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AMMONIA AND UREA METABOLISM IN RELATION TO GILL FUNCTION AND ACID–BASE BALANCE IN A MARINE ELASMOBRANCH, THE SPINY DOGFISH (*SQUALUS ACANTHIAS*)

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Summary

Nitrogenous waste excretion in resting dogfish occurred largely (>90 %) as urea-nitrogen (urea-N) efflux across the gills, with a very small urea efflux *via* the kidney. Ammonia excretion, almost entirely at the gills, accounted for less than 3 % of total nitrogen excretion. Given the extremely high blood urea levels (approximately 640 mmol-N l^{-1}) 'retained' for osmoregulation, and blood ammonia levels (approximately 80 μmol-N l^{-1}) comparable to those of teleosts, the gills of resting dogfish were exceptionally impermeable to both urea and ammonia. Experiments investigated the origins of these low permeabilities and the responses of urea-N and ammonia-N excretion and acid–base status to 6 h infusions with iso-osmotic solutions of NaCl (control), NH_4Cl, $NaHCO_3$, urea and its analogues thiourea and acetamide. NaCl had no effects, whereas NH_4Cl loading caused intense acidosis and marked elevation of acidic equivalent, ammonia-N and urea-N excretion rates, the latter despite unchanged blood levels of urea-N. Apparent branchial ammonia permeability increased greatly. Acidosis resulted from both stimulated urea production and branchial NH_3 loss, the former making the larger contribution. $NaHCO_3$ loading caused intense alkalosis, a marked elevation of basic equivalent excretion and a moderate stimulation of urea-N excretion. Blood urea-N levels were again unchanged. Infusion of urea itself raised blood urea-N levels, but initially reduced branchial urea-N excretion. Acetamide and thiourea infusions both moderately elevated branchial urea-N excretion. We suggest that the low ammonia permeability may arise metabolically from an ammonia scavenging system in the gills, that a 'back-transport' mechanism in the gills may contribute to the low urea permeability, and that the dissociation between blood urea-N levels and excretion rates may reflect urea production at extrahepatic sites. These studies demonstrate that urea synthesis in the dogfish is linked more to nitrogen availability than to acid–base status.

Key words: ammonia, urea, elasmobranch, dogfish, *Squalus acanthias*, ornithine–urea cycle, gill permeability, kidney function, nitrogenous waste excretion, acid–base balance.

Introduction

Since the classic work of Homer Smith (1929, 1936), it has been recognized that marine elasmobranchs retain large amounts of urea, thereby raising the osmolality of their body fluids slightly above that of the ambient sea water and facilitating the uptake of free water by osmosis across the gills. Sharks therefore avoid the problem of marine teleosts, which must drink sea water to replace branchial losses and thereby incur the burden of substantial NaCl loading. The elasmobranch strategy for osmoregulation in sea water is generally considered to be less costly than that of marine teleosts, although this view has been challenged recently on theoretical grounds (Kirschner, 1993). Nevertheless, the strategy is clearly a successful one, for elasmobranchs have thrived for millions of years in the upper trophic levels of marine ecosystems.

At present, general belief holds that urea is produced in the liver and retained in the blood by the very low permeability of the gills and by reabsorptive mechanisms in the kidney (Perlman and Goldstein, 1988; Mommsen and Walsh, 1991). Elasmobranchs such as the spiny dogfish *Squalus acanthias* possess a full hepatic complement of the enzymes for both the ornithine–urea cycle (OUC) and uricolysis (reviewed by Wood, 1993). *In vitro* activity measurements indicate that the OUC is the dominant pathway of urea production, a conclusion supported by pilot *in vivo* studies (Schooler *et al.* 1966). The OUC in elasmobranchs differs from that of higher vertebrates in that the first enzyme of the cycle is carbamoyl phosphate synthetase III (CPS III), rather than the CPS I of lungfish, amphibians and mammals, so the proximate substrates are

glutamine and bicarbonate, rather than ammonia and bicarbonate (Anderson, 1980; Mommsen and Walsh, 1989, 1991).

The low effective permeability of the kidney to urea has been well studied (see Hickman and Trump, 1969, for a review of the earlier literature). In brief, retention appears to be facilitated by an active transport mechanism (Schmidt-Nielsen and Rabinowitz, 1964; Schmidt-Nielsen *et al.* 1972; Hays *et al.* 1977) superimposed on an anatomically complex countercurrent exchanger (Lacy *et al.* 1985; Lacy and Reale, 1991). In contrast, little is known about the very low effective permeability of the gills to urea. The only detailed studies are those of Boylan and colleagues (summarized by Boylan, 1967), but these were performed on air-exposed, artificially irrigated dogfish. Boylan (1967) concluded that there was no evidence of transport mediation and that the low gill permeability to urea was of structural origin. This conclusion remains the subject of conjecture (Griffith, 1991; Wood, 1993), for it is difficult to understand how an epithelium can sustain a urea concentration gradient of 300–400 mmol l^{-1} while allowing the free passage of respiratory gases. In view of the above, the present study addresses some current uncertainties about nitrogenous waste excretion in marine elasmobranchs.

First, it is commonly stated that urea-N is the major form of nitrogenous waste excreted by marine elasmobranchs (e.g. Perlman and Goldstein, 1988). However, a system designed to *retain* urea may not be designed to *excrete* it well. Recently, Mommsen and Walsh (1991) speculated that ammonia-N excretion may be quantitatively more important than urea-N excretion under many circumstances in marine elasmobranchs. Therefore, our first objective was to quantify the rates of urea-N and ammonia-N excretion under resting conditions in *Squalus acanthias*, to partition the fluxes between gills and kidney, and to examine how these rates might change in response to loading with ammonia-N (as NH_4Cl) and urea-N (as urea).

A second related objective was to examine the responses of a CPS-III-based ureagenic system to experimental substrate loading with ammonia (as NH_4Cl) and bicarbonate (as $NaHCO_3$), for comparison with those of higher vertebrates with a CPS I system. In mammals, this whole area has become polarized (e.g. Halperin *et al.* 1986; Knepper *et al.* 1987, *versus* Atkinson and Camien, 1982; Atkinson and Bourke, 1984) because of the controversial views of Atkinson and colleagues that ureagenesis is driven mainly by the needs of acid–base regulation (i.e. HCO_3^- removal) rather than for nitrogenous waste excretion. Recently, Atkinson (1992) has proposed that these same arguments can be extended to ureagenic fish. We therefore examined the responses of ammonia and urea excretion, acid–base status and net acidic (or basic) equivalent excretion in dogfish during and after 6 h of infusion with $NaHCO_3$ or NH_4Cl.

A final objective was to re-examine the very low gill urea permeability, using intact unanaesthetized animals (in contrast to Boylan, 1967). To this end, we infused *Squalus acanthias* for 6 h with two urea analogues, thiourea and acetamide, both of which are known to act as simple competitive blockers of urea transport in some mammalian systems (Marsh and Knepper, 1992). If a 'back-transport' mechanism at the gills contributes to urea retention, then the predicted result of competitive inhibition would be an increase in urea excretion. These latter studies served as a basis for a more detailed *in vitro* investigation of gill urea permeability in *Squalus acanthias* (P. Pärt, P. A. Wright and C. M. Wood, in preparation).

Materials and methods

Experimental animals

Spiny dogfish (*Squalus acanthias* L., 1.25–2.30 kg) were obtained by angling or trawling in Berkely Sound, British Columbia, Canada, in July and August 1992 and 1993. At Bamfield Marine Station, the fish were held for 1–3 weeks prior to experimentation in large outdoor tanks served with running sea water at the experimental temperature (12±1 °C), salinity (30±2 ‰) and pH (7.90±0.15). During this period, the fish were not fed so as to eliminate the exogenous fraction of nitrogen waste production (Wood, 1993).

Each dogfish was anaesthetized with MS-222 (0.2 g l^{-1}), placed on an operating table, weighed and fitted with an indwelling anterior mesenteric artery catheter for repetitive blood sampling and test infusions by the method of Graham *et al.* (1990). In addition, male animals only were fitted with a urinary papilla catheter for continuous collection of urine, as described by Wood and Patrick (1994). We could not collect urine reliably from females because the ureters remain divided until close to the end of the papilla. In male dogfish, urinary catheterization was successful in approximately 50 % of the animals; if the catheter was not working after 24 h, it was removed. Fish were allowed to recover for 48 h in their experimental chambers served with flowing sea water at 1 l min^{-1}. These chambers were oblong wooden boxes (total volume 40 l) sealed with polyurethane and fitted with a volume calibration scale and internal perimeter aeration so that the chambers could be operated as closed systems for flux measurements. The aeration ensured thorough mixing and maintained water P_{O_2} above 130 mmHg (1 mmHg= 0.1333 kPa). Tests with added ammonia demonstrated that aeration did not cause any volatilization loss.

Experimental series

Seven experimental series were performed, each with the same basic infusion, sampling and flux measurement protocol. The limited number of dogfish with working urinary catheters was distributed so as to ensure that each series contained 2–3 such animals, allowing an evaluation of the importance of the renal contribution in each treatment. During periods of box closure for the purpose of flux measurements, the water volume was set to 16 l kg^{-1}. Infusions were performed using a Gilson Minipuls peristaltic pump at a nominal rate of 3 ml kg^{-1} h^{-1}; the molarity of the solutions was chosen so as to approximate the measured osmolality

(930–970 mosmol kg^{-1}) of the blood plasma in these dogfish. All infusion solutions were prepared using chemicals obtained from Sigma.

Series i: no infusion

This series (N=9) served as a control for the effects of blood sampling and box closure alone. No infusion was performed. At the start of the experiment, the box was closed for a 3–5 h 'pre-infusion control' flux measurement, with water samples (50 ml) drawn at the start and end, a blood sample (300 μl) taken at the midpoint, and urine was collected throughout. The box was then thoroughly flushed by filling (to 40 l) and partially emptying (to 8 l) three times over a 15 min period, without exposing the fish to air. Flux measurements and urine collection then resumed for the 6 h 'infusion' period. Water samples were drawn at 1 h intervals for total ammonia-N (T_{Amm}) and urea-N measurements, and at 2 h intervals (i.e. at 2, 4 and 6 h) for pH and titratable alkalinity (T_{Alk}) measurements. Blood and urine samples were also taken at 2, 4 and 6 h. The box was then flushed again as above, a new water sample taken, and urine collection restarted to cover a 12 h overnight 'post-infusion' period. Final water, blood and urine samples were collected at 18 h.

Water samples were analyzed immediately for pH and T_{Alk}, and frozen at −20 °C for later determination of T_{Amm} and urea-N concentrations. Blood samples, obtained by centrifugation at 9000 ***g*** for 2 min, were analyzed immediately for arterial pH (pHa) and true plasma CO_2 concentration (Ca_{CO_2}), and plasma samples, obtained by centrifugation at 9000 ***g*** for 2 min, were frozen at −20 °C for later determination of T_{Amm} and urea-N concentrations. Urine samples were analyzed immediately for volume, pH and titratable acidity minus bicarbonate concentration [$TA-HCO_3^-$], and then frozen at −20 °C for later determination of T_{Amm} and urea-N concentrations.

Series ii: NaCl infusion

This series (N=6) served as a control for the possible additional effects of infusion using 500 mmol l^{-1} NaCl, a solution which is acid–base neutral, infused at 3.010±0.045 ml kg^{-1} h^{-1}=1505±23 μmol kg^{-1} h^{-1} for 6 h. Flux measurements and water, blood and urine sampling were performed in an identical fashion to series i.

Series iii: NH_4Cl infusion

This series (N=7) provided the ammonia substrate for ureagenesis by the infusion of 500 mmol l^{-1} NH_4Cl at 3.380±0.183 ml kg^{-1} h^{-1}=1690±92 μmol kg^{-1} h^{-1} for 6 h. The protocol was otherwise identical to that of series ii.

Series iv: $NaHCO_3$ infusion

This series (N=6) provided the bicarbonate substrate for ureagenesis by the infusion of 500 mmol l^{-1} $NaHCO_3$ at 3.167±0.124 ml kg^{-1} h^{-1}=1584±62 μmol kg^{-1} h^{-1} for 6 h. The protocol was otherwise identical to that of series ii.

Series v: urea infusion

This series (N=6) evaluated the effects of urea loading by the infusion of 1000 mmol l^{-1} urea (2000 mmol l^{-1} urea-N) at 3.122±0.130 ml kg^{-1} h^{-1}=3122±130 μmol kg^{-1} h^{-1} for 6 h. The protocol was otherwise identical to that of series ii, except that acid–base measurements were not performed on the blood, urine and water samples.

Series vi: thiourea infusion

This series (N=6) examined the effects of this possible competitive blocker of urea transport by the infusion of 1000 mmol l^{-1} thiourea at 3.044±0.046 ml kg^{-1} h^{-1}= 3044±46 μmol kg^{-1} h^{-1} for 6 h. The protocol was otherwise identical to that of series v.

Series vii: acetamide infusion

This series (N=7) examined the effects of another possible competitive blocker of urea transport by the infusion of 1000 mmol l^{-1} acetamide at 3.048±0.116 ml kg^{-1} h^{-1}= 3048±116 μmol kg^{-1} h^{-1} for 6 h. The protocol was otherwise identical to that of series v.

Analytical techniques

Water

T_{Amm} was determined by the salicylate–hypochlorite method (Verdouw *et al.* 1978) and urea-N concentration by the diacetyl monoxime method (Price and Harrison, 1987). Water pH was measured with a Radiometer GK2401C combination electrode and T_{Alk} by titration to pH 4.00 as described by McDonald and Wood (1981). The change in net acidic equivalent concentration was calculated as the sum of the T_{Alk} and T_{Amm} changes, signs considered, as outlined by Maetz (1973).

Blood

Arterial pH was measured with a Radiometer E5021 microelectode system thermostatically controlled to the experimental temperature; plasma Ca_{CO_2} was determined by the method of Cameron (1971). Plasma T_{Amm} was measured enzymatically by the glutamate dehydrogenase method of Mondzac *et al.* (1965), using a Sigma kit and the same standards as for water T_{Amm}, and plasma urea-N concentration was assayed by the diacetyl monoxime method, after dilution to the same range (0–200 μmol urea-N l^{-1}) as the water assay.

Urine

Urine volume was determined gravimetrically, urine pH, T_{Amm} and urea-N concentration were measured as for blood, and [$TA-HCO_3^-$] was quantified as a single value using the double-titration procedure recommended by Hills (1973) and described by McDonald and Wood (1981).

Calculations

The partial pressure of CO_2 (Pa_{CO_2}) and the bicarbonate concentration [HCO_3^-] in arterial blood plasma were calculated from pHa and Ca_{CO_2} measurements by means of the Henderson–Hasselbalch equation and appropriate constants (αCO_2, pK′) for dogfish plasma from Boutilier *et al.* (1984).

Similarly, the partial pressure of ammonia (P_{NH_3}) and ammonium ion concentration [NH_4^+] in blood plasma and water were calculated from the respective pH and T_{Amm} measurements in the two media using the Henderson–Hasselbalch equation as detailed by Wright and Wood (1985). In the absence of elasmobranch data, constants (αNH_3, pK_{Amm}) were taken from the study of Cameron and Heisler (1983) on trout, with appropriate adjustments for ionic strength. The difference (ΔP_{NH_3}) between simultaneous determinations of P_{NH_3} in blood and water was calculated as a measure of the NH_3 diffusion gradient. The ammonium ion gradient ($\Delta[NH_4^+]$) was calculated in an analogous fashion.

Whole-body flux rates (in μmol kg^{-1} h^{-1}) of ammonia-N ($J_{Amm\text{-}N}$), urea-N ($J_{Urea\text{-}N}$) and net acidic equivalents (J_H) from the animal to the water were calculated in the standard fashion from changes in concentration in the water (μmol l^{-1}) multiplied by volume (l) and factored by time (h) and mass (kg). Similarly, flux rates through the urine were calculated from the urine flow rate (UFR; ml kg^{-1} h^{-1}) and the concentration in the urine (μmol ml^{-1}); the UFR was calculated from the volume of urine collected over a period divided by time (h) and mass (kg). The urinary fluxes were added to the fluxes from the animal to the water to give the whole-body flux rates in dogfish bearing urinary catheters.

Data are expressed as means ± 1 S.E.M. (N). The significance of changes within each experimental series was assessed using Student's two-tailed paired t-test at $P \leqslant 0.05$, with the t-value adjusted for multiple comparisons using the Bonferroni procedure (Nemenyi *et al.* 1977).

Results

Nitrogen metabolism and acid–base status under control conditions

There was no significant change in any measured parameter over the time course of the 'no infusion' protocol (series i), indicating that the blood sampling and flux measurement procedures did not alter the variables under study. In order to produce the best overall representation of control conditions, the data for each fish of series i (grand means) were averaged with the pre-infusion values from each fish of the other six series so as to produce the means reported in Tables 1 and 2. Values for a typical teleost, the rainbow trout *Oncorhynchus mykiss*, are included in Table 1 for comparison (see Discussion).

Urea-N levels in the blood of resting dogfish were about 8000 times greater than ammonia-N concentrations (T_{Amm}), and urea-N was clearly the dominant excretory nitrogen product on a whole-body basis (Table 1). Indeed ammonia-N excretion rates were extremely low, despite positive ΔP_{NH_3} and $\Delta[NH_4^+]$ gradients from blood to water (Table 1). Resting dogfish were in acid–base balance, as indicated by typical control values for arterial blood and a mean value of J_H which was not significantly different from zero.

UFR was quite low, yet urinary urea-N concentrations (Table 2) were only about 20 % of blood levels (see Table 1), so urea was strongly reabsorbed at the kidney. Urinary T_{Amm} was about three times higher than blood T_{Amm} (Table 2), but urinary ammonia-N excretion was negligible. Urine pH was extremely acidic, and there was a consistent net acidic equivalent excretion (J_H) in the urine, made up almost entirely of the [$TA - HCO_3^-$] component.

Fig. 1 summarizes resting rates of urea-N and ammonia-N excretion through the gills and kidney for all animals where measurements were made simultaneously. Clearly, urea-N excretion through the gills was the predominant pathway, accounting for over 90 % of the total excretion. Urea-N excretion through the kidney was a small but significant component (6.5 %), and ammonia-N excretion through both gills and kidney accounted for a negligible fraction (<3 %) of the total nitrogen budget.

Table 1. *Parameters of nitrogen metabolism and acid–base balance in the dogfish* Squalus acanthias *under control conditions; a comparison with comparable measurements in a teleost, the rainbow trout* Oncorhynchus mykiss

	Squalus acanthias	*Oncorhynchus mykiss**
Arterial blood		
pHa	7.881±0.013 (26)	7.840±0.016
Pa_{CO_2} (mmHg)	1.29±0.07 (26)	3.05±0.10
[HCO_3^-] (mmol l^{-1})	4.40±0.20 (26)	7.51±0.20
Urea-N (mmol-N l^{-1})	636±12 (47)	5.21±0.19
T_{Amm} (μmol-N l^{-1})	81±13 (47)	60±6
Whole-body fluxes		
$J_{Urea\text{-}N}$ (μmol-N kg^{-1} h^{-1})	549±31 (47)	32±5
$J_{Amm\text{-}N}$ (μmol-N kg^{-1} h^{-1})	28±5 (45)	240±35
J_H (μmol kg^{-1} h^{-1})	11±18 (26)	–
Blood–water gradients		
P_{NH_3} (nmHg)	25±5 (25)	18±2
[NH_4^+] (μmol l^{-1})	78±12 (25)	57±5

Values are means ± S.E.M. (N).
*Values for *Oncorhynchus mykiss* are taken from Wilkie and Wood (1991, 1995).

Table 2. *Urinary parameters of nitrogen excretion and acid–base balance in the dogfish* Squalus acanthias *under control conditions*

Urea-N (mmol-N l^{-1})	126±30 (16)
T_{Amm} (μmol-N l^{-1})	290±49 (16)
[$TA–HCO_3^-$] (mmol l^{-1})	49.9±10.4 (10)
pH of urine	5.754±0.067 (13)
Urine flow rate (ml kg^{-1} h^{-1})	0.210±0.036 (16)
$J_{Urea\text{-}N}$ (μmol-N kg^{-1} h^{-1})	26.04±6.01 (16)
$J_{Amm\text{-}N}$ (μmol-N kg^{-1} h^{-1})	0.06±0.01 (16)
$J_{TA–HCO_3}$ (μmol kg^{-1} h^{-1})	10.48±2.26 (10)
J_H (μmol kg^{-1} h^{-1})	10.58±2.29 (10)

Values are means ± S.E.M. (N).

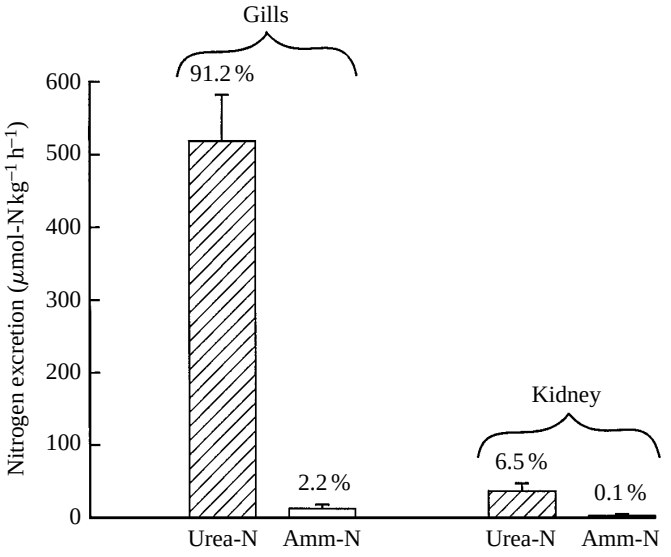

Fig. 1. The partitioning of resting nitrogen excretion between ammonia-N and urea-N at the gills and kidney of male dogfish *Squalus acanthias* fitted with urinary catheters. The percentages were calculated assuming that the total ammonia-N and urea-N excretion through the two routes was 100 %. Means + 1 S.E.M. (N=10).

Responses to NaCl, NH_4Cl and $NaHCO_3$ infusion

Infusion of 500 mmol l^{-1} NaCl (approximately 9000 μmol kg^{-1} over 6 h) had no effect on ΔP_{NH_3} (Fig. 2A), $\Delta[NH_4^+]$ (not shown), plasma T_{Amm} (Fig. 2B) or plasma urea-N level (Fig. 2C). However, infusion of 500 mmol l^{-1} NH_4Cl at the same rate caused a progressive increase in plasma T_{Amm} which reached about 3.6 mmol l^{-1} by 6 h. In consequence, ΔP_{NH_3} rose to about 220 nmHg and $\Delta[NH_4^+]$ to about 3.2 mmol l^{-1}. Plasma urea-N concentration remained unaltered. $NaHCO_3$ infusion did not influence plasma T_{Amm} or urea-N levels, but caused a small rise in ΔP_{NH_3} because of its alkalizing influence on the blood (see below). All effects had disappeared by the 18 h post-infusion sample.

NaCl infusion had minimal influence on blood acid–base status, causing only a small rise in pHa at 6 h (Fig. 3A). However NH_4Cl and $NaHCO_3$ loading exerted large, generally opposite, effects, the former causing acidosis and the latter alkalosis. Thus, pHa dropped to about 7.40 with NH_4Cl loading and rose to about 8.25 with $NaHCO_3$ loading, relative to pre-infusion values of 7.80–7.90 (Fig. 3A). These changes occurred in concert with respective decreases (to <1 mmol l^{-1}) and increases (to approximately 14 mmol l^{-1}) in plasma $[HCO_3^-]$ relative to control levels of 4–5 mmol l^{-1} (Fig. 3B). Pa_{CO_2} did not change during NH_4Cl infusion, but the alkalosis of $NaHCO_3$ loading was complicated by a 30 % increase in Pa_{CO_2} to about 1.7 mmHg (Fig. 3C). All these changes developed quite rapidly and were stable from 2 to 6 h of infusion, with complete recovery by 18 h. It is noteworthy that the acidosis of NH_4Cl infusion actually attenuated the resultant rise in ΔP_{NH_3}, which otherwise (i.e. had a control pHa of 7.88 been maintained) would have reached about 800 nmHg rather than 220 nmHg (Fig. 2A). In contrast, the alkalosis of $NaHCO_3$ infusion was totally responsible for the small rise in ΔP_{NH_3}, which otherwise would have remained unchanged.

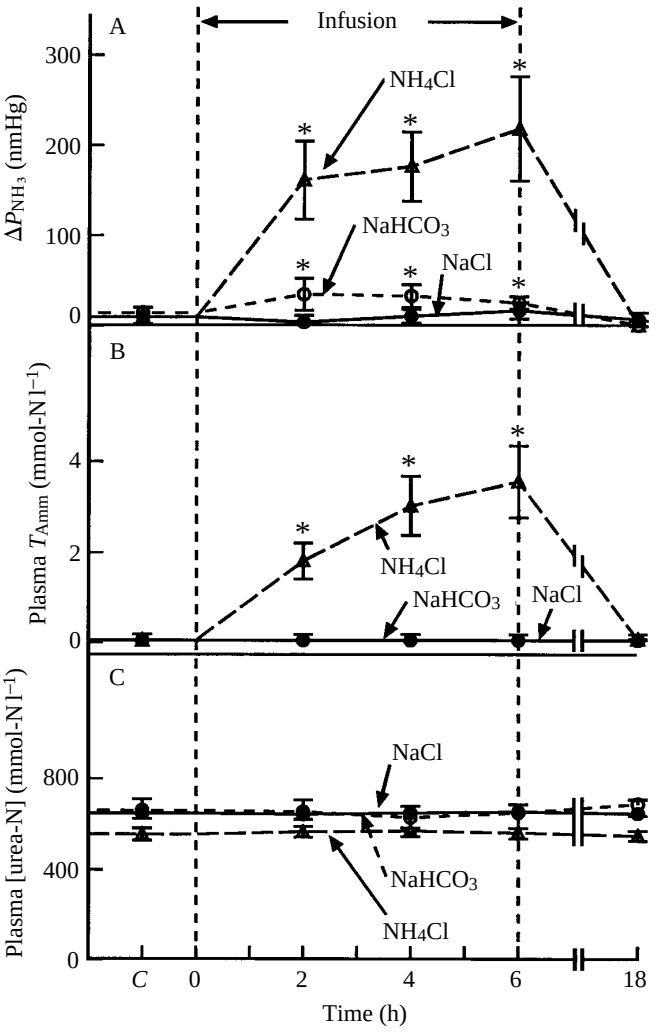

Fig. 2. The influence of NaCl, NH_4Cl and $NaHCO_3$ infusions on (A) the diffusion gradient for ammonia from blood to water, P_{NH_3}; (B) plasma total ammonia-N concentration, T_{Amm}; and (C) plasma urea-N concentration in *Squalus acanthias*. Dogfish were infused for 6 h with 500 mmol l^{-1} NaCl (N=6), NH_4Cl (N=7) or $NaHCO_3$ (N=6) at a nominal rate of 3 ml kg^{-1} h^{-1}. Asterisks indicate means significantly different ($P \leqslant 0.05$) from the respective pre-infusion control values (C) in each group. Means ± 1 S.E.M.

NaCl infusion caused no disturbance of whole-body acid–base exchange (i.e. J_H; Fig. 4A), ammonia-N excretion (i.e. $J_{Amm\text{-}N}$; Fig. 4B) or urea-N excretion (i.e. $J_{Urea\text{-}N}$; Fig. 4C) to the environment. This infusion control therefore demonstrated that there was a stable background against which to examine the possible influence of NH_4Cl and $NaHCO_3$ on these parameters.

NH_4Cl loading activated a highly effective excretion of net acidic equivalents to the environment (i.e. positive J_H by the convention used here; Fig. 5A). The stimulation was significant by 2–4 h, and by 4–6 h, J_H had reached almost 1000 μmol kg^{-1} h^{-1}, relative to an NH_4Cl infusion rate of

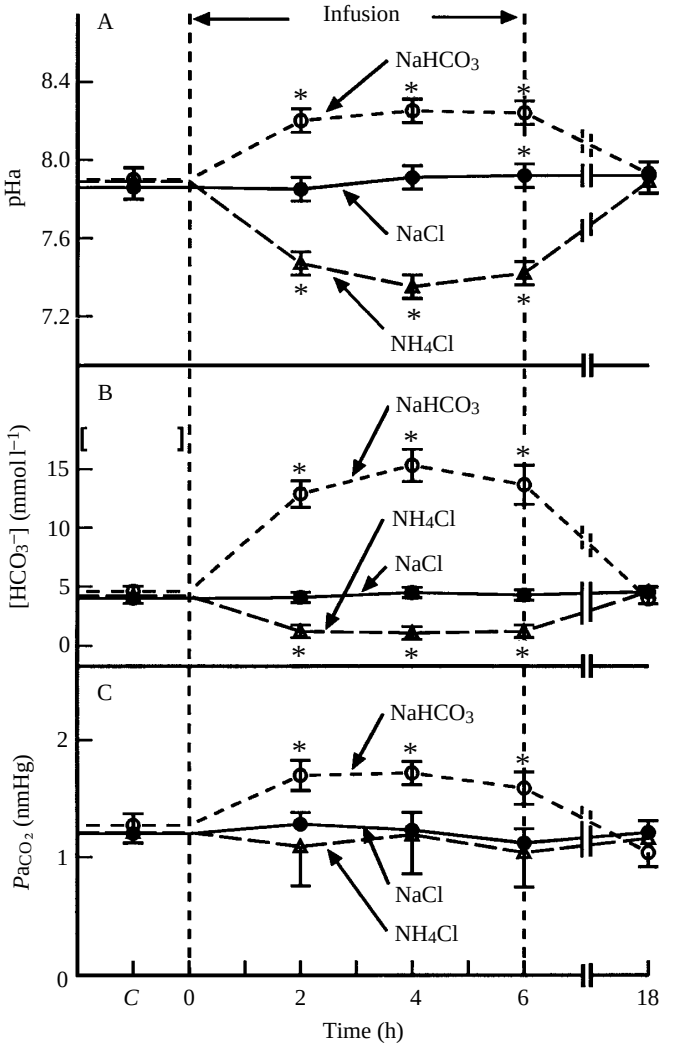

Fig. 3. The influence of NaCl, NH_4Cl and $NaHCO_3$ infusions on (A) arterial blood pH, pHa; (B) plasma bicarbonate concentration, $[HCO_3^-]$; and (C) carbon dioxide tension, Pa_{CO_2}, in *Squalus acanthias*. Other details as in legend of Fig. 2.

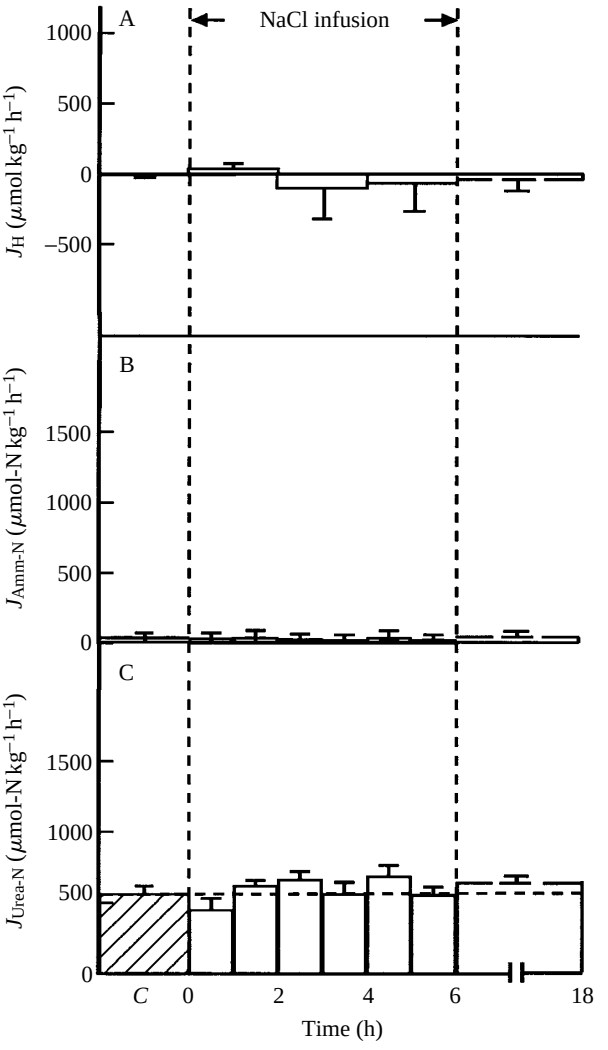

Fig. 4. The influence of NaCl infusion (500 mmol l^{-1} for 6 h at a nominal rate of 3 ml kg^{-1} h^{-1}) on (A) the whole-body rate of net acidic equivalent excretion to the environment, J_H; (B) the whole-body rate of ammonia-N excretion to the environment, $J_{Amm\text{-}N}$, and (C) the whole-body rate of urea-N excretion to the environment, $J_{Urea\text{-}N}$, in *Squalus acanthias*. There were no significant differences ($P \leqslant 0.05$) from the respective pre-infusion control values (*C*, hatched bars and dashed horizontal line). Means + 1 S.E.M. (N=6).

1690±92 μmol kg^{-1} h^{-1}. A slight elevation persisted during the 12 h post-infusion flux in some fish, but was not significant overall. By 18 h, cumulative net acidic equivalent excretion amounted to 44 % (4420 μmol kg^{-1}) of the infused NH_4Cl burden (10 140 μmol kg^{-1}). Urinary data from three animals (not shown) revealed a negligible change in urinary J_H, so the response occurred almost entirely at the gills.

Ammonia-N excretion increased rapidly in response to NH_4Cl loading; $J_{Amm\text{-}N}$ was significantly elevated even during the first hour of infusion, and from 2 to 6 h stabilized at approximately 1250 μmol kg^{-1} h^{-1} (Fig. 5B). A slight elevation remained significant during the post-infusion period. Over 18 h, the cumulative increase in $J_{Amm\text{-}N}$ accounted for 72 % (7300 μmol kg^{-1}) of the load infused as NH_4Cl. Overall, this cumulative elevation in $J_{Amm\text{-}N}$ was significantly greater than that in J_H; the difference was particularly pronounced during the first few hours of infusion. Thus, at least a portion (approximately 28 %) of the stimulated ammonia-N excretion probably occurred in the form of NH_3 alone (i.e. 7300 − 4420 = 2880 μmol kg^{-1}) rather than as NH_4^+ or NH_3+H^+. This response occurred almost entirely at the gills; urinary $J_{Amm\text{-}N}$ did increase about 30-fold (N=3, data not shown), presumably in response to the increase in plasma T_{Amm} (Fig. 2B), but this amounted to less than 0.3 % of the cumulative whole-body $J_{Amm\text{-}N}$.

NH_4Cl infusion also stimulated a pronounced elevation in urea-N excretion (Fig. 5C), but over a somewhat slower time course than that in $J_{Amm\text{-}N}$. $J_{Urea\text{-}N}$ first became significantly increased at 1–2 h, stabilized at about 1200 μmol kg^{-1} h^{-1} from 3 to 6 h, and remained substantially elevated during the 12 h post-infusion period. Over 18 h, the cumulative increase in $J_{Urea\text{-}N}$ amounted to 6055 μmol kg^{-1}, or about 60 % of the

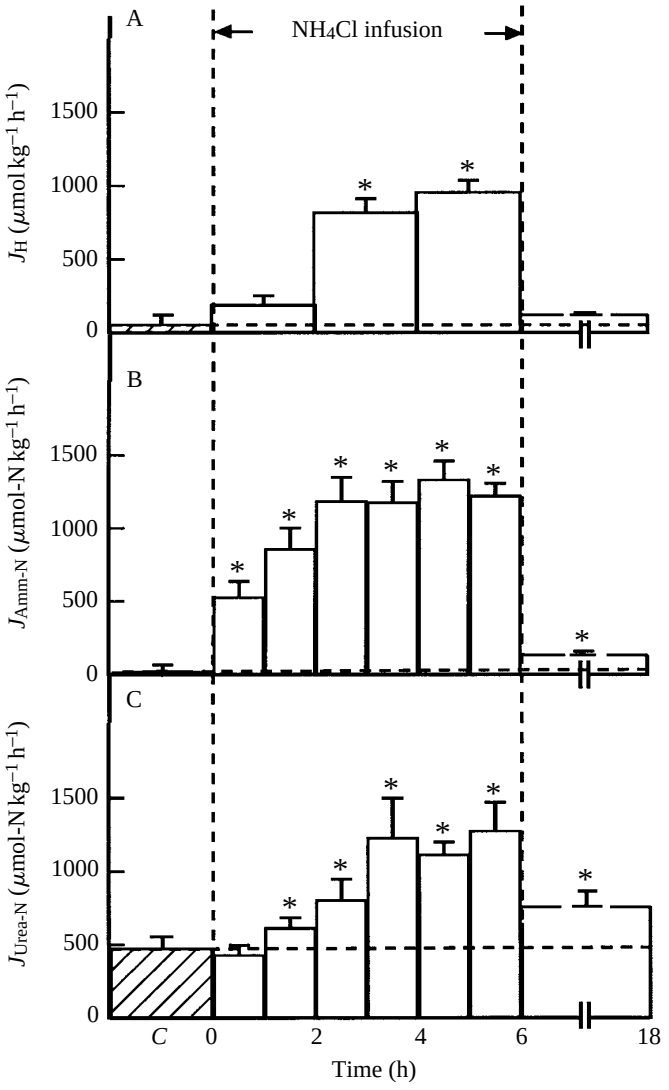

Fig. 5. The influence of NH_4Cl infusion (500 mmol l^{-1} for 6 h at a nominal rate of 3 ml kg^{-1} h^{-1}) on (A) the whole-body rate of net acidic equivalent excretion to the environment, J_H; (B) the whole-body rate of ammonia-N excretion to the environment, $J_{Amm\text{-}N}$, and (C) the whole-body rate of urea-N excretion to the environment, $J_{Urea\text{-}N}$, in *Squalus acanthias*. Asterisks indicate significant differences ($P \leqslant 0.05$) from the respective pre-infusion control values (*C*, hatched bars and dashed horizontal lines). Means + 1 S.E.M. (N=7).

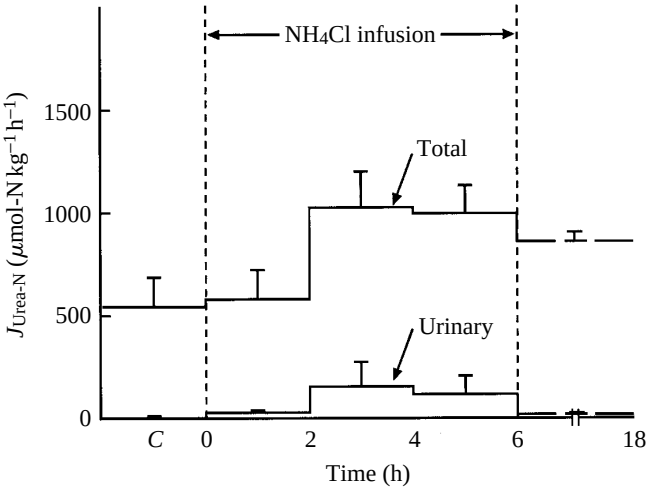

Fig. 6. The influence of NH_4Cl infusion (500 mmol l^{-1} for 6 h at a nominal rate of 3 ml kg^{-1} h^{-1}) on the total (i.e. whole-body rates) and urinary rates of urea-N excretion to the environment, $J_{Urea\text{-}N}$, in three male *Squalus acanthias* fitted with urinary catheters. For both total and urinary excretion, the mean rates during the infusion period were significantly elevated ($P \leqslant 0.05$) relative to respective pre-infusion control values (*C*). Means + 1 S.E.M. (N=3).

exogenous nitrogen load presented as NH_4Cl. Thus, the dual response of elevated $J_{Amm\text{-}N}$ (Fig. 5B) and elevated $J_{Urea\text{-}N}$ (Fig. 5C) was more than adequate to excrete all of the infused nitrogen load, together accounting for 132 % of the infused nitrogen.

In the case of $J_{Urea\text{-}N}$, the renal response did appear to be important, though less so than that of the the gills. Fig. 6 illustrates that increased urinary $J_{Urea\text{-}N}$ accounted for about 20 % of the elevation in whole-body $J_{Urea\text{-}N}$ measured in three dogfish with urinary catheters. Note that these marked increases in $J_{Urea\text{-}N}$ across both gills and kidney occurred in the absence of any detectable change in plasma urea-N concentration (Fig. 2C).

Infusion of $NaHCO_3$ at a measured rate of 1584±62 μmol kg^{-1} h^{-1} activated a net excretion of basic equivalents (i.e. negative J_H; Fig. 7A) at an even higher rate than the acid-excreting mechanism turned on by NH_4Cl loading (cf. Fig. 5A). The response was significant in the first 2 h, reached almost 1100 μmol kg^{-1} h^{-1} by 4–6 h, and remained significant during the 12 h post-infusion period. The cumulative excretion of basic equivalents over 18 h amounted to 7963 μmol kg^{-1}, about 84 % of the infused bicarbonate load (9505 μmol kg^{-1}). The urinary response, measured in two animals only (data not shown), was negligible, so this excretion of basic equivalents occurred entirely at the gills.

Statistically significant increases in $J_{Amm\text{-}N}$ occurred during two periods of $NaHCO_3$ infusion (Fig. 7B), but these changes were so small as to be negligible relative to the whole-animal nitrogen budget. They probably resulted from the elevation of ΔP_{NH_3} across the gills which accompanied the blood alkalosis (Fig. 2A). There was no urinary response.

$NaHCO_3$ infusion caused an increase in $J_{Urea\text{-}N}$ (Fig. 7C), but the response was much less pronounced than with NH_4Cl loading (cf. Fig. 5C). The elevation was significant at 3–5 h and did not persist during the post-infusion period; overall, it amounted to 1055 μmol kg^{-1} or 11 % of the infused load. As with NH_4Cl loading, the urinary response accounted for about 20 % of the elevation in whole-animal $J_{Urea\text{-}N}$ (N=2, results not shown).

Responses to acetamide, thiourea and urea infusion

Infusions of acetamide and thiourea had no detectable effect on plasma urea-N concentrations (Fig. 8A,B), but infusion of urea itself, at a measured rate of 6244±260 μmol-N kg^{-1} h^{-1}, caused a gradual increase in blood urea levels

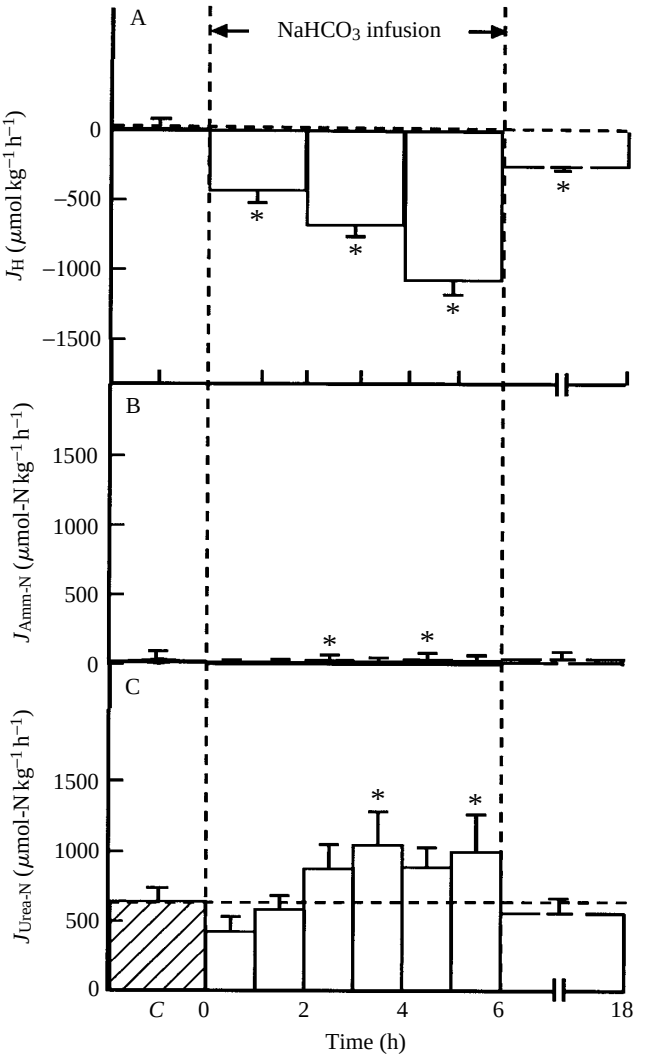

Fig. 7. The influence of $NaHCO_3$ infusion (500 mmol l^{-1} for 6 h at a nominal rate of 3 ml kg^{-1} h^{-1}) on (A) the whole-body rate of net acidic equivalent excretion to the environment, J_H; (B) the whole-body rate of ammonia-N excretion to the environment, $J_{Amm\text{-}N}$, and (C) the whole-body rate of urea-N excretion to the environment, $J_{Urea\text{-}N}$, in *Squalus acanthias*. Asterisks indicate significant differences ($P \leqslant 0.05$) from the respective pre-infusion control values (*C*, hatched bars and dashed horizontal lines). Means ± 1 S.E.M. (N=6).

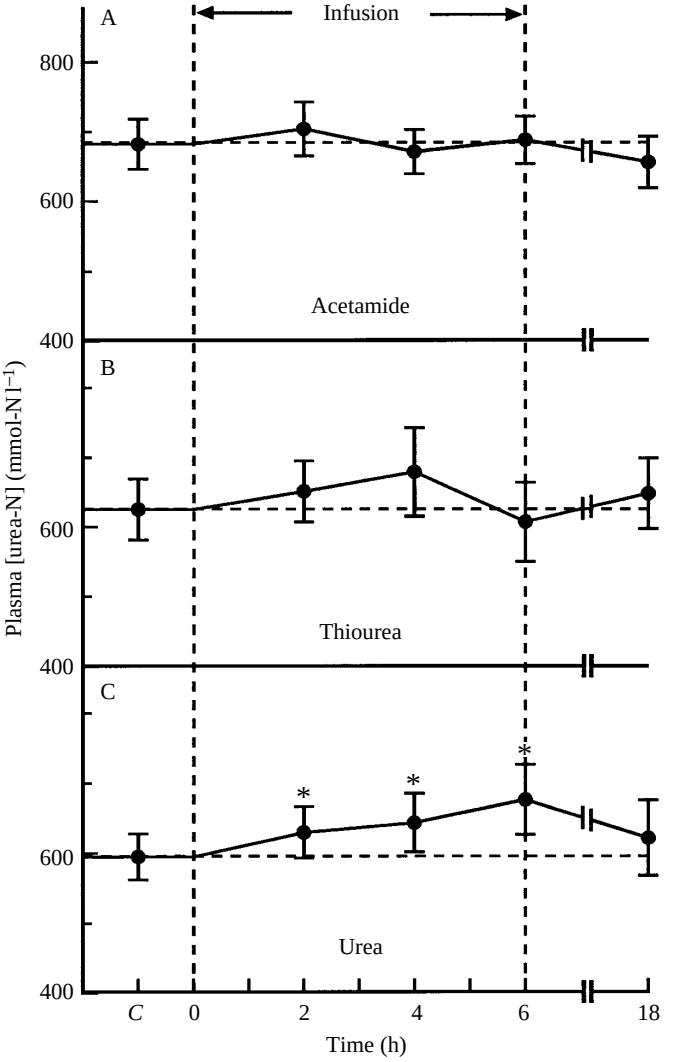

Fig. 8. The influence of the infusion of (A) acetamide (N=7), (B) thiourea (N=6) and (C) urea (N=6) on plasma urea-N concentrations in *Squalus acanthias*. Dogfish were infused for 6 h with 1000 mmol l^{-1} solutions at a nominal rate of 3 ml kg^{-1} h^{-1}. Asterisks indicate means significantly different ($P \leqslant 0.05$) from the respective pre-infusion control values (*C*, dashed horizontal lines) in each group. Means ±1 S.E.M.

(Fig. 8C). This effect was significant at all sample times during infusion, and by 6 h plasma urea levels had increased by 82 mmol-N l^{-1}, about 15 % of the control level in this group. The increase was corrected by 18 h. Acetamide and thiourea concentrations in the blood were not measured, but if they distributed in a similar fashion to urea, then the internal levels achieved were probably equal to about 15 % of the blood urea concentration. None of these infusions had any effect on plasma T_{Amm} or $J_{Amm\text{-}N}$ excretion through gills or kidney (results not shown).

Both acetamide and thiourea caused increases in $J_{Urea\text{-}N}$. The increases amounted to 20–40 % of the pre-infusion $J_{Urea\text{-}N}$ and were significant at 2–3 h and 5–6 h with acetamide (Fig. 9A) and at 3–5 h with thiourea (Fig. 9B); they did not persist during the post-infusion period. Both drugs caused a two- to threefold increase in urinary $J_{Urea\text{-}N}$ (measured in two dogfish in each group, results not shown), which accounted at most for one-quarter of the whole-animal response, so the larger effects were exerted on the gills.

Urinary urea-N excretion exhibited no response to urea infusion (N=2). Surprisingly, the infusion of urea itself also did not increase whole-body $J_{Urea\text{-}N}$ (Fig. 9C), despite a nitrogen loading rate four times greater than with NH_4Cl and despite significant increases in plasma urea-N levels (Fig. 8C). Indeed, a significant 40 % *inhibition* of $J_{Urea\text{-}N}$ occurred during the first 2 h of urea infusion (Fig. 9C). As with the responses to NH_4Cl and $NaHCO_3$ loading, changes in the excretion of urea across

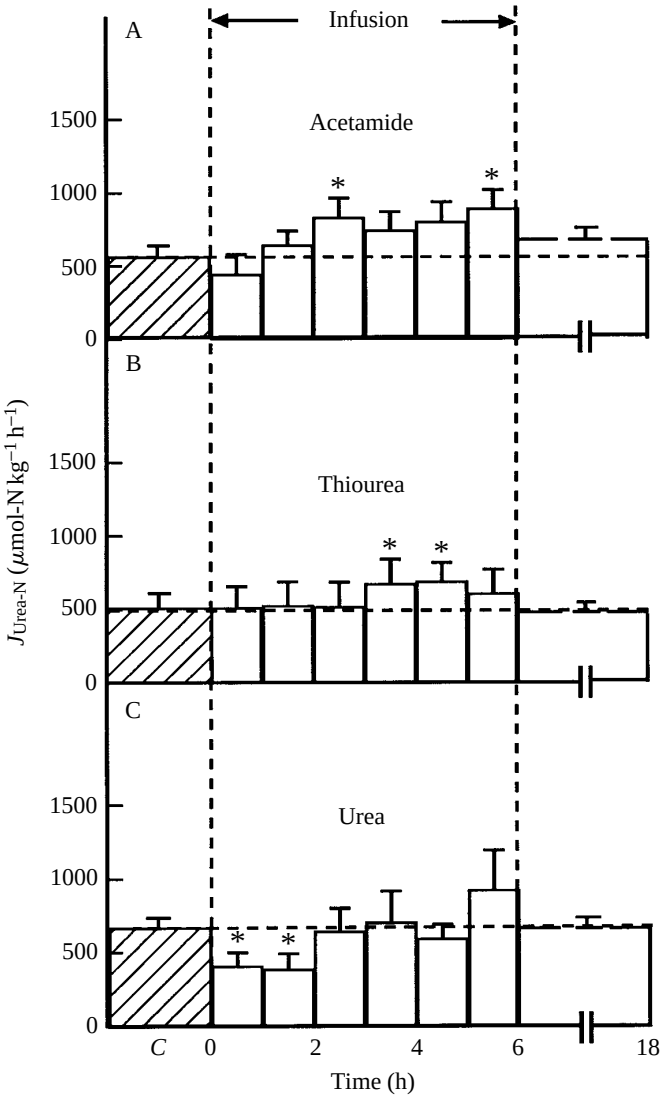

Fig. 9. The influence of the infusion of (A) acetamide (N=7), (B) thiourea (N=6) and (C) urea (N=6) on the whole-body rate of urea-N excretion to the environment, J_{Urea-N}, in *Squalus acanthias*. Dogfish were infused for 6 h with 1000 mmol l^{-1} solutions at a nominal rate of 3 ml kg^{-1} h^{-1}. Asterisks indicate means significantly different ($P \leqslant 0.05$) from the respective pre-infusion control values (*C*, hatched bars and dashed horizontal lines) in each group. Means +1 S.E.M.

the gills appear to be dissociated from changes in plasma urea-N concentrations.

Discussion

Control measurements

Arterial blood acid–base status (Table 1) was alkalotic relative to many early studies (e.g. Hodler *et al.* 1955; Cross *et al.* 1969; King and Goldstein, 1983) but similar to that reported in several more recent investigations on *Squalus acanthias* in which unrestrained animals, chronic cannulation and temperature-controlled electrodes were employed (e.g. Swenson and Maren, 1987; Claiborne and Evans, 1992). Urinary parameters were similar to those of the earlier studies in terms of the fixed acid pH of the urine (approximately 5.7), its high [TA−HCO_3^-] (approximately 50 mmol l^{-1}) and its low NH_4^+ concentration (<1 % of [TA−HCO_3^-]). However, UFR was only about 50 % of the earlier measurements, probably reflecting the less stressful conditions of our experiments. Plasma levels and whole-body excretion rates of urea-N were comparable to those reported in a variety of marine elasmobranchs including *Squalus acanthias* (reviewed by Perlman and Goldstein, 1988; Shuttleworth, 1988; Wood, 1993). In contrast, plasma T_{Amm} was considerably lower (<25 %) than previously reported in this species (e.g. Cross *et al.* 1969; Robertson, 1975; Kormanik and Evans, 1986), probably reflecting the fact that the present study is the first to measure T_{Amm} by chronic cannulation in unrestrained dogfish. Sampling disturbance is well known to elevate blood ammonia levels markedly in fish (Wood, 1993). Indeed, the measured resting value of arterial plasma T_{Amm} in the dogfish (approximately 80 μmol l^{-1}) was comparable to that (50–200 μmol l^{-1}) measured by cannulation in a number of teleosts (Wood, 1993), including the rainbow trout *Oncorhynchus mykiss* (e.g. Wilkie and Wood, 1991, 1995; Table 1).

The present results demonstrate a convincing dominance (98 %:2 %) of urea-N over ammonia-N excretion, with the vast majority of the former occurring at the gills (Fig. 1; Table 1). The low urea loss in the urine is in agreement with the well-documented, highly efficient reabsorption of urea by the kidney (see Introduction). At least for resting, moderately fasted dogfish under control conditions, the present findings argue against the suggestion of Mommsen and Walsh (1991) that J_{Amm-N} may be larger than J_{Urea-N}. Indeed, as will be argued below, the dogfish appears actively to prevent ammonia excretion. Trimethylamine oxide (TMAO) excretion, while quite low, may actually be a larger component of nitrogen balance than J_{Amm-N}, for Goldstein and Palatt (1974) reported TMAO losses that were about 10–20 % of urea losses in several elasmobranchs, including *Squalus acanthias*.

There are only a few measurements of J_{Amm-N} in marine elasmobranchs for comparison with the very low present value (28 μmol kg^{-1} h^{-1}), and almost none where J_{Urea-N} was determined simultaneously. For *Scyliorhinus canicula*, Payan and Maetz (1973) reported a J_{Amm-N} of 120 μmol-N kg^{-1} h^{-1} (no J_{Urea-N} value), but their animals were obviously stressed and plasma T_{Amm} was 13 times higher than in the present study. A resting J_{Amm-N} for *Scyliorhinus stellaris* of about 50 μmol kg^{-1} h^{-1} (no J_{Urea-N} or plasma T_{Amm} values) may be estimated from the study of Heisler *et al.* (1988). For *Squalus acanthias*, rates of 17 μmol kg^{-1} h^{-1} (Evans, 1982) and 60 μmol kg^{-1} h^{-1} (Claiborne and Evans, 1992) have been reported as control values for ‘pups’ and adult animals respectively (no J_{Urea-N} or T_{Amm} measurements). The one report of simultaneous J_{Amm-N} and J_{Urea-N} determinations in *Squalus acanthias* is an abstract (Goldstein and Forster, 1962), with methods described only as ‘combined divided box-

catheterization experiments'. Here $J_{Amm\text{-}N}$ was about $150\,\mu mol\,kg^{-1}\,h^{-1}$ relative to a $J_{Urea\text{-}N}$ of about $570\,\mu mol\,kg^{-1}\,h^{-1}$ and the great majority of both fluxes took place through the gills rather than the kidney. Apart from the much higher $J_{Amm\text{-}N}$, these values are similar to the present data. Overall, we conclude that branchial urea excretion is the dominant route of nitrogen excretion and that $J_{Amm\text{-}N}$ is very low, especially when precautions against experimental disturbance are taken.

The permeability of the gills to ammonia and urea

A comparison of the present nitrogen metabolism data for *Squalus acanthias* with measurements made in an ammoniotelic teleost *Oncorhynchus mykiss* (Wilkie and Wood, 1991, 1995) by identical methods is instructive (Table 1). Plasma urea-N concentration, which determines the diffusion gradient from blood to water, is 122 times greater in the elasmobranch, yet $J_{Urea\text{-}N}$ is only 17 times greater. Branchial urea permeability is obviously much lower in the elasmobranch than in the teleost, in agreement with the original findings of Smith (1929, 1936). Indeed, using data from Table 1 and gill areas tabulated by Hughes and Morgan (1973) for appropriately sized specimens of the two species, we calculate that the urea permeability of the gills is 13.5 times higher in *Oncorhynchus mykiss* ($8.67\times10^{-7}\,cm\,s^{-1}$) than in *Squalus acanthias* ($6.46\times10^{-8}\,cm\,s^{-1}$). The latter figure is almost identical to the value of $7.5\times10^{-8}\,cm\,s^{-1}$ reported by Boylan (1967) for *Squalus acanthias*.

A more surprising conclusion is that the same calculation for T_{Amm} (i.e. combining NH_3 and NH_4^+) yields a 22-fold higher gill ammonia permeability ($5.84\times10^{-4}\,cm\,s^{-1}$) in the teleost than in the elasmobranch ($2.65\times10^{-5}\,cm\,s^{-1}$) because the two species have similar blood T_{Amm} values, but the dogfish exhibits a much lower $J_{Amm\text{-}N}$. Note that the conclusion would be identical (22-fold lower ammonia permeability in the dogfish) if the calculation were based on ΔP_{NH_3} gradients only (cf. Evans and More, 1988). This low resting permeability to ammonia seems to have been overlooked previously, probably because of the general focus on low urea permeability in the elasmobranch gill. Thus, the elasmobranch manifests 'normal permeability' to O_2 and CO_2, yet selectively low permeability to a third respiratory gas NH_3, as well as to urea. Given the different physico-chemical properties of ammonia *versus* urea (see Wood, 1993), we propose that the explanation should be sought in biochemical and/or transporter mechanisms, rather than in the physical properties of the membranes. Note that ammonia permeability increases twofold (based on T_{Amm} gradients) to sixfold (based on ΔP_{NH_3} gradients) in the first 2 h of NH_4Cl infusion (calculated from Figs 2 and 5), prior to any change in apparent urea permeability. An ammonia retention mechanism that works well under resting conditions becomes less efficient in the face of ammonia loading.

Urea-N is essentially a metabolic dead-end because of the absence of endogenous urease in vertebrates. In elasmobranchs, metabolically useful nitrogen appears to be transported through the circulation as ammonia-N, rather than as amino-acid-N (Mommsen and Walsh, 1991), so there is a danger of ammonia-N loss from the blood across the gills. Nitrogen limitation *via* starvation impairs the osmoregulatory ability of elasmobranchs by compromising their ability to maintain plasma urea-N levels (Haywood, 1973; Leech *et al.* 1979). A gill biochemically poised to prevent ammonia-N excretion during fasting (Table 1; Fig. 1), as well as to minimize urea-N excretion, may be an important design feature of elasmobranchs. In this regard, we have measured significant activity (approximately 10 % of liver levels) of the ammonia-scavenging enzyme glutamine synthetase in the gill tissue of *Squalus acanthias* (P. A. Wright, P. Pärt and C. M. Wood, unpublished results). Glutamine synthetase activity is similarly high in the kidney of *Squalus acanthias* (King and Goldstein, 1983), which may explain the very low urinary $J_{Amm\text{-}N}$ (Fig. 1; Table 2). Since glutamine synthetase is the key nitrogen-trapping enzyme for urea production *via* both uricolysis and the CPS-III-based OUC (Mommsen and Walsh, 1989, 1991; Wood, 1993), this would provide a synthetic pathway to help maintain internal urea-N levels.

Glutamine produced in the gill and kidney could be shipped to the liver to fuel urea production. However, the possibility of urea synthesis directly within the gill and kidney tissues themselves is worthy of consideration; to our knowlege, the idea has never been tested experimentally. Certainly, such a scheme would help explain the curious dissociation between rates of $J_{Urea\text{-}N}$ and blood urea-N levels observed in the present study. Branchial $J_{Urea\text{-}N}$ increased greatly in response to nitrogen substrate loading by NH_4Cl infusion (Fig. 5C; also in the urine, Fig. 6) and moderately in response to HCO_3^- substrate loading by $NaHCO_3$ infusion (Fig. 7C), without detectable change in plasma urea-N level (Fig. 2C). Conversely, when plasma urea-N level was raised by infusion of exogenous urea (Fig. 8C), $J_{Urea\text{-}N}$ initially dropped and in fact never increased significantly (Fig. 9C). In this regard, it is relevant that the inhibitory effects of physiological concentrations of urea on both glutamine synthetase and the OUC (specifically CPS III) have been documented *in vitro* in *Squalus acanthias* (Anderson, 1981, 1986; Shankar and Anderson, 1985). Assays for the complete complement of OUC and uricolytic enzymes in gills and kidney, and for the possible urea synthetic abilities of isolated tissues or cells, are needed to cast further light on these ideas.

Low gill permeability to urea in elasmobranchs, attributed by Boylan (1967) to structural features alone, may in fact be more complex. Using a perfused gill preparation, we have confirmed that the apical membrane permeability of the gill cells is very low relative to the basolateral permeability (P. Pärt, P. A. Wright and C. M. Wood, in preparation). However, in addition, we find *in vitro* evidence for an inwardly directed 'back-transport' mechanism in the gills which appears to limit urea excretion (P. Pärt, P. A. Wright and C. M. Wood, in preparation). Indeed, in his artificially irrigated and air-exposed live dogfish preparation, Boylan (1967) reported a non-linear increase of branchial $J_{Urea\text{-}N}$ in response to very large (two- to threefold increases) elevations of blood urea.

This could be interpreted as saturation of a 'back-transporter', though Boylan argued for a physical change in membrane structure. However, he did note that a large elevation of $J_{Urea\text{-}N}$ observed at high temperature could be explained by the failure of a 'back-transport' mechanism.

The present observations of moderately elevated $J_{Urea\text{-}N}$ at the gills in response to infusion of the urea analogues thiourea and acetamide (Fig. 9) at internal levels no more than 15 % of blood urea provides evidence that a 'back-transport' mechanism may be operative *in vivo*. By this scheme, thiourea and acetamide would compete with urea for the inwardly directed transporter (probably on the basolateral membrane of the epithelium), thereby allowing more urea to leak out by diffusion to the water. In other systems, these compounds act as simple low-potency competitive blockers of urea transport with inhibitor constants close to the affinity constant of the carrier(s) for urea itself (Marsh and Knepper, 1992). In the dogfish, the observed two- to threefold elevation of urinary urea excretion demonstrates their effectiveness, though the efficiency of renal reabsorption remained high. The present results, suggesting a 'back-transport' mechanism for urea-N retention in the elasmobranch, may be contrasted with the tidepool sculpin (*Oligocottus maculosus*), a teleost which does not retain substantial levels of urea-N in the body fluids (Wright *et al.* 1995). $J_{Amm\text{-}N}$ was 10-to 20-fold greater than in *Squalus acanthias*, acetamide, thiourea and phloretin had no effect on the much lower rate of $J_{Urea\text{-}N}$, and unidirectional flux measurements indicated that simple diffusion alone explained urea-N movements across the gills.

Responses of acid–base status and nitrogen metabolism to NH_4Cl and $NaHCO_3$ infusion

The observed alkalosis (Fig. 3) was the expected response to $NaHCO_3$ infusion. The most surprising feature was the exceptional tolerance of *Squalus acanthias*. For example, *Oncorhynchus mykiss* exhibited a comparable blood alkalosis when infused with $NaHCO_3$ (Goss and Wood, 1990) at only 26 % of the rate employed in the present study (1584 μmol kg^{-1} h^{-1}). The explanation lies not in blood and tissue buffer capacities, which are comparable in the two species, but rather in the extremely high capacity of the elasmobranch gill to transfer acid–base equivalents (in this case base) to the environment, as reviewed by Heisler (1988). Indeed, the base transfer rates recorded here (approximately 1100 μmol kg^{-1} h^{-1}) are amongst the highest ever reported for fish gills. A small additional factor may be the capacity of elasmobranchs to remove HCO_3^- metabolically through the OUC. The measured basic equivalent excretion across the gills (Fig. 7A) eliminated 84 % of the infused HCO_3^- load over the 18 h period of the experiment, and elevated ureagenesis removed an additional 11 % (Fig. 7C).

The gill acid–base regulatory mechanisms appear to cope almost as well with acidosis as with alkalosis. During NH_4Cl infusion (Fig. 3), the rate of excretion of acidic equivalents reached almost 1000 μmol kg^{-1} h^{-1} (Fig. 5A). The cumulative excretion of acidic equivalents (44 % of the load) over the 18 h experimental period was not as large as for basic equivalents during $NaHCO_3$ loading (84 %), but this probably reflects the fact that NH_4Cl acidosis was indirect, as explained below. The full details of gill acid–base transport in elasmobranchs remain to be worked out, but modulation of Na^+/'acid' and Cl^-/'base' exchangers appears to be involved and branchial carbonic anhydrase plays a critical role (Payan and Maetz, 1973; Evans, 1982; Swenson and Maren, 1987). The renal contribution is generally agreed to be small or negligible (e.g. Hodler *et al.* 1955; Cross *et al.* 1969; King and Goldstein, 1983), in agreement with the present results.

NH_4Cl was infused as a neutral solution, so the development of severe acidosis in the blood (Fig. 3) must have resulted from metabolic and/or transport events in the animal. In teleosts, injected ammonium salts also cause acidosis, apparently entirely due to NH_3 loss across the gills, which leaves behind 'free' metabolic acid (e.g. Cameron and Kormanik, 1982; Cameron and Heisler, 1983). In the elasmobranch, the origin appears to be more complex, reflecting a ureagenic rate linked largely to nitrogen availability, discussed below, and the limited permeability of the gills to ammonia, discussed above. Over the 18 h period of the experiment, NH_3 removal by elevated urea production (Fig. 5C) would have converted about 60 % of the infused NH_4Cl to 'free' metabolic acid (e.g. Knepper, 1988), while branchial NH_3 excretion (i.e. the amount by which cumulative $J_{Amm\text{-}N}$ exceeded cumulative J_H) would have converted only 28 % to 'free' acid. Loss of NH_3 to the tissues may also have contributed to the acidosis.

In view of the very large body pool of urea, one must be cautious in interpreting changes in excretion rates as being equivalent to changes in production rates. Earlier, we suggested that at least a portion of the elevated $J_{Urea\text{-}N}$ observed in response to NH_4Cl and $NaHCO_3$ loading may have been made at extrahepatic sites (gills, kidney), where excretion is most likely to reflect production. Regardless of the tissues involved, if we do interpret elevated urea excretion as representing elevated production, then ureagenesis in *Squalus acanthias* is extremely sensitive to substrate loading. Even though ammonia is not the direct substrate of either the CPS-III-based OUC or uricolysis, it was clearly a more potent stimulant (Fig. 5C) than bicarbonate (Fig. 7C) when the two were infused in approximately equimolar loads. The explanation probably lies in the very high affinity of elasmobranch glutamine synthetase for NH_3 (K_m= 15 μmol l^{-1}), together with its unusual intramitochondrial location in association with CPS III (Shankar and Anderson, 1985). Glutamine synthetase thereby serves as a high-affinity trap for ammonia, chanelling it directly into the OUC (and/or uricolysis) as mitochondrial glutamine. In contrast, the modest effect of bicarbonate loading on ureagenesis probably reflects a much higher K_m (lower affinity) of CPS III for HCO_3^-. We are aware of no determinations of this value in elasmobranchs, but it is in the millimolar range in mammalian CPS I systems (Meijer *et al.* 1990) and in two teleosts which express a CPS-III-based OUC (Walsh *et al.* 1989; Wood *et al.* 1994).

Atkinson and colleagues (e.g. Atkinson and Camien, 1982; Atkinson and Bourke, 1984) have argued that urea synthesis in higher vertebrates is geared primarily to the needs of acid–base regulation (i.e. metabolic removal of HCO_3^-) rather than for nitrogen excretion and have recently proposed that the same ideas may be extended to ureagenic fish (Atkinson, 1992). These arguments have met strong theoretical and experimental challenges in mammalian physiology (e.g. Halperin *et al.* 1986; Knepper *et al.* 1987; Marsh and Knepper, 1992). Recently, these arguments have been critically examined in two unusual ureagenic teleosts which exhibit a CPS-III-based OUC, the gulf toadfish (*Opsanus beta*; Walsh *et al.* 1989; Barber and Walsh, 1993) and the Lake Magadi tilapia (*Oreochromis alcalicus grahami*; Wood *et al.* 1989, 1994). The conclusion of both studies was that although urea production was responsive to bicarbonate supply and therefore affected by acid–base status, it was much more sensitive to nitrogen supply. The present results on *Squalus acanthias* are entirely consistent with this conclusion. Thus, $NaHCO_3$ loading (Fig. 7C), which created alkalosis (Fig. 3), moderately stimulated urea production, in accord with Atkinson's views. However, urea production was stimulated to a much greater extent by NH_4Cl loading (Fig. 5C), despite the development of profound acidosis (Fig. 3). The latter should inhibit urea synthesis according to the Atkinson viewpoint. Clearly, there is a need for further research in this area; experiments in which acid–base status is manipulated by changing environmental P_{CO_2} without appreciable alteration to nitrogen load or HCO_3^- load would be especially instructive.

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