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Renal responses of trout to chronic respiratory and metabolic acidoses and metabolic alkalosis

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Wood, Chris M., C. Louise Milligan, and Patrick J. Walsh. Renal responses of trout to chronic respiratory and metabolic acidoses and metabolic alkalosis. *Am. J. Physiol.* 277 (*Regulatory Integrative Comp. Physiol.* 46): R482–R492, 1999.—Exposure to hyperoxia (500–600 torr) or low pH (4.5) for 72 h or $NaHCO_3$ infusion for 48 h were used to create chronic respiratory (RA) or metabolic acidosis (MA) or metabolic alkalosis in freshwater rainbow trout. During alkalosis, urine pH increased, and [titratable acidity (TA) − HCO_3^-] and net H^+ excretion became negative (net base excretion) with unchanged NH_4^+ efflux. During RA, urine pH did not change, but net H^+ excretion increased as a result of a modest rise in NH_4^+ and substantial elevation in [TA − HCO_3^-] efflux accompanied by a large increase in inorganic phosphate excretion. However, during MA, urine pH fell, and net H^+ excretion was 3.3-fold greater than during RA, reflecting a similar increase in [TA − HCO_3^-] and a smaller elevation in phosphate but a sevenfold greater increase in NH_4^+ efflux. In urine samples of the same pH, [TA − HCO_3^-] was greater during RA (reflecting phosphate secretion), and [NH_4^+] was greater during MA (reflecting renal ammoniagenesis). Renal activities of potential ammoniagenic enzymes (phosphate-dependent glutaminase, glutamate dehydrogenase, α-ketoglutarate dehydrogenase, alanine aminotransferase, phospho*enol*pyruvate carboxykinase) and plasma levels of cortisol, phosphate, ammonia, and most amino acids (including glutamine and alanine) increased during MA but not during RA, when only alanine aminotransferase increased. The differential responses to RA vs. MA parallel those in mammals; in fish they may be keyed to activation of phosphate secretion by RA and cortisol mobilization by MA.

renal ammoniagenesis; phosphate; titratable acidity; glutamine; cortisol

EARLY STUDIES indicated that the kidney plays a small but significant role in acid-base balance in freshwater teleost fish (8, 26, 27, 42, 67). In more recent quantitative studies wherein fish were subjected to standardized acid-base disturbances, urinary excretion typically accounted for <50% of net acid or base excretion by the whole animal, with the greater fraction occurring across the gills (4, 6, 7, 16, 43, 64, 65). However, in circumstances such as exposure to low environmental pH, where acid loading through the gills was the source of the acid-base disturbance, renal acid excretion accounted for all the compensation that occurred (30, 35, 37). In general, the freshwater teleost kidney responds to systemic acidosis by an increased output of acidic equivalents in the form of both titratable acidity (TA) and NH_4^+ and to systemic alkalosis by an increased output of basic equivalents in the form of HCO_3^-, responses that parallel those classically described in the mammalian kidney (24, 44, 50, 56).

However, in the mammalian kidney it is now well documented that the renal response to chronic respiratory acidosis differs from that to chronic metabolic acidosis (e.g., Refs. 9, 22, 24, 32, 46, 47). In particular, the latter appears to be a more powerful stimulant of net renal acid excretion because it induces a greater production and excretion of NH_4^+, whereas the response to respiratory acidosis relies more heavily on increased TA excretion in the form of phosphate ($H_2PO_4^-$). The first objective of the present study was to test whether the same difference occurred in freshwater fish by directly comparing the renal acid-base responses of rainbow trout to the two types of acidoses. Inasmuch as the first series of experiments confirmed that this same type of differential response to metabolic vs. respiratory acidosis occurred in the trout as in the mammal, the second objective was to characterize some of the possible mechanisms involved. To this end, a second experimental series focused on the activities of enzymes potentially involved in ammoniagenesis in the kidney and the mobilization of the ammoniagenic substrate glutamine from extrarenal sites. This series also assessed potential differences in the appearance of ammonia, inorganic phosphate, cortisol, and individual amino acids in the blood plasma in the two types of acidoses as possible explanations for the differential response.

It should be noted that the standard mammalian technique for inducing respiratory acidosis (high environmental P_{CO_2}) is unsuitable for fish, because elevated P_{CO_2} lowers the pH of the water, creating a mixed stimulus. Similarly, the standard mammalian tool for inducing metabolic acidosis (NH_4Cl administration) is also unsuitable, because teleost fish are predominantly ammoniotelic, and do not make urea by the ornithine-urea cycle. We therefore employed two treatments (high water P_{O_2}, low water pH) that have previously been shown to induce respiratory and metabolic acidoses in fish and that are more relevant to the natural situation. Environmental hyperoxia (water $P_{O_2} > 500$ Torr; as commonly occurs from photosynthesis of freshwater algal blooms) does not alter water pH but causes internal CO_2 retention by slowing ventilation and

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vasoconstricting the gills (25, 68, 69). A pure respiratory acidosis ensues with clear evidence of renal compensation (64). Low environmental pH (4.0–5.0; as occurs from acidic runoffs and discharges), when applied in hard water, induces pure metabolic acidosis, again with clear evidence of renal compensation (35, 37). In this situation, the renal response is likely maximized, because branchial acid excretion is blocked by such low external pH, and the gills become the site of acid loading. For the sake of comparison, the response to metabolic alkalosis was also studied. However, high water pH is not "symmetrical" to low water pH in its effects on the acid-base status of fish, because alkaline water serves as an infinite CO_2 sink, thereby producing profound respiratory alkalosis rather than a clear metabolic alkalosis (66). Therefore, the third treatment employed was $NaHCO_3$ infusion, which has been shown to induce pure metabolic alkalosis and an accompanying renal response in freshwater trout (16).

MATERIALS AND METHODS

Experimental Animals

Rainbow trout (*Oncorhynchus mykiss*; 1–2 yr old, 195–400 g) were obtained from Spring Valley Trout Farm, Petersburg, Ontario, and acclimated for 2–4 wk to the experimental medium, dechlorinated Hamilton tap water [14 ± 1°C; 0.6 Na^+, 0.7 Cl^-, 1.8 Ca^{2+}, 0.04 K^+ meq/l; titration alkalinity (to pH 4.0) 1.9 meq/l; total hardness 140 mg/l as $CaCO_3$; pH 8.0]. The fish were starved for 1 wk before surgery, which was performed under MS-222 anesthesia (100 mg/l). *Series 1* and *2* were performed on different batches of trout, two years apart, from the same source.

In *series 1*, trout were fitted with dorsal aortic catheters for blood sampling (51) and internal urinary bladder catheters for collection of ureteral urine (70). With this catheterization technique, modification of urine composition by the bladder is prevented (16), allowing study of kidney function alone. In *series 2*, only internal urinary bladder catheters were implanted. The fish were allowed to recover for 48 h on flow-through water supply (>300 ml/min) in darkened Plexiglas boxes of the design described by McDonald (35). These boxes were placed on a wet table thermostated to 14 ± 1°C. Each fish box consisted of an inner 2-l chamber, which confined and restrained the trout, and an outer 10-l chamber, which contained most of the water volume. An airlift pump at the rear of the inner chamber provided continuous aeration and recirculation (>500 ml/min) so the boxes could be operated as closed systems. Throughout the recovery and subsequent experimental periods, urine was collected continuously into covered vials by means of a slight siphon (3–4 cm).

Series 1

After 48 h recovery, urine was collected over 8- to 12-h periods for 24–48 h to assure that urine flow rate (UFR) was normal. Trout were then subjected to one of three experimental treatments, each with its simultaneous control.

Acid exposure (metabolic acidosis). For experimental fish (n = 13) the inflow was changed over to a thermostated, recirculating reservoir fitted with a Radiometer TTT80 pH controller; the reservoir contained 250 l of water for each batch of four fish. The water in the reservoir was titrated to a mean pH of 4.0 with HCl and vigorously aerated for 24 h before use to return P_{CO_2} to undetectable levels. Measured pH in the boxes averaged 4.5 (range = 4.2–4.8), reflecting the alkalinizing influence of the fish. Water overflowing from the boxes was returned to the reservoir, which was changed over every 24 h. Urine samples were collected from each fish over 12-h intervals through to 72 h of low pH exposure. Blood samples (400 µl) for measurement of acid-base status were taken at 60 h. Control fish (n = 12) were treated identically, but the recirculating reservoir was kept at pH 8.0.

Hyperoxia (respiratory acidosis). For experimental fish (n = 12), the 12-l flux boxes were closed, and the aeration driving the airlift pump was changed over to pure O_2, which effected a rapid increase of measured water P_{O_2} in the boxes from normoxic (~130 torr) to hyperoxic levels (500–600 torr). Hyperoxia was maintained for 72 h, and the water in the fish boxes was renewed at 12-h intervals by flushing with hyperoxic water from the thermostated reservoir, which was also gassed with O_2. Urine samples were also collected at 12-h intervals, and blood samples were drawn at 60 h. Control trout (n = 13) were treated identically, but compressed air was used to drive the airlift pumps throughout.

$NaHCO_3$ infusion (metabolic alkalosis). For experimental fish (n = 12), an infusion with 140 mmol/l $NaHCO_3$ at a rate of 3.0 $ml \cdot kg^{-1} \cdot h^{-1}$ (i.e., 420 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) via the arterial catheter was maintained for 48 h by means of a Gilson peristaltic pump. Infusion rate was monitored gravimetrically and kept within 5% of nominal values. Control fish (n = 12) received a comparable infusion with 140 mmol/l NaCl. Urine samples were collected over intervals of 6–10 h, and blood samples were drawn at 40 h. The fish boxes received a flow of fresh normoxic water throughout.

Series 2

After 48 h recovery, experimental trout were subjected to either the acid exposure (metabolic acidosis; n = 10) or hyperoxia (respiratory acidosis; n = 10) regimes outlined for *series 1*, the only difference being that urine samples were collected over 24-h rather than 12-h intervals. A control group of trout (n = 10) were kept in fresh normoxic water (pH 8.0) throughout. At 72 h, trout were rapidly killed without disturbance by adding a lethal solution of MS-222 to the fish boxes (final concentration 750 mg/l; pH adjusted to 8.0 with NaOH). A blood sample (1.0–2.0 ml) was drawn by caudal puncture from the haemal arch and centrifuged at 10,000 *g* for 30 s to separate the plasma. The liver, kidney, and a 5-g sample of white muscle were rapidly excised and freeze-clamped in liquid N_2. Samples were stored at −70°C for later analysis of enzymes (in tissues) or amino acid, cortisol, phosphate, and ammonia concentrations (in plasma).

Analytical Techniques

In *series 1*, acid-base status [pHa, arterial P_{CO_2} (Pa_{CO_2}), plasma true concentration of HCO_3^- ($[HCO_3^-]$)] of arterial blood samples was measured by standard radiometer electrode techniques (16). Urine samples were analyzed for volume (by weighing), pH, and net titratable acidity ($TA - HCO_3^-$) immediately after collection and frozen for later analysis of total ammonia by the salicylate hypochlorite method (58). Total inorganic phosphate was measured by the phosphomolybdate reduction method by means of a Sigma kit in the last two urine samples from each fish (which bracketed the blood acid-base measurements). Urine [$TA - HCO_3^-$] was measured as a single value by means of the double titration procedure recommended by Hills (24) and detailed elsewhere (16, 64). The endpoint of the titration was pH 7.9, representing the control arterial pH (pHa) measured in these trout. Titrants used were standardized 0.02 N HCl and 0.02 N NaOH delivered by Gilmont microburettes, and pH was

measured during the titration with a radiometer microelectrode (E5021) coupled to a radiometer PHM 71 acid-base analyzer.

In *series 2*, urine samples were analyzed for volume, total inorganic phosphate, and total ammonia as in *series 1*. Terminal plasma samples were assayed for total inorganic phosphate (phosphomolybdate reduction method again), total ammonia (L-glutamatic dehydrogenase method via Sigma kit), cortisol (ICN Immunocorp RIA with standards diluted to match protein concentrations in rainbow trout plasma), and amino acids. For the latter, a 100-μl plasma sample was mixed with 150 μl HPLC-grade acetone and 40 μl of 10 mM α-aminobutyric acid (Sigma) in 0.1 mM HCl (internal standard), then centrifuged at 10,000 *g* for 5 min. The supernatants were derivatized with phenylisothiocyanate (PICT; Sigma) in accordance with the Waters Pico-Tag method (13). Aliquots (25 μl) of the supernatant were dried under nitrogen, and then 20-μl aliquots of 1:1:3 triethylamine:methanol: water were added, the samples were mixed, and then dried again. Aliquots (20 μl) of freshly prepared 1:1:1:7 triethylamine: water:PICT:methanol were added, and the samples were incubated at room temperature for 20 min. The reaction was stopped by drying the samples under a stream of nitrogen for 90 min. The pellet was dissolved in 1 ml of 5 mM sodium phosphate (pH 7.4) in 5% acetonitrile, and filtered through a 20-μm nylon filter to remove particulates. Aliquots (20 μl) of the derivatized samples were injected onto a reverse-phase column (CSC sil, 80Å/ODS2, 25 cm) at 40°C and separated with a 7–60% acetonitrile gradient. Derivatized amino acids were detected at 254 nm with a Beckman ultraviolet/visible light detector. Standards consisting of a mixture of amino acids at a concentration of 1.25 mM were derivatized and run on the HPLC with each set of samples to aid in peak identification and quantification.

White muscle, liver, and kidney samples were assayed for glutamine synthetase (GSase) and phosphate-dependent glutaminase (GLNase) activity, whereas glutamate dehydrogenase (GDH), α-ketoglutarate dehydrogenase (α-KGDH), phospho*enol*pyruvate carboxykinase (PEPCK), and alanine aminotransferase (AlaAT) activities were measured in kidney only. For determination of enzyme activities, frozen tissues were homogenized on ice in 4 volumes of homogenization buffer (20 mM K_2HPO_4, 10 mM HEPES, 0.5 mM EDTA, 1 mM dithiothreitol, 50% glycerol, pH 7.5) in a Polytron (Brinkman) homogenizer. Homogenates were centrifuged at 13,000 *g* for 5 min, and crude supernatants were used directly in enzyme assays. Methods used were as previously published for GDH, PEPCK, and AlaAT (39, 60), GSase (61), and phosphate-dependent GLNase (15). The method used for α-KGDH was a radiometric adaptation of the method used by Sanadi (48) as follows: tissue homogenate (50 μl) was incubated in 1 ml of a solution of 66 mM KPO_4 buffer (pH 7.2), 0.1 mM Coenzyme A (SH), 3.3 mM L-cysteine, 0.33 mM NAD^+, 1 mM α-ketoglutarate, and 0.5 μCi α-[1-^{14}C]oxoglutarate in a rubber-septum-sealed vial with a center well containing filter paper. At the end of 20 min, 0.2 ml of hyamine hydroxide was injected through the septum onto the filter paper, and then the reaction was terminated by injection of 0.1 ml 70% perchloric acid. The vial was then shaken for 90 min to release the CO_2 formed from the reaction and trap it on the filter paper. The paper was then counted by liquid scintillation, and the activity was calculated based on the specific activity of the substrate. The assay was linear with respect to time out to 30 min, with doubling or halving of the amount of supernatant used.

Calculations and Statistical Analysis

Urine flow rates (UFR) and excretion rates were expressed on a weight-specific basis. The urinary excretion rate of each substance was calculated as the product of the urine concentration and UFR. Total renal output of acidic equivalents was taken as the sum of the $[TA - HCO_3^-]$ and NH_4^+ components (24). At the urine pH values measured in the present study, NH_4^+ accounted for >90%, and usually 95–100%, of the total ammonia present, and therefore was taken as equal to the latter.

Data have been expressed as means ± SE(*n*), where *n* represents the number of fish, except in Figs. 2 and 3, in which each urine sample was tabulated separately, so that *n* represents the number of urine samples. Data were transformed to match variance ratios where indicated by the *F* test, and then differences significant at $P \leq 0.05$ were evaluated by Student's *t*-test (2-tailed) with the Bonferroni correction applied for multiple comparisons (41).

RESULTS

Series 1

This series focused on the renal response to the three different types of acid-base disturbance in terms of urinary acid or base excretion. The data from the parallel control fish exhibited no significant differences amongst the three treatments (apart from UFR) and therefore have been combined. For all three experimental treatments, maximum and apparently stable renal acid-base responses were seen in the final two periods of urine collection (32–40 and 40–48 h for metabolic alkalosis; 48–60 and 60–72 h for respiratory acidosis and metabolic acidosis). Because there were no significant differences within a treatment between these two periods (which bracketed the arterial blood acid-base measurement), data for each fish were averaged over these two collections.

Arterial blood acid-base measurements (Table 1) after 40 h of $NaHCO_3$ infusion indicated a state of pure metabolic alkalosis with an elevation in pHa by ~0.3 units and a doubling of plasma $[HCO_3^-]$ relative to controls. After 60 h of exposure to environmental hyperoxia, the arterial acid-base status was indicative

Table 1. *Arterial acid-base status in response to experimental treatments in series 1*

	Control ($n = 37$)	Metabolic Alkalosis ($NaHCO_3$ Infusion) ($n = 13$)	Respiratory Acidosis (P_{O_2} = 500–600 torr) ($n = 12$)	Metabolic Acidosis (Water pH = 4.5) ($n = 12$)
pHa	7.887 ± .015	8.164 ± .099*	7.743 ± .022*	7.338 ± .108*†
Pa_{CO_2}, torr	3.06 ± .17	3.01 ± .76	8.50 ± .76*	2.51 ± .44†
$[HCO_3^-]$ mmol/l	8.49 ± .50	16.75 ± 1.19*	19.02 ± 1.20*	2.16 ± .31*†

Data are means ± SE; *n* = no. of animals. pHa, Arterial pH; Pa_{CO_2}, arterial P_{O_2}; $[HCO_3^-]$, concentration of HCO_3^-. *$P \leq 0.05$ vs. control; †$P \leq 0.05$ vs. respiratory acidosis.

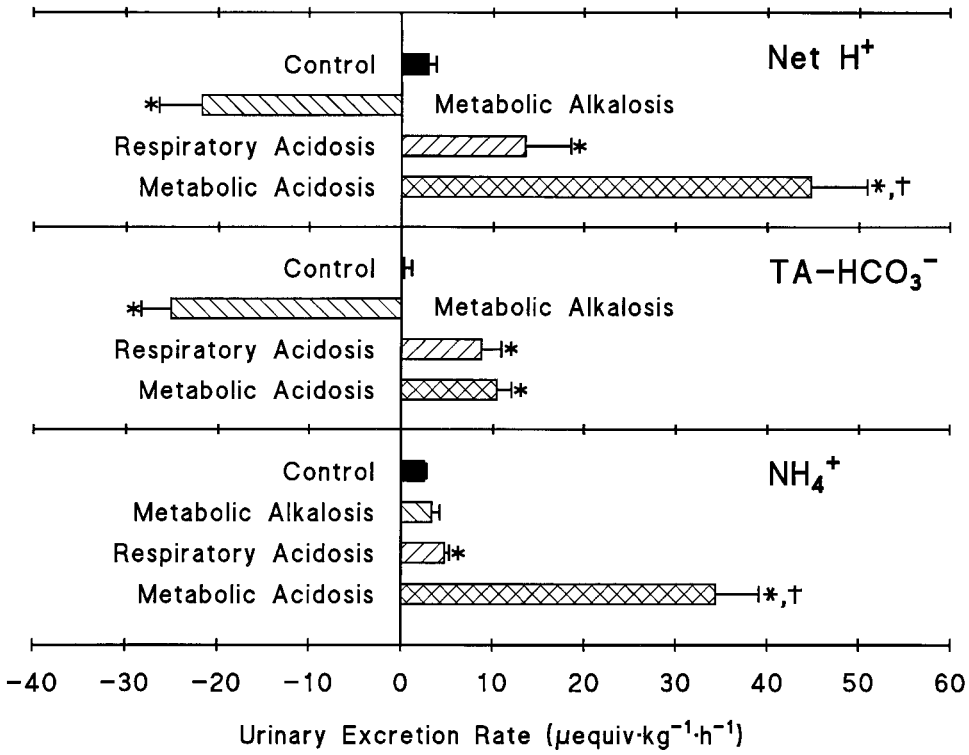

Fig. 1. Urinary excretion rates of net H^+ [i.e., net acidic equivalents (positive) or net basic equivalents (negative)] and of its individual components, [titratable acidity (TA) − HCO_3^-] and [NH_4^+], in rainbow trout of *series 1* under control conditions (n = 37), during metabolic alkalosis (at 32–48 h of $NaHCO_3$ infusion, n = 12), during respiratory acidosis (at 48–72 h of environmental hyperoxia, n = 12), and during metabolic acidosis (at 48–72 h exposure to low environmental pH, n = 13). Data are means ± SE. *P < 0.05 vs. respective control value; †P < 0.05, metabolic acidosis vs. respiratory acidosis.

of partially compensated respiratory acidosis, with a 2.8-fold elevation in Pa_{CO_2}, a 2.2-fold elevation in plasma [HCO_3^-], and a 0.15-unit depression in pHa. After 60 h exposure to low water pH, trout exhibited a classic metabolic acidosis in the arterial blood, with a 0.55-unit decrease in pHa associated with a 75% decrease in plasma [HCO_3^-] but unchanged Pa_{CO_2}.

Under control conditions, rainbow trout exhibited a small positive net H^+ excretion through the kidney, made up almost entirely of an NH_4^+ component (Fig. 1). During metabolic alkalosis, net H^+ excretion became highly negative at about −22 μmol·kg^{-1}·h^{-1} (i.e., net basic equivalent excretion), caused entirely by a negative [TA − HCO_3^-] excretion (i.e., HCO_3^- > TA). NH_4^+ excretion did not change. During both respiratory acidosis and metabolic acidosis, net H^+ excretion became highly positive, with the response being 3.3-fold greater during the latter (45 vs. 14 μmol·kg^{-1}·h^{-1}). The [TA − HCO_3^-] component became positive (i.e., TA > HCO_3^-) and was approximately equal at ~9 μmol·kg^{-1}·h^{-1} in the two acidotic treatments, so the larger net H^+ excretion under metabolic acidosis was the result of a sevenfold higher NH_4^+ response (34 vs. 5 μmol·kg^{-1}·h^{-1}).

Table 2 summarizes the components of urine acidity. Mean urine pH, which averaged ~7.2–7.3 in control fish, remained unchanged during respiratory acidosis but was elevated to 7.9 in response to metabolic alkalosis and lowered to 6.5 in response to metabolic acidosis. Mean [TA − HCO_3^-], which was not significantly different from zero in control fish, became significantly negative in response to metabolic alkalosis, and significantly positive in response to both respiratory acidosis and metabolic acidosis. [TA − HCO_3^-] concentrations in the urine were virtually identical in these two acidotic treatments. Inorganic phosphate concentrations were low and highly variable in control fish, and even lower during metabolic alkalosis, although the difference was not significant, reflecting this variability. Both respiratory acidosis and metabolic acidosis caused substantial elevations in inorganic phosphate concentrations. Despite the similar [TA − HCO_3^-] concentrations in these two treatments, the phosphate elevation was 50% greater during respiratory acidosis than during meta-

Table 2. *Components of urine acidity and urine flow rate in response to experimental treatments in series 1*

	Control (n = 37)	Metabolic Alkalosis ($NaHCO_3$ Infusion) (n = 13)	Respiratory Acidosis (Po_2 = 500–600 torr) (n = 12)	Metabolic Acidosis (Water pH = 4.5) (n = 12)
Urine pH	7.258 ± .051	7.910 ± .129*	7.122 ± .147	6.464 ± .139*†
[TA − HCO_3^-], mmol/l	0.129 ± .238	−4.379 ± .566*	3.219 ± .776*	3.559 ± .539*
[Phosphate], mmol/l	0.21 ± .10	0.04 ± .03	7.10 ± .42*	4.75 ± .54*†
[NH_4^+], mmol/l	0.84 ± .02	0.59 ± .07*	1.73 ± .07*	11.65 ± .54*†
UFR, ml·kg^{-1}·h^{-1}	3.105 ± .109‡	5.725 ± .201*	2.746 ± .271	2.950 ± .299

Data are means ± SE; n = no. of animals. TA, Titratable acidity; [Phosphate], phosphate concentration; [NH_4^+], concentration of NH_4^+; UFR, urine flow rate. *P < 0.05 vs. control; †P < 0.05 vs. respiratory acidosis; ‡n = 25 because NaCl infusion controls (for metabolic alkalosis) were excluded from mean.

bolic acidosis. Assuming a pK (negative log of dissociation constant) of 6.8, at the measured urine pHs urinary phosphate was sufficient to explain about 55% of titratable acidity in the urine during respiratory acidosis and >85% of the total during metabolic acidosis. Urinary [NH_4^+] was quite uniform under control conditions at ~0.8 mmol/l, fell by 30% during metabolic alkalosis, and approximately doubled during respiratory acidosis, representing ~35% of urine net acid content However, the greatest response was seen during metabolic acidosis, where mean urinary [NH_4^+] increased 14-fold and was by far the largest contributor (77%) to urine net acid content. UFR was unaffected by the various acid-base disturbances. The significantly higher UFR during metabolic alkalosis was simply a response to the volume loading of the 3 ml·kg^{-1}·h^{-1} infusion of 140 mmol/l $NaHCO_3$; a virtually identical elevation (to 5.910 ± 0.151 ml·kg^{-1}·h^{-1}, n = 12) was seen in the control fish infused with 140 mmol/l NaCl at the same rate (data not shown).

The relationships between urine pH and the two major components of urine acid-base content in individual urine samples are compared for the control (357 samples), metabolic alkalosis (75 samples), respiratory acidosis (72 samples), and metabolic acidosis (86 samples) treatments in Fig. 2 ([TA − HCO_3^-]) and Fig. 3 ([NH_4^+]). Relative to control, urine pH was distributed over a higher pH range during metabolic alkalosis. However, in samples compared at the same pH, [TA − HCO_3^-] was significantly more negative (Fig. 2) during alkalosis, whereas [NH_4^+] was unchanged (Fig. 3). During respiratory acidosis, the distribution of urine pH was similar to that in control fish. However, when compared at the same pH, [TA − HCO_3^-] was significantly greater (by ~2-fold) than in control fish at all pHs below 7.6 (Fig. 2). Urinary [NH_4^+] was also significantly elevated at several pHs during respiratory acidosis, but this difference was far less pronounced (Fig. 3). The opposite situation occurred during metabolic acidosis, although here the distribution of urine pHs was shifted to a markedly lower range. When compared at the same pH, urinary [TA − HCO_3^-] was not significantly different from control (Fig. 2), whereas [NH_4^+] was elevated manyfold, tending to increase in an exponential manner at the lowest pHs (Fig. 3).

Series 2

This series focused on the mechanisms behind the different renal responses to respiratory vs. metabolic acidosis. Measurements over 24-h periods demonstrated the same basic patterns seen in *series 1*: a preferential stimulation of phosphate excretion by respiratory acidosis and of NH_4^+ excretion by metabolic acidosis, with the greatest responses occurring on *day 3* (Fig. 4). Note, however, that the magnitudes of the renal responses to both types of acidosis were less than half those seen in *series 1* (c.f. Fig. 1 and Table 2), despite the fact that the experimental treatments were the same.

Plasma concentrations of both inorganic phosphate and ammonia, measured in terminal samples at 72 h, were significantly elevated by ~1.5-fold and 2-fold,

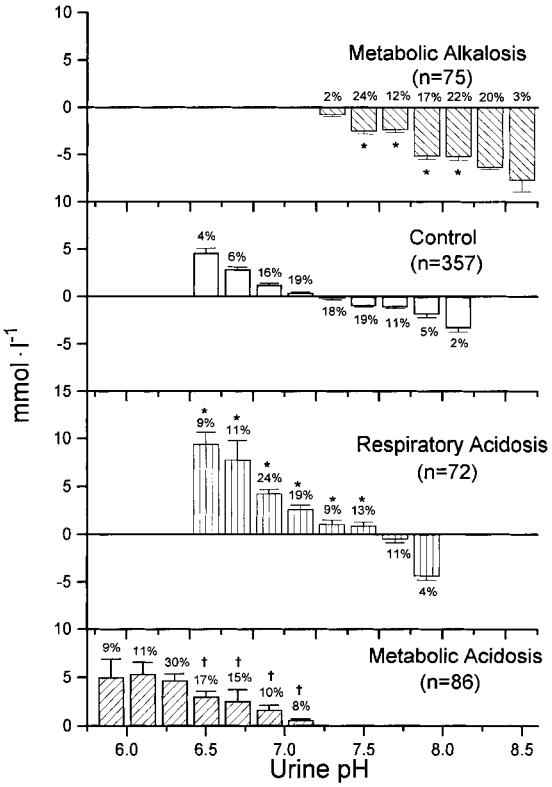

Fig. 2. Comparison of relationships between urine pH and [TA − HCO_3^-] in individual urine samples (6- to 12-h collections) from rainbow trout of *series 1* under control conditions (357 samples from 74 fish), metabolic alkalosis (75 samples from 12 fish), respiratory acidosis (72 samples from 12 fish), and metabolic acidosis (86 samples from 13 fish). Percentage of urine samples within each pH category is shown for each treatment. Control group includes preexposure collections plus all collections over experimental periods from all 3 control groups. Experimental groups include all collections made during 48 h of $NaHCO_3$ infusion (for metabolic alkalosis), during 72 h of environmental hyperoxia (for respiratory acidosis), and during 72 h of low pH exposure (for metabolic acidosis). Data are means ± SE. *P < 0.05 vs. respective control value; †P < 0.05, metabolic acidosis vs. respiratory acidosis.

respectively, during metabolic acidosis, but were not significantly altered by respiratory acidosis (Table 3). Thus the differential excretion patterns of these two major urinary buffers was not reflective of their appearance patterns in the blood plasma. Cortisol concentrations were substantially elevated during metabolic acidosis (and highly variable) but were unaffected during respiratory acidosis (Table 3).

Examination of enzymes possibly involved in the ammoniagenic response revealed no changes at extrarenal sites in response to either respiratory or metabolic acidosis. GLNase activities were low and GSase was below detection (detection limit = 0.04 U/g) in white muscle, but both were substantially higher in liver (Table 4).

In contrast, enzyme activities in the kidney itself changed markedly during metabolic acidosis, with significant elevations in GLNase, GDH, α-KGDH, and AlaAT activities (Table 5). However, during respiratory acidosis, only the activity of the latter enzyme increased significantly. PEPCK tended to increase during both treatments, but the changes were not significant.

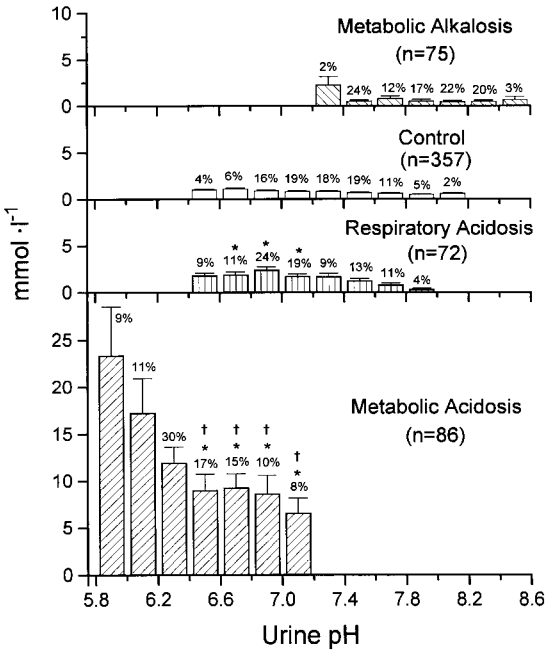

Fig. 3. Comparison of relationships between urine pH and $[NH_4^+]$ in individual urine samples (6- to 12-h collections) from rainbow trout of *series 1* under control conditions (357 samples from 74 fish), metabolic alkalosis (75 samples from 12 fish), respiratory acidosis (72 samples from 12 fish), and metabolic acidosis (86 samples from 13 fish). Percentage of urine samples within each pH category is shown for each treatment. Control group includes preexposure collections plus all collections over experimental periods from all 3 control groups. Experimental groups include all collections made during 48 h of $NaHCO_3$ infusion (for metabolic alkalosis), during 72 h of environmental hyperoxia (for respiratory acidosis), and during 72 h of low pH exposure (for metabolic acidosis). Data are means ± SE. *$P < 0.05$ vs. respective control value; †$P < 0.05$, metabolic acidosis vs. respiratory acidosis.

GSase activities in kidney tissue remained below detection.

Examination of plasma amino acid profiles revealed little change during respiratory acidosis apart from small decreases in valine, leucine, and isoleucine concentrations. However, during metabolic acidosis, there were marked increases in the concentration of most of the possible substrates for renal ammoniagenesis (Table 6). The total concentration of amino acids in the blood plasma approximately doubled, with the largest contributions coming from taurine, threonine, and lysine. Notably, although alanine and glutamine levels both increased in accord with most of the other amino acids, the change in the former was larger. Aspartate, glutamate, and hydroxyproline remained extremely low and did not change; arginine was undetectable.

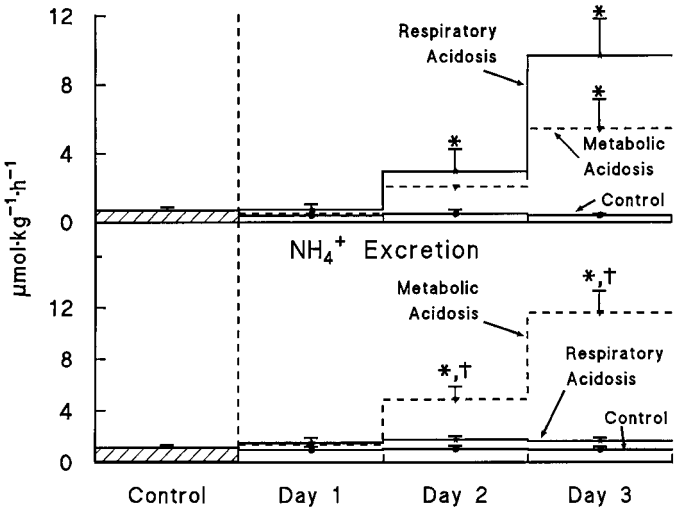

Fig. 4. Daily urinary excretion rates of total inorganic phosphate and NH_4^+ in rainbow trout of *series 2* on control day and during 3 days of subsequent exposure to control conditions ($n = 10$) or respiratory acidosis (environmental hyperoxia, $n = 10$), or metabolic acidosis (low environmental pH, $n = 10$). Data are means ± SE. *$P < 0.05$ vs. simultaneous control value; †$P < 0.05$, metabolic acidosis vs. respiratory acidosis.

Table 3. *Plasma concentrations of ammonia, inorganic phosphate, and cortisol in response to experimental treatments in series 2*

	Control ($n = 10$)	Respiratory Acidosis (P_{O_2} = 500–600 torr) ($n = 10$)	Metabolic Acidosis (Water pH = 4.5) ($n = 10$)
Ammonia, μmol/l	168 ± 21	109 ± 8	337 ± 57*†
Phosphate, mmol/l	2.28 ± .11	2.07 ± .12	3.04 ± .18*†
Cortisol, ng/ml	9.60 ± 2.84	12.87 ± 4.26	67.07 ± 13.95*†

Data are means ± SE; n = no. of animals. *$P < 0.05$ vs. control; †$P < 0.05$ vs. respiratory acidosis.

DISCUSSION

Comparison to Other Studies on Fish and Mammals

The blood acid-base responses (Table 1) of rainbow trout in the present study to treatments designed to induce metabolic acidosis (acid exposure), respiratory acidosis (hyperoxia), and metabolic alkalosis ($NaHCO_3$) were virtually identical to previous reports (see introduction). Qualitatively, the renal acid-base responses were also similar, in terms of the relative importance of NH_4^+, TA (as inorganic phosphate), and HCO_3^- excretion in the respective treatments. However, quantitatively, there were some differences. For example, although a longer $NaHCO_3$ infusion period was employed in the present study, the net rate of excretion of basic equivalents in the urine at 32–48 h (Fig. 1) was only ~70% of that recorded at 24–32 h in an otherwise identical experiment (16). Similarly, in *series 1* and *2*, net H^+ excretion rates at 48–72 h of exposure to mean pH 4.5

Table 4. *Enzyme activities in white muscle and liver in response to experimental treatments in series 2*

	Control ($n = 10$)	Respiratory Acidosis (P_{O_2} = 500–600 torr) ($n = 10$)	Metabolic Acidosis (Water pH = 4.5) ($n = 10$)
White muscle			
Glutamine synthetase*	ND	ND	ND
Glutaminase*	0.040 ± .010	0.057 ± .013	0.050 ± .013
Liver			
Glutamine synthetase*	0.35 ± .03	0.27 ± .03	0.32 ± .06
Glutaminase*	0.263 ± .023	0.303 ± .027	0.287 ± .017

Data are means ± SE; n = no. of animals. *All units are μmol substrate → product·g^{-1}·min^{-1}. ND, not detectable. There are no significant differences at $P \leq 0.05$.

Table 5. *Enzyme activities in kidney in response to experimental treatments in series 2*

	Control ($n = 10$)	Respiratory Acidosis (P_{O_2} = 500–600 torr) ($n = 10$)	Metabolic Acidosis (Water pH = 4.5) ($n = 10$)
Glutamine synthetase‡	ND	ND	ND
Glutaminase‡	0.110 ± .007	0.106 ± .009	0.138 ± .009*†
Glutamate dehydrogenase‡	27.60 ± 2.96	29.72 ± 2.71	34.59 ± 1.53*
α-Ketoglutarate dehydrogenase‡	0.021 ± .006	0.039 ± .009	0.066 ± .010*
Phospho*enol*pyruvate carboxykinase‡	0.45 ± .12	0.74 ± .10	0.73 ± .17
Alanine amino transferase‡	28.92 ± 3.35	43.62 ± 5.03*	39.49 ± 3.51*

Data are means ± SE; n = no. of animals. *$P \leq 0.05$ vs. control; †$P \leq 0.05$ vs. respiratory acidosis. ‡All units are μmol substrate → product · g^{-1} · min^{-1}. ND, not detectable.

were ~90% (Fig. 1) and 35% (Fig. 4), respectively, of those measured in trout exposed for the same period to a slightly lower pH (4.2) (37). On the other hand, net H^+ excretion rates at 48–72 h of exposure to an environmental P_{O_2} of 500–600 torr were ~200% (*series 1*, Fig. 1) and 80% (*series 2*, Fig. 4) of those recorded in a similar exposure (64). Water quality and size of the trout were very similar in all these studies. Thus the reasons (nutritional status, season, stock differences?) for this quantitative variation are unclear. Nevertheless, the variation likely reflects more or less reliance on the gills (the predominant organ for excreting acidic or basic equivalents) in different batches of fish. That notable variation occurred even between the two series of the present study, in which all the fish originated from the same brood stock, but two years apart, emphasizes the fact that simultaneous comparisons within single stocks of fish (as in each of the present series) are essential for discerning treatment differences.

Despite this variability, the most important finding of the present study is its high level of agreement with patterns described in mammalian physiology. Considering the great phylogenetic distance between freshwater fish and air-breathing mammals, their completely different environments, and their very different anatomies (presence of gills and absence of loop of Henle in fish), the general similarity of their renal responses to acid-base disturbances is remarkable (c.f. Refs. 45, 47). These include the reliance on HCO_3^-, TA, and NH_4^+ as the primary excreted acid-base equivalents during metabolic alkalosis, respiratory acidosis, and metabolic acidosis, respectively (Fig. 1, Table 2), the relationships of these variables with urine pH (Figs. 2, 3), the upregulation of many of the same ammoniagenic enzymes in the kidney during metabolic acidosis (Table 5, Fig. 5), and the potential involvement of cortisol (Table 3) and amino acid mobilization (Table 6) in the latter response.

A few studies on freshwater teleosts (26, 30, 42) have suggested that their renal acid-base physiology might be quite similar to that of mammals with a variable urinary acid-base content, a dependence on carbonic anhydrase for acid secretion and HCO_3^- reabsorption, and a capacity for ammoniagenesis. However, most mechanistic research on this topic in fish has concentrated on marine elasmobranchs, which appear to be very different from mammals in having an acid-secreting kidney that lacks carbonic anhydrase, produces a urine of fixed low pH (~5.8) and low volume, has limited ammoniagenic capacity, and exhibits little flexibility or capacity for dealing with acid-base disturbances (11, 17, 29, 34, 53, 54). Similarly, several studies on the marine teleost kidney suggest that the acid-base

Table 6. *Plasma amino acid concentrations in response to experimental treatments in series 2*

Amino Acids‡	Control ($n = 10$)	Respiratory Acidosis (P_{O_2} = 500–600 torr) ($n = 10$)	Metabolic Acidosis (Water pH = 4.5) ($n = 10$)
Arginine	ND	ND	ND
Aspartate	0.001 ± .001	0.009 ± .009	0.000 ± 0.00
Glutamate	0.024 ± .003	0.029 ± .009	0.023 ± .002
Hydroxyproline	0.022 ± .002	0.026 ± .005	0.030 ± .004
Serine	0.108 ± .006	0.104 ± .006	0.171 ± 0.019*†
Asparagine	0.117 ± .004	0.114 ± .010	0.190 ± .011*†
Glutamine	0.331 ± .019	0.284 ± .011	0.412 ± .027*†
Glycine	0.274 ± .015	0.282 ± .021	0.517 ± .059*†
β-Alanine	0.010 ± .004	0.018 ± .007	0.028 ± .009*
Histidine	0.126 ± .011	0.147 ± .020	0.278 ± .030*†
Taurine	0.324 ± .062	0.284 ± .030	0.733 ± .119*†
Threonine	1.023 ± 0.97	0.914 ± .072	2.436 ± .301*†
Alanine	0.363 ± .027	0.333 ± .027	0.705 ± .055*†
Proline	0.021 ± .005	0.027 ± .008	0.074 ± .013*†
Tyrosine	0.040 ± .005	0.029 ± .006	0.075 ± .011*†
Valine	0.702 ± .046	0.595 ± .023*	0.780 ± .059†
Methionine	0.095 ± .006	0.084 ± .010	0.176 ± .012*†
Leucine	0.317 ± .021	0.267 ± .013*	0.350 ± .029†
Isoleucine	0.524 ± .030	0.409 ± .013*	0.633 ± .050†
Phenylanine	0.117 ± .006	0.112 ± .010	0.172 ± .014*†
Tryptophan	0.107 ± .012	0.101 ± .013	0.258 ± .021*†
Lysine	0.575 ± .043	0.501 ± .034	1.344 ± .090*†
Total	5.221 ± .248	4.668 ± .198	9.394 ± 0.534*†

Data are means ± SE; n = no. of animals. *$P \leq 0.05$ vs. control; †$P \leq 0.05$ vs. respiratory acidosis. ‡All concentrations are mmol/l. ND, not detectable.

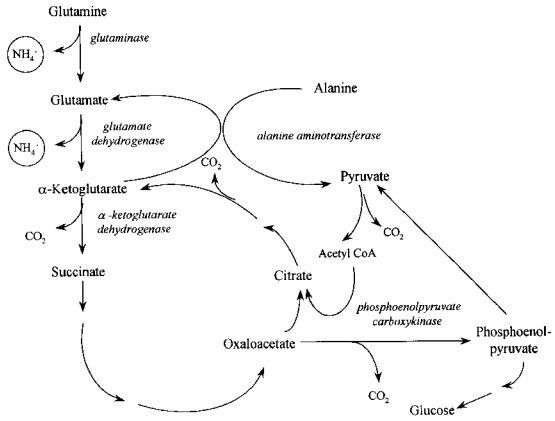

Fig. 5. Proposed model by which increased activity levels of renal enzymes tabulated in Table 5 contribute to increased renal ammoniagenesis in freshwater trout kidney during metabolic acidosis.

regulatory capacity is again low, constrained by the low urine flow rate in seawater and by an absence of carbonic anhydrase (34, 36). In contrast, the present study provides further evidence that the freshwater teleost kidney has similar mechanisms and comparable flexibility to the mammalian kidney. Its UFR and acid-base excreting capacity are greater than those of seawater fish, although the latter is clearly lower than that in mammals, reflecting the predominance of the gills (see introduction).

Differential NH_4^+ Response to Metabolic vs. Respiratory Acidosis

The much greater urinary net H^+ excretion in the form of NH_4^+ in freshwater trout during metabolic acidosis relative to respiratory acidosis (Figs. 1, 3, and 4; Table 1) is in direct accord with mammalian findings (46, 47). In acidotic mammals, the fraction of urinary NH_4^+ resulting from filtration of plasma is small, and the increased urinary NH_4^+ excretion is known to reflect greatly increased metabolic production of ammonia in the kidney. The same appears to be true in the trout despite the elevation in plasma [NH_4^+] during metabolic acidosis (Table 3). For example, using a glomerular filtration rate (GFR) measured in trout under comparable conditions [5.2 $ml \cdot kg^{-1} \cdot h^{-1}$; (16)], the calculated filtered load of NH_4^+ could explain <20% of the measured NH_4^+ excretion rate for the metabolic acidosis treatment in *series 2* (Fig. 4).

In the present study, at least part of the difference between the responses to respiratory vs. metabolic acidosis may have been a result of the more intense extracellular pH depression in the latter (0.15 vs. 0.55 units; Table 1). On the basis of earlier studies (25, 68, 69), the extent of pHa depression even early during hyperoxia in trout is rarely much greater than that measured here, because Pa_{CO_2} buildup occurs slowly and progressively, and compensating [HCO_3^-] accumulation essentially keeps pace. Regardless, in mammals there is abundant evidence that the chronic renal response is mediated by factors other than a simple change in extracellular pH, and that renal NH_4^+ production and excretion are much higher during chronic metabolic acidosis, even when the extracellular pH depression is identical to that during chronic respiratory acidosis (9, 12, 33). The more meaningful comparison is at the same urine pH (44, 45); Fig. 3 highlights the dramatic difference between the two treatments when the comparison is made on this basis in the present study. The original "diffusion-trapping" model of Pitts (44) for ammonia distribution into urine has now been superseded by more mechanistic analyses (20, 22, 31) but nevertheless still provides a useful description of the overall process. This model certainly fits the exponential increase of urinary [NH_4^+], inasmuch as urine pH fell during metabolic acidosis (Fig. 3). Using the Henderson-Hasselbalch equation with appropriate values for pK′ (negative log of apparent dissociation constant) and ammonia solubility in trout plasma (5), and assuming an intracellular pH 0.5 units below extracellular pH (69), the observed urinary [NH_4^+] values can be explained by equilibration with a compartment that has a concentration of NH_4^+ ~20-fold higher than that measured in plasma during metabolic acidosis (Table 3). The difference is only 5- to 10-fold during the other treatments, which supports the idea of a stimulation of ammoniagenesis, and therefore elevated [NH_4^+], in the renal tubule cells during metabolic acidosis. Enzymatic profiles in the kidney (Table 5, Fig. 5; see below) also support this interpretation. King and Goldstein (30) similarly concluded that ammoniagenesis was stimulated in the kidney of goldfish subjected to acute low pH exposure.

Although the preferential stimulation of NH_4^+ production and excretion by metabolic vs. respiratory acidosis is widely recognized in mammalian physiology, there appears to be no agreement on the explanation. Simpson (50) and Pitts (44) pointed out that chronic respiratory acidosis does not lower urine pH to the same extent as chronic metabolic acidosis; as a result of the much higher HCO_3^- filtration in the former, more of the H^+ ions secreted are used for HCO_3^- reabsorption and less are available to trap NH_3. Furthermore, Krapf et al. (32) demonstrated that chronic metabolic acidosis is in fact a more powerful inducer of the Na^+/H^+ antiporter in the rat proximal tubule, where most HCO_3^- reabsorption occurs. However, these observations do not explain [NH_4^+] differences at the same urine pH, or differences in [NH_4^+] vs. [TA]. Sabatini and Kurtzman (47) attributed the difference in ammoniagenesis to a reduction of renal blood flow during respiratory acidosis, although most other workers claim that glutamine supply is not limiting (22, 44). Rodriguez-Nichols et al. (46) reported a stimulation and a blunting, respectively, of ammoniagenic capacity in the tubules of rats subjected to chronic metabolic and respiratory acidosis. Other workers have interpreted these results as indicating that a decrease in intracellular or intramitochondrial [HCO_3^-] during metabolic acidosis stimulates key ammoniagenic enzymes, whereas an increase during respiratory acidosis inhibits them (22, 49). Interestingly, although a stimulatory role for corticosteroids in renal ammoniagenesis is widely recognized, possible differences in this parameter during the two types of acidosis appear to have been overlooked (see *Perspectives*).

Differential TA Response to Respiratory vs. Metabolic Acidosis

In chronic respiratory acidosis, the majority of the H^+ ions secreted by the kidney are employed in reabsorbing the increased filtered HCO_3^- load; only those that are not consumed in this process appear in the net H^+ excretion measurement. Using the measured elevation in plasma HCO_3^- concentration during respiratory acidosis in *series 1* (Table 3) and the same GFR as assumed earlier (16), ~55 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ of H^+ ions were secreted for HCO_3^- reabsorption in addition to the 14 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ that appeared in the urine as [TA − HCO_3^-] and NH_4^+ (Fig. 1). Viewed on this basis, the renal response to chronic respiratory acidosis was

greater than that to chronic metabolic acidosis in the same series (45 μmol·kg^{-1}·h^{-1}; Fig. 1). Furthermore, TA clearly comprised the larger fraction of excreted H^+ during respiratory acidosis, in contrast to metabolic acidosis.

In mammals, all other things being equal, the ratio of TA to NH_4^+ in the urine depends on the availability of titratable buffer, principally phosphate; i.e., the rate of H^+ secretion increases to match titratable buffer availability, thereby keeping urine pH more or less constant (45, 50, 56). Phosphate supply increases primarily as a result of decreased tubular reabsorption from the glomerular filtrate; phosphate secretion does not occur (40). In the rainbow trout, the increased appearance of phosphate (and perhaps other unmeasured buffers) in the urine appears to be the dominant feature of the renal response to respiratory acidosis, such that large increases in [TA − HCO_3^-] occur without significant lowering of urine pH (Fig. 2, Table 2). Similar patterns have been reported previously (43, 64). Indeed, the relative stimulation of TA excretion over NH_4^+ excretion during respiratory acidosis appears to be more marked in the trout (Figs. 1 and 4) than in mammals (9, 45).

In this regard, fish may have an advantage relative to mammals, namely the ability to secrete phosphate into the urine, as well as to add it by glomerular filtration. Using assumed GFR values and a clearance ratio (CR) analysis, Wheatly et al. (64) concluded that phosphate handling by the trout kidney changed from net reabsorption (CR $<$ 1.0) to net secretion (CR $>$ 1.0) during respiratory acidosis to the extent that plasma phosphate levels were significantly depleted. In the present study, using measured plasma phosphate concentrations (Table 3) and the same GFR as assumed earlier, the CR for phosphate during respiratory acidosis would be 1.0 (*series 2*) to 1.8 (*series 1*), suggesting net secretion. In all other treatments, net reabsorption occurred as indicated by CR $<$ 1.0. The significant increase in plasma phosphate level (Table 3), and associated increase in filtered load during metabolic acidosis likely contributed to the modest increase in urinary phosphate and [TA − HCO_3^-] excretion in this treatment (Figs. 1, 2, 4; Table 2). The ability of marine fish kidneys to secrete phosphate has long been known (21, 23, 52); recently, the molecular basis of the phenomenon, a Na^+-dependent phosphate cotransporter (NaPi-II), has been characterized in the nephron of the euryhaline winter flounder (63). Renal phosphate secretion has also now been directly documented in freshwater goldfish (28) and rainbow trout (3) subjected to phosphate loading. We speculate that respiratory acidosis may preferentially activate NaPi-II in its secretory mode in freshwater fish.

Response to Metabolic Alkalosis

The flexibility of the trout kidney was highlighted by its very different response to metabolic alkalosis vs. respiratory acidosis, conditions in which the elevations in plasma [HCO_3^-] were very similar (Table 1). The elevations in filtered HCO_3^- loads were likely also very similar. Rather than reabsorbing all of this filtered HCO_3^- as in respiratory acidosis (and excreting additional H^+), the kidney in metabolic alkalosis allowed approximately one half of the load to be excreted on a net basis (Fig. 1). Phosphate and ammonia secretion were not activated (Table 1). Very similar patterns have been seen in mammals, and modulation of the H^+ secretion/HCO_3^- reabsorption rate by both extracellular and intracellular P_{CO_2} as well as intracellular pH have been proposed as possible explanations (18, 45, 47). As in mammals, the process appears to be dependent on carbonic anhydrase in freshwater fish, because acetazolamide impaired renal H^+ secretion/HCO_3^- reabsorption in two catfish species (26, 42).

Mechanism of Renal Ammoniagenesis

In mammals, the dominant feature of the renal response to metabolic acidosis is a markedly increased production of ammonia from glutamine by the kidney itself. In the rainbow trout, the significant rise in plasma glutamine concentration (Table 6) and the renal activities of many of the enzymes involved in deamidation, deamination, and subsequent oxidation or gluconeogenesis from the carbon skeleton of glutamine (Table 5; Fig. 5) is consistent with the mammalian response (1, 14, 44, 45, 55, 59, 62). However, unlike mammals there were no detectable changes in GSase and GLNase activities in white muscle or liver indicative of increased glutamine production at extrarenal sites (Table 4), and glutamine was not the principal amino acid in blood plasma (Table 6; see also Ref. 38). Indeed during metabolic acidosis, many other amino acids increased to a greater extent, including alanine. Similar patterns were reported in acid-exposed brown trout (19) and may result from the well-known action of cortisol in driving proteolysis (57). Nevertheless, it is important to note that amino acid concentrations in plasma are not necessarily indicative of amino acid turnover rates. In this regard, the apparent mobilization during metabolic acidosis of taurine, a relatively inert amino acid, is notable. Although often considered an indicator of cell damage, this is unlikely to be the case in the present study. In freshwater fish, taurine appears to be used primarily as an osmolyte that is mobilized in response to situations such as low pH exposure, where ions are lost across the gills (19).

Glutamine metabolism appears to be very different in teleosts than in mammals, with GSase levels generally much lower than GLNase levels in most organs (Tables 4, 5; and Ref. 10). Although we were unable to detect GSase activity in either white muscle (Table 4) or kidney (Table 5), it should be noted that the detection limit of our assay was 0.04 U/g; using a more sensitive assay, Chamberlin et al. (10) reported levels of 0.01 and 0.08 U/g in these two tissues, respectively, in lake trout. Furthermore, in the absence of hepatic ureagenesis, the teleost kidney does not have to "compete" with the liver for glutamine as in mammals, but rather with skeletal muscle, where glutamine serves as an important metabolic fuel (10). Alanine rather than glutamine is often considered to be the principal vehicle for the transport of metabolic nitrogen in fish plasma (66).

Figure 5 proposes a model, only slightly modified from that generally accepted in mammals (1, 4, 44), by

which the increased activities of kidney enzymes measured in *series 2* (Table 5) would contribute to increased ammoniagenesis from both glutamine and alanine during metabolic acidosis. In contrast to the normal organization in the mammalian kidney, where net alanine synthesis occurs (59), AlaAT (a "near-equilibrium reaction") would catalyze the transfer of amino-nitrogen from alanine to α-ketoglutarate, thereby refueling the glutamate deamination reaction. Notably, this is the only kidney enzyme that also increased in activity during respiratory acidosis (Table 5), when a modest rise in renal ammonia output occurred. This model does not exclude contributions of amino-nitrogen by parallel transamination reactions from many of the other amino acids that also increased during metabolic acidosis (Table 6). King and Goldstein (30) provided evidence of a dominant role for transamination of aspartate in the kidney of the goldfish, although this amino acid was negligible in trout plasma (Table 6).

Perspectives

In mammals, both cortisol and aldosterone have been implicated in the renal ammoniagenic and H^+ secretory responses, respectively, during metabolic acidosis (18, 47, 62). In teleost fish, cortisol alone subserves both gluco- and mineralocorticoid functions. Notably, plasma cortisol levels increased significantly during metabolic acidosis but not during respiratory acidosis (Table 3), consistent with previous reports (2, 69). Indeed, during a comparable low pH exposure in trout, cortisol turnover rates increased to a much greater extent than simple plasma concentrations (2). We speculate that cortisol mobilization may be the key factor responsible for the fundamentally different response of the trout kidney to metabolic vs. respiratory acidosis. Future studies may profitably look at the role of this hormone in reorganizing both renal and extrarenal metabolism during metabolic acidosis.

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