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RENAL REGULATION OF ACID-BASE BALANCE IN A FRESHWATER FISH (1)

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ABSTRACT Intra-arterial injection of a fixed acid load caused only a
short-lived (< 2h) disturbance of blood pH but a long lived (2-3 days)
elevation of urinary acid excretion in freshwater trout (Salmo gairdneri).
The renal response comprised an immediate increase in acid output in the
form of titratable acidity minus bicarbonate, and a slower rise in acid
output in the form of ammonia. The total elevation in urinary acid
efflux over 72h was such that no other mechanism besides renal function
is needed to explain the ultimate compensation of this experimental
acid-base disturbance.

In terrestrial vertebrates, both lungs and kidneys are important organs
of acid-base balance, the former acting rapidly to regulate blood P_{CO_2} and
the latter more slowly to adjust blood HCO_3^- (Pitts, '74). Because of the
high solubility in water of CO_2 relative to O_2, water-breathing fish are
unable to regulate blood P_{CO_2} by variations in ventilation; instead they
rely on much slower adjustments of blood HCO_3^- (Cameron and Randall, '72;
Randall and Cameron, '73; Janssen and Randall, '75; Eddy et al., '77). The
latter control is usually attributed to ionic exchange mechanisms (Na^+ vs H^+
or NH_4^+; Cl^- vs HCO_3^- or OH^-) on the gill epithelia of freshwater fish, although
definitive evidence is lacking (Maetz and García-Romeu, '64; Kerstetter et al.,
'70; Kerstetter and Kirschner, '72; Maetz, '73; De Renzis and Maetz, '73;
Cameron, '76). The possible role of the kidney in acid-base balance is commonly
ignored (Cameron and Randall, '72; De Renzis and Maetz, '73; Hoar, '75; Eddy et
al., '77) or discounted (Maetz and García-Romeu, '64; Janssen and Randall, '75;

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Cameron, '76; Eddy, '76). However recently we have shown that the output of acid by the teleost kidney is highly variable and can approximate that of man under resting conditions (Cameron and Wood, '78). In the present report, we provide the first evidence that the kidney of a freshwater fish can dynamically regulate acid-base status by excreting all of a fixed acid load injected into the bloodstream.

MATERIALS AND METHODS Rainbow trout (Salmo gairdneri; 140-301 g.) were acclimated to the experimental temperature (10-18° C) for at least 3 weeks. Chronic dorsal aortic and urinary bladder catheters were implanted under MS-222 anaesthesia (Wood and Randall, '73a, b; Cameron and Wood, '78); the fish were allowed to recover in darkened experimental chambers served with dechlorinated, aerated freshwater. The chambers confined but did not phys-ically restrain the fish. Continuous urine collection and periodic blood sampling from the respective catheters were performed without disturbance to the animals. Blood and urine pH's were measured with a Radiometer micro-electrode system thermostatted to the experimental temperature. Total renal acid output was calculated as urinary (NH_4^+ + titratable acid (TA) - HCO_3^-) concentrations x urine flow rate (Hills, '73). NH_4^+ was determined by a micro-modification of Solorzano's method ('69). TA-HCO_3^- was determined as a single value in the double titration procedure recommended by Hills ('73). The acidic (HCl) and basic titrants (NaOH) were both .02N, and the final titration end-point was taken as the blood pH measured during the period of urine collection.

An initial series of experiments indicated that a pre-experimental starvation period was required to remove the influence of feeding history on renal acid output. A second series revealed that acid output was signifi-cantly elevated during the first 24h after surgery but remained stable there-

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after for at least 5 days. Therefore in the following injection experiments, the trout were starved for 7 days prior to surgery, and the 12h pre-injection control was taken 36-48h post-surgery. In the experimental group (n=10), 0.5 ml/100 g of .02N HCl in 120 mM NaCl (ie. 10 uEq acid/100g) was infused into the dorsal aortic catheter over a 10 min period at time 0 (48h post-surgery), and washed in with 0.2 ml/100g of 120 mM NaCl. The fish struggled briefly during infusion, but only one mortality (in 17 such experiments) occurred. The control group were similarly infused with 0.7 ml/100g of 120 mM NaCl.

RESULTS Parameters of urinary acid output were highly variable between fish, but relatively consistent in individuals from time to time. Consequently, all statistical comparisons employ the paired Student's two-tailed t-test, using each fish as its own control. In starved, fully recovered trout, total acid excretion by the kidney ranged from -0.2 uEq/100g/h (ie. net base efflux) to + 0.8 uEq/100g/h. The average values were low, +0.15 ± 0.15 (6) uEq/100g/h in the control group, + 0.22 ± 0.08 (10) uEq/100g/h in the experimental group ($\overline{x}$ ± 1 S.E,N), reflecting a low urinary NH_4^+ level and a concentration of TA-HCO_3^- which was not significantly different from 0 (figs. 1, 2). In the control fish, there were no significant effects associated with infusion of the saline vehicle except for a decrease in total renal acid excretion at 0-4h post injection (fig. 1). The latter was caused by a significant decrease to negative values of TA-HCO_3^- efflux at this time, probably due to the diuretic effect of the fluid load (fig. 1).

In the experimental group, total renal acid excretion rose significantly in the first 4h after acid injection, and remained significantly elevated until 48h (fig. 2). The response comprised an immediate increase in TA-HCO_3^- efflux, and a much slower rise in NH_4^+ efflux. There was also evidence of moderately elevated urinary acidification: urinary H^+ concentration increased

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gradually, eventually reaching a level approximately twice the pre-injection control, and remained significantly elevated from 12 to 48h post-injection (fig. 2). A significant diuresis also occurred over the first 12h (fig. 2).

Relative to pre-injection control levels, the increase in total renal acid output over 72h averaged 14.9 ± 5.1 uEq/100g ($\bar{x} \pm 1$ S.E.) in the 6 individual fish of the experimental group for which complete data sets were available. This figure is comparable to the original injected acid load (10 uEq/100g). Thus no other mechanism besides kidney function is needed to explain the ultimate compensation of this experimental acid-base disturbance. Short-term regulation was probably accomplished by intra-cellular buffering of the added protons, for at 2h, arterial blood pH was identical to the pre-injection control value, and remained so for the rest of the experimental period, whereas renal compensation occurred over a period of 2-3 days (fig. 2).

DISCUSSION In essentially every aspect, the renal response of this freshwater teleost to a fixed acid load ("metabolic acidosis") was similar to that of the mammalian system (Pitts, '74). Thus renal competence in acid-base regulation seems to have occurred early in the phylogenetic scale and cannot be attributed to the water-breathing/air-breathing transition (Howell, '70;

FIGURE LEGENDS

1 Control group (N=6). Changes in (A) total renal acid excretion, (B) renal NH_4^+ excretion, (C) renal $TA-HCO_3^-$ excretion, (D) urine flow, and (E) hydrogen ion concentrations in urine (bars) and arterial blood plasma (circles), following intra-arterial infusion of the saline vehicle (0.7 ml/100g of 120 mM NaCl) at time 0. Each point is the mean of 6 values. Asterisks indicate means significantly different (p < 0.05) from pre-infusion controls by Student's paired two-tailed t-test.

2 Experimental group (N=10). Changes in same parameters as in figure 1 following intra-arterial infusion of acid (10 uEq/100g of HCl carried in 0.7 ml/100g of 120 mM NaCl) at time 0. Each point is the mean of 6-10 values. Asterisks as in figure 1.
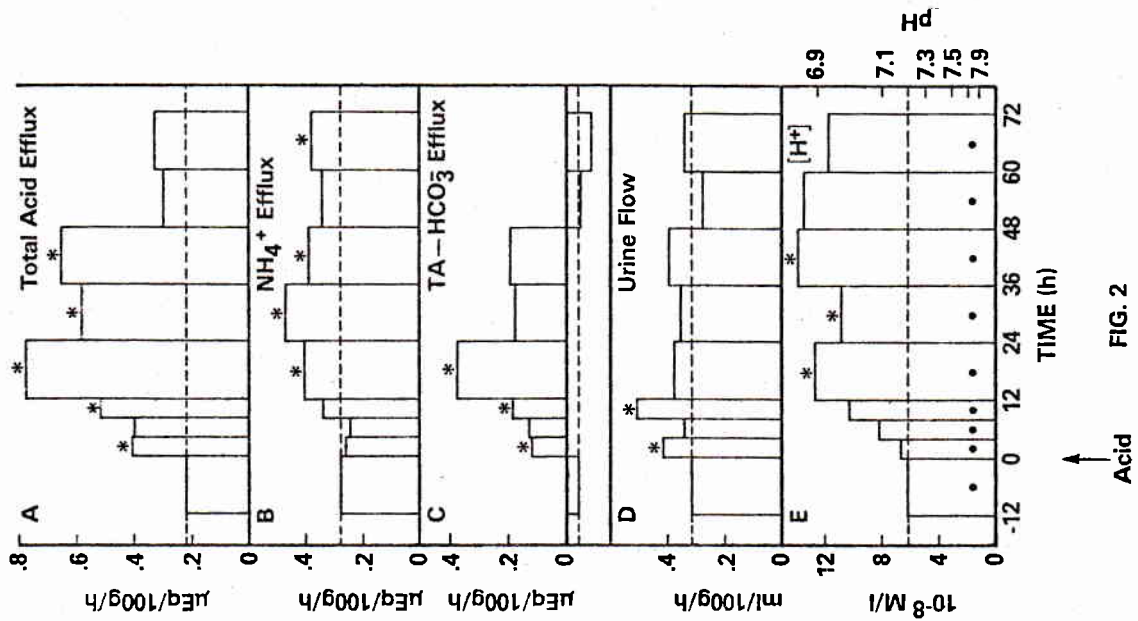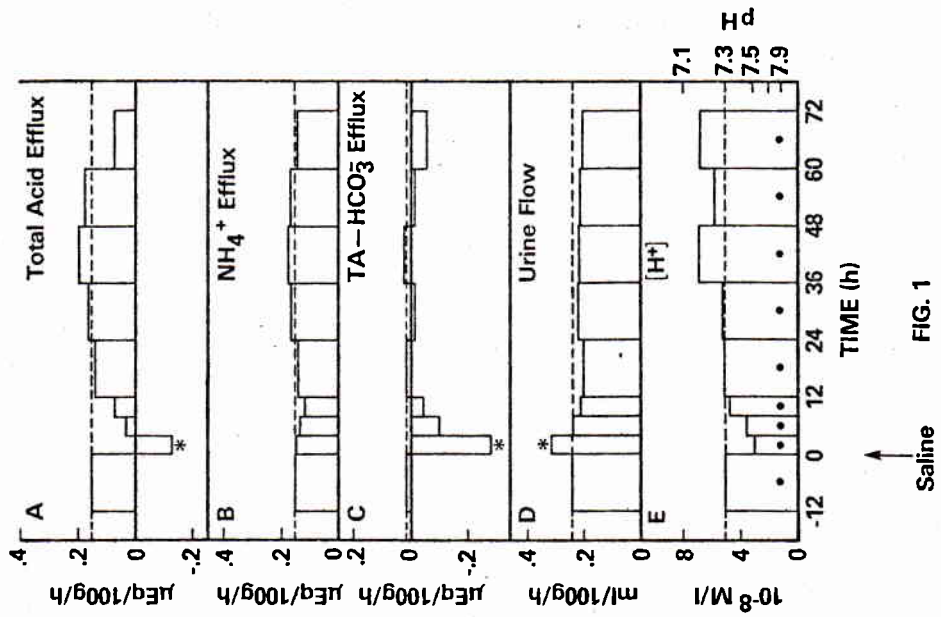
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FIG. 1

FIG. 2

Cameron and Wood, '78). This finding necessitates a re-evaluation of the conclusions of all earlier studies which have attributed acid-base regulation wholly to branchial ionic exchange mechanisms in freshwater fish (see INTRODUCTION). However, the present results are based only on a fixed acid acidosis. We do not wish to imply that branchial mechanisms are necessarily unimportant in correcting other forms of acid-base disturbance in freshwater fish. Indeed experiments currently in progress on the responses of trout to environmental hypercapnia indicate that while urinary acid output does rise, the magnitude of the renal response is insufficient to fully account for the total increase in extracellular HCO_3^- which corrects this "respiratory acidosis".

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