Renal function and acid–base regulation in two Amazonian erythrinid fishes: *Hoplias malabaricus*, a water breather, and *Hoplerythrinus unitaeniatus*, a facultative air breather[1]

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The function of the kidney in ion, water, and acid excretion was investigated in two erythrinid fishes, the water-breathing *Hoplias malabaricus* and the facultative air-breathing *Hoplerythrinus unitaeniatus*. Chronic catheterization of the urinary papilla and the dorsal aorta provided information on the urinary parameters and blood acid–base status. By monitoring total flow of urine, pH, and concentrations of Na^+, Cl^-, ammonia, titratable acidity, and lactate, the total renal flux of water, various ions, and total acid was computed. The kidneys of both species were found capable of acidifying urine, creating gradients of up to 620:1 for H^+ ion, and contributing substantially to steady-state acid excretion. There was no significant increase in lactate or total acid efflux from urine during postoperative (metabolic) acidosis. Respiratory (hypercapnic) acidosis caused a compensatory increase in blood HCO_3^-, and an increase in branchial Na^+ uptake (presumably by Na–H exchange), but no change in ammonia excretion. There was no renal response in one *Hoplias* to hypercapnia, but an increased acid excretion in one *Hoplerythrinus*. The behavior of the urinary excretion system appears in various respects similar to the higher vertebrates. There was no obvious correlation between renal parameters and air breathing in these two species.

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On a étudié le rôle du rein dans l'excrétion des ions, de l'eau et des acides chez deux poissons érythrinidés, *Hoplias malabaricus*, à respiration aquatique, et *Hoplerythrinus unitaeniatus* à respiration aérienne facultative. Le cathétérisme chronique des papilles urinaires et de l'aorte dorsale ont permis de recueillir des données sur les paramètres urinaires et l'équilibre acide–base du sang. On a mesuré l'écoulement total d'urine, ce qui a permis d'évaleur le pH, les concentrations de Na^+, de Cl^- et d'ammoniaque, l'acidité et le lactate titrables, l'écoulement total d'eau dans le rein, les concentrations d'ions divers et la concentration totale d'acide. Chez les deux espèces, les reins peuvent acidifier l'urine, créer des gradients allant jusqu'à 620:1 dans le cas de l'ion H^+, et favoriser une excrétion d'acide relativement stable. L'acidose (métabolique) postopératoire ne cause pas d'augmentation importante de l'excrétion du lactate ou de la fraction acide totale. L'acidose respiratoire (hypercapnie) entraîne une augmentation compensatoire des ions HCO_3^- dans le sang et une augmentation de l'absorption du Na^+ par les branchies (sans doute dans des échanges Na–H); l'excrétion d'ammoniaque ne subit toutefois pas de changements. Un spécimen d'*Hoplia* est resté sans réaction à l'hypercapnie; un spécimen d'*Hoplerythrinus* a réagi à l'hypercapnie en excrétant plus d'acide. Le système excréteur urinaire se comporte donc à bien des points de vue, chez ce poisson, comme chez les vertébrés supérieurs. Il n'y a pas de corrélation très marquée entre les paramètres rénaux et la respiration aérienne chez ces deux espèces.

[Traduit par le journal]

Introduction

Past investigations of the function of the teleost kidney have largely focussed on its function in water and ion regulation (Hickman and Trump 1969) and nitrogen excretion (Forster and Goldstein 1969). However the latter role may be intimately bound up with another function, namely acid–base regulation, which has not been investigated in the teleosts. In higher vertebrates, the kidney is responsible for long-term regulation of

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blood pH, for example during chronic hypercapnic acidosis, shorter term adjustments being achieved mainly by ventilation and shifts in intracellular and extracellular buffer stores (Davenport 1974). In elasmobranchs, the kidney has been shown to contribute to steady-state acid excretion, but exhibits a negligible response to acute experimental acidosis (Cross *et al.* 1969). So far, all that is known about any possible acid–base role of the teleost kidney is that the urine pH tends to be somewhat below that of the blood (Hickman and Trump 1969), and decreases further after hypoxic stress (Hunn 1969), but without information on buffering capacity, total acidity, etc., one cannot assess the contribution of the urine to acid excretion.

On the other hand, there is information indicating that the gill of teleosts is an important acid–base regulating organ. In the goldfish, there is a branchial exchange of Na^+ ions for either NH_4^+ or H^+ ions, and of Cl^- ions for either HCO_3^- or OH^- ions, presumably in the "chloride" cells of the gills (Maetz and Garcia-Romeu 1964; Maetz 1973; De-Renzis and Maetz 1973). Similarly, these mechanisms may occur in the trout (Kerstetter *et al.* 1970; Kerstetter and Kirschner 1972). Furthermore, in the grayling, Na^+ and Cl^- uptake vary in a manner that would tend to compensate hypercapnic acidosis; i.e. the ratio of sodium uptake (H^+ excretion) to Cl^- uptake (OH^- excretion) rises in response to hypercapnia, leading to a net accumulation of HCO_3^- ions in the blood (Cameron 1976). It has been known for some time that most of the ammonia excretion takes place through the gills rather than the kidney in teleosts (Smith 1929).

Since the opportunity for ion exchange with the external medium declines as an animal evolves onto the land, it might be expected that the acid–base regulating ability of the kidney evolved in concert with other adaptations for terrestrial living. We therefore selected for this study two closely related fish, one a strict water breather (traíra, *Hoplias malabaricus*) and the other a facultative air breather (jejú, *Hoplerythrinus unitaeniatus*), both of which are common in the Amazon basin. (The jejú breathes air with a modified swim bladder served by a branch of the coeliac artery.) Our objectives were to study the steady-state acid excretion by the kidney in these two species, to assess their capacity to acidify urine, and to see if there was any response of renal acid excretion to acid loading in the blood.

Methods and Materials

All experiments were performed on board the *R/V Alpha Helix* during October 1976. Specimens of traíra, *Hoplias malabaricus*, and jejú, *Hoplerythrinus unitaeniatus* (both suborder Charcoidea, family Erythrinidae), weighing 250–750 g were purchased from fishermen in the neighborhood of Lake Janaucá, about 50 km upstream on the Rio Solimões from Manaus, Brazil. They were held without feeding in running water from the Solimões at temperatures ranging from 28 to 32°C, and a mean Na^+ concentration of 0.220 $mM \cdot \ell^{-1}$ and chloride of 0.135 $mM \cdot \ell^{-1}$.

Experimental Procedures

Dorsal aortic cannulation was performed as described for trout by Smith and Bell (1964) using urethane or MS222 anesthesia. Cannulae were filled with Cortland saline (Wolf 1963). The urinary papilla was catheterized as described by Wood and Randall (1973*b*). After surgery, the fish were transferred to lucite holding chambers supplied with running water and vigorous aeration. The chambers contained a water volume of 2500–3000 ml, with a comparable volume of air above the water. The whole unit could be sealed by taping a lid in place.

Urine was continuously collected in small covered polyethylene vials over regular intervals (generally 2 h). Samples were analysed for Na^+, Cl^-, and ammonia concentrations, and pH, total volume, lactate (in some cases), and total titratable acidity (*TA*). In one experiment, records of periodic urination were obtained by leading the outflow of the urinary catheter through a drop counter connected to a strip-chart recorder. In fish with patent aortic catheters, small blood samples were periodically taken for measurement of blood pH, total CO_2, and lactate concentration. Concentrations of Na^+, Cl^-, and ammonia in blood were usually measured on larger samples at the end of each experiment.

Hypercapnic acidosis was induced by bubbling sealed chambers with mixtures of 2% or 4% CO_2 in air, supplied by gas mixing pumps (Wösthoff). External pH also changed because of this treatment, from the usual values of 6.4–6.8 to 0.2–0.4 U lower. In one series of experiments, the time course of changes in blood pH and total CO_2 in response to hypercapnia was recorded in fish without urinary catheters.

Measurements of gill ammonia fluxes (in fish with urinary catheters) or whole body ammonia fluxes (no catheters) were performed by sealing off the chambers with a known volume of water and following the increase in ammonia concentration with time. External ammonia levels varied from 0 to 1 m*M*, and over this range fluxes were independent of concentration. Changes in external pH were measured simultaneously. At the pHs recorded (6–7) virtually all (>99.5%) of the ammonia exists in the ionized form (NH_4^+), so loss to the air of the nonionized form (NH_3) was negligible.

Measurements of whole animal Na^+ flux rates were performed by adding $^{22}NaCl$ to the sealed chambers and following the decrease in radioactivity and change in total Na^+ concentration at ½-h intervals for 2 h. At the end of the 2-h period, a second aliquot of the isotope was added, 4% CO_2 was introduced into the chambers, and after a ½-h mixing period, measurements were resumed for a further 2 h. At the end of the experiment, blood samples were taken for measurement of specific radioactivity of the blood. Since the blood specific radioactivity did not exceed 10% of the specific radioactivity of the external medium, influx calculations were performed as outlined by Kirschner (1970), ignoring backflux. External Na^+ levels were approximately 0.15 $mM \cdot \ell^{-1}$ (representative of the extremely low levels in the natural environment of these fish) and did not vary greatly over the period of the experiments. For the control and the hypercapnic period, the four flux measurements were subjected to regression analysis, and the difference between slopes (rates) for each period compared using analysis of covariance.

Measurements of O_2 consumption were obtained in non-catheterized fish by flow-through respirometry in a blackened chamber. A thermostatted microelectrode (Radiometer-Copen-

hagen) was used for P_{O_2} determinations. In these experiments, the fish were denied access to the air, so all O_2 consumption occurred from water.

The right set of gill arches from one traíra and one jejú of comparable size were preserved in 2% glutaraldehyde and 3% formalin in Cortland saline. Branchial dimensions were determined later by the weighted averages technique of Muir and Hughes (1969) as modified by Wood (1974).

Analytical Techniques

Sodium concentrations were measured using a flame photometer attachment for a spectrophotometer (Beckman DU-2), referring samples to a NaCl standard. Chloride analyses were performed with an amperometric titrator (Buchler–Cotlove). A micromodification of the method of Solorzano (1969) was used for the assay of total ammonia concentrations (NH_3 plus NH_4^+). A commercial reagent kit (Sigma 826-UV) was used for lactate analyses. Blood and water pHs were measured at the experimental temperature with a microelectrode (Radiometer-Copenhagen) and a macroelectrode (Fisher), respectively. Total CO_2 content was determined by the method of Cameron (1971). Estimates of P_{CO_2} and HCO_3^- levels in blood were obtained from pH and total CO_2 data by use of the Henderson–Hasselbach equation (Davenport 1974) with appropriate values for αCO_2 (Severinghaus 1965) and pK' (Albers 1970). A few direct measurements of P_{CO_2} in blood, water, and air were made with a Severinghaus microelectrode (Radiometer-Copenhagen). Total titratable acidity (*TA*) was measured by titrating a urine sample back to the blood pH with 0.010 *N* NaOH, which was standardized with 0.010 *N* HCl. In some cases where the blood pH was not measured at the time of urine collection, the urine samples were titrated to the average pH determined from earlier measurements on the same fish, or from measurements on other fish of the same species under similar conditions. The total renal H^+ concentration was then calculated as *TA* plus total ammonia concentration (Davenport 1974). Total urinary ammonia concentration is considered effectively equal to NH_4^+ ion concentration, for at the pH of the urine (≤7.4), at least 99% of total ammonia exists as NH_4^+ and therefore carries a proton. Urine titration curves were slightly sigmoid, as shown in Fig. 1; most had an inflection point at about pH 6.8 to 7.0. As an approximation of their buffer capacity (slope), a buffer index (*BI*) was calculated as *TA* divided by the difference between blood and urine pH.

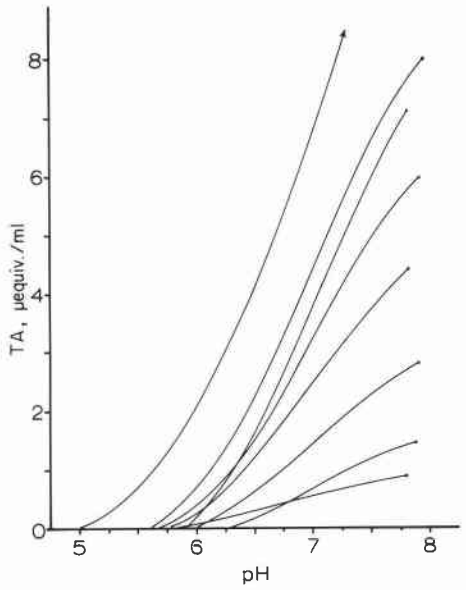

FIG. 1. Titration curves for some representative urine samples from traíra and jejú. Samples were titrated with 0.010 *N* NaOH back to the blood pH.

Results

Blood Composition

Concentrations of various ions and pH of the blood of resting fish are given in Table 1 for 15 traíra and 17 jejú; these values exhibit some marked differences from normal teleost patterns. Na^+ and Cl^- levels were unusually low in both species (cf. Holmes and Donaldson 1969) and there appeared to be a rather large anion deficit. This deficit was particularly large in traíra, where both Cl^- and HCO_3^- values were significantly below the jejú values. Though no analyses were performed, the blood seemed to have a high protein content, and the volume of TCA (tricarboxylic acid) precipitate was large. Anionic proteins could account for the anion deficit if they were highly acidic and thereby help reduce the ion pumping requirements in the dilute waters inhabited by these fish. We have no definitive explanation of the anion deficit.

Arterial pHs were unusually high in both species. This phenomenon was especially pronounced in jejú, where the value was significantly greater than in traíra and in other Amazon fish at a comparable temperature (Rahn and Garey 1973). Since the pH of neutrality (pN) at 30°C is 6.9165, the OH^-–H^+ ratio for traíra is 53:1, and for jejú 89:1, compared with 24:1 for the turtle, 40:1 for the frog (Howell 1970), and 32:1 for grayling and trout (Cameron 1976; Randall and Cameron 1973). The pH difference between jejú and traíra appears to be due to the higher HCO_3^- concentrations in the former, for calculated arterial P_{CO_2} values were virtually identical in the two species (≈5 torr, 1 torr = 133.322 Pa). Plasma ammonia levels were also higher in both species than in other teleosts (cf. Payan and Matty 1975). Blood lactate concentrations were low in all fully recovered fish.

Renal Function in Resting Fish

Recovery from surgery was rapid in these species, with urine flow rates stabilizing in a few hours, and blood pH reaching stable values in 2 to 6 h. Table 2 contains a summary of urine characteristics and urinary effluxes for eight traíra and six jejú after at least a 6-h recovery following surgical implantation of aortic and urinary catheters. There was considerable variability in the concentrations of all measured parameters both within individual fish and among different fish of the same species. The urinary effluxes were somewhat less variable, since there was a tendency for flows and concentra-

TABLE 1. Mean blood values (±SE) for traíra and jejú after at least 6 h recovery from surgery

	Traíra	Jejú	P*
Na^+	110.4±4.1 (11)	120.4±7.6 (5)	NS†
Cl^-	72.8±4.9 (11)	87.0±2.9 (8)	<0.05
HCO_3^-	8.73±0.35 (3)	11.97±1.02 (6)	<0.05
NH_4^+	0.85±0.17 (12)	0.86±0.09 (10)	NS
Lactate	0.59±0.40 (3)	1.00 (1)	NS
pH	7.78+0.03 (10) −0.04	7.87+0.02 (11) −0.02	<0.02

NOTE: All concentrations in milliequivalents per litre; numbers of animals in parentheses.
*Student's two-tailed *t*-test.
†Not significant.

tions to be inversely related. Urine pH ranged as low as 4.99 in one 2-h collection from traíra, and as low as 6.40 from one jejú during the resting, recovered period. Urinary total CO_2 concentrations were always below the sensitivity of the analytical method (i.e. <1 $mM \cdot \ell^{-1}$), as were urinary urea concentrations (<0.5 $mM \cdot \ell^{-1}$).

Virtually every parameter was significantly different between the two species (Table 2) except the mean flow of urine. Effluxes of NH_4^+ and ΣH^+ (*TA* + NH_4^+) were significantly different for the two species if data from J10 were eliminated. This fish had atypically high rates of *TA* and NH_4^+ excretion, which were not seen for any other jejú. The role of the kidney in acid excretion was obviously greater in traíra, and this difference was reflected in all associated parameters: i.e. lower urine pH, higher urine *BI*, higher urinary concentrations of *TA*, NH_4^+, and ΣH^+, and higher net effluxes of *TA*, NH_4^+, and ΣH^+.

Urinary losses of Na^+ and Cl^- were higher in jejú than traíra, though the data were extremely variable (Table 2). Comparisons of the whole animal Na^+ efflux rates in Table 5 with the renal effluxes in Table 2 reveal that urinary Na^+ losses were only about 4% of the total for traíra, on the average, and about 10% for jejú.

Casual observation suggested that the flow of urine was intermittent. The phenomenon was directly observed in traíra T12 which voided urine in four 'bursts' during a 4-h period. The bursts ranged from 33 to 138 s in duration, and produced 2.02, 0.38, 2.24, and 0.31 ml at intervals of almost exactly 1 h. The catheter in this fish was inserted less than 1 cm beyond the opening of the urinary papilla. No comparable observations were made on jejú, but it is our impression that urination is periodic in this species also.

An inadvertent demonstration of the volume regulating function of the kidney was provided by jejú J4. This fish had patent aortic and urinary catheters, and a series of 0.4-ml blood samples was drawn during the period or urine collection. The consequent reduction of urine volume is shown in Fig. 2.

Partitioning of Acid and Ammonia Excretion

In addition to the higher urinary excretions of NH_4^+, *TA*, and ΣH^+ in traíra, there was also a significant difference in the proportion of ΣH^+ excretion occurring as NH_4^+ (Table 3; the anomalous data from J10 have been omitted). *TA* was relatively more important in traíra, though it comprised less than 30% of the ΣH^+ efflux in either species. Absolute *TA* excretion was over five times higher in traíra than in jejú. Gill ammonia effluxes were extremely variable both within individual fish and among different fish of the same species, but the mean values were comparable in traíra and jejú (Table 3). However, largely because of the lower absolute NH_4^+ excretion in the urine, the fraction of total ammonia excretion by the gill was significantly higher in jejú. The mean renal excretion was less than 10% of the total ammonia excretion for both species.

Changes in water pH were monitored during the ammonia flux experiments. Values fluctuated considerably from time to time, but the long-term trend was consistently upward (seven of eight jejú, seven of eight traíra) at a mean rate of 0.06 pH units per hour in both species.

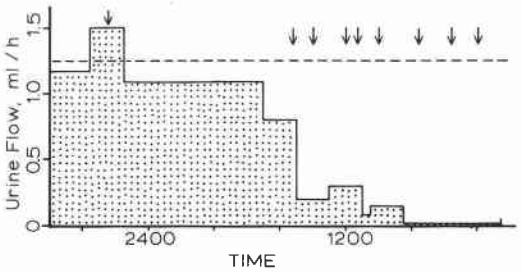

FIG. 2. Influence of serial withdrawal of 0.4-ml blood samples (indicated by arrows) on the urine flow of jejú J4 (324 g). The broken line represents mean urine flow for all jejú corrected to that weight (cf. data in Table 2).

TABLE 2. Mean urinary parameters for eight traíra and six jejû after at least 6 h recovery from surgery. *N* is the number of complete data sets used in the table, with mean values shown for each fish

Fish	*N*	Flow ml/100 g · h^{-1}	pH	*BI*, μequiv/ml · pH^{-1}	Concentrations, μequiv/ml				Effluxes, μequiv/100 g · h^{-1}			
					TA	NH_4^+	Na^+	Cl^-	NH_4^+	ΣH^+	Na^+	Cl^-
Traíra												
T11	3	0.52	6.35	1.72	2.47	3.59	7.10	4.08	1.74	2.85	3.48	1.94
T12	7	0.20	5.90	2.45	5.44	15.49	2.48	0.65	3.38	4.45	0.40	0.08
T8	5	0.14	5.80	4.75	9.51	27.84	6.79	0.45	3.63	4.95	0.93	0.05
T9	4	0.55	6.01	1.34	2.46	10.61	2.45	0.15	5.89	7.27	1.36	0.08
T7	5	0.30	6.32	1.97	3.23	7.80	6.10	4.53	2.45	3.49	1.77	1.20
T13	4	0.26	6.06	3.64	6.19	21.45	2.09	0.41	6.60	8.37	0.54	0.08
T15	2	0.06	5.10	4.01	10.84	13.49	14.08	0.44	1.15	1.95	0.96	0.03
T6	4	0.14	5.86	7.39	13.87	26.44	4.03	1.56	3.48	5.32	0.66	0.09
$\bar{x}\pm$SE		0.27 ±0.06	5.73 +0.28 −0.17	3.41 ±0.71	6.75 ±1.50	15.70 ±3.17	5.64 ±1.40	1.53 ±0.62	3.54 ±0.67	4.83 ±0.77	1.26 ±0.35	0.44 ±0.26
Jejú												
J3	2	0.38	6.73	0.64	0.59	3.46	—	4.05	1.12	1.36	—	4.35
J4	5	0.24	7.18	1.21	0.82	10.51	—	2.25	1.91	2.07	—	0.79
J8	1	0.50	7.19	0.54	0.33	3.84	18.16	14.74	1.91	2.08	9.09	7.34
J9	6	0.55	7.02	0.57	0.40	2.88	1.45	1.11	1.42	1.63	0.58	0.53
J10	2	0.34	6.79	2.79	3.22	16.16	3.77	0.90	5.33	6.50	1.34	0.31
J11	4	0.29	6.95	1.37	1.33	5.70	23.97	25.25	1.59	1.94	7.65	8.30
$\bar{x}\pm$SE		0.38 ±0.05	6.94 +0.09 −0.07	1.19 ±0.35	1.12 ±0.45	7.09 ±2.14	11.84 ±4.47	8.05 ±4.04	2.21 ±0.64	2.60 ±0.79	4.67 ±1.77	3.60 ±1.47
*P**		NS	<0.01	<0.05	<0.01	0.05<*P*<0.1	NS	0.05<*P*<0.1	†	†	0.05<*P*<0.1	<0.05

*Student's two-tailed *t*-test.
†Omitting data of J10, $P < 0.05$.

TABLE 3. Partitioning of total acid excretion in urine between *TA* and NH_4^+, and partitioning of NH_4^+ excretion between the gill and the kidney. All NH_4^+ and ΣH^+ flux data are expressed as microequivalents per 100 g per hour. *TA* can be obtained by subtracting urinary NH_4^+ from ΣH^+. Significance assessed using unpaired *t*-test

	Urinary efflux		% total acid excretion as NH_4^+	Gill efflux of ammonia	% total NH_4^+ excretion by kidney
	NH_4^+	ΣH^+			
Traíra					
T11	1.74	2.85	61%	—	—
T12	3.38	4.45	76	61.4	5.2%
T8	3.63	4.95	73	37.3	8.9
T9	5.89	7.27	81	43.8	11.9
T7	2.45	3.49	70	27.4	8.2
T13	6.60	8.37	79	63.8	9.4
T15	1.15	1.95	59	28.8	3.8
T6	3.48	5.32	65	25.1	12.2
$\bar{x}$	3.54	4.83	71	41.1	8.5
Jejú					
J3	1.12	1.36	82	—	—
J4	1.91	2.07	92	23.1	7.7
J8	1.91	2.08	92	100.6	1.9
J9	1.42	1.63	87	49.1	2.8
J11	1.59	1.94	82	48.6	3.2
$\bar{x}$	1.59	1.82	87	55.4	3.9
*P**	<0.05	<0.02	<0.01	NS	<0.05

*Two-tailed Student's *t*-test.

Postoperative Acidosis

A brief, but often severe acidosis followed the anaesthesia and surgery necessary to implant catheters in both species. Blood samples taken immediately after surgery had a pH of 7.2 to 7.6, but pH returned to normal by 6 h. In Fig. 3*a* the time course of pH correction is illustrated for several fish in which the acidosis was particularly pronounced. The pH depressions were associated with depressions of blood total CO_2 content, which returned to normal over a similar period (Fig. 3*b*). The latter indicates an acidosis of largely metabolic origin (i.e. displacement of HCO_3^- by fixed metabolic acid), and this was confirmed by measured blood lactate levels of 9 to 14 $mM \cdot \ell^{-1}$ immediately postoperatively, in contrast with resting levels of <1 $mM \cdot \ell^{-1}$ (Table 1). A slight respiratory acidosis (i.e. elevated P_{CO_2}) may also have occurred in some individuals, but there were no consistent variations in calculated arterial P_{CO_2} (Pa_{CO_2}) values.

Data from urinary collections made during this period of metabolic acidosis (i.e. the 6 h following surgery) are summarized in Table 4. There was no elevation of urinary H^+ excretion, and in fact none of the means were significantly different from the resting data in Table 2. Lowest urine pH values during this period were 5.18 in traíra and 5.69 in jejú. In many of the individual fish, there occurred a slight postoperative diuresis, but the variance in lumped data obscured the trend.

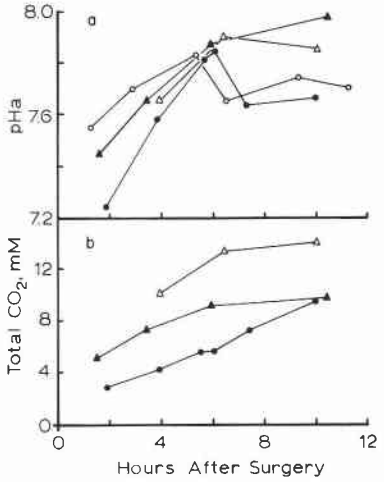

FIG. 3. Time course of arterial blood pH (pHa) and total CO_2 content following anaesthesia and surgery. Open circles = traíra T6, solid circles = T10; open triangles = jejú J7, solid triangles = J5.

Lactate dynamics during recovery are illustrated by the data of traíra T6 in Fig. 4. Note that although blood lactate was still considerably elevated at 3 h, the urine lactate had returned to nearly control levels. The blood lactate level at the first sample period for T6 was 13.9 $mM \cdot \ell^{-1}$, and the control value was 0.22 $mM \cdot \ell^{-1}$. Assuming a blood volume of 4% of body weight, and an equivalent lactate space, this 426-g fish would have had a total lactate

TABLE 4. Mean urinary parameters for six traíra and four jejú during the first 6 h following surgery. Units as in Table 2. None of the differences between these data and the resting data in Table 2 are statistically significant (*t*-test)

					Concentrations				Effluxes			
Fish	*N*	Flow	pH	*BI*	*TA*	NH_4^+	Na^+	Cl^-	NH_4^+	H^+	Na^+	Cl^-
Traíra												
T11	5	0.84	6.79	0.83	0.90	1.43	4.70	2.83	1.27	2.14	5.01	2.58
T12	3	0.42	5.96	0.46	0.95	3.40	2.17	1.50	1.43	1.86	0.97	0.61
T9	1	0.43	5.18	2.41	6.30	9.99	12.44	0.38	4.30	7.01	5.35	0.16
T7	2	0.67	5.98	2.86	5.00	7.13	6.95	3.70	4.60	7.89	4.65	2.47
T13	4	0.23	5.95	2.31	4.82	16.03	3.81	0.26	3.72	4.77	0.39	0.06
T6	2	0.58	5.74	4.91	9.34	16.67	7.70	2.14	2.30	3.64	1.06	0.29
$\bar{x}\pm$SE		0.53 ±0.09	5.70 +0.29 −0.17	2.30 ±0.65	4.55 ±1.32	9.11 ±2.59	6.30 ±1.48	1.80 ±0.56	2.94 ±0.60	4.55 ±1.02	2.91 ±0.95	1.03 ±0.48
Jejú												
J4	1	0.36	5.80	0.65	1.36	6.87	—	—	2.45	2.94	—	—
J9	2	0.47	7.38	0.74	0.23	2.88	0.63	0.04	1.37	1.47	0.30	0.12
J10	4	0.61	6.63	3.39	4.48	15.93	5.87	0.85	8.48	10.70	3.67	0.46
J11	3	0.87	7.17	0.44	0.21	3.05	13.76	9.10	2.82	3.03	11.16	6.89
$\bar{x}\pm$SE		0.58 ±0.11	6.32 +0.63 −0.25	1.31 ±0.70	1.57 ±1.01	7.18 ±3.08	6.75 ±3.81	3.33 ±2.89	3.78 ±1.60	4.54 ±2.09	5.04 ±3.21	2.49 ±2.20

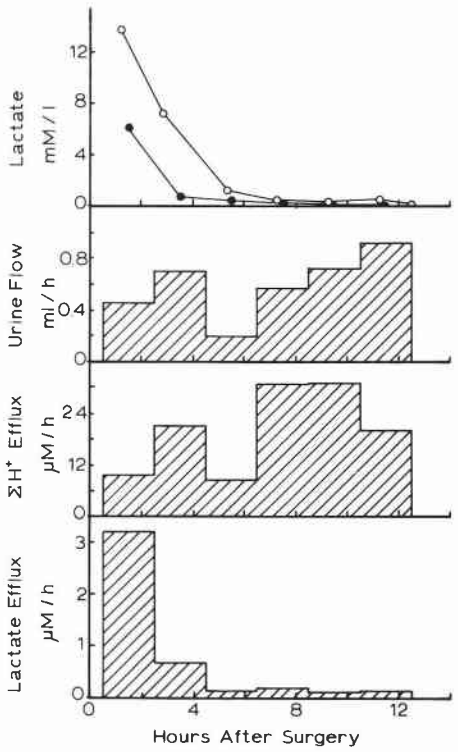

FIG. 4. Changes in blood and urine lactate concentrations (open and filled circles, respectively), urine flow, ΣH^+ efflux, and urinary lactate efflux in traíra T6 (426 g) following anaesthesia and surgery.

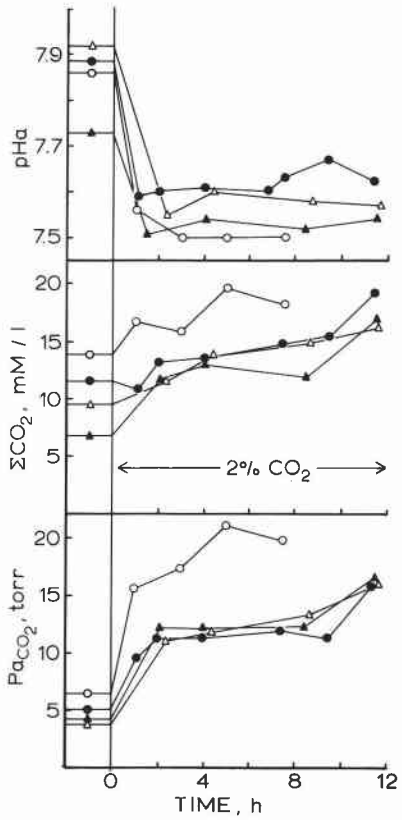

FIG. 5. Time course of arterial pH (pHa), total CO_2, and arterial P_{CO_2} (calculated) following onset of experimental hypercapnia. Open circles = jejú J4, solid triangles = traíra T10, solid circles = jejú J6, and open triangles = jejú J5.

excess of 234 μmol. In the first 8 h postoperation, 8.33 μ*M* were excreted in the urine, or only 3.6% of the excess. In a second traíra, T13, lactate excretion by the kidney in the first 6.5 h totalled only 3.2 μ*M*.

Hypercapnic Acidosis

The response of blood pH, total CO_2 and Pa_{CO_2} (calculated) to 2% CO_2 (15 torr) is shown in Fig. 5 for three jejú and one traíra. Arterial pH dropped rapidly, reaching a minimum by about 2 h, and stayed constant or increased slightly over the ensuing 10 h. Total CO_2 (largely plasma HCO_3^-), on the other hand, gradually increased throughout the experimental period. Pa_{CO_2} followed a similar trend, and in three of the fish did not surpass 15 torr until 11 h. This was due to the slow time course of P_{CO_2} increase in the chambers after the introduction of 2% CO_2, a fact confirmed by direct measurements of P_{CO_2} in blood, water, and air. Nevertheless, the experiment did illustrate an active compensation of the respiratory acidosis by HCO_3^- accumulation in the blood, for pH remained stable or increased slightly in the face of increasing Pa_{CO_2}. If compensation had not occurred, the minimum values of pH, the maximum values of total CO_2, and the maximum values of Pa_{CO_2} would all have coincided.

Unidirectional fluxes of Na^+ and net effluxes of ammonia were measured during a more severe hypercapnic acidosis (4% CO_2, 30 torr) in five traíra and four jejú (Table 5). The fish were not catheterized, so the figures in Table 5 represent whole animal flux rates. There was a significant increase in Na^+ influx in the jejú, but no other significant change. This increase in Na^+ influx roughly corresponded to the net increase in ammonia efflux seen for the jejú, but the latter was not statistically significant.

Urinary ΣH^+ efflux was measured during extended hypercapnia (4% CO_2) in one jejú and one traíra (Fig. 6). There was a slight drop in ΣH^+ excretion in the traíra, but a large increase in the jejú, manifested in both *TA* and NH_4^+ effluxes. The jejú also had atypically high urinary Na^+ and Cl^- losses.

Urinary Relationships

Complete data sets for both species (all postoperative and resting state data except that taken

TABLE 5. Whole animal Na^+ and ammonia fluxes ($\bar{x} \pm SE$) for five traíra and four jejú during control and hypercapnic (4% CO_2) periods. All fluxes in microequivalents per 100 g per hour

	Sodium			Ammonia
	Influx	Efflux	Net flux	net flux
		Traíra		
Control	27.85±6.15	33.00±3.31	−5.15±4.13	−43.6±5.9
Hypercapnic	32.04±7.79	39.91±7.06	−7.87±3.10	−43.8±5.1
*P**	NS	NS	NS	NS
		Jejú		
Control	44.56±0.44	42.69±1.94	1.87±2.34	−65.4±10.4
Hypercapnic	49.85±1.56	48.28±3.04	1.57±3.73	−73.0±10.3
*P**	<0.02	NS	NS	NS

*Covariance analysis for Na^+ fluxes; paired Student's *t*-test for ammonia fluxes.

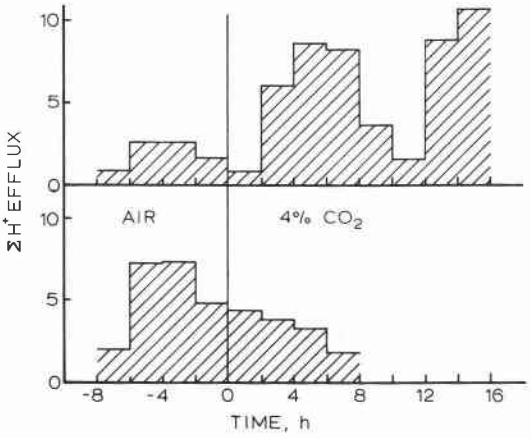

FIG. 6. Response of urinary acid excretion to hypercapnia in a jejú (upper panel, J11, 310 g) and a traíra (lower, T6, 426 g).

during hypercapnia) were subjected to correlation analysis to test for relationships between variables. The results for several urinary parameters plus blood pH are shown in Table 6. In jejú, there was a strong positive correlation between Na^+ and Cl^- concentrations in the urine, but essentially no such relationship in traíra (the weak positive correlation here is in fact due to 3 anomalous data pairs out of 49). In neither species were the concentrations of these ions related to flow.

In traíra, parameters of urine acidity (i.e. NH_4^+, *TA*, ΣH^+, *BI*, and pH) tended to decrease as urine flow increased, so that ΣH^+ efflux was independent of flow. However, in jejú, the flow–concentration relationships were less well defined, so ΣH^+ efflux was positively correlated with flow. In neither species was there a relationship between blood pH and any parameter of urine acidity. In both species, the two components of urine acidity, NH_4^+ and *TA*, were strongly correlated with each other (and of course with ΣH^+), indicating that these vary in concert. *TA* and *BI* were also strongly correlated in both animals, but despite this relationship, urine pH still dropped with increasing *TA*. Two relationships of particular importance were seen only in the traíra data: the negative correlation between urine NH_4^+ concentration and pH, and the positive correlation between urine Cl^- concentration and pH. (Similar interactions for NH_4^+ and Cl^- with other parameters of urine acidity are also apparent from Table 6.)

Respiratory Parameters

Total secondary lamellar surface area was over twice as large in the strict water breather, traíra, as in the facultative airbreather, jejú (Table 7). These areas are high relative to that of an active temperate species of similar predatory habits, the rainbow trout (Table 7). Such high surface areas, even in the air breather, may be adaptations to the frequently hypoxic environment of these erythrinids. The difference between traíra and jejú was expressed in a greater total number of gill filaments, a greater weighted mean filament length, and a greater weighted mean lamellar spacing in the former; however, weighted mean surface areas of an individual lamella were virtually identical. Despite this difference in respiratory surface area, routine O_2 consumption (entirely from water) was comparable in the two species: jejú, 59.1 ± 5.6 (4) ml $O_2 \cdot kg^{-1} \cdot h^{-1}$; and traíra, 49.7 ± 3.3 (4) ($\bar{x}$ ± SE (*N*)).

The observed frequency of air breathing in jejú was variable: some animals did not breathe air at all, others at intervals as short as a minute. According to Stevens and Holeton (personal communication), this variability is reflected in O_2 consumption data when access to air is provided, resting animals taking anywhere from 0 to 50% of their O_2 from the air. During experimental hypercapnia, the frequency of air breathing noticeably increased,

TABLE 6. Correlation analysis for urinary flow (ml/100 g · h^{-1}), urine pH_u, concentrations (NH_4^+, Na^+, Cl^-, *TA*, H^+, (μequiv/ml)), buffer index (*BI*, (μequiv/ml · pH^{-1})), ΣH^+ efflux (μequiv/100 g · h^{-1}), and blood pH (pH_b) for traíra (14–52 data sets from nine fish) and jejú (14–35 data sets from nine fish)

	Flow	pH_u	pH_b	NH_4^+	*TA*	ΣH^+	*BI*	Na^+	Cl^-
				Traíra					
pH_u	+0.47***								
pH_b	—	—							
NH_4^+	−0.52***	−0.56***	—						
TA	−0.53***	−0.63***	—	+0.84***					
ΣH^+	−0.37**	−0.61***	—	+0.98***	+0.92***				
BI	−0.48***	−0.48***	—	+0.69***	+0.94***	+0.90***			
Na^+	—	—	—	—	+0.40**	—	—		
Cl^-	—	+0.47***	—	−0.43**	−0.31*	−0.45**	—	+0.29*	
ΣH^+ efflux	—	−0.36**	—	+0.42**	+0.32*	+0.40**	+0.32*	—	−0.36**
				Jejú					
	Flow	pH_u	pH_b	NH_4^+	*TA*	ΣH^+	*BI*	Na^+	Cl^-
pH_u	—								
pH_b	—	—							
NH_4^+	−0.39*	—	—						
TA	—	−0.40*	—	+0.80***					
ΣH^+	−0.34*	—	—	+0.99***	+0.88***				
BI	—	—	—	+0.86***	+0.96***	+0.91***			
Na^+	—	—	—	—	—	—	—		
Cl^-	—	—	—	—	—	—	—	+0.97***	
ΣH^+ efflux	+0.35*	—	—	+0.60***	+0.73***	+0.65***	+0.74***	—	—

NOTE: Only significant correlations given; *r* significant at the 5% level (*), 1% level (**), or 0.1% (***).

TABLE 7. Branchial dimensions of a traíra, a jejú, and a rainbow trout of comparable size

	Traíra	Jejú	Rainbow trout†
Weight, g	374	395	340
Total number of filaments	3162	2018	1672
Filament length, mm*	5.69	4.53	7.56
Lamellar spacing, no./mm*	58.66	50.09	39.03
Total no. of lamellae	1 055 098.0	457 906.0	493 509.0
Lamellar surface area, mm^2*	0.2094	0.2163	0.1764
Total lamellar surface area, mm^2/g body weight	591	251	256

*Weighted averages.
†Wood 1974.

though it remained variable, and was not precisely recorded.

Discussion

Branchial and Urinary Fluxes

Urine flows and Na^+ and Cl^- loss rates were variable in both traíra and jejú (Tables 2, 4). To a large extent, this variability may reflect differences in the condition of experimental animals. While an effort was made to select fish in good condition, all the animals used had suffered some degree of abrasion or scale loss during capture and handling. As urine flows in fresh water reflect total body permeability (Hickman 1965), somewhat lower urine

TABLE 8. A comparison of some renal and metabolic parameters for selected fish and man

Species	Urine pH	Urine flow ml/100 g · h^{-1}	Urinary effluxes, μequiv/100 g · h Na^+	Cl^-	NH_4^+	ΣH^+	$\dot{V}O_2$, ml/kg · h^{-1}	Total ammonia efflux, μequiv/100 g · h^{-1}
Traíra	5.73	0.27	1.26	0.44	3.54	4.83	49.7	44.6
Jejú	6.94	0.38	4.67	3.60	2.21	2.60	59.1	57.6
Trout*	7.23	0.42	3.58	4.16	1.98	—	38.7‖	24.3
Dogfish†	5.78	0.05	12.0§	12.0§	0.03	3.07	43.0¶	8.4†**
Man‡	5.40	0.08	11.0	12.0	1.80	3.60	215.0	1.8

*Fromm 1963.
†Cross *et al.* 1969.
‡Altman and Dittmer 1972; Pitts 1974.
§Burger 1967.
‖Cameron and Davis 1970.
¶Lenfant and Johansen 1966.
**Murdaugh and Robin 1967.

flows (and salt losses) than those in Table 2 might be expected had all the fish been in perfect condition.

Nevertheless most of the variables measured in this study are in good agreement with comparable data for other teleosts in the literature. What is surprising is the remarkable temperature compensation shown by most parameters. In particular, the following resting state values at 30°C in the erythrinids are comparable with data at 5–18°C in temperate teleosts: urine flow and Na^+ and Cl^- loss rates (Mackay 1974; Hunn 1969; Hickman and Trump 1969; Hickman 1965), branchial Na^+ flux rates for fish at such low environmental Na^+ concentrations (Wood and Randall 1973*a*) branchial ammonia excretion (Maetz 1973), urinary ammonia excretion (Fromm 1963), and oxygen consumption (Cameron and Davis 1970). Similarly, the partitioning of ammonia and Na^+ losses between gills and kidney agrees with the ratios reported at much lower temperatures (Fromm 1963; Maetz 1972; Wood and Randall 1973*a*).

Urine flow in both species appeared intermittent; to our knowledge, intermittent urination has not previously been documented in teleosts. In view of recent studies showing a resorptive capability in the teleost bladder (e.g. Beyenbach and Kirschner 1975), we believe the present urinary data represent true 'voided' urine rather than ureteral urine collected by continuous drainage. The degree of urine modification in the bladder and the influence of catheter placement on urine composition need a great deal more attention in future studies.

Resting Acid Excretion by the Kidney

The major finding of this study is that the teleost kidney plays an important role in steady state acid excretion. In spite of the very different natures of the animals, the present two Amazonian erythrinids, the marine dogfish (Cross *et al.* 1969), and the standard man (Altman and Dittmer 1972; Pitts 1974) all exhibit very similar rates of urinary acid efflux on a weight-adjusted basis (Table 8). For the erythrinids and the dogfish, the ratios of acid excretion to metabolic rate are about the same, whereas for man the ratio is much lower, owing to the higher metabolic rate. Part of this difference may be due to the strictly carnivorous, high-protein diet of the fish as opposed to the catholic diet of man. The relationship between total ammonia excretion and metabolic rate in fish and man is also pointed out in Table 8.

The upward shift in the pH of the water in the closed chamber experiments with catheterized fish tends to indicate a net excretion of base by the gills, so that net acid excretion by the whole animal may be less than the urinary figure. However this result must be interpreted with caution, for as Maetz (1973) has pointed out, if there is a significant branchial ammonia excretion in the nonionized form (NH_3), then water pH could increase in the absence of a net base excretion. Calculated NH_3 gradients were favourable for diffusive outflux at the gills in most of our experiments, so this possibility cannot be ignored.

Urinary Acidification

The teleost kidney, especially that of traíra, shows considerable similarities to the mammalian kidney in the mechanisms of urinary acidification. The minimum urine pH values observed were 4.99 in a resting traíra, and 5.69 in a postoperative jejú. These figures reflect urine:blood H^+ gradients of about 620:1 and 120:1 respectively. The former compares favourably with the maximum gradient of 800:1 exhibited by the mammalian kidney (Davenport 1974). NH_4^+ accounted for about 71% of total urinary acid excretion in traíra and about 87% in jejú, compared with 50–71% in normal man (Pitts 1974). This situation contrasts with the elasmobranch kidney, which excretes only 1% of total H^+ as NH_4^+ (Cross *et al.* 1969) (Table 8). The

advantage in using NH_4^+ may lie in Na^+ conservation, which is important to the freshwater teleost and the terrestrial mammal but not to the marine dogfish. Protons trapped as NH_4^+ do not contribute appreciably to urine acidity; consequently the tubular Na^+–H^+ exchange which is thought to account for Na^+ reabsorption will not be inhibited by low urine pH (Davenport 1974). The close correlation between urinary *TA* and *BI* (Table 6) is similar to the mammalian pattern, and indicates that the excretion of *TA* is dependent upon the availability of appropriate buffers (Pitts 1974). The main mammalian urine buffer is phosphate, with a p*K* of about 6.8; the similarity to buffer curves shown in Fig. 1 may be significant. The inverse relationship between urine pH and NH_4^+ concentrations (Table 6) in traíra is also commonly seen in the mammalian kidney. This is thought to indicate that ammonia is produced in the nonionic form NH_3, and can freely diffuse through the kidney; the lower the urine pH, the greater is the sink into which NH_3 diffuses to be trapped as NH_4^+ ions (Pitts 1974).

The positive correlation between urinary Cl^- concentration and pH (Table 6) does not usually occur in mammals, however, where high HCO_3^- reabsorption (i.e. high urine acidity and low pH) is commonly associated with high Cl^- excretion (Davenport 1974). In mammals the mechanism is complex and apparently does not involve a direct HCO_3–Cl^- interaction at the tubular level (Pitts 1974), so this may not reflect a real difference in renal physiology.

The sodium data are somewhat confusing, in that Na^+ concentrations of urine samples were not correlated with any other variable except *TA* (in traíra only). That is, if sodium conservation is effected by Na–H exchange, one would expect an inverse correlation between Na^+ concentration and various parameters of urine acidity, which does not appear (Table 6). The weak correlation between Na^+ concentration and urine flow in traíra may also be spurious, and could have been caused by one animal (T11). The mechanism of Na conservation needs further investigation.

Postoperative Acidosis

There was no demonstrable response of the kidney in terms of acid excretion to metabolic acidosis following surgery. There was some excretion of lactate in urine, but the total efflux was only a few percent of the lactate excess (Fig. 4). This is not unexpected, since the lactate can be remetabolized to pyruvate and oxidized at a later time, resulting in a far greater energy yield. Compensation for this acute metabolic acidosis is probably due largely to the metabolism of lactic acid by the liver rather than to acid excretion.

Hypercapnic Acidosis

In a number of aquatic animals, it is now established that during hypercapnic acidosis, blood pH is gradually returned to normal by an elevation of plasma HCO_3^- concentrations (Cameron and Randall 1972; Truchot 1975; Janssen and Randall 1975; Cameron 1977). Although the present experiments were less than ideal owing to the slow time course of the P_{CO_2} rise in the experimental chambers, they did indicate a similar pattern (i.e. active regulation of the extracellular pH by HCO_3^- accumulation (Fig. 5)).

Branchial influx of sodium increases during hypercapnia in the grayling (Cameron 1976) and the blue crab (Cameron 1977). Since this increase is concomitant with an increase in blood HCO_3^-, increased excretion of H^+ (or NH_4^+) is hypothesized as the mechanism of regulation. In the present study, small increases in Na^+ influx also occurred during the hypercapnia, though these were significant in jejú, not traíra (Table 5). The reduction in external pH during hypercapnia may have inhibited Na^+ uptake via Na^+–H^+ exchange to some extent, obscuring the branchial response to blood acidosis. The pH change was fairly small (0.2–0.4 U) in these already acid waters, but further experiments with buffered media would be worthwhile.

The data are then consistent with the general hypothesis of pH regulation via control of Na^+–H^+ and Cl^-–HCO_3^- exchanges, but the changes observed in this study were small and not conclusive. Comparison of grayling and blue crab responses (Cameron 1976, 1977) indicates that there may be a variable lag time before a large response in Na^+ uptake is seen, and it might have been interesting to continue the sodium flux experiments for several more hours. It is also perhaps worth noting that the natural environment of these fishes, which includes shallow ponds, flooded areas, and swampy lakes, may have diurnal variations in P_{CO_2} of 15–20 torr; consequently short-term changes may be ignored by the regulatory system.

The renal response to hypercapnia was assessed in only one traíra and one jejú (Fig. 5). Unfortunately the latter showed abnormally high Na^+ and Cl^- loss rates in the urine (T6 and J11, Table 2). The jejú responded with a large increase in the renal H^+ excretion, and traíra with a slight decrease. On the basis of this scanty evidence, it remains to be convincingly demonstrated whether or not there is a renal role in the compensation of hypercapnic acidosis. Certainly the physiological capability is

there; i.e. the kidney can strongly acidify urine, and the rate of urinary H^+ excretion is not fixed, but varies with time in a given individual. What is not clear is whether there is any link between these temporal variations and the variations in blood pH.

Comparison of Traíra and Jejú

The lower resting acid excretion by the kidney of jejú may reflect a difference in net metabolic acid production and (or) differences in renal function. We have no information on the former, but several factors may contribute to the latter. The jejú kidney exhibits a lower capacity to secrete H^+ ions against a gradient, and a lower buffer capacity in the urine (*BI*, Table 2). Furthermore, the higher HCO_3^- concentrations of jejú blood (Table 1) may inhibit renal acid excretion (cf. Davenport 1974).

At the outset we expected that we might see a difference between the two species related to air breathing in one and not the other, perhaps a greater role of the kidney in ammonia and acid excretion in jejú since the gills are smaller (Table 7), and the opportunities for carrying out these functions at the gills may be at least occasionally limited by air exposure. In fact, the results indicate the opposite: renal acid excretion and percent of total ammonia excretion by the kidney were both higher in the water-breathing traíra (Table 3). It must be noted, however, that the gills of jejú are competent in satisfying the animal's total O_2 requirements, and under the conditions of our resting state determinations, O_2 uptake from air breathing was probably minimal. It remains possible that the jejú kidney can play a more significant role under environmental conditions which necessitate air breathing. In this regard it is interesting that in the one jejú studied during extended hypercapnia (which stimulated air breathing), total urinary H^+, and NH_4^+ effluxes increased markedly (Fig. 6).

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