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THE MECHANISMS OF ACID–BASE AND IONOREGULATION IN THE FRESHWATER RAINBOW TROUT DURING ENVIRONMENTAL HYPEROXIA AND SUBSEQUENT NORMOXIA. II. THE ROLE OF THE KIDNEY

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Abstract. Plasma ionic status and renal excretion of acidic equivalents and electrolytes were continuously monitored in the freshwater rainbow trout (*Salmo gairdneri*) during 24 h normoxia (P_{IO_2} = 120–150 torr; control); 72 h hyperoxia (P_{IO_2} = 500–600 torr), and 24 h return to normoxia. Plasma $[Cl^-]$ progressively declined in approximate equivalence to the rise in $[HCO_3^-]$ which compensated the respiratory acidosis of hyperoxia, while $[Na^+]$ increased only slightly. $[Ca^{2+}]$ and $[K^+]$ rose, [phosphate] declined, and $[NH_4^+]$ was unchanged. During normoxic recovery, the $[Na^+]$, $[Cl^-]$ and $[HCO_3^-]$ changes were reversed, $[K^+]$ and $[Ca^{2+}]$ showed further elevations, and $[NH_4^+]$ increased sharply.

Renal acid output increased greatly during hyperoxia with elevations in both NH_4^+ and titratable components, though the latter predominated due to a marked elevation of phosphate excretion. Renal efflux rates of other electrolytes were generally homeostatic for ECF composition, with increased Na^+, K^+, and Ca^{2+} effluxes, and decreased Cl^- efflux. Clearance calculations indicated that net tubular reabsorption increased for Cl^-, fell for Na^+ and K^+, and changed over to marked net secretion for phosphate, while net ammonia secretion increased. Most trends were reversed upon return to normoxia. The critical role of phosphate in urinary electrolyte balance and acid–base regulation is emphasized. The net renal excretion of acidic equivalents accounted for only 7–10% of the total compensation observed for the whole animal during hyperoxia. The kidney contributed primarily in conserving ECF HCO_3^- and secondarily in balancing branchial exchanges.

Acid–base balance
Hyperoxia
Phosphate
Plasma electrolytes
Renal function
Salmo gairdneri
Titratable acidity

Over a 72 h period the rainbow trout completely compensated the respiratory acidosis associated with environmental hyperoxia by accumulating HCO_3^- in intra-

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cellular and extracellular fluids (Hōbe *et al.*, 1984). This compensation was achieved by the effective removal of over 7000 $\mu Eq \cdot kg^{-1}$ of acidic equivalents, of which approximately 2/3 were lost from the ECFV and 1/3 from the ICFV. Over 24 h of normoxic recovery, the majority of these acidic equivalents were rapidly restored, at least in the ECFV. The mechanism was presumably exchange with the external environment *via* either gills or kidney.

Historically, the gills have been considered pre-eminent in acid–base regulation in freshwater teleosts, and the possible role of the kidney has been neglected or discounted (Heisler, 1980, 1982). Nevertheless, several studies have reported at least a significant capacity for renal acid or base excretion (Cameron and Wood, 1978; Wood and Caldwell, 1978; Kobayashi and Wood, 1980; Cameron, 1980). Three recent investigations have actually compared the contribution of the kidney to that of the gills, and concluded that the renal response was clearly significant, accounting for 20–50% of the total exchange of acidic equivalents with the environment (McDonald and Wood, 1981; Cameron and Kormanik, 1982a,b). Thus, in the present study, renal function was continuously monitored in the trout over the experimental regime of hyperoxia and subsequent normoxic recovery. Since branchial exchanges were simultaneously recorded (Wood *et al.*, 1984) and changes in the acidic equivalent pool within the animal assessed (Hōbe *et al.*, 1984), the overall role of the kidney could be accurately quantified. A second objective was to examine renal handling of major electrolytes by comparison of levels in plasma and urine in order to detect any interactions between renal acid and ion excretion. Such interactions are characteristic of mammalian urine (*e.g.*, Hills, 1973; Pitts, 1974).

Materials and methods

Experimental animals and protocol. The treatment of rainbow trout (*Salmo gairdneri*) prior to experimentation, the general test conditions and the details of Series I have been outlined in the preceding paper (Hōbe *et al.*, 1984). The plasma ion data were obtained from Series I, and the renal data from Series III, in which the fish were subjected to an identical hyperoxic regime. Series III was designed specifically for simultaneous measurements of the fluxes of electrolytes and acidic equivalents *via* the kidney (present study) and gills (Wood *et al.*, 1984). Arterial cannulation and blood sampling were purposely avoided because of the known sensitivity of branchial and renal flux rates to these disturbances (*e.g.*, Cameron and Wood, 1978; McDonald *et al.*, 1983).

Series I. Plasma levels of osmolality, Na^+, K^+, Ca^{2+}, Cl^-, total ammonia, and phosphate were measured on arterial blood samples drawn from individual trout (mean wt = 284 ± 16 g; n = 12) prior to, (control, 'C' in figures), at 5, 24, 48 and 72 h of hyperoxia, and after 5 and 24 h of recovery in normoxia.

Series III. Renal measurements were performed on individual trout (mean

wt = 281 ± 12 g; n = 12) fitted with urinary catheters (Wood and Randall, 1973) to separate renal from branchial fluxes. The catheter tip was located within the urinary bladder, thus providing largely ureteral urine (*cf.* Beyenbach and Kirschner, 1975). The catheter drained outside the fish chambers into covered vials by a siphon of 7 cm (see diagram in McDonald, 1983). Total urine production was collected over two successive 12 h periods of normoxia (control) immediately prior to hyperoxia, six consecutive 12 h experimental periods during the 72 h of hyperoxia, and two final periods during normoxic recovery. Each 12 h urine collection was analysed for volume, pH, and concentrations of TA-HCO_3^- (titratable acidity minus bicarbonate), total ammonia, phosphate, Na^+, K^+, Ca^{2+}, and Cl^-.

Analytical techniques and calculations. Plasma was obtained from blood samples centrifuged at 9000 g for 2 min. Plasma osmolality and [Cl^-], and urine pH and [TA-HCO_3^-] were determined on freshly collected samples; all others were frozen for later analysis. Plasma osmolality was measured by vapour pressure osmometry (Wescor Model 5100B), and plasma and urine [Cl^-] by coulometric titration (Radiometer CMT10). [Na^+], [K^+] (Eel Mark II) and [Ca^{2+}] (Coleman 20) in plasma and urine were determined by flame photometry, appropriate swamping being used to remove the interference of Na^+ on K^+ and Ca^{2+} emission. Micro-modifications of commercial diagnostic kits were used for the colorimetric assay of total inorganic phosphate in plasma and urine (phosphomolybdate reduction method; Sigma, 1981) and total ammonia in plasma (l-glutamic dehydrogenase/NAD method; Sigma, 1982). Total ammonia in urine was determined by a micromodification of the salicylate–hypochlorite method of Verdouw *et al.*, (1978). Different ammonia assays were used for plasma and urine because the simpler salicylate–hypochlorite method occasionally gave spurious values for plasma in our hands. The two assays were cross-validated.

Urine pH and [TA-HCO_3^-] were determined as described in Wood and Caldwell (1978) and Kobayashi and Wood (1980). [TA-HCO_3^-] was measured as a single value in the double titration procedure recommended by Hills (1973) using a Radiometer pH micro-electrode (Type E5021) coupled to a Radiometer PHM-71 acid–base analyser. The HCl and NaOH titrants employed were both 0.02 N. As trout in Series III were not implanted with blood sampling cannulae, the final end point of the titrations was taken as the mean control pHa for Series I animals (*cf.* McDonald and Wood, 1981).

The urinary efflux of each electrolyte was calculated as the product of the urine concentration times the flow rate (UFR). Total renal output of acidic equivalents was taken as the sum of the TA-HCO_3^- and NH_4^+ components (Hills, 1973). At the urine pH values recorded in the present study, NH_4^+ accounted for 96–100% of the total ammonia present, and therefore was assumed equal to the latter.

Statistical analysis. Methods follow those outlined in Hōbe *et al.* (1984). As each fish served as its own control, Student's paired two-tailed *t*-test was used to

test the results of both Series I and III. In the latter, urine data for the two initial 12 h normoxic periods were averaged for individual fish to produce a control value, and data transformed where necessary to match variance ratios.

Results

Plasma electrolytes – Series I. Plasma osmolality and concentrations of Na^+, Cl^-, K^+ and Ca^{2+} prior to, during, and following hyperoxic exposure are shown in fig. 1. Overall, the most pronounced change was in $[Cl^-]$ which progressively declined by 14 $mEq \cdot L^{-1}$ during hyperoxia (fig. 1C) and thereby mirrored the simultaneously measured rise in plasma $[HCO_3^-]$ (*cf.* fig. 1D of Hōbe *et al.*, 1984). The slight rise in $[Na^+]$ during hyperoxia was only significant at 72 h with a net change of $+4$ $mEq \cdot L^{-1}$ (fig. 1B). $[K^+]$, $[Ca^{2+}]$ and osmolality all increased significantly during the first 5 h, a change which was only sustained throughout the hyperoxic period by $[K^+]$ (fig. 1D). Overall, these increases were small (2–9%); this, combined with the general constancy of plasma protein and MCHC (*cf.* table 1 of Hōbe *et al.*, 1984), suggests that major ECF–ICF compartmental water shifts did not occur.

On reinstatement of normoxia, plasma $[Cl^-]$ and $[Na^+]$ returned to control levels within 24 h (fig. 1B, 1C). However, $[Ca^{2+}]$ (fig. 1E), $[K^+]$ (fig. 1D) and osmolality (fig. 1A) increased further, remaining above control values at 24 h in the case of the latter two. While the $[Ca^{2+}]$ and osmolality changes were again small, $[K^+]$ rose to a level about 35% above control after 5 h. Interestingly, the calculated 'anion gap' (*i.e.* $[Na^+ + K^+ + Ca^{2+} - Cl^- - HCO_3^-]$, representative of unmeasured negative electrolytes in the system (*cf.* Wilkes *et al.*, 1981), which hitherto had remained fairly constant, increased sharply at 5 h return to normoxia. This observation may reflect a greater negative charge carried on plasma proteins at this period of abnormally high blood pH (*cf.* fig. 1C of Hōbe *et al.*, 1984). In absolute terms, the observed changes in measured electrolytes were too small to explain the increases in osmolality recorded during hyperoxia and return to normoxia (fig. 1A), but the discrepancy was within the cumulative error of the measurements.

Total circulating levels of ammonia and inorganic phosphate merit special attention by virtue of their distinctive role in H^+ buffering in the urine (see below). The control plasma phosphate level of 2.47 ± 0.11 (12) $mM \cdot L^{-1}$ underwent a progressive reduction totalling 20% over 72 h of hyperoxia (fig. 2A). Phosphate decreased even further 5 h after re-institution of normoxia, with no evidence of restoration by 24 h. Plasma ammonia, on the other hand, remained essentially constant throughout hyperoxia at the control level of 172 ± 28 (12) $\mu M \cdot L^{-1}$, but underwent a highly significant 3-fold increase during 24 h of normoxic recovery (fig. 2B). Over the range of blood pHa observed (7.6–8.1; *cf.* fig. 1C of Hōbe *et al.*, 1984), the dominant species in the plasma would be HPO_4^{2-} (86–95%) and NH_4^+ (99–96%), respectively.

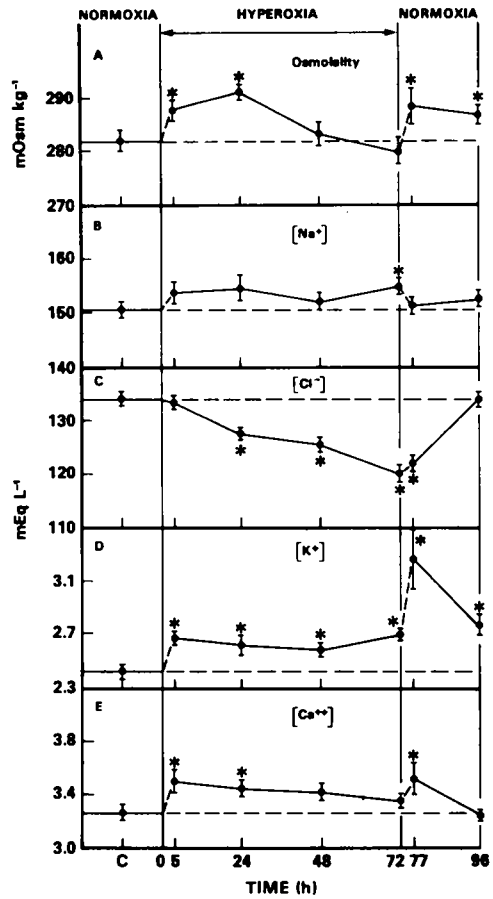

Fig. 1. Time-dependent changes in plasma electrolyte status of rainbow trout prior to, (normoxic control; 'C'), during, and following (normoxic recovery) hyperoxic exposure at 12–14 °C in Series I. Asterisks represent significant differences ($P < 0.05$) from normoxic control values indicated by broken lines. Values are means ± SEM, n = 12 at C = 48 h, 10 at 72 and 77 h, and 7 at 96 h.

Renal efflux rates – Series III. Mean urine flow rate under normoxia was 2.35 ± 0.15 (12) ml · kg^{-1} · h^{-1}, and rose slightly during hyperoxia with significant increases at 36–48 h and 60–72 h (table 1). More substantial increases (+45%) occurred upon reinstatement of normoxia.

The various components of urine acidity are illustrated in fig. 3. Under control normoxic conditions, trout produced a urine of pH ≈7.6 which carried essentially no net excretion of acidic equivalents, since a slightly negative [TA-HCO_3^-] efflux was balanced by a small positive NH_4^+ efflux. On exposure to hyperoxia, the net renal efflux of acidic equivalents increased dramatically, reaching a peak of 18.61 ± 2.31 (12) μEq · kg^{-1} · h^{-1} at 24–36 h, thereafter declining to a level not significantly different from control in the final 12 h (fig. 3A). The overall acid efflux primarily reflected changes in the titratable component (fig. 3B). NH_4^+ efflux

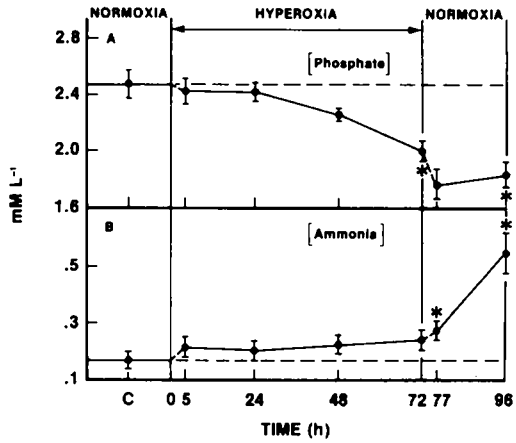

Fig. 2. Changes in plasma (A) total inorganic phosphate and (B) total ammonia during hyperoxia and normoxic recovery in rainbow trout in Series I. Note values are expressed in mM rather than mEq. See legend of fig. 1 for other details.

TABLE 1

Measured urine flow (UFR) and estimated glomerular filtration rate (GFR) in rainbow trout prior to, during, and following exposure to hyperoxia in Series III. Values significantly different from normoxic controls ($P < 0.05$) are designated by asterisks. Means ± SEM; n = 12.

	Normoxia		Hyperoxia						Normoxia	
Period:	1	2	3	4	5	6	7	8	9	10
Time (h):	−[24–12]	−[12–0]	0–12	12–24	24–36	36–48	48–60	60–72	72–84	84–96
UFR	2.42	2.28	2.31	2.67	2.54	2.78*	2.69	2.79*	3.40*	2.85*
($ml \cdot kg^{-1} \cdot h^{-1}$)	±0.26	±0.16	±0.13	±0.27	±0.18	±0.30	±0.30	±0.25	±0.50	±0.29
GFR[a]	4.28	4.04	4.09	4.73	4.50	4.92	4.76	4.94	6.02	5.04
($ml \cdot kg^{-1} \cdot h^{-1}$)										

[a] GFR estimated as 1.77 × UFR for use in clearance calculations of figs. 6 and 7. See text for details.

increased up to 2.5-fold from 12 h onwards (fig. 3C), but contributed only 12% of the total response. Urinary acidification during hyperoxia was accompanied by a sharp drop in urine pH (fig. 3D), the greatest depression (at 12–24 h) pre-empting the period (24–36 h) of maximum TA-HCO_3^- and net acid efflux. This presumably reflected the sequential availability of urinary buffers (see below).

On normoxic recovery, a marked changeover to net base efflux occurred over the initial 12 h but control rates were re-established within 24 h (fig. 3A). This was entirely attributable to the titratable component since NH_4^+ excretion remained elevated at the hyperoxic level (fig. 3C). Urine pH rose to *ca.* 8.1 during this period (fig. 3D).

The renal effluxes of major electrolytes are illustrated in fig. 4. Under resting normoxic conditions, Na^+ and Cl^- were lost at similar rates (*ca.* 19 $\mu Eq \cdot kg^{-1} \cdot h^{-1}$),

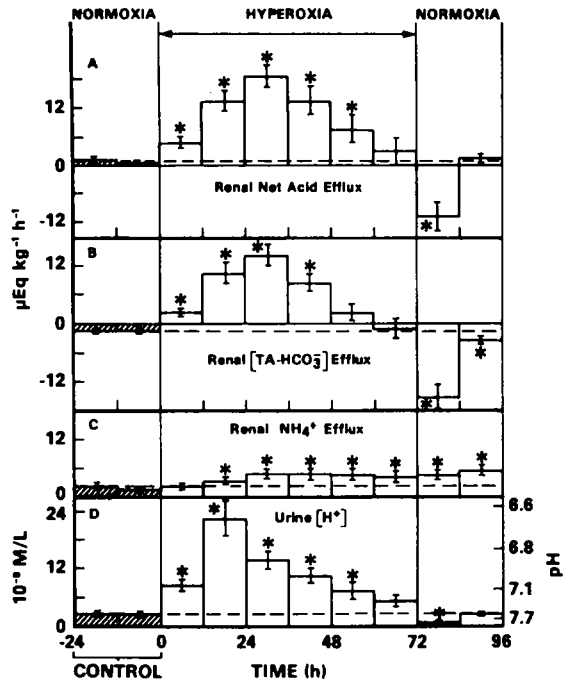

Fig. 3. Time-dependent changes in the renal efflux rates of various components of urine acidity of rainbow trout prior to (normoxic control), during, and following (normoxic recovery) hyperoxic exposure at 12–14 °C in Series III. Collections were made on a 12 hourly basis. A. Net excretion of acidic equivalents, the sum of (B) and (C). B. Titratable acid minus bicarbonate excretion, measured as a single component. C. Ammonium excretion (nontitratable acid). D. Urine $[H^+]$ or pH. Asterisks represent significant differences ($P < 0.05$) from the normoxic control value (broken line), taken as the mean of the two control periods. Values are means ± SEM, n = 12 throughout.

K^+ and Ca^{2+} at much lower rates, and phosphate excretion was negligible. As renal output of acidic equivalents increased during hyperoxia, Na^+ efflux doubled (fig. 4A), K^+ efflux rose by 50% (fig. 4D), and Cl^- efflux fell by 35% (fig. 4B). The most pronounced change, however, was in phosphate excretion, which increased from 0.56 ± 0.22 (12) to 25.16 ± 2.19 (12) $\mu M \cdot kg^{-1} \cdot h^{-1}$ during the peak hyperoxic response (fig. 4C), in concert with the rise in $[TA\text{-}HCO_3^-]$ excretion (fig. 3B), a point considered in greater detail below. After a small initial increase, Ca^{2+} efflux showed no consistent trend for the remainder of hyperoxia (fig. 4E).

On return to normoxia, when net renal base efflux commenced, Na^+ (fig. 4A), phosphate (fig. 4C) and Cl^- (fig. 4B) excretion returned to control levels within 12 h, with some evidence of overshoot in the latter (fig. 4B). However, K^+ and Ca^{2+} effluxes remained significantly elevated above resting values (fig. 4D,E).

The excretion of the two major urinary buffers increased during hyperoxia, though phosphate (fig. 4C) to a much greater extent than ammonia (fig. 3C). This explained the much greater contribution of the titratable component (fig. 3B) to the now elevated net excretion of acidic equivalents. At 24–36 h hyperoxia,

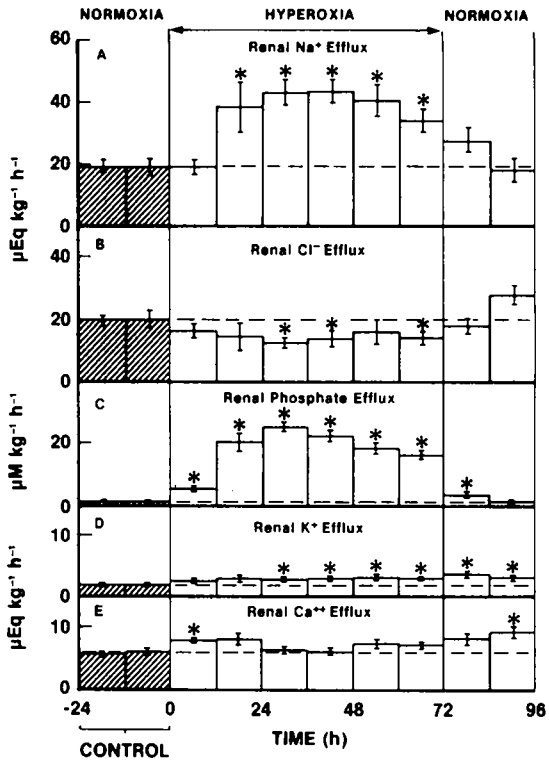

Fig. 4. Changes in the renal excretion rates of major electrolytes during hyperoxia and normoxic recovery in rainbow trout in Series III. Note the phosphate data are expressed in μM rather than in μEq. See legend of fig. 3 for other details.

peak renal phosphate efflux was 5 times NH_4^+ efflux. Note that the most acidic urine pH's occurred at 12–24 h (fig. 3D), prior to the peak excretion of both buffers at 24–36 h, suggesting that the acid excretion mechanism was initially limited by the availability of buffers.

H^+ can be accepted by HPO_4^{2-} to form $H_2PO_4^-$. The relative proportions of mono- and dibasic phosphate excreted can be calculated from the measured urine pH by means of the Henderson–Hasselbalch equation. Unfortunately, there is considerable uncertainty as to the correct value for pK_2, which is particularly sensitive to specific ionic interactions, varying considerably in human urine (Hills, 1973). We have therefore simply elected to use the standard urinary value of 6.8, which seemed reasonable from our titration curves. The analysis (fig. 5A) showed that at the peak of renal acid excretion (24–36 h hyperoxia, fig. 3A), when total phosphate excretion was also at its maximum (fig. 4C), only about half of the latter had been titrated to $H_2PO_4^-$. Thereafter, despite the continuation of relatively high total phosphate efflux (fig. 4C), the $H_2PO_4^-$ component progressively decreased (fig. 5A) as urine pH rose (fig. 3D). This probably explained the parallel decrease of TA-HCO_3^- efflux (fig. 3D). Regression of TA-HCO_3^- efflux against $H_2PO_4^-$ efflux

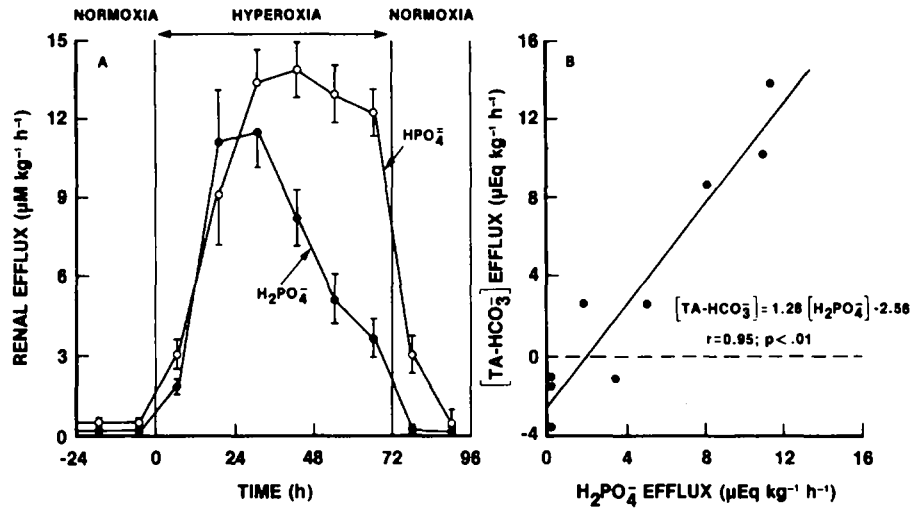

Fig. 5. (A) Changes in the renal excretion rates of mono- (closed symbols) and dibasic (open symbols) components of total inorganic phosphate during hyperoxia and normoxic recovery in rainbow trout in Series III. Note that the data are expressed in μM, rather than in μEq. Values are plotted at the midpoint of each collection interval. See text for details of calculation; see legend of fig. 3 for other details. (B) The regression relationship between mean renal titratable acidity minus bicarbonate efflux ($TA\text{-}HCO_3^-$) and mean renal monobasic phosphate efflux ($H_2PO_4^-$) in the trout of Series III. Each point represents a separate collection period in the entire normoxia–hyperoxia–normoxia experimental regime. The value for the first 12 h of return to normoxia (*i.e.*, 72–84 h) has been omitted, because of the highly negative $TA\text{-}HCO_3^-$ efflux at this time. See legend of fig. 3 for other details.

produced a highly correlated relationship ($r = 0.95$, $P < 0.01$) of slope 1.28 and negative intercept (fig. 5B), the latter representing HCO_3^- efflux at zero $H_2PO_4^-$ efflux. HCO_3^- efflux should fall rapidly to zero as $H_2PO_4^-$ excretion increases, so had TA, rather than $TA\text{-}HCO_3^-$, been measured and used in the regression, the true slope would probably have been very close to 1.0 and the intercept close to zero.

Renal clearances – Series I and III. The concept of clearance (Koch, 1965; Vander, 1975) provides a useful tool for analyzing the renal handling of electrolytes. The clearance ratio (CR) relates the clearance (C) of a substance (X) to the glomerular filtration rate (GFR) – that is, to the clearance of an inert marker which is neither secreted nor reabsorbed by the tubules:

$$CR_X = \frac{C_X}{GFR} \tag{1}$$

where

$$C_X = \frac{[X]u \cdot UFR}{[X]p} \tag{2}$$

in which u and p refer respectively to urine and plasma concentrations. Thus, by substitution:

$$CR_X = \frac{[X]u \cdot UFR}{[X]p \cdot GFR} = \frac{X_{excreted}}{X_{filtered}} \quad (3)$$

The clearance ratio therefore gives a direct index of how the substance X is handled by the tubules. If CR_X is <1, then *net* reabsorption must occur; if >1, then *net* secretion must occur, though the index does not quantitatively separate the relative contributions of simultaneous secretory and reabsorptive processes (Koch, 1965). The CR_X also effectively distinguishes between changes in excretion rate due to variations in plasma concentration or GFR (CR_X is constant) from those caused by alterations in tubular processes (CR_X changes when % net reabsorption or % net secretion vary).

In freshwater teleosts, there is a linear relationship between GFR and UFR because a constant proportion of the water filtered at the glomeruli is reabsorbed by the tubules (Hickman, 1965; Hickman and Trump, 1969), a relationship which has been validated in *Salmo gairdneri* over a wide range of urine flows and metabolic rates (Hofmann and Butler, 1979). In the present analysis, GFR has been conservatively estimated as 1.77 × UFR (see table 1) from Holmes and Stainer (1966). It should be remembered in the remaining analysis that conclusions reached on renal electrolyte handling ultimately depend on the value taken for GFR.

Clearance ratios over the experimental regime are illustrated in fig. 6. Note that the scale is logarithmic to encompass the wide range of CR observed. Under normoxia, CR_{Na^+} and CR_{Cl^-} were very low (0.03 = 97% net reabsorption) as was $CR_{phosphate}$ (0.06 = 94% net reabsorption), indicating very strong reabsorptive processes for these ions. CR_{K^+} (0.19 = 81%) and $CR_{Ca^{2+}}$ (0.44 = 56%) also indicated net reabsorption, though if 40% of total plasma Ca^{2+} were protein bound as in mammals, true $CR_{Ca^{2+}}$ would be close to 1.0, indicating no net secretion or reabsorption. NH_4^+, with $CR_{NH_4^+} = 2.82$, was the only species undergoing net secretion.

During hyperoxia, CR_{Na^-} approximately doubled, while CR_{Cl^-} fell by *ca.* 35% (fig. 6), changes corresponding closely to those in excretion rates (fig. 4A,B). Thus decreased tubular Na^+ reabsorption and increased Cl^- reabsorption were largely responsible for the measured changes in renal efflux rates of these ions; the contribution of increased plasma [Na^+] (fig. 1B) and decreased plasma [Cl^-] (fig. 1C) were minor. CR_{K^+} increased only slightly during hyperoxia (fig. 6); here small decreases in reabsorption and increases in plasma [K^+] (fig. 1D) and GFR (table 1) all contributed to the 50% increase in renal K^+ excretion (fig. 4D). $CR_{Ca^{2+}}$ rose slightly at the start of hyperoxia, thereafter showing no consistent trend, similar phenomena to those in the excretion rate (fig. 1E). The most remarkable changes occurred for $CR_{phosphate}$ which rapidly increased from 0.06 under normoxia to *ca.* 2.0 during hyperoxia. Thus phosphate, formerly strongly reabsorbed, underwent significant net secretion. This change in renal handling was entirely responsible

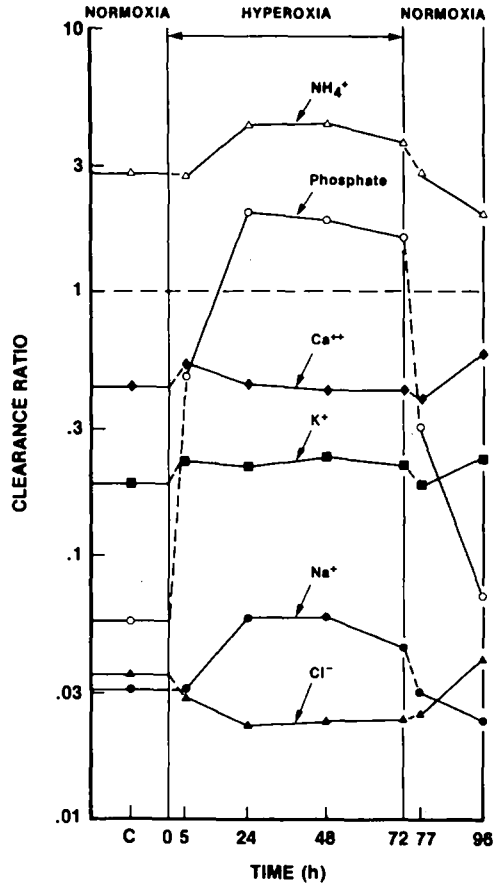

Fig. 6. Time-dependent changes in the apparent renal clearance ratios for major electrolytes in the rainbow trout prior to (normoxic control; 'C'), during, and following (normoxic recovery) hyperoxic exposure at 12–14 °C. The formulae for calculation of the clearance ratio are given in the text and were based on the assumption that GFR = 1.77 UFR. Plasma data were taken from Series I and urinary data from Series III, with calculations performed at the times of plasma sampling using the following association: 'C' plasma *vs* mean −24 h − 0 h urine; 5 h plasma *vs* 0–12 h urine; 24 h plasma *vs* mean 12–36 h urine; 48 h plasma *vs* mean 36–60 h urine; 72 h plasma *vs* 60–72 h urine; 77 h plasma *vs* 72–84 h urine; 96 h plasma *vs* 84–96 h urine. Note that the clearance ratio scale is logarithmic. Ratios below 1.0 indicate net tubular reabsorption and above 1.0, net secretion.

for the dramatic elevation of phosphate excretion (fig. 4C) which occurred at this time of similarly elevated renal acid excretion. Indeed, plasma phosphate levels actually fell (fig. 2A), perhaps a direct result of this stimulated renal efflux. $CR_{NH_4^+}$ increased to *ca.* 4.4 during hyperoxia, reflecting at least a doubling of the net NH_4^+ secretion rate, and explaining most of the observed 2.5-fold increase in renal NH_4^+ output which contributed 21% of the total renal acid efflux (fig. 3C).

During normoxic recovery, when the urine became a pathway for the restoration of acidic equivalents (*i.e.*, net base output; fig. 3A), the tubular handling of Na^+,

Cl^-, and phosphate rapidly returned to the control situation (fig. 6), thereby explaining the observed changes in renal efflux rates (fig. 4A–C). $CR_{NH_4^+}$ actually fell well below the control level, though still representative of net secretion. The continued high NH_4^+ excretion at this time (fig. 3C) was therefore due to a combination of elevated plasma levels (fig. 2B) and GFR (table 1). $CR_{Ca^{2+}}$ and CR_{K^+} first decreased slightly and then rose at the end of the recovery period. The elevations in Ca^{2+} and K^+ excretion at this time (fig. 4D,E) resulted from a combination of GFR, plasma concentration, and reabsorptive effects.

The clearance ratio concept could not be directly applied to renal HCO_3^- processing as HCO_3^- was not measured in the urine. However, total urinary acidic equivalent excretion was recorded, so a similar analysis could be performed on the basis that one HCO_3^- equalled one negative acidic equivalent. Total HCO_3^- filtration was estimated as GFR × plasma $[HCO_3^-]$ (fig. 1D of Hōbe *et al.*, 1984). The clearance ratio would be meaningless in this case, assuming both negative and positive values. However, the arithmetic sum of HCO_3^- filtration and *net* acidic equivalent *excretion* estimates the *net* renal *secretion* rate of acidic equivalents (fig. 7). This parameter could reflect either H^+ secretion or HCO_3^- reabsorption which are indistinguishable but functionally equivalent in terms of acid–base balance.

The analysis (fig. 7) shows that the control rate of net renal acid secretion was about $30\,\mu Eq \cdot kg^{-1} \cdot h^{-1}$ and just balanced HCO_3^- filtration, resulting in negligible excretion of acidic equivalents. During hyperoxia, HCO_3^- filtration increased progressively to almost 4-fold the control level by 72 h as the animal accumulated HCO_3^-. Net renal acid secretion rose to an even greater extent so that HCO_3^- excretion was prevented and net acid excretion (the difference between the two curves) became significantly positive. At 72 h, the calculated net acid secretion rate was greater than $120\,\mu Eq \cdot kg^{-1} \cdot h^{-1}$. Thus, the true magnitude of the renal response was much greater than indicated by the excretion figures alone (fig. 3A), and its most important contribution was in preventing passive renal HCO_3^- loss. Rather surprisingly, on return to normoxia, net renal acid secretion did not cease, though it did fall at a faster rate than HCO_3^- filtration, resulting in net base efflux. Overall, acid secretion and HCO_3^- filtration rates appeared to be relatively well matched.

Discussion

Plasma and urinary electrolytes

Renal and plasma data, employed for comparative purposes such as the calculation of clearance ratios, were taken from separate experimental series (I and III), subjected to identical hyperoxic regimes. In our experience, this approach was preferable to drawing blood and urine samples from the same fish, for urinary parameters are far more variable, and far more affected by blood sampling, than are plasma parameters. Urine was continuously collected by bladder catheterization, a technique which would tend to reduce any reabsorptive or secretory function of the bladder.

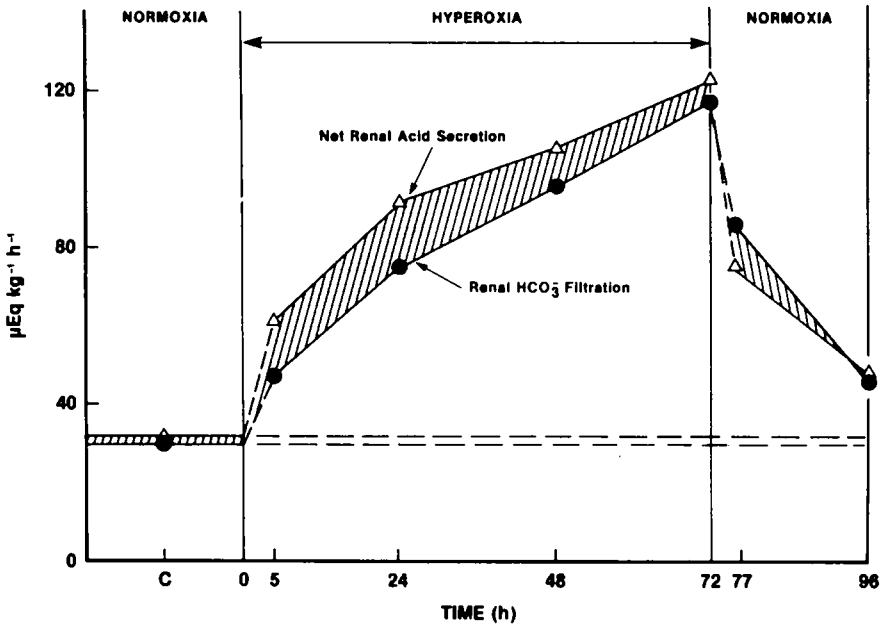

Fig. 7. A comparison of time-dependent changes in the apparent renal filtration rate of bicarbonate and the apparent renal acid *secretion* rate during hyperoxia and normoxic recovery in the rainbow trout. The difference between the two curves at any time (cross-hatched) represents the net renal *excretion* rate of acidic equivalents. The methods of calculation are outlined in the text. Plasma data were taken from Series I (Hōbe *et al.*, 1984) and urinary data from Series III, with other details as in the legend of fig. 6.

The trout bladder *in vitro* is capable of salt and water movement (Lahlou and Fossat, 1971), though its role in acid–base regulation has never been assessed. Since a method for continuous discrete collection of naturally voided urine in freshwater fish has yet to be developed, the function of the bladder *in vivo* remains to be elucidated. In the absence of such a method, it seems likely that bladder catheterization will yield data which tend to underestimate the homeostatic efficiency of the urinary system. The urine collection technique was not anaerobic. While this may have resulted in minor changes in pH, the estimation of net acidic equivalent excretion would not have been affected because of the double titration methodology employed (see Hills, 1973, for details).

The major change observed in plasma ionic status during hyperoxic compensation was a progressive fall in $[Cl^-]$ almost equivalent to the rise in $[HCO_3^-]$, while $[Na^+]$ increased only very slightly (fig. 1). Lloyd and White (1967) reported almost identical effects in trout during the compensation of a rather similar respiratory acidosis due to high environmental P_{CO_2}. However, unlike the trout, the white sucker showed only partial pHa compensation over 72 h hyperoxia, plasma HCO_3^- accumulation was not as great, and plasma $[Na^+]$ as well as $[Cl^-]$ fell (Wilkes

et al., 1981). Whatever the mechanisms, the results in each case reflect the constraints of electroneutrality, with Na^+, Cl^-, and HCO_3^- being the principal ionic species which are manipulated in the plasma during acid–base adjustment.

As the subsequent paper demonstrates (Wood *et al.*, 1984), the principal mechanism responsible for these changes in plasma electrolytes was not renal excretion, but rather modulation of branchial ionic exchanges. Indeed, renal flux rates in general opposed the influence of these and other factors, thereby tending to minimize disturbances in the ECF. Thus during hyperoxia, when branchial Na^+ balance became more positive, and Cl^- balance negative, renal Na^+ efflux increased and Cl^- efflux decreased, with reversal of these trends upon return to normoxia (fig. 4). Similarly, as plasma $[K^+]$ and $[Ca^{2+}]$ levels rose during hyperoxia (fig. 1), probably due to H^+ exchange with the intracellular and bone compartments (Ladé and Brown, 1963; Ruben and Bennett, 1981; McDonald and Wood, 1981), and branchial Ca^{2+} uptake (Wood *et al.*, 1984), the renal efflux rates of these ions increased (fig. 4). The only plasma electrolyte for which renal adjustments may have been causative, rather than preventative, of extracellular disturbance, was phosphate. While branchial phosphate fluxes were not measured, the massive increases in renal phosphate excretion associated with acid output during hyperoxia (figs. 4,5) probably contributed to the observed 20% fall in plasma phosphate levels (fig. 2). Indeed, this elevated renal phosphate excretion over 72 h amounted to approximately twice the calculated total ECF phosphate pool.

The contribution of the kidney to acid–base regulation

Pronounced compensatory changes occurred in the renal excretion of acidic equivalents during both the acidosis of hyperoxia and the alkalosis of normoxic recovery (fig. 3). However, enhanced renal acid excretion over 72 h (-693 $\mu Eq \cdot kg^{-1}$) accounted for only 9.6% of the total calculated change in the acidic equivalent pool (-7252 $\mu Eq \cdot kg^{-1}$; see table 3 of Hōbe *et al.*, 1984). Relative to the simultaneously measured branchial fluxes (see table 2 of Wood *et al.*, 1984), the renal contribution was again minor (*i.e.*, 6.8% hyperoxia; 2.0% recovery).

However, these figures underestimate the true magnitude and importance of the renal contribution. As the analysis in fig. 7 illustrates, the net renal acid *secretion* rate increased to a much greater extent than the net renal acid *excretion* rate, thereby effecting HCO_3^- reabsorption and preventing passive renal HCO_3^- loss during hyperoxia. Without such a response (admittedly based largely on calculated data), the marked compensatory accumulation of HCO_3^- in intra- and extracellular fluid compartments (see Hōbe *et al.*, 1984) would have been impossible. In a preliminary study of renal function during hyperoxia in trout, Wood and Jackson (1980) reached a similar conclusion, although they were unable to demonstrate a significant increase in acidic equivalent excretion, probably because of their low n number and high inter-animal variability.

Indeed, at 72 h, the net renal *secretion* rate of acidic equivalents was *ca.* 122 $\mu Eq \cdot kg^{-1} \cdot h^{-1}$ (fig. 7) compared to a peak branchial *excretion* rate of *ca.*

214 μEq · kg^{-1} · h^{-1} (at 12–24 h; see fig. 1 of Wood *et al.*, 1984). From the data of Nash, Henderson and Brown (1980), scaled by the surface area : mass conversion of Webb (1978), we estimate that the total surface area of the renal tubules is about half that of the gills (Hughes and Morgan, 1973). On this basis, the capacities of the renal tubular and the branchial epithelia for the net transport of acidic equivalents appear comparable.

The mechanisms of renal acid–base regulation

There was relatively close matching between net renal acid secretion and HCO_3^- filtration rates over a wide range of the latter during hyperoxia and normoxic recovery (fig. 7). By analogy with the mammalian kidney (Hills, 1973; Pitts, 1974), these responses could be explained by specific stimulatory effects of elevated tubular Pi_{CO_2} (and/or depressed pHi) on H^+ secretion, superimposed on normal glomerulo-tubular balance, the latter due to the automatic influence of varying HCO_3^- load on the transtubular electrochemical gradient affecting H^+ movement. Note also that as HCO_3^- filtration increased, net renal acid excretion (*i.e.*, the difference between the filtration and secretion curves in fig. 7) fell, despite the continued availability of urinary buffers (see below). As the filtered HCO_3^- load rose, increasingly more of the stimulated H^+ secretion was devoted to HCO_3^- reabsorption, so less was available for urinary acidification. Ultimately, the maximum renal HCO_3^- reabsorptive capacity may limit the maximum level of plasma [HCO_3^-] and thus the maximum pH compensation which can be maintained. The same phenomenon is seen in man during the compensation of chronic respiratory acidosis (Hills, 1973). If this model is correct, then during chronic mineral acidosis where plasma [HCO_3^-] and therefore HCO_3^- filtration rates are reduced, urinary acidification and net renal excretion of acidic equivalents should both be enhanced. Indeed, McDonald and Wood (1981) reported peak excretion rates almost 4-fold greater than those of the present study and much lower urine pH's (*ca.* 6.25) in trout with plasma [HCO_3^-] reduced to *ca.* 2 mEq · L^{-1} by chronic HCl exposure (*vs* 7–24 mEq · L^{-1} during hyperoxia; fig. 1 of Hōbe *et al.*, 1984).

As in mammals, the principal urinary buffers in trout were ammonia and phosphate. The majority of ammonia entered the urine by secretion rather than filtration (fig. 6), presumably by diffusion along a P_{NH_3} gradient (Pitts, 1974). The fall in urine pH during hyperoxia should enhance this gradient thereby tending to automatically increase renal NH_4^+ output. However, the response in renal NH_4^+ output was slow, lagging behind urine pH (fig. 3), suggesting that a gradual increase in ammoniogenesis during chronic acidosis was a more important factor. This slow response agrees with other studies on the trout kidney (Wood and Caldwell, 1978; Kobayashi and Wood, 1980) and is again analogous with the mammalian situation. Once stimulated, the ammoniogenesis seemed to persist since renal NH_4^+ excretion remained elevated during normoxic recovery (fig. 3C), despite the rise in both urine pH (fig. 3D) and blood pH (see fig. 1C of Hōbe *et al.*, 1984) at this time.

Phosphate was responsible for buffering virtually all the titratable acid in the

urine (fig. 5B). The remarkable changeover from net tubular phosphate reabsorption to net secretion (fig. 6) permitted the large increase in TA-HCO_3^- efflux during hyperoxia (fig. 3B); TA excretion was apparently dictated by the availability of phosphate buffer as in mammals (Pitts, 1974). However, net phosphate secretion does not occur in mammals (Mudge *et al.*, 1973), where phosphaturia during acidosis reflects changes in filtration and reabsorption only. Phosphate secretion has been well documented in marine fish, and at least one other freshwater teleost, the eel (Hickman and Trump, 1969).

The mechanism responsible for this switch in the renal handling of phosphate in fish is unknown. Curiously, phosphate clearance seemed to be well in excess of that required for acid excretion. At best, only about half of the total phosphate efflux was titrated to the monobasic form (fig. 5A) and phosphate excretion remained high (fig. 4C) while TA-HCO_3^- excretion fell in the later stages of hyperoxia (fig. 3B). One wonders whether this apparent inefficiency would have been seen had the urinary bladder been functional in urinary modification.

Renal interactions between acid–base and ionoregulation

In mammals, elevated H^+-secretion and net acidic equivalent excretion are associated with both increased Na^+ and K^+ reabsorption and reduced Cl^- reabsorption due to the nature of the exchange processes in the renal tubular cells (Pitts, 1974). Thus, increased acidic equivalent secretion is associated with reduced Na^+ and K^+ but elevated Cl^- excretion and *vice versa*. However, previous studies on teleosts have been unable to detect these ionic reciprocities in the urine (Hunn, 1969; Cameron and Wood, 1978; Kobayashi and Wood, 1980; Cameron, 1980; McDonald and Wood, 1981; Hōbe *et al.*, 1984). The present results explain this dilemma and point to the key importance of phosphate secretion in teleosts as the source of the difference.

The clearance ratio analysis of fig. 6 shows that Na^+ and K^+ reabsorption decreased and Cl^- reabsorption increased during the elevated acidic equivalent excretion of hyperoxia, events reflected in efflux rates (fig. 4), and exactly opposite to the mammalian situation. Construction of a charge balance for the urine illustrates that the major change during hyperoxia was the massive increase in phosphate (fig. 8). At most, only half of the phosphate was titrated to the monobasic form (fig. 5A), so HPO_4^{2-} contributed disproportionately to the total negative charge. Whatever the molecular mechanisms involved, this great secretion of negative charge must electrically constrain increased Na^+ and decreased Cl^- excretion, especially in view of the only moderate rise in the NH_4^+ component (fig. 8). Such a strategy should not be viewed as disadvantageous, because in the teleost fish, unlike the mammal, there is simultaneous involvement of a second organ, the gills, in both acid–base and ionic balance. Indeed, the elevated renal Na^+ losses and decreased renal Cl^- losses in the trout during hyperoxia helped compensate for exactly opposite events occurring at the gills (*cf.* fig. 3 of Wood *et al.*, 1984), thereby contributing to overall homeostasis.

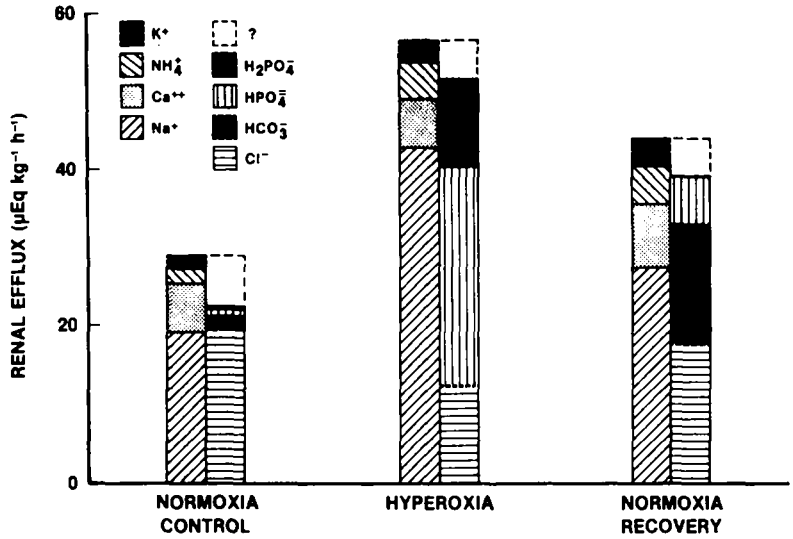

Fig. 8. Excretion charge balance for the urine of rainbow trout in Series III under normoxia (control), at 24–36 h hyperoxia (time of maximum urinary net efflux of acidic equivalents), and during the first 12 h of normoxic recovery (*i.e.*, 72–84 h; time of maximum urinary net efflux of negative acidic equivalents). HCO_3^- was calculated from TA-HCO_3^- on the assumption that $H_2PO_4^-$ accounted for all TA.

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References

Beyenbach, K. W. and L. B. Kirschner (1975). Kidney and urinary bladder functions of the rainbow trout in Mg and Na excretion. *Am. J. Physiol.* 229: 389–393.

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

Cameron, J. N. (1980). Body fluid pools, kidney function, and acid–base regulation in the fresh water catfish *Ictalurus punctatus*. *J. Exp. Biol.* 86: 171–185.

Cameron, J. N. and G. A. Kormanik (1982a). Intra- and extracellular acid–base status as a function of temperature in the fresh water channel catfish, *Ictalurus punctatus*. *J. Exp. Biol.* 99: 127–142.

Cameron, J.N. and G.A. Kormanik (1982b). The acid–base responses of gills and kidneys to infused acid and base loads in the channel catfish, *Ictalurus punctatus*. *J. Exp. Biol.* 99: 143–160.

Heisler, N. (1980). Regulation of the acid–base status in fishes. In: Environmental Physiology of Fishes, edited by M.A. Ali. New York, Plenum Press, pp. 123–162.

Heisler, N. (1982). Transepithelial ion transfer processes as mechanisms for fish acid–base regulation in hypercapnia and lactacidosis. *Can. J. Zool.* 60: 1108–1122.

Hickman, C.P., Jr. (1965). Studies on renal function in freshwater teleost fish. *Trans. R. Soc. Can.* 3: 213–236.

Hickman, C.P., Jr., and B.F. Trump (1969). The kidney. In: Fish Physiology. Vol. I, edited by W.S. Hoar and D.J. Randall. New York. Academic Press.

Hills, A.G. (1973). Acid–base Balance – Chemistry, Physiology, Pathophysiology. Baltimore, Williams and Wilkins Co.

Hōbe, H., P.R.H. Wilkes, R.L. Walker, C.M. Wood and B.R. McMahon (1984). Acid–base balance, ionic status, and renal function in resting and acid-exposed white suckers (*Catostomus commersoni*). *Can. J. Zool.* (in press).

Hōbe, H., C.M. Wood and M.G. Wheatly (1984). The mechanisms of acid–base and ionoregulation in the freshwater rainbow trout during environmental hyperoxia and subsequent normoxia. I. Extra- and intracellular acid–base status. *Respir. Physiol.* 55: 139–154.

Hofmann, E.L. and D.G. Butler (1979). The effect of increased metabolic rate on renal function in the rainbow trout, *Salmo gairdneri*. *J. Exp. Biol.* 82: 11–23.

Holmes, W.N. and I.M. Stainer (1966). Studies on the renal excretion of electrolytes by the trout (*Salmo gairdneri*). *J. Exp. Biol.* 44: 33–46.

Hughes, G.M. and M. Morgan (1973). The structure of fish gills in relation to their respiratory function. *Biol. Rev.* 48: 419–475.

Hunn, J.B. (1969). Chemical composition of rainbow trout urine following acute hypoxic stress. *Trans. Am. Fish. Soc.* 98: 20–22.

Kobayashi, K. and C.M. Wood (1980). The response of the kidney of the freshwater rainbow trout to true metabolic acidosis. *J. Exp. Biol.* 84: 227–244.

Koch, A. (1965). Kidney function and body fluids. In: Physiology and Biophysics, edited by T.C. Ruch and H.D. Patton. Philadelphia, W.B. Saunders Co.

Ladé, R.J. and E.B. Brown (1963). Movement of potassium between muscle and blood in response to respiratory acidosis. *Am. J. Physiol.* 204: 761–764.

Lahlou, B. and B. Fossat (1971). Mécanisme du transport de l'eau et du sel à travers la vessie urinaire d'un poisson téléostéen en eau douce, la truite arc-en-ciel. *C.R. Acad. Sci. (Paris)* 273: 2108–2110.

Lloyd, R. and W.R. White (1967). Effect of high concentrations of carbon dioxide on the ionic composition of rainbow trout blood. *Nature (London)* 216: 1341–1342.

McDonald, D.G. and C.M. Wood (1981). Branchial and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri*, at low environmental pH. *J. Exp. Biol.* 93: 101–118.

McDonald, D.G. (1983). The interaction of environmental calcium and low pH on the physiology of the rainbow trout, *Salmo gairdneri*. I. Branchial and renal net ion and H^+ fluxes. *J. Exp. Biol.* 102: 123–140.

McDonald, D.G., R.L. Walker and P.R.H. Wilkes (1983). The interaction of environmental calcium and low pH on the physiology of the rainbow trout, *Salmo gairdneri*. 2. Branchial ionoregulatory mechanisms. *J. Exp. Biol.* 102: 141–155.

Mudge, G.H., W.O. Berndt and H. Valtin (1973). Tubular transport of urea, glucose, phosphate, uric acid, sulfate, and thiosulfate. In: Handbook of Physiology. Section 8. Renal Physiology, edited by S.R. Geiger. Baltimore, Williams and Wilkins, pp. 587–652.

Nash, J., I.W. Henderson and J.A. Brown (1980). The number and size of glomeruli in the kidneys of fishes, with observations on the morphology of the renal tubules of fishes. *Am. J. Anat.* 47: 425–445.

Pitts, R.F. (1974). Physiology of the Kidney and Body Fluids, 3rd Ed. Chicago, Yearbook Medical Publishers.

Ruben, J.A. and A.F. Bennett (1981). Intense exercise, bone structure and blood calcium levels in vertebrates. *Nature (London)* 291: 411–413.

Sigma (1981). The colorimetric determination of inorganic phosphorus in serum and urine at 620–700 nM. Sigma Technical Bulletin 670. St. Louis, MO, Sigma Chemical Co.

Sigma (1982). The quantitative ultraviolet determination of ammonia in plasma at 340 nM. Sigma Technical Bulletin 170-UV. St. Louis, MO, Sigma Chemical Co.

Vander, A.J. (1975). Renal Physiology. New York, McGraw-Hill.

Verdouw, H., C.J.A. van Echteld and E.M.J. Dekkers (1978). Ammonia determinations based on indophenol formation with sodium salicylate. *Water Res.* 12: 399–402.

Webb, P.W. (1978). Fast-start performance and body form in seven species of teleost fish. *J. Exp. Biol.* 74: 211–226.

Wilkes, P.R.H., R.L. Walker, D.G. McDonald and C.M. Wood (1981). Respiratory, ventilatory, acid–base and ionoregulatory physiology of the white sucker *Catostomus commersoni*: the influence of hyperoxia. *J. Exp. Biol.* 91: 239–254.

Wood, C.M. and D.J. Randall (1973). The influence of swimming activity on water balance in the rainbow trout (*Salmo gairdneri*). *J. Comp. Physiol.* 82: 257–276.

Wood, C.M. and F.H. Caldwell (1978). Renal regulation of acid–base balance in a freshwater fish. *J. Exp. Zool.* 205: 301–307.

Wood, C.M. and E.B. Jackson (1980). Blood acid–base regulation during environmental hyperoxia in the rainbow trout (*Salmo gairdneri*). *Respir. Physiol.* 42: 351–372.

Wood, C.M., M.G. Wheatly and H. Hōbe (1984). The mechanisms of acid–base and ionoregulation in the freshwater rainbow trout during environmental hyperoxia and subsequent normoxia. III. Branchial exchanges. *Respir. Physiol.* 55: 175–192.