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INTRACELLULAR AND EXTRACELLULAR ACID–BASE STATUS AS A FUNCTION OF TEMPERATURE IN THE FRESHWATER CHANNEL CATFISH, *ICTALURUS PUNCTATUS*[1]

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SUMMARY

The relationship between acid–base status and temperature was studied in the channel catfish, *Ictalurus punctatus*. The change in blood pH with temperature had a slope of −0·0132/°C and involved both a decrease in total CO_2 at higher temperatures, and a significant rise in arterial P_{CO_2}. The acid–base changes in the intracellular compartment were similar to those in the blood, except that for red and white muscle the slope of the change in pH with temperature had a slightly higher value (−0·0185 and −0·0147, respectively), and for heart muscle it had a smaller value (−0·0117).

The net whole-body excretion of acid or base in response to temperature change was relatively small: 0·40 m-mole.kg^{-1} net OH^- was excreted in response to an increase from 22 to 31 °C, and 0·31 m-mole.kg^{-1} net H^+ was excreted in response to change from 25 to 15 °C. In both cases approximately half was excreted renally and half branchially.

Using information on the volumes and buffer capacities of the various body fluid compartments as well as the information above, the ratio of imidazole to phosphate intracellular buffers was calculated to be 5·1 to 1. The amount of intercompartmental (active) transfer required to make temperature adjustments is strongly dependent on the buffer ratio, and on the P_{CO_2}. Without the observed changes in P_{CO_2} with temperature, the transfer requirement would have been 3 to 4 times larger.

INTRODUCTION

Although the inverse relationship between temperature and the pH of fish blood has been known for about 15 years (Rahn, 1967) and subsequently verified for many species, complete acid–base parameters have only been measured in a few. In order for pH to decline at increasing temperature, either the total CO_2 must decline, or

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the P_{CO_2} rise, or a combination of both must occur. Those studies done to date on fish have shown a variable pattern: in the rainbow trout, the pH decrease is accompanied by a decline in total CO_2, with virtually no change in P_{CO_2} (Randall & Cameron, 1973), while in the marine dogfish there is a substantial increase in the P_{CO_2}, with a slight rise in the total CO_2 as well (Heisler, 1978; Heisler, Neumann & Holeton, 1980). The pattern observed in other water-breathers is intermediate, with some small rise in P_{CO_2} and a decline in total CO_2 (Cameron & Batterton, 1978; Cameron, 1978; Heisler, 1980). The air-breathing ectotherms appear to have a more predictable response, with the P_{CO_2} rising at higher temperatures at a rate that results in a constant total CO_2 content (Jackson, Palmer & Meadow, 1974). In the air-breathing ectotherms the ventilatory control of P_{CO_2} is apparently a primary regulator, but the high ventilation requirement in water in order to supply oxygen needs precludes ventilation as a means of regulating P_{CO_2} in water-breathers.

The changes in total CO_2 content are dependent upon changes in strong ion differences (SID), and are generally thought to involve linked ion exchanges in the gills (Maetz & Garcia-Romeu, 1964; De Renzis & Maetz, 1973; Cameron, 1976). Adjustment of pH by change in SID takes longer and requires more energy than if the change were effected by change in P_{CO_2}.

Even fewer data have been published on the intracellular pH in fishes: the dogfish *Scyliorhinus stellaris* has been investigated in some detail (Heisler, Weitz & Weitz, 1976; Heisler, 1978; Heisler & Neumann, 1980; Heisler *et al.* 1980), and preliminary data have been published for *Synbranchus marmoratus* (an air-breather) and *Cyprinus carpio* (carp) (reviewed by Heisler, 1980). These data generally show that changes in intracellular pH parallel those in the blood, and indicate some role for intra-/extracellular transfers in the adjustment to temperature.

The objectives of this study, then, were to investigate the changes in intra- and extracellular acid–base status in a further species of teleost fish as a function of temperature, to assess the relative importance of changes in P_{CO_2} and SID in bringing about pH adjustment, and to measure the branchial and renal acid–base-related excretion during temperature adjustments.

MATERIALS AND METHODS

All the experiments were performed with channel catfish, *Ictalurus punctatus* Rafinesque, obtained from the Adams Fish Farm, Angleton, Texas. The fish were maintained in the laboratory without feeding, in running dechlorinated Port Aransas tap water, and were usually used within two weeks after purchase. The composition of the tap water was similar to local surface waters, with Na^+ and Cl^- averaging about 3 m-equiv. l^{-1}, and Ca^{2+} about 1·6 m-equiv. l^{-1}. The fish ranged in weight from 490 to 1500 g, and were held at temperatures ranging from 22 to 25 °C, at a partial pressure of CO_2 (P_{CO_2}) less than 0·5 torr.

The protocol for most of the experiments was as follows: after at least two days' acclimation to the laboratory, a catheter was surgically placed in the dorsal aorta using the method of Soivio, Nyholm & Westman (1975), under 1:10000 MS 222 anaesthesia. At the same time, a urinary catheter was also implanted using a method

slightly modified from Wood & Randall (1973). The urinary catheter was constructed from polyethylene tubing (PE 60), flared slightly at one end, and enlarged twice about 5 and 10 mm from the tip by heating to form spherical enlargements or 'blebs', that served to make the catheter fit snugly in the ureters. A 1·5 × 2 cm piece of surgical rubber sheet was laid over the papilla externally, its corners fastened with sutures, and the rubber glued to the fish with a cyanocrylate cement. This procedure avoided the use of a ligature around the urinary papilla, which in our experience caused rapid tissue erosion and early failure of the catheters by leakage or loss. Using our procedure, the catheters remained patent for several days.

Surgery usually took about 15–30 min, after which the fish were placed in darkened lucite chambers provided with a copious flow of well-aerated water. At least 24 h recovery was allowed in these chambers before starting any experimentation. As a measure of full recovery from surgery, the blood pH was measured, and if it had not reached stable values after 24 h a second full day of recovery was allowed. At the end of the recovery period, measurements of urine volume flow were made continuously at 2 h intervals (longer at night), and the urine samples were saved for analysis of pH, total CO_2 (C_T), titratable acidity (TA), and ammonia concentration. At various intervals, usually at the mid-point of the urine collection periods, blood samples were taken from the dorsal aortic catheter for analysis of plasma pH, C_T and P_{CO_2} Following 24 h of these control measurements, the temperature of the chamber system in which the fish were held was changed over about a 30 min period to a higher or lower value, at which the fish were held for another 24–48 h. The same blood and urine measurements were continued throughout this second temperature treatment.

At least once during the day following surgery, the fresh flow to the chamber was cut off, and the closed volume re-circulated with constant aeration. The volume of the closed recirculation system was about 3–6 times that of the fish. During these periods, the total ammonia concentration and change in titratable acidity were measured in the water at half-hourly intervals for calculation of net branchial acid–base flux. Titratable acidity was measured by titrating water samples to a pH of 4·000 with 0·010 N-HCl, so that differences from one interval to the next represent changes in the SID of the bath. This procedure was repeated on the second day after surgery, and again at several intervals following the temperature change. Some fish were put through the same protocol, except that no temperature change was made.

For intracellular pH measurements, approximately 10 μCi of [^{3}H]mannitol and 4 μCi of ^{14}C-labelled 5,5-dimethyl-2,4-dioxazolidinedione (DMO) were infused over a 2 min period into the dorsal aorta in a total volume of 0·25 ml saline. The blood (plasma) concentrations and acid–base parameters were measured at half-hourly intervals beginning 1 h later, and at 4 h a final blood sample was taken and the fish quickly killed by a sharp blow on the skull. The blood samples were taken from undisturbed fish via the catheters, so that no acidosis produced by struggling (as in 'grab and stab' sampling) interfered with the results. Samples of white muscle, red muscle, heart and brain were quickly dissected out, weighed fresh, and placed in an oven at 65 °C for drying to constant weight. Six replicates of white muscle, 5 of

red muscle were processed, but due to their small size the heart and brain each yielded only a single value.

Analytical methods and calculations

The acid–base measurements for plasma and urine were made with routine electrode techniques (pH, P_{CO_2}), except that the C_T was determined using a conductometric apparatus constructed by one of us, based on methods outlined by Maffly (1968). The estimation of titratable acidity (TA) in the urine was made by titrating an aliquot of the 2 h collection to the pH of the blood at the mid-point of the collection interval. In a few samples of urine which had pH values higher than blood, titration with base was performed, but since there was no significant titratable base present other than bicarbonate, data from these titrations were not used. Total ammonia concentration of the urine and water samples was measured using the phenolhypochlorite method (Solorzano, 1969).

The mean whole-body intracellular pH was calculated on the basis of the total DMO dose, the concentration in plasma at 4 h (previously determined to be adequate time for equilibration, Cameron, 1980), and the extracellular space determined by extrapolation of the mannitol clearance data to zero time, according to formulas given by Cameron (1980). The dried individual tissue samples were combusted (Packard Sample Oxidizer, Model 306) so that the ^{3}H and ^{14}C radioactivity could be assayed separately, whereas the plasma samples could be counted directly. All radioactivity assays were performed with a liquid scintillation counter (Packard 3255) using the external standardization method, and correcting all samples for counting efficiency.

The calculation of pH_i for each tissue was done according to the formula

$$pH_i = pK_{DMO} + \log\left((DMO_i/DMO_e)(1 + 10^{pH-pK_{DMO}}) - 1\right)$$

where DMO_e = the concentration of DMO per kg plasma water (water content determined by drying aliquots of plasma samples), DMO_i = the concentration of DMO per kg intracellular water, pK of DMO from Heisler *et al.* (1976), and the pH_e was the plasma pH determined at the end of the 4 h equilibration period. In order to calculate the concentration of DMO per kg intracellular water, the [^{3}H]-mannitol activity of the tissue sample was used, along with the final plasma concentration, to correct each tissue sample for trapped extracellular fluid. These corrections were about 7% in the white muscle samples, but much higher for other tissues (see below).

In order to determine the non-bicarbonate buffer value of blood at varying haematocrit, samples were taken from 12 fish and either analysed at their normal haematocrit (which varied from 10 to 32%) or at a lower haematocrit produced by removing some of the cells by centrifugation, followed by re-suspension of the remaining cells. The samples were equilibrated at 0·2, 0·4, 1·0 and 2·0% CO_2 in air, and at each gas tension their pH and C_T were measured as described above. The slope of the plot of pH vs. C_T is the buffer value, β, in m-mole/pH, or slykes. Tissue samples were treated somewhat differently, according to the method of Bate-Smith (1938): fish were rapidly sacrificed, samples of tissue quickly dissected out and weighed

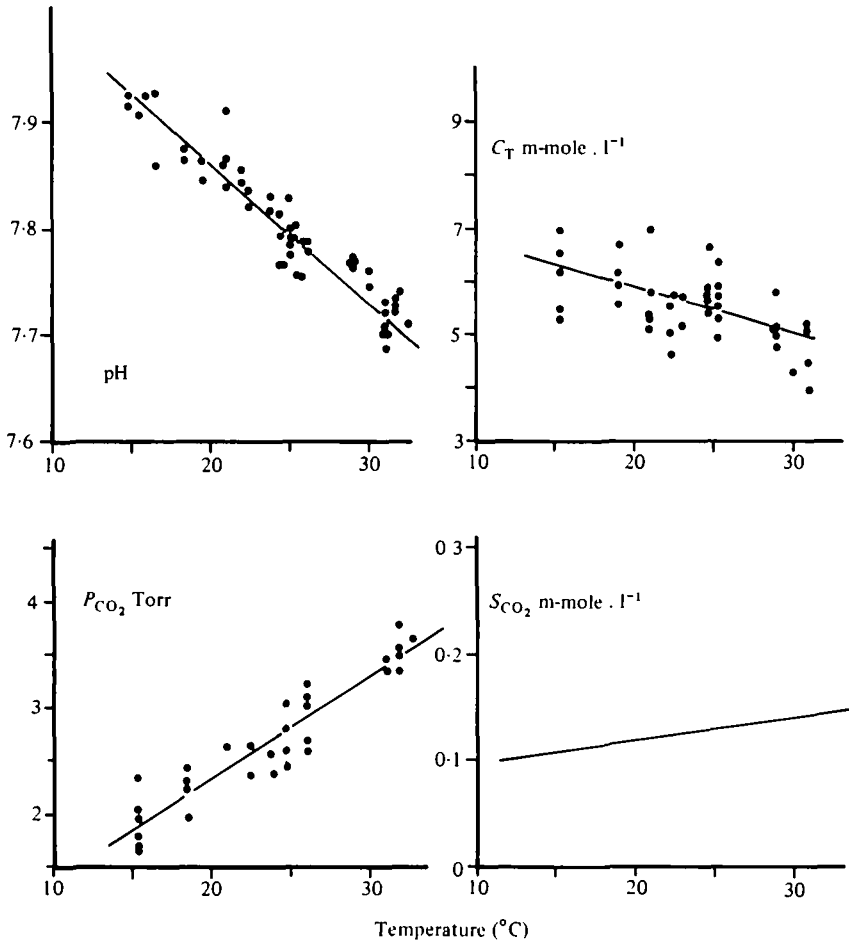

Fig. 1. The relationship between blood acid–base parameters and temperature for catfish held at least 24 h at the test temperature. Equations for regression lines are given in text; the line for S_{CO_2} is calculated from the P_{CO_2} regression line and the CO_2 solubility.

immediately, and the tissue then ground in 10 ml of iced 0·9% NaCl with 20 strokes of a glass tissue homogenizer. These homogenates were then equilibrated in a stirred titration vessel with CO_2-free nitrogen, enough NaOH added to bring their pH to about 8·0, then titrated with HCl to pH 6·5. The slope of the titration curve between 7·1 and 7·5 was taken as the buffer value, since this was the approximate range of intracellular pH.

RESULTS

Blood acid–base parameters

The values for arterial pH, total CO_2 and P_{CO_2} obtained from 61 fish are shown in Fig. 1. Numbers of determinations shown in Fig. 1 vary slightly, since not all data sets were complete; each point represents the mean of from 1 to as many as 12

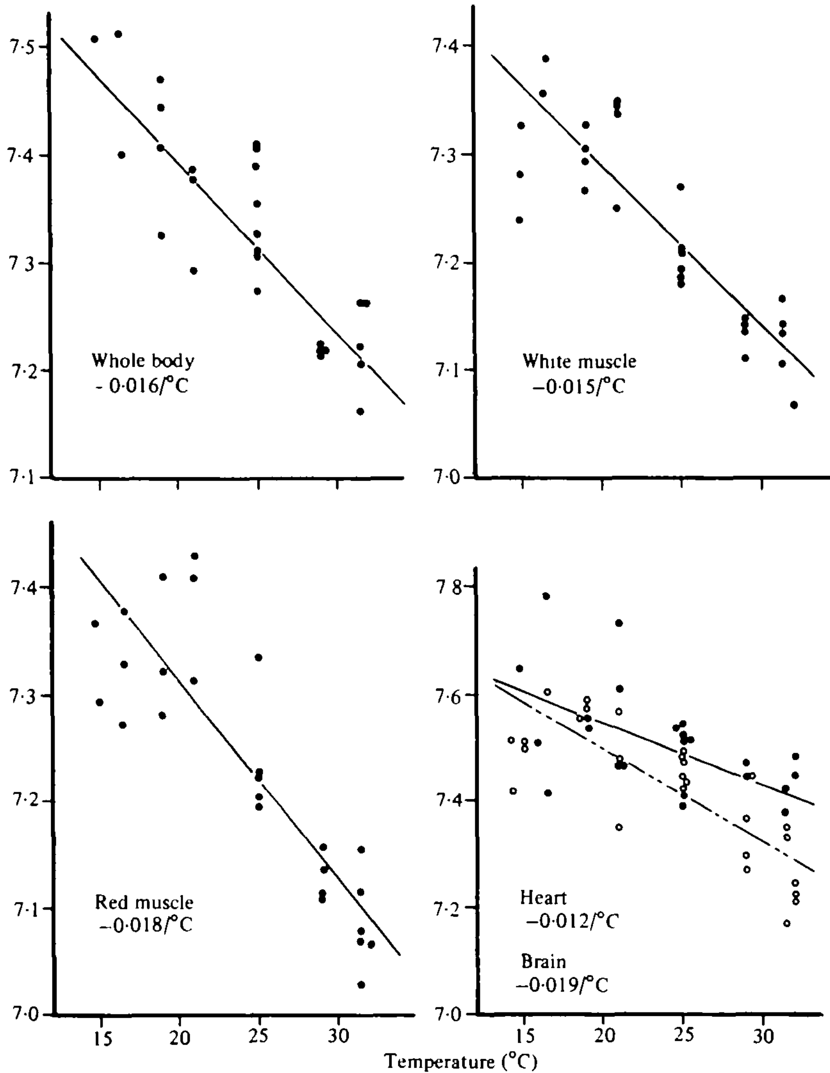

Fig. 2. Relationship of intracellular pH and temperature. Regression equations are given in the text. Each point represents the mean of six determinations for white muscle, five for red muscle, and single values for heart and brain.

Table 1. *Regression statistics for intracellular pH determinations. The equations are in the form* $Y = a+bX$, *where* X = *temperature* (°C)

Y	a	b	$\pm$S.D.$_b$	N
White muscle	7·5836	−0·0147	0·00136	27
Red muscle	7·6832	−0·0185	0·00205	26
Heart	7·7785	−0·0117	0·00362	27
Brain	7·8928	−0·0187	0·00362	28
Mean whole body	7·7073	−0·0157	0·00270	28

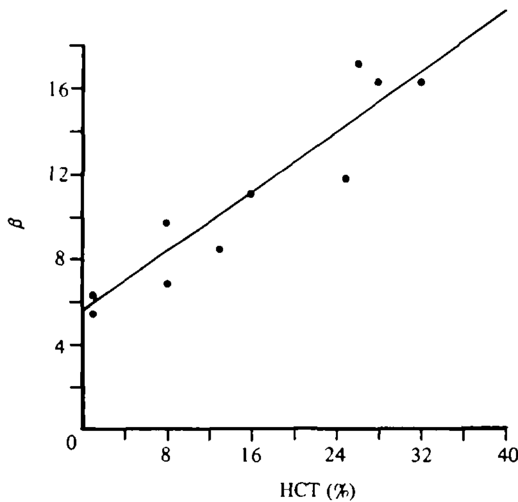

Fig. 3. The relationship between the non-bicarbonate buffer value, β, in m-mole.pH^{-1}, for true plasma from whole blood of varying haematocrit.

measurements from one fish. The regression equations describing the fish mean values were:

$$pH = 8{\cdot}120 - 0{\cdot}0132\,(\text{temp.}), \quad r = 0{\cdot}95$$
$$C_T = 7{\cdot}585 - 0{\cdot}0858\,(\text{temp.}), \quad r = 0{\cdot}60$$
$$P_{CO_2} = 0{\cdot}445 + 0{\cdot}0953\,(\text{temp.}), \quad r = 0{\cdot}98$$

where r is the correlation coefficient (all are significant at the 1% level). In the bottom right panel of Fig. 1, the value for the concentration of dissolved CO_2 (S_{CO_2}) is shown as calculated from the regression line for P_{CO_2} and temperature, and the solubility coefficient for CO_2 at each temperature (Dejours, 1975).

Intracellular acid–base parameters

The data for the calculated mean whole-body intracellular pH (pH_i) are given in Fig. 2, along with data obtained from individual tissue samples of white muscle, red muscle, heart and brain. The statistics for the regression lines on temperature are given in Table 1. Only the slope for heart muscle is significantly different from the rest (5% level, analysis of covariance). While the difference in temperature slope between the mean whole body pH_i and the individual white muscle pH_i values was not significant, the line for the mean whole body pH_i was shifted about 0·1 units upward, thus overestimating the pH of both red and white muscle. Heart and brain had the highest pH_i values, so that at 25 °C the means were: blood 7·803, mean whole body 7·315, heart 7·486, brain 7·396, red muscle 7·221 and white muscle 7·216. That the mean whole-body estimate falls roughly in the middle of the various tissue values is probably coincidental, since the majority of the intracellular compartment consists of white muscle, the rest contributing only a minor fraction (heart and brain less than 1% each).

Table 2. *Total CO_2 pools in a 1 kg catfish at 15, 22 and 31 °C. The values for pH_e, C_{Te} and P_{CO_2} were measured; pH_i data are from Fig. 2 for white muscle; C_{Ti} values were calculated as in Cameron (1980), and the pools – $\sum C_{Ti}$ and $\sum C_{Te}$ – from the above and the volumes of intracellular and extracellular spaces*

	15 °C		22 °C		31 °C
pH_e	7·929		7·832		7·704
pH_i	7·364		7·260		7·128
C_{Te}, m-mole.l^{-1}	6·30		5·50		4·75
C_{Ti}, m-mole.l^{-1}	1·80		1·56		1·35
P_{CO_2}, torr	1·86		2·53		3·40
$\sum C_{Ti}$, m-mole	0·885		0·770		0·667
$\sum C_{Te}$, m-mole	1·178		1·029		0·888
$\sum_{pool}$, m-mole	2·063		1·799		1·555
Δ, m-mole		0·264		0·244	
$\sum_{pool}$, constant P_{CO_2}	2·805		1·799		1·156 (1)
Δ, m-mole		1·006		0·643	
$\sum_{pool}$, constant S_{CO_2}	2·292		1·799		1·499 (2)
Δ, m-mole		0·493		0·300	

(1) These calculations show the pool change from 15 °C if the P_{CO_2} were to remain constant at the 15 °C value.
(2) The pool changes from 15 °C if the product of P_{CO_2} and CO_2 solubility (i.e. S_{CO_2}) remained constant.

Buffer values

The relationship between the haematocrit and the buffer value of the whole blood is shown in Fig. 3, the data in which are described by the linear regression: $\beta = 5{\cdot}76 + 0{\cdot}34$ Hct, $r = 0{\cdot}94$. The mean of the white muscle determinations ($N = 5$ fish, 12 determinations) was 35 ± 2 slykes ($\pm$ s.d.), or 35 m-mole.kg $H_2O^{-1}.pH^{-1}$ between 7·1 and 7·5. (At a range of 6·6 to 7·1, the buffer slopes of the tissue titrations averaged 55% higher.)

Tissue water and ECF volumes

The fractional water contents of the four tissues studied plus plasma, by drying to constant weight were (mean ± S.E.) ($N = 34$ fish):

White muscle	0·7913 ± 0·0018
Red muscle	0·6721 ± 0·0093
Heart	0·8301 ± 0·0032
Brain	0·8289 ± 0·0025
Plasma	0·9511 ± 0·0029

The fractional ECF of the tissues, expressed as a percentage of the total tissue water were:

White muscle	6·92 ± 0·26
Red muscle	18·02 ± 1·08
Heart	44·28 ± 1·92
Brain	13·41 ± 0·57

The very large ECF fraction for heart was probably due to the trabecular nature of the teleost heart (Cameron, 1975), and the analysis of the heart whole, which probably led to retention of blood in the spongy tissue. The red muscle also has a high lipid content, which was quite apparent from the oiliness of the dried tissues, and probably accounted for the low fractional water content of that tissue.

CO_2 pool calculations

In order to assess the CO_2 pool size at various temperatures, some model calculations were carried out, utilizing the data given above, and the procedure described below. Mean values for extra- and intracellular pH are given in Table 2 at 15, 22 and 31 °C, representative temperatures in this study. The pH_e data are taken from Fig. 1 and the pH_i data used were the white muscle values given in Fig. 2. Also shown are the total CO_2 concentrations measured in the ECF (Fig. 1) and calculated for the ICF, as described in Cameron (1980). The P_{CO_2} values are also taken from Fig. 1.

In order to use these data to calculate the total CO_2 pool size, first the extracellular pool size was calculated by multiplying its concentration by the volume of the pool (186 ml.kg^{-1}, Cameron, 1980). A similar procedure was used for the intracellular pool (493 ml.kg^{-1}), and the sum of these two pools was the total (Σ). Then this was done for each temperature, the differences (Δ) between 15 and 22 °C, and between 22 and 31 °C were calculated, and are given in Table 2. Thus there was a 0·264 m-mole.kg^{-1} increase in the total CO_2 pool going from 22 to 15 °C, and a decrease of 0·244 m-mole.kg^{-1} going from 22 to 31 °C. Nearly all of the total CO_2 present is in the form of HCO_3^-, a 'volatile' base, but these numbers may or may not correspond to the measured net H^+ or OH^- excretion following temperature change, depending on the composition of the intracellular buffer pool (see Discussion).

As a measure of the contribution of changes in the various parameters, two further sets of calculations were carried out. The first employed the supposition that P_{CO_2} had not changed, and the resulting pool sizes and the magnitude of temperature-induced changes under these hypothetical conditions are also given in Table 2. Finally, the calculations were repeated using the same data except that the product of P_{CO_2} and solubility (i.e. S_{CO_2}) was held constant. The calculated pool changes would have been considerably larger had either of these conditions been met, which highlights the effect of variations of the P_{CO_2} in reducing the required acid–base-related fluxes.

Acid–base excretion following temperature change

The effects of temperature elevation were studied in 10 fish that had been acclimated to holding temperatures of 22 ± 1 °C. These fish were catheterized on Day 1, control measurements made at 22 °C on Day 2, then further control measurements on the morning of Day 3, following which the temperature was increased in about ½ h to 31 °C. Further measurements were made throughout Day 3, and finally 24 h after the temperature change on Day 4. The net H^+ or OH^- excretion from kidneys was measured by monitoring urine flow rate, pH, titratable acidity, total CO_2 and ammonia concentration. Branchial measurements were made as described above, and included ammonia and titratable acid excretion. The results of the net acid excretion measurements are shown in Fig. 4.

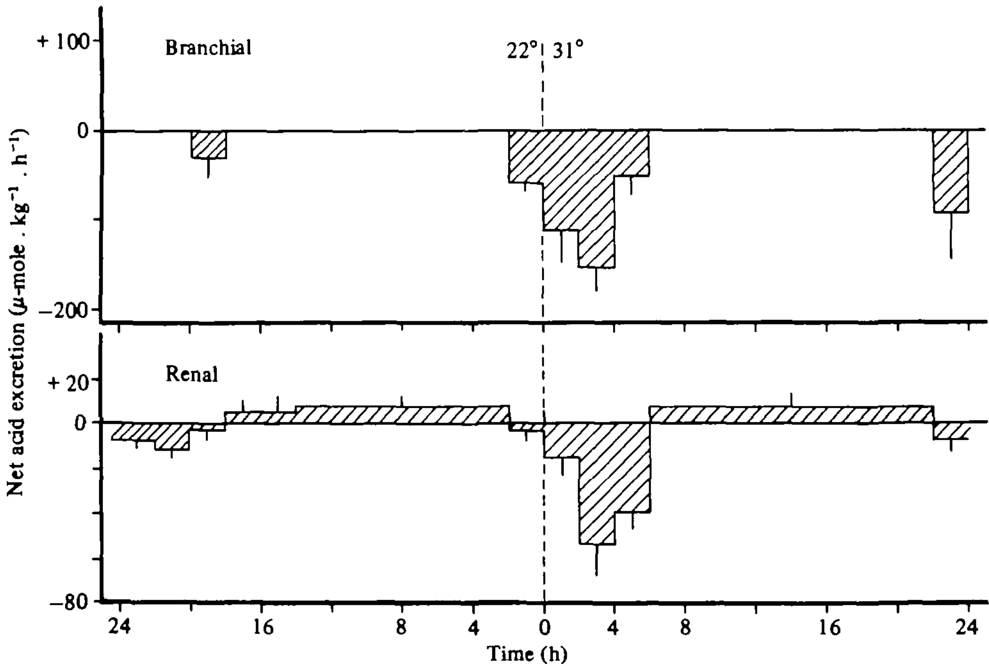

Fig. 4. Net acid–base excretion via the gills (upper panel) and kidneys (lower panel) before and after a change from 22 to 31 °C at 0 h. The vertical bars indicate 1 S.E. of the mean for each interval.

The increase in net OH^- excretion following the temperature increase was statistically significant for the gills at 0–2 h and 2–4 h, and for the kidneys from 0 to 6 h. The total excreted by the gills was obtained by summing the difference between the observed rates from 0 to 4 h and the control rate, taken as the average of the control periods just prior to the temperature increase, and 24 h afterwards. A total of 0·18 m-mole.kg^{-1} net OH^- was excreted above the control rate, and after similar calculations the renal pathway contributed an additional 0·22 m-mole.kg^{-1}, for a total of 0·40 m-mole.kg^{-1} net OH^- excreted. This is about 60% more than predicted by the CO_2 pool calculations in Table 2.

Similar experiments were carried out with 12 fish acclimated to 24 ± 1 °C, with a control period at 25 °C, and a temperature decrease on Day 3 to 15 °C. The results of these experiments are shown in Fig. 5, and whereas there was again a significant change in the branchial excretion rate from 0 to 6 h following the temperature change ($P < 0.05$, one-way ANOVA), the renal data show a small but significant switch from net OH^- to net H^+ excretion that persisted, rather than any pulse of net H^+ output. The summed contribution of the gills was 0·166 m-mole.kg^{-1} net H^+ excreted, and if the 24 h value for the kidneys is averaged with the control rate for purposes of computing a baseline value, the renal pathway then contributed another 0·14 m-mole.kg^{-1}, for a total of 0·306 m-mole.kg^{-1} net H^+ excreted, or about 19% less than predicted for a 10 °C change using values from Table 2.

Unlike the response to infused acid and base loads (Cameron & Kormanik, 1982), the response to temperature did not involve any change in ammonia excretion by

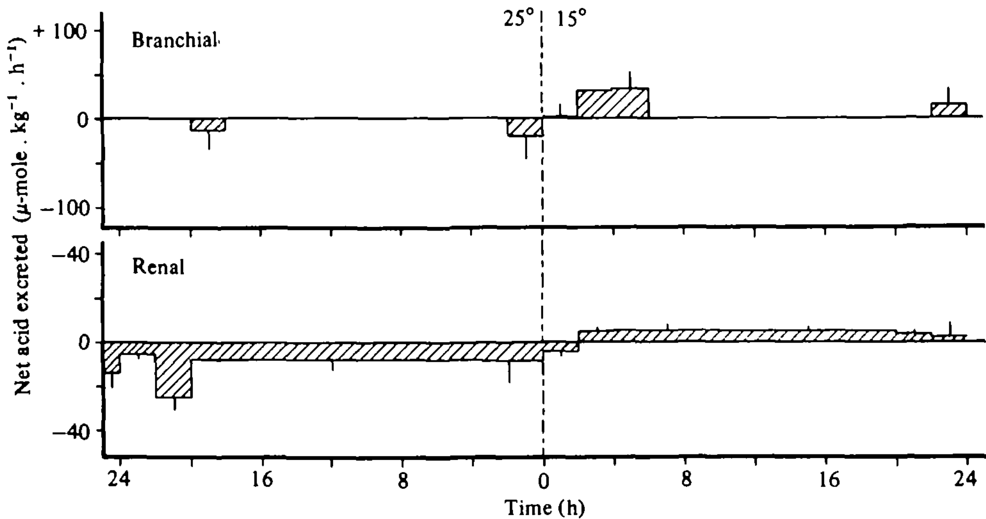

Fig. 5. Net acid-base excretion via the gills (upper panel) and kidneys (lower panel) before and after a change from 25 to 15 °C at 0 h. The vertical bars indicate 1 S.E. of the mean.

either gills or kidneys. There were no significant changes in the rate of ammonia excretion by either route during temperature increase or temperature decrease (one-way ANOVA). The principal change in the urine following temperature increase was a rise in the urine bicarbonate concentration, and after a temperature decrease, a rise in titratable acid. With our methods the branchial response manifests itself as a change in titratable acidity (or alkalinity) of the water, but is not chemically identified.

Before and after the temperature change treatments, blood acid-base parameters were measured at frequent intervals (pH, P_{CO_2} and C_T), and conformed to the patterns shown in Fig. 1. Following the step increase or decrease in temperature, the new values were generally reached in less than 4 h, and remained stable for the remainder of the experiments. In a few cases the experiments were carried on for another day, and no difference was found between 24 h and 48 h values. Thus it appears that the adjustments of the acid-base pool to temperature change were complete within 4-6 h.

DISCUSSION

The general pattern of blood acid-base regulation as temperature changed was similar to that described for several other species of fish (Rahn & Baumgardner, 1972; Cameron, 1978; Heisler, 1980), with a slope of pH_e vs. temperature very close to that often observed, i.e. −0·013 pH/°C. The inverse relationship between pH and temperature was accomplished in the channel catfish by a greater increase in P_{CO_2} and a smaller decrease in C_T than is usually described for teleosts, and so the channel catfish does not really maintain a 'constant content' system' like an air-breathing ectotherm (Jackson *et al.* 1974), nor do they have a 'constant partial pressure' system. The data given here resemble somewhat the data given for 'adult'

dogfish (*Scyliorhinus stellaris*) (Heisler *et al.* 1976), in which there was an increase in C_T at higher temperatures and an even larger increase in P_{CO_2}.

The P_{CO_2} of the blood in a water-breather is not thought to be under active control by gill ventilation, as it is by lung ventilation in the air-breathers. The evidence for this is partly that the ratio of ventilation to CO_2 production declines in the air-breather at high temperature (Jackson *et al.* 1974) so that the P_{CO_2} gradient in the lung (and arterial P_{CO_2}) rises, whereas the ratio of ventilation to CO_2 production (or oxygen consumption) does not change at high temperatures in water-breathers (Randall & Cameron, 1973; Cameron & Batterton, 1978). The critical difference here is that oxygen availability in water dictates a very high rate of ventilation in the water-breather relative to the CO_2 excretion requirements, and so the ventilation rate is partially uncoupled from CO_2.

What then is the probable mechanism for the rise seen in the P_{CO_2} as the temperature changes? Since the total ventilation apparently does not change, there must be changes in either the blood flow in the gill, or the effectiveness of the blood and water flow distribution in the gills. It is, however, a bit puzzling that such effects have not been seen as a reduction in the efficiency of oxygen exchange. In the rainbow trout, the oxygen gradient across the gills apparently does not change between 6·8 and 20 °C (Randall & Cameron, 1973), but there are some changes in O_2 extraction in a water-breathing crab at high temperature (Cameron & Batterton, 1978). The change in P_{CO_2} in the rainbow trout is not as great as in the catfish, however, so the possibility of a reduction in oxygen extraction in this study, parallel to the increase in the CO_2 gradient, should not be ruled out.

Intracellular pH values

The use of the 'mean whole body' method for estimating intracellular pH has been criticized on several grounds, principally that the number derived is not representative of any particular tissue compartment, and that a tissue compartment deviating from the mean whole-body value will bias the estimate in a non-linear way (Manfredi, 1963; Waddell & Bates, 1969). The error resulting from non-homogeneity can be shown to be rather small by a simple model calculation, and in any case the same criticism may be applied to the use of individual tissue determinations by the DMO method, since the intracellular compartment is itself heterogeneous. The comparison conducted in the present study, then, was of some interest, since to our knowledge values obtained by both the mean whole-body method and individual tissue analysis have not been compared in any poikilotherm. The mean whole-body values (Table 1, Fig. 2) were biased upwards by about 0·1 pH unit, but yielded a reasonably faithful estimate of the changes in pH_i of the individual tissues as temperature changed.

Aside from heterogeneity of the various tissue pH_i values, other errors can affect the mean whole-body estimate. Excretion or metabolic breakdown of the DMO seems the most probable, since the other variables that enter into the calculation (total dose administered, final plasma radioactivity, and final plasma pH) may be measured fairly precisely. In an earlier study it was determined that the mean whole-body estimates reach a plateau in 2–4 h, giving an estimate of mixing time

(Cameron, 1980), but the rate of excretion or metabolism of DMO has not been measured in any fish.

The individual tissue values for pH_i (Table 1, Fig. 2) all conform in general to the pH–temperature relationship described for blood, except that pH_i is about 0·5 units below the blood. Although most of the individual temperature slopes were not significantly different from one another (except heart), the pattern of red muscle > white muscle > heart, as reported for the dogfish (Heisler *et al.* 1976) and carp (Heisler, 1980), was observed. It is tempting to speculate that the difference for heart muscle may be related to the much higher concentration of myoglobin (acting as a protein buffer), but that argument would seem to be contradicted by the results from red muscle, which also has higher myoglobin content, and a greater temperature slope. The biochemical consequences of these different pH–temperature relationships are completely unknown, but the differences are sufficiently great that they should be noted by those conducting investigations into pH effects on enzymes of various tissues.

The CO_2 pool and the acid–base pool

The calculations of the total CO_2 pool at various temperatures as shown in Table 2 agree satisfactorily with pool calculations conducted in an earlier study using the mean whole-body method (Cameron, 1980), and give an idea of the change in total CO_2 content attendant upon temperature changes. Since nearly all of the total CO_2 at these physiological pH values exists as bicarbonate ion, the adjustment to temperature would appear to require considerable transfer of bicarbonate between the intra- and extracellular compartments, and between the extracellular compartment and the environment. These transfers, of course, would be much greater were there no change in the P_{CO_2}, as shown by the alternate calculations in Table 2. This suggests that one 'function' of the variations in P_{CO_2} is to reduce the total energy requirement for an adjustment to temperature change, since the transfer processes are almost certainly energy-requiring.

In order to relate the total CO_2 changes to net acid–base change, the composition of the intracellular buffering system must be taken into account. Following the analysis of Heisler & Neumann (1980), the change in intracellular bicarbonate following a temperature change can be described by:

$$\Delta[HCO_3^-]_i = \Delta[HCO_3^-]_{NB} - \Delta[HCO_3^-]_{i\to e}, \qquad (1)$$

where $\Delta[HCO_3^-]_{NB}$ is the amount of bicarbonate generated or consumed by the non-bicarbonate buffers, and $\Delta[HCO_3^-]_{i\to e}$ is the amount transferred from intra- to extracellular compartment. The change due to non-bicarbonate buffers may in turn be calculated for a binary buffer system as:

$$\Delta[HCO_3^-]_{I+II} = \beta_I(\Delta pK_I - \Delta pH) + \beta_{II}(\Delta pK_{II} - \Delta pH), \qquad (2)$$

where the βs are the buffer values due to the buffers I and II, the ΔpKs the changes in the pKs of the two buffers with temperature, and the ΔpH the observed change in pH. The two principal buffers in tissue are the phosphates, with ΔpK values of about $-0{\cdot}002/°C$, and the imidazole moiety of histidine residues, with a ΔpK of

about $-0.02/^{\circ}C$. If the relative values of buffering due to each type are known, then the $i \rightarrow e$ transfer component can be calculated, as has been done for the dogfish (Heisler & Neumann, 1980). In the present study these values were not known, and further, since the change in pH lay between the temperature slopes for the *pK*s of the two types of buffer, we could not predict whether there would be a net increase or a net decrease in intracellular HCO_3^- due to the NB buffers, without knowing the proportion of each.

We could, however, approach the problem from the opposite direction. Using the observed net acid–base excretion (Figs. 4 and 5), the measured total tissue buffering, and the data given in Table 2, we could calculate the amounts of the two buffer types, using an additional equation:

$$\beta_{I} + \beta_{II} = 35 \quad (3)$$

and solving equations (3) and (1), with (2) substituted into (1) for β_I and β_{II}, which are the values for imidazole and phosphate, respectively. The observed net H^+ or OH^- excretion was taken as the amount transferred from intra- to extracellular compartments, values from Table 2 used for calculating the change in the intracellular compartment, and white muscle values of pH_i were used. This approach ignores the non-bicarbonate buffer contribution in the extracellular fluid, but since the volume and buffer value (particularly of the interstitial fluid, which is the bulk of the ECF) is small, the error is minor. The results of this calculation gave an imidazole buffer value of 29.3 and of phosphate 5.7, or a ratio of 5.1 to 1. This is slightly higher than that reported by Heisler & Neumann (1980), who found ratios of about 3.7 to 1 for dogfish white muscle. In view of the approximations necessary in our approach, and the experimental uncertainties inherent in the data, the agreement seems quite reasonable. A change of only 2.7 m-mole.l^{-1} in the buffers, such that β_{IM} were 26.6 and β_{Ph} were 8.4 (a ratio of 3.2 to 1) would lead to a net transfer from intra- to extracellular compartments of zero, which highlights the importance of the buffer ratio in determining transfer requirements, and overall acid–base state.

In all of the foregoing discussion, it is understood that when speaking of bicarbonate transfer one could just as well mean OH^- transfer, or H^+ transfer in the opposite direction. Experimentally it is impossible to distinguish between these alternative processes, and any of the alternatives must also be understood in terms of strong ion transfer between intra- and extracellular compartments. For example, the transfer of a Na^+ ion from ECF to ICF without an accompanying strong anion or counter-movement of a strong cation will necessarily result in an increase in the SID, and consequently in the intracellular HCO_3^- concentration, regardless of whether there is an actual HCO_3^- transport. Thus it may be technically incorrect to speak of HCO_3^- transfer, as Stewart (1978) has emphasized, but it is a harmless convenience so long as the convention is understood. The terms 'net H^+ excretion' and 'net OH^- excretion' have been used here to indicate the end result without specifying the actual species transported.

Taken as a whole, then, the pattern of extra- and intracellular pH regulation in response to a temperature increase in the catfish may be summarized as follows: the pH of the blood decreases in a fashion similar to the change in the p*K* of water,

involving a decrease in the total CO_2 (mostly bicarbonate), and varying degrees of increase of P_{CO_2}. The decrease in plasma pH is similar to the decrease in intracellular pH, which also involves a decrease in intracellular HCO_3^- (or SID), and presumably a proportionate rise in the intracellular P_{CO_2}. The changes in intracellular HCO_3^- appear to be brought about by passive changes in the p*K*s of the dominant non-bicarbonate buffers, phosphates and imidazole compounds, and by transfer of strong ions (bicarbonate, in effect) between the intra- and extracellular compartments. Several features of the system appear to combine to operate in such a way as to minimize the degree of active inter-compartmental transport.

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