounted ion species are not involved. This theoretical condition is rather well reflected by the present experimental data, where 91 % of the transfer in acid–base relevant ions is balanced by analysed counter ions.

Bicarbonate infusion

Bicarbonate infusions were performed in order to test the possibility that the somewhat incomplete compensation of the hypercapnia-induced plasma pH shift (1 % CO_2, short-term exposure, Claiborne & Heisler, 1984*b*) was primarily due to a rate limitation of transbranchial ion exchange processes rather than to an upper limit of bicarbonate concentration for the bicarbonate-retaining and resorbing structures.

Although $NaHCO_3$ infusions transiently restored and even overshot control plasma pH values, in spite of continuing hypercapnia because of the considerable increase in plasma bicarbonate, the additional bicarbonate administered to the fish was not retained, but was mostly released to the environment within 24 h. Plasma bicarbonate, which was approximately 19 mmol l^{-1} prior to the first infusion, established a new equilibrium at about 23 mmol l^{-1} (equivalent to a pH compensation of approx. 54 %). This value was reattained 24 h after the second infusion without any further elevation of plasma bicarbonate.

The elimination of the infused bicarbonate is documented in the pattern of transepithelial ion fluxes. Immediately after infusion, the bicarbonate equivalent release rose significantly ($P < 0·05$), equivalent to a net ΔHCO_3^- efflux of 6·0 mmol kg^{-1} during the 24 h following infusion no. 1 (calculated from the extrapolated pre- and the postinfusion transfer rates), and 5·4 mmol kg^{-1} after infusion no. 2 (calculated from the extrapolated transfer rates between hours 83 and 96, and the rates after infusion). These changes in net HCO_3^- transfer were mainly responsible for the reversal of the cumulative H^+-equivalent transfer observed after each infusion

(Fig. 6), which was about 6·5 mmol kg^{-1}, a value not significantly different ($P < 0{\cdot}5$) from the amount of infused $NaHCO_3$. The Na^+ infused together with the bicarbonate was also eliminated to the ambient water. There was a net release of 6·4 and 5·8 mmol kg^{-1} of Na^+ during the 24 h after each respective infusion; this was, within statistical limits, identical to the infused load. The Cl^- flux was little affected by the infusions and associated elimination processes: after the standard reversal of Cl^- uptake to Cl^- efflux during hypercapnia, the efflux remained essentially unaltered during both infusion periods, indicating that the activity of the HCO_3^-/Cl^- exchange mechanism was not affected by the transient increases in plasma $[HCO_3^-]$ and subsequent elimination of bicarbonate.

Maximal plasma bicarbonate concentration

Relevant studies in carp and other fish species indicate that the plasma bicarbonate concentration in fish does not rise above certain values, which seem to range from 20–28 mmol l^{-1} (Table 1). There is only a slight correlation between the bicarbonate concentration achieved during hypercapnia, and the time of exposure. However, long-term acclimation studies are scarce, and it may well be that the acclimation time is a limiting factor for a final, slow further increase in plasma bicarbonate. This factor has not yet been clarified, since the results of the present long-term hypercapnia acclimation, as well as data gathered during hyperoxia-induced hypercapnia in *Scyliorhinus* (Heisler, Holeton & Toews, 1981; see also Heisler, 1984, 1986*b*), suggest that final plasma bicarbonate adjustment can be a rather delayed process.

For any given state of acclimation to hypercapnia, however, the bicarbonate concentration cannot freely be elevated beyond a certain threshold level, as demonstrated by the present study. This seems to be corroborated by the maximal reported bicarbonate concentration values found in other fish species (Table 1). It has recently been postulated (Heisler *et al.* 1982; Heisler, 1984, 1986*c*) that lower vertebrates generally may have some maximum extracellular $[HCO_3^-]$ which can be attained. Any rise in $[HCO_3^-]$ exceeding this level (22–28 mmol l^{-1}) will result in loss of HCO_3^- to the environment. As shown by comparing the bicarbonate infusion experiments and the high level hypercapnia experiments, this bicarbonate threshold may be readjusted at different levels of P_{CO_2}.

According to these findings, the extent of pH compensation during hypercapnia is very much a function of the individual control (normocapnia) bicarbonate concentration. Fishes with low initial bicarbonate (e.g. *Scyliorhinus*) are clearly capable of increasing their $[HCO_3^-]$ to a greater extent than animals with high control bicarbonate levels (e.g. *Synbranchus*) before the level of maximal bicarbonate is attained (see Table 1). The capability of compensation, however, is not linearly correlated with the control bicarbonate concentration. This is due to the fact that pH is exponentially related to P_{CO_2} and $[HCO_3^-]$. Thus, the increase in plasma P_{CO_2} by a certain factor requires a rise in $[HCO_3^-]$ by the same factor in order to achieve complete compensation (for details see Heisler, 1986*c*). Therefore, the extent of compensation is directly dependent on the ratio of the control and maximal bicarbonate concentrations in relation to the relative increase in P_{CO_2}.

Table 1. *Extracellular pH compensation (%) in fishes during hypercapnia, plasma bicarbonate concentrations (mmol l^{-1}) during control conditions ($[HCO_3^-]_c$) and after exposure to hypercapnia for variable time periods ($[HCO_3^-]_{hyp}$)*

Species	Cause of hypercapnia	$[HCO_3^-]_c$	$[HCO_3^-]_{hyp}$	pH Compensation (%)	Exposure time (h)	Reference
Scyliorhinus stellaris	Ambient	7·6	20	85	8	Heisler, Weitz & Weitz (1976)
		6	19	85	4	Randall, Heisler & Drees (1976)
Scyliorhinus stellaris	Hyperoxia-induced	5·3	20	95	25	Heisler *et al.* (1981); Heisler (1984)
		5·3	24	90	144	
Squalus acanthias	Ambient	8	21	64	5	Cross *et al.* (1969)
Conger conger	Ambient	5·0	22	90	10	Toews, Holeton & Heisler (1983)
			22	90	30	
Ictalurus punctatus	Ambient	7	21	90	30	Cameron (1980)
Cyprinus carpio	Ambient	13·8	22	40	48	Claiborne & Heisler (1984*b*)
		11·4	28	45	96	Claiborne & Heisler (this study)
		13·0	26	80	456	Claiborne & Heisler (this study)
		12·0	23	55	120	Claiborne & Heisler (this study)
Cyprinus carpio	Hyperoxia-induced	12·5	21	40	72	N. A. Andersen, J. B. Claiborne & N. Heisler (unpublished)
Salmo gairdneri	Ambient	9	27	95	75	Janssen & Randall (1975)
		3·8	23	90	>168	Eddy, Lomholt, Weber & Johansen (1977)
	Hyperoxia-induced	7·0	25·8		72	Hōbe, Wood & Wheatly (1984)
Synbranchus marmoratus	Transition water to air breathing	24	24	0	18	Heisler (1982)
		24	24	0	100	

We conclude that carp (*Cyprinus carpio*) regulate their plasma acid–base status during exposure to high ambient CO_2 in a fashion at least qualitatively similar to that used by other fish. Complete plasma pH compensation, however, can only be achieved by this species when exposed to mild hypercapnia (<1 % CO_2) for several weeks. Hypercapnic stresses of greater magnitude aggravate the extracellular acid–base disturbances, when elevation of the plasma [HCO_3^-] is limited well below the level required for complete pH compensation. Infusion of HCO_3^- can restore plasma pH to control values only transiently and does not permanently affect the amount of accumulated extracellular HCO_3^-. This provides evidence for some maximal [HCO_3^-] 'set-point' in this species. The intra- and extracellular [HCO_3^-] adjustments in the carp are mainly due to activity of the HCO_3^-/Cl^- exchange mechanism (HCO_3^- uptake linked to a Cl^- efflux) between the fish and the environment, which clearly predominates over NH_4^+/Na^+ and/or H^+/Na^+ transfer processes of lesser magnitude.

Financial support for this study was provided by a faculty research grant to JBC from Georgia Southern College, and by a grant to NH by the Deutsche Forschungsgemeinschaft. The authors wish to acknowledge gratefully the skilful technical assistance of Mr G. Forcht and Mrs S. Glage. Thanks also to Mr R. Culp for critical discussions of this work.

REFERENCES

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

CAMERON, J. N. (1980). Body fluid pools, kidney function, and acid–base regulation in fresh water catfish *Ictalurus punctatus*. *J. exp. Biol.* **86**, 171–185.

CAMERON, J. N. (1985). The bone compartment in a teleost fish, *Ictalurus punctatus*: size, composition and acid–base response to hypercapnia. *J. exp. Biol.* **117**, 307–318.

CAMERON, J. N. & HEISLER, N. (1983). Studies of ammonia in the rainbow trout: physico-chemical parameters, acid–base behaviour and respiratory clearance. *J. exp. Biol.* **105**, 107–125.

CAMERON, J. N. & RANDALL, D. J. (1972). The effect of increased ambient CO_2 on arterial CO_2 tension, CO_2 content and pH in rainbow trout. *J. exp. Biol.* **57**, 673–680.

CLAIBORNE, J. B. & HEISLER, N. (1984*a*). A minicomputer program for the rapid graphical and statistical analysis of laboratory data. *Int. J. bio-med. Comput.* **15**, 51–60.

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

CROSS, C. E., PACKER, B. S., LINTA, J. M., MURDAUGH, H. V., JR & ROBIN, E. D. (1969). H^+ buffering and excretion in response to acute hypercapnia in the dogfish *Squalus acanthias*. *Am. J. Physiol.* **216**, 440–452.

DERENZIS, L. G. & MAETZ, J. (1973). Studies on the mechanism of the chloride absorption by the goldfish gill: Relation with acid–base regulation. *J. exp. Biol.* **59**, 339–358.

EDDY, F. B., LOMHOLT, J. P., WEBER, R. E. & JOHANSEN, K. (1977). Blood respiratory properties of rainbow trout (*Salmo gairdneri*) kept in water of high CO_2 tension. *J. exp. Biol.* **67**, 37–47.

EVANS, D. H. (1980*a*). Osmotic and ionic regulation by freshwater and marine fishes. In *Environmental Physiology of Fishes* (ed. M. A. Ali), pp. 93–122. New York: Plenum Press.

EVANS, D. H. (1980*b*). Kinetic studies of ion transport by fish gill epithelium. *Am. J. Physiol.* **283**, R224–R230.

EVANS, D. H. (1984). The role of gill permeability and transport mechanisms in euryhalinity. In *Fish Physiology*, vol. XB (ed. W. S. Hoar & D. J. Randall), pp. 315–401. Orlando, New York: Academic Press.

EVANS, D. H. (1986). The role of branchial and dermal epithelia in acid–base regulation in aquatic animals. In *Acid-Base Regulation in Animals* (ed. N. Heisler), pp. 139–172. Amsterdam: Elsevier Biomedical Press.

HEISLER, N. (1978). Bicarbonate exchange between body compartments after changes of temperature in the larger spotted dogfish (*Scyliorhinus stellaris*). *Respir. Physiol.* **33**, 145–160.

HEISLER, N. (1980). Regulation of the acid–base status in fish. In *Environmental Physiology of Fishes* (ed. M. A. Ali), pp. 123–162. New York: Plenum Press.

HEISLER, N. (1982*a*). Intracellular and extracellular acid–base regulation in the tropical fresh water teleost fish *Synbranchus marmoratus* in response to the transition from water breathing to air breathing. *J. exp. Biol.* **99**, 9–28.

HEISLER, N. (1982*b*). Transepithelial ion transfer processes as mechanisms for fish acid–base regulation in hypercapnia and lactacidosis. *Can. J. Physiol.* **60**, 1108–1122.

HEISLER, N. (1984). Acid–base regulation in fishes. In *Fish Physiology* vol. XA (ed. W. S. Hoar & D. J. Randall), pp. 315–401. Orlando, New York: Academic Press.

HEISLER, N. (1985). Branchial ion transfer processes as mechanisms for fish acid–base regulation. In *Transport Processes, Iono- and Osmoregulation* (ed. R. Gilles & M. Gilles-Baillien), pp. 177–193. Berlin Heidelberg: Springer-Verlag.

HEISLER, N. (1986*a*). Buffering and transmembrane transfer mechanisms. In *Acid-Base Regulation in Animals* (ed. N. Heisler), pp. 3–47. Amsterdam: Elsevier.

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

HEISLER, N. (1986*c*). Comparative aspects of acid–base regulation. In *Acid-Base Regulation in Animals* (ed. N. Heisler), pp. 397–450. Amsterdam: Elsevier.

HEISLER, N. (1986*d*). Mechanisms and limitations of fish acid–base regulation. In *Fish Physiology: Recent Advances* (ed. S. Nielson & S. Holmgren). London: Croom-Helm Publishers Ltd (in press).

HEISLER, N., HOLETON, G. F. & TOEWS, D. P. (1981). Regulation of gill ventilation and acid–base status in hyperoxia-induced hypercapnia in the larger spotted dogfish (*Scyliorhinus stellaris*). *Physiologist* **24**, 305.

HEISLER, N. & NEUMANN, P. (1977). Influence of sea water pH upon bicarbonate uptake induced by hypercapnia in an elasmobranch (*Scyliorhinus stellaris*). *Pflügers Arch. ges. Physiol.* **368**, Suppl. R19.

HEISLER, N., WEITZ, H. & WEITZ, A. M. (1976). Hypercapnia and resultant bicarbonate transfer processes in an elasmobranch fish (*Scyliorhinus stellaris*). *Bull. europ. Physiopath. Respir.* **12**, 77–85.

HÖBE, H., WOOD, C. M. & 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. & HEISLER, N. (1983). Contribution of net ion transfer mechanisms to the acid–base regulation after exhausting activity in the larger spotted dogfish (*Scyliorhinus stellaris*). *J. exp. Biol.* **103**, 31–46.

HOLETON, G. F., NEUMANN, P. & HEISLER, N. (1983). Branchial ion exchange and acid–base regulation after strenuous exercise in rainbow trout (*Salmo gairdneri*). *Respir. Physiol.* **51**, 308–318.

JANSSEN, R. G. & RANDALL, D. L. (1975). The effect of changes in pH and P_{CO_2} in blood and water on breathing in rainbow trout, *Salmo gairdneri*. *Respir. Physiol.* **25**, 235–245.

MAETZ, J. & GARCIA-ROMEU, F. (1964). The mechanism of sodium and chloride uptake by the gills of a freshwater fish *Carassius auratus*. Evidence for NH_4^+/Na^+ and HCO_3^-/Cl^- exchange. *J. gen. Physiol.* **47**, 1209–1226.

RANDALL, D. J., HEISLER, N. & DREES, F. (1976). Ventilatory response to hypercapnia in the larger spotted dogfish *Scyliorhinus stellaris*. *Am. J. Physiol.* **230**, 590–594.

SOIVIO, A., WESTMAN, K. & NYHOLM, K. (1975). Improved method of dorsal aorta catheterization: haematological effects followed for three weeks in rainbow trout (*Salmo gairdneri*). *Finnish Fish. Res.* **1**, 11–12.

THOMAS, S., FIEVET, B., BARTHELEMY, L. & PEYRAUD, C. (1983). Comparison of the effects of exogenous and endogenous hypercapnia on ventilation and oxygen uptake in the rainbow trout (*Salmo gairdneri*). *J. comp. Physiol.* **151**, 185–190.

THOMAS, S. & LE RUZ, H. (1982). A continuous study of rapid changes in blood acid–base status of trout during variations of water P_{CO_2}. *J. comp. Physiol.* **148**, 123–130.

TOEWS, D. P., HOLETON, G. F. & HEISLER, N. (1983). Regulation of the acid–base status during environmental hypercapnia in the marine teleost fish *Conger conger*. *J. exp. Biol.* **107**, 9–20.

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

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