J. exp. Biol. **107**, 9–20 (1983)
Printed in Great Britain © *The Company of Biologists Limited 1983*

REGULATION OF THE ACID-BASE STATUS DURING ENVIRONMENTAL HYPERCAPNIA IN THE MARINE TELEOST FISH *CONGER CONGER*

BY D. P. TOEWS*, G. F. HOLETON† AND N. HEISLER

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

(*Received 11 January 1983–Accepted 13 April 1983*)

SUMMARY

Specimens of *Conger conger* (L.) were exposed to environmental hypercapnia in a closed recirculating seawater system. Arterial plasma pH, P_{CO_2} and bicarbonate concentration, as well as the net transfer of bicarbonate and ammonia between fish and ambient seawater, were monitored for 30 h of hypercapnia. The initial hypercapnia-induced reduction of arterial pH by about 0·4 pH units was restored to near control values within 10 h of hypercapnia by compensatory elevation of plasma bicarbonate concentration. The continuous rise in extracellular bicarbonate from about 5 to 22 mM during this time was the result of two different mechanisms. Initially, there was a net bicarbonate transfer from the intracellular space to the extracellular compartment until the net uptake of bicarbonate from the seawater started. The amount of bicarbonate originally transferred to the extracellular space was then returned to the intracellular compartment and finally the changes in both extracellular and intracellular pH were compensated by bicarbonate taken up from the environmental seawater. Since the ammonia excretion was not increased during hypercapnia and the pattern of plasma electrolyte concentrations does not favour the H^+/Na^+ ion exchange mechanism, it is concluded that the additional bicarbonate is gained by active HCO_3^-/Cl^- ion exchange against the electrochemical gradient between fish and seawater.

INTRODUCTION

The effect of hypercapnia on the acid-base status of fish has been studied in a number of species and the general conclusion which has emerged is that the initial reduction of plasma pH is usually restored to values close to those observed during normocapnic control periods (e.g. Lloyd & White, 1967; Cross *et al.* 1969; Cameron & Randall, 1972; Janssen & Randall, 1975; Eddy, 1976; Eddy, Lomholt, Weber & Johansen, 1977; Heisler, Weitz & Weitz, 1976*a*; Börjeson, 1976; Randall, Heisler & Drees, 1976; for review see Heisler, 1982*a*). This compensation of the increased P_{CO_2} is achieved by accumulation of bicarbonate in the extracellular compartment. The intracellular pH in the few species studied is even more completely compensated

* Present address: Department of Biology, University of Acadia, Wolfville, Nova Scotia, Canada.
† Deceased.
Key words: Hypercapnia, ion transfer, extracellular pH, fish.

than pH in the extracellular space (*Scyliorhinus stellaris*, Heisler, 1980; *Ictalurus punctatus*, Cameron, 1980). In the exceptional tropical airbreather *Synbranchus marmoratus* the intracellular pH is almost completely restored by elevated bicarbonate concentration, whereas the extracellular pH remains uncompensated at 0·6 units below the control values during airbreathing-induced hypercapnia (Heisler, 1982*b*).

The origin of the additional bicarbonate required for compensation of the respiratory acidosis is unknown for most of the studied species. The relatively small quantity accumulated in the intracellular space of hypercapnic *Synbranchus* is primarily produced by intracorporeal non-bicarbonate buffering. Only a small quantity is gained by ion exchange with the environmental water ($\sim$0·7 mmol kg^{-1} body water, Heisler, 1982*b*). The only other data available on the contribution of net ion transfer to pH compensation in hypercapnia have been collected in elasmobranch fish. Cross *et al.* (1969) failed to demonstrate transepithelial ion transfer as a source for the observed large bicarbonate increase during hypercapnia in the spiny dogfish (*Squalus acanthias*). The technique which they used, whereby arterio-venous total CO_2 difference is multiplied by cardiac output, however, is relatively insensitive. They concluded that the additional bicarbonate was produced by non-bicarbonate buffering of CO_2 in the body fluids.

In the larger spotted dogfish (*Scyliorhinus stellaris*), however, a large proportion of the rise in the bicarbonate content of the body fluids during hypercapnia was demonstrated to have been gained from the environmental water ($\sim$4·4 mmol kg^{-1} body water; Heisler *et al.* 1976*a*). Most of the net bicarbonate uptake was utilized to increase the bicarbonate concentration of the poorly buffered extracellular space and about one quarter was transferred to the intracellular body compartment (Heisler *et al.* 1976*a*). Also, measurements of the intracellular pH in white muscle, red muscle and heart muscle of *Scyliorhinus* after 5 days of hypercapnia revealed apparent intracellular CO_2 buffer lines (Heisler, 1980), which were significantly steeper than those expected from the chemical buffer values of the respective intracellular compartments (Heisler & Neumann, 1980), suggesting a net transfer of bicarbonate into the intracellular space.

The aim of the present study was to evaluate the extent of transepithelial net bicarbonate uptake and its role as a mechanism for the acid-base regulation in a marine teleost fish (*Conger conger*).

MATERIALS AND METHODS

Specimens of *Conger conger* (weight 0·75–2·1 kg, mean 1·35 ± 0·35 kg) were caught in the Bay of Naples and kept for periods ranging from one week to several months in large, well-aerated seawater aquaria at temperatures between 14 and 17 °C. The animals were fed small fish until about 3 days prior to the experiments when they were transferred into smaller temperature controlled tanks (17 ± 0·5 °C, volume about 60–100 l $fish^{-1}$) which were flushed with fresh seawater at a rate of >400 l $fish^{-1}$ day^{-1}).

About 18–36 h (in two cases 42 h) prior to the experiments, the fish were anaesthetized in aerated urethane/seawater solution (20 g l^{-1}) until reactivity ceased and then placed ventral side up on an operating rack. Anaesthesia and oxygen supply were maintained during surgery by irrigating the gills through the mouth with a

generous flow of oxygenated seawater containing 2·5–3 g l^{-1} urethane. The body cavity was opened by a 3–4 cm long mid-ventral incision and a polyethylene catheter (PE 50) was introduced into a small gastric or splenic artery through an incision about 3–5 mm from the vessel's origin on the dorsal aorta. The catheter was pushed forward into the dorsal aorta for 2–7 cm and tied in place in the small vessel with a fine polyamide thread. The PE 50 catheter in the vessel was cut off 5–10 mm from the entrance into the vessel and fitted snugly into a short piece (7–10 mm) of PE 90 catheter, which, in turn, was fitted into larger thick-walled and unkinkable PVC tubing (1 mm i.d., 1·8 mm o.d.). The PVC tubing was led out of the body cavity through an additional small mid-ventral incision caudal to the larger operating incision. The body wall was closed by two layers of atraumatic sutures and the wound was made additionally waterproof with cyanoacrylate glue. The PVC catheter was secured to the body wall at the small incision by two circular sutures. After surgery the fish were revived by flushing the gills with fresh seawater until the fish showed a normal ventilation pattern.

Experimental apparatus

After recovery from anaesthesia, the animals were introduced into a seawater recirculation system (8–16 l) which was flushed for 6–8 h with 1–2 l min^{-1} of fresh seawater. The recirculation system consisted of a fish box, a pump and an oxygenator system with bubble trap (Heisler, 1978). The water was thermostatted to 17 ± 0·2 °C and aerated in the oxygenator at a rate of >8 l min^{-1} with CO_2-stripped air. The water was circulated in the system at a rate of about 5–6 l min^{-1}. This procedure provided a water P_{O_2} of higher than 130 mmHg throughout the experiment and a water P_{CO_2} of lower than 0·7 mmHg throughout the control period.

Net transfer of bicarbonate* between fish and environment was continuously measured by a 'Δ-bicarbonate measurement system' (Heisler, 1978). The idea of this system is to eliminate all influences of changing P_{CO_2} and temperature on the pH of the environmental water. pH in the water of the recirculation system is considerably affected by the normal fluctuations of P_{CO_2} from 0·5 to 0·7 mmHg during the normocapnic control period (equivalent to a change in pH of ~0·15 pH units), and by the increase in P_{CO_2} to about 8 mmHg during the hypercapnic period. However, when water P_{CO_2} and temperature are standardized, then all changes in water pH are related to excretion or uptake of HCO_3^- (OH^-, or H^+ in opposite direction) by the particular fish. For the purpose of this standardization water from the fish system was pumped by a roller pump through three glass columns with fritted glass bottoms, connected in series and therein equilibrated by bubbling with an extremely constant gas mixture of 1 % CO_2 in nitrogen at 30 ± 0·05 °C. The gas mixture was provided by a gas mixing pump, selected for particularly high long-term stability (Wösthoff, Bochum, F.R.G.). The water, thus standardized for P_{CO_2} and temperature, was fed over an electrode chain consisting of a special pH glass electrode and a double electrolyte bridge Ag/AgCl reference with sleeve diaphragm (drift less than 0·001 units per 24 h), before it was returned to the fish system. The electrodes were connected to the recording system *via* high-impedance precision isolation amplifiers (Model

* The term 'bicarbonate transfer' and similar terms are descriptively used, although the mechanisms may involve transfer of OH^- ions, or transfer of H^+ in the opposite direction; they are indistinguishable by the methods used.

87, Knick, Berlin, F.R.G.) in order to prevent ground loops and to minimize external electromagnetic disturbances by establishing a high common mode rejection. Th recirculating water was grounded with a platinum electrode near the site of the sensing electrode. For further details and standardization of the experimental apparatus see Heisler (1978), Heisler, Forcht, Ultsch & Anderson (1982), Holeton & Heisler (1983).

Procedure

After the supply of fresh seawater to the fish recirculation system was stopped, and the system thereby closed for non-volatile substances, the control release of bicarbonate and ammonia was determined for 8–14 h (in two cases for up to 36 h). When the bicarbonate release was steady and three blood samples withdrawn at 30-min intervals also showed steady state conditions of the plasma acid-base parameters, the gas supply to the oxygenator system was switched from air to a gas mixture of 1 % CO_2 in air. This time was defined as time zero for all succeeding measurements. Blood samples (~0·4 ml) were withdrawn from the dorsal aorta and water samples (~2 ml) were taken from the recirculation system 15 and 30 min, 1, 2, 3, 4, 6, 8, 10, 23, 26 and 30 h (in some cases 33 h) after time zero. Part of the sample was analysed immediately for plasma pH, P_{CO_2} and P_{O_2} using appropriate microelectrodes (Radiometer) as described previously (Heisler, Weitz & Weitz, 1976*b*; Heisler, 1978). Plasma bicarbonate was calculated by application of the Henderson-Hasselbalch equation using values for CO_2 solubility (α_{CO_2}) and pK'_1 reported for human plasma (Severinghaus, 1965), because of the similarity in ionic strength. Plasma was separated from the red cells by centrifugation (16 000 ***g***) for 1 min, decanted and saved for later [Na^+] and [K^+] analysis (flame photometry, IL 343), and determination of [Cl^-] (coulometric titration, Radiometer CMT10). Occasionally a small sample of plasma was deproteinized by addition of perchloric acid and analysed for plasma lactate concentration (Benadé & Heisler, 1978). The ammonia concentration in the water samples was determined after alkalinization (pH > 12) using gas-sensitive ammonia electrodes (HNU Systems, Inc.). The changes in the amounts of ions in the environmental water were expressed in relation to the absolute volume of water in the body fluids of the fish and then expressed as mmol kg^{-1} body water.

The CO_2 buffer curves of *Conger* blood and true plasma were established by equilibration of samples from a pool of three animals, with four different gases with P_{CO_2} values of 0·73–0·83, 1·45–1·55, 2·85–2·95 and 6·75–6·85 mmHg in air. Plasma was separated from the erythrocytes by centrifugation in sealed haemotocrit glass tubes. pH was determined by using the same technique as described above. Total CO_2 was measured in whole blood and true plasma using techniques similar to those described by Cameron (1971). The bicarbonate concentration was calculated by subtraction of the physically dissolved component calculated from the equilibration P_{CO_2} and α_{CO_2} reported for human plasma (Severinghaus, 1965).

RESULTS

Upon switching the oxygenator of the fish system from CO_2-free air to 1 % CO_2 in air, P_{CO_2} in the sea water of the fish box rose from 0·6–0·7 mmHg to about 8·2 mmHg

within a few minutes and remained steady throughout the rest of the experiment. The rise in seawater P_{CO_2} caused plasma P_{CO_2} to increase, with a slight delay, to about 10 mmHg (Fig. 1, upper panel). Associated with the rise in plasma P_{CO_2} was a sharp fall in plasma pH from 7·85 to 7·45 (Fig. 1, middle panel). After 0·5 h of hypercapnia, pH started to recover towards control values and levelled off at 0·05 pH units below the original value after 10 h of hypercapnia. This recovery of pH was the result of a considerable increase in plasma bicarbonate concentration from about 5 to 22 mM within the first 10 h of hypercapnia (Fig. 1, lower panel).

The major plasma electrolytes (Na^+, Cl^- and K^+) showed small initial concentration increases after the onset of hypercapnia. Sodium recovered control values within half an hour and remained unchanged for the rest of the hypercapnia period (Fig. 2, upper panel). Plasma potassium exhibited a slow, non-significant fall to values slightly

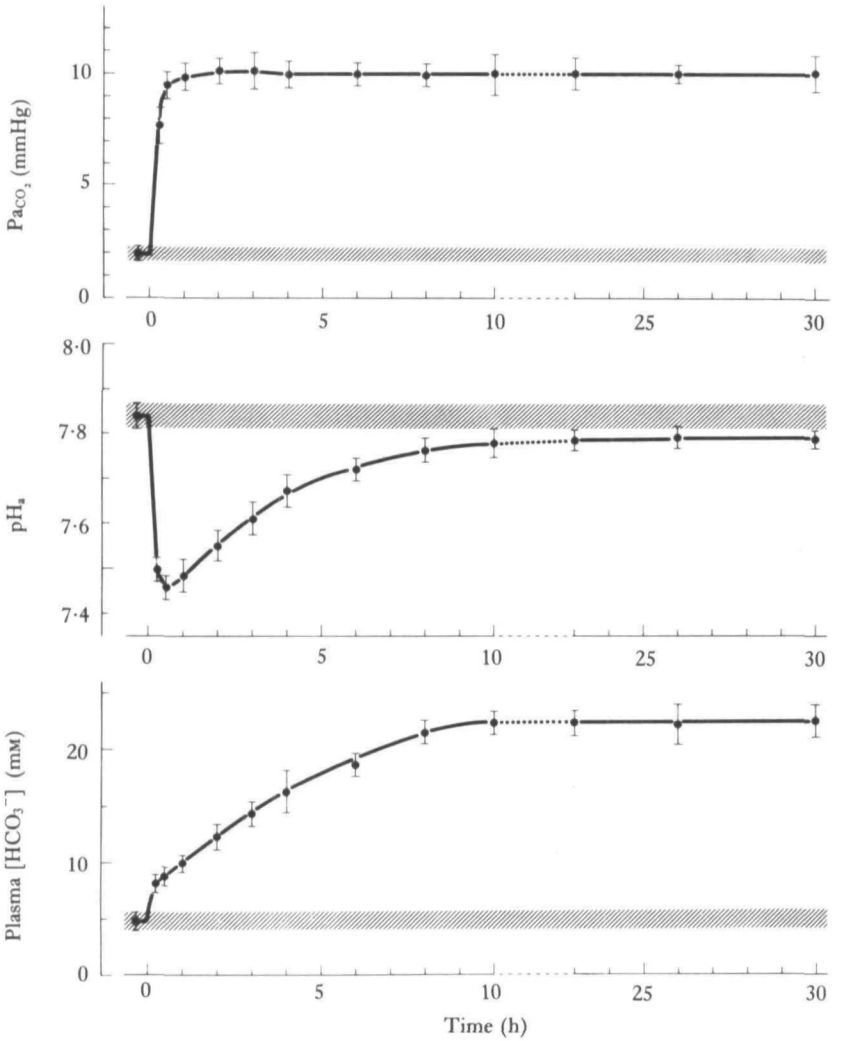

Fig. 1. Plasma P_{CO_2}, pH and bicarbonate concentration in dorsal aortic blood of *Conger* as a function of time after onset of environmental hypercapnia ($\bar{x}\pm$S.E., $N = 10$). Shaded areas represent control values.

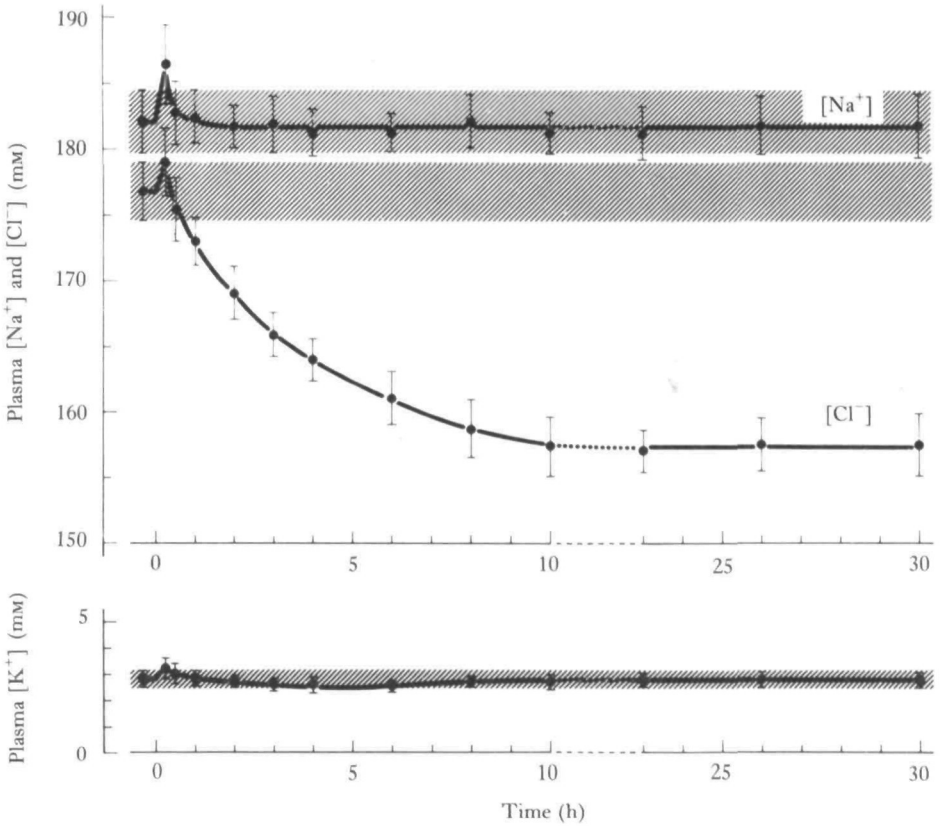

Fig. 2. Plasma Na^+, K^+ and Cl^- concentration in dorsal aortic blood of *Conger* as a function of time after onset of environmental hypercapnia ($\bar{x} \pm$ S.E., $N = 10$). Shaded areas represent control values.

below controls within 5 h, but reattained the prehypercapnic values after 10 h (Fig. 2, lower panel). Plasma chloride concentration dropped below control values within half an hour and continued to fall to values about 20 mM below the controls during the first 10 h of hypercapnia (Fig. 2, middle panel). Plasma lactate averaged $0{\cdot}7 \pm 0{\cdot}3$ mM ($\bar{x} \pm$ S.D.) and no significant differences could be found between control and hypercapnic periods.

During the control period the seawater pH continuously increased as a result of the rise in the seawater bicarbonate concentration. Bicarbonate was released by the fish at a rate of $2{\cdot}78\,\mu mol\,min^{-1}\,kg^{-1}$ body water, slightly less than the rate of ammonia release of $3{\cdot}22\,\mu mol\,min^{-1}\,kg^{-1}$ body water, resulting in an H^+ excretion – as the difference between ammonia and bicarbonate release – of $0{\cdot}44\,\mu mol\,min^{-1}\,kg^{-1}$ body water (Fig. 3). Upon exposure of the fish to environmental hypercapnia, the release of bicarbonate stopped after a delay of about 15–30 min, and reversed into a bicarbonate gain until about 10–12 h after the onset of hypercapnia when the bicarbonate concentration of the water started to rise again. Similarly, the release of ammonia to the sea water stopped initially, but continued at a slightly lower rate during the first 10 h, reattaining the control rate only at the end of the experimental period. This pattern of bicarbonate and ammonia release resulted in a net (hypercapnic minus control) H^+ ion excretion of about $5{\cdot}3\,mmol\,kg^{-1}$ body water, which

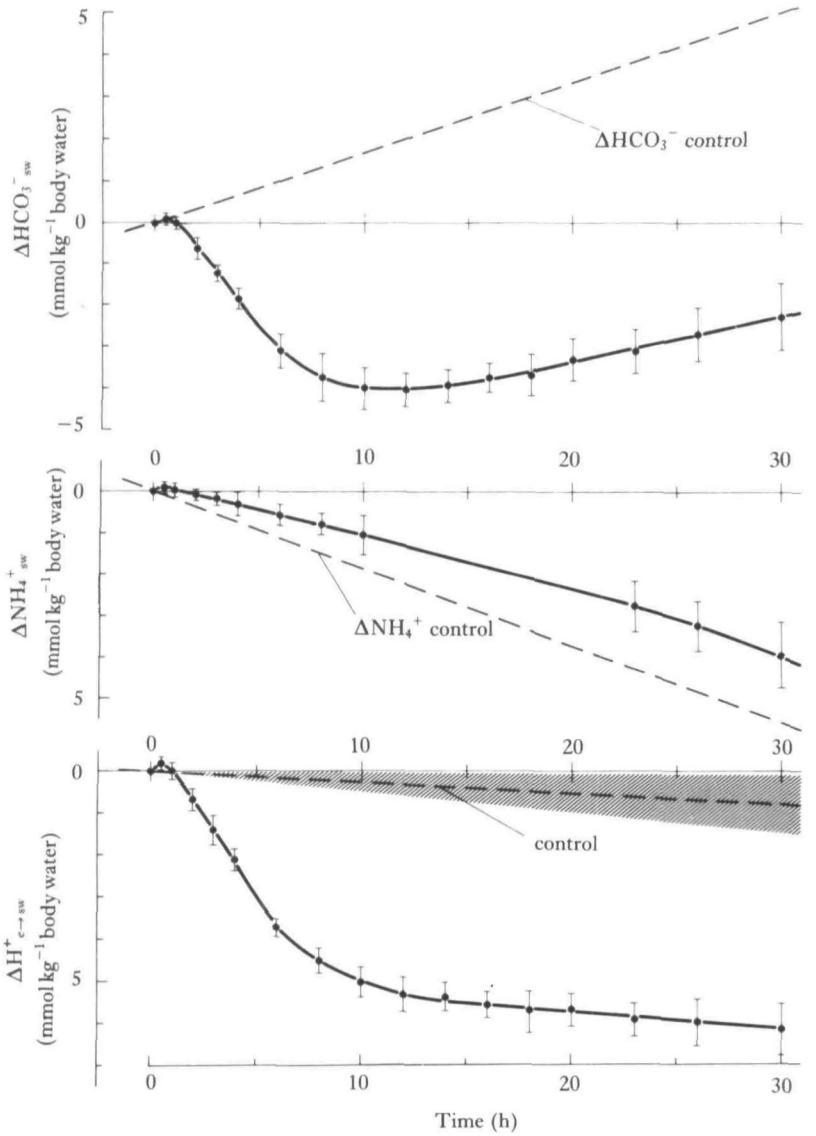

Fig. 3. Changes in bicarbonate and NH_4^+ content in the environmental water of *Conger* after onset of environmental hypercapnia. $\Delta H^+{}_{c\rightarrow sw}$ (lower panel) represents the difference of the above two ions and is the H^+ excretion by the animal ($\bar{x} \pm$ S.E., $N = 10$).

is equivalent to a net gain of bicarbonate by the animal from the environment.

The non-bicarbonate buffer values of oxygenated whole blood (Ht = 18 %) and plasma in contact with erythrocytes (true plasma) were determined to be as follows: whole blood, $\beta_b = 6{\cdot}3$ mequiv l^{-1} pH^{-1}; true plasma, $\beta_{pl} = 7{\cdot}7$ mequiv l^{-1} pH^{-1}.

The haematocrit decrease which occurred throughout the experiment (1–2 %) was statistically insignificant and the mean value for control and hypercapnic periods was $15{\cdot}5 \pm 3{\cdot}9$ % ($\bar{x} \pm$ S.D.).

DISCUSSION

The respiratory acidosis resulting from environmental hypercapnia in *Conger* is almost completely compensated (about 90 % of the expected change in plasma pH at constant bicarbonate concentration) by a roughly four-fold elevation of the extracellular bicarbonate concentration. This appears to be characteristic of most fish species. The time course of compensation in the marine teleost *Conger* is similar to that found in marine elasmobranchs (*Squalus acanthias*, Cross *et al.* 1969; *Scyliorhinus stellaris*, Heisler *et al.* 1976*a*), but is much faster than in freshwater teleosts (e.g. *Salmo gairdneri*: 70 h, Janssen & Randall, 1975; 22–24 h, Eddy, 1976; Eddy *et al.* 1977; *Ictalurus punctatus*: 24 h, Cameron, 1980). This comparison suggests that differences exist for the compensation of respiratory acidosis between marine and freshwater fish rather than between elasmobranch and teleost fish species, and that these differences may be related to the contrasting ionic and osmotic gradients.

The observed increase in plasma bicarbonate concentration by more than 17 mM after 30 h of hypercapnia cannot be attributed to extracellular non-bicarbonate buffering. Since non-bicarbonate buffer values of blood and extracellular fluid are generally low in fish and since the plasma pH during compensated hypercapnia is restored to values very close to the original ($\Delta pH = -0{\cdot}05$), the amount of bicarbonate produced is negligible ($\sim 0{\cdot}03$ mmol kg^{-1} body water) compared to the observed increase in the total amount of extracellular bicarbonate of about $3{\cdot}4$ mmol kg^{-1} body water*. In addition, intracellular non-bicarbonate buffering of CO_2 does not contribute to the eventual complete extracellular pH compensation, since even more than the additionally accumulated $\sim 3{\cdot}4$ mmol kg^{-1} body water of bicarbonate was gained by the fish through transepithelial ion transfer processes. This suggests that part of the net bicarbonate uptake from the environment was utilized for the compensation of the intracellular acidosis. This type of regulation, where additional bicarbonate is transferred from the extracellular compartment to the well-buffered intracellular space, has been found in other fish (*Scyliorhinus stellaris*: Heisler *et al.* 1976*a*; Heisler, 1980; *Synbranchus marmoratus*: Heisler, 1982*b*) and amphibians (*Bufo marinus*: Toews & Heisler, 1982; *Siren lacertina* and *Amphiuma means*: Heisler *et al.* 1982) during steady state hypercapnia.

During the transition from normocapnia to steady state hypercapnia, however, the well-buffered intracellular compartments are involved in the acid-base regulation of the extracellular space. This becomes evident when the fish is modelled as a two-compartment system (intracellular and extracellular space) which is surrounded by the closed third compartment, the environmental water (Fig. 4)†. Immediately after the onset of hypercapnia (time '0', Fig. 4) the amount of extracellular bicarbonate ($\Delta HCO_3^-{}_e$) is sharply increased in spite of the fact that during the first 0·5 h there is a net bicarbonate loss to the environment ($\Delta HCO_3^-{}_{sw \rightarrow e}$, Fig. 4). The extracellular bicarbonate increase is the result of a considerable net transfer from the intracellular to the extracellular compartment ($\Delta HCO_3^-{}_{e \leftrightarrow i}$, Fig. 4). During the first hour, more

* For calculations see Heisler (1982*b*). The extracellular space volume is assumed to be 20 % of the body water, a value similar to values determined in other teleost fish (Heisler, 1982*b*; Cameron, 1980).

† Model calculations assuming the extracellular space to be constantly 20 % of the body water according to formulae presented by Heisler (1982*b*) and Holeton & Heisler (1983).

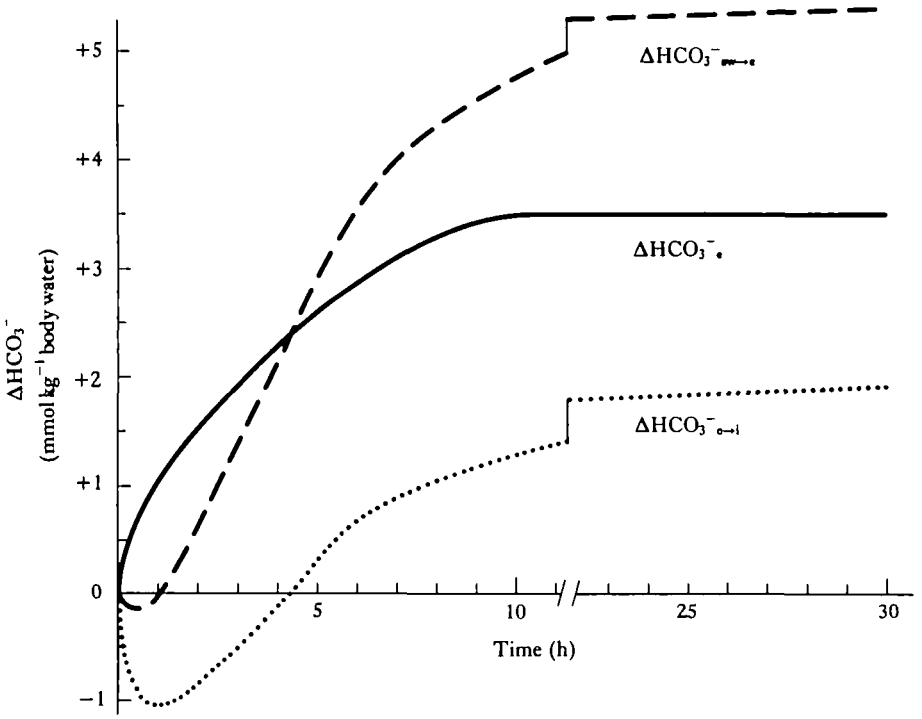

Fig. 4. Change in the amount of extracellular bicarbonate ($\Delta HCO_3^-{}_e$) and cumulative net transfer of bicarbonate between environmental seawater and extracellular space ($\Delta HCO_3^-{}_{sw\rightarrow e}$), and between extracellular and intracellular space ($\Delta HCO_3^-{}_{e\rightarrow i}$) of *Conger* after onset of environmental hypercapnia (calculations from model, see text).

than 1 mmol kg^{-1} body water of bicarbonate is transferred to the extracellular space. About 0·5 h after the onset of CO_2 exposure, the animal starts to gain bicarbonate from the environment and recovers the amount originally lost to the water after the first hour ($\Delta HCO_3^-{}_{sw\rightarrow e}$, Fig. 4). At about the same time the intracellular space starts to recover the bicarbonate transferred to the extracellular space. This process is complete about 4·5 h after the onset of hypercapnia ($\Delta HCO_3^-{}_{e\rightarrow i}$, Fig. 4). The net bicarbonate gained later from the environment ($\Delta HCO_3^-{}_{sw\rightarrow e}$) is utilized to further compensate the respiratory acidosis in both the extracellular and intracellular compartments.

The initial net loss of bicarbonate by the animal should be considered as a lack of adjustment. It may be explained on the basis of a threshold for plasma bicarbonate, which is not yet adjusted to the new hypercapnic condition, a situation analogous to that found in the mammalian kidney (e.g. Pitts, Ayer & Schiess, 1949; Rector, Seldin, Roberts & Smith, 1960). The loss of bicarbonate would thus be due to the discrepancy between the still low (normocapnic) bicarbonate threshold and the suddenly increased plasma bicarbonate concentration resulting from the transfer from the intracellular compartment. In turn, this net transfer of bicarbonate from intracellular to extracellular space may be the result of a similar overflow mechanism at the cell membrane level. With the onset of intracorporeal hypercapnia the intracellular bicarbonate concentration is elevated to a great extent as a result of non-bicarbonate buffering of CO_2 (Heisler & Neumann, 1980; Heisler, 1980). If the

active mechanism responsible for the regulation of the intracellular bicarbonate concentration is not yet readjusted to the new condition, this, in relative terms, muc more pronounced increase in intracellular – as compared to extracellular – bicarbonate concentration may be responsible for the observed transfer to the extracellular space.

The mechanisms by which there is a net bicarbonate uptake of about 5·3 mmol kg^{-1} body water from the environmental water cannot be easily explained. It seems evident that the ammonia mechanism does not contribute to any degree. The release of ammonia is not increased but is even slightly depressed during the early phases of hypercapnia. If *Conger* is similar to the trout (Holeton, Neumann & Heisler, 1983; Cameron & Heisler, 1983), in that at low environmental ammonia concentration a sizeable proportion of the produced ammonia is eliminated by non-ionic diffusion, the depression of ammonia release could be explained on the basis of the reduced absolute partial pressure difference between plasma and seawater as a consequence of the decreased plasma pH. Part of the ammonia produced would therefore be stored in the body and the ammonia concentration would build up until a new steady state for non-ionic ammonia elimination is attained, or, as shown in trout (Cameron & Heisler, 1983), active excretion of NH_4^+ would take over to compensate for the reduced non-ionic diffusion. An alternative mechanism might be a reduction of ammonia production, possibly directing the metabolism to different nitrogenous endproducts induced by the fall in intracellular pH or by the direct interaction with CO_2.

The other two mechanisms in question, active ion exchanges of H^+ against Na^+, or HCO_3^- against Cl^-, cannot easily be distinguished. Fluxes of the respective counter ions of H^+ or HCO_3^- (Na^+ or Cl^-, respectively) cannot be precisely determined against the background of seawater concentrations. The plasma ion concentration pattern (Fig. 3), however, does not favour the H^+/Na^+ ion exchange as the operative mechanism, in that plasma sodium concentration remains constant throughout the experiment. Accordingly, net H^+/Na^+ exchange could have taken place only with a concurrent extension of the extracellular space by about 10%, and the *de novo* dissociation of CO_2 to H^+ and HCO_3^- in the fish. Water shifts of such extent are not expected since extracellular pH is – and intracellular pH is very probably – restored to almost normal, and thus the dissociation of large non-diffusible molecules (proteins) is more or less constant.

The decrease of plasma chloride concentration almost balances the increase in plasma bicarbonate. Since there is a net bicarbonate transfer to the intracellular compartment and since Na^+ – the major candidate for a co-transfer ion – remains constant in the plasma, the chloride difference is very probably the result of an electroneutral transepithelial ion exchange with HCO_3^- against the electrochemical gradient (transepithelial potential difference: positive – see Evans, 1979).

The data obtained in hypercapnic *Conger* clearly illustrate the importance of transepithelial net bicarbonate transfer as a regulatory mechanism to maintain the acid-base integrity in marine teleosts. The net amount of bicarbonate taken up from the environment (~5·3 mmol kg^{-1} body water) is even larger than the prehypercapnic total bicarbonate content of the fish. Equally large quantities of bicarbonate are gained from the environment in the elasmobranch *Scyliorhinus* during environmental (Heisler *et al.* 1976*a*) and hyperoxia induced hypercapnia (Heisler, Holeton & Toews, 1981), and after strenuous exercise (Holeton & Heisler, 1983). Transfer of bicarbonate between

environmental water and fish also contributes considerably to the acid-base regulation after strenuous exercise in the freshwater teleost fish *Salmo gairdneri* (Holeton *et al.* 1983). The absolute quantities of bicarbonate-equivalent ions transferred between fish and water after changes of temperature are smaller, but are still very important for the adjustment of the acid-base status in *Scyliorhinus stellaris* (Heisler, 1978) and *Ictalurus punctatus* (Cameron & Kormanik, 1982). Because of the physical limitations imposed on a fish by the aquatic environment, P_{CO_2} is not readily adjusted (Rahn, 1966) and energetic problems would develop with long term hyperventilation of the viscous medium. Considering also that the extracellular space is generally weakly buffered, it is not surprising that the transfer of ions between body compartments and the environment is probably the most important and widespread mechanism involved in fish acid-base regulation.

The respiratory acidosis resulting from environmental hypercapnia in *Conger* is almost completely compensated in the extracellular compartment by the accumulation of bicarbonate originating from the environmental water. Bicarbonate produced by intracellular non-bicarbonate buffering contributes to the extracellular compensation only during a transitory period of about 4 h after the onset of hypercapnia. After this period, bicarbonate gained from the environmental water is also transferred to the intracellular compartment for intracellular pH compensation. The ammonia mechanism does not appear to contribute to the net uptake of bicarbonate from the environment. Based on the plasma ion balance the uptake is very probably performed by a transepithelial HCO_3^-/Cl^- ion exchange mechanism against the electrochemical gradient.

The authors gratefully acknowledge the support by the Stazione Zoologica at Naples and the skilful technical assistance of Mr G. Forcht and Mrs S. Glage. Life was also partially sustained by 'Il Drago' and the timely activities of the animals outside our barred windows. Supported by Deutsche Forschungsgemeinschaft.

REFERENCES

Benadé, A. J. S. & Heisler, N. (1978). Comparison of efflux rates of hydrogen and lactate ions from isolated muscles *in vitro*. *Respir. Physiol.* **32**, 369–380.

Börjeson, H. (1976). Some effects of high carbon dioxide tension on juvenile salmon (*Salmo salar* L.). *Acta Univ. Ups. Nova Acta Regiae Soc. Ups.* Ser. V.C. **383**, 1–35.

Cameron, J. N. (1971). Rapid method for determination of total carbon dioxide in small blood samples. *J. appl. Physiol.* **31**, 632–634.

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 rainbow trout: physico-chemical parameters, acid-base behaviour and respiratory clearance. *J. exp. Biol.* **105**, 107–125.

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

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.

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.

Eddy, F. B. (1976). Acid-base balance in rainbow trout (*Salmo gairdneri*) subjected to acid stresses. *J. exp. Biol.* **64**, 159–171.

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. (1979). *Fish*. In *Comparative Physiology of Osmoregulation in Animals*, (ed. G. M. O. Maloiy), pp. 305–390. London, New York, San Francisco: Academic 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 fishes. In *Environmental Physiology of Fishes*, (ed. M. A. Ali), pp. 123–162. New York: Plenum Press.

HEISLER, N. (1982*a*). Transepithelial ion transfer processes as mechanisms for fish acid-base regulation in hypercapnia and lactacidosis. *Can. J. Zool.* **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., FORCHT, G., ULTSCH, G. F. & 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.

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. (1980). The role of physico-chemical buffering and of bicarbonate transfer processes in intracellular pH regulation in response to changes of temperature in the larger spotted dogfish (*Scyliorhinus stellaris*). *J. exp. Biol.* **85**, 99–100.

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

HEISLER, N., WEITZ, H. & WEITZ, A. M. (1976*b*). Extracellular and intracellular pH with changes of temperature in the dogfish *Scyliorhinus stellaris*. *Respir. Physiol.* **26**, 249–263.

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**, 303–318.

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

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

PITTS, R. F., AYER, J. L. & SCHIESS, W. A. (1949). The renal regulation of acid-base balance in man. III. The reabsorption and excretion of bicarbonate. *J. clin. Invest.* **28**, 35–44.

RAHN, H. (1966). Aquatic gas exchange: Theory. *Respir. Physiol.* **1**, 1–12.

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.

RECTOR, F. C., JR., SELDIN, D. W., ROBERTS, A. D., JR. & SMITH, J. S. (1960). The role of plasma CO_2 tension and carbonic anhydrase activity in the renal reabsorption of bicarbonate. *J. clin. Invest.* **39**, 1706–1721.

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

TOEWS, D. P. & HEISLER, N. (1982). The effects of hypercapnia on intracellular and extracellular acid-base status in the toad *Bufo marinus*. *J. exp. Biol.* **97**, 77–86.