J. exp. Biol. 108, 25–43 (1984)
Printed in Great Britain © The Company of Biologists Limited 1984



# ACID-BASE REGULATION AND ION TRANSFERS IN THE CARP (*CYPRINUS CARPIO*) DURING AND AFTER EXPOSURE TO ENVIRONMENTAL HYPERCAPNIA

BY J. B. CLAIBORNE* AND NORBERT HEISLER

*Abteilung Physiologie, Max-Planck-Institut für experimentelle Medizin, D-3400 Göttingen, F.R.G.*

*Accepted 7 June 1983*

SUMMARY

Acid-base balance and ion transfers were studied in the carp, *Cyprinus carpio* L., during and after 48 h of exposure to environmental hypercapnia ($P_{CO_2} \sim 7{\cdot}5$ Torr). Plasma pH, $P_{CO_2}$, $[HCO_3^-]$, and net transfers of $HCO_3^-$, $NH_4^+$, $Cl^-$ and $Na^+$ between the fish and the environmental water were measured periodically throughout the experiment.

Over the first 8 h of hypercapnia, plasma $P_{CO_2}$ increased by 7·6 Torr with a concurrent decrease in plasma pH of 0·28 units. Plasma $[HCO_3^-]$ was slowly elevated from about 14 to 22 mM after 48 h, at which point 50 % of the pH depression expected at constant bicarbonate concentration had been compensated. The net amount of $H^+$ transferred to the water was 3·3 mmol $kg^{-1}$ fish, representing a 115 % increase in the rate of cumulative $H^+$ efflux, and inducing an elevation of both intracellular and extracellular $[HCO_3^-]$. $Cl^-$ transfer was reversed from a net uptake to a net efflux, while net $Na^+$ influx was increased slightly.

Following hypercapnia, plasma pH returned to control values within 1 h, while the plasma $[HCO_3^-]$, which was elevated during hypercapnia, fell continuously to reattain pre-hypercapnic control values after 20 h. The $[HCO_3^-]$ decrease was due to the net gain of $H^+$ ions from the water during this period. $Cl^-$ transfer returned to a net uptake, while the original $Na^+$ influx was reversed to a net loss.

Acid-base regulatory responses in the carp are qualitatively similar to those observed in other fish, though the time required for compensatory pH adjustment is longer. It is concluded that alterations in the rates of $Cl^-/HCO_3^-$ and $Na^+/H^+$ exchanges during hypercapnia and $Na^+/H^+$ exchange following hypercapnia, play a significant role in the compensation of respiratory acid-base disturbances in these animals.

## INTRODUCTION

The nature of blood acid-base regulation in fish during hypercapnia has been studied in several marine and freshwater species (e.g. Lloyd & White, 1967; Heisler, Weitz & Weitz, 1976; Eddy, Lomholt, Weber & Johansen, 1977). The general pattern

* Present address: Department of Biology, Georgia Southern College, Statesboro, Georgia 30460 U.S.A.

Key words: Acid-base regulation, *Cyprinus carpio*, ammonia elimination, hypercapnia, ion transfer, pH compensation.

of response to an elevation in plasma $P_{CO_2}$ (and the concomitant depression of plasma pH) observed in these animals is a compensatory increase of the plasma $HCO_3^-$ concentration. The augmentation of $[HCO_3^-]$ induces an increase in arterial pH to values near or identical to control levels (see review by Heisler, 1982*a*). The time course of the $HCO_3^-$ build-up may be of the order of several hours to several days, depending on the species examined (e.g. Janssen & Randall, 1975; Heisler *et al.* 1976).

In both teleosts and elasmobranchs, extracellular acid-base balance may involve a combination of $HCO_3^-$, $H^+$ and $NH_4^+$ transfers between the animal and the environment, predominantly occurring at the gills (Heisler *et al.* 1976; Cameron, 1980; Evans, 1982; Holeton, Neumann & Heisler, 1983; Heisler, 1982*b*). $HCO_3^-$ (or $H^+$) movements between extracellular and intracellular compartments also play a role in the initial stages of pH compensation following the onset of hypercapnia (Heisler *et al.* 1976; Cameron, 1980).

The underlying mechanisms behind the branchial ion transfers of $HCO_3^-$, $H^+$ and $NH_4^+$ have not been studied in such detail. It has been proposed that the transbranchial ion exchange processes – i.e. $Na^+/H^+$, $Na^+/NH_4^+$, and $Cl^-/HCO_3^-$ exchange – that are thought to occur in teleosts and elasmobranchs (Maetz & Garcia-Romeu, 1964; Maetz, 1973; Evans, 1977; Claiborne, Evans & Goldstein, 1982) are involved with acid-base regulation as well as ion regulation in these fish (DeRenzis & Maetz, 1973; Cameron, 1976; Evans, 1982; Heisler, 1982*a*). Utilizing the freshwater arctic grayling, *Thymallus arcticus*, Cameron (1976) showed that the uptake of $Na^+$ during hypercapnia was enhanced at the same time that $Cl^-$ influx was inhibited. This result might be expected if the efflux of $H^+$ (and/or $NH_4^+$) and $HCO_3^-$ were linked to the uptake of $Na^+$ and $Cl^-$, respectively. In contrast, Perry, Haswell, Randall & Farrell (1981) recently found that hypercapnia had no effect on the rates of branchial NaCl influx in the trout, *Salmo gairdneri*. They proposed that transbranchial $Na^+/H^+$ ($NH_4^+$) and $Cl^-/HCO_3^-$ exchanges were not involved with blood acid-base regulation during or following hypercapnia.

The purpose of the present experiment was two-fold: (1) to examine the extracellular acid-base response of the freshwater carp during and after environmental hypercapnia, and (2) to determine the net rates of $HCO_3^-$, $H^+$, $NH_4^+$, and NaCl movements between the animal and the surrounding media, and the contribution of these transfer processes towards the regulation of the overall acid-base status of the fish.

## MATERIALS AND METHODS

### *Maintenance and preparation of the animals*

Carp (*Cyprinus carpio*; weight $1{\cdot}783 \pm 0{\cdot}092$ kg, $\bar{x} \pm$ S.E., $N = 8$) were obtained from a local fish hatchery and maintained for at least 1 month in large aquaria (100 l fish$^{-1}$) at $15 \pm 0{\cdot}5$ °C supplied with dechlorinated and well aerated local tap water at a rate of >100 l fish$^{-1}$ day$^{-1}$. The fish were subjected to a standardized 12 h light/12 h dark cycle and regularly fed with commercial carp chow.

At least 30 h, and in some cases up to 5 days prior to the beginning of an experiment, fish were anaesthetized in MS 222 (1:10 000), placed on an operating rack, and the gills were immediately irrigated with aerated water from a reservoir to which MS 222

1 : 20 000) had been added. Implantation of a dorsal aortic cannula was accomplished by the methods described by Ultsch, Ott & Heisler (1981) as modified from those of Soivio, Westman & Nyholm (1972) and Soivio, Nyholm & Westman (1975). Briefly, a short length of PE 60 tubing with several notches cut near one end to allow enhanced blood flow once implanted, was threaded onto a piece of straight wire until the sharpened end of the wire just extended past the notched end of the tubing. This assembly was then inserted through the roof of the mouth of the anaesthetized fish into the dorsal aorta, the wire removed, and the cannula advanced into the artery by about 7 cm. A hollow grommet was implanted in the thin tissue on the side of the mouth, and the free end of the cannula was fed through the grommet to the outside. The tubing was held in place by one shallow suture in the roof of the mouth and two sutures tied around the cannula on either side of the grommet and hardened with a drop of cyanoacrylate glue. Using a small adapter, an additional length of PE tubing filled with Ringer solution (Cortland Ringer solution, glucose and $HCO_3^-$ free; 100 i.u. $ml^{-1}$ heparin) was connected to the original cannula. The entire length of tubing was flushed briefly with Ringer solution and the open end was then plugged. The fish was placed in the experimental chamber filled with fresh water, and after regular opercular movements had resumed (usually within 20 s) the top of the chamber was replaced and the flow of fresh water maintained. This method of implantation allowed unrestrained movement of the animals, and held the cannula in position for the duration of the experimental procedures (and well over 3 weeks in several fish returned to a holding tank).

### *Experimental set-up*

A closed water recirculation system (Heisler *et al.* 1976; Heisler, 1978) with a total volume of 19 l was utilized in these experiments (Fig. 1). The water in the system was pumped through a large aeration column (6 l) through a Plexiglas box (12 l) containing the fish, and then returned to the column. Water was circulated at approximately 5 l $min^{-1}$ and thermostatted to $15 \pm 0{\cdot}5$ °C. Input gas for the aeration column was controlled through two gas flow meters with differential pressure regulators, permitting precise mixing of air and $CO_2$ bubbled through the water. $[NH_4^+]$ of the water was monitored throughout the experiments with an ammonia electrode (HNU Systems Inc., Newton, Massachusetts) which was mounted in a Plexiglas, thermostatted chamber ($30 \pm 0{\cdot}05$ °C), with a volume of 2 ml. The fluid within the chamber was constantly stirred with a magnetic bar and sealed from the atmosphere with an 'O'-ring. Ports in the chamber near the electrode tip allowed the rapid change of solutions in contact with the membrane. Water from the recirculation system was transported to the ammonia electrode with a roller pump and mixed with a small volume of 10 mM-NaOH also pumped into the chamber in order to alkalinize (pH > 12) the sample and convert all ammonium ions to physically dissolved ammonia. The electrode was connected to a microprocessor/ionalyser (Orion Research; model 901) and corresponding digital printer (model 951). After an initial slope calibration of the electrode assembly using 0·1 and 1·0 mM-$NH_4Cl$ standards, the base-line calibration of the electrode was readjusted (with 0·1 mM-$NH_4Cl$) prior to every water ammonia measurement. The sequential calibration and sampling process was accomplished automatically by a programmable microprocessor/clock which controlled the roller

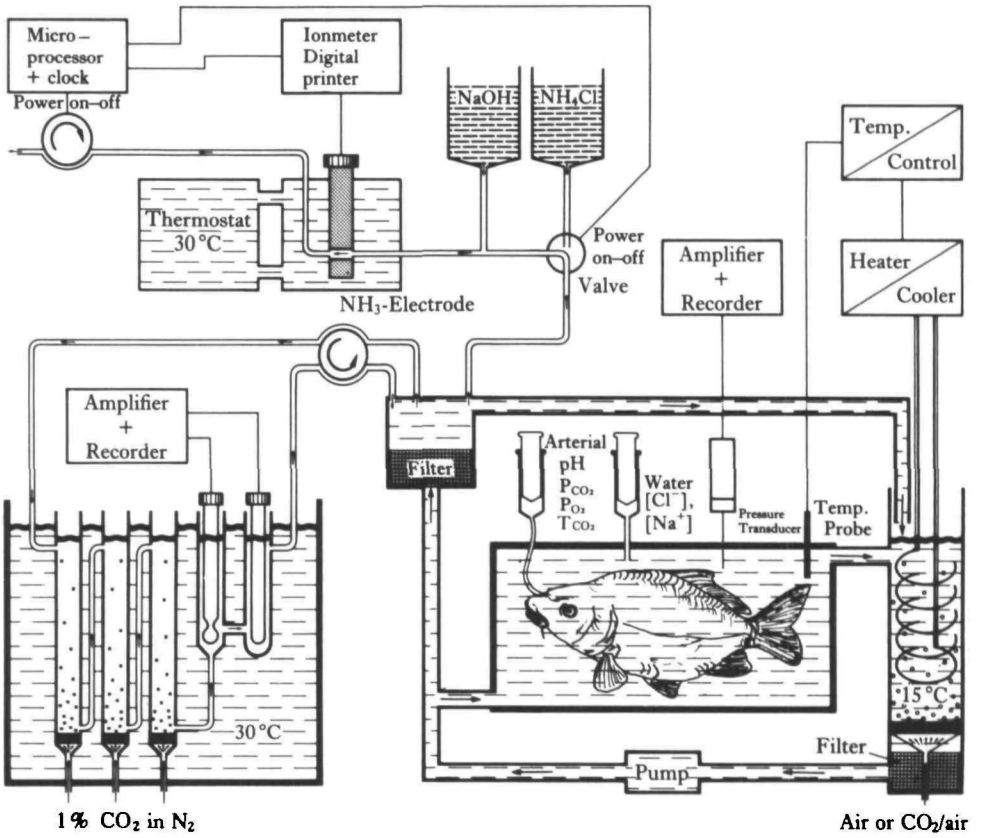


Fig. 1. Experimental apparatus. The closed recirculation system consisted of an animal box, gas equilibration column and recirculation pump. Water $HCO_3^-$ and $NH_4^+$ concentrations were measured using a'Δ-bicarbonate measurement system' (lower left) and an ammonia sensitive electrode assembly (upper left), respectively. Water samples were periodically saved for ion analysis. Blood samples were collected through a dorsal aortic cannula. See text for details. PT, pressure transducer.

pump, ionalyser, printer and two small-volume, 12 V d.c. valves connecting delivery lines from the experimental water and the $NH_4Cl$ standard. This system allowed automatic analysis of water samples for up to 7 days of preprogrammed collection times with a resolution of 0·01 mM-$NH_4^+$.

Another roller pump constantly drew water from the fish chamber to a 'Δ-bicarbonate' monitoring system (Heisler, 1978). This consisted of three glass columns connected in series and submerged in a thermostatted bath (30 ± 0·05 °C). Each column was vigorously bubbled with 1 % $CO_2$ delivered from a gas mixing pump selected for particularly long-term stability (Wöstoff). Water was pumped from the fish chamber through this series of columns, and before being returned to the fish system, through two smaller tubes containing a spherical pH glass electrode and a double electrolyte bridge (Ag/AgCl) sleeve diaphragm reference electrode. Both electrodes were adapted to the ion strength of the system water and selected for very low drift (<0·001 pH units/24 h). The electrodes were connected *via* a high impedance isolation amplifier (Knick, Berlin, F.R.G.) to a chart recorder. Utilizing this system, water from the fish chamber was standardized for temperature and $P_{CO_2}$, and

all changes in pH were then due to changes in bicarbonate concentration of the water. Bicarbonate concentrations were calculated using the Henderson-Hasselbalch equation (Heisler, 1978). The electrodes were initially calibrated with two phosphate buffers (pH ~ 6·8, pH ~ 7·4) and prior to (and occasionally after) each experiment, the baseline calibration was rechecked by measuring in triplicate the total $CO_2$ content of the water ($T_{CO_2}$) conductometrically (Capni-Con II; Cameron Instruments Inc., Port Aransas, Texas). In practice, electrode drift was very low throughout the experiments, and when the slope calibration was rechecked with phosphate buffers, a variation of less than 0·010 pH units was usual even after several months.

In most experiments, a water-filled tube (PE 60) was inserted through the top of the fish chamber and submerged below the water surface. The other end of the tube lead to a pressure transducer (Statham Instruments Inc., P23BB) connected to an amplifier and chart recorder. This allowed fish swimming activity to be continuously monitored, and made possible the important precaution of not beginning experiments until the animals had regained their normal quiescence (sometimes requiring several days post-operation).

### *Protocol*

Experiments were divided into three successive parts: (1) a control period usually 12 h or more in length, (2) a 48-h hypercapnic period, and (3) a post-hypercapnic period, again 48 h in length. During the hypercapnic period, the recirculation system was bubbled with a mixture of approximately 1 % $CO_2$ in air (water $P_{CO_2}$ ~ 7·5 Torr, $P_{O_2}$ ~ 120 Torr), while during control and post-hypercapnic periods, the water was equilibrated with air ($P_{CO_2}$ ~ 1·3 Torr, $P_{O_2}$ > 120 Torr). In most experiments, the recirculation system was flushed between each period with fresh water (gassed with the appropriate $CO_2$/air combination) in order to prevent the build-up of excreted ammonia. During all periods, water [$HCO_3^-$] and [$NH_4^+$] were monitored and water samples were saved for ion analysis. [$Na^+$] was determined by flame photometry (Instrumentation Laboratory Model 343), and [$Cl^-$] was measured with a system consisting of a solid state $Cl^-$-sensitive electrode and reference connected to a microprocessor/ionalyser, printer and timer/microprocessor, which was automatically calibrated with NaCl standard after every second measurement. Immediately before the beginning of the hypercapnic period, three successive control blood samples were withdrawn at 15-min intervals. During hypercapnia and post-hypercapnia, blood samples were taken at 0, 0·5, 1, 2, 4, 8, 20, 28 and 48 h after the respective change in water $P_{CO_2}$. Each sample (total volume at least 0·4 ml) was analysed for plasma $P_{CO_2}$, $P_{O_2}$, pH and $T_{CO_2}$. $P_{CO_2}$, $P_{O_2}$ and pH were measured with the appropriate microelectrodes (Radiometer, Copenhagen) according to the methods described by Heisler (1978). A small portion of the blood sample was centrifuged briefly in haematocrit tubes, the haematocrit recorded, and samples of plasma were analysed in duplicate for $T_{CO_2}$ using a Capni-Con II (Cameron Instruments Inc.) which was calibrated with $K_2CO_3$ standards.

### *Calculations*

Plama $HCO_3^-$ concentration was calculated by subtracting dissolved $CO_2$ ($\alpha_{CO_2} \times T_{CO_2}$) from $T_{CO_2}$ using values calculated from those for human plasma reported by

Severinghaus (1965). $\Delta HCO_3^-{}_w$, $\Delta NH_4^+{}_w$, $\Delta Cl^-{}_w$, and $\Delta Na^+{}_w$ (in mmol $kg^{-1}$ fish) were computed for all time periods by multiplying the measured concentration of the specified ion (mmol $l^{-1}$) by the total volume of water within the recirculation system (starting volume−volume of water samples removed) and correcting for the weight of the animal. $\Delta H^+{}_{e\rightarrow w}$ is the paired difference between $\Delta HCO_3^-{}_w$ and $\Delta NH_4^+{}_w$ for each sample. Control cumulative ion transfer rates (in mmol $h^{-1}$ $kg^{-1}$; Table 1) were calculated by dividing the appropriate net ion concentration changes by the length of the control period (usually 12 h). 'Net Δ' values are the difference between control and experimental means for each time period. Statistical analysis was performed utilizing Student's *t* test for paired or unpaired data where appropriate. Calculations were carried out on a minicomputer (Digital, PDP 11/03-L) using specially designed software (Claiborne & Heisler, 1983).

## RESULTS

Control values for acid-base and electrolyte parameters for the carp in normocapnic water are presented in Table 1. Plasma pH, $P_{CO_2}$, $[HCO_3^-]$ and $[Cl^-]$, as well as net transfers of $HCO_3^-$ and ammonia, were very similar to those reported earlier for this species (Ultsch *et al.* 1981). The mean control $P_{O_2}$ measured in the present experiments was lower than that presented by Ultsch and coworkers but was not statistically different due to the large variation between individual animals in the earlier study. The mean $P_{O_2}$ reported here is concordant with values obtained from free-swimming, undisturbed carp ($P_{O_2} = 37{\cdot}5 \pm 6{\cdot}3$ Torr, $\bar{x} \pm$ S.E., $N = 6$; J. B. Claiborne & N. Heisler, unpublished results; $P_{O_2} \sim 25$ Torr, N. Heisler & P. Neumann, unpublished results).

The changes in blood acid-base status following exposure to environmental hypercapnia are shown in Fig. 2. Plasma $P_{CO_2}$ increased significantly from approximately 4·8 to 8·8 Torr ($P < 0{\cdot}01$, $N = 7$) over the first 30 min and finally rose to 12·6 Torr after 48 h. After 8 h of hypercapnia, plasma pH had decreased from about 7·87 to 7·59

Table 1. *Control values for acid-base and electrolyte parameters in* Cyprinus carpio

| Parameter | |
|---|---|
| Dorsal aortic plasma | |
| $P_{CO_2}$ (Torr) | 4·75 ± 0·26 (7) |
| $P_{O_2}$ (Torr) | 44·4 ± 5·9 (7) |
| pH | 7·87 ± 0·05 (7) |
| $[HCO_3^-]$ (mM) | 13·8 ± 1·0 (7) |
| Cumulative ion transfer rates (mmol $h^{-1}$ $kg^{-1}$) | |
| $\Delta HCO_3^-{}_w$ | 0·100 ± 0·039 (8) |
| $\Delta NH_4^+{}_w$ | 0·17 ± 0·03 (8) |
| $\Delta H^+{}_{e\rightarrow w}$ | 0·07 ± 0·02 (8) |
| $\Delta Cl^-{}_w$ | −0·027 ± 0·021 (5) |
| $\Delta Na^+{}_w$ | −0·018 ± 0·028 (5) |

x ± S.E., number of experiments in parentheses.
'Δ' ion transfer values represent the cumulative appearance of the specified ion in the environmental wat
Negative values indicate a net uptake from the water.

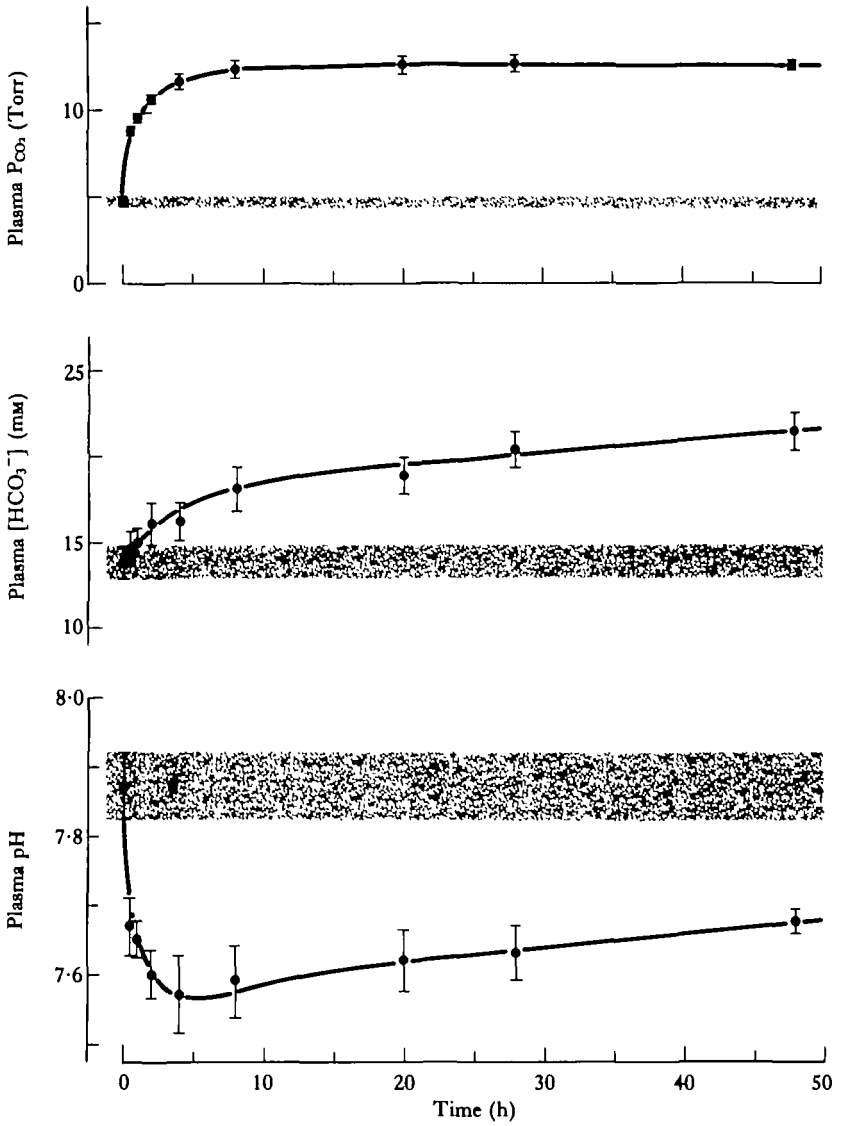


Fig. 2. Plasma $P_{CO_2}$, $[HCO_3^-]$ and pH in dorsal aortic blood following the onset of environmental hypercapnia ($\bar{x} \pm$ S.E., $N = 7$). Shaded areas represent $\bar{x} \pm$ S.E. of the pre-hypercapnic control values.

($P < 0{\cdot}001$, $N = 7$), and then recovered to 7·68 at the end of the 48 h. This value was still significantly below that of the control measurement. Plasma $HCO_3^-$ concentration had increased from 13·8 to 21·6 mM ($P < 0{\cdot}001$, $N = 7$) by the end of the hypercapnic exposure.

The blood acid-base modulation following the return of carp to normal ambient $CO_2$ concentrations after hypercapnic exposure is shown in Fig. 3. Though plasma $P_{CO_2}$ falls rapidly (by 4·7 Torr over the first 30 min), it returns to pre-hypercapnic levels only after 20 h. A significant increase (0·14 units in the first 30 min of post-hypercapnia, $P < 0{\cdot}001$, $N = 6$) brought plasma pH to control values for the

remainder of the experimental period. Plasma [$HCO_3^-$] declined slowly and, in parallel with $P_{CO_2}$, was significantly elevated from pre-hypercapnic levels until after 20 h of normocapnic exposure.

Hypercapnia induced a reduction in the rate of $HCO_3^-$ excretion and an increase in ammonia transfer (Fig. 4). The control $HCO_3^-$ excretion of 0·10 mmol $h^{-1}$ $kg^{-1}$ was significantly reduced by some 60 % to 0·04 mmol $h^{-1}$ $kg^{-1}$ between hours 16 to 28 of hypercapnia. $NH_4^+$ efflux was significantly increased from 0·17 to 0·32 mmol $h^{-1}$ $kg^{-1}$ during the first 16 h of this period. These changes produced a 115 % increase in

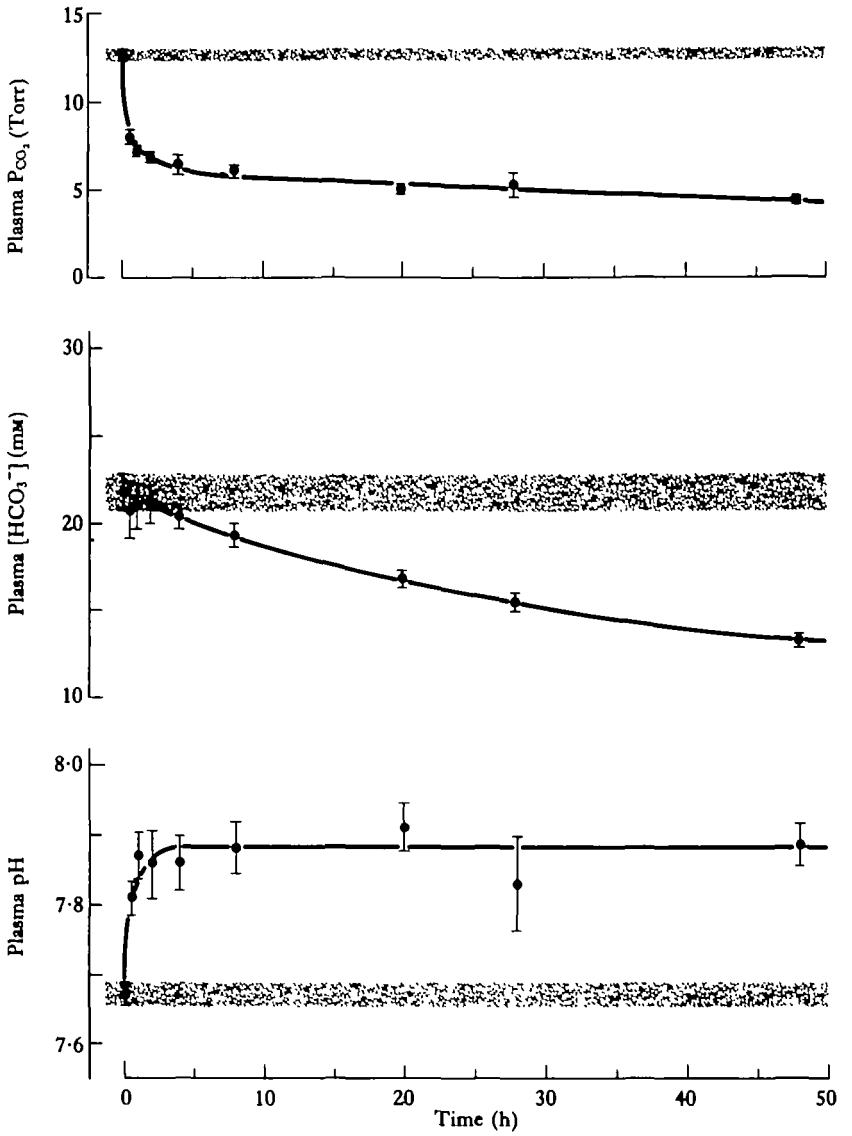


Fig. 3. Plasma $P_{CO_2}$, [$HCO_3^-$] and pH in dorsal aortic blood after return from hypercapnia to normocapnia. ($\bar{x} \pm$ S.E., $N = 6$). Shaded areas represent $x \pm$ S.E. of final hypercapnic values taken prior to returning the external media from high $CO_2$ to normal aeration.

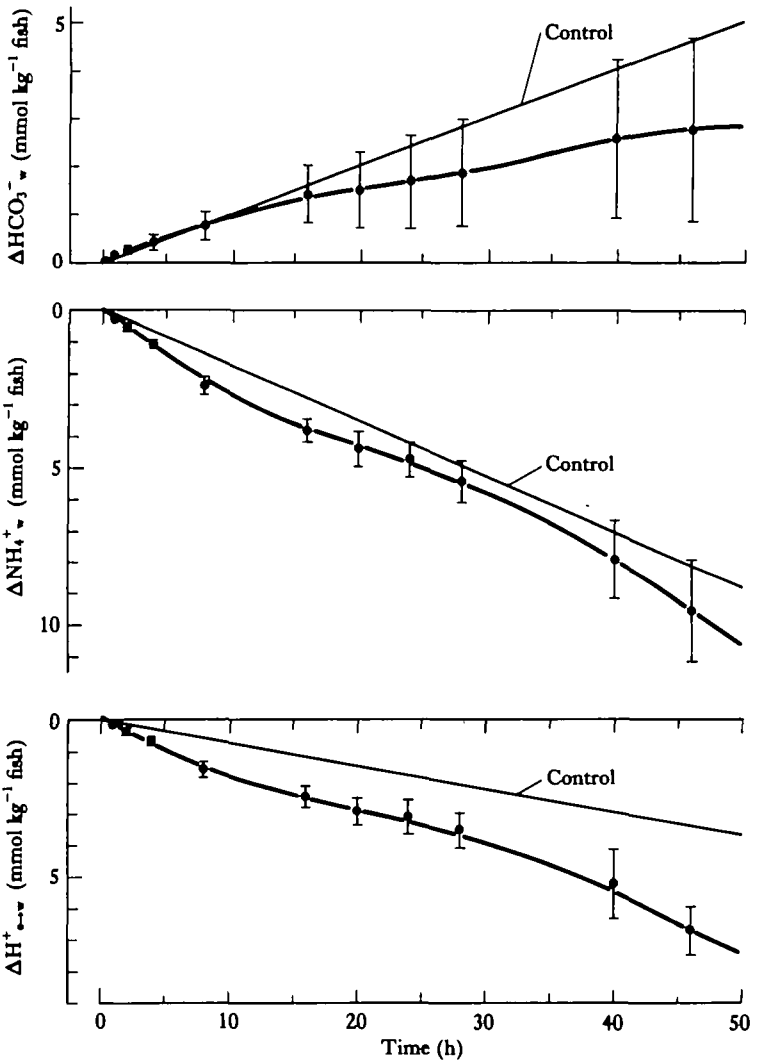


Fig. 4. Changes in water $HCO_3^-$ and $NH_4^+$ concentrations during hypercapnia. The control line was determined over a 12-h period prior to the start of hypercapnia. In this and the following figures, the control line has been extended as a reference over the subsequent experimental periods. Cumulative $H^+$ efflux ($\Delta H^+_{e\to w}$) is calculated as the difference between the release of $NH_4^+$ and $HCO_3^-$ ($\bar{x} \pm$ S.E., $N = 8$ for control lines, $N = 6$ for experimental points).

cumulative $\Delta H^+$ transfer measured at hour 20. $\Delta H^+$ efflux for the entire hypercapnic period averaged $0{\cdot}15 \pm 0{\cdot}02$ mmol h$^{-1}$ kg$^{-1}$ ($N = 6$); well above the control rate of $0{\cdot}07 \pm 0{\cdot}02$ ($N = 8$) (Table 1, Fig. 4).

When the environmental water was returned to normal (low $CO_2$) aeration after hypercapnia, net $HCO_3^-$ loss increased to control rates within 2 h (Fig. 5). Ammonia efflux was immediately reduced from that measured during hypercapnia and averaged $0{\cdot}09 \pm 0{\cdot}03$ mmol h$^{-1}$ kg$^{-1}$ ($N = 5$) at the midpoint of the post-hypercapnic period (approximately 50 % less than the control rate). After 2 h of post-hypercapnia, $H^+$ was transferred to the water at a rate of $0{\cdot}03$ mmol h$^{-1}$ kg$^{-1}$ for the next 38 h; this

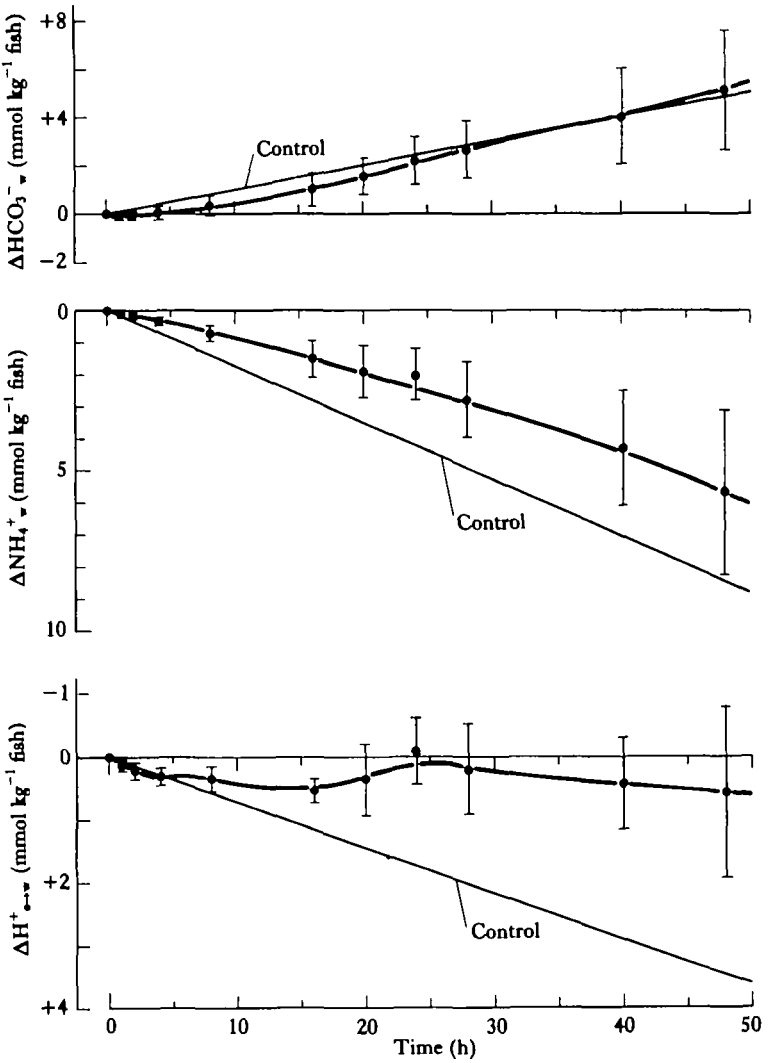


Fig. 5. Changes in water $HCO_3^-$, $NH_4^+$ and $\Delta H^+_{c\to w}$ after return from environmental hypercapnia to normocapnia ($\bar{x} \pm$ S.E., $N = 8$ for control lines, $N = 5$ for experimental points). For further explanations see legend to Fig. 4.

rate is significantly ($P < 0{\cdot}02$, $N = 5$) less than the control rate (Table 1).

Fig. 6 shows the effect of hypercapnia on the rate of net $Cl^-$ and $Na^+$ movements. The control $Cl^-$ uptake rate of 0·03 mmol $h^{-1}$ $kg^{-1}$ was significantly reversed to a $Cl^-$ efflux of 0·05 mmol $h^{-1}$ $kg^{-1}$ during this period. A six-fold increase in $Na^+$ uptake (to $0{\cdot}11 \pm 0{\cdot}03$ mmol $h^{-1}$ $kg^{-1}$) was noted between hours 8 to 20. During the post-hypercapnic period (Fig. 7), net $Cl^-$ efflux was reduced to control uptake rates once again, while the $Na^+$ influx of the previous period became a net loss to the environmental water. The maximum rate of efflux occurred between hours 2 and 20 when n$Na^+$ loss averaged 0·04 mmol $h^{-1}$ $kg^{-1}$

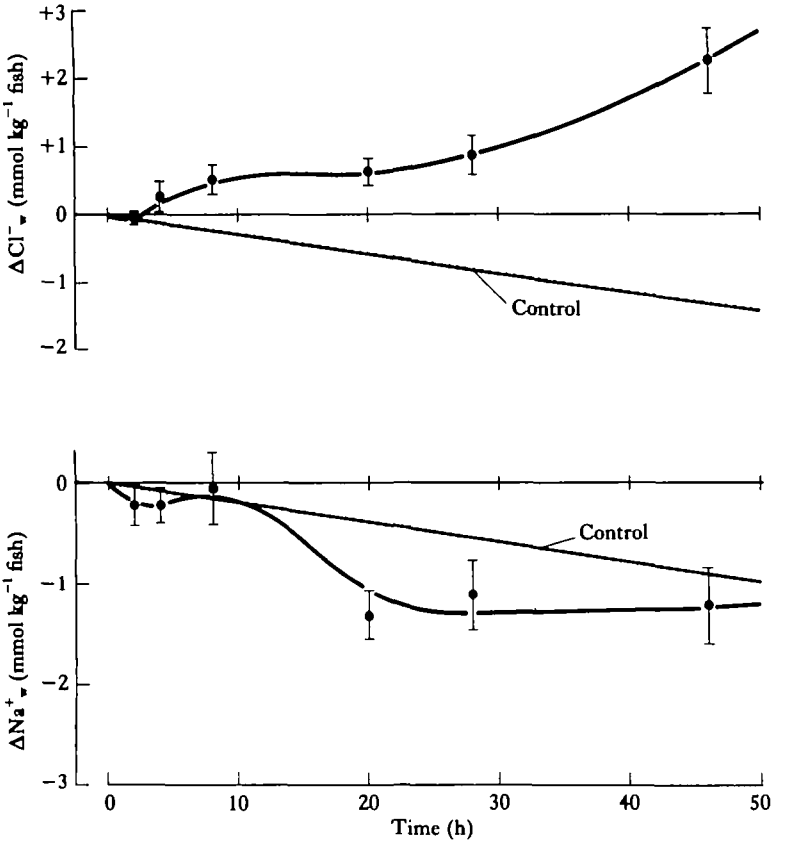


Fig. 6. Changes in water $Cl^-$ and $Na^+$ concentrations during the hypercapnic period ($\bar{x} \pm$ S.E., $N = 5$ for control lines, $N = 7$ for experimental points).

## DISCUSSION

As reported in the Results, the low control plasma $P_{O_2}$ measured in the experimental animals prior to hypercapnia was very similar to the average value observed in free-swimming fish acclimated to a large flow-through system for several months. Likewise, the low rates of ammonia and $HCO_3^-$ excretion observed in control animals (Table 1) represent a slight net $H^+$ excretion which agrees with the results from other species (Heisler, 1982*a*). We believe that care in handling, preparation and the time allowed for the acclimation of the experimental animals is of the utmost importance. It is unfortunate that some studies have overlooked the necessity of allowing the fish to reach a true 'control', steady-state status; a level which must be attained before experimental results become meaningful.

Alteration in ambient $P_{CO_2}$ rapidly affected the acid-base status of the carp. At the onset of hypercapnia, plasma $P_{CO_2}$ was quickly elevated while plasma pH was depressed. Over the next 48 h, plasma [$HCO_3^-$] slowly increased and partially compensated for the decline in pH. This type of response has been reported in several freshwater and seawater species, though some quantitative differences exist. The rapidity and degree of pH compensation achieved appear to be very dependent on the species studied. *Salmo gairdneri* has been reported to reach control blood pH levels

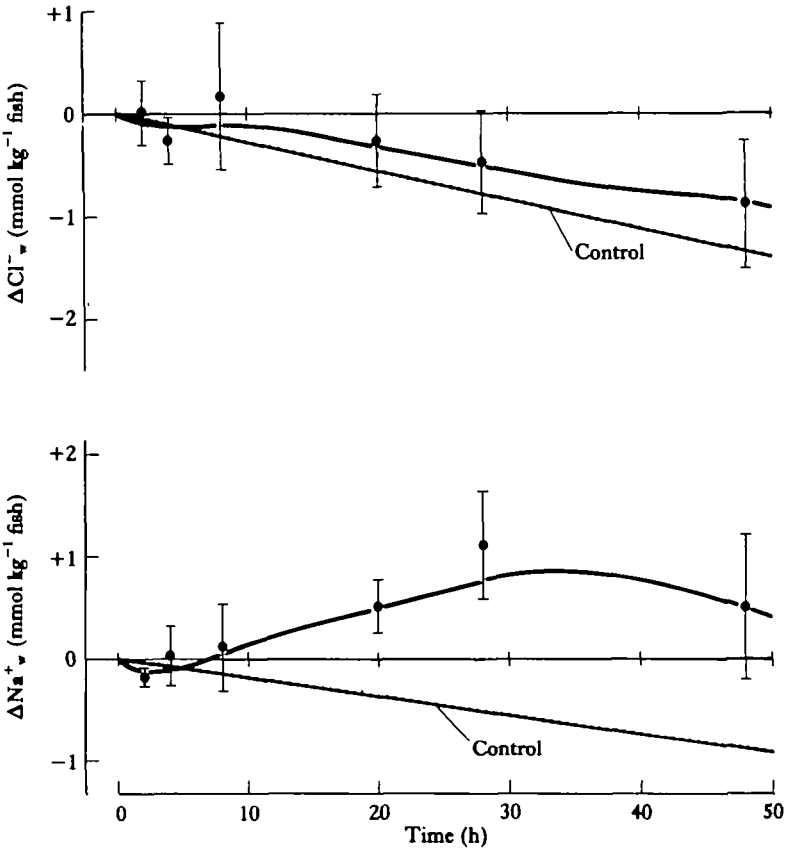


Fig. 7. Changes in water $Cl^-$ and $Na^+$ concentrations during the post-hypercapnic period. ($\bar{x} \pm$ S.E., $N = 5$ for control lines, $N = 7$ for experimental points).

within 22 h (Eddy *et al.* 1977) or may require as long as 3 days (Janssen & Randall, 1975), this discrepancy perhaps being due to the different ion concentrations of the environmental water in these two studies (Heisler, 1982*a*). The freshwater catfish, *Ictalurus punctatus*, requires about 24 h to compensate both the intracellular and extracellular pH after the onset of hypercapnia (Cameron, 1980), while the seawater teleost, *Conger conger* has been reported to attain nearly total compensation only 10 h after the onset of hypercapnia (Toews, Holeton & Heisler, 1983). In contrast, it appears that the carp requires at least several days to compensate for the increased respiratory acidosis. After 48 h of hypercapnia, the plasma pH had risen to a value of 7·68. If plasma bicarbonate levels had remained constant during this period, the pH would have fallen to about 7·48. In other words, the extracellular [$HCO_3^-$] increase during hypercapnia compensated for about 1/2 of the possible pH depression (Fig. 2). Indeed, we have recently found that in free-swimming carp, 2 weeks may be required to regain total compensation of plasma pH during mild (<1 % $CO_2$) hypercapnic exposure (J. B. Claiborne & N. Heisler, unpublished results). It is likely that the $P_{CO_2}$ of the natural environment of the carp fluctuates significantly. The habitat of this fish is commonly a pond or lake subject to variable rates of mixing and some thermostratification, both possibly leading to increased $CO_2$ tensions (see review by

Heisler, 1980). The short-term tolerance of a reduced extracellular pH in these animals (rather than an immediate and total compensatory response, presumably at the cost of additional metabolic energy consumption) might be interpreted as an adaptive advantage to a species which may encounter intermittent exposure to hypercapnia in its natural environment.

Another explanation for the apparent lack of total compensation during the hypercapnic period may be that the carp have approached a maximal threshold of plasma $HCO_3^-$ which can be retained. In the aquatic salamander, *Siren lacertina*, plasma $[HCO_3^-]$ did not rise above about 25 mM during hypercapnia even though plasma pH was depressed by more than 0·3 pH units (Heisler, Forcht, Ultsch & Anderson, 1982). Increasing external $[HCO_3^-]$ or infusion of $HCO_3^-$ did not alter the final steady-state plasma $[HCO_3^-]$. It was postulated that *Siren* was not able to adjust the maximal threshold of plasma $[HCO_3^-]$ retention to a higher level. Recent evidence from our laboratory suggests that carp plasma $[HCO_3^-]$ also cannot be maintained above ~25 mM – even during a hypercapnic stress greater than the present study (5 % $CO_2$) or following infusion of $NaHCO_3^-$ (5 mmol $kg^{-1}$) (J. B. Claiborne & N. Heisler, unpublished results). Whether a maximal $HCO_3^-$ 'set-point' governs pH compensatory responses in the carp remains to be seen, but it is surely a point of interest to be pursued in future investigations.

When carp were returned to low $CO_2$ aeration, plasma pH rose to normal levels within the first 30 min. This pH increase was caused by the initially rapid fall of plasma $P_{CO_2}$ within the same time period. As the $P_{CO_2}$ continued to decline slowly, plasma $[HCO_3^-]$ was reciprocally reduced, allowing the extracellular pH to remain relatively constant for the remaining 47 h (Fig. 3). These animals did not exhibit the over-shoot of plasma pH (due to the rapid decrease in plasma $P_{CO_2}$ without a concurrent modulation of plasma $[HCO_3^-]$) observed in *Salmo gairdneri* (Eddy *et al.* 1977) or the elasmobranch, *Scyliorhinus stellaris* (Heisler *et al.* 1976). This may be due to the moderate extent of plasma $[HCO_3^-]$ adjustment attained by the carp (an increase of approximately 8 mM *vs* about 19 mM in the trout) during the 48-h hypercapnic period.

The acid-base response of *Cyprinus* during and after environmental hypercapnia is presented in a pH – $[HCO_3^-]$ diagram in Fig. 8. From control values (point A), a minimal plasma pH value was recorded at hour 4 of hypercapnia (point B) due to a rapid increase in plasma $P_{CO_2}$. During the remainder of this period, the plasma $P_{CO_2}$ essentially followed the 12 Torr $P_{CO_2}$ isopleth while an increase in $[HCO_3^-]$ induced an elevation in plasma pH (points B–C). One hour after normal ambient $CO_2$ levels were reinstated, pH had returned to control values due to a sharp drop in plasma $P_{CO_2}$ (point D). At the end of this period, all parameters were normal once again (point E). Qualitatively, this pattern of response is similar to that described for the trout (Janssen & Randall, 1975) or for the freshwater gar (*Lepisosteus oculatus*, Smatresk & Cameron, 1982), but, again the degree of compensation by accumulation of $HCO_3^-$ during hypercapnia is reduced in the carp.

The measurement of $HCO_3^-$ and ammonia transfers (Figs 4, 5) permits a deeper understanding of the regulatory processes involved in the extracellular acid-base adjustments during ambient $CO_2$ changes. The total $H^+$ excretion ($\Delta H^+_{e \to w}$) is calculated as the difference between the amount of $HCO_3^-$ and $NH_4^+$ excreted at any given time (Heisler *et al.* 1976). It should be noted that, utilizing the present

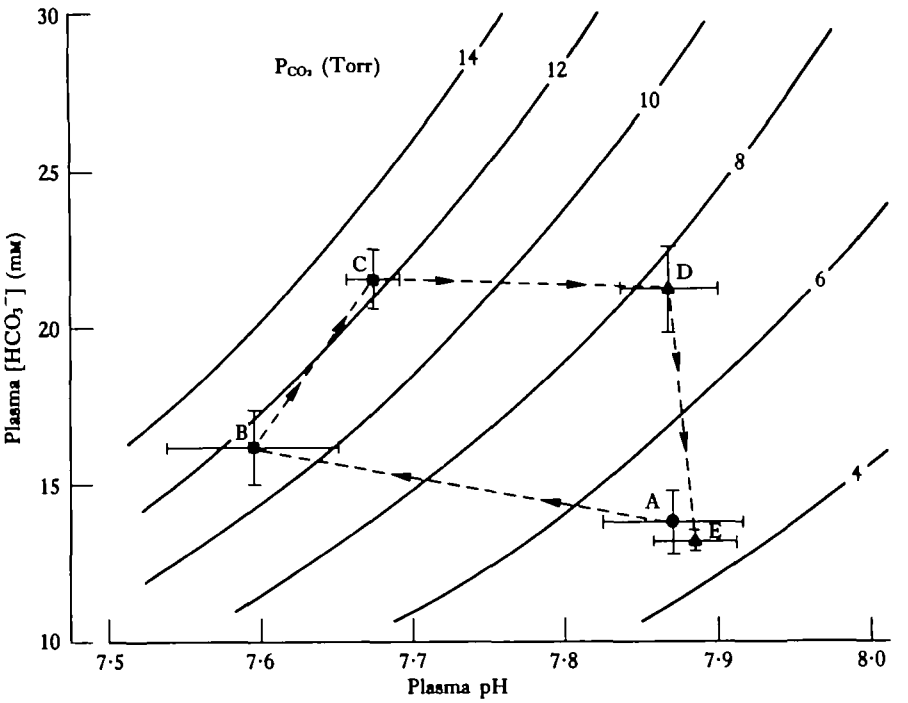


Fig. 8. pH – $[HCO_3^-]$ diagram depicting plasma acid-base changes during and after environmental hypercapnia. $P_{CO_2}$ isopleths were calculated using the Henderson-Hasselbalch equation with $\alpha$ and pK′ values extrapolated from the data of Severinghaus (1965). Point A represents control values prior to hypercapnia. Points B and C show changes during hypercapnia, while D and E indicate post-hypercapnia measurements ($\bar{x} \pm$ S.E., $N = 7$ for points A, B and C, $N = 6$ for D and E). See text for details.

methods, the efflux of $HCO_3^-$ is not discernible from the uptake of $H^+$ or the loss of $OH^-$. Likewise, the differential determination of the amounts of $NH_3$ and $NH_4^+$ released to the ambient water is not possible since 99 % of all $NH_3$ excreted by the fish will quickly be converted into the ionized form, $NH_4^+$ (because the pK′ for the $NH_3/NH_4^+$ equilibrium is about 9·6, Cameron & Heisler, 1983). When calculating net $H^+$ transfer it is not necessary to know whether ammonia has been excreted as $NH_3$ gas or $NH_4^+$; effectively all $NH_3$ transferred will be immediately ionized, and therefore produce equimolar amounts of $HCO_3^-$ which must be subtracted from the measured $HCO_3^-$ efflux, while all ammonia excreted as $NH_4^+$ represents the loss of a proton and must be deducted from the net base excretion as well. It should also be noted that the cumulative and net transfers reported here represent 'whole body' fluxes of these ions. We found it very difficult to separate branchial and renal ion transfers (by the collection of urine through a urinary catheter) for the extent of the recovery, control, hypercapnic and post-hypercapnic periods without inducing stress in the experimental animals. Numerous reports have shown that transfers of acid-base and strong ions occur predominantly at the gills (see reviews by Evans, 1980*b*; Heisler, 1980; also, Cameron, 1980; Evans, 1982). It is probable that the observed ion transfers in the present study are also mainly due to transbranchial fluxes.

Extracellular $[HCO_3^-]$ was elevated by ~55 % (an increase of about 8 mM) during

the hypercapnic period; the net $\Delta H^+$ transferred from the animal into the water during this period was approximately 3·3 mmol $kg^{-1}$ fish. If the extracellular space of *Cyprinus* is of the order of 20 % (e.g. Cameron, 1980; Heisler, 1982*b*), then the increased rate of $H^+$ loss (or $HCO_3^-$ gain) during hypercapnia can easily account for the increase in plasma $[HCO_3^-]$. In fact, the observed increase in $HCO_3^-$ represents only about 50 % of the net $H^+$ transferred, indicating that additional $HCO_3^-$ must have been sequestered intracellularly. This is not surprising since the initial pH depression and subsequent pH compensation of the intracellular compartment mirrors the response of the extracellular space during hypercapnia (Heisler *et al.* 1976; Cameron, 1980). The net $H^+$ excreted during this period was ~78 % as much as that reported for *Conger conger* (approximately 4·2 mmol $kg^{-1}$ fish) (Toews *et al.* 1983). The fact that the marine teleost voided this quantity of $H^+$ within 10 h, as opposed to 46 h in the carp, may be an indication of the relative difference in compensation rates between freshwater and marine fish. The marine toadfish, *Opsanus beta*, has a net $H^+$ excretion rate of about 0·5 mmol $h^{-1}$ $kg^{-1}$ measured after 3–5 h of hypercapnia (5 % $CO_2$; calculated from Evans, 1982), a value which is more than six times greater than that of the carp. Likewise, the ureotelic elasmobranch *Scyliorhinus stellaris* also transfers $H^+$ to the ambient water approximately six times faster than the carp during the first few hours of hypercapnic stress (Heisler *et al.* 1976). Marine teleost fish are known to exchange ions across the gills at a much higher turn-over rate than freshwater species (see review by Evans, 1980*b*). Since it has been shown that at least one seawater teleost excretes $Cl^-$ branchially, in exchange for external $HCO_3^-$ (Kormanik & Evans, 1979), and takes up external $Na^+$ in exchange for serosal $H^+$ (Evans, 1982), the cellular mechanisms required to compensate for hypercapnic acidosis may be immediately available to these fish. Though the rate of net $H^+$ transfer is much slower, the present data indicate that the freshwater carp might also utilize a $HCO_3^-$ uptake, as well as a net $H^+$ loss, to regulate internal pH during hypercapnia. The discrepancy between the rates of net $H^+$ transfer in the carp and the marine species reported above may well be due to the much greater availability of counter-exchange ions in the ambient seawater. Indeed, initial investigations from our laboratory indicate that when carp are adapted to an elevated ambient $[NaHCO_3]$ of 3 mM, the net rate of $H^+$ transfer during hypercapnia is twice as great as that reported in the present study (J. B. Claiborne & N. Heisler, unpublished results).

Within 2 h after the return to normal ambient $CO_2$ tensions, $\Delta H^+$ excretion from the carp was reduced by some 80 % from the hypercapnic rate of transfer. For the remainder of this period, the $H^+$ flux was equal to only 40 % of the control $H^+$ loss (Fig. 5). The net $\Delta H^+$ loss became negative (equal to a net $HCO_3^-$ efflux) and the total loss after 48 h was about $-2{\cdot}9$ mmol $kg^{-1}$ fish. Expressed another way, ~90 % of the $HCO_3^-$ taken up during hypercapnia was transferred back to the water during the experimental post-hypercapnia period. Again, assuming an extracellular space of about 20 %, the net $HCO_3^-$ loss can account for the observed drop in plasma $[HCO_3^-]$ (and a parallel decrease in intracellular $[HCO_3^-]$) during this period. To our knowledge, the only other study which followed the net movements of $H^+$ during a post-hypercapnic period was that of Heisler *et al.* (1976). After hypercapnia, the seawater elasmobranch, *Scyliorhinus* voided approximately the same amount of $HCO_3^-$ as had been gained during hypercapnia. As was the case for hypercapnic

fluxes, the rate of $HCO_3^-$ transfer by the shark during post-hypercapnia was much faster than that exhibited by the carp in the present study – again, perhaps because of differences between seawater and freshwater ambient ion concentrations which are available for exchange.

The mechanisms governing the movements of $H^+$, $OH^-$, $HCO_3^-$ and $NH_4^+$ during acid-base adjustment in fish are more difficult to ascribe. Over the last few decades, several studies have implied that there is a linkage between acid-base and strong ion (mainly $Na^+$ and $Cl^-$) transfers. The efflux of $HCO_3^-$ (or $OH^-$) has been proposed to occur in exchange for external $Cl^-$ (Krogh, 1939; Maetz & Garcia-Romeu, 1964; Cameron, 1978), and the excretion of $H^+$ and/or $NH_4^+$ is thought to be linked to the uptake of ambient $Na^+$ (Maetz, 1973; Evans, 1980*a*). These counter-transport mechanisms can be inhibited or even reversed when exposed to the proper gradients for the ions involved (Maetz & Garcia-Romeu, 1964; Evans, 1977). Cameron (1976)

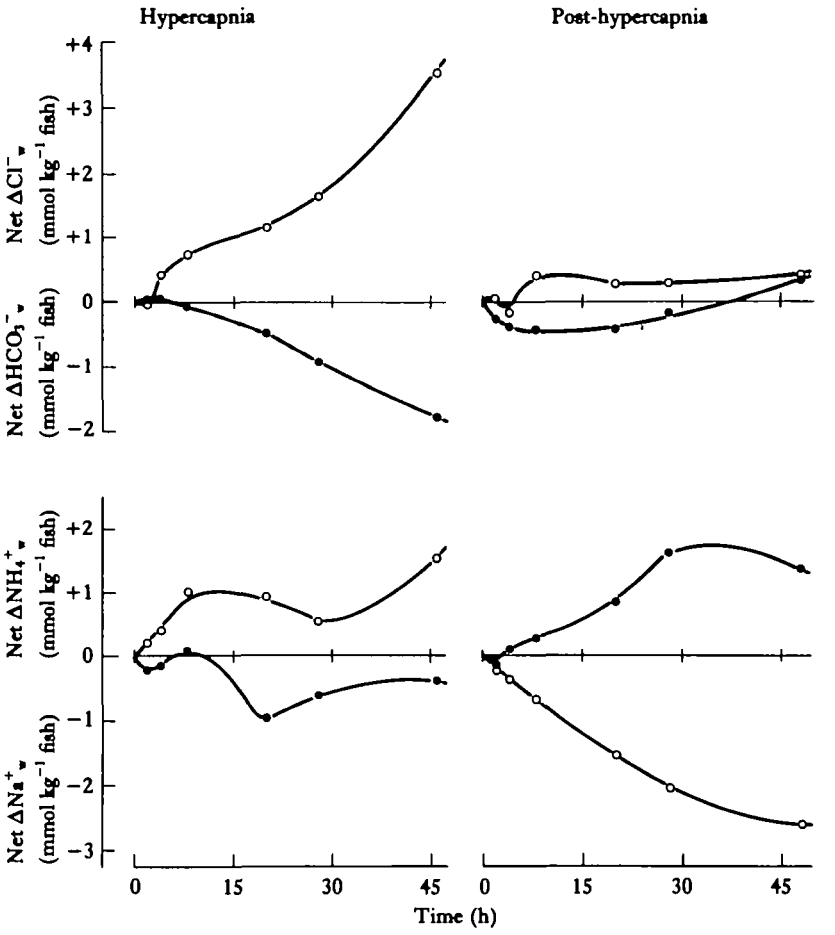


Fig. 9. Net changes in water $Cl^-$, $HCO_3^-$, $Na^+$ and $NH_4^+$ concentrations during and after hypercapnia. Net values are calculated as the difference between control and experimental means for each time period. Key to symbols: in upper diagram, —○— = net $\Delta Cl^-$, —●— = net $\Delta HCO_3^-$; in lower diagram, —○— = net $\Delta NH_4^+$, —●— = net $\Delta Na^+$. See text for details.

showed that in the hypercapnic arctic grayling the ratio of net $Na^+$ to $Cl^-$ uptake was elevated above control levels. In the light of these results, the author proposed a key role for $Na^+/H^+$ transfers during hypercapnic acidosis. In contrast, Perry *et al.* (1981) have recently hypothesized that hypercapnic pH compensation occurs in *S. gairdneri* without the contribution of transbranchial or renal $Na^+/H^+$ ($NH_4^+$) or $Cl^-/HCO_3^-$ exchanges. They also proposed that these exchanges, during hypercapnia, were not limited by the low ambient NaCl levels in their experimental water. In the present experiments, net $Cl^-$ uptake was reversed to a net excretion during hypercapnia. Net $Na^+$ influx increased significantly for a portion of this period (Fig. 6). In fish returned to normocapnia, $Cl^-$ excretion returned to a rate of net uptake similar to the control, while net $Na^+$ influx became a net loss (Fig. 7). Clearly, transfers of both ions have been modified during the two experimental periods. When the 'net $\Delta$' changes for $Cl^-$, $HCO_3^-$, $Na^+$ and $NH_4^+$, (the difference between the experimental average for each collection period and the mean control amounts transferred for that period) are compared (Fig. 9), a relationship between these transfers becomes apparent. Though we feel hesitant to make a quantitative comparison of flux rates, it would appear that $HCO_3^-$ transfers are mirrored in a reciprocal fashion by $Cl^-$ movements. Likewise, modifications in the direction of net $\Delta Na^+$ flux is correlated with variation in ammonia excretion at least during the post-hypercapnic period. The difficulty in determining the ratio of $NH_3$ to $NH_4^+$ transferred (as discussed above) makes a quantitative analysis more difficult. Cameron & Heisler (1983) have proposed that ammonia excretion from *Salmo gairdneri* under normal conditions can be attributed solely to the loss of ammonia by nonionic diffusion. Only when external ammonia gradients favour the inward diffusive movement of $NH_3$ does $Na^+/NH_4^+$ exchange become necessary. One can only speculate as to what form of ammonia is excreted by the carp during and after hypercapnia. If, as in the trout, a considerable percentage of the total ammonia efflux from the fish is due to non-ionic diffusion of $NH_3$ to the water and its ionization to $NH_4^+$, the concentration of ambient $HCO_3^-$ will increase equimolarly. This effect might explain the apparent imbalance of $Cl^-$ excretion and $HCO_3^-$ uptake during hypercapnia (Fig. 9). Furthermore, $H^+$ transferred out of the animal in exchange for $Na^+$ could be hidden behind the loss and ionization of $NH_3$ (Holeton *et al.* 1983). This may be the case between hours 8 and 20 of hypercapnia when the uptake of $Na^+$ is increased six-fold without a corresponding elevation in ammonia efflux (Fig. 9).

During the post-hypercapnic period, the rate of ammonia excretion was below control levels (Fig. 9). A net decrease in the amount of $NH_3$ transferred to the water should induce an equimolar net decrease in ambient $HCO_3^-$ (due to the relative increase in $H^+$ in the water available to combine with $HCO_3^-$). Since net $\Delta HCO_3^-$ did not decline during this period, either additional $HCO_3^-$ was transferred from the fish to the water, or more $H^+$ was taken up from the external media. Because of the reversal of $Na^+$ transfer from a net uptake to a net loss measured during this period, we would propose that the efflux of $Na^+$ is related to a net gain of $H^+$ which is masked by a decrease in $NH_3$ loss. If, on the other hand, a large part of the ammonia transfer was due to $Na^+/NH_4^+$ transport, then one would have to postulate a net $NH_4^+$ uptake in exchange for the loss of $Na^+$ during the post-hypercapnic period. Clearly, the uptake of a potentially toxic ion from the environment solely for the gain of a proton

would not seem energetically appropriate. Following hypercapnia, $H^+/Na^+$ exchange is a more viable alternative.

The present data indicate that $Cl^-/HCO_3^-$ exchange occurs predominantly during hypercapnia, while $Na^+/H^+$ exchange takes place after hypercapnia; both mechanisms allowing the animal to gain $HCO_3^-$ or $H^+$, but also transferring $Cl^-$ and $Na^+$ in the opposite direction from that usually proposed for freshwater teleosts (Heisler, 1980; Evans, 1980*b*). It is important to note that because of the relative concentrations of these ions, only moderate changes in extracellular $[Cl^-]$ or $[Na^+]$ were required to elicit large acid-base effects. For example, during hypercapnia the $Cl^-$ lost to the water represents ~15 % of the total extracellular $Cl^-$ pool (a net loss of 3·5 mmol $kg^{-1}$ fish over 46 h as compared to a control plasma $[Cl^-]$ of ~114 mM; Ultsch *et al.* 1981), while net $HCO_3^-$ taken up from the media increased by about 115 % (3·3 mmol $kg^{-1}$ fish) and plasma $[HCO_3^-]$ was elevated by ~55 %. In order to maintain osmotic balance, it is likely that $Cl^-$ and $Na^+$ lost during the experimental periods would be regained after the acid-base stress had passed.

## CONCLUSION

Acid-base regulation during and after respiratory acidosis in the carp is similar to that of other species, although the time course of plasma $[HCO_3^-]$ adjustment is longer. Serosal pH is compensated by 50 % during a 48-h hypercapnic exposure. Regulation of plasma pH is linked to the modulation of $HCO_3^-$ and $H^+$ transfers between the fish and the environment. Hypercapnia induces an increase in intracellular and extracellular $[HCO_3^-]$ due to an enhancement of $Cl^-/HCO_3^-$ and $Na^+/H^+$ exchange. Following hypercapnia, plasma $[HCO_3^-]$ is decreased by the uptake of $H^+$ from the water *via* $Na^+/H^+$ transport. These transfers compensate internal pH when acid-base balance has been perturbed.

The authors gratefully acknowledge the skilful technical assistance of Mr G. Forcht and Mrs S. Glage. This research was supported by the Deutsche Forschungsgemeinschaft.

## 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. (1978). Regulation of blood pH in teleost fish. *Respir. Physiol.* **33**, 129–144.

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

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

CLAIBORNE, J. B., EVANS, D. H. & GOLDSTEIN, L. (1982). Fish branchial $Na^+/NH_4^+$ exchange is via basolateral $Na^+$-$K^+$-activated ATPase. *J. exp. Biol.* **96**, 431–434.

CLAIBORNE, J. B. & HEISLER, N. (1983). A minicomputer program for rapid graphical and statistical analysis of laboratory data. *Int. J. Bio-Med. Comput.* (in press).

DERENZIS, G. & MAETZ, J. (1973). Studies on the mechanisms 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. (1977). Further evidence for $Na/NH_4$ exchange in marine teleost fish. *J. exp. Biol.* **70**, 213–220.

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

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

EVANS, D. H. (1982). Mechanisms of acid extrusion by two marine fishes: the teleost, *Opsanus beta*, and the elasmobranch, *Squalus acanthius*. *J. exp. Biol.* **97**, 289–299.

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.

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

HEISLER, N. (1982*b*). 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., WEITZ, H. & WEITZ, A. M. (1976). Hypercapnia and resultant bicarbonate transfer processes in an elasmobranch fish (*Scyliorhinus stellaris*). *Bull. Europ. Physiopathol. Respir.* **12**, 77–85.

HEISLER, N., FORCHT, G., ULTSCH, G. R. & ANDERSON, J. F. (1982). Acid-base regulation in response to environmental hypercapnia in two aquatic salamanders, *Siren lacertina* and *Amphiuma means*. *Respir. Physiol.* **49**, 141–158.

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**, 303–318.

JANSSEN, R. G. & RANDALL, D. J. (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.

KORMANIK, G. A. & EVANS, D. H. (1979). $HCO_3^-$ stimulated $Cl^-$ efflux in the Gulf toadfish acclimated to seawater. *J. exp. Zool.* **208**, 13–16.

KROGH, A. (1939). *Osmotic Regulation in Aquatic Animals*. Cambridge: Cambridge University Press.

LLOYD, R. & WHITE, W. R. (1967). Effect of high concentrations of carbon dioxide on the ionic composition of rainbow trout blood. *Nature, Lond.* **216**, 1341–1342.

MAETZ, J. (1973). $Na^+/NH_4^+$, $Na^+/H^+$ exchanges and $NH_3$ movement across the gill of *Carassius auratus*. *J. exp. Biol.* **58**, 255–275.

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

PERRY, S. F., HASWELL, M. S., RANDALL, D. J. & FARRELL, A. P. (1981). Branchial ionic uptake and acid-base regulation in the rainbow trout, *Salmo gairdneri*. *J. exp. Biol.* **92**, 289–303.

SEVERINGHAUS, J. (1965). Blood gas concentrations. In *Handbook of Physiology, Section 3, Respiration*, pp. 1475–1487. Washington, D.C.: American Physiological Society.

SMATRESK, N. J. & CAMERON, J. N. (1982). Respiration and acid-base physiology of the spotted gar, a bimodal breather. II. Responses to temperature change and hypercapnia. *J. exp. Biol.* **96**, 281–294.

SOIVIO A., NYHOLM, K. & WESTMAN, K. (1975). A technique for repeated sampling of the blood of individual resting fish. *J. exp. Biol.* **63**, 207–217.

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

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.