J. exp. Biol. **165**, 97–110 (1992)
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RECOVERY FROM ACUTE HAEMOLYMPH ACIDOSIS IN UNFED LOCUSTS

II. ROLE OF AMMONIUM AND TITRATABLE ACID EXCRETION

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Accepted 28 November 1991

Summary

In this study we characterized acid, ammonium and total urate excretion in the faecal pellets of unfed locusts (*Schistocerca gregaria*) and examined the effect of haemolymph acidosis (HCl injections into the haemocoel) on net acid and nitrogen excretion. In unfed, uninjected locusts, the pH of the urinary pellets was less than 5, and ammonium was excreted at three times the rate of total urate. Ammonium was present primarily as a precipitate, indicating that ammonium excretion is compatible with water conservation in this desert locust. Ammonium excretion was increased by HCl injections, theoretically accounting for 15 % of the acid equivalents removed from the haemolymph during recovery from acute acid loads. Luminal pH in the hindgut was affected by feeding state but not by haemolymph acidosis. HCl injections did not affect faecal pellet pH or the excretion rates of bicarbonate, titratable acid, pellet buffer compounds (urate, inorganic phosphate), Na^+, K^+, Ca^{2+}, Mg^{2+} or Cl^-. In unfed locusts, the low rate of excretion and low pH of faecal pellets may limit the capacity to increase titratable acid excretion after acid-loading.

Introduction

In terrestrial insects, regulation of the levels of haemolymph ions is primarily controlled by the renal system (Malpighian tubule–hindgut complex, reviewed by Phillips, 1981). Transport of non-volatile acid–base equivalents by the renal system has been proposed to be a fundamental component of haemolymph H^+ and HCO_3^- regulation in insects (Phillips *et al.* 1986). While several studies have documented the capacity for components of the locust renal system to transport acid–base equivalents (Thomson *et al.* 1988*a*; Irvine *et al.* 1988; Lechleitner *et al.* 1989; Stagg *et al.* 1991), no studies of any insect have quantified acid excretion by

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Key words: acid–base regulation, ammonium, nitrogen excretion, acid excretion, *Schistocerca gregaria*.

the complete renal apparatus. In this study we provide the first quantitative measure of faecal acid excretion for unfed locusts (*Schistocerca gregaria*) and examine the effect of haemolymph acidosis (HCl injection into the haemocoel) on acid excretion in the faecal pellets. In a companion paper, we have described the effect of the HCl injections on haemolymph acid–base status, haemolymph buffer compounds and transfer of acid equivalents to the alimentary lumen (Harrison *et al.* 1992).

It has been known for some time that locusts excrete acidic faecal pellets, and that the hindgut can acidify luminal contents (Phillips, 1961). More recently, it has been shown that this acidification is due to active, apparent proton secretion by the locust rectum (Thomson *et al.* 1988*a*,*b*; Thomson 1990). The locust ileum can also secrete acid and reabsorb bicarbonate (Irvine *et al.* 1988; Lechleitner *et al.* 1989; Thomson *et al.* 1991). While apparent proton secretion by the rectum is not affected by changes in serosal pH or P_{CO_2} *in vitro* (Thomson, 1990; Thomson *et al.* 1988*a*), titratable acid secretion by the rectum and ileum *in vitro* is modified by cyclic AMP and/or factors isolated from locust corpora cardiaca (Audsley, 1990; Thomson *et al.* 1991). Thus, the potential exists for the hindgut to regulate haemolymph pH *via* hormonal modulation of titratable acid secretion.

Thomson *et al.* (1988*a*) reported that the pH of rectal contents decreases after injection of acid into the haemocoel of *S. gregaria*. However, in this previous study, locusts were fed up to the point of acid injection and then were starved (Speight, 1967). Thus, it is not clear whether the decrease in the pH of the rectal lumen represents a response to haemolymph acidosis or to feeding state. To differentiate these alternatives, we (1) examined the effect of feeding state on the luminal pH in the hindgut, (2) tested the effect of acid injection on acid excretion in locusts previously unfed for 12 h, and (3) compared acid excretion in HCl- and NaCl-injected locusts. The NaCl injections did not affect haemolymph acid–base status (Harrison *et al.* 1992).

Acid excretion is intimately related to the excretion of nitrogenous wastes. In locusts, urate or uric acid has been found to be the primary nitrogenous waste (Brown, 1937; Chauvin, 1941). At the pH of locust Malpighian tubule fluid (>6.8, Stagg *et al.* 1991), the majority of the total urate (Ur_{tot}, urate+uric acid) secreted by the tubules will be in the form of urate. Acid secretion by the hindgut may then elevate the ratio of uric acid to urate, increasing net acid secretion.

Ammonium, derived from *in situ* oxidation of amino acids, is actively secreted into the hindgut lumen of locusts (Thomson *et al.* 1988*b*; Lechleitner, 1988). Ammonium excretion is functionally equivalent to acid excretion if (1) HCO_3^- is produced stoichiometrically with ammonium in the renal tissue and (2) HCO_3^- is transferred to the haemolymph while ammonium is secreted to the lumen. For this to occur (1) the amino acids oxidized by the renal tissue must be primarily neutral, (2) the glutamate generated during amino acid catabolism must be oxidized, and (3) NH_4^+ secretion to the lumen must not be accompanied by HCO_3^- (or OH^-) excretion (Walser, 1986). The amino acids that support short-circuit current and NH_4^+ production in the rectum and ileum are primarily neutral amino acids

(proline, alanine, glutamine, asparagine, serine; Chamberlin and Phillips, 1982; Thomson *et al.* 1988*b*; Peach and Phillips, 1991). It is not known whether amino acid oxidation is generally complete in the locust hindgut; however, the enzymes necessary for complete amino acid oxidation *via* glutamate dehydrogenase are present at high activities in rectal tissue (Chamberlin and Phillips, 1983). *In vitro*, rectal NH_4^+ transport occurs primarily in exchange for Na^+, in parallel with acid secretion. Therefore, the data collected suggest that NH_4^+ excretion in locusts is equivalent to acid excretion from the haemolymph.

Quantification of acid excretion in insect faecal pellets requires estimation of the quantity of protons bound to buffers, including precipitated buffers such as uric acid. It cannot be assumed that all excreted Ur_{tot} is in the acid form, because urate salts such as ammonium or potassium urate also have low solubilities (similar to that of uric acid) and may be present in urinary precipitates (Porter, 1963; Minnich, 1972; Shoemaker and McClanahan, 1975; Dantzler, 1978). When acid excretion is quantified in uricotelic animals, it is sometimes possible to assume that $HPO_4^{2-}/H_2PO_4^-$ and urate/uric acid are the only quantitatively important buffer systems in the excretory pellets (Long and Skadhauge, 1980). However, information on the buffer compounds present in insect faecal pellets is not currently available. Therefore, we have developed a direct measure of the titratable acidity of the faecal pellets of locusts, which included measurement of protons bound to precipitated buffers such as uric acid. We also tested the hypothesis that urate and phosphate are the dominant buffers in locust faecal pellets and examined the effect of acid-loading on inorganic cation and chloride excretion to characterize further the ionic composition of locust excretory pellets.

Materials and methods

General techniques

Adult, female locusts (*Schistocerca gregaria* Forskål) were maintained, selected and fed synchronously as previously described (Harrison *et al.* 1992). Locusts were placed in individual cartons on a wire mesh platform so that faecal pellets dropped to a collection sheet. Data for unfed, uninjected locusts refer to pellets collected 12–24 h after cessation of feeding. Locusts were then injected with 10 μmol of HCl or NaCl as described previously (Harrison *et al.* 1992). As recovery of haemolymph acid–base status from acid-loading occurred within 24 h (Harrison *et al.* 1992), pellets were recovered for 24 h after injections of HCl or NaCl.

All pellets were collected within 5 min of voiding. Pellets were weighed and then divided into subsamples for analyses. Some portions were weighed and dried to constant mass at 40 °C for measurement of water content. We measured the effects of acid-loading on faecal pellet production rate, water content, pH, total carbon dioxide content (dissolved $CO_2+HCO_3^-$, C_{CO_2}), titratable acidity and inorganic phosphate content by comparing these variables among HCl- and NaCl-injected animals over the same time course. Because of high inter-individual variability, we tested the effects of HCl and NaCl injections on (1) total ammonia (Amm_{tot},

$NH_4^+ + NH_3$), (2) total urate (Ur_{tot}, uric acid+urate) and (3) inorganic ion (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-) excretion by comparing excretion rates during the 12 h before injections with the rates 24 h after injections for individual locusts. Excretion rates (J, μmol h^{-1}) were calculated from pellet wet masses and concentrations.

Owing to limitations on sample sizes, all variables could not be measured for individual animals. Experiments were performed in series: for each series, half the locusts were injected with HCl and half with NaCl, and one or more variables were measured. Pellet pH, C_{CO_2} and water content were measured for each animal during the first series. Inorganic phosphate was measured during the second series. Titratable acidity, Amm_{tot}, Ur_{tot}, inorganic cations and chloride were each measured for individual animals from two additional series. The sample sizes reported throughout represent the number of animals used; when individual locusts excreted multiple pellets, values were averaged.

Acid–base techniques

The pH of faecal pellets was measured with a glass microelectrode (Harrison *et al.* 1990) after addition of less than 1 μl of distilled water to a 1–2 mg portion of the pellet. The C_{CO_2} of pellets was measured by gas chromatography (Harrison *et al.* 1990). The pH and C_{CO_2} of pellets were measured from 10 s to 3 min from pellet collection. While the possibility exists that some CO_2 loss may have occurred in pellets exposed to air for the longest periods, pellet C_{CO_2} values were very low (<2 mmol l^{-1}) even when pellets were analyzed and collected immediately (under 10 s) after being voided. Because the HCO_3^- content of the pellets was so low, errors of 100 % in measurement of pellet C_{CO_2} would represent errors in total acid excretion of less than 0.1 %. The pH of the luminal contents of the hindgut was measured as previously described (Harrison *et al.* 1992).

Titratable acidity of the solubilized faecal pellet (TA_{sp}, mequiv kg^{-1}) was calculated as the equivalents of base necessary to titrate 1 kg of bicarbonate-free, diluted pellet to pH 7.0 (the approximate pH of Malpighian tubule fluid, Stagg *et al.* 1991) at 21 °C. Pellet samples assayed for TA_{sp} were frozen on dry ice within 5 min of being voided by the animal. Later, the pellet was diluted and vortexed in 1500 times its volume of 100 mmol l^{-1} KCl to solubilize precipitated buffers in a solution of a similar ionic composition to Malpighian tubule fluid. Total urate concentrations after dilution were below 0.1 mmol l^{-1}, ensuring that all the urate was in solution (Porter, 1963). The samples were centrifuged (5 min at 1500 g) to remove insoluble plant material and cuticle. Preliminary trials indicated that removal of the insoluble material had no effect on the measured buffer value. After centrifugation, 1 ml of the supernatant was acidified to a pH below 4 with 10 mmol l^{-1} HCl and stirred for 2 h to liberate all bicarbonate. The solution was then titrated to pH 7.0 with 1 mmol l^{-1} KOH using a Radiometer PHM84 research pH meter, TTT80 titrator, ABU80 Autoburette and GK473901 combined pH electrode. Calculated TA_{sp} was corrected for the quantity of base necessary to titrate an equivalent volume of 100 mmol l^{-1} KCl over the same pH range.

Biochemical analyses

Pellet fragments analyzed for Amm_{tot} and Ur_{tot} were placed in 19 times their volume of 5 % trichloroacetic acid (TCA) and frozen to prevent loss of NH_3. This solution was later diluted 1500 times with distilled water and the pellet dispersed and dissolved by vortexing. We analyzed the supernatant for Amm_{tot} with an enzymatic assay modified from Kun and Kearney (1974) and for Ur_{tot} with an enzymatic Sigma kit (procedure 685). Pellets analyzed for inorganic phosphate concentration were diluted 19 times with 25 % TCA and frozen immediately after collection. We measured inorganic phosphate concentrations, $[P_i]$, as previously described (Harrison *et al.* 1990).

Pellets were too dry to allow direct measurement of Amm_{tot} and Ur_{tot} in a supernatant. Therefore, to estimate the fraction of Ur_{tot} and Amm_{tot} present as a precipitate, we measured the Amm_{tot} and Ur_{tot} in the supernatant fractions of pellets diluted and vortexed with water to total dilutions of 10, 20, 1000 or 1500 times.

Inorganic ion analyses

Na^+, K^+, Mg^{2+} and Ca^{2+} concentrations were determined by atomic absorption spectrophotometry (Techtron AA model 120). Samples were diluted in an appropriate ionization buffer (Na^+, distilled water; K^+, 21.7 mmol l^{-1} NaCl; Mg^{2+}, 1.5 % EDTA; Ca^{2+}, 0.5 % $LaCl_3$). Chloride concentration was determined by electrometric titration (Ramsay *et al.* 1955). Preliminary experiments indicated that ashing had no effect on the release of any of the ions from the pellets, so samples were analyzed without ashing.

Calculations and statistics

Because the pK of NH_3 is over 9 and the pH of urinary pellets was below 5, $[NH_4^+]$ in pellets was assumed to be equal to Amm_{tot}. We calculated $[HCO_3^-]$ from C_{CO_2}, by assuming that the P_{CO_2} in the rectal lumen and haemolymph were equal and by using the Henry's law constant for carbon dioxide measured by Harned and Davis (1943) for a NaCl solution with a similar temperature and ionic concentration to the urinary pellets (1 mol l^{-1} NaCl, 21 °C, 0.2251 mmol kg^{-1} kPa^{-1}).

Data satisfied assumptions of normality (Kolmogorov's test) and heteroscedasticity (F_{max} test), so differences between treatments were tested for significance ($P<0.05$) with *t*-tests or paired *t*-tests.

Results

Average concentrations of inorganic ions, Amm_{tot} and Ur_{tot} in urinary pellets

In the urinary pellets of unfed locusts, we found the cations NH_4^+, Ca^{2+}, and K^+ in highest concentration, followed by Na^+ and Mg^{2+} (Table 1). Amm_{tot} values were three times higher on a molar basis than Ur_{tot} values (Table 1). Only 15 % of

Table 1. *The average concentrations of measured inorganic ions, total ammonia and total urate in the urinary pellets of locusts unfed for 18–36 h*

	Cations				Anions and potential anions		
	Mean	S.E.	*N*		Mean	S.E.	*N*
NH_4^+	263	27.3	64	Total urate	85	16.0	24
K^+	258	51.3	26	HPO_4^{2-}	30	3.0	24
Ca^{2+}	220	32.0	28	Cl^-	17	5.1	21
Na^+	128	25.6	26	HCO_3^-	1	0.5	25
Mg^{2+}	10	1.3	26				

Pre-injection values are pooled with values for HCl- and NaCl-injected animals (all values are given in mmol kg^{-1} wet mass; pellets averaged 25 % water).

the negative charges necessary to balance the measured cations were accounted for by urate, HPO_4^{2-} and Cl^-, even assuming that total urate is all in the form of urate (Table 1).

Low fractions (≤20 %) of Amm_{tot} and Ur_{tot} were present in the supernatant fractions of pellets diluted 10 times or 20 times with distilled water, suggesting that the majority of both nitrogenous wastes are present as precipitates in the locust faecal pellets. Pellets diluted 1000 times or 1500 times yielded similar values for Amm_{tot} and Ur_{tot}, indicating that these dilutions completely solubilize these nitrogenous wastes.

Effect of HCl and NaCl injections on acid, nitrogen and inorganic ion excretion

Only 53 % of all locusts excreted a faecal pellet during the 24 h after injections, and the proportion of locusts excreting pellets did not differ between HCl- and NaCl-injected animals (HCl-injected, 26 of 56, NaCl-injected, 23 of 36; χ^2-test of independence, χ^2=2.68). For those locusts excreting pellets, there were no differences between HCl- and NaCl-injected animals in the number of pellets excreted, pellet mass, water content, pH, C_{CO_2}, TA_{sp}, titratable acid excretion (J_{ta}) or [P_i] (Table 2). Locusts unfed for 12–24 h had a J_{ta} of 0.28 μequiv h^{-1} (S.E.=0.038, N=8), not significantly different from that of HCl- or NaCl-injected animals (t-tests; uninjected *vs* HCl-injected, t=0.54; uninjected *vs* NaCl-injected, t=0.66).

Acid injection increased pellet Amm_{tot} and J_{amm}, while NaCl injection had no effect on total ammonia excretion (Fig. 1; paired t-tests; HCl effects: on Amm_{tot}, t=2.45; on J_{amm}, t=2.38; NaCl effects: on Amm_{tot}, t=0.75; on J_{amm}, t=1.30). Neither HCl nor NaCl injection affected Ur_{tot} or J_{ur} (Fig. 2; paired t-tests, HCl effects: on Ur_{tot}, t=0.80, on J_{ur}, t=1.14; NaCl effects: on Ur_{tot}, t=0.57; on J_{ur}, t=1.66). Neither HCl nor NaCl had a measurable effect on the excretion of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^- or P_i (Table 3).

Table 2. *Some components of acid excretion that did not differ between HCl- and NaCl-injected animals*

	HCl-injected			NaCl-injected			
	Mean	S.E.	*N*	Mean	S.E.	*N*	*t*-value
Number of pellets per 24 h	3.0	0.26	26	3.2	0.40	23	0.45
Pellet mass (mg)	9.6	1.71	26	8.5	1.27	23	0.52
Water content (%)	26	6.6	15	25	5.3	13	0.44
pH	4.65	0.083	9	4.66	0.067	16	0.26
C_{CO_2} (mmol kg^{-1})	2.5	0.92	9	0.9	0.34	16	0.89
TA_{sp} (mequiv kg^{-1})	226	26.1	18	268	28.5	15	0.83
J_{ta} (μequiv h^{-1})	0.26	0.025	18	0.30	0.028	15	0.61
$[P_i]$ (mmol kg^{-1})	30	4.6	10	30	6.3	11	0.11

C_{CO_2}, total CO_2 content; TA_{sp}, titratable acidity of faecal pellet; J_{ta}, rate of excretion of titratable acid; $[P_i]$, concentration of inorganic phosphate.
t-tests, $P>0.05$.

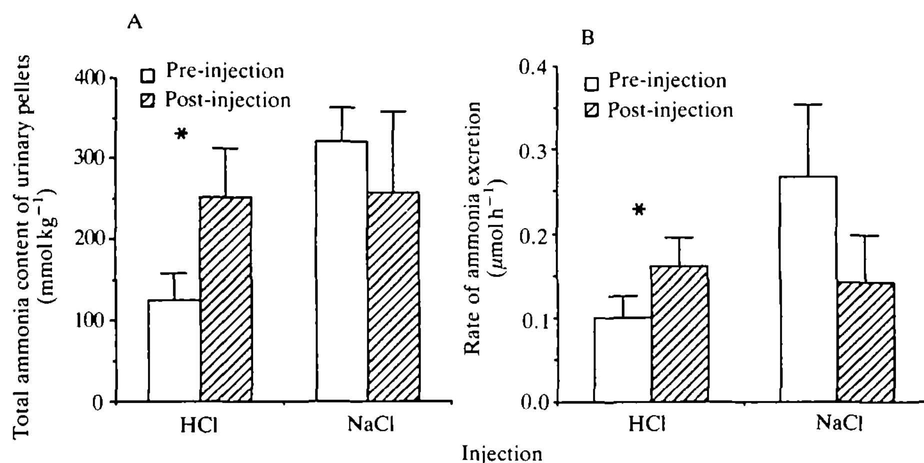

Fig. 1. The effect of HCl and NaCl injections into the haemocoel on the concentration of total ammonia in the urinary pellets (A, Amm_{tot}) and total ammonia excretion rate (B, J_{amm}) in unfed *Schistocerca gregaria*. HCl, but not NaCl injections increased Amm_{tot} and J_{amm} (asterisks indicate significant difference between pre- and post-injection values by paired *t*-tests, mean and standard error, $N=17$).

Contribution of Ur_{tot} and P_i to faecal buffering

The buffer value of solubilized faecal pellets averaged 115 mequiv kg^{-1} pH unit^{-1} between pH 5 and 7 (the approximate pH difference between secreted Malpighian tubule fluid and rectal contents). The contribution of Ur_{tot} and P_i to buffering across this pH range (B) was calculated as:

$$B = (h_7 - h_5)C,$$

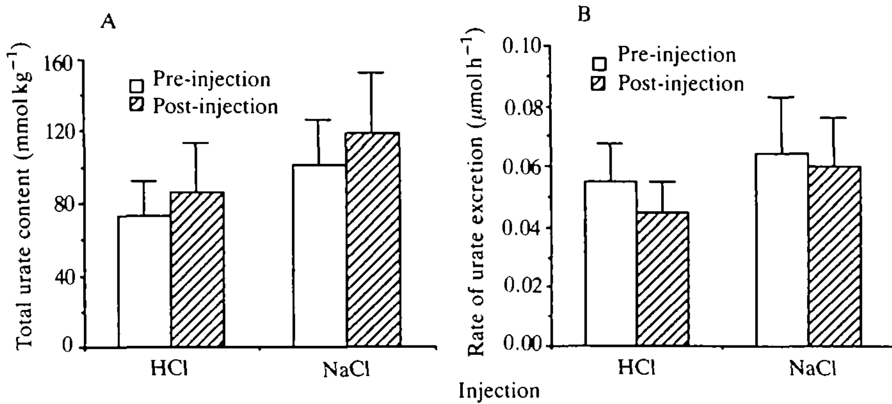

Fig. 2. The effect of HCl and NaCl injections into the haemolymph on the concentration of total urate (A, Ur_{tot}) and total urate excretion rate (B, J_{ur}) in unfed *Schistocerca gregaria*. Urate excretion did not differ between the two treatments (paired *t*-tests, mean and standard error, N=17).

Table 3. *The effect of HCl or NaCl injection on the concentration and rate of excretion of some inorganic ions in the faecal pellets of* Schistocerca gregaria

	HCl-injected				NaCl-injected			
	Pre-injection		Post-injection		Pre-injection		Post-injection	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Pellet mass (mg)	11.5	1.67	8.4	1.82	7.3	1.81	4.6	0.80
$[Na^+]$ (mmol kg^{-1})	243	34.7	399	100.7	436	173.8	332	87.0
J_{Na} (μmol h^{-1})	0.21	0.037	0.19	0.037	0.24	0.083	0.16	0.060
$[K^+]$ (mmol kg^{-1})	433	54.4	399	55.4	279	50.2	223	41.1
J_K (μmol h^{-1})	0.35	0.050	0.26	0.065	0.22	0.070	0.092	0.022
$[Mg^{2+}]$ (mmol kg^{-1})	8	1.5	11	1.6	15	3.5	15	2.9
J_{Mg} (μmol h^{-1})	0.007	0.0011	0.006	0.0013	0.007	0.0018	0.006	0.0020
$[Ca^{2+}]$ (mmol kg^{-1})	226	35.8	315	53.5	219	68.6	197	45.6
J_{Ca} (μmol h^{-1})	0.18	0.033	0.15	0.021	0.11	0.021	0.11	0.035
$[Cl^-]$ (mmol kg^{-1})	15	6.6	16	7.2	16	7.8	17	7.3
J_{Cl} (μmol h^{-1})	0.013	0.0040	0.009	0.0052	0.007	0.0038	0.007	0.0036

N=12 for HCl-injected animals, N=9 for NaCl-injected animals.

There were no significant effects of either treatment on any of the variables listed (paired *t*-tests, P>0.05).

where h_7 and h_5 are the fractional dissociation of the compound at pH 7 and 5, respectively, and C is the average concentration in the faecal pellet. Fractional dissociation (h) was calculated as:

$$h = 1/(1 + 10^{pK - pH})\ \text{(Albers, 1970)}.$$

Table 4. *Luminal pH of the hindgut of fed* Schistocerca gregaria *and those starved for 24 h*

	Unfed		Fed		
	Mean	S.E.	Mean	S.E.	*t*
Rectum	5.11	0.204	5.70	0.091	2.33
Ileum	5.48	0.305	6.24	0.182	2.20
Malpighian tubules	7.03	0.024	6.89	0.094	1.10

Fed locusts were kept without food for 12 h and then fed lettuce *ad libitum* for 2 h.

The value listed for Malpighian tubules is the pH at the point of Malpighian tubule entry to the alimentary lumen.

Luminal pH was significantly lower in the ileum and rectum of unfed animals (*t*-test, $P<0.05$, $N=7-8$).

Assuming a pK for uric acid of 5.75 and for phosphate of 6.8 (Hitchings, 1978; Robinson and Stokes, 1959), a buffer value of 230 mequiv kg^{-1} from pH 5 to 7, $Ur_{tot}=85$ mmol kg^{-1} and $P_i=30$ mmol kg^{-1} (Table 1), Ur_{tot} accounts for 29 % and inorganic phosphate for 8 % of the buffer value of solubilized faecal pellets.

Effect of the feeding state on luminal pH in the hindgut

There was no effect of feeding state on the pH at the point of Malpighian tubule entry to the alimentary canal (Table 4). However, in the rectum and ileum, luminal pH was significantly lower for locusts unfed for 24 h than in recently fed animals (Table 4).

Discussion

Ammonium is excreted at high rates in the faecal pellets of unfed *S. gregaria*. Since the majority of the ammonium is present as a precipitate, and pellets contain only 25 % water, ammonium excretion is compatible with water conservation in this desert locust. Acute haemolymph acidification increased ammonium excretion but not titratable acid excretion in the faecal pellets.

Amm_{tot} *and* Ur_{tot} *excretion*

In unfed *S. gregaria*, ammonium was excreted at over three times the molar rate of total urate. The high rate of ammonium excretion seems surprising in the light of the general perception that only aquatic insects excrete substantial amounts (Chapman, 1982). Total ammonia has been shown to be the predominant nitrogenous waste in blowfly larvae (Brown, 1936; Prusch, 1972) and cockroaches (Mullins and Cochran, 1972), but terrestrial insects are still generally held to secrete nitrogen primarily as uric acid or as metabolites of uric acid such as allantoin (Cochran, 1985). However, Chauvin (1941) reported that mature *S. gregaria* fed on lettuce excreted ammonium at twice the molar rate of uric acid.

Table 5. *Rates of excretion of titratable acid (J_{ta}) and total ammonia (J_{amm}) in* Schistocerca gregaria in vivo *compared with reported values for components of the renal system* in vitro *(recta and ilea) and* in vivo *(Malpighian tubules)*

	J_{ta} (μequiv h^{-1})	J_{amm} (μmol h^{-1})
Unfed *in vivo* (this study)	0.26	0.15
Rectum*	1.20	0.39
Ileum	0.60†	0.60‡
Malpighian tubules§	0.09	0.05

* Thomson *et al.* (1988*b*), pH 7.0 bilaterally.
† Thomson *et al.* (1991), pH 7.0 bilaterally.
‡ Lechleitner (1988).
§ Stagg *et al.* (1991).

Ammonium was reported to be a much smaller component of nitrogen excretion in *Melanoplus bivittatus* grasshoppers (Brown, 1937); however, in Brown's study, pellets were oven-dried before analysis, which can lead to loss of NH_3 (Mullins and Cochran, 1972). It is also not clear whether in Brown's studies the pellets were diluted sufficiently to release all precipitated ammonium. These two technical difficulties appear to have plagued many of the early studies of insect nitrogen excretion, suggesting that the importance of total ammonia as a nitrogenous waste in terrestrial insects may have been generally underestimated. The forms of nitrogenous wastes excreted can also vary with feeding or developmental state in insects (Chauvin, 1941; Razet, 1961); it is possible that the high rate of excretion of total ammonia found in our study was related to the necessity for acid excretion in fasted locusts (see below).

The rates of secretion of total ammonia that have been documented for locust ilea and recta *in vitro* are more than sufficient to account for the high concentrations of NH_4^+ in the urinary pellets (Thomson *et al.* 1988*b*; Lechleitner, 1988). Average J_{amm} under these *in vivo* conditions was only about 14 % of the J_{amm} estimated for the complete locust renal system *in vitro* (1.09 μmol h^{-1}; Table 5). J_{amm} may be reduced under these unfed *in vivo* conditions relative to *in vitro* due to (1) depletion of luminal amino acids necessary for J_{amm} (Thomson *et al.* 1988*b*), (2) depletion of luminal ions, particularly chloride, necessary to support the primary ATP utilisation pathways in the hindgut (Phillips *et al.* 1986; Irvine *et al.* 1988), or (3) depression of epithelial metabolism under unfed conditions.

The finding that ammonium can be excreted as a precipitate in a low-water-content urinary pellet contrasts with the general perception that conversion of ammonium to uric acid facilitates water conservation (Campbell, 1991). However, excretion of ammonium as a precipitate has been reported for a variety of vertebrates, particularly in the presence of urate (Porter, 1963; Minnich, 1972; Shoemaker and McClanahan, 1975). We are unaware of previous studies which document precipitated ammonium in excess of the concentration of urate.

$Ammonium_2$ urate is unlikely to occur since the pK for the dibasic urate salt is 10.3 (Hitchings, 1978) and dibasic urate–ammonium salts have not been observed to occur at pH 7.0 *in vitro* (McNabb and McNabb, 1980). Given the large number of unidentified anions present in the pellets (Table 1), it seems likely that a large portion of the ammonium was precipitated with organic anions.

Is NH_4^+ excretion functionally equivalent to acid excretion from haemolymph?

From these studies, it cannot be definitively determined whether ammonium excretion is functionally equivalent to acid excretion from haemolymph. The acid–base effects of amino acid catabolism depend on the amino acid and the extent of oxidation (Atkinson and Camien, 1982; Walser, 1986). For example, glutamine deamination to glutamate by glutaminase, with subsequent NH_4Cl secretion into the urine, does not affect organismal acid–base status (Atkinson and Camien, 1982; Walser, 1986). However, a number of results from this and other studies suggest that ammonium excretion may be equivalent to acid excretion under these *in vivo* conditions. (1) The hindgut of *S. gregaria* catabolizes primarily neutral amino acids in the support of short-circuit current and ammonium production *in vitro* (Chamberlin and Phillips, 1982; Thomson *et al.* 1988*b*; Peach and Phillips, 1991). If the glutamate derived from neutral amino acids is oxidized, bicarbonate will be produced in equimolar quantities to ammonium (Walser, 1986). Glutamate will support substantial rates of oxygen consumption by rectal mitochondria and glutamate dehydrogenase is present at high activities in rectal tissue, indicating that the hindgut has considerable capacity for oxidation of glutamate (Chamberlin and Phillips, 1983). (2) Bicarbonate concentrations in the faecal pellets are less than 0.5 % of ammonium concentrations under these *in vivo* conditions (Table 1). Since complete oxidation of neutral amino acids in the hindgut is suggested by the high capacity for glutamate oxidation, bicarbonate may be produced and transferred to the haemolymph at a rate roughly equivalent to the ammonium excretion rate. (3) Acute acidification of the haemolymph increases ammonium excretion, without affecting bicarbonate or titratable acid excretion (Table 2, Fig. 1). If bicarbonate and ammonium derived from amino acid oxidation were both transferred to the lumen, HCO_3^- would combine with luminal protons, producing CO_2 and reducing titratable acidity.

Titratable acid excretion

In unfed locusts, regardless of injection status, the pH of the urinary pellets was very low (4.7). Unfed locusts may excrete acid due to metabolic acid production associated with the protein catabolism known to occur under these conditions (Hill and Goldsworthy, 1970). There was no increase in J_{ta} after HCl or NaCl injections.

The J_{ta} (6.2 μequiv day^{-1}, 0.26 μequiv h^{-1}) measured in these experiments was only 14 % of the calculated J_{ta} for locust renal system summed from *in vitro* and *in vivo* experiments (Table 5). However, in the recta (Thomson *et al.* 1988*b*), J_{ta} decreases with decreasing luminal pH. At the average pH of the rectal lumen under these conditions (5.5, Harrison *et al.* 1992), J_{ta} of the rectum is reduced to

7 % of its maximum (Thomson *et al.* 1988*b*). At the pH of the faecal pellets (below 5), proton backflux is predicted to exceed J_{ta} (Thomson *et al.* 1988*b*). The *in vitro* data suggest that, in unfed locusts, the pH of the rectal lumen is near the minimum attainable and cannot be further decreased in response to acid-loading.

In theory, J_{ta} could be increased after acid-loading by an increase in the concentration of buffers in the hindgut lumen. However, pellet buffer value, TA_{sp}, Ur_{tot} and $[P_i]$ did not increase after HCl injections, indicating that an increase in the concentration of luminal buffers was not a mechanism for increasing acid excretion under these conditions. Interestingly, urate and phosphate accounted for a relatively small percentage (37 %) of faecal buffering between pH 5 and 7. Even if all total urate is in the form of uric acid at pH 5, as is suggested to occur in bird faecal pellets (Lonsdale and Sutor, 1971), unmeasured buffer compounds accounted for 57 % of the total faecal buffer value.

The mechanisms responsible for the effect of haemolymph acidosis on ammonium excretion in locusts are unclear. Excretion of total ammonia by the rectum *in vitro* and the Malpighian tubules *in vivo* is unaffected by acute changes in serosal or luminal pH or cyclic AMP concentration (Thomson *et al.* 1988*b*; Stagg *et al.* 1991).

Role of urinary acid excretion in haemolymph acid–base regulation

Locusts recovered from injections of 10 μmol of HCl into the haemocoel by transferring acid equivalents out of the haemolymph (Harrison *et al.* 1992). Approximately 75 % of the acid equivalents were transferred to the lumens of the midgut and crop. If ammonium excretion is functionally equivalent to acid excretion under these conditions, the increase in ammonium excretion accounted for a further 15 % of the injected acid load. The data suggest that in insects, as in many vertebrates (Sullivan, 1986), an increase in renal ammonium excretion may be an important mechanism of compensation for extracellular acidosis.

Previously, it was reported that the pH of *S. gregaria* rectal contents decreases during the 24 h after injection of HCl into the haemolymph when locusts are fed up to the point of acid injection and thereafter starved (Thomson *et al.* 1988*a*). It appears that this decrease in luminal pH is a response to feeding state rather than to haemolymph acidosis (Table 4). Hormonal regulation of ion transport by insect renal systems in association with feeding cycles is well-known (Phillips, 1981). Both cyclic AMP and peptide factors isolated from the locust corpora cardiaca strongly affect hindgut titratable acid secretion (Audsley, 1990; Thomson *et al.* 1991), suggesting that variation of urinary J_{ta} may be important in the maintenance of acid–base homeostasis during changes in feeding state.

While the role of urinary acid excretion in haemolymph acid–base regulation was less important than acid equivalent transfer to the alimentary lumen in this study, urinary net acid excretion may be quantitatively more important under other physiological conditions. In fed locusts, urinary pellet production is orders of magnitude greater and luminal pH values in the hindgut are more alkaline, increasing the potential for the hindgut epithelia to increase J_{ta} in response to acid-

loading. Also, as in vertebrates, larger changes in urinary net acid excretion may be observed with chronic than with acute acid loads (Sullivan, 1986).

This research was supported by NATO and Killam PDF's to J.F.H. and an NSERC operating grant to J.E.P. M. Kennedy helpfully reviewed the manuscript.

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