BODY FLUID POOLS, KIDNEY FUNCTION, AND ACID-BASE REGULATION IN THE FRESHWATER CATFISH *ICTALURUS PUNCTATUS**

BY JAMES N. CAMERON

Departments of Zoology and Marine Studies, The University of Texas at Austin, Port Aransas Marine Laboratory, Port Aransas, Texas, U.S.A. 78373

SUMMARY

Resting catfish excreted a urine with electrolyte concentrations about $\frac{1}{10}$ that of blood, with very low concentrations of ammonia and lactic acid, only slightly acid pH, and a small resultant net base excretion.

At 26 °C, the intracellular pH was normally 0·51 units lower than the extracellular pH. Both were depressed following the onset of hypercapnia, but there was compensation for both by 24 h, so that pH_i was near control values. Consequently the whole body CO_2 pool increased from 1·95 to 4·77 m-equiv kg^{-1} during hypercapnia, with about 1·50 m-equiv by active means, and the other 1·32 by passive (non-bicarbonate) buffering.

The renal response to hypercapnic acidosis was to switch from a small net base excretion to a net acid excretion, which persisted for up to 10 h, and accounted for about 14% of the total active bicarbonate compensation.

There was no statistically significant renal response to an infused HCl load, but the whole body base excretion rate declined so as to indicate that about 25% of the infused load was excreted in the first 2 h, presumably by the gills.

The catfish kidney appears to participate in acid-base regulation in a significant, but quantitatively minor way, implying that the gills are the principal site of ion transfer in regulating the whole body SID.

INTRODUCTION

It is now well established that nearly all poikilothermic animals regulate their internal pH so as to maintain a nearly constant OH/H ratio over a wide temperature range (Reeves, 1977), and that at any given temperature they defend a pH set-point from various disturbances such as activity and hypercapnia (Cameron & Randall, 1972; Wood, McMahon & McDonald, 1977). It has also been established that the principal mechanism by which the pH and bicarbonate are maintained in water-breathing animals is not ventilatory control of P_{CO_2}, as in air-breathers, but adjustment of the strong ion difference (SID) (Cameron, 1976, 1978; Randall & Cameron, 1973). The SID can be adjusted by changes in the relative rates of Cl^-/HCO_3^- and

Na^+/H^+ exchange, both at the gills and in the kidney. That these exchanges in the gills could regulate internal pH has been recognized for some time, but only recently have any data been provided to show that they do in fact influence internal (blood) pH (Cameron, 1976; DeRenzis & Maetz, 1973). In the case of the Arctic grayling, changes in ion uptake at the gills could apparently account for all of the compensatory adjustment of bicarbonate in response to hypercapnic acidosis (Cameron, 1976).

The evidence that pH regulation is a branchial, rather than renal, process has been largely indirect, however, and somewhat presumptive, since it is difficult to show that changes in Na^+ and Cl^- uptake are causally linked to H^+ (or NH_4^+) and HCO_3^- excretion. Until recently there was almost no information on the role of the kidney in pH regulation, and the little information we now have seems contradictory. Cross *et al.* (1969) reported that there was essentially no response of the dogfish shark's kidney to hypercapnic acidosis, and more recent confirmation of this has been provided by Heisler *et al.* (1976*b*). Cameron & Wood (1978) found that two Amazonian teleosts were capable of excreting a strongly acid urine (as low as pH 4·99), but neither species appeared to respond to a mixed post-operative acidosis by increasing net renal H^+ excretion. On the other hand, Wood & Caldwell (1978) have reported recently that an injected mineral acid (HCl) load is almost completely handled by the kidney of the rainbow trout over 2–3 days.

To provide further data for teleosts, the following series of experiments were performed with the freshwater channel catfish, *Ictalurus punctatus*. First the major body fluid pools were measured by conventional means, and the normal urine output was measured and characterized in acid-base terms. The acid-base characteristics of the extracellular fluid (ECF) were determined from plasma samples, both in control and hypercapnic situations, and in response to injected mineral acid loads. Then the 5,5-dimethyl-2,4-oxazolidinedione (DMO) distribution method was used to attempt an assessment of the acid-base changes in the intracellular fluid (ICF) in response to hypercapnia. From these various studies, a model of whole body CO_2 stores and their response to acid-base disturbance was constructed in order to assess the proportional contribution of the kidney.

METHODS AND MATERIALS

Channel catfish weighing between 500 and 1050 g were obtained from a commercial fish farm, and maintained without feeding in running de-chlorinated Port Aransas tap water. The ambient temperature ranged from 19 to 26 °C during the year in which the experiments were carried out.

Blood sampling was accomplished in free-swimming fish by implantation of a PE50 catheter into the caudal artery. The catheter was put in under MS222 anaesthesia (1 : 10000), using a trocar and stylet for insertion. Since the tap water was quite hard, acidic effects of MS222 were insignificant. Urine sampling was performed by continuous collection from a catheter inserted in the urogenital papilla. This catheter was constructed from polyethylene tubing (Clay-Adams PE50), with the tip flared, two or three side-holes placed near the tip with a hot needle, and a larger diameter sleeve glued about 2 cm from the tip with cyanoacrylate cement. This larger sleeve

also had flared ends, and was used to stitch the whole catheter securely to the skin and the base of the anal fin. Where the catheter was inserted in the papilla, a fine ligature was also placed to prevent leakage or siphoning. All fish were allowed recovery overnight before any experiments were performed following surgery. Return to a stable blood pH was used as a second criterion for recovery.

The pH of blood and urine samples was measured with a microelectrode apparatus (Radiometer-Copenhagen), and total CO_2 (C_T) was measured with a modification of a conductometric method described by Bruins (1963) and Maffly (1968). This method depends upon acidification of samples to convert CO_3^{2-} and HCO_3^- to CO_2, transport of the CO_2 onto a dilute NaOH absorbing column by a CO_2-free (N_2) carrier gas stream, and measurement of a conductivity difference due to Na_2CO_3 formation using a Wescan differential conductivity meter. Ammonia analyses were made with the phenolhypochlorite method (Solorzano, 1969). Sodium and potassium were measured with a flame photometer, chloride with an amperometric titrator (Buchler), and osmolarity with a freezing point osmometer (Advanced Instruments).

In order to calculate the net renal acid-base output, timed urine collections were made, usually at 2 h intervals, except overnight, when 4–8 h intervals were used. The volume and time of each sample were noted to give urine flow in ml h^{-1}. During each sampling interval, usually near the midpoint, blood pH was measured; this pH was used as the reference pH for titration of urine samples. After the urine pH was measured, an aliquot was titrated to that reference pH with 0·01 N-NaOH in an automatic titrator (Radiometer-Copenhagen), and the results expressed as titratable acidity (TA) in μequiv l^{-1}. Another aliquot was used for measurement of total CO_2, as described above, and urine HCO_3^- was calculated from pH and C_T. The remainder of the sample was immediately frozen for later analysis of total ammonia concentration ($NH_3 + NH_4^+$) using the method of Solorzano (1969). The total urine H^+ concentration, ΣH^+, was then calculated as $TA + NH_3 - HCO_3^-$ (where NH_3 includes NH_4^+, all in μequiv l^{-1}), and net renal H^+ flux, J_n^{H+}, in μequiv 100 g^{-1} h^{-1}, was calculated as: (Flow, ml 100 g^{-1} h^{-1}) $\times$ (ΣH^+). At physiological pH, ammonia is 99 + % ionized as NH_4^+, so only a slight error is caused by treating it all as NH_4^+.

The urine pH values measured were slightly higher, and bicarbonate slightly lower than bladder urine because these values were measured equilibrated with air, rather than a probable *in situ* P_{CO_2} of about 3 torr. Anaerobic collection is of no use, since the low rates of urine flow in the catheters lead to equilibration of the urine with air due to the high CO_2 permeability of polyethylene tubing. It is not a significant error, however, since the slight decrease in titratable acidity offsets the slightly lower bicarbonate in the calculations of net renal acid excretion.

For the study of pool dynamics, measurements of total body water, ICF and ECF volumes, and intracellular pH were needed. The total body water content (TBW) was determined by blotting freshly killed fish dry and weighing, then freeze-drying to constant weight and re-weighing. The extracellular fluid volume was determined either with labelled inulin or mannitol; in either case by injection of a known dose and periodic sampling to determine the back-extrapolated zero time concentration. In several fish, the ECF was determined simultaneously with ^{3}H-inulin and ^{14}C-mannitol; the mannitol space averaged 7·0% higher, and all subsequent inulin results were corrected to the mannitol space.

Mean whole body pH was estimated indirectly using the DMO distribution method (Waddell & Bates, 1969). After at least overnight recovery from anaesthesia and surgery involved in arterial catheter placement, 0·3 ml of a solution containing approx. 4 μCi of [^{14}C]DMO and 10 μCi [^{3}H]mannitol (or [^{3}H]inulin) (all from New England Nuclear) dissolved in heparinized saline (plus traces of ethanol and ethyl acetate) was infused over about 2 min. The infusion was followed by 0·3 ml of saline. Subsequently, blood samples were taken at regular intervals, and at each interval the plasma pH, plasma C_T, and total ^{3}H and ^{14}C radioactivity were measured. The latter were measured on a Packard liquid scintillation spectrometer using both internal and external standarization for quench and dual-label correction. For each fish the ECF volume was determined as described above from ^{3}H data (by extrapolation of zero-time concentration); the ICF volume for each fish was calculated as the TBW − ECF. These volumes were then used in the calculation of intracellular pH (pH$_i$) according to the formula:

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

where DMO$_i$ is calculated as the total dose infused less the plasma concentration times the ECF volume, divided by the ICF volume; and using a pK for DMO at 22 °C of 6·20 (Heisler, Weitz & Weitz, 1976a). This calculation involves two assumptions: (1) that the concentration of DMO in plasma is representative of the ECF, and (2) that the distribution of DMO in the ICF is uniform. There are, of course, variations in pH$_i$ from tissue to tissue in fish (Heisler et al., 1976a) as well as other animals (Waddell & Bates, 1969) but the deviations from uniformity cause not very serious errors in the estimation of the (arithmetic) mean pH$_i$ in the direction of the higher pH in the system (Waddell & Bates, 1969). For example, in a fish with a total ICF of 50 ml/100 g, if the true pH$_i$ were 7·35 for 40 ml, and 7·15 for 10 ml, the arithmetic mean pH$_i$ would be 7·31, and the DMO-estimated pH$_i$ would be 7·32. Similarly, if half the ICF were at 7·15 and half at 7·35, the DOM-estimated mean pH$_i$ would be 7·26, rather than 7·25. These errors are within the error limits of the method.

Once the pH$_i$ has been calculated, the intracellular total CO$_2$ (C_{T_i}) concentration can also be calculated by manipulation of the Henderson–Hasselbalch equation for ECF and ICF:

$$C_{T_i} = (10^{(pH_i - pK)} + 1)(C_{T_e})/(10^{(pH_e - pK)} + 1),$$

with a further assumption that partial pressure of CO$_2$ is equal inside and out.

There have been no studies on the rate of uptake and excretion, or on the time needed for equilibration of DMO in fish. The DMO data, however, did show a rapid mixing and equilibration phase, followed by a nearly flat plateau phase; these results are similar to what is conventionally obtained with compartment tracers (Milhorn, 1966) and are similar to the kinetics described by Hinke & Menard (1978), although the time taken for catfish to reach the plateau in vivo, about 2 h, was naturally longer than the 30 min observed for their barnacle muscle fibres in vitro. For the present study, no values prior to 2 h post-infusion were used for any further analysis, and 8 h was arbitrarily selected as the useful limit for each experiment, since calculated pH$_i$ will steadily rise due to the slow elimination of DMO. When longer

Table 1: *Normal values for blood plasma and urine in* 10 *resting catfish at* 26 °C

(All data given as mean ± S.E. All ion concentrations in m-mole l^{-1}, fluxes in μequiv 100 $g^{-1} h^{-1}$.)

| | Concentrations | | |
	Blood	Urine	Urine Fluxes
pH	7·75 ± 0·01	7·40 ± 0·18	—
P_{CO_2}, torr	2·5 − 3·0	—	—
C_r	7·44 ± 0·29	4·02 ± 0·81	2·10 ± 0·61
Na^+	141·5 ± 3·6	12·98 ± 1·44	7·27 ± 0·92
K^+	3·14 ± 0·23	6·32 ± 1·28	2·82 ± 0·61
Cl^-	109·7 ± 1·6	9·13 ± 1·66	5·06 ± 0·99
NH_4^+	0·37 ± 0·03	0·69 ± 0·12	0·34 ± 0·06
Lactate	0·37	0·18	
mOsm	259 ± 3	46·3 ± 4·3	
			J_n^{H+} − 1·48 ± 0·43

periods were of interest, experiments were staggered so that only 8 h data from each was used.

External hypercapnia was induced by passing mixtures of CO_2 and air through a stripping column (Fry, 1951), so that the water entering experimental chambers came to a P_{CO_2} of 10 ± 1 torr. For all other experiments, the stripping column was rapidly flushed with air to get rid of excess CO_2 in the incoming tap water, resulting in P_{CO_2} below the measurement limits (0·5 torr).

Infusions of mineral acid were performed by infusing 1·0 N-HCl in a teleost Ringer solution at a rate of 30 μl min^{-1} with a Sage Model 375 infusion pump. More rapid infusions often caused severe trauma to blood vessels and haemolysis. The dose employed was 1·0 m-equiv kg^{-1}.

RESULTS

Body fluid compartments

The total body water content (TBW) of 14 catfish was 68·0 ± 0·60 g 100 g^{-1} (± S.E.). The ECF volume determined from 25 catfish was 18·7 ± 0·9 g 100 g^{-1}, and so the mean ICF volume, by difference, was 49·3 g 100^{-1}.

Normal kidney output

The normal concentrations of various substances in both blood and urine of catfish at 26 °C are given in Table 1. These data are derived from 14 fish, with a number of determinations per fish ranging from 4 to 27, over time spans ranging from 8 h to 56 h. The mean urine flow rate was 0·50 ± 0·045 ml 100 $g^{-1} h^{-1}$ (± S.E., $N = 78$). The urine had a pH only slightly below the blood pH, which yielded a net resting base excretion, due to the moderate bicarbonate concentration, low ammonia concentration, and low titratable acidity in the resting urine. The lactate concentration was only measured in a few samples, and was always very low. The normal urine electrolytes (Na^+, K^+, and Cl^-) were quite variable in concentration, both in time in any given fish, and among fish. On the whole, the urine represents a significant path of electrolyte loss.

The results of simple correlation analyses of complete data sets are given in Table 2. Interestingly, there was no significant correlation between either urine pH and Na^+

Table 2. *Correlations among blood pH (pH$_B$), plasma C$_T$ (C$_{TB}$) and several urine variables for 14 catfish*

(Only those correlations significant at the 5 % level are shown; asterisks denote $P < 1$ %.
The analysis was performed on 76 complete data sets.)

	Flow	pH$_B$	C$_{TB}$	pH$_u$	Na+	K+	Cl-	NH$_3$	TA	HCO$_3^-$
pH$_B$	—									
C$_{TB}$	—	—								
pH$_u$	—	—	0·42							
Na+	—	—	—	—						
K+	−0·43*	—	0·38	—	−0·38					
Cl-	—	—	—	—	0·94*	—				
NH$_3$	—	—	—	−0·78*	—	−0·49*	—	—		
TA	—	—	0·42*	−0·88*	—	—	—	0·66*		
HCO$_3^-$	—	—	—	0·79*	—	0·37	—	−0·58*	−0·58*	
ΣH+	—	—	—	−0·77*	—	—	—	0·62*	0·68*	−0·88*

concentration, nor urine pH and Cl⁻, as one might expect if the urine pH was controlled by either a Na/H or Cl/HCO₃ exchange mechanism. The various acid-base variables, such as TA, pH, HCO₃⁻ and ΣH⁺ were highly correlated with each other, which means principally that TA (which was not chemically identified in this study) and ammonia excretion tend to rise together, driving off bicarbonate and resulting in higher ΣH⁺ and urinary acid excretion. There was no correlation between flow and either Na⁺ or Cl⁻, although diuresis in other animals is often accompanied by reduced resorption of these ions. Part of the explanation may lie in the low tendency of the channel catfish to show marked diuresis; the urine flow rates were fairly stable even after handling and surgery, and the range of flows examined here for resting, recovered fish was only 0·26 to 0·87 ml 100 g⁻¹ h⁻¹, with the fairly low variance given above.

The response to hypercapnia: blood and urine

Following the onset of hypercapnia ($\approx$ 10 torr), there was a reduction of blood pH, for which there was gradual compensation over the next 10–13 h, accompanied by an increase in C_T (Fig. 1). There was an immediate renal response, as shown in Fig. 1 with net renal H⁺ flux changing from negative to positive in the first hour. The net excretion remained acid for 8 h, and was not statistically the same as control values until 10 h after the onset of hypercapnia. The integrated renal response, taken as the time-weighted sum of the area above the dotted line in Fig. 1, amounted to 20·5 μequiv 100 g⁻¹ over the 10 h compensation period. This response was evident in each of the ten fish studied, but there was no clear pattern of increase in either TA or ammonia; one or the other was elevated in different fish, but the net H⁺ excretion was elevated in all 10 fish studied. Although the C_T of plasma continued to increase slowly beyond 10 h, I found no evidence that the renal response continued longer. A small change persisting over a longer time would, however, be nearly impossible to detect in view of the inherent variability of J_n^{H+} (Fig. 1).

During hypercapnia, the correlations amongst blood and urine variables were quite similar to those for the control data shown in Table 2, except that some correlations were reinforced, such as among acid-base variables, and a significant correlation between pH$_B$ and urine ΣH⁺ was evident.
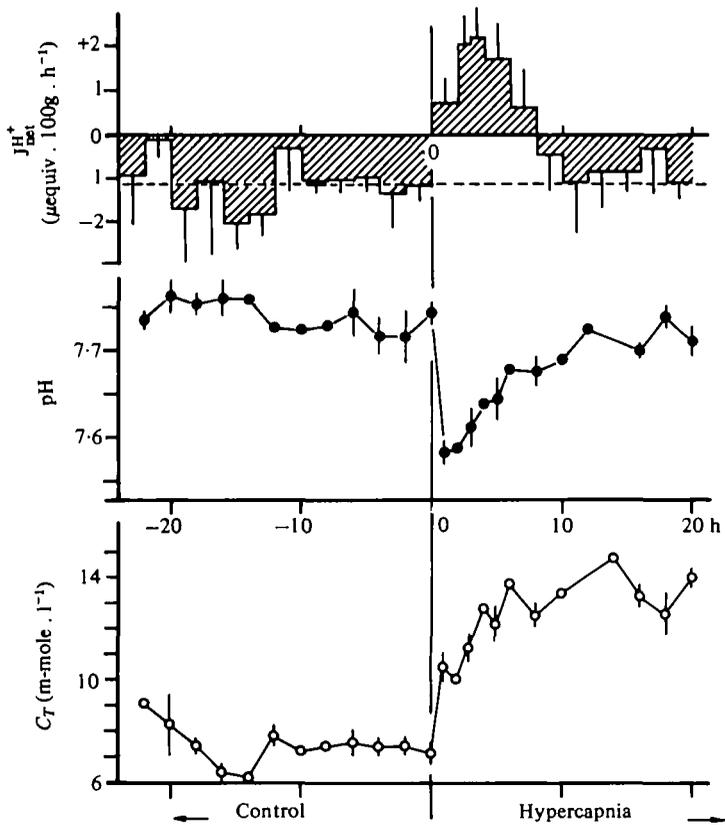
Fig. 1. Blood and urine acid-base variables for 10 catfish during a control and a hypercapnic period, showing the time course of pH and C_T compensation, and the time course of the renal response. The vertical bars are plus or minus one standard error.

Intracellular pH

The values of intracellular pH determined from DMO studies of 12 fish at 19–22 °C are shown in Fig. 2 as a function of time before and after the onset of hypercapnia (10 torr). The rise in pH_i in the first 2 h reflects the time taken for equilibration of the infused DMO, and is similar to the time needed for equilibration of inulin and mannitol amongst body fluid compartments. The DMO concentration appeared to reach a plateau at about 2 h – the 2·25 h and 3 h values were not significantly different from each other – so a 2 h period was routinely allowed for equilibration before any data were used. The mean control value for the pH of blood was $7·865 \pm 0·017$ ($\pm$ s.d.), $N = 22$; and the mean control value for pH_i was $7·354 \pm 0·059$, $N = 18$, giving a mean difference of 0·511 pH units. The difference in pH_B between these data and those shown in Fig. 1 yields a temperature slope of $-0·018$ pH/°C, which is consistent with a large body of literature (Cameron, 1978; Reeves, 1977).

Following the onset of hypercapnia, pH_B dropped by about 0·22, and pH_i about 0·13, and thereafter rose slowly over the next 24 h. All values for 4–8 h (1–5 h after
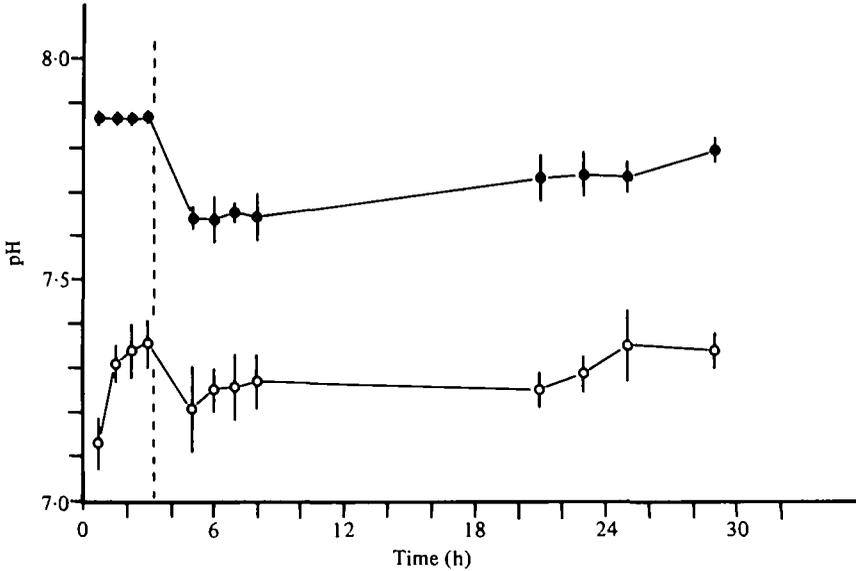
Fig. 2. Intracellular (○) and extracellular pH (●) for 12 catfish at 19–22 °C. Vertical bars are one standard deviation. The dotted vertical line shows the beginning of the hypercapnic period ($P_{CO_2} \approx 10$ torr).

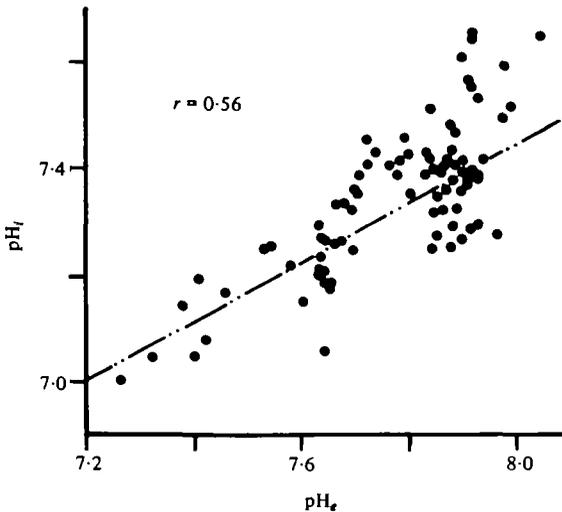Fig. 3. Scatter diagram of pH_i and pH_e (= pH_B), showing the correlation coefficient (r), and the least squares regression line (see text) drawn through the points. $N = 65$.

the beginning of the hypercapnic period, Fig. 2) are significantly different from the control (0–3 h) data ($P < 0.01$). By 21 and 23 h, the pH_B was stable, but still significantly different from controls. By 25 h, the pH_i was statistically the same as the initial control value. At 25 h, the mean pH_i was 7.351 ± 0.088, $N = 14$; and the mean pH_B was 7.739 ± 0.035, $N = 11$, for a difference of 0.388 pH units.

From these 12 fish, 65 individual data pairs were taken after the 2 h equilibration

Table 3. *An analysis of the total CO_2 pools of ECF and ICF for a hypothetical 100 g catfish*

(Calculations are described in the text.)

	ECF	ICF	Total
Normal			
Volume, ml	18·6	49·3	68·0
C_T m-mole l^{-1}	5·63	1·82	—
Total pool, μmole	105	90	195
Hypercapnic			
C_T, m-mole l^{-1}	12·05	5·10	—
Total pool, μmole	225	251	476
Change: μmole	+120	+161	+281
Passive buffering*	32	99	131
Active compensation	88	62	+150
Renal contribution			21
			(14%)

* Buffer value, β, was measured as 10 for ECF, and was assumed to be 20 for ICF (see text).

period, and the data are plotted in Fig. 3 with a fitted least squares regression line, where $pH_i = 3\cdot100 + 0\cdot5426\ pH_B$. The correlation coefficient shown is significant at the 1% level.

The whole body CO_2 pool

An analysis of the whole body CO_2 pools, and the principal changes caused by hypercapnia, is given in Table 3, using data derived from the experiments described above. The TBW measurements, minus the extracellular space (taken as mannitol space), provide a measurement of the intracellular water, or ICF, of 49·3 g/100 g. For both the ICF and the ECF, then, the total CO_2 content can be calculated as the CO_2 concentration times the compartment volume. The C_T values used for the ECF were taken from the experiments shown in Fig. 1 at 25–26 °C, producing a change in the ECF C_T of 120 μequiv 100 g^{-1}. Some of this change, however, will be due to the passive nonbicarbonate buffer capacity of the plasma proteins; *i.e.* as P_{CO_2} increases and reacts to form HCO_3^- and H^+, the H^+ is buffered by proteins, and C_T increases some by purely passive processes. Thus in order to assess the active component of compensation, the amount of C_T increase due to this passive buffering should be subtracted from the total increase in C_T. The mean buffer capacity, β, for catfish blood was found to be 10 slykes (unpublished data), and with a pH change immediately following the onset of hypercapnia of about 0·2 (Figs 1 and 2), the passive component is 32 μmole. The active component, by difference, is 88 μmole (Table 3). A similar analysis has been carried out for the ICF, except that C_T has been calculated from pH_i data obtained at 19–22 °C (Fig. 2), and corrected downward by $-0\cdot018$ pH/°C (Cameron, 1978; Heisler *et al.*, 1976a; Reeves, 1977). The C_T calculations were carried out as described above. Estimation of the active and passive components is more difficult for the ICF, since there is no buffer value known for the whole body ICF compartment of fish. The value for β of 20 was arbitrarily selected for this exercise. It seems a reasonable value in comparison to various published mammalian muscle values (Heisler & Piiper, 1971), and is consistent with the change of pH_i immediately after the onset of the hypercapnia, which is about half that of pH_B (Fig. 2) which might indicate roughly twice the buffer capacity

(Waddell & Bates, 1969). Using a β of 20, the calculated active compensation of the ICF (Table 3) is 62 μmole, making a total of 150. From data presented in Fig. 1, we have already seen that the renal response amounts to some 21 μequiv excreted, and so accounts for about 14% of the total active compensation. Higher or lower values of β for the ICF will affect this percentage, of course; the extreme values would be 23% if all the ICF buffering was passive, and 8% for an ICF β value of zero.

Mineral acid infusion

The infusion of 1000 μequiv kg^{-1} of HCl into the caudal artery over a 20- to 30-min period caused a depression of blood pH of approx. 0·2 units, and a variable, but small, decrease in C_T of up to 1 m-mole l^{-1}. This disturbance had invariably disappeared completely after 2 h, with blood pH not statistically different from pre-injection values (10 fish). In order to document the handling of this fixed acidosis, two sets of experiments were conducted: in the first, the animals were fitted with blood and urine catheters, and urine collections made for a 12 to 24 h control period. At the end of the control period, the HCl was infused as above, and blood and urine collections continued for up to 36 h afterward. In the second set of experiments, the animals were confined to a small closed-circuit but well-aerated chamber system (Cameron, 1976), with a total water volume of about 2·5 l. At 30-min intervals, water samples were taken and titrated to pH 4·0 with 0·010 N-HCl. Changes in titrant required to reach pH 4 from one interval to the next plus measured changes in ammonia were expressed as net acid or base excretion of the animal on a weight specific basis, taking into account the changing bath volume due to sample removal. Resting (control) acid excretion by the whole animal (with no urinary catheter) was followed for 2 to 3 h, after which HCl infusions were given as in the first set of experiments. The whole body acid excretion was followed for another five hours after the infusion.

There was no marked and reproducible renal response to the HCl infusions in the 5 catfish studied. Resting renal $J_n{}^{H+}$ was $-1\cdot10\pm0\cdot41$ μequiv 100 g^{-1} h^{-1} ($\bar{x}\pm$ S.E.) for the control periods, and $+0\cdot26\pm0\cdot68$ for the post-infusion period, which was not significant (by paired t test). Net excretion did become more acidic in 4 of the 5, but there was high variability in all of the data.

The whole body acid excretion response of 5 further fish is shown in Fig. 4, where it can be seen that (1) the resting $J_n{}^{H+}$ in all fish was negative (i.e. there was a net resting base excretion), and (2) there was a short transient reduction of the net resting base excretion which lasted only about as long as the blood pH depression, or about 2 h. It is difficult to quantify this response; the changes in slope were significant in 4 of the 5 cases (by analysis of covariance, $P < 0\cdot05$), and projection of the initial slopes showed that about 200 to 300 μequiv kg^{-1} less base had appeared after several hours than would have been the case if there had been no change in excretion rate. That is, 20 to 30% of the acid infused appears to have been excreted during the first 2 h, the other 70 to 80% has not. Clearly, any significant excretion had occurred across the gills, and not via the kidney, since the renal difference pre- and post-infusion was only 13·6 μequiv kg^{-1} h^{-1}, and that was non-significant.

Since the HCl load disappears from the blood in 2 h, and only at most $\frac{1}{3}$ of it appears outside the fish, then the balance must be buffered either in the intracellular
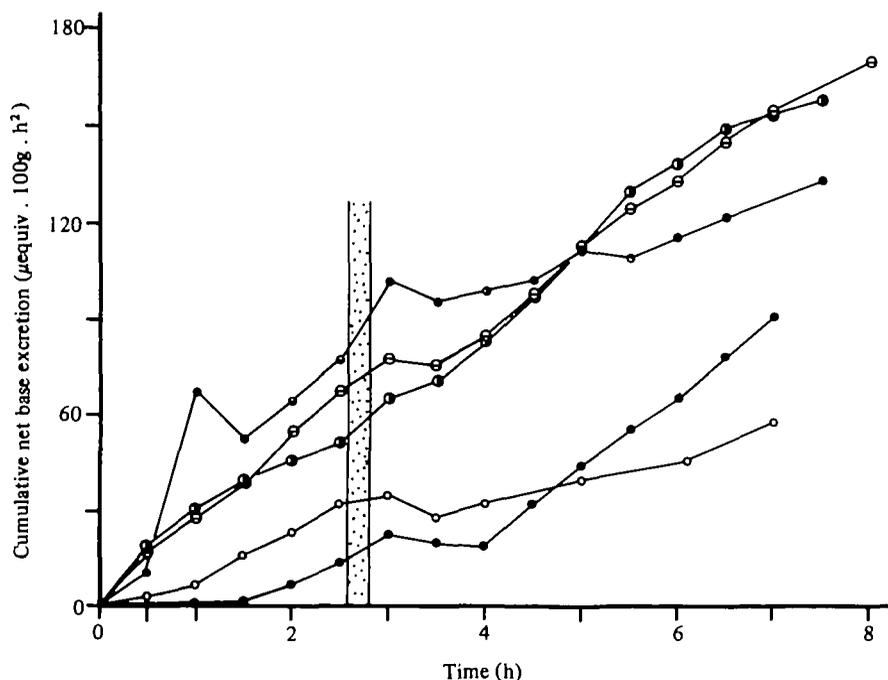
Fig. 4. Cumulative net base excretion for five catfish before and after infusion of 100 μequiv 100 g⁻¹ of HCl (vertical stippled area).

or bone compartments. If there is a slow excretion of the balance either via kidney or gill, the present methods are not sensitive enough to detect it, since any difference would be less than the natural variability of J_n^{H+}.

<div align="center">DISCUSSION</div>

Methods

Most of the methods employed in this study have been used for physiological investigations in fishes before, and most are generally considered routine procedures. The determination of fluid compartment volumes, for example, using radioactive marker substances, has been carried out for a large number of fish species (Hickman & Trump, 1969). It is generally agreed that inulin and mannitol yield slightly different values for their respective distribution spaces, and that neither exactly measures the extracellular fluid space, but both markers are widely used to approximate the ECF volume, given those caveats. It has been reported that inulin under some conditions is lost through the gills (Beyenbach & Kirschhner, 1976) but this is unimportant for ECF determination so long as the zero-time extrapolation method is being used.

It must be remarked that, in the present study, urine values obtained are probably somewhere intermediate between those for bladder (voided) urine and tubular urine, since the collecting catheters tend to reduce the residence time in the bladder by a variable amount. The urinary bladder has been shown to have some resorptive function in fish (Renfro, 1975), but its possible acid-base function has not been investigated.

The greatest number of assumptions, and the greatest uncertainty, are associated with the application of the DMO distribution method to the measurement of pH_i. This method, as originally described by Waddell and Butler (1959) for skeletal muscle, and later reviewed by Waddell and Bates (1969) and Hinke and Menard (1978), has been shown to provide an estimate of pH_i that agrees quite closely with micro-electrode studies, with pH_i calculated using the CO_2 method, and with other weak acid and weak base distribution studies. The meaning of 'mean whole body pH_i' has been debated variously (Waddell & Bates, 1969; Manfredi, 1963), and it has been pointed out that in multi-compartment systems with different pH's in the different compartments, DMO will yield a value closer to the higher pH (see calculations above, and Waddell & Bates, 1969), but that error is small. There has been as yet no direct validation of the method in teleost fish, but two arguments can be made in favour of its probable validity: first, agreement with other methods has been obtained in tissues from a phylogenetically diverse group of animals (Hinke & Menard, 1978), and second, the tissue-to-tissue variations of pH_i found in fish (Heisler *et al.* 1976a) and other animals (Waddell & Bates, 1969) are not that large, and are not likely to alter the 'mean' pH_i by a significant amount. Finally, the data show that the method provides consistent data from any individual with time, and from one fish to another; the method is sensitive to changes produced with various experimental treatments; and the bulk of the ICF is contained in a single tissue type, the white skeletal muscle, in teleosts.

Results

The fluid and electrolyte functions of the catfish kidney appear to be much like those of other teleosts (Hickman & Trump, 1969), and in many respects similar to the closely related brown bullhead, *Ameirus nebulosus* (Hodler *et al.* 1955). Sodium and chloride resorption are moderately effective in *Ictalurus*; the flow rates are quite typical for freshwater teleosts at the relatively high temperatures of this study, and the resting values of lactate, NH_3, and TA are fairly low. The normal acid output of the kidney was negative, indicating a net base excretion of $14.8\ \mu$equiv kg^{-1} h^{-1} for these fasted catfish. Acid-base values have been published for only a few other teleosts: two Amazonian teleosts were recently reported to have fairly high rates of resting acid excretion, 26 and $48\ \mu$equiv kg^{-1} h^{-1} (Cameron & Wood, 1978); the rainbow trout had only $2\ \mu$equiv kg^{-1} h^{-1} acid excretion (Wood & Caldwell, 1978), and goldfish showed a net base excretion (Dejours, Armand & Verreist, 1968).

The blood acid-base changes following initiation of external hypercapnia, as shown in Fig. 1, have a time course quite similar to that of the compensation described now for several species of fish (Cameron, 1976; Cameron & Randall, 1972; Eddy *et al.* 1977). In a study of the responses of the Arctic grayling to hypercapnia, I suggested that the entire compensatory increase in the bicarbonate reserves was accomplished by increases in Na$^+$/H$^+$ exchange relative to Cl$^-$/HCO$_3^-$ exchange in the gills (Cameu, 1976), in accord with evidence for these linked exchanges (Maetz & Garcia-Romeu, 1964) and for their participation in pH regulation (DeRenzis & Maetz, 1973). Until now, the importance of the kidney in hypercapnic compensation had not been determined. The present study demonstrates conclusively that there is a renal com-

ponent to the process, but that it is a small part of the total compensation (Fig. 1, Table 3). The urinary acid output is sustained for as long as the blood pH is disturbed, but only 14% of the calculated pool increase comes about via renal action (Table 3).

There is some uncertainty in the exact figure, since the active component of the compensation depends on the accuracy of each of the pool size calculations, and upon the accuracy of the value used for β, the buffer capacity of the intra- and extracellular compartments. The error involved in estimation of plasma β is probably small; there is not that much variability among various individual catfish (unpublished observations), and a value of 10 slykes is similar to published values for other teleosts (Dejours, 1975). Also, the passive component is only about one quarter of the total for the plasma compartment. Errors in estimation of the intracellular buffer value will have a larger effect, however, since the pool is smaller, and the buffer capacity is bound to be larger. The value of 20 was chosen as an average of published values for mammalian muscle, and on the basis of relative changes in pH_B and pH_i (Fig. 2), but there are great differences related to method of measurement of β, and the particular tissue examined (Heisler & Piiper, 1971). In the most extreme case, a buffer value between 30 and 40 slykes, which is not impossible, would mean that all the intracellular compensation was passive, and the renal contribution to the total would rise to 23%. If this were the case, however, pH_i would not drop immediately in hypercapnia (Fig. 2) and then recover; the pattern of response seen supports the idea of a small initial pH drop due to intermediate β, then a secondary recovery due to an active component.

The response to infused HCl is a bit more difficult to assess, especially when comparing the results of this study to those of Wood and Caldwell (1978) on the rainbow trout. In this present study, the change in renal acid excretion was not statistically significant, but even if it had been, not more than 50 or 60 μequiv of the 1000 μequiv kg^{-1} infused could be accounted for in the urine, whereas monitoring of the whole body acid excretion rates indicate that 200 to 300 μequiv kg^{-1} of the load is excreted in this time (Fig. 4). In compensating fixed acid loads, as well as hypercapnic acidosis, then, the catfish appears to rely primarily upon the gills, and to a minor extent also upon the kidney. This is quite different from what Wood and Caldwell (1978) reported for the trout. They found that essentially all of a much smaller HCl load, only 100 μequiv kg^{-1} h^{-1}, could be accounted for in the urine during a 48 h period. They did not measure whole body acid excretion, however.

Retention of H$^+$ in tissue compartments for fairly long periods has been reported for elasmobranchs (Piiper, Meyer & Drees, 1972), and it is likely that much of the infused H$^+$ is sequestered in tissues, presumably to be released and excreted slowly over a long period. It is possible that the rate of compensation of acid-base disturbances is related to the normal rates of branchial ion uptake or exchange, and to the ionic content of waters normally inhabited. Correlated with their higher rates of ion flux, the catfish compensate for external hypercapnia in only about 12 h (Fig. 1), as opposed to 24–72 h for trout (Cameron & Randall, 1972; Janssen & Randall, 1975), and 24 h or more in grayling (Cameron, 1976). Lower ionic concentrations in the environment may favour less 'leaky' fish, and a correspondingly greater role for the kidney.

The intracellular pH data presented here are the first published for any teleost, fish, so far as I know, and along with Heisler *et al.* (1976*a*) data on dogfish, and some unpublished data on the air-breathing *Synbranchus marmoratus* (Heisler *et al.* 1978; Heisler, personal communication) constitute all that is known of intracellular pH regulation in water-breathing animals. *Synbranchus marmoratus* is a rather special case, since it lives in extremely ion-poor waters of the Amazon basin, and switches from water-breathing at a low blood P_{CO_2}, to air-breathing, with an attendant much higher P_{CO_2}. The intracellular pH tends to remain nearly constant under these *in vivo*, open system conditions. Both the catfish and the dogfish (*Scyliorhinus*) maintain pH_i 0·4 to 0·5 units below pH_B, which is less than that for *Synbranchus*, but similar to mammalian tissues (Adler, Roy & Relman, 1965). The intra- and extracellular pH values tend to be correlated fairly closely in both catfish and dogfish, but neither show evidence of any plateau region over which pH_i is held constant (Heisler, 1975), in contrast to *Synbranchus* (Heisler *et al.* 1978).

The bulk of evidence now supports the major role of branchial regulation of internal pH in most water-breathers, and future work will probably focus on the ionic mechanisms responsible for this regulation. It is extremely difficult, for example, to distinguish between an increase in H^+ excretion, and either a reduction in HCO_3^- excretion, or HCO_3^- uptake. Experiments with artificial media, specific blocking agents, and perhaps some perfused organ systems may shed light on the mechanisms, and upon the control of these processes that regulate internal pH.

REFERENCES

ADLER, S., ROY, A., & RELMAN, A. S. (1965). Intracellular acid-base regulation. I. The response of muscle cells to changes in CO_2 tension or extracellular bicarbonate concentrations. *J. Clin. Invest.* **44**, 8–20.

BEYENBACH, K. W. & KIRSCHNER, L. B. (1976). The unreliability of mammalian glomerular markers in teleostean renal studies. *J. exp. Biol.* **64**, 369–378.

BRUINS, E. H. (1963). Determination of carbon dioxide using a high frequency oscillator. *Anal. Chem.* **35**, 934–936.

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

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

CAMERON, J. N. & 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.

CAMERON, J. N. & WOOD, C. M. (1978). Renal function and acid-base regulation in two Amazonian Erythrinid fishes: *Hoplias malabaricus*, a water-breather, and *Hoplerythrinus unitaeniatus*, a facultative air-breather. *Can. J. Zool.* **56**, 917–930.

CROSS, E. C., PACKER, B. S., LINTA, J. M., MURDAUGH, H. V. & ROBIN, E. D. (1969). H^+ buffering and excretion in response to acute hypercapnia in the dogfish *Squalus acanthias*. *Amer. J. Physiol.* **216**, 440–451.

DEJOURS, P. (1975). Principles of comparative respiratory physiology. *Amer. Elsevier, N.Y.* 253 pp.

DEJOURS, P., ARMAND, J. & VERREIST, G. (1968). Carbon dioxide dissociation curves of water and gas exchange of water-breathers. *Respir. Physiol.* **5**, 23–33.

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**, 339–358.

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

FRY, F. E. J. (1951). A fractionating column to provide water of various dissolved oxygen content. *Can. J. Technol.* **29**, 144–146.

HEISLER, N. (1975). Intracellular pH of isolated rat diaphragm muscle with metabolic and respiratory changes of extracellular pH. *Respir. Physiol.* **23**, 243–256.

HEISLER, N. & PIIPER, J. (1971). The buffer value of rat diaphragm muscle tissue determined by P_{CO_2} equilibration of homogenates. *Respir. Physiol.* **12**, 169–178.

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

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

HEISLER, N., WEITZ, H., WEITZ, A. M. & NEUMANN, P. (1978). Comparison of the acid-base regulation in hypercapnia between a marine elasmobranch fish (*Scyliorhinus stellaris*) and a facultative air breathing fish (*Symbranchus marmoratus*). *The Physiologist* **21**(4), 42. (Abstract).

HICKMAN, C. P., JR. & TRUMP, B. F. (1969). The kidney. In *Fish Physiology*, vol. 1 (eds W. S. Hoar and D. J. Randall), pp. 91–239. New York: Academic.

HINKE, J. A. M. & MENARD, M. R. (1978). Evaluation of the DMO method for measuring intracellular pH. *Respir. Physiol.* **33**, 31–40.

HODLER, J. E., HEINEMANN, H. O., FISHMAN, A. P. & SMITH, H. W. (1955). Urine pH and carbonic anhydrase activity in the marine dogfish. *Amer. J. Physiol.* **183**, 155–162.

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.

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

MAFFLY, R. H. (1968). A conductometric method for measuring micromolar quantities of carbon dioxide. *Analyt. Biochem.* **23**, 252–262.

MANFREDI, F. (1963). Calculation of total body intracellular pH in normal human subjects from the distribution of 5,5-dimethyl-2,4-oxazolidinedione (DMO). *J. Lab. Clin. Med.* **61**, 1005–1014.

MILHORN, H. T., JR. (1966). *The Application of Control Theory to Physiological Systems*, vi + 368 pp. Philadelphia: Saunders.

PIIPER, J., MEYER, M. & DREES, F. (1972). Hydrogen ion balance in the elasmobranch *Scyliorhinus stellaris* after exhausting activity. *Respir. Physiol.* **16**, 290–303.

RANDALL, D. J. & CAMERON, J. N. (1973). Respiratory control of arterial pH as temperature changes in rainbow trout *Salmo gairdneri. Amer. J. Physiol.* **225**, 997–1002.

REEVES, R. B. (1977). The interaction of body temperature and acid-base balance in ectothermic vertebrates. *A. Rev. Physiol* **39**, 559–586.

RENFRO, J. L. (1975). Water and ion transport by the urinary bladder of the teleost *Pseudopleuronectes americanus. Amer. J. Physiol.* **228**, 52–62.

SOLORZANO, L. (1969). Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnol. Oceanogr.* **14**, 799–801.

WADDELL, W. J. & BATES, R. G. (1969). Intracellular pH. *Physiol Rev.* **49**, 285–329.

WADDELL, W. J. & BUTLER, T. C. (1959). Calculation of intracellular pH from the distribution of 5,5-dimethyl-2,4-oxazolidinedione (DMO): application to skeletal muscle of the dog. *J. clin. Invest.* **38**, 720–729.

WOOD, C. M. & CALDWELL, F. H. (1978). Renal regulation of acid-base balance in a freshwater fish. *J. exp. Zool.* **205**, 301–307.

WOOD, C. M., MCMAHON, B. R. & MCDONALD, D. G. (1977). An analysis of changes in blood pH following exhausting activity in the starry flounder, *Platichthys stellatus. J. exp. Biol.* **69**, 173–186.