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PHYSIOLOGICAL RESPONSES OF THE CRAYFISH *PACIFASTACUS LENIUSCULUS* TO ENVIRONMENTAL HYPEROXIA

II. ROLE OF THE ANTENNAL GLAND IN ACID–BASE AND ION REGULATION

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Summary

Handling of electrolytes and acidic equivalents by the antennal gland was monitored in freshwater crayfish (*Pacifastacus leniusculus*) during control normoxia (P_{O_2} = 148 mmHg; 1 mmHg = 133·3 Pa), 72 h of hyperoxia (P_{O_2} = 500 mmHg) and 24 h of recovery. A preparation was developed for direct collection of urinary flow (UFR) which was confirmed using inulin clearance ($\dot{C}_{IN}$). Renal effluxes of Na^+, Cl^-, Mg^{2+}, K^+, Ca^{2+}, sulphate, phosphate, titratable and nontitratable acidity and CO_2 were monitored. These were used in conjunction with filtration rates to calculate net rates of reabsorption.

UFR was elevated by around 50 % during hyperoxia. Contributing to this were an increase in $\dot{C}_{IN}$ and a reduction in $[IN]_u$:$[IN]_e$. Excretion of Na^+ ($\dot{E}_{Na}$) tended to increase initially whereas $\dot{E}_{Cl}$ tended to decrease after 36 h. $\dot{E}_K$ and $\dot{E}_{Mg}$ showed a similar profile to that of $\dot{E}_{Na}$ with increases averaging 60 %. The increases in excretion of Ca^{2+}, phosphate and sulphate were more pronounced (threefold). In control crayfish, 65–95 % of filtered electrolytes were reabsorbed at the antennal gland except for ammonia which was secreted. Electrolyte reabsorption increased during hyperoxia; percentage increases varied from 70 % (Na^+, Cl^-) to 150 % (sulphate, phosphate) with HCO_3^- showing a fourfold increase above control values. Ammonia secretion correspondingly increased. Control urine was acid with respect to the haemolymph and became increasingly acidified during initial hyperoxic exposure. Net renal proton excretion largely reflected (90 %) ammonia excretion; both were approximately doubled during hyperoxia. Unlike the situation in mammals, ammonia appears to be more important than phosphate in buffering urine pH. Urinary parameters generally required 24 h for complete recovery when normoxia was reinstated.

Renal net efflux and reabsorption rates were compared with branchial net and unidirectional influx rates from an earlier part of this study. Branchial and renal net effluxes of Na^+ and Cl^- had similar vectors and magnitudes during the control period but tended to counter each other during hyperoxia. Renal reabsorption rates of these ions were threefold greater than branchial influx rates, confirming increased transporting capability. Both epithelia exhibited a net H^+ efflux during

Key words: antennal gland, acid–base balance, ion regulation, crayfish.

hyperoxia; the kidney contributed only 10% of the whole-animal response. Possible mechanisms of renal postfiltrational electrolyte and acidic equivalent processing are discussed.

Introduction

In the preceding paper (Wheatly, 1989) it was shown that the extracellular acidosis occasioned by hyperoxia in the crayfish *Pacifastacus leniusculus* (Dana) was compensated by metabolic [HCO_3^-] accumulation. This was accompanied by transbranchial exchange of acidic equivalents and electrolytes. In addition to the gills, decapods possess antennal glands (kidneys) which are primarily used for volume regulation in marine species since the urine produced is isosmotic (Riegel & Lockwood, 1961). In crayfish the antennal glands are unique in their ability to excrete a dilute urine (see reviews by Riegel, 1972; Mantel & Farmer, 1983); this is associated with the appearance of a morphologically distinct region of the distal tubule (Kamemoto & Tullis, 1972) where Na^+ is actively reabsorbed by Na^+/K^+-ATPase (Peterson & Loizzi, 1974) followed by Cl^-. Active solute reabsorption also occurs in the bladder (Kamemoto *et al.* 1962).

In higher vertebrates, urinary acid/base and electrolyte excretion are linked *via* exchange processes in the renal tubular cells (Hills, 1973). The specific activity of carbonic anhydrase (CA) in the crayfish antennal gland greatly exceeds that reported in marine species (Henry & Cameron, 1982; Wheatly & Henry, 1987), suggesting that it serves to provide counterions for Na^+ and Cl^- reabsorption from an intracellular pool of CO_2. This implies that the antennal gland may be involved in acid–base regulation in the freshwater crayfish. Two previous studies have failed to demonstrate a comparable role in marine species (Cameron & Batterton, 1978; Wheatly, 1985).

The purpose of this study was therefore to correlate postfiltrational processing of electrolytes and acidic equivalents in the crayfish kidney during hyperoxia and recovery and compare any compensatory role with that of the gills. To do this, a non-invasive method for urine collection had to be developed.

Materials and methods

Experimental animals and protocol

Experiments were performed on adult intermoult crayfish *Pacifastacus leniusculus leniusculus* (Dana) (of either sex and mean mass 40·1 ± 3·9 g) maintained as previously outlined (Wheatly, 1989). The present paper describes findings from two experimental series. Series 3 used a preparation for direct collection of urine to measure effluxes of acidic equivalents and electrolytes for 48 h of control normoxia, 72 h of hyperoxia and 48 h of recovery. Series 4 used clearance of radiolabelled inulin to calculate urine flow rate (UFR) and subsequently determine electrolyte filtration and reabsorption rates under an identical experimental regime. Two of the eight animals used in series 4 were not fitted with a urinary

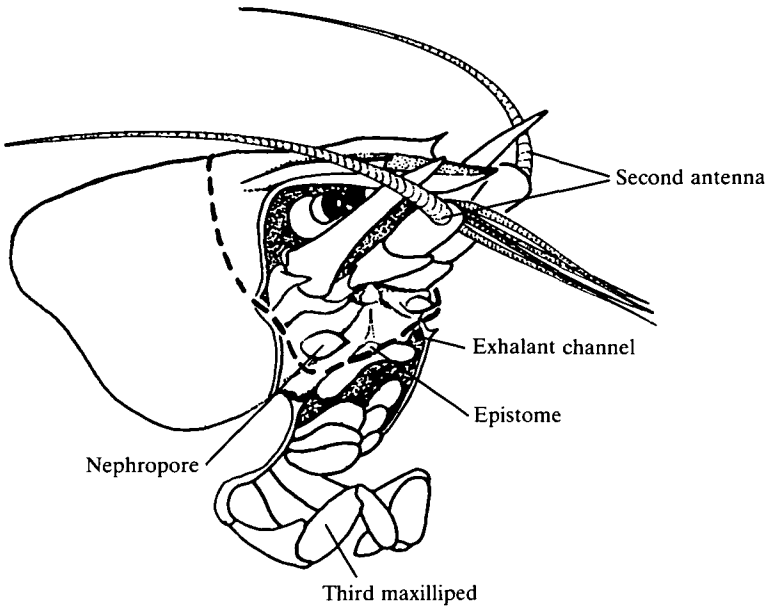

Fig. 1. Anteroventral view of the crayfish to show positioning of the urinary collection device. The broken line indicates the location of the contoured balloon with respect to the nephropore openings and the exhalant channel. Mandibular palps were removed and maxillipeds were deflected downwards during surgery.

collection device (UCD) to demonstrate that the procedure had no effect on inulin clearance.

Since crustaceans typically urinate intermittently and postfiltrational processing continues in the bladder, techniques for urine collection involving aspiration (Pritchard & Kerley, 1970) or internal cannulation of the ducts (Cameron & Batterton, 1978) were considered inappropriate. Initial attempts to cannulate the nephropores externally, as demonstrated in crabs (Holliday, 1977; Wheatly, 1985), were unsuccessful. Wherever this technique has previously been used in crayfish it has involved amputation of the chelae (Ono & Kamemoto, 1969; Tyler-Jones & Taylor, 1986). We wanted to avoid this procedure since our experiment required accurate determination of extracellular fluid volume (ECFV).

In preparing crayfish for urine collection, the flagellum of each second antenna was removed and the spines of the acumen, exopodite of the second antenna, and postantennal wing of the epistome were all filed down to prevent puncture of the collection bag. The crayfish was weighed and the chelae banded. A Qualatex grade 11 balloon, whose end had been cut to fit the contours of the anterior part of the protocephalic region, was then glued, with cyanoacrylate adhesive, ventrally to the epistomal ridge just posterior to the nephropore openings and dorsally just behind the eye orbits (Fig. 1). Before sealing the UCD at the sides, the balloon was flushed with nitrogen, which served the dual purpose of checking for leaks and, more importantly, preventing aeration of urine samples. Positioning of the

balloon did not obstruct the mouthparts or occlude the exhalant branchial openings. Preliminary experiments indicated that complete recovery from surgical manipulation required 1 week. Patency and control urinary function were then ensured for 3–4 weeks (T. Toop & M. G. Wheatly, unpublished observations). Every 24 h mixed urine was removed from the balloon and UFR estimated from volume. A 26 gauge needle attached to a syringe was inserted through a resealing dental dam sampling port glued onto the surface of the balloon, which prevented subsequent leakage. The urine was analysed for acid–base parameters, electrolytes and inulin concentration.

In series 3, collected urine (u) was immediately assayed for pH, total carbon dioxide content (Cu_{CO_2}) and titratable acidity minus bicarbonate ($TA - HCO_3^-$). The remainder was frozen and subsequently used to determine levels of ammonia, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, phosphate and sulphate.

Crayfish in series 4 were surgically prepared for postbranchial haemolymph removal 1 week prior to experimentation as outlined by Wheatly (1989). In this series, 48 h prior to the commencement of hyperoxia, the bath water was adjusted to a fixed volume (250 ml) with fresh water which was aerated and equilibrated to the experimental temperature. Each crayfish was then injected *via* the pericardial septum with 3 μCi of [^{3}H]methoxyinulin (New England Nuclear; ECF marker repurified on Sephadex G-50 according to P. J. Walsh & C. M. Wood, personal communication) in 20 μl of crayfish saline. Every 24 h for the 7 days of the experiment, the haemolymph, bath water and urine (in animals fitted with UCDs) were sampled and assayed for [^{3}H]inulin radioactivity.

Analytical procedures

Urine pH (pHu) was determined on a 50-μl subsample using an IL 20985 liquid junction capillary electrode attached to a 213 blood gas analyser. Cu_{CO_2} was measured on a 40-μl subsample using the Capnicon (Cameron Instruments Inc.). Urine P_{CO_2} and bicarbonate concentration [$HCO_3^- + CO_3^{2-}$] were calculated using the Henderson–Hasselbalch equation (see McMahon *et al.* 1978) and values for pK_1' and αCO_2 for a solution of comparable chlorinity (Harvey, 1974).

[$TA - HCO_3^-$] was determined on 500 μl of fresh urine using the double titration procedure recommended by Hills (1973) and the IL pH microelectrode and blood gas analyser described above. The HCl and NaOH titrants used were both 0·002 mol l^{-1} and the end point of the titration was taken as the corresponding mean haemolymph pH determined in series 1 (see Wheatly, 1989). Frozen samples were thawed and urine [ammonia] was determined on a 10-fold dilution using a micromodification of the phenolhypochlorite method of Solorzáno (1969). Urine acidic equivalent concentration was taken as the sum of the [$TA - HCO_3^-$] and [ammonia] components (Hills, 1973).

Urine inorganic cation concentrations were determined using atomic absorption spectrophotometry (Perkin Elmer model 5000). Urine subsamples were diluted 1 in 1000 (Na^+) or 1 in 30 (K^+) with 0·1 % CsCl or 1 in 30 (Ca^{2+} and Mg^{2+}) with 0·1 % $LaCl_3.6H_2O$ to suppress interference in the air/acetylene flame. [Cl^-] wa

determined on a 25-μl subsample of undiluted urine by coulometric titration (Radiometer CMT 10). Total inorganic phosphate was measured on a twofold dilution of urine using a micromodification of the phosphomolybdic acid method of Atkinson *et al.* (1973). Inorganic sulphate was determined on a fivefold dilution of urine using the turbidometric method of Jackson & McCandless (1978).

For experimental series 4, samples of injection stock (20 μl), haemolymph (20 μl), bath water (100 μl) and urine (20 μl) were diluted in 5 ml of fluor (ScintiVerse E; Fisher) and ^{3}H radioactivity was measured on a scintillation counter (Beckman LS 5801).

Calculations

UFR was expressed in ml kg^{-1} h^{-1} and also in % BW day^{-1} (where BW is body mass in grams).

Urinary effluxes ($\dot{E}_X$) were calculated in μequiv kg^{-1} h^{-1} as:

$$\dot{E}_X = [X]_u \times UFR\ , \tag{1}$$

where $[X]_u$ is urine electrolyte concentration in μequiv ml^{-1}.

Haemolymph inulin radioactivity ($[IN]_e$) was plotted semilogarithmically (ln) *versus* time for individual crayfish and the rate constant, K (% h^{-1}), calculated every 24 h by linear regression (after Harris & Kormanik, 1981) using radioactivities at the start and end of each collection period. ECFV (inulin space) was calculated in ml at the time the injection was made as:

$$ECFV = \frac{IN_{injected}}{[IN]_{eo}}\ , \tag{2}$$

where $IN_{injected}$ is injected dose in counts min^{-1} and $[IN]_{eo}$ is haemolymph inulin radioactivity at zero time (in counts min^{-1} ml^{-1} haemolymph) obtained by extrapolating the initial (48 h) linear portion of ln^{3}H radioactivity *versus* time back to zero time.

The rate of inulin clearance, $\dot{C}_{IN}$, was then calculated in ml kg^{-1} h^{-1} as:

$$\dot{C}_{IN} = \frac{K \times ECFV}{BW}\ . \tag{3}$$

Urine was simultaneously assayed for [^{3}H]inulin radioactivity, $[IN]_u$, enabling UFR to be calculated in ml kg^{-1} h^{-1} according to Kormanik & Harris (1981) as:

$$UFR = \frac{\dot{C}_{IN}}{[IN]_u/[IN]_e}\ . \tag{4}$$

Filtration rate of each electrolyte, $\dot{F}_X$, in μequiv kg^{-1} h^{-1} was calculated as:

$$\dot{F}_X = [X]_e \times \dot{C}_{IN}\ , \tag{5}$$

where $[X]_e$ is the extracellular or haemolymph concentration in μequiv ml^{-1} determined on series 3 crayfish. These values were identical to those reported for series 1 crayfish (Wheatly, 1989).

Finally, rate of reabsorption ($\dot{R}_X$) or secretion ($\dot{S}_X$ if $\dot{E}_X > \dot{F}_X$) was calculated from $\dot{E}_X$ and $\dot{F}_X$ by difference according to Kirschner (1967) as:

$$\dot{R}_X = \dot{F}_X - \dot{E}_X \quad (6)$$

or

$$\dot{S}_X = \dot{E}_X - \dot{F}_X . \quad (7)$$

Statistical analysis

Data are expressed throughout as mean ± S.E.M. (number of observations) and compared using Student's two-tailed paired *t*-tests with 5 % as the fiducial limit.

Results

Urinary electrolyte and acidic equivalent effluxes

Under control conditions urine was collected at rates of $4{\cdot}30 \pm 0{\cdot}26$ ml kg^{-1} h^{-1} in series 3 (Fig. 2). During the first 24 h of hyperoxia UFR virtually doubled; there was a slight accommodation with time although values remained 50 % above control levels. UFR remained elevated during the initial 24 h of recovery normoxia, thereafter returning to control levels.

Control urine concentrations of electrolytes were as follows (in mequiv l^{-1}): Na^+, $22{\cdot}0 \pm 4{\cdot}7$; K^+, $0{\cdot}57 \pm 0{\cdot}14$; Ca^{2+}, $1{\cdot}55 \pm 0{\cdot}17$; Mg^{2+}, $1{\cdot}24 \pm 0{\cdot}06$; Cl^-, $17{\cdot}2 \pm 3{\cdot}8$; phosphate, $0{\cdot}18 \pm 0{\cdot}03$; sulphate, $0{\cdot}37 \pm 0{\cdot}11$; $TA - HCO_3^-$, $0{\cdot}23 \pm 0{\cdot}02$; and ammonia, $1{\cdot}74 \pm 0{\cdot}11$. Urinary effluxes of Na^+ and Cl^- were comparable under control conditions ($92{\cdot}7 \pm 20{\cdot}1$ and $73{\cdot}5 \pm 9{\cdot}5$ μequiv kg^{-1} h^{-1}, respectively; Fig. 2) but responded differently to hyperoxia. $\dot{E}_{Na}$ tended to increase over the initial 48 h of hyperoxia, thereafter recovering, whereas $\dot{E}_{Cl}$ tended to become reduced after 36 h of hyperoxia. Both $\dot{E}_{Na}$ and $\dot{E}_{Cl}$ were significantly reduced initially on return to normoxia but subsequently recovered. Control urinary effluxes of the remaining haemolymph electrolytes were small by comparison (Fig. 3). $\dot{E}_K$ and $\dot{E}_{Mg}$ showed a similar response to hyperoxia to $\dot{E}_{Na}$; excretion increased over the first 24 h of hyperoxia (by 44 % and 80 %, respectively) and decreased on recovery (by around 45 %) before returning to control values. $\dot{E}_{Ca}$ exhibited a different trend; levels remained significantly elevated (by up to threefold) throughout hyperoxia and the first 24 h of recovery. Urinary phosphate excretion trebled during initial hyperoxic exposure, recovering partially after 48 h, somewhat resembling the profile exhibited by $\dot{E}_K$ and $\dot{E}_{Mg}$. Renal sulphate efflux showed a similar trend but with a different time course; peak excretion rates (three times control) were approached between 24 and 48 h.

Significant changes were also observed in urinary acid–base parameters during hyperoxia (Fig. 4). Under control conditions the urine was more acid (pH 7·60) than the haemolymph (7·91; see Wheatly, 1989). During the first 24 h of hyperoxia the urine pH became significantly more acidic although control levels were re

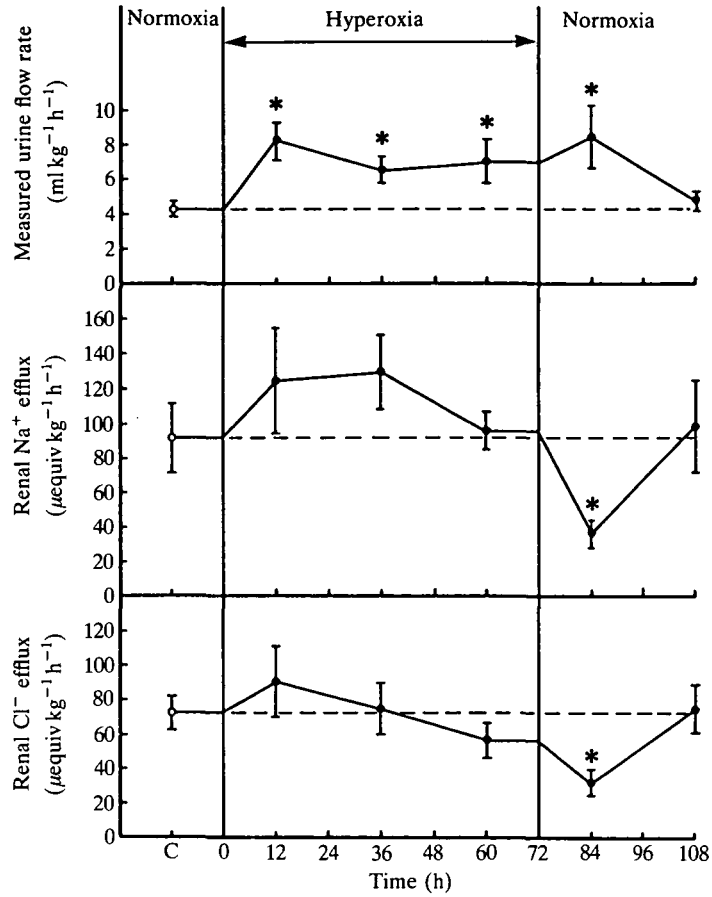

Fig. 2. Time-dependent changes in measured urine flow rate (UFR) and urinary effluxes of Na^+ and Cl^- during control normoxia (C, mean of two 24-h control collection periods), hyperoxia and recovery in *Pacifastacus leniusculus* (series 3) at 12°C. Values are expressed as mean ± S.E.M. ($N = 8$) and asterisks denote significant differences from control. Values have been plotted at the midpoint for each collection period. Dashed line indicates that the parameter did not vary significantly from settled values over the course of 5 days in preliminary experiments in control crayfish.

established within 48 h. Renal CO_2 efflux trebled initially, settling at a value which was double the control level for the duration of hyperoxia; control rates were rapidly re-established upon recovery. Around 90 % of the control net renal proton excretion was attributable to the nontitratable component, i.e. ammonia. Renal net proton efflux during hyperoxia and recovery mirrored ammonia efflux. Both doubled initially, progressively accommodated and recovered immediately upon return to normoxia. Renal [$TA - HCO_3^-$] efflux remained at three times the control value for the duration of hyperoxia, once again recovering rapidly when normoxia was reinstated.

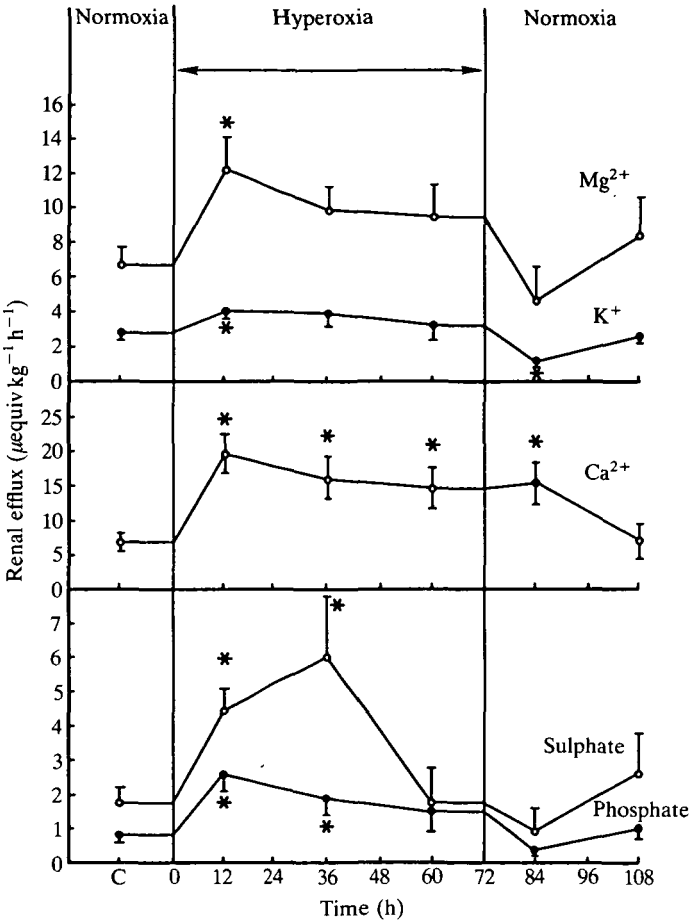

Fig. 3. Time-dependent changes in urinary effluxes of Mg^{2+}, K^+, Ca^{2+}, sulphate and phosphate. Consult legend to Fig. 2 for further details.

Inulin clearance and urine flow rate

In series 4 control crayfish, the rate constant (K) of inulin clearance was $0{\cdot}71 \pm 0{\cdot}13\ \% \ h^{-1}$. During hyperoxia K varied from $1{\cdot}71 \pm 0{\cdot}26\ \% \ h^{-1}$ in the initial sample to $1{\cdot}82 \pm 0{\cdot}29\ \% \ h^{-1}$ in the final sample. During initial recovery K was $1{\cdot}12 \pm 0{\cdot}16\ \% \ h^{-1}$, falling to control levels of $0{\cdot}75 \pm 0{\cdot}10\ \% \ h^{-1}$ by the 24–48 h sample. The ECFV of the crayfish in series 4 was calculated as $13{\cdot}8 \pm 1{\cdot}5$ ml which was $34{\cdot}8 \pm 2{\cdot}2\ \%$ BW.

Control $\dot{C}_{IN}$ was $4{\cdot}5 \pm 0{\cdot}6\ ml\,kg^{-1}\,h^{-1}$ ($N = 8$) ($10{\cdot}7 \pm 1{\cdot}4\ \%\ BW\,day^{-1}$). $\dot{C}_{IN}$ increased progressively throughout hyperoxia (Fig. 5), the rise becoming significant between 24 and 48 h and reaching peak values of 70% above control. $\dot{C}_{IN}$ remained elevated for the first 24 h of normoxic recovery. $[IN]_u:[IN]_e$ was around 1·6 in control crayfish. This ratio tended to decrease during hyperoxia to levels of around 1·4 but returned to the control value upon recovery. To avoid aspirating

bladder urine, the mean $[IN]_u$ from the six cannulated crayfish was used to calculate UFR in the uncannulated animals. Calculated rates in the cannulated and uncannulated crayfish (Table 1) were similar, indicating that the presence of the urinary collection device did not interfere with inulin clearance rate. The combined control calculated UFR was $2{\cdot}6 \pm 0{\cdot}3$ $ml\,kg^{-1}\,h^{-1}$ ($N = 8$) ($6{\cdot}9 \pm 1{\cdot}0\,\%\,BW\,day^{-1}$) which agreed favourably with collected rates. UFR progressively increased throughout hyperoxia (Fig. 5) to peak values which were double the control rates and which remained elevated for 24 h of recovery. Therefore, similar trends in UFR were observed in both experimental series, although flow rates were generally lower in series 4 crayfish (Table 1).

Calculated rates of reabsorption and secretion

Table 2 summarizes the postfiltrational reprocessing of various electrolytes by

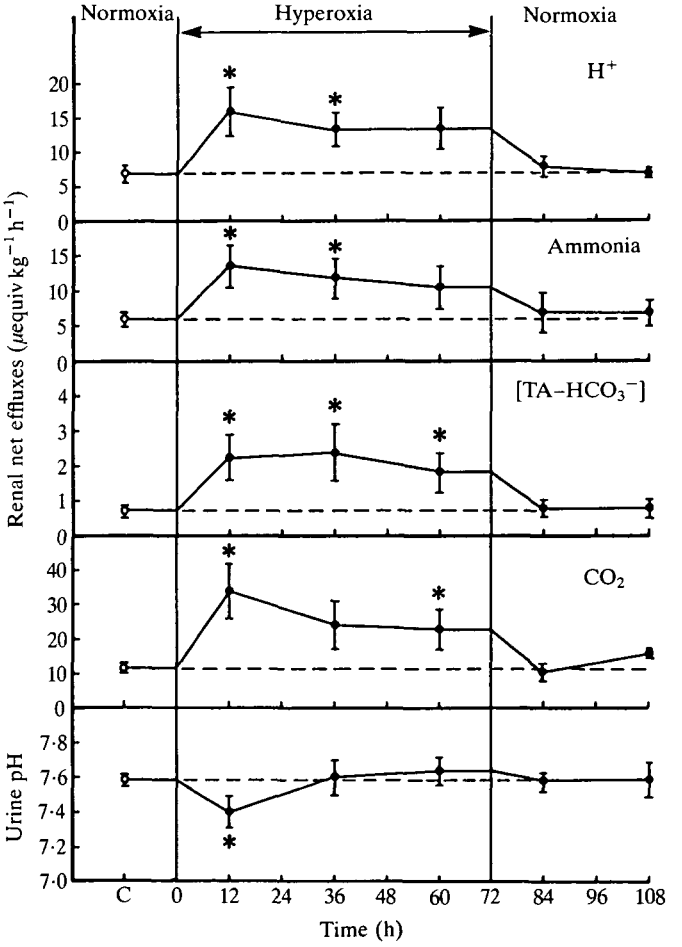

Fig. 4. Time-dependent changes in urinary effluxes of net acid, ammonia, titratable acidity minus bicarbonate [TA – HCO_3^-] and total CO_2 and urine pH. Consult legend to Fig. 2 for further details.

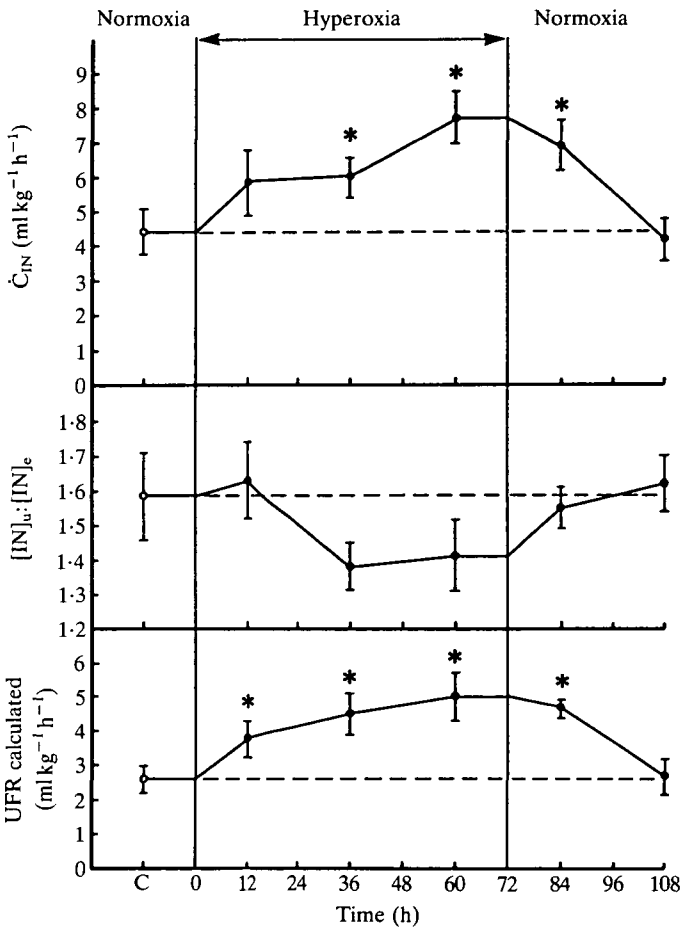

Fig. 5. Time-dependent changes in inulin clearance rate ($\dot{C}_{IN}$), urine to haemolymph inulin concentration ratio ($[IN]_u$:$[IN]_e$) and calculated urine flow rate (UFR) in *Pacifastacus leniusculus* (series 4) at 12°C. Consult legend to Fig. 2 for other details.

examining $[X]_u$:$[X]_e$. With the exception of ammonia, which is secreted, all other electrolytes had urine to haemolymph ratios below 1·6 (the value for inulin, see above) suggesting that they are reabsorbed. Under control conditions the ions most effectively reabsorbed were phosphate and sulphate (97 %) closely followed by Na^+, Ca^{2+} and Cl^- at 95 %. K^+, Mg^{2+} and HCO_3^- were less effectively reabsorbed (85, 72 and 65 %, respectively).

Under control conditions $\dot{R}_{Na}$ and $\dot{R}_{Cl}$ each approached 1000 μequiv kg^{-1} h^{-1} (Fig. 6) which was an order of magnitude greater than the rate of reabsorption of any other ion. During hyperoxia, both progressively increased to peak values between 48 and 72 h, 60–80 % above control levels which were re-established within 48 h of recovery. The remaining haemolymph cations exhibited similar trends except that $\dot{R}_K$ peaked within 48 h of hyperoxia and the increase in $\dot{R}_{Mg}$ was less pronounced (only 50 %). During the initial 24 h of recovery, reabsorption of

Table 1. *Comparison of urine flow rates (UFR) in control* Pacifastacus leniusculus *at 12 °C and the effect of 72 h of hyperoxic exposure followed by 48 h of normoxic recovery*

		Urine flow rate ($ml\,kg^{-1}\,h^{-1}$)				
	Control	Hyperoxia			Recovery	
Technique		0–24 h	24–48 h	48–72 h	0–24 h	24–48 h
Collected series 4	3·21 ± 0·84 (8)	4·07 ± 1·29	4·38 ± 1·04	5·68 ± 0·63	4·99 ± 0·79	3·03 ± 0·77
Collected series 3	4·30 ± 0·26 (8)	8·36 ± 1·00	6·64 ± 0·87	7·12 ± 1·33	8·52 ± 1·85	4·92 ± 0·46
Calculated series 4						
Cannulated	2·46 ± 0·59 (6)	3·78 ± 0·47	3·89 ± 0·44	4·56 ± 0·85	4·14 ± 0·46	2·77 ± 0·62
[Uncannulated]	[2·91] (2)	[3·86]	[6·17]	[6·16]	[4·87]	[2·53]
Combined	2·59 ± 0·31 (8)	3·80 ± 0·42	4·53 ± 0·99	5·09 ± 0·76	4·76 ± 0·23	2·70 ± 0·47

UFR was either measured by collection or calculated from inulin clearance.

Values in parentheses are number of experiments; values in square brackets are mean values calculated for uncannulated animals using urine inulin concentration taken from cannulated crayfish.

Table 2. *Urine:haemolymph ratios for the major haemolymph electrolytes in control normoxic* Pacifastacus leniusculus *at 12 °C (N = 8)*

Electrolyte	$[X]_u:[X]_e$
Na^+	0·088 ± 0·011
K^+	0·302 ± 0·062
Ca^{2+}	0·092 ± 0·008
Mg^{2+}	0·539 ± 0·075
Cl^-	0·090 ± 0·002
Phosphate	0·050 ± 0·010
Sulphate	0·063 ± 0·015
Ammonia	3·092 ± 0·612
HCO_3^-	0·575 ± 0·027

all these ions remained high; recovery again occurred within 48 h. The time course of $\dot{R}_{phosphate}$ paralleled that of $\dot{R}_{Na}$ except that peak values were 150 % above the control level. $\dot{R}_{sulphate}$ exhibited a linear increase with time in hyperoxia, becoming significantly elevated 24 h earlier than $\dot{R}_{phosphate}$ but with peak values again increased by 150 %. However, control reabsorption rates were re-established more rapidly. $\dot{R}_{HCO_3}$ was significantly elevated throughout the hyperoxic period with peak reabsorption rates of four times control values. Reabsorption rates remained elevated for the initial 24 h of recovery.

Control ammonia secretion rate was low compared with rates of reabsorption (Fig. 7). $\dot{S}_{ammonia}$ increased progressively during hyperoxia, becoming significantly elevated (50 % above control) in the final collection period. This trend was rapidly and immediately reversed upon recovery when the rate of secretion dropped significantly below control levels and subsequently recovered.

Discussion

UFR measurement techniques

Close agreement between UFR calculated from $\dot{C}_{IN}$ and from collected flow (Table 1) in series 4 crayfish confirms that the UCD developed in this study was satisfactory. Agreement between $\dot{C}_{IN}$ values in cannulated and uncannulated crayfish suggests that the UCD did not affect UFR. The combined calculated and collected value for UFR ($7{\cdot}1 \pm 1{\cdot}0\,\%$ BW day^{-1}) agrees with flow rates determined by direct external cannulation in another crayfish study (Tyler-Jones & Taylor, 1986). Earlier studies estimating UFR by weight gain following nephropore blockage (e.g. Maluf, 1941) have consistently yielded lower values, confirming that increased back pressure reduces filtration. Flow rates calculated in previous investigations are not strictly comparable with the present study if $[IN]_u$: $[IN]_e$ was determined on aspirated bladder urine. In marine species, water is

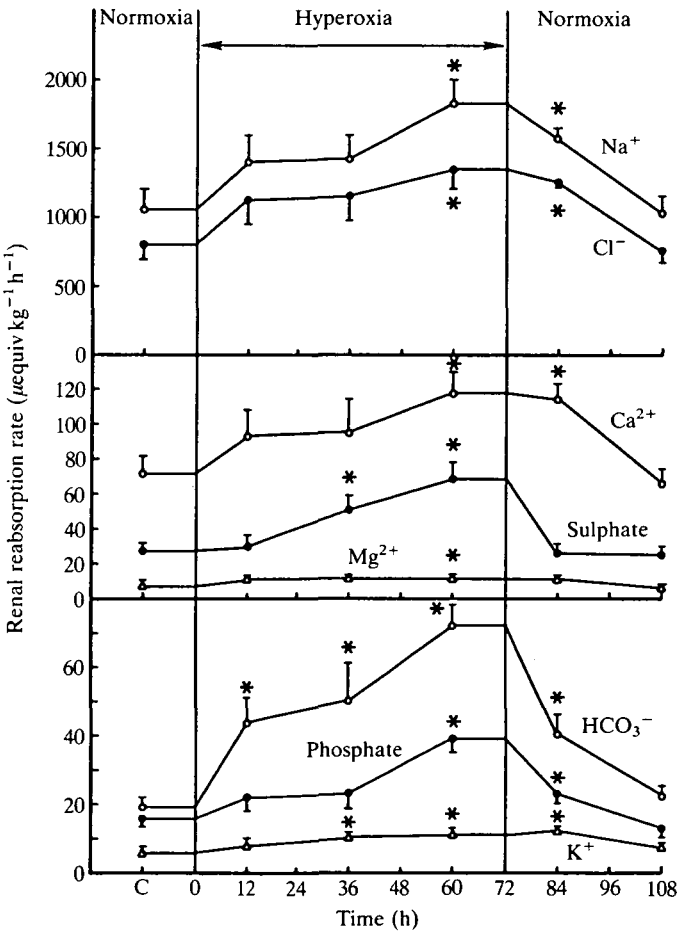

Fig. 6. Time-dependent changes in the calculated rate of renal reabsorption of the major haemolymph electrolytes. Consult legend to Fig. 2 for other details.

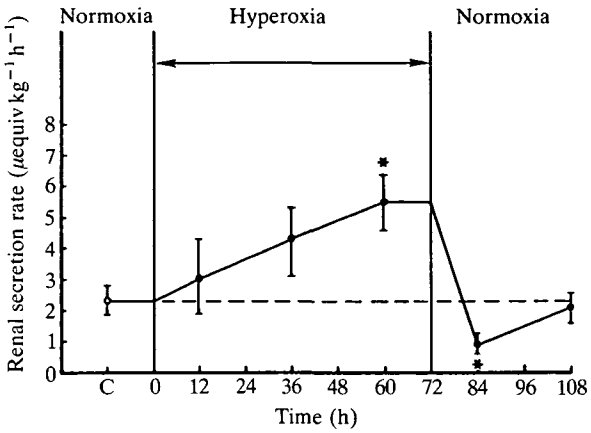

Fig. 7. Time-dependent changes in the calculated rate of renal secretion of ammonia. Consult legend to Fig. 2 for other details.

reabsorbed in the bladder so that aspirated urine typically has a lower $[IN]_u$:$[IN]_e$ value than voided urine (see Wheatly, 1985) and this would tend to overinflate UFR. The reverse situation occurs in the crayfish. The $[IN]_u$:$[IN]_e$ in voided urine (1·6, present study and Tyler-Jones & Taylor, 1986) was much lower than values previously reported for aspirated urine (2·5, Riegel, 1961; Pritchard & Kerley, 1970). We are presently unable to explain this discrepancy. The fact that $[IN]_u$:$[IN]_e$ is above unity indicates *net* reabsorption of water in the antennal gland despite selective pressure to eliminate a water load; presumably this is an unavoidable consequence of active solute reabsorption. There was, however, good agreement between $\dot{C}_{IN}$ determined in this study and values reported by Tyler-Jones & Taylor (1986) and Riegel (1961). Pritchard & Kerley (1970), however, reported lower values when monitoring the appearance of radioinulin in the bath water rather than disappearance from the haemolymph.

The increase in UFR (Figs 2, 5; Table 1) was similar though more pronounced than that reported in a comparable hyperoxic study in trout (Wheatly *et al.* 1984). Two factors contributed to this diuresis: a reduction in net water reabsorption ($[IN]_u$:$[IN]_e$ decreased) and an increased clearance ($\dot{C}_{IN}$, Fig. 5). The latter is generally attributed to an increase in haemolymph volume or pressure. In a later part of this study we found no evidence for an increase in circulating volume (M. G. Wheatly, R. Morrison, T. Toop & L. C. Yow, in preparation) and we did not measure intracardiac pressure (equivalent to antennary artery filtration pressure) in this study. However, in a parallel study on rock crabs (M. G. Wheatly, in preparation) hyperoxia was accompanied by a maintained bradycardia which has been correlated with increased ventricular pressure in decapods (Burggren *et al.* 1985; deFur *et al.* 1985). An alternative explanation for the increase in UFR is that the permeability of the filtration membrane is altered by a diuretic hormone. Norfolk & Craik (1980) similarly concluded that a diuretic factor may be

involved in increasing UFR in crabs in dilute sea water, since the increase could not be explained by changes in arterial pressure.

Urinary electrolyte excretion

Urine electrolyte concentrations agreed with previous determinations in voided crayfish urine (Tyler-Jones & Taylor, 1986); lower levels have been reported in aspirated urine (e.g. Pritchard & Kerley, 1970) consistent with reduced flow rates. One common feature is that $[Cl^-]_u$, is consistently lower than $[Na^+]_u$, suggesting that minor anions contribute more to net negativity than minor cations do to net positivity. However, the resulting anion gap (i.e. $Na^+ + K^+ + Mg^{2+} + Ca^{2+} - Cl^- - PO_4^{2-} - SO_4^{2-} - HCO_3^-$) is relatively small, indicating that the major urinary electrolytes were identified in this study.

The antennal gland appears to act in concert with the gills (Wheatly, 1989) in the control whole-body regulation of certain ions such as Na^+ and Cl^- which undergo net losses of around 100 μequiv kg^{-1} h^{-1} at each epithelium. Renal Ca^{2+} excretion is small compared to net branchial loss. In addition, renal excretion of other ions (e.g. K^+ and Mg^{2+}) is offset by net branchial uptake. The dynamics of whole-body ion regulation appear to alter during experimental hyperoxia. For example, renal excretion of Na^+ and Cl^- countered branchial net fluxes, and Mg^{2+} was handled similarly, constituting a reversal of the control characteristics. Calcium was exceptional in that the kidney predominated over the gills in terms of whole-body efflux during the experiment.

Urinary acid–base excretion

In contrast to marine species (Cameron & Batterton, 1978; Wheatly, 1985), control crayfish produce a urine that is acid with respect to the haemolymph. Our measurements agree with an isolated measurement from Maluf's historic article (1941) which reports a pH for bladder urine of 7·5. Moreover, the components of urine acidity differ in magnitude and in some cases vector. In a marine crab, such as *Cancer* (Wheatly, 1985), the alkaline pH is primarily attributable to a titratable alkalinity efflux (5·0 μequiv kg^{-1} h^{-1}) which is negligibly countered by ammonia efflux (0·2 μequiv kg^{-1} h^{-1}). In crayfish, urine acidity is primarily determined by ammonia efflux combined with a small excretion of titratable protons (Fig. 4). Although the magnitude of renal ammonia efflux differs between these two species, whole-animal excretion rates are comparable (Wheatly, 1989, in preparation). Control freshwater fish also produce an acid urine (Wheatly *et al.* 1984). Calculating the ratio of nontitratable to titratable effluxes emphasizes the relative importance of ammonia in the renal excretion of acid in freshwater species. This value is around 7·39 in crayfish and 1·5–6·5 in trout; both are considerably in excess of the typical mammalian value (1–2·5).

Our data suggest that the crayfish antennal gland, by increasingly acidifying the urine (Fig. 4), assisted the gills in correcting the extracellular hyperoxic acidosis. The magnitude of the total renal response was only 500 μequiv kg^{-1} which is approximately 10 % of the net branchial H^+ loss (Wheatly, 1989), agreeing with

other studies on the relative role of the gills and kidney in freshwater species (Heisler, 1984). H^+ was excreted predominantly as ammonia although the titratable component of urine acidity also increased.

Renal postfiltrational electrolyte reprocessing mechanisms

Although the two primary exchange epithelia exhibited comparable net electrolyte fluxes, the antennal gland had a greater capacity for ion reabsorption. Control values for $\dot{R}_{Na}$ and $\dot{R}_{Cl}$ (Fig. 6) significantly exceeded corresponding values for branchial unidirectional influx ($J_{in}^{Na} = 263$ and $J_{in}^{Cl} = 306\ \mu equiv\ kg^{-1}\ h^{-1}$, Wheatly, 1989). If the ion uptake mechanisms are equally energy-dependent then, as Kirschner (1967) suggests, the costliest part of hyperosmotic regulation should be operating the kidneys. The difference in ion-transporting capability is further amplified if one considers the relative surface area of the two exchange organs. The branchial surface area of a 35-g crayfish can be estimated as $140\ cm^2$ (McMahon & Wilkens, 1983). Based on Maluf's original observations (1941) the combined exchange area of the nephric tubules is approximately $3{\cdot}76\ cm^2$. The exchange area ratio for gills:kidney is therefore 40:1. If the antennal gland achieves three times the net ion reabsorption with one-fortieth of the surface area, then the specific activity of transport enzymes per unit area should be 120 times greater. Wheatly & Henry (1987) confirmed a threefold discrepancy between antennal-gland- and gill-specific activities of Na^+/K^+-ATPase and carbonic anhydrase expressed per gram of tissue homogenate protein. The ratio of ammonia loss at the gills to that at the kidney (65:2·3) may also reflect relative exchange areas (all remaining diffusional characteristics being equal). The nephric tubule is less elongated in marine species which may explain why their renal ammonia effluxes are lower than that of the crayfish. Electrolyte reabsorption and secretion rates showed a more pronounced response to hyperoxia than did excretion rates. Just as for net flux rates, the antennal gland and gills (Wheatly, 1989) exhibited opposing trends with respect to unidirectional influx rates of Na^+ and Cl^- during hyperoxia. In other cases (e.g. ammonia) epithelia worked in concert although an earlier response time was generally seen at the gills.

Together with inulin clearance (Fig. 5) the present data would suggest that glomerular filtration and tubular reabsorption rate are balanced in an attempt to regulate circulating electrolyte levels. Fluid balance, however, did not appear to be regulated by feedback control, as is typical in the mammalian nephron, although UFR partially recovered in series 3 crayfish (Fig. 2). In fact the $[IN]_u:[IN]_e$ ratio suggested that fluid reabsorption decreased. The primary stimulus for tubular fluid reabsorption in mammals is increased plasma colloid osmotic pressure (COP). This stimulus may be less effective in crustaceans since they excrete protein in the urine (Kirschner & Wagner, 1965).

Renal mechanisms of acid–base regulation

The renal response to respiratory acidosis in the crayfish was urinary acidification and net HCO_3^- reabsorption which assisted in alkalinizing the blood.

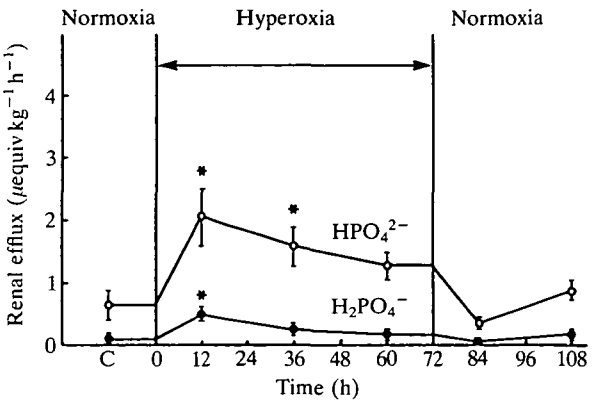

Fig. 8. Time-dependent changes in renal excretion of monobasic (closed symbols) and dibasic (open symbols) components of total inorganic phosphate. Details of calculations are given in the text. Consult legend to Fig. 2 for other experimental details.

Although this is the same overall response seen in mammals (Marsh, 1983), there appear to be some differences between the mechanisms involved. In mammals, the two buffers involved in tubular acid secretion (which ultimately form new HCO_3^- in the blood) are phosphate (titratable acidity) and ammonia (non-titratable acidity). In the tubular lumen, the dibasic phosphate (HPO_4^{2-}) accepts a proton, forming monobasic $H_2PO_4^-$ which is then excreted. When total phosphate excretion in the present study was resolved into dibasic and monobasic components (Fig. 8) by means of the Henderson–Hasselbalch equation (at urine pH and assuming a pK_1' of 6·8) it transpired that $H_2PO_4^-$ only accounted for 10% of the [TA – HCO_3^-] efflux. It would therefore constitute a minute fraction of the TA flux given that urinary CO_2 is very high. This strongly suggests that other urinary buffers, perhaps including urinary proteins (Kirschner & Wagner, 1965; M. G. Wheatly, unpublished observations), contribute to titratable acidity.

The urinary NH_3/NH_4^+ buffer pair appears to play the more important role in crayfish during hyperoxia. Renal ammonia excretion (Fig. 4) was precipitated by the fall in urine pH which increased diffusional loss of NH_3 by increasing the P_{NH_3} gradient. After protonation, NH_4^+ is typically excreted as a neutral salt. Since renal Cl^- efflux remained constant (Fig. 2) it is unlikely that NH_4^+ was excreted as NH_4Cl. However, sulphate excretion (Fig. 3) paralleled ammonia excretion obeying a 1:2 stoichiometry, making $(NH_4)_2SO_4$ excretion a distinct possibility. Electrolyte handling by cells in the antennal gland has been confirmed by subsequent intracellular determinations (M. G. Wheatly & E. C. Vevera, in preparation). Cellular ammonia and sulphate levels were elevated whereas phosphate level remained unchanged.

Presumably proton secretion into the lumen from the renal tubule cell is analogous in crustaceans and vertebrates, occurring *via* apical carrier-mediated electroneutral ion exchange, i.e. H^+/Na^+. Basolateral HCO_3^- reabsorption will include newly formed HCO_3^- and filtered HCO_3^-. Both will acidify the urine but

the latter will not change circulating HCO_3^- levels. Although HCO_3^- reabsorption (Fig. 6) adapts to the filtered load, any which escapes reabsorption will raise pH, thereby increasing the driving force for proton secretion. A reduction in ECFV which occurs initially during hyperoxia (M. G. Wheatly, R. Morrison, T. Toop & L. C. Yow, in preparation) is also a powerful stimulus for proton secretion, again acting *via* the Na^+/H^+ counter-transporter mechanism. However, the principal stimulus for renal acidification is P_{CO_2}, which increased in the renal cells (M. G. Wheatly, R. Morrison, T. Toop & L. C. Yow, in preparation) and might increase the driving force for proton secretion by lowering cell pH.

From the studies which exist, monobasic phosphate would appear to be the major urinary buffer in fish, as in mammals. Trout showed a long-lived urinary acidification during hyperoxia (Wheatly *et al.* 1984); however, the net acid efflux reflected changes in titratable acidity, not ammonia levels. Furthermore, the TA efflux was stoichiometrically equivalent to $H_2PO_4^-$ efflux, which was increased *via* a switch from net phosphate reabsorption to secretion.

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