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THE ACID–BASE RESPONSES OF GILLS AND KIDNEYS TO INFUSED ACID AND BASE LOADS IN THE CHANNEL CATFISH, *ICTALURUS PUNCTATUS*

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SUMMARY

Acid and base loads infused into the dorsal aorta of the freshwater channel catfish (*Ictalurus punctatus* Rafinesque) were excreted by both gills and kidneys. The gills excreted 2–3 times as much as the kidneys.

After a 2 mmol.kg^{-1} load of $NaHCO_3$, the gills excreted 50% of the net OH^- load in the first 5 h, while the kidneys excreted 18% in the first 6 h. After a 2 mmol.kg^{-1} load of NH_4Cl, the gills excreted 32% of the load in 6 h, and 16% was excreted renally in 20 h. There was no evidence of tissue damage after either $NaHCO_3$ or NH_4Cl infusions, whereas infusion of 2 mmol.kg^{-1} HCl or 1 mmol.kg^{-1} of L(+)-lactic acid caused significant kidney damage and extensive tissue necrosis within 24 h.

After both $NaHCO_3$ and NH_4Cl infusions, the majority of the load had been transferred to the intracellular compartment within 2 h. From there it was excreted slowly, presumably by transfer back through the extracellular compartment. Due to the relative compartment volumes and buffer values, the change in intracellular pH was less than 0·05 units, while the blood pH was changed by as much as 0·3 units.

INTRODUCTION

The relative importance of gills and kidneys in acid-base regulating processes is not yet well established for the fishes as a whole. In the elasmobranchs the kidney and rectal gland do not appear to be important (Cross *et al.* 1969; Heisler, 1980; Heisler, Weitz & Neumann, unpublished observations), but the studies on teleost fish have provided no clear answer. Hickman & Trump (1969) reported numerous low pH values for fish urine, indicating an acidification capability. In a study of two Amazonian fishes, however, only one showed a renal response to a mixed post-operative blood acidosis (Cameron & Wood, 1978). In the rainbow trout, Wood & Caldwell (1978) reported that all of an infused HCl load (0·1 mmol.kg^{-1}) was excreted renally, but Kobayashi & Wood (1980) reported only a few percent of an infused lactate load was excreted renally. Finally, the kidney of the freshwater channel

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catfish is only partly responsible for compensation of an acidosis produced by environmental hypercapnia (Cameron, 1980).

On the basis of volume output alone (Hickman & Trump, 1969), the renal contribution to acid-base regulation in marine fish would not be expected to be large, since highly concentrated acid or base would have to be excreted. In the freshwater fish, however, the urine volume is considerable, and the evidence cited above made it seem likely that the kidney would participate in handling of various sorts of acid-base disturbances. The objective of the present study, then, was to assess the branchial and renal responses of the freshwater channel catfish to various infused acid and base loads, including HCl, NH_4Cl, L(+)-lactic acid, and $NaHCO_3$. We have also attempted to follow the distribution of the loads in various body compartments following infusion.

MATERIALS AND METHODS

Freshwater channel catfish (*Ictalurus punctatus* Rafinesque) were obtained from a commercial catfish farm near Angleton, Texas, and transported to the laboratory at Port Aransas, where they were held in running de-chlorinated city tap water. The fish weighed from 495 to 1100 g, were not fed, and were usually used within 3 weeks. The temperature of the holding tanks varied from 20 to 24 °C.

At least 24 h before the start of any experiment, each fish was fitted with two catheters: one in the dorsal aorta, and one in the urinary tract. The catheterization methods are described in the preceding paper (Cameron & Kormanik, 1982), except that in a few fish an alternate method was used to introduce the dorsal aortic catheter. The urinary catheterization provides tubular urine, not true bladder urine, since the urine is continuously siphoned out. The urinary bladder is very thin-walled and distensible, with no vascularization, but we have not been able to estimate the natural residence time.

Following surgery, the animals were placed in narrow lucite chambers, darkened with plastic sheet, and provided with approximately 500 ml. min^{-1} of water at 22 ± 1 °C. The usual experimental protocol was as follows: after an initial 24 h recovery period, during which blood pH was measured several times to ascertain that the post-operative acidosis had disappeared, control measurements were made. These consisted of blood pH, total CO_2 (C_T), partial pressure of CO_2 (P_{CO_2}), and sometimes blood ammonia; urine pH, urine volume per hour, urine ammonia content, urine C_T, and titratable acidity; and the water temperature. In addition, for a 2 h period, the chamber was connected to a pump and recirculation and aertation apparatus, so that it could function as a closed system with a volume about 3–5 times that of the fish. During these recirculation periods, the ammonia content and titratable acidity of the water were measured at half-hourly intervals to assess branchial acid-base output. The ammonia concentration was not allowed to build up during the recirculation periods to concentrations that might inhibit ammonia excretion, nor was the oxygen content allowed to fall to levels that affect metabolism. The P_{CO_2} of the water was kept low by vigorous aeration, and checked with a CO_2 electrode.

These control measurements were repeated on the morning of the following day (2 days post-operative) for a 2 h period. At the end of the second control period, the experimental infusion was begun, using a syringe infusion pump attached to the dors

aortic catheter. The infusions were performed over a 30–60 min period, during which the fish chambers were configured as recirculation systems. The same measurements on water, blood and urine were then followed for another day. After each 2 h period of recirculation, the chambers were flushed for 10–20 min, then re-sealed, to arrest the build-up of ammonia. Usually 2 or 3 recirculation periods of 2 h each were made immediately after the infusions, and the measurements then repeated the day following the infusion (day 3 post-operative). Blood measurements were made at 5, 30 and 60 min after the end of the infusions, and thereafter at least once during each urine collection period. Urine collections were made for 2 h intervals over the entire experiment, except that longer collections were made overnight.

Analytical methods

Methods for blood measurements were routine blood gas procedures, except for total CO_2 (C_T), which was performed with a conductometric apparatus constructed by one of us (J.N.C.) and based on modifications of the principle described by Maffly (1968). It depends on conversion of all forms of CO_2 (including carbonate and bicarbonate) to dissolved CO_2 gas by acidification, and ultimate detection of the CO_2 by re-absorption into dilute alkali, where it is measured by a differential conductivity detector. Blood ammonia was measured using the phenolhypochlorite method on microlitre samples of blood diluted appropriately with double-distilled water (Solorzano, 1969).

The urine volume was measured by weighing 2 h collections in tared vials, and the flow rate was calculated on the basis of $ml.kg^{-1}.h^{-1}$. Urine ammonia concentration was also measured with the phenolhypochlorite method. The urine pH was measured only to determine which samples contained titratable acidity (relative to the blood pH during the collective interval). For those with pH values lower than blood, the urine was diluted with 3 volumes of distilled water and titrated to the blood pH as recommended by Hills (1973) with 0·01 N-NaOH. In samples with pH values at or above the blood pH, titration with HCl did not show any significant non-bicarbonate base contribution, so TA for these samples was simply recorded as zero. The net renal H^+ excretion was calculated as the ammonia concentration plus the titratable acidity minus the total CO_2 (which is > 95% bicarbonate) times the hourly flow rate (Hills, 1973). In many cases this was a negative number, indicating a net OH^- excretion via the kidneys.

The ammonia concentration of the water during recirculation periods was measured using the phenolhypochlorite method, and the TA was measured by tirtrating 3 ml water samples to a pH of 4·000 with 0·01 N-HCl using a syringe micrometer burette. This method had a reproducibility of $\pm 0{\cdot}1$ %, determined by replicate titrations, and by addition of known amounts of acid or base to the recirculation systems. The overall sensitivity of our system, then, was about 10 μequiv of net H^+ or OH^- excreted into a volume just over 3 l. Excretion of buffers, such as amino acids, proteins, etc., would lead us to an overestimate of net OH^- excretion. The net H^+ or OH^- excretion was calculated as the change in TA per h plus the ammonia excretion per h, since the ammonia is present as NH_4^+ at the normal water pH of about 7·8. Negative numbers indicate a net OH^- excretion (or H^+ uptake) by the animal.

Lactate was analysed by the standard spectrophotometric measurement of con-

version of NAD to NADH by LDH, measured as the change in absorbance at 340 nm (Sigma reagents).

For some of the calculations of distribution of the infused loads, data on intra- and extracellular fluid compartment volumes were taken from earlier studies (Cameron, 1980; Cameron & Kormanik, 1982). In addition, one series of measurements was made to try to detect a change in intracellular pH after the infusion of NH_4Cl. The ammonia infusions were made in the manner described above, then 3–4 h afterwards, a second infusion of approx. 4 μCi of ^{14}C-labelled DMO (5,5-dimethyl-2,4-dioxazolidinedione) and 10 μCi of ^{3}H-labelled mannitol (New England Nuclear) was made into the dorsal aorta. Then at 1 h intervals, samples of blood were taken to allow the mannitol distribution space (an estimate of the extracellular fluid volume, ECF) at zero time to be calculated, and to allow the calculation of the equilibrated DMO concentration in plasma. After the 4 h sample, the fish were treated as described in the preceding paper.

Infusion solutions

Ammonium chloride was infused at neutral pH in a concentration of 2 M; a dose of 2 mmol.kg^{-1} then required an infusion of 1 ml.kg^{-1}. $NaHCO_3$ was similarly infused as a 2 M solution. L(+)-lactic acid (Sigma Chemical) was infused as a 3 M solution in Cortland saline, with a total dose of 1 mmol.kg^{-1}, and HCl as a 2 M solution in distilled water. The rate of infusion of each of these was between 6 and 20 μl.min^{-1} directly into the dorsal aorta for 30–60 min. Concentrated solutions at very low flow rates were chosen in preference to more dilute solutions in order to avoid the complication of volume loading, and subsequent diuresis.

RESULTS

HCl infusions

The results of five HCl infusions were similar to those in an earlier study (Cameron, 1980), except that in this study, observations were made over a longer period of time. In every case in which 1 mmol.kg^{-1} or more of HCl was infused, there was evidence of kidney and other systemic tissue damage. In all five fish studied, the urine flow gradually decreased to only a small fraction of the normal flow after 24 h, and in four of the fish there was blood in the urine by the day following infusion. Obvious and extensive tissue necrosis caudal to the catheter tip location in the dorsal aorta became obvious by the day following infusion: The blood pH had returned to control values in all of the fish by 4–6 h, but only a small percentage of the load had been excreted ($< 20\%$). Due to this tissue damage problem, the HCl treatment was discontinued.

Lactic acid infusions

The administration of 1 mmol.kg^{-1} of L(+)-lactic acid produced a depression of blood pH and C_T, and a transient elevation of the plasma lactate concentration (Fig. 1). The plasma lactate concentrations and pH were only significantly different from control values during the first hour after infusion. There was a slight ($P < 0.05$) elevation of the net branchial H^+ excretion, which amounted to about 16 μmol of acid

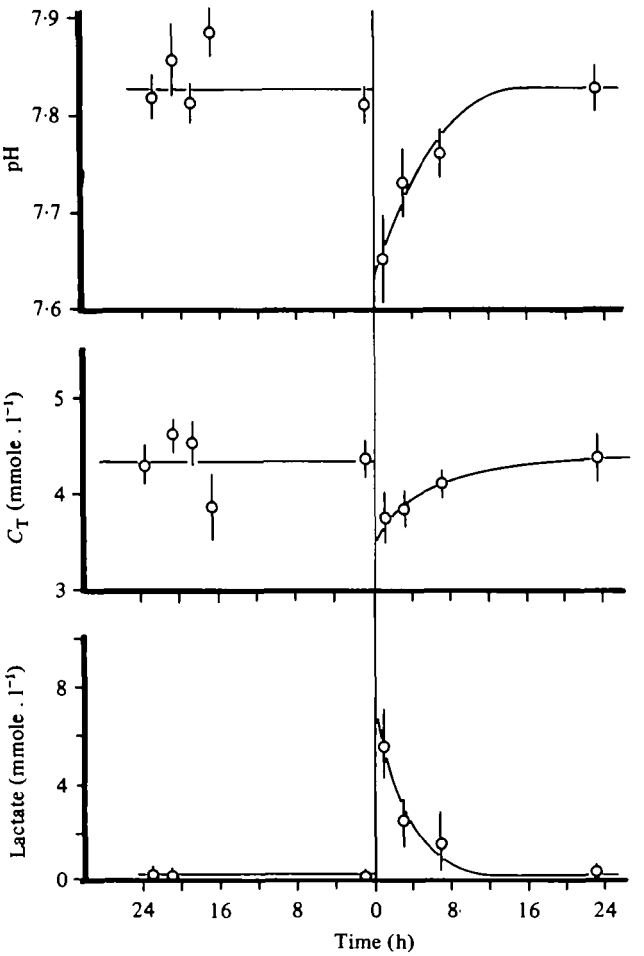

Fig. 1. The time course of plasma lactate concentration before and after the infusion of L(+)-lactic acid (bottom panel), along with the time course of plasma pH (top) and total CO_2 (middle). Infusion at 0 h. (N = 5 fish, ±S.E.).

(1·6%), and a slight (non-significant) increase in the renal net H^+ excretion. Renal lactate excretion amounted to 2·4 μmol during the first 4 h (0·24% of the load), then fell to undetectable values for the next 24 h.

As with the NaCl infusions, there was evidence of kidney and tissue damage in 4 of the 5 fish treated, and evidence of some haemolysis in all of them, in spite of the infusions being made very slowly over a fairly long period (40 min or more). In three of the fish, blood appeared in the urine within 2 h, and by 24 h, 4 of the 5 showed extensive tissue necrosis caudal to the catheter tip location in the dorsal aorta. This treatment was therefore also discontinued.

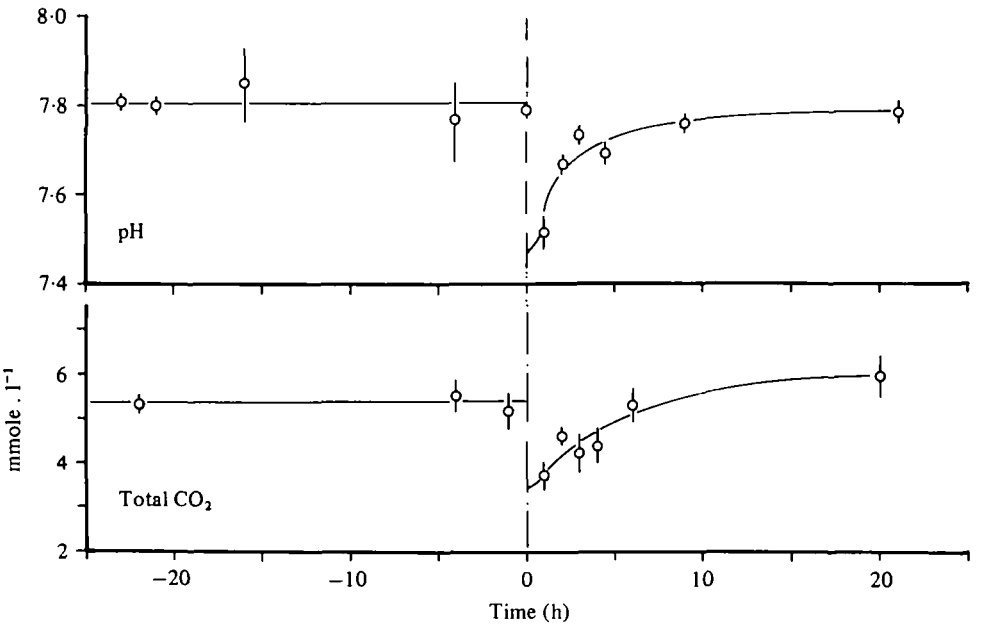

Fig. 2. The time course of plasma pH and total CO_2 (C_T) before and after the infusion of 2 mmol.kg^{-1} NH_4Cl. Each point represents the mean ± S.E. of measurements from eight fish.

NH_4Cl infusions

Since NH_4Cl is a neutral salt, relatively large quantities could be infused without obvious stress to the fish, and in none of the 8 fish treated was there the slightest evidence of kidney damage, tissue damage, or haemolysis. Diffusive loss of NH_3 across the body surface, however, leaves an equimolar proton load behind, and so in effect the ammonia is used as a proton carrier to administer a mineral acid load.

The time course of blood acid-base change is shown in Fig. 2, and the time course of changes in the ammonia ($NH_3 + NH_4^+$) concentration in plasma in Fig. 3. If we assume that the NH_4Cl concentration is uniform throughout the extracellular space (186 ml.kg^{-1}, Cameron, 1980), then the peak concentration of 2·26 mmol.l^{-1} observed at ½ h after the end of the infusion means that only 0·42 mmol (21%) of the infused ammonia is present in the ECF, and the rest has either been excreted or shuttled into the intracellular compartment.

The branchial responses were rapid and pronounced (Fig. 4). The branchial ammonia excretion rose immediately to about 6 times the basal rate, and remained significantly elevated for 3 h. The total excess ammonia excretion during this period, i.e. the difference between the observed post-infusion and the mean control rates, was 1·07 mmol, accounting for 53% of the infused load. Excess ammonia was probably excreted at a much lower rate during subsequent hours, but due to the inherent variability of ammonia excretion, it could not be statistically demonstrated.

The response of branchial net H^+ excretion was also rapid and pronounced (Fig. 4), rising to almost 200 μmol.kg^{-1}.h^{-1}, and remaining significantly elevated for at least 6 h. The rate had returned to control values by 24 h. During the first 6 h after in-

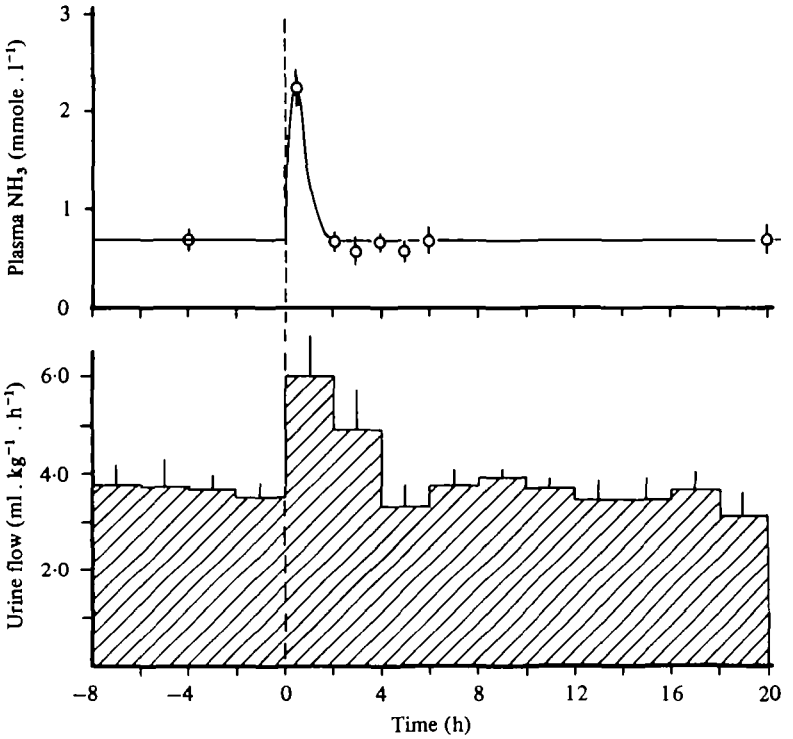

Fig. 3. The time course of plasma ammonia concentration (upper panel) and urine flow rate (lower panel) following the infusion of NH_4Cl (N = 8 fish).

fusion, a total of 0·65 mmol.kg^{-1} acid in excess of the Day 0 control rate were excreted, or about 32·5% of the infused load. The effective acid load is produced, of course, by diffusive loss of NH_3, and not by direct excretion of NH_4^+ (possibly also by diffusion). With our methods, excretion of NH_4^+ would appear as net H^+ excretion, whereas NH_3 excretion would not. During the first hour after infusion, ammonia excretion exceeded net H^+ excretion by nearly 4·5 to 1, confirming diffusive loss of NH_3 leading to acidosis. The net H^+ efflux is reasonably well correlated with the severity of this resulting acidosis (cf. Figs. 2, 4).

The renal responses to the NH_4Cl infusions (Fig. 5) were smaller and more prolonged than the branchial responses. Ammonia excretion rates rose from a control mean of 8·0 ± 1·3 μmol.kg^{-1}.h^{-1} to over 60, and remained significantly elevated ($P < 0.05$) until 12 h after infusion. During this time a total excess ammonia excretion of 221 μmol.kg^{-1} was observed, or 11·1% of the infused load. The renal net H^+ excretion was also significantly elevated, amount to 314 μmol.kg^{-1} over the first 20 h (15·7% of the load), which was roughly half as much as was excreted by the gills. Interestingly, renal excretion of both ammonia and net H^+ remained significantly elevated after the blood pH (Fig. 2) had returned to control values. The infusion caused a slight diuresis, with the mean urine flow rate rising from the control value of 3·75 ± 0·14 (± S.E.) ml.kg^{-1}.h^{-1} to 5·99 ± 0·82 and 4·91 ± 0·78 during the first and second 2 h periods following the infusion. Later flow rates were not significantly

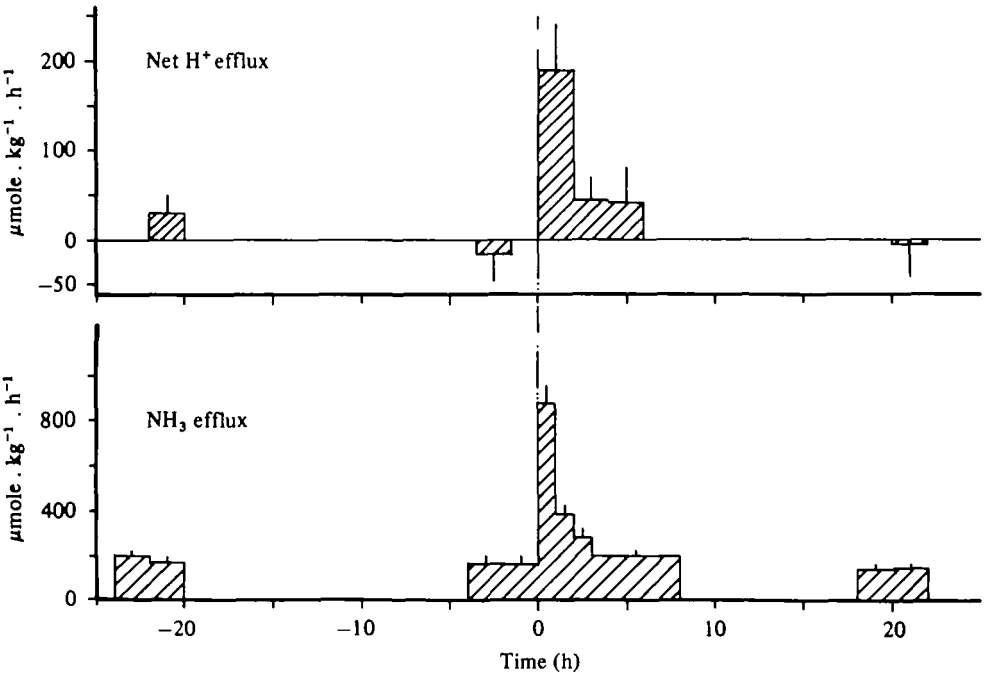

Fig. 4. Branchial excretion of ammonia (lower panel) and net H^+ excretion (upper panel) before and after the infusion of NH_4Cl (N = 8 fish). Negative values signify a net OH^- excretion, or H^+ uptake.

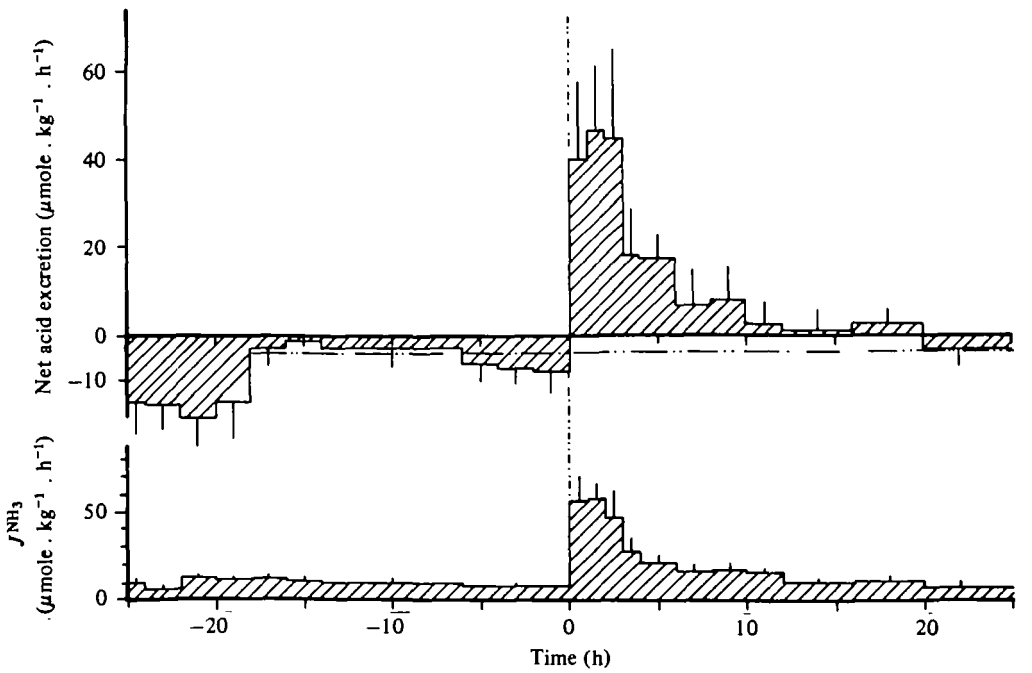

Fig. 5. Renal excretion of ammonia and net H^+ before and after infusion of NH_4Cl (N = 8 fish). Negative values signify a net OH^- excretion.

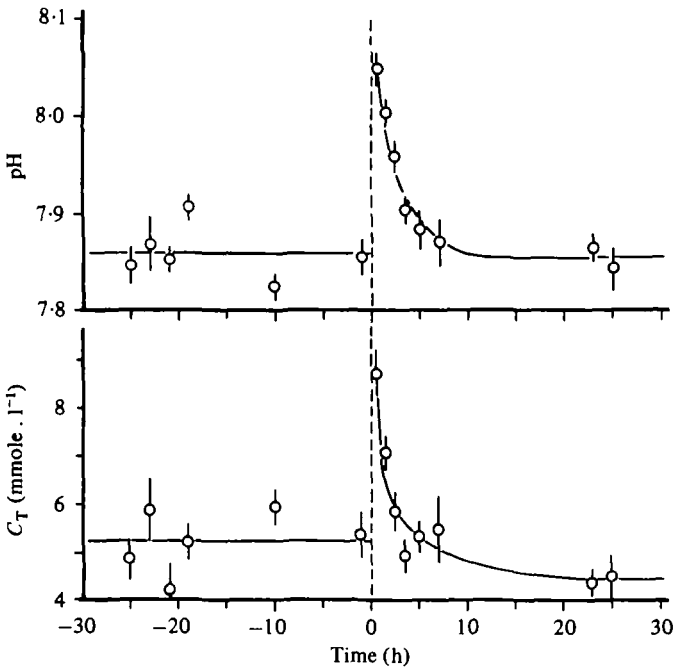

Fig. 6. The time course of plasma pH and C_T before and after infusion of 2 mmol.kg^{-1} of $NaHCO_3$ (N = 11 fish).

different from controls. Most of the increased net H^+ excretion took place via the increased ammonia excretion, as is evident from Fig. 5; there was never much titratable acidity (less than 2 mmol.l^{-1}), and although the urine pH dropped as low as 5·35, it was very poorly buffered at that pH.

In one series of experiments (N = 4), both the blood and urine calcium concentrations were measured by atomic absorption spectrophotometry. There were no significant differences between the control and the post-infusion measurements, apparently ruling out any participation of the bone or other carbonate buffer pool.

To summarize the responses to NH_4Cl infusion, there was rapid clearance of the ammonia from the blood, and a blood acidosis that lasted several hours. Branchial and renal net H^+ excretion rose significantly, with about 32·5% of the load excreted across the gills in the first 6 h, and 15·7% across the kidneys during the first 20 h. Excretion of the ammonia load was more rapid (which caused the acidosis), with 53·4% appearing across the gills in the first 3 h, and only 11·1% via the kidneys over 12 h. There remains a balance of 35·5% of the ammonia load, and 51·8% of the developed proton load, that was either retained, or excreted at rates not distinguishable from controls over a longer period of time.

$NaHCO_3$ infusions

The administration of 2 mmol.kg^{-1} of $NaHCO_3$ caused an immediate alkalosis (Fig. 6). By 4–5 h after the infusion, however, values for pH and C_T were not significantly different from controls. There also appeared to be some slight overshoot in the recovery, as the 24 h values were slightly below the pre-infusion means.

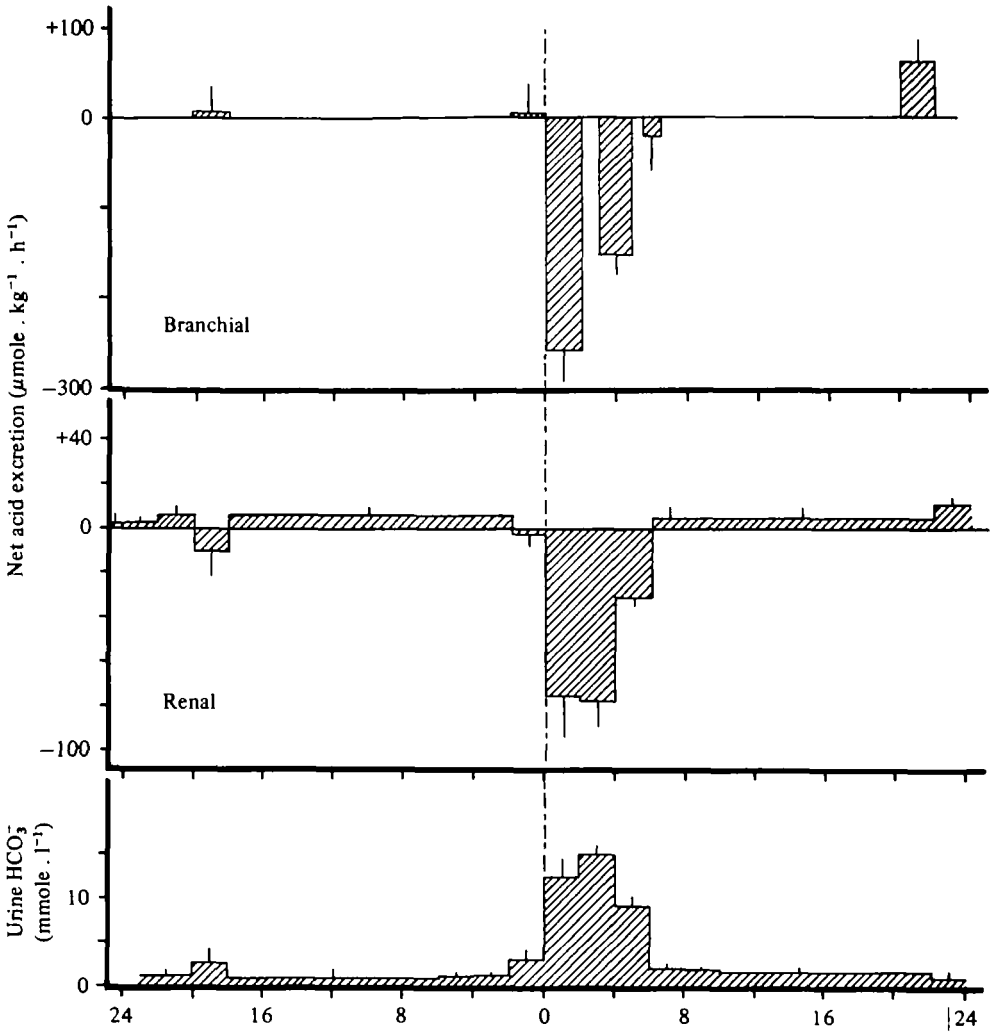

Fig. 7. The response of gills and kidneys to infusion of $NaHCO_3$. Upper panel: branchiial net H^+ excretion before and after infusion. Middle: renal net H^+ excretion over the same time period. Bottom: The concentration of HCO_3^- in the urine. (N = 11 fish).

The branchial response was immediate, with the fish switching from a small net H^+ excretion during the control periods to a net OH^- excretion of 260 μmol.kg^{-1}.h^{-1} during the first 2 h post-infusion (Fig. 7). Branchial excretion was only measured during the first 5 h after the infusion, but the total net OH^- excreted during that time was 1·02 mmol.kg^{-1}, or 50·1 % of the load. As with the NH_4Cl infusions, the intensity of net OH^- excretion appeared to be correlated with the degree of alkalosis in the blood (cf. Figs. 6, 7).

The renal response was also immediate, but smaller than the branchial response (Fig. 7). The maximum rate observed was 76·8 μmol.kg^{-1} net OH^- between 2 and 4 h, and the renal excretion dropped to control values after 6 h. The total excess net OH^- excreted during the 6 h post-infusion was 367 μmol.kg^{-1}, or 18·4 % of the load,

making a total of branchial plus renal excretion of 68·5%. The remaining 31·5% was probably either excreted slowly, at rates not statistically distinguishable from the control rates, or simply remained sequestered in the intracellular compartment.

Neither branchial nor renal ammonia excretion showed any significant change during or after the $NaHCO_3$ infusion, in spite of the increase in pH, which would increase the partial pressure of ammonia in the blood. The net OH^- excretion across the gills was manifested by changes in TA, and across the kidneys by an increase in the urine pH and HCO_3^- concentration. In fact, the kidneys not only appeared to inhibit HCO_3^- resorption, but actually to concentrate HCO_3^-, since the urine HCO_3^- reached 15·2 mmol.l^{-1} after 2–4 h (Fig. 7) when the blood concentration was only between 5 and 6 mmol.l^{-1} (Fig. 6). The extent of the diuresis following $NaHCO_3$ administration was less than that following NH_4Cl infusion: a 19% increase between 0 and 2 h, and a 21% increase between 2 and 4 h. By 4 h urine flow rate had returned to control values.

Calculation of acid and base load distribution

Since we measured the total load infused, the amount excreted as a function of time after the infusion, and the various blood parameters, we could calculate the distribution of the load in different body fluid compartments with time. The fluid compartment volumes required for these calculations were: V_{ECF} = 186 ml.kg^{-1}, V_{ICF} = 493 ml.kg^{-1} (Cameron, 1980), blood volume (V_b) = 40 ml.kg^{-1} (Holmes & Donaldson, 1969), and by difference, interstitial fluid volume (V_{ISF}) = 146 ml.kg^{-1}. The non-bicarbonate buffer value for blood, β_{NB_e}, was related to haematocrit by the equation: $\beta_{NB_e} = 0{\cdot}33\ Hct \pm 5{\cdot}76$ (Cameron & Kormanik, 1982), with units of ΔBase, mmol.pH^{-1}. The acid or base load in the ECF was then calculated using the equation:

$$\Delta B_{ECF} = (\Delta pH_e . \beta_{NB_e} . V_b) + (\Delta[HCO_3^-]_e . V_b) + (\Delta[HCO_3^-]_e . V_{ISF} . F_{Donnan}).$$

The non-bicarbonate buffering contribution of the interstitial fluid was ignored, since protein content is generally very low, and the results would not have been significantly affected. The Donnan ratio for HCO_3^- was arbitrarily assumed to be 1·05.

The acid or base load remaining in the ICF was then the total infused, minus the sum of excretion plus the remaining ECF load. For the $NaHCO_3$ experiments, the ECF and ICF loads as a function of time after infusion aere shown in Fig. 8, middle. The final calculation was to estimate the change in intracellular pH caused by the ICF load, which follows the equation:

$$\Delta pH_i = (\Delta B_i - \Delta[HCO_3^-]_i . V_{ICF})/(\beta_{NB_i} . V_{ICF}),$$

where ΔB_i = the ICF load, and β_{NB_i} is the non-bicarbonate buffer value for the ICF. The value for β_{NB_i} was assumed to be equal to the white muscle value of 35 mmol.pH^{-1} (Cameron & Kormanik, 1982), and as an approximation, $\Delta[HCO_3^-]_i$ was assumed to occur in proportion to the change in $\Delta[HCO_3^-]_e$ observed in Figs. 2 and 6. Using these simplifying approximations, the expected changes in pH_i were calculated and are shown in Fig. 8, bottom.

The procedure for calculating the effective load in the case of the NH_4Cl infusions

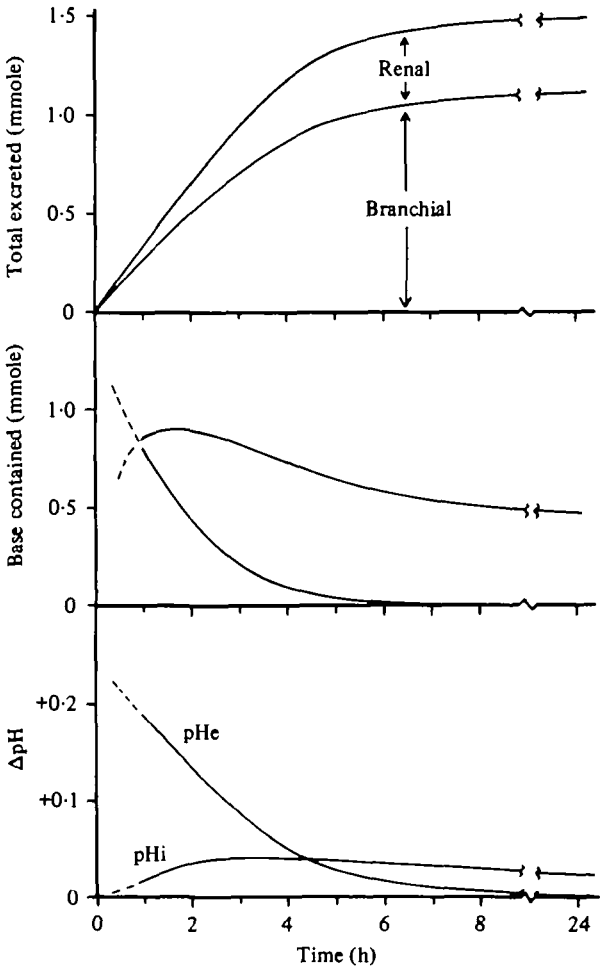

Fig. 8. Summary diagram of the fate of infused base. The cumulative excretion of net OH^- by gills and kidneys is shown in the top panel. The middle panel shows the total base load contained in the intracellular compartment (ICF) and extracellular compartment (ECF), calculated as explained in the text. The bottom panel shows the change in pH from control values, as measured for the ECF and calculated (see text) for the ICF. Data are taken from Figs. 6 and 7.

is complicated, since an effective H^+ load is only generated by an excess of NH_3 excretion over net H^+ excretion. This 'expressed load' was calculated by summing the differences between ammonia and net H^+ excretion for each time period (both gills and kidneys). That is, if NH_3 is excreted, it will subsequently combine with a proton in the external water, and with our methods we will measure zero net H^+ excretion. In that case the ammonia excretion all contributes to the expressed H^+ load. If NH_4^+ is excreted, or NH_3 and a proton simultaneously, we would measure net H^+ excretion equal to the total ammonia excretion, and in this case no contribution to the expressed H^+ load. Calculations of both the total ammonia load and the expressed net H^+ load in the ICF, along with the ECF load calculated as described above, are shown in Fig. 9 (middle) for the NH_4Cl experiments. The expected changes

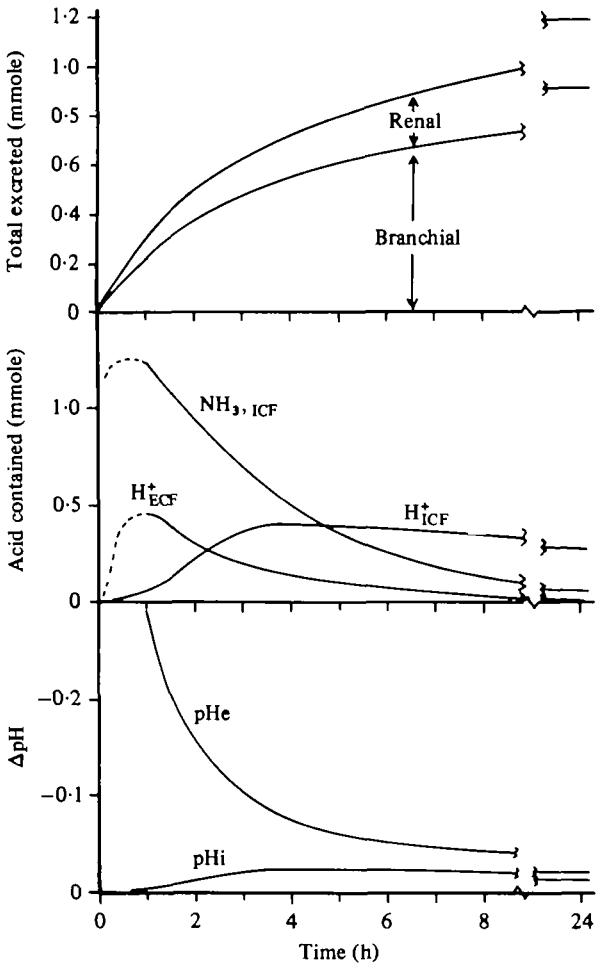

Fig. 9. Calculations similar to those in Fig. 8 for NH_4Cl infusions. Top panel: cumulative excretion, showing the total, and the proportion excreted by gills and kidneys. Middle: Total acid load contained in the ECF and ICF, calculated as explained in the text. The uppermost line shows the intracellular ammonia load as a function of time. Bottom: The time course of the change from control pH in the ECF (measured) and the ICF (calculated, see text). Data from Figs. 2, 4, 5.

in pH_i were also calculated, using the net H^+ load in the ICF, and are shown along with the observed pH_e values in Fig. 9 (bottom panel).

The distribution patterns shown in Figs. 8 and 9 are similar in that the amount of the load in the ECF declines fairly rapidly after the infusion, reaching below 10% by 3 h after $NaHCO_3$ infusion and 5 h after NH_4Cl, but the load in the ICF peaks after 2–4 h, and declines fairly slowly thereafter. The total ammonia load in the ICF after NH_4Cl infusion reaches a high value after just 1 h (Fig. 9, middle), but the expressed net H^+ load peak follows by several hours due to the difference in ammonia and net H^+ excretion rates. The slow uptake and release of the loads in the ICF lead to very small changes in pH_i, less than 0·05 in our calculations, due in part to the much larger volume and buffer value of the ICF compared to the ECF.

The intracellular pH values determined experimentally for red muscle, white muscle, heart and brain in 4 fish at 6 h after NH_4Cl infusion (2 mmol.kg^{-1}) were not significantly different from control measurements. With the precision of the DMO method, the predicted differences (above) of 0·05 or less would not be detected.

Thus, treatments producing relatively severe alkalosis and acidosis in the blood apparently produce only small transients in the intracellular pH, in spite of the fact that the intracellular compartment sequesters up to 60% of the load. This is due both to the larger volume and superior buffer value of the intracellular compartment.

DISCUSSION

Methodology

Aside from the usual technical difficulties of implanting urinary catheters and maintaining them over the several days needed to complete the experiments, there are two possible sources of error in the urinary measurements that should be mentioned. First, there could be differences between the tubular urine collected by such catheters and true bladder urine; and secondly, the urine could not be collected anaerobically.

No systematic studies have been conducted to test the difference between tubular urine and bladder urine, but Renfro (1975) has demonstrated water and ion resorptive functions in the bladder of a marine teleost. There may also be some capability for acid-base ion transfer in the urinary bladder. The channel catfish has an extremely thin-walled bladder whose volume could vary widely, depending on distension. Since we do not know the *in vivo* volume, we could not estimate the residence time for urine in the bladder.

In this study, as in all previous ones of fish, urine drained slowly from the fish to a collecting vessel via thin-walled polyethylene tubing. The gas permeability of this tubing is such that by the time the urine reaches the collecting vessel, it has equlibrated with the atmosphere, so it is of no avail to collect it under oil, except possibly as an extra precaution against evaporative loss. By performing all the pertinent measurements, however, on air-equilibrated urine, i.e. ammonia concentration, bicarbonate concentration, and titratable acidity, no significant error in the estimation of net H^+ excretion is incurred, since the loss of CO_2 would cause offsetting reductions in both TA and HCO_3^- concentration. Equilibration of the urine to the correct *in vivo* renal P_{CO_2} (which is not known) would make no noticeable difference.

The branchial measurements of net H^+ or OH^- excretion have a few more pitfalls, and methods used have varied amongst investigators. Heisler (1978) in particular, has favoured the use of a continuous, closed measurement system, measuring pH of water equilibrated to a constant P_{CO_2}. If such a system is of large volume, then sensitivity becomes a problem which can only be partly overcome by the most careful attention to electrode calibration and long-term drift. Even so, ammonia measurements must be made at discrete intervals. If, on the other hand, the volume is made relatively small, then accumulation of ammonia, and possibly other excretory products, will quickly reach levels that will affect results. In his dogfish work, Heisler (1980) did not find a significant contribution of ammonia, but this is certainly not true for teleosts, since the ammonia component is an important part of the overall acid-base excretion.

These considerations led to the use of a small-volume system in the present work, in which measurements were made over discrete intervals of $\frac{1}{2}$ h for a 2 h period. After each 2 h period, the entire system was completely flushed with fresh water, re-sealed, and measurements begun anew. The titrations could be performed with very high reproducibility, and since the water titration curve is very steep at pH 4, small drift errors in the pH electrode make almost no difference. Excretion of other titratable products, however, such as amino acids, is a possible source of error. The procedure, then, prevents ammonia accumulation, but provides a discontinuous measurement of the time course of events.

Comparative aspects

In studies of the effects of acid and base loads, the loads have been achieved by a variety of methods, and the total dosage has varied widely. Cameron & Wood (1978) took advantage of a mixed (respiratory and metabolic) acidosis following surgery, but could not quantify the total acid load. Wood & Caldwell (1978) administered 0·1 m-equiv.kg^{-1} of H^+ as 5 ml.kg^{-1} of 0·02 N-HCl, directly into the dorsal aorta over a 10 min interval. Kobayashi & Wood (1980) infused an average of 1·36 mmol.kg^{-1} of L(+)-lactic acid as 5 ml.kg^{-1} of a 0·27 M solution in 15 min. Eddy (1976) used a dose of about 0·2 mmol HCl.kg^{-1} injected into the dorsal aorta in 1 to 2 min (a procedure which, when repeated by us, caused rapid death of the fish). Cameron (1980) employed hypercapnia, which led to a (calculated) load of 2·8 m-equiv H^+.kg^{-1}, induced over several hours: this load was similar to that employed by Heisler *et al.* (1976) with dogfish. In the present study, loads of 2·0 mmol.kg^{-1} of $NaHCO_3$ and NH_4Cl were infused into the dorsal aorta over 30–60 min periods, and an expressed net H^+ load of 0·58 m-equiv.kg^{-1} was developed in response to the NH_4Cl over 4 h. It should not be ignored that the type of acid, the site and rate of administration, as well as the total dose employed might influence the responses measured subsequently. By comparison, acid loads administered in mammalian studies are often 6–12 m-equiv H^+.kg^{-1} or even larger, although the greater buffer capacity of mammalian blood and tissues renders this appropriate. In some of our early studies, larger loads were administered, but they usually led to the death of the fish, even when administered very slowly. Another important difference between fish and mammals is that the former cannot hyperventilate in order to reduce P_{CO_2} and thereby ameliorate the pH change during metabolic acidosis.

A final difficulty in comparing the studies published to date is the disparity in species employed for the studies. Not only do the species differ, but we have results for freshwater teleosts (Kobayashi & Wood, 1980; Cameron, 1980) which typically have a copious urine output, marine teleosts (McDonald *et al.* 1982) whose urine output is typically very small, and marine elasmobranchs (Heisler *et al.* 1976; Heisler 1980), whose urine output is low and whose nitrogen metabolism is very different. These various fishes also include those whose gills carry on active salt uptake from dilute environments, those whose gills extrude salts at a high rate, and the elasmobranchs that are almost osmotically neutral. It should therefore not be any great surprise that results show a similar diversity, with the urinary contribution much greater in the freshwater fishes.

Partitioning of excretion: gills vs. *kidneys*

In the channel catfish, significant excretion of net H^+ and OH^- loads occurs both via the kidneys and the gills. In response to the $NaHCO_3$ loading, the gills excreted just over 50% of the infused load, the kidneys 18%, and the remaining 32% was not accounted for by our experimental measurements. This means that the kidneys accounted for 26% of the observed excretion, and the gills 74%. In response to the NH_4Cl loading, the gills excreted about 33% of the load, the kidneys 16%, and 52% of the load was not observed during the experimental periods. In this case, the kidney accounted for one third of the observed excretion. These results are in accord with the recent work of Kobayashi & Wood (1980), who found 6% of the proton load from infused lactacidosis and 12–25% of the proton load from hypoxic lactacidosis were excreted renally in rainbow trout, but they contradict earlier work of Wood & Caldwell (1978), who reported that all of an infused HCl load was excreted via the kidneys. The present work also agrees fairly well with the estimate of 14% excretion of the net H^+ load by the kidney during hypercapnic acidosis in the channel catfish (Cameron, 1980). The recent work by McDonald *et al.* (1982) on the marine lemon sole, and the earlier work of Cross *et al.* (1969) and Heisler *et al.* (1976) on marine dogfish both indicate no significant contribution of the renal pathway, but this was to be expected, as discussed above, since urine flow rates are low in marine fish, and the dogfish is not ammonotelic.

While the present work was not addressed to the question of renal mechanism, it does demonstrate clearly that the teleost kidney is capable of both acidification and alkalinization of the urine. The range of urine pH values found in this study was 5·35 to 8·70 (with blood pH in the range of 7·6 to 8·0), indicating a capability of concentrating H^+ more than 100:1 during acidosis, and OH^- about 9:1 during alkalosis. Presumably the acidification takes place via selective resorption of Na, K and Cl, leading to changes in the strong ion difference in both blood and urine. We may speculate that the control of the selective resorption lies in the intracellular pH of the renal tubule cells.

Neither was the study directed toward the actual cellular mechanisms of branchial acid-base ion transfer, but we assume that these processes occurred via the ion exchanges already documented in fish gills, i.e. Na^+-for-H^+ and Cl^--for-HCO_3^- (or OH^-) (Maetz & Garcia-Romeu, 1964; DeRenzis & Maetz, 1973; Cameron, 1976). The rates of acid-base transfer reported here are fairly high in relation to the reported ion fluxes across freshwater gills (cf. Maetz, 1971), but the channel catfish lives in and was studied in water of higher salt content (3–5 $mmol.l^{-1}$ NaCl), and has higher Na and Cl fluxes than the rainbow trout and goldfish (J. N. Cameron, unpublished data). This suggests that the rate of acid-base compensation following disturbance may be related to the ionic content of the ambient water, and to the permeability of the fish's gills. For example, the time required for compensation of acidosis was much shorter for the channel catfish (present study; Cameron, 1980) than for the rainbow trout in water of lower salt content (Cameron & Randall, 1972; Janssen & Randall, 1975). The extreme example may be that of *Synbranchus marmoratus*, a fish which lives in the extremely ion-poor waters of the Amazon basin, and does not appear to compensate

extracellular acidosis at all (Heisler, 1980, and personal communication). A final point related to the branchial transfers is that the present study appears to provide further evidence for excretion of ammonia in the non-ionized form, NH_3, since excretion of NH_4^+, either by ionic diffusion or exchange would not have resulted in an acidosis.

Distribution of acid-base loads within the body

The present study confirms for a freshwater teleost what has been earlier demonstrated for marine fish (Heisler, 1980) and for mammals (Gonzalez & Clancy, 1975), namely that the majority of any load infused into the blood or extracellular fluids rapidly enters the intracellular compartment (Figs. 8, 9). Due to the much greater buffer capacity of the ICF, the change in pH_i is considerably less than that for the ECF, and at 6 h after infusion with NH_4Cl no significant pH_i difference could be detected.

In this study, the distribution analyses (Figs. 8, 9) were performed using the buffer value of white muscle as representative of the whole intracellular compartment, and based on the assumption of a uniform distribution of the load in the ICF. Neither of these is exactly correct, of course, but the bulk of the ICF is contained in the white muscle, and the model was only intended to indicate the approximate magnitude of the change in pH_i if the load were buffered in the ICF. The value for white muscle buffering of 35 slykes is somewhat lower than a survey of white muscle values of various fishes given recently by Castellini & Somero (1981), but they determined their buffer slopes between pH 6 and 7, whereas in this study we took the slopes between 7·1 and 7·5, values more representative of the normal pH_i of white muscle (Heisler *et al.* 1976; Cameron & Kormanik, 1982). These slopes between 6 and 7 are substantially greater, which accounts for some of the discrepancy.

At the end of the 24 h study period after the infusions, there was, both in the case of acid and base infusions, a considerable portion of the load not accounted for. This portion was clearly not in the ECF, since all blood parameters had returned to normal (Figs. 2, 6), and the presence of such a load would have been evident. We can only assume that either the balance was excreted slowly during the interval between 6 and 24 h, in which case our methods would not have been sensitive enough to distingush it against the background of a normally variable resting rate; or that it remained in the intracellular compartment (cf. Figs. 8, 9). A third possibility is that part of the load may have been buffered by the bone compartment, which in mammals has a mobile CO_2 pool that can be affected by short-term acid-base changes (Poyart *et al.* 1975). Since neither the Ca concentration nor the total Ca excretion rate changed in either blood or urine, we assume that the skeletal compartment did not participate in buffering in our experiments.

Acid-base responses to disturbances induced by infusion, then, involves the parallel pathways of both renal and branchial excretion in the channel catfish, as well as a complex shuttling of the load from the ECF to the ICF, then back again as it is excreted.

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