

BRANCHIAL ION UPTAKE IN ARCTIC GRAYLING: RESTING VALUES AND EFFECTS OF ACID-BASE DISTURBANCE

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SUMMARY

1. Techniques for the measurement of unidirectional flux rates in fish which require no anaesthesia or surgery are described.

2. Resting values for Cl^- uptake at 10 and 17 °C were 8.03 ± 1.11 and $13.52 \pm 0.95 \mu\text{-equiv. } 200 \text{ g}^{-1} \text{ h}^{-1}$ (\pm S.E.), respectively; and for Na^+ the rates were 15.49 ± 0.40 and 26.30 ± 0.36 , respectively.

3. Hypercapnic acidosis caused an increase in Na^+ uptake, presumably through Na^+/H^+ (or NH_4^+) exchange. It is suggested that this is a compensation mechanism leading to the increase in blood buffering observed in response to hypercapnia.

4. Alkalosis was observed following acute temperature increase and was accompanied by an increase in the rate of $\text{Cl}^-/\text{HCO}_3^-$ exchange and also by an increase in Na^+/H^+ exchange.

5. The role of these branchial ion exchange mechanisms in overall acid-base regulation is discussed.

INTRODUCTION

In a theoretical discussion of CO_2 exchange and pH regulation in aquatic poikilotherms, Rahn (1967) has proposed that OH^-/H^+ ratio, rather than $[\text{H}^+]$, is maintained at a constant value as temperature changes. An inverse relationship between blood pH and temperature is now well established for a number of groups, including crustacea (Howell *et al.* 1973; Truchot, 1973), amphibia (Howell, 1970), and fish (Howell, 1970; Randall & Cameron, 1973). Rahn (1967) suggested, and a recent reviewer (Albers, 1970) implies, that the decrease in pH at higher temperatures is accomplished by decreasing ventilation in order to increase the P_{CO_2} of the blood, as occurs in air-breathers. A constant bicarbonate concentration is implied. Randall & Cameron (1973) have recently demonstrated that the P_{CO_2} of the blood of rainbow trout does not increase significantly over a wide temperature range, but that instead the HCO_3^- concentration varies inversely with temperature. These changes in HCO_3^- with increased temperature were not accompanied by any changes in the ratio of ventilation to oxygen uptake (and presumably CO_2 excretion), nor does increased ambient CO_2 produce a lasting change in ventilation (Janssen, 1973; D. J. Randall & J. N. Cameron, unpublished data).

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Hence ventilation and its effects on CO_2 diffusion loss do not appear to have anything to do with regulating pH as the temperature changes. Neither does ventilation appear to participate in pH regulation in the face of environmental hypercapnia. Cameron & Randall (1972) detected no change in the CO_2 gradient between blood and water at elevated ambient CO_2 levels but found pH compensation occurring through an increase in plasma HCO_3^- concentration after 24 h.

An alternative mechanism for pH and bicarbonate regulation must then be sought, and recent work on the exchange of HCO_3^- for Cl^- and on exchange of Na^+ for H^+ or NH_4^+ in the gill suggests that these ion exchanges may possibly play a key role (Maetz & Garcia-Romeu, 1964; Kerstetter & Kirschner, 1972; DeRenzi & Maetz, 1973). An excess excretion of HCO_3^- over H^+ (in exchange for Cl^- and Na^+ , respectively) would lead to acidification of the blood, and to decreased buffering in response to alkalosis. Conversely, an excess of H^+ excretion over HCO_3^- excretion would lead to compensation of acidosis. The dependence of the rates of these branchial exchanges on external ion concentrations has been demonstrated (Kerstetter & Kirschner, 1972; Maetz & Garcia-Romeu, 1964), and depletion of ions internally has been shown to lead to pH imbalance (DeRenzi & Maetz, 1973), but so far there has been no demonstration that acid-base disturbances lead to compensatory changes in the exchange rates. A primary purpose of the present study was to investigate the effects of acid-base disturbance on the rates of $\text{Cl}^-/\text{HCO}_3^-$ exchange and on Na^+/H^+ (NH_4^+) exchange.

The second objective of this study was to determine the quantitative rates of HCO_3^- excretion and ion fluxes under conditions that were as physiological as possible. It was of particular interest to determine whether the direct excretion of bicarbonate could account for a significantly large percentage of total CO_2 excretion; if so, the assumptions of an earlier simulation model (Cameron & Polhemus, 1974) would not hold, and the dynamics of the CO_2 excretion system in the gill would have to be re-examined. It was also of interest to measure the resting rates of ion fluxes under carefully defined conditions of handling and treatment, since such details are seldom generously described in the literature (*cf.* DeRenzi & Maetz, 1973; Kerstetter & Kirschner, 1972; Maetz & Garcia-Romeu, 1964) and are known profoundly to affect the blood pH, electrolytes, and water loss (Hickman & Trump, 1969; Houston *et al.* 1971; Wood, 1971). A considerable effort was therefore devoted to developing techniques for minimizing handling effects and defining the physiological state of the animals being measured.

MATERIALS AND METHODS

Symbols used: \mathcal{J}_i , \mathcal{J}_o , \mathcal{J}_n Influx, outflux, and net flux – superscripts refer to ion species.

\dot{V}_{O_2} , \dot{V}_{CO_2} Oxygen consumption, CO_2 excretion rates. Others defined in text.

Experiments were carried out between October 1973 and February 1975 on Arctic grayling (*Thymallus arcticus* Pallas) obtained from the Chena River, interior Alaska, by electroshocking or by hook-and-line. Mortality from both methods was less than 3%, and any fish appearing unhealthy after a few days was discarded. In most cases

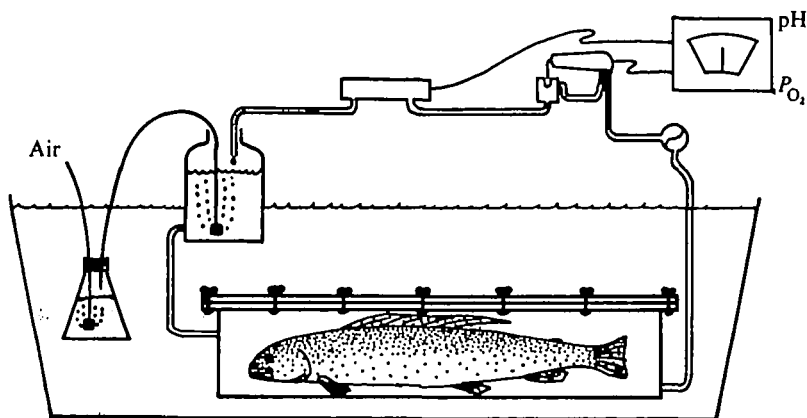


Fig. 1. Recirculating chamber apparatus used in the bulk of experiments. Three air-washing flasks similar to the one shown were used. Temperature jackets for oxygen and pH electrodes are shown at top centre. Water bath was temperature-controlled.

the fish were held in filtered recirculating river water at 10 °C, but some were held at 16 °C (see below). Natural levels of sodium and chloride were very low (≤ 0.1 mM), so for analytical convenience, NaCl was added to the holding tanks to bring concentrations of both ions to 0.9–1.2 m-equiv./l, still well within the natural range for fresh waters inhabited by grayling. All tests were carried out with water from the holding tanks. The fish were fed brine shrimp and chopped liver, but not in the 1–3 days before tests.

Recirculating chamber apparatus

The main components of the chamber developed for the experiments are shown in Fig. 1. The holding chamber is constructed of blackened 12 mm Perspex and has a volume of about 1 l (without fish). Water is pumped with a tubing pump from the chamber through the temperature jackets of a pH electrode and an oxygen electrode, then into an aeration reservoir, and finally back into the chamber. Oxygen tension is maintained by bubbling air through an air stone in the reservoir, the air first having passed through three washing flasks for humidification. The entire apparatus is immersed in a temperature bath, including the air-washing flasks. Total volume of the system with a 200 g fish in the chamber was usually between 1200 and 1400 ml.

This apparatus allows measurement of not only the salt (Na^+ and Cl^-) fluxes but also of the oxygen consumption rates by monitoring flow through the chamber and the P_{O_2} difference between inflow and outflow. Since resting values for grayling were known from previous work (J. N. Cameron, unpublished data), it could be assessed whether or not the animal was in a resting state. Typical results are shown in Fig. 2, an experiment measuring the effects of temperature change (see Results).

Another important feature was that the animals were never anaesthetized, nor were any surgical procedures necessary. Handling of the animals was very limited: animals were gently transferred from the holding tank to a large bucket of water and from there to the chamber by cradling the fish gently in the hands. With practice, the fish could be lifted and quickly transferred without struggling and without having to actually grasp the fish, as there seemed to be a moment's lag in their response. Those

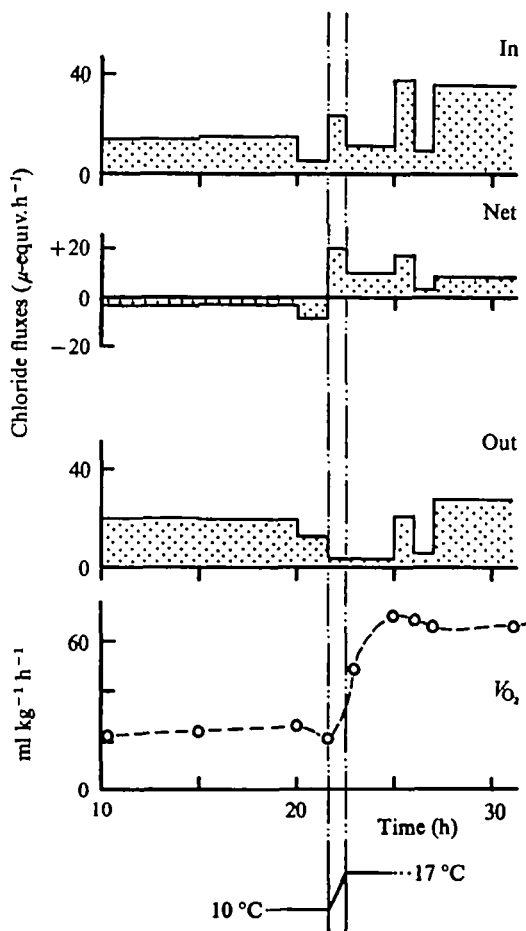


Fig. 2. Results of a typical experiment, showing Cl^- flux rates and O_2 consumption during 10 or 12 h before and after temperature was increased from 10 to 17 °C. The variability, especially just following the temperature rise, is fairly typical, as is the 'overshoot' in O_2 consumption.

fish that did struggle and were handled less gently were returned to the holding tanks for at least 2 weeks.

In some of the early experiments, fish that received rougher handling sustained high net loss rates of Cl^- , and after a time, the influx rates also rose, probably due in part to internal Cl^- depletion. Later, we actually used the net flux rate as a measure of our handling skill; poor handling could cause an increase of two- to five-fold in the rate of salt loss, and so these fish were rejected.

Protocol for the temperature experiments

Protocol for the first set of experiments on the effects of temperature increase was as follows: 500 ml of water from the holding tanks was added to the chamber, followed by introduction of the fish, as explained above. The lid was fastened, then the chamber and the remainder of the system was also filled, and the total water volume was noted. $\frac{1}{2}$ –1 h was allowed for acclimation to the chamber, and 1–3 μCi of Na^{86}Cl

was then added to the system. After a suitable mixing interval, periodic sampling was begun. For each interval, the chamber flow rate, inflow and outflow oxygen tensions and temperature were measured; samples were taken for analysis of the total chloride and ^{36}Cl radioactivity. Total chloride analyses were performed immediately so that we were constantly aware of the status of the animal.

After about 24 h at 10 °C, provided the usual initial loss of chloride had been stopped for at least 12 h, the temperature was raised to 17 °C during a period of $\frac{1}{2}$ –1 h. The sampling routine was then continued for about another 24 h.

As controls, one series of fish was followed at 10 °C for about 48 h and a second series at 17 °C for 48 h. This latter group had been acclimated to 16 ± 0.5 °C for 2–4 weeks.

To document the alkalosis occurring after a temperature increase, catheters were implanted in the dorsal aortae of six fish, following the method of Smith & Bell (1964). After 1–3 days recovery, these animals were put through the procedure outlined above, except that approximately 0.2 ml of blood was taken for pH measurement at each sampling interval. Samples were replaced with an equivalent amount of saline having Cl^- identical to that of plasma.

Protocol for hypercapnia experiments

The methods for the series of experiments testing the effects of hypercapnia were like those for the temperature experiments, except that instead of changing the temperature after the first 24 h, the air was replaced by a mixture of 1% CO_2 in air ($P_{\text{CO}_2} = 7.5$ torr). This mixture was provided by a gas mixing pump (Wösthof), and was checked for accuracy against standard gases.

Sodium fluxes were also measured in this series and in a second temperature series using ^{22}Na . The only modifications necessary were the taking of extra samples for total sodium and ^{22}Na radioactivity analyses, and changes in the isotope counting methods for dual-labelled samples.

At the end of each experiment, the following data were collected: fish weight, final chamber volume, haematocrit, plasma chloride, plasma sodium, plasma specific radioactivity of ^{36}Cl and ^{22}Na .

Chemical and isotope analyses

Oxygen tensions and pH were measured using temperature-jacketed electrodes (Radiometer-Copenhagen) incorporated into the chamber system. Chloride and sodium analyses were done by electrometric titration (Buchler-Cotlove) and atomic absorption spectrophotometry (Perkin-Elmer), respectively. ^{36}Cl activity was assayed by liquid scintillation counting, ^{22}Na by a crystal scintillation detector. In dual-labelled samples, a curve was established for subtraction of the sodium radioactivity from the liquid scintillation counts to give chloride radioactivity.

Calculations

Sodium and chloride influx rates were calculated according to Kirschner's (1970) equation:

$$J_i = \frac{(dQ^* \text{ Vol}) - (J_n X_i)}{(X_o - X_i) t}$$

Table 1. *Chloride flux data for 10 and 17 °C controls*

Parameter	10 °C	17 °C
N	9	4
Wt	205 ± 12	169 ± 12
\dot{V}_{O_2}	50.9 ± 3.5	44.7 ± 5.7
\dot{V}_i	9.21 ± 1.02	13.52 ± 0.11
\dot{y}_e	17.19 ± 0.27	17.74 ± 0.92
\dot{J}_n	-7.78 ± 0.30	-4.22 ± 0.44
\dot{y}_i/\dot{y}_e	0.54	0.76
% Exchange	2.8 ± 0.7	3.9 ± 0.5
Cl ⁻ Space	26.5 ± 1.4	

Weight in g, \dot{V}_{O_2} = oxygen consumption in ml O₂ kg⁻¹ h⁻¹ fluxes in μ -equiv. 200 g⁻¹ h⁻¹. ' % Exchange ' is the proportion of \dot{V}_{CO_2} occurring as Cl⁻-HCO₃⁻ exchange (see text). Chloride space in ml 100 g⁻¹. All values mean ± s.e.

where dQ^* is the change in radioactivity during the sample interval, t , in hours; Vol in ml; \dot{J}_n = net flux in μ -equiv. t⁻¹; X_t and X_0 are the specific radioactivities (cpm μ -equiv.⁻¹) of plasma and water, respectively. The specific radioactivity of plasma was measured at the end of each experiment, then calculated for each sample interval by apportioning the total radioactivity that had disappeared from the bath at each time. Net flux was calculated from the change in concentration and bath volume, and outflux by subtraction. After calculating the flux rates for each time interval, a table was made up showing the elapsed time and cumulative flux. A regression analysis then provided a slope (rate) and a standard deviation for each treatment period. These are the flux rates shown in Tables 1-5. Statistical comparisons of flux rates were made by covariance analysis.

Chloride space was calculated as: $[Q_I^* - Q_F^*] V / Q_P^*$ where Q_I^* and Q_F^* are the initial and final radioactivities of Cl⁻ in the bath (cpm ml⁻¹), V = bath volume (ml), and Q_P^* is the radioactivity per ml of plasma (cpm ml⁻¹) at the end of the experiment. All computations and statistical analyses were performed with programs written for a 32K mini-computer (Nova 800, Data General).

RESULTS

Resting flux rates

Table 1 contains a summary of data obtained in the recirculating chamber apparatus from the fish acclimated and tested for 24-48 h at 10 °C. The first 1-4 h of each experiment were not included in the data analysis, since oxygen consumption and flux rates were often somewhat elevated during the initial period. Using the mean data for each fish, the proportion of CO₂ excretion occurring via Cl⁻/HCO₃⁻ exchange was calculated, based on the assumption that (1) $RQ = 0.9$, and (2) there is a 1:1 stoichiometry between Cl⁻ uptake and HCO₃⁻ excretion (DeRenzi & Maetz, 1973).

Variability of the flux rates over time was fairly large, as has been reported previously (Mackay, 1974). As a consequence, trends or small changes in flux rates could often be detected only by statistical analysis of many observation periods.

A similar summary for 4 fish tested and acclimated to 17 °C is also given in Table 1. Chloride influx was somewhat higher, and the net loss was a little less than at 10 °C.

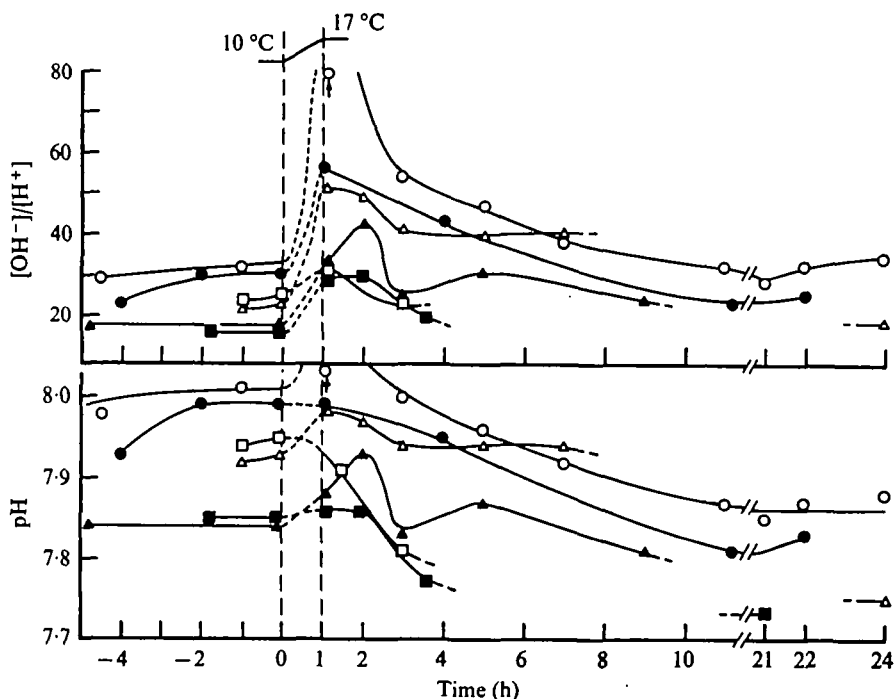


Fig. 3. Time course of pH (bottom) and OH^-/H^+ (top) ratio before and after temperature increase in 6 fish. Fish were acclimated to 10 °C.

The proportion of CO_2 excretion occurring as $\text{HCO}_3^-/\text{Cl}^-$ exchange increased from 2.8–3.9%, but this difference was not statistically significant.

Effects of temperature increase on blood pH

The pH of samples of grayling blood equilibrated *in vitro* at a constant P_{CO_2} of 2.26 torr changed only 0.040 pH units between 5 and 20 °C, a dpH/dT of 0.0027 °C. The time course of pH and OH^-/H^+ ratio following temperature change in 6 grayling is shown in Fig. 3. Data points immediately following the temperature increase resemble the *in vitro* data, resulting in a relative alkalosis at 17 °C. Compensation occurred over the next 24 h, however, so that the final pH was about 0.12 pH units lower than at 10 °C, yielding an *in vivo* slope of 0.017, which is the same as has been reported for rainbow trout (Randall & Cameron, 1973) and most other poikilotherms (Reeves, 1972). At this point the original OH^-/H^+ ratio was restored to the normal range. The severe immediate alkalosis (pH 8.2) following temperature change in one fish is unexplained and was not typical.

The passive pH change expected from the Henderson–Hasselbach equation as temperature is changed from 10 to 17 °C and total CO_2 is held constant is small, probably from about 8.02 to 7.99 based on the pK' -temperature relationship for other fish (Albers, 1970). This is not known precisely because data relating pK' for grayling blood and temperature are not available. In summary, though, relative alkalosis is a result of increased temperature in grayling; in order to conform to a constant OH^-/H^+ ratio, HCO_3^- must be reduced by about 15% in response to a 7 °C temperature rise.

Table 2. *Summary of effects of temperature increase on Cl⁻ flux. Control groups were acclimated to the test temperature, Group B was acclimated to 10 °C. Fluxes expressed in μ -equiv. 200 g⁻¹ h⁻¹, \pm S.E.*

Group	N	10 °C		17 °C	
		\mathcal{J}_i	$\mathcal{J}_i/\mathcal{J}_o$	\mathcal{J}_i	$\mathcal{J}_i/\mathcal{J}_o$
(A) 10 °C Controls	9	9.21 \pm 1.02	0.54	—	—
(B) Temperature change	9	7.47 \pm 1.27	0.66	20.20 \pm 2.36	1.59
(C) 17 °C Controls	4	—	—	13.52 \pm 0.95	0.76

Difference between A and B at 10 °C not significant. All other influx rates significantly different from each other (*t* test, 0.01 level).

Table 3. *Sodium and chloride flux rates for controls and temperature change group (10 and 17 °C, respectively). Rates derived from four experiments, with approximately 24 h data for each treatment. Mean weight = 178 g. All values in μ -equiv. h⁻¹ 200 g⁻¹, \pm S.E.*

	Net Flux		Influx		Outflux	
	Na ⁺	Cl ⁻	Na ⁺	Cl ⁻	Na ⁺	Cl ⁻
Control, 10 °C	-1.27 \pm 0.25	-5.90 \pm 0.34	15.49 \pm 0.40	5.06 \pm 0.27	16.13 \pm 0.38	10.96 \pm 2.31
T change, 17 °C	7.91 \pm 0.11	6.21 \pm 0.12	26.30 \pm 0.36	13.07 \pm 0.42	18.39 \pm 0.38	6.57 \pm 0.42
Change, <i>P</i>	+, < 0.001	+, < 0.001	+, < 0.001	+, < 0.001	+, < 0.01	-, < 0.001
Ratio of $\mathcal{J}_{\text{net}}^{\text{Na}} : \mathcal{J}_{\text{net}}^{\text{Cl}}$	= 1.472				2.801	
Ratio of $\mathcal{J}_{\text{in}}^{\text{Na}} : \mathcal{J}_{\text{in}}^{\text{Cl}}$	= 3.062				2.012	
$[\mathcal{J}_{\text{net}}^{\text{Na}} - \mathcal{J}_{\text{net}}^{\text{Cl}}]$	= 4.631				1.705	
					10 °C	
					17 °C	

Effects of temperature increase in chloride fluxes

Branchial influx of chloride and the ratio of influx to outflux increased after temperature increase in all nine fish studied (Table 2). Following the temperature increase net flux of chloride averaged 7.56 μ -equiv. 200 g⁻¹ h⁻¹, which was also significantly greater than in either the 10 ° or 17 °C controls. There seemed to be a trend for higher influx rates in the first 12 h following temperature increase than in the second 12 h, which would match the time course of alkalosis shown in Fig. 3, but the large inherent variability in fluxes prevented statistical demonstration of such a trend.

Efflux rates (branchial+renal) and temperature were not related, but efflux was weakly correlated with oxygen consumption ($R = 0.36$, $P < 0.05$).

Effects of temperature increase on Na⁺ and Cl⁻ fluxes

When the changes in Na⁺ and Cl⁻ uptake were measured simultaneously in a separate series of experiments, the results were more complicated (Table 3). Although the rate of Cl⁻/HCO₃⁻ exchange was also elevated in these experiments, and the ratio of $\mathcal{J}_i^{\text{Na}}/\mathcal{J}_i^{\text{Cl}}$ decreased, the rate of Na⁺ uptake increased by a larger absolute amount. The four-fold increase in influx-to-outflux ratio for chloride is consistent with the idea that increased HCO₃⁻ excretion is occurring to compensate for the relative alkalosis, but the Na⁺ uptake data indicate that an even greater amount of H⁺ (or NH₄⁺) exchange is occurring. Influx-to-efflux ratio for sodium rose from 0.95 to 1.42. At the

Table 4. *Effects of hypercapnic acidosis on chloride flux values. Values shown are means \pm S.E. for the number of experiments shown in parentheses. For details of treatment see text*

Treatment	\bar{J}_i	\bar{J}_o	\bar{J}_i/\bar{J}_o	\bar{J}_n
Control, 1	11.75 ± 0.67 (7)	18.04 ± 0.65 (7)	0.65	-6.06 ± 0.39
1% CO ₂	5.03 ± 0.11	7.71 ± 0.25	0.65	-2.88 ± 0.26
Control, 2	7.98 ± 0.13 (5)	5.62 ± 0.17 (5)	1.42	3.32 ± 0.14

Table 5. *Sodium and chloride flux rates for controls and hypercapnic treatment (1% CO₂). Rates were calculated from each of four experiments, with approximately 24 h data under each treatment. Means expressed as μ -equiv. 200 g⁻¹ h⁻¹, \pm S.E.*

	Net Flux		Influx		Outflux	
	Na ⁺	Cl ⁻	Na ⁺	Cl ⁻	Na ⁺	Cl ⁻
Normal	-1.55 ± 1.30	-5.73 ± 1.73	15.12 ± 1.40	8.65 ± 1.62	18.33 ± 1.83	14.38 ± 2.77
1% CO ₂	14.93 ± 0.67	-4.27 ± 0.61	25.94 ± 1.30	6.35 ± 1.85	11.02 ± 1.42	9.34 ± 1.33
Change, <i>P</i> +,	< 0.001	+, n.s.	+, < 0.001	-, < 0.10	-, < 0.05	-, $0.05 < P < 0.10$
Ratio of $\bar{J}_{Na}^{Na} : \bar{J}_{Cl}^{Cl}$	= Normal: 1.49, 1% CO ₂ : 1.14					
Ratio of $\bar{J}_{Na}^{Na} : \bar{J}_{Na}^{Cl}$	= Normal: 1.75, 1% CO ₂ : 4.09					
$\bar{J}_{Na}^{Na} - \bar{J}_{Na}^{Cl}$	= Normal: 4.18, 1% CO ₂ : 19.20					

same time the oxygen consumption rose from 31.4 ± 6.3 at 10 °C to 48.4 ± 3.1 ml kg⁻¹ h⁻¹ at 17 °C.

Effect of hypercapnia on chloride fluxes

Results of seven experiments testing the effects of hypercapnic acidosis (1% CO₂, or 7.5 torr) on chloride fluxes alone are summarized in Table 4. In two of these experiments fluxes were studied for an initial control period (approx. 24 h) and a subsequent period of hypercapnia (approx. 24 h). In the other 5, the initial control period was shortened to 6–12 h, depending on how rapidly fluxes stabilized. Following the hypercapnic period (12–18 h) a second control period was employed. The reduction of influx between the first control period and the hypercapnic period was significant overall and in five of the seven individual experiments. Influx increased following return to the control conditions, but was significantly greater in only 3 of the 5 individual experiments.

Effect of hypercapnia on Na⁺ and Cl⁻ fluxes

A series of four experiments was performed measuring both Na⁺ and Cl⁻ fluxes over a 24-h control period and a subsequent 24-h hypercapnic period at 10 °C (Table 5). As in the previous series where Cl⁻ flux alone was measured, changes in the chloride fluxes were small in response to hypercapnia; they were not quite significant statistically in this series. The changes in sodium fluxes were quite pronounced, however, with a large increase in the branchial influx and a significant decrease in the total (branchial+renal) efflux. The ratio of sodium-to-chloride efflux decreased,

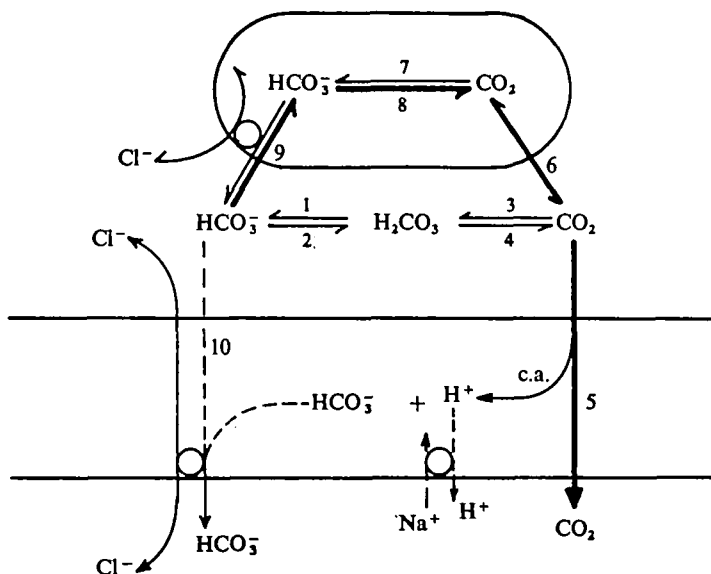


Fig. 4. Principle pools and reaction pathways for CO₂ in the fish gill. Oval shape at top represents erythrocyte in plasma; middle region is gill epithelium, with the water medium toward the bottom. Individual reactions are numbered. c.a. = carbonic anhydrase.

sodium-to-chloride influx ratio increased more than two-fold, and there was an increased net gain of sodium relative to chloride.

DISCUSSION

(A) Methodology

Ion fluxes in teleosts have been studied in three fundamentally different types of apparatus: aquarium preparations using intact fish; perfusion apparatuses using intact, anaesthetized fish; and various forms of isolated gill preparations. Choice of method depends on experimental aims, but from the standpoint of evaluating the quantitative role of various processes in the physiological functioning of a whole fish, the isolated gill method seems clearly inappropriate, and will not be discussed further here.

The second method, as described by Kerstetter, Kirschner & Rafuse (1970) was the first one I tried, since the small bath volume has distinct advantages. I found that the fish sustained high loss rates of salts, that evaporation was a constant problem, and in general that conditions were so unphysiological that the method was unsuitable. The recirculating chamber used subsequently allows natural ventilation, allows monitoring of oxygen consumption, minimizes the need for handling and abrasion of the fish, and does not require anaesthesia or surgery. The fish could also be held in it for long periods, so no experiment had to begin until it was determined that salt loss rates were not excessive and that the animal had reached the resting state (determined by \dot{V}_{O_2}). Disadvantages of the system are the larger volume, which reduces sensitivity to small changes, and the lack of discrimination between renal and branchial ion efflux. Since ion uptake was of primary interest, I judged it more important to avoid the stresses associated with bladder catheterization or blockage.

(B) Role of $\text{Cl}^-/\text{HCO}_3^-$ exchange in CO_2 excretion

The conversion of plasma bicarbonate to CO_2 via carbonic anhydrase in the erythrocyte (or gill tissue) and the subsequent diffusive movement of CO_2 is the principal pathway for excretion of CO_2 (Reaction 9-8-6-5, Fig. 4). Data presented in this study indicate that only 2.8 to 3.9% of the total \dot{V}_{CO_2} occurs via the branchial $\text{Cl}^-/\text{HCO}_3^-$ exchange pathway (Table 1), given that all the Cl^- uptake is in exchange for HCO_3^- as indicated by the recent work of DeRenzi & Maetz (1973). It has not been established whether the source of HCO_3^- for exchange is the plasma pool or HCO_3^- generated from CO_2 within the epithelium.

Comparison with data from other fishes is extremely difficult due to differences in method, lack of temperature data, and the lack of simultaneous measurement of \dot{V}_{O_2} or \dot{V}_{CO_2} in other published work. The chloride fluxes reported per unit weight (200 g) in the goldfish range from 32 to 100 $\mu\text{-equiv. h}^{-1}$ in some studies (DeRenzi & Maetz, 1973; Maetz & Garcia-Romeu, 1964), but as low as 2 $\mu\text{-equiv. h}^{-1}$ in another (Mackay, 1974). Kerstetter & Kirschner (1972) report rates of 22–39 $\mu\text{-equiv. } 200 \text{ g}^{-1} \text{ h}^{-1}$ for rainbow trout using their perfusion method, compared to 8 and 13 for grayling (Table 1). A problem with such comparisons is that fish have frequently been acclimated to low Cl^- levels (0.1 mM or less), which may depress plasma $[\text{Cl}^-]$, and have then been tested at higher concentrations, often about 1 mM (DeRenzi & Maetz, 1973; Kerstetter & Kirschner, 1972).

With these problems in mind, it does not seem safe at present to say whether branchial $\text{Cl}^-/\text{HCO}_3^-$ exchange activity is greater in goldfish than in grayling or trout.

(C) Role of branchial ion exchange in acid-base regulation

The adjustment of buffer status in response to hypercapnic acidosis is clearly not passive; and from *in vitro* measurements, calculations using the Henderson-Hasselbach equation, and studies of more complex buffer systems (Reeves, 1972), the adjustment of buffer status following temperature change is not passive either. Ventilatory control of P_{CO_2} as temperature changes was ruled out by the work of Randall & Cameron (1973) and seems also ruled out in the case of hypercapnic acidosis (Janssen, 1973; D. J. Randall & J. N. Cameron, unpublished results). A consideration of the pathways for CO_2 and HCO_3^- reactions (Fig. 4) would suggest that alteration of the relative rates of branchial $\text{Cl}^-/\text{HCO}_3^-$ exchange and Na^+/H^+ (NH_4^+) exchange offers a mechanism by which the buffer capacity of the blood can be adjusted at constant P_{CO_2} .

In the present study, respiratory acidosis or alkalosis was induced independently of ion concentrations. The significant response of Na^+ uptake to hypercapnic acidosis (Table 5) is strongly indicative of a key rôle for branchial Na^+/H^+ exchange in acid-base regulation. A Davenport diagram of acid-base events occurring during hypercapnia is shown in Fig. 5. Point A was established using data from the grayling (this study). The slopes of AB and AC were taken from Cameron & Randall's (1973) data for rainbow trout. The initial response to hypercapnia is a shift from A to B, following the *in vitro* buffer line (Cameron & Randall, 1972; Janssen, 1973). Over the next 24 h, acid-base status shifts along the P_{CO_2} isoline toward Point C, yielding an A-to-C *in vivo* buffer line. The mechanism proposed for increase of bicarbonate buffering is

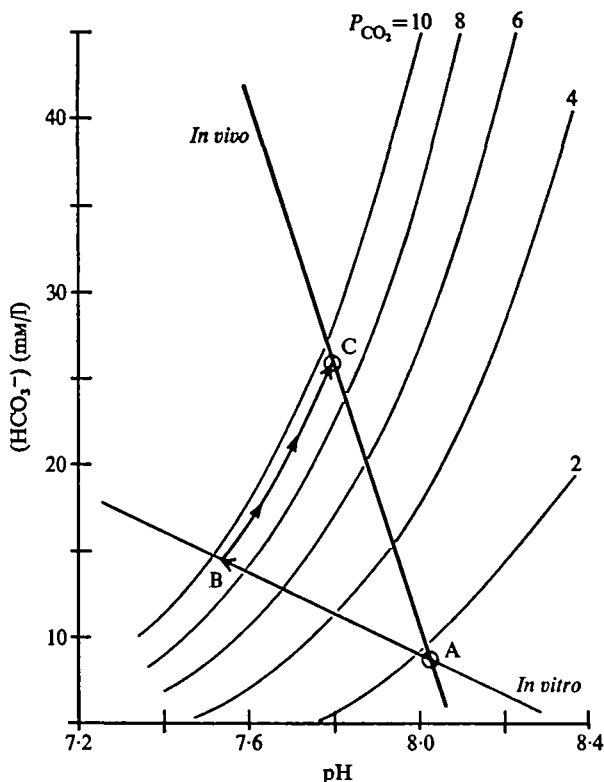


Fig. 5. Davenport diagram showing acid-base events caused by hypercapnia. The initial response is a shift from A to B along the *in vitro* buffer line (Cameron & Randall, 1972; Janssen, 1971), followed by an increase in HCO_3^- leading to C, at which point the original pH is partially restored. Retention of metabolic CO_2 and excretion of H^+ ions resulting from ionization via Na^+-H^+ exchange is presumably the mechanism operating (see Table 5, text).

retention of HCO_3^- formed from metabolic CO_2 , and excretion of H^+ ions in exchange for Na^+ at the gill. Stoichiometric equivalence of Na^+ uptake for H^+ , or its functional equivalent, NH_4^+ , is assumed (Maetz, 1973).

Assuming that HCO_3^- space is about the same as Cl^- space, or 25 % of body volume, we see that a 200 g fish needs to increase HCO_3^- concentration from about 8.5 to 25 mM in 25 % of 200 (= 50) ml of fluid, for a total increase of 875 μM HCO_3^- , and needs to excrete the same amount of excess H^+ ions. The difference between Na^+ and Cl^- uptake increased from 6.47 $\mu\text{-equiv. 200 g}^{-1} \text{ h}^{-1}$ to 19.59 $\mu\text{-equiv. 200 g}^{-1} \text{ h}^{-1}$, a change of 13.12 (Table 5). Since there was no change in the rate of oxygen consumption, presumably there was no change in the rate of metabolic acid production, so that this entire increase can be attributed to buffer readjustment. At this rate, 66 h would be required for grayling to achieve the degree of compensation implied by the *in vivo* slope AC (Fig. 6). Janssen (1973) has shown that the compensation process in the rainbow trout continues up to 72 h, and it seems likely that the grayling have a similar time requirement. Compensation would occur more rapidly if there is significant renal H^+ secretion. Reduction of total Na^+ efflux (Table 5) may be due to increased renal resorption of Na^+ in exchange for H^+ . We do not at present have sufficient data to show how the acid secretion is partitioned between the gill and the kidney, but the

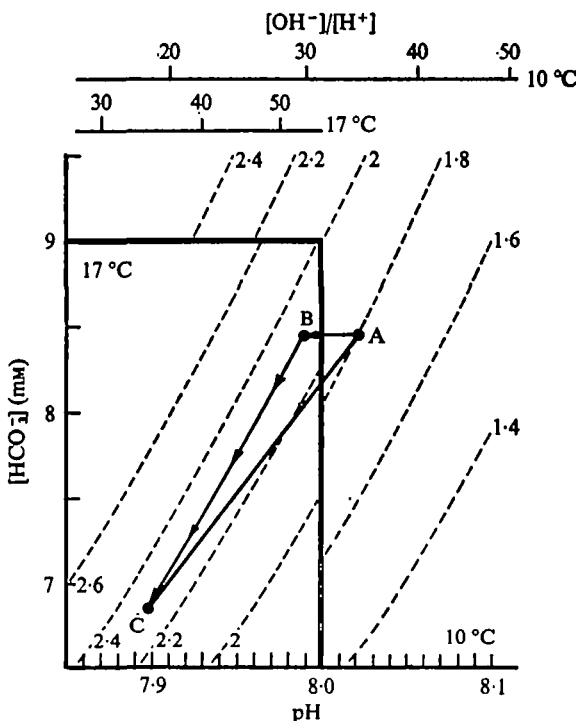


Fig. 6. Davenport diagram showing acid-base events caused by a temperature increase. The normal animal acclimated to 10 °C has values corresponding to point A, with an OH^-/H^+ ratio of about 33. The passive pH change caused by pK' temperature-dependence causes a shift to B, with $\text{dpH}/^\circ\text{C} \approx 0.003$. Subsequent reduction of HCO_3^- by exchange for Cl^- leads to a new steady state at C, which yields the same OH^-/H^+ ratio of 33. Broken lines with numbers show P_{CO_2} isolines. Data for pH taken from Fig. 3, P_{CO_2} from rainbow trout (Randall & Cameron, 1973), and pK' from Albers (1970) and Randall & Cameron (1973).

present study indicates that most, if not all, of it is carried out by the gill during hypercapnic acidosis.

The results from the thermal alkalosis experiments (Tables 2 and 3) are not so clear. On the one hand, the $\text{Cl}^-/\text{HCO}_3^-$ exchange was stimulated by thermal alkalosis, and the ratio of sodium uptake to chloride uptake fell, as predicted by the hypothesis. On the other hand, the absolute increase in sodium uptake was greater than the increase in Cl^- , which argues against the hypothesis. A further complication is that the rate of metabolism rose 54 % (as measured by \dot{V}_{O_2}), and so presumably the rate of metabolic acid production would also rise. Temperature *per se* undoubtedly has other effects on salt balance, such as permeability changes. Whether the observed increase in $\text{Cl}^-/\text{HCO}_3^-$ exchange activity contributed to compensation of the alkalosis, then, depends on knowing not only what is shown in Table 3, but also upon knowing the rate of metabolic generation of excess H^+ and whether there is any change in the excretion of H^+ or HCO_3^- by the kidney. Acid-base events following temperature increase are summarized as a Davenport diagram in Fig. 6, but whether branchial ion exchange mechanisms are responsible for the compensatory shift (B to C) cannot be stated at present.

Of course the control of the rates of these branchial ion exchange mechanisms is not

simply a matter of internal acid-base status. Uptake of both sodium and chloride has been shown to depend upon concentrations of the ions, both internally (DeRenzi & Maetz, 1973) and externally (Maetz & Garcia-Romeu, 1964; Kerstetter *et al.* 1970; Maetz, 1971). The total excretion of CO_2 has also been shown to depend on the availability of external chloride (Dejours, 1969). Conversely, HCO_3^- loading has been shown to stimulate Cl^- uptake, although my own analysis of one of these reports (Kerstetter & Kirschner, 1972) shows that the difference is not statistically significant; and in the other (Maetz & Garcia-Romeu, 1964), it is difficult to say what the effect of the HCO_3^- injection was: does intraperitoneally injected HCO_3^- diffuse as the charged ion into the blood, leading to alkalosis, or does it react with H^+ locally and then diffuse into the blood as CO_2 , causing acidosis?

In summary, the following points emerge: first, that the rates of salt flux in unstressed, resting fish are low in fresh water, leading to long pool turnover times; second, the normal rate of $\text{Cl}^-/\text{HCO}_3^-$ exchange does not constitute a major pathway for CO_2 excretion, amounting to only a few percent of the total; and third, at least in the case of hypercapnic acidosis, the relative rates of $\text{Cl}^-/\text{HCO}_3^-$ exchange and Na^+/H^+ exchange change in a manner leading to compensation of the acidosis. The possibility remains that alkalosis can also be compensated for in this manner, but a more complete picture of renal activity and effects of temperature on metabolic acid production is needed to settle this point. It is tempting to speculate that the basic mechanisms at work in the fish gill are the same as those now studied intensively in the kidney of higher vertebrates (Steinmetz, 1974), and that one of the major physiological changes during the course of the evolution of land animals was a transfer of this entire function from the gill to the kidney.

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REFERENCES

- ALBERS, C. (1970). Acid-base balance. In *Fish Physiology*, vol. 4 (ed. W. S. Hoar and D. J. Randall), pp. 173-208. New York: Academic Press.
- CAMERON, J. N. & POLHEMUS, J. A. (1974). Theory of CO_2 exchange in trout gills. *J. exp. Biol.* **50**, 183-94.
- 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**, 673-80.
- DERENZIS, G. & MAETZ, J. (1973). Studies on the mechanism of chloride absorption by the goldfish gill: relation with acid-base regulation. *J. exp. Biol.* **59**, 339-58.
- DEJOURS, P. (1969). Variations of CO_2 output of a fresh-water teleost upon change of the ionic composition of the water. *J. Physiol., Lond.* **202**, 113P-14P.
- HICKMAN, C. P., Jr., & TRUMP, B. F. (1969). The kidney. In *Fish Physiology*, vol. 1 (ed. W. S. Hoar and D. J. Randall), pp. 91-239. New York: Academic Press.
- HOUSTON, A. H., MADDEN, J. A., WOODS, R. J. & MILES, H. M. (1971). Variations in the blood and tissue chemistry of brook trout, *Salvelinus fontinalis*, subsequent to handling, anesthesia and surgery. *J. Fish Res. Bd. Can.* **28**, 635-42.
- HOWELL, B. J. (1970). Acid-base balance in the transition from water breathing to air breathing. *Fedn Proc. Fedn Am. Soc. exp. Biol.* **29**, 1130-4.
- HOWELL, B. J., RAHN, H., GOODFELLOW, D. & HERREID, C. (1973). Acid-base regulation and temperature in selected invertebrates as a function of temperature. *Am. Zool.* **13**, 556-63.
- JANSSEN, R. G. (1973). The relationship between the arterial blood acid-base status and ventilation in rainbow trout. M.Sc. Thesis, Univ. of British Columbia. October, 1973. 80 pp.

- KERSTETTER, T. H. & KIRSCHNER, L. B. (1972). Active chloride transport by the gills of rainbow trout (*Salmo gairdneri*). *J. exp. Biol.* **56**, 263-72.
- KERSTETTER, T. H., KIRSCHNER, L. B. & RAFUSE, D. (1970). On the mechanisms of sodium ion transport by the irrigated gills of rainbow trout (*Salmo gairdneri*). *J. gen. Physiol.* **56**, 342-59.
- KIRSCHNER, L. B. (1970). The study of NaCl transport in aquatic animals. *Am. Zool.* **10**, 365-76.
- MACKAY, W. C. (1974). Effect of temperature on osmotic and ionic regulation in goldfish, *Carassius auratus* L. *J. cell. comp. Physiol.* **88**, 1-19.
- MAETZ, J. (1971). Fish gills: mechanisms of salt transfer in fresh water and sea water. *Phil. Trans. R. Soc. Lond. B* **262**, 209-249.
- MAETZ, J. (1973). $\text{Na}^+/\text{NH}_4^+$, Na^+/H^+ exchanges and NH_3 movement across the gills of *Carassius auratus*. *J. exp. Biol.* **58**, 255-75.
- MAETZ, J. & GARCIA-ROMEU, F. (1964). The mechanism of sodium and chloride uptake by the gills of fresh water fish *Carassius auratus*. II. Evidence for $\text{NH}_4^+/\text{Na}^+$ and $\text{HCO}_3^-/\text{Cl}^-$ exchange. *J. gen. Physiol.* **47**, 1209-1226.
- RAHN, H. (1967). Gas transport from the external environment to the cell. In *Development of the Lung*, (ed. A. V. S. de Reuck and R. Porter), pp. 3-23. Ciba Found. Symp. London: Churchill.
- RANDALL, D. J. & CAMERON, J. N. (1973). Respiratory control of arterial pH as temperature changes in rainbow trout, *Salmo gairdneri*. *Am. J. Physiol.* **225**, 997-1002.
- REEVES, R. B. (1972). An imidazole alphastat hypothesis for vertebrate acid-base regulation: tissue carbon dioxide content and body temperature in bullfrogs. *Resp. Physiol.* **14**, 219-36.
- SMITH, L. S. & BELL, G. R. (1964). A technique for prolonged blood sampling in free-swimming salmon. *J. Fish. Res. Bd Can.* **21**, 711-17.
- STEINMETZ, P. R. (1974). Cellular mechanisms of urinary acidification. *Physiol. Rev.* **54**, 890-957.
- TRUCHOT, J. P. (1973). Temperature and acid-base regulation in the shore crab, *Carcinus maenas*. *Resp. Physiol.* **17**, 11-20.
- WOOD, C. M. (1971). The influence of swimming activity on sodium and water balance in the rainbow trout (*Salmo gairdneri*). MSc. Thesis, University of British Columbia. 83 pp.