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ACID–BASE VARIABLES IN MALPIGHIAN TUBULE SECRETION AND RESPONSE TO ACIDOSIS

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Summary

1. Malpighian tubule fluid from *Schistocerca gregaria* adults, starved for 1 day, was collected *in situ* by cannulation of the gut, both before and after injecting 10 μmol of HCl or NaCl into the haemocoel.

2. Haemolymph pH at the neck remained depressed by 0.3 units for at least 6 h in HCl- as compared to NaCl-injected locusts. A lower haemolymph pH persisted near the acid injection site for several hours.

3. The pH of tubule fluid remained about 0.5 units more acid than haemolymph under all conditions. Thus, net tubular acid secretion was proportional to haemolymph acid–base status.

4. The greater acidity of tubular fluid after acid injection was associated with lower estimated bicarbonate concentrations and higher P_{CO_2}, without any change in total CO_2 when compared to controls.

5. The combined contribution of bicarbonate, phosphate and urate to total buffering capacity of tubular fluid was estimated to be 75%, with bicarbonate responsible for 55% of the total.

6. The maximum rate of acid removal by all Malpighian tubules of starved locusts, including H^+ trapped in ammonium ions, was calculated to be very small in relation to the acid load injected into the haemocoel.

Introduction

Regulation of acid–base balance in terrestrial insects has been a neglected area. Phillips *et al.* (1986) proposed that the process is probably similar to that in vertebrates: i.e. the respiratory (tracheal) system regulates haemolymph P_{CO_2} and the excretory system (consisting of Malpighian tubules and hindgut) regulates haemolymph bicarbonate levels. As a result, haemolymph pH is maintained within narrow limits as was recently reported for locusts during rest, temperature change and activity (Harrison, 1988, 1989; Harrison *et al.* 1991).

Indeed, during hopping and recovery at 35°C, regulation of pH back to resting

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values can largely be explained by increased tracheal ventilation (Harrison *et al.* 1991). In contrast, recovery from acidosis caused by injection of HCl into desert locusts is almost entirely due to an increase in haemolymph bicarbonate levels (J. F. Harrison, C. J. H. Wong and J. E. Phillips, in preparation). Although the source of this additional haemolymph bicarbonate has not been firmly established, the excretory system of the locust seems a likely source. A significant fraction of the acid removed from the haemocoel within a few hours of acid injection appears (temporarily at least) in the midgut contents (J. F. Harrison, C. J. H. Wong and J. E. Phillips, in preparation). This transfer may be partially mediated by the Malpighian tubules.

It is now firmly established that locust ileum and rectum secrete H^+ and NH_4^+ and absorb OH^- and HCO_3^-; moreover, these processes can be substantially modified by cyclic AMP and by corpora cardiaca and ventral ganglia factors (Thomson *et al.* 1988*a*,*b*, 1991; Lechleitner *et al.* 1989; Audsley, 1991). However, the potential contribution of Malpighian tubules to acid–base regulation has not been investigated in any terrestrial insect. Even simple measurements of the primary determinants of acid–base excretion (i.e. the concentrations of CO_2, levels of bicarbonate, phosphate, ammonia and organic acids, and total buffering capacity) have never been measured in tubule secretion. The exception is some *in vitro* values for tubular bicarbonate concentrations in corixids living in alkaline lakes, an unusual situation for most insects (Cooper *et al.* 1987). Previous relevant information for terrestrial insects is restricted to some isolated estimates of pH, urate or phosphate levels in fluid collected from Malpighian tubules isolated *in vitro* (reviewed by Phillips, 1981; also O'Donnell *et al.* 1983; Andrusiak *et al.* 1980; Hanrahan *et al.* 1984). In no insect has the response of tubular acid–base variables to changes in haemolymph pH been investigated. In this paper we describe such a study, using tubules of the desert locust cannulated *in situ* and with acidosis initiated by HCl injection into the haemocoel.

Materials and methods

Animals

Adult female *Schistocerca gregaria* (Forskål), 2–3 weeks past their final moult, were used in all experiments. Locusts were maintained as previously described (Thomson *et al.* 1988*a*).

Collection of Malpighian tubule fluid in situ

Animals were restrained under dry cotton to prevent any visual stimulus. An incision was made in the third and fourth abdominal segments, and the gut was gently lifted with glass hooks. The gut was ligated under the anterior Malpighian tubules just anterior to the point of tubule entry and at the posterior end of the ileum. The ileum was then partially severed near the tubule junction and the gut contents were gently teased out with a glass hook. A glass cannula (1.0 mm wide)

was inserted into the anterior ileum and secured with surgical thread. (The end of the cannula was thinly coated with wax to ensure a tight seal.) The gut and cannula were then gently inserted back into the abdomen and the incision sealed with wax. The cannula and gut were then rinsed with 100 mmol l^{-1} KCl to ensure no blockage was present and the rinse solution was removed with a syringe. Visual inspection indicated that very few tubules of the total (250), if any, were damaged or severed by this ligation procedure. This was confirmed by checking for concentrated dye emanating from cut tubules of locusts previously injected with amaranth, which is quickly concentrated (>100×) in the tubule lumen. Rates of tubule fluid production from the full complement of 250 tubules were calculated by measuring the fluid advancement through the glass cannula at intervals and calculating volume knowing the inside diameter of the tubing.

Experimental protocols

We first determined in preliminary experiments that locusts survived indefinitely following injection of 25 μl of 0.4 mol l^{-1} HCl into their haemocoel and showed no obvious change in their normal activities during this insult. This temporarily lowers haemolymph pH to the lower end of the spectrum of *in situ* values reported during various challenges (e.g. exercise).

Locusts were placed in individual containers and fed lettuce *ad libitum* for 2 h at 35 °C the day before cannulation. Animals were then starved for 18–24 h at 20 °C (the experimental temperature) with access to cotton soaked with distilled water, and then cannulated as above. It is worth noting that locusts commonly experience even larger daily temperature shifts overnight in their natural desert habitat.

The influence of haemolymph acidosis on the composition of Malpighian tubule fluid was assessed as follows. When 20 μl of tubular fluid had collected in the cannula (usually after 2–3 h), this fluid was drawn into a length of fine PE 20 tubing by a Hamilton syringe. This constituted the pre-injection sample. Acidification was initiated by injecting 25 μl of 0.4 mol l^{-1} HCl into the abdominal haemocoel through the seventh or eighth intersegmental membrane. A further 20 μl of tubular secretion was collected after another 2–3 h. This constituted the post-injection sample on the same locust. A second 20 μl post-injection sample was often collected, but no significant differences in solute composition were observed compared to the first post-injection sample. Only data for the first period are, therefore, included in this report. Since our method precluded reabsorption of tubule fluid in the midgut and hindgut, the resulting reduction in haemolymph volume must be considered. In most later experiments, 20 μl was collected before, and another 20 μl of tubule fluid collected after, injection of acid or NaCl into the haemocoel. This represents roughly 10 % of haemolymph volume. No more than 20 % of haemolymph volume was estimated to be secreted in any of our long experiments lasting up to 8 h. Preparations continued to secrete at low rates for 1–2 days.

As a control for possible diuresis resulting from fluid injection, other locusts

were treated in the same way, but they were injected with 25 μl of 0.4 mol l^{-1} NaCl. Finally, a third group was not injected but was otherwise treated identically to assess the changes in composition of tubular secretion and haemolymph with time due to hindgut cannulation.

Acid–base measurements

Tubular fluid pH was measured with glass microelectrodes on 1–2 μl samples as previously described (J. F. Harrison, C. J. H. Wong and J. E. Phillips, in preparation). Total CO_2 was measured on 4 μl samples using the technique of Boutilier *et al.* (1985). Bicarbonate concentration and P_{CO_2} were calculated from total CO_2 and pH assuming that carbonic acid pK values and CO_2 solubility coefficients were identical to those for locust haemolymph (Harrison, 1988).

The method of Chamberlin and Phillips (1982) was used to verify that collection of fluid from a cannula did not result in CO_2 loss. As before, an incision was made in the third and fourth abdominal segments. Instead of placing a cannula in the gut, ligatures were made just anterior and posterior to the point of tubule entry. Fluid was allowed to collect in the distended sac, and pH and total CO_2 determinations were made on fluid collected by puncturing the sac with a Hamilton syringe. In preliminary experiments, the pH and total CO_2 values estimated in this way were similar to values determined by cannulation.

Biochemical measurements

Total urate and inorganic phosphate concentrations were determined on 5 μl samples of tubular fluid diluted 100-fold with distilled water. Urate concentrations were determined spectrophotometrically at 520 nm with a commercial kit (Sigma) and uric acid stock solutions as standards. Inorganic phosphate was determined spectrophotometrically at 820 nm by the technique of Chen *et al.* (1956). Total ammonia (ammonia+ammonium) concentrations were determined by the enzymatic assay of Kun and Kearny (1974). The non-bicarbonate buffer value (β) for tubular secretion was determined using samples diluted 100-fold with 100 mmol l^{-1} KCl. Dilute samples were acidified with 20 mmol l^{-1} HCl until the pH was below 4.0. Samples were then stirred for 2 h to drive off CO_2 and HCO_3^-. Buffer value was then determined from pH 6.4 to 7.0 by titrating with 2 mmol l^{-1} KOH using a PHM 84 research pH meter, TTT 80 titrator and ABU 80 autoburette (Radiometer, Copenhagen, Denmark).

Statistics

All data are presented as mean±standard error (S.E.) with N indicating the number of locusts. Paired t-tests were used to determine significant differences in composition of secretion caused by acid injection. Where paired t-tests were not appropriate, significance of difference between means was determined by one-way analysis of variance, ANOVA, with $P<0.05$ accepted as significant.

Results

Time course of fluid secretion

The rate of fluid secretion (J_v) by insect Malpighian tubules isolated *in vitro* generally falls with time (reviewed by Maddrell, 1980). We therefore first followed J_v with time for the full complement of locust tubules in starved animals, as determined by hindgut cannulation *in situ* (Fig. 1). The initial mean J_v over the first 2 h varied between 8 and 12 $\mu l\,h^{-1}$ in different experiments and gradually fell by 20–40 % over 6 h. These rates are within the range of values previously reported for the full complement of *Schistocerca* tubules using *in vitro* and other *in vivo* methods (reviewed by Phillips, 1981).

The injections of 25 μl of 0.4 $mol\,l^{-1}$ NaCl or HCl into locusts between the second and third hour after cannulation had no measurable effects on J_v compared to uninjected controls (Fig. 1). There was no statistical difference between J_v values for the three treatment groups except for HCl-injected locusts at the third hour.

Time course of tubular acid–base variables in uninjected locusts

The pH of tubular fluid from starved, uninjected locusts did not change significantly over the first 7 h and averaged 6.63 (Fig. 2). This is 0.61 pH units below the initial cannulated haemolymph value of 7.24, which fell slightly to 7.11 over 6 h of cannulation (Fig. 3). Initial values in tubular fluid over the first 2 h were: total CO_2 (8.9 $mmol\,l^{-1}$), P_{CO_2} (7.3 kPa) and [HCO_3^-] (6.5 $mmol\,l^{-1}$), and these did not change significantly over 8 h (Fig. 4).

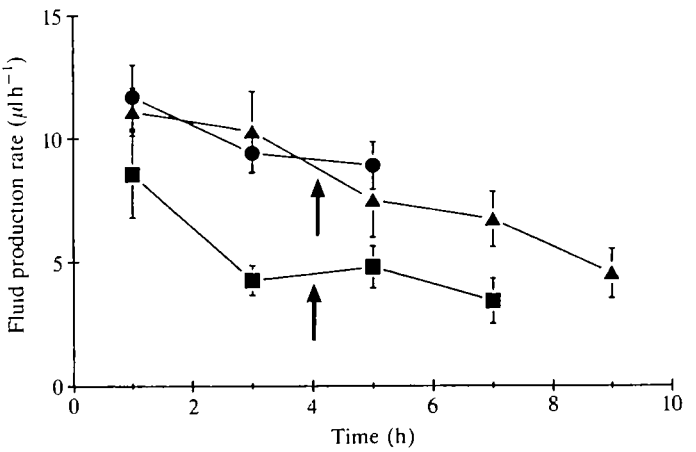

Fig. 1. Fluid production rates (J_v) for the full complement of Malpighian tubules of starved locusts cannulated *in situ* at 20°C. Mean±S.E. (N=10–20) for J_v over an equal period before and after injection (arrows) (i.e. 2 h total period). Values for the three treatment groups [uninjected (●), HCl-injected (■) and NaCl-injected (▲)] are not significantly different except for the HCl-injected group at 3 h after cannulation.

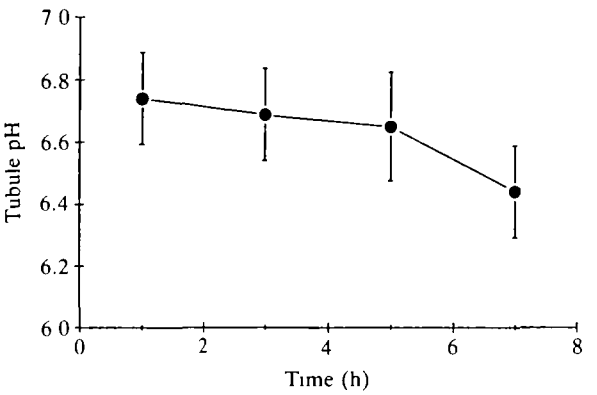

Fig. 2. Tubule fluid pH in uninjected locusts cannulated *in situ* at 20°C (mean±S.E., N=5–9). The apparent decrease in pH over 8 h is not significant (P>0.5).

Effect of HCl or NaCl injection on haemolymph acid–base variables

Before cannulation, locust haemolymph pH averaged 7.28. Injection of 25 μl of 0.4 mol l^{-1} NaCl into the abdomen of locusts (control) did not significantly change haemolymph pH, which remained above 7.2 for at least 5 h after injection (Fig. 3). Similarly, haemolymph total CO_2, P_{CO_2} and [HCO_3^-] were not significantly changed by NaCl injection and remained close to 8.0 mmol l^{-1}, 2.4 kPa and 7.5 mmol l^{-1}, respectively (Fig. 5).

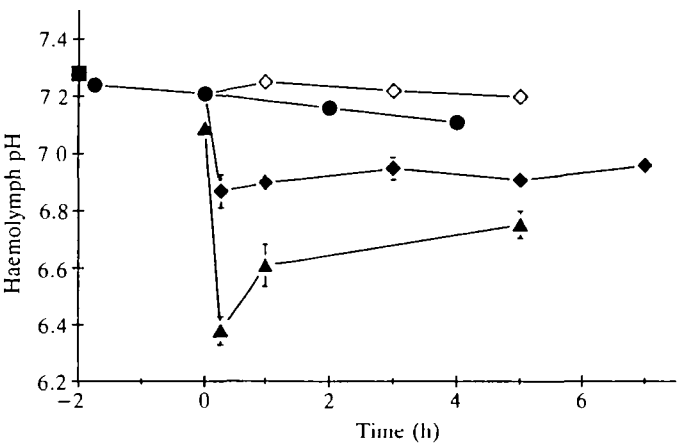

Fig. 3. Haemolymph pH relative to the time of injection (0 h) for different experimental groups of locusts starved for 1 day at 20°C. Mean±S.E. (N=4–9). Values are for haemolymph collected at the neck (all symbols except ▲) or near the abdominal injection site (▲). Locusts were injected with 25 μl of 0.4 mol l^{-1} NaCl (◇) or 0.4 mol l^{-1} HCl (◆) or uninjected (●). The pH of haemolymph sampled from the neck prior to cannulation is also given (■). HCl injection caused a significant decrease (P<0.001) in haemolymph pH within 15 min (first sample) of injection. Haemolymph pH remained significantly depressed for 5 h after HCl injection.

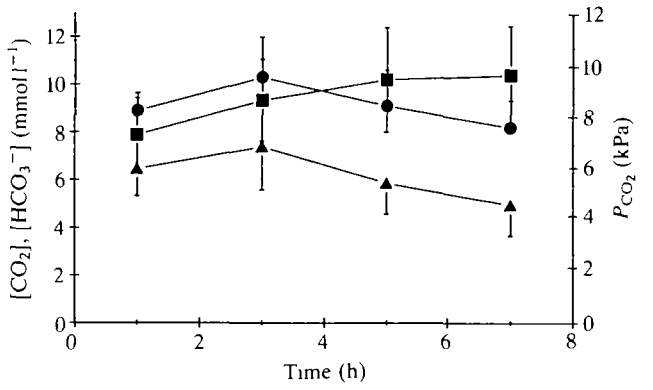

Fig. 4. Total CO_2 (●), P_{CO_2} (■) and HCO_3^- concentration (▲) in Malpighian tubule fluid from uninjected, starved animals at 20°C as a function of time after cannulation. Mean±S.E., N=5. There was no significant change in any variable with time.

Within 15 min of injecting 25 μl of 0.4 mol l^{-1} HCl into the abdomen, haemolymph pH fell by 0.34 units to 6.87, as measured at the neck (30–50 μl sample) and to 6.4 as measured by abdominal sample near the site of injection (Fig. 3). Apparently, complete mixing in the haemolymph compartment is not attained over several hours. Abdominal injection of HCl had a marked effect on the acid–base variables of haemolymph collected at the neck (Fig. 6). Initial values before injection were similar to those for the NaCl-injected group (Fig. 5). Over the first 15 min after HCl injection, total CO_2 fell significantly by over 30% to 5.2 mmol l^{-1}, P_{CO_2} increased temporarily but not significantly, and calculated $[HCO_3^-]$ fell by 40% to 4.2 mmol l^{-1}. Total CO_2, $[HCO_3^-]$ (Fig. 6) and pH (Fig. 3) all remained significantly depressed 7 h after injection.

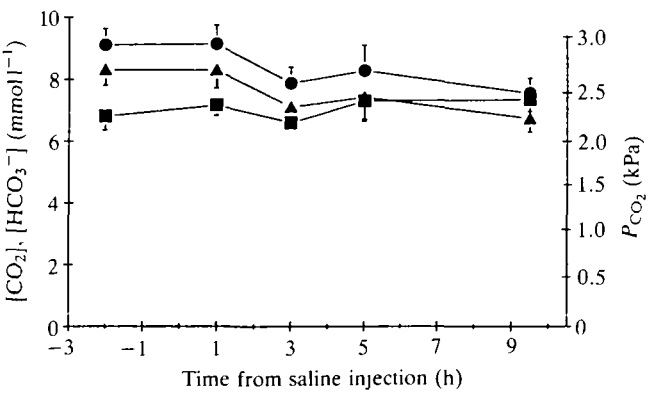

Fig. 5. Total CO_2 (●), P_{CO_2} (■) and HCO_3^- (▲) concentration in haemolymph collected at the neck from NaCl-injected, starved locusts at 20°C as a function of time (injection at 0 h). Mean±S.E. (N=5–9). There was no significant change in any of these variables due to injection, or with time after injection.

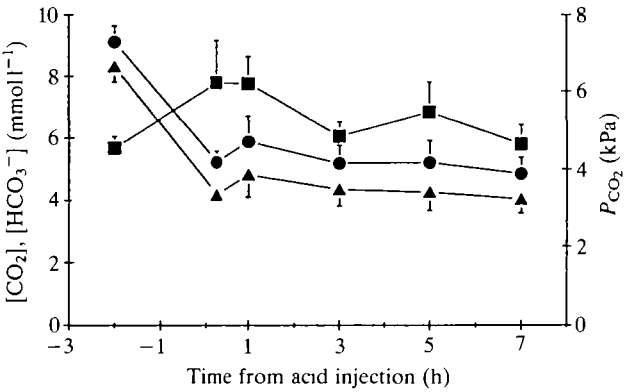

Fig. 6. Total CO_2 (●), P_{CO_2} (■) and HCO_3^- (▲) concentration in haemolymph collected at the neck from HCl-injected starved animals at 20°C as a function of time (injection at 0 h). Mean±S.E. (N=5–8). P_{CO_2} did not change significantly after acid injection (P=0.13); but both total CO_2 and HCO_3^- levels significantly decreased (P=0.028, 0.007, respectively) after injection and remained depressed for the next 7 h.

In summary, changes in haemolymph acid–base variables (measured at the neck) caused by acid injection were sustained for at least 7 h. Subsequent studies on the composition of tubule fluid were normally made within 4 h (at most) of acid injection.

Effect of haemolymph acidosis on the composition of Malpighian tubule fluid

Acid injection into the haemocoel initially caused a significant decrease in the pH of tubular fluid by 0.47 units compared to NaCl-injected controls (Fig. 7). However, tubular fluid pH in the NaCl-injected controls also gradually fell with

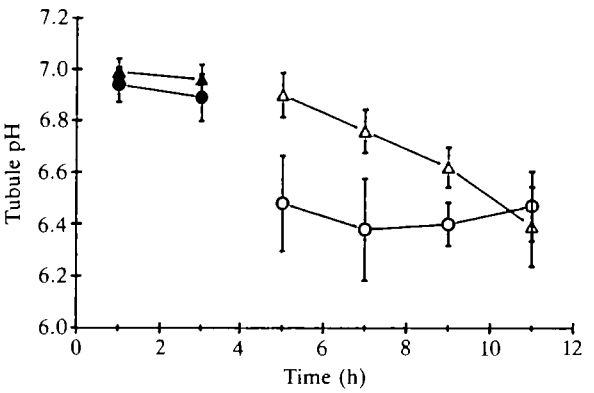

Fig. 7. The pH of Malpighian tubule fluid prior to injection (filled symbols) and after injection (open symbols) with either NaCl (triangles) or HCl (circles). Mean±S.E. (N=10–14). The pH decreased significantly (P=0.001) immediately after HCl injection relative to NaCl-injected controls.

Table 1. *Effect of HCl and NaCl injection into the haemocoel on acid–base status of Malpighian tubule fluid in* Schistocerca gregaria *at 20°C*

	HCl-injected		NaCl-injected	
	Pre-injection	Post-injection	Pre-injection	Post-injection
pH	6.89±0.07	6.42±0.1*	6.91±0.08	6.81±0.07
Total CO_2 (mmol l^{-1})	6.2±0.8	5.5±0.7	11.8±1.3	9.1±0.9*
P_{CO_2} (kPa)	3.2±0.4	6.8±1.5	6.0±0.7	5.6±0.5
$[HCO_3^-]$ (mmol l^{-1})	5.1±0.7	3.2±0.4*	9.8±1.3	7.3±0.9*
Non-bicarbonate buffer value (β) (mequiv l^{-1} pH unit^{-1})	9.5±1.0	10.4±1.2	10.4±1.1	15.5±2.6*

Pre-injection samples were secreted over a 2- to 3-h period before injection, post-injection samples were secreted over a 2- to 3-h period after injection.

Mean±S.E. (N=9–14).

*Significantly different from pre-injection value, paired t-test, $P<0.05$. Non-bicarbonate buffer value for the pH range 6.4–7.0.

time so that after 10–12 h there was no difference between the acid- and salt-injected groups. Thus, the cannulation/injection procedure itself caused a slow increase in tubule acidity. Therefore, in the remaining series of experiments, we compared the composition of Malpighian tubule fluid pooled over approximately 2.0–3.0 h before injection with tubular fluid collected within 3 h after injection from the same locust.

The effect of injecting either 10 μmol of NaCl or HCl on tubular acid–base variables is shown in Table 1. Before injection, the tubule fluid pH of 6.89 was similar in acid- and NaCl-injected groups. Acid, but not NaCl, injection caused a significant decrease in tubule fluid pH by 0.47 units to 6.42. Total CO_2 did not change significantly in the acid-injected group, so that the effect of increased tubular acidification was to titrate HCO_3^- (which fell significantly from 5.1 to 3.2 mmol l^{-1}) to CO_2. As a result, tubule fluid P_{CO_2} doubled after acid injection. There is no indication from these results that Malpighian tubules contributed additional HCO_3^- to the haemolymph after acid injection.

By comparison, the NaCl-injected controls showed no change in P_{CO_2} after injection. However, total CO_2 and $[HCO_3^-]$ in tubule fluid were initially higher in this group and both declined slightly but significantly after injection.

There is no indication that acid injection stimulated long-term active acid secretion by Malpighian tubules, because the final pH differences between haemolymph and tubular fluid were not significantly different in uninjected, NaCl-injected and HCl-injected groups (Table 2). After 4 h, tubule fluid was on average 0.56–0.75 pH units more acid than haemolymph, regardless of treatment. The lower tubule fluid pH initially observed (2 h) after acid injection (reported in Table 1 and Fig. 7) may simply reflect the greater haemolymph acidity of the former group rather than enhanced acid secretory capability. In particular, different pH values of haemolymph collected from the abdomen and neck (Fig. 3)

Table 2. *pH determinations of haemolymph (neck sample) and tubule fluid at the end of experiments on three groups of locusts at 20°C*

	Uninjected	HCl-injected	NaCl-injected
Haemolymph pH (1)	7.15±0.04	6.95±0.01	7.12±0.03
Tubule pH (2)	6.39±0.21	6.40±0.11	6.51±0.11
Difference (1−2)	0.75±0.18	0.56±0.11	0.60±0.13

Measured on fluids collected between 3 and 6 h after injection and 6–9 h after cannulation. Mean±S.E. (N=9–14). The pH difference across the tubule wall was not significantly different between treatments.

make it difficult to decide the effective pH experienced by tubules after acid injection.

Tubular fluid buffer values

The rate of acid secretion is determined not only by tubular pH but also by the buffer capacity (β), which might change following acid injection. We measured the non-bicarbonate buffer value (β) for Malpighian tubule fluid over the pH range 6.4–7.0 observed in earlier experiments (Table 1). Titration indicated a constant value for β between pH 6.4 and 7.0 (data not shown). The value of β was 9.5 mequiv l^{-1} pH unit^{-1} before injection, and this was unchanged after HCl injection. However, NaCl injection caused a significant increase in β to 15.5 mequiv l^{-1} pH unit^{-1}. The nature of this increased buffer capacity was investigated by measuring levels of major potential buffers previously reported in tubule fluid, namely phosphates and urates. Moreover, ammonia will trap secreted H^+ to form NH_4^+. Indeed, enhanced ammonia production by vertebrate proximal tubules is a major mechanism for removing excess acid (Pitts, 1973). We therefore also measured total ammonia concentrations in locust tubular fluid, as discussed in the next section. Although total amino acid concentrations in locust tubule fluid are very high (50 mmol l^{-1}, mostly proline; Chamberlin and Phillips, 1982), we found that the β value for an artificial amino acid mixture resembling *in vivo* values reported by Chamberlin and Phillips was negligible.

Phosphate, urate and total ammonia levels in tubule fluid

As shown in Table 3, phosphate levels in tubule fluid increased with time in uninjected locusts, and this was significantly enhanced (fourfold increase) by NaCl injection but not HCl injection. This difference in phosphate secretion may account for the greater β value of tubular fluid from NaCl- as opposed to HCl-injected locusts (Table 1). However, the contribution of phosphate to acid removal depends on the form of this anion that is actively secreted by tubules, and this is unknown (reviewed by Phillips, 1981). Average urate concentrations varied from 1.6 to 5.3 mmol l^{-1} in the three treatment groups prior to injection. Urate levels decreased significantly from 5.3 to 2.2 mmol l^{-1} following HCl injection (Table 3). We measured urate levels of 0.21±0.05 mmol l^{-1} (N=12) in haemo-

Table 3. *Composition (mmol l^{-1}) of* Schistocerca gregaria *Malpighian tubule fluid collected* in situ *at 20°C*

	Uninjected		HCl-injected		NaCl-injected	
	Pre	Post	Pre	Post	Pre	Post
Urate	1.6±0.4	1.6±0.5	5.3±0.8	2.2±0.2*	3.6±0.4	3.8±1.3
Phosphate	2.7±0.7	4.8±1.2*	4.5±1.0	6.7±1.0*	4.5±1.2	16±3.9*
Ammonia	5.2±1.0	8.1±1.8*	4.6±0.7	6.7±0.9*	5.3±0.9	6.2±1.3

Mean values±S.E. (N=9–21 locusts) for fluid collected by cannulation over 2–3 h before (Pre) and 2–3 h after (Post) HCl or NaCl injection, and during same time periods for uninjected locusts.

* Indicates significant differences from pre-injection values in the same animals.

lymph of unfed locusts. Thus, in our experiments locusts secrete urate against large concentration gradients similar to those first reported by O'Donnell *et al.* (1983) using *in vitro* tubules. Total ammonia concentration was 4–5 mmol l^{-1} in tubular fluid prior to injection in all three groups. While there was a small but significant increase in total ammonia to 6.7 mmol l^{-1} following acid injection, this could reflect a change with time because a similar significant change with time was observed in uninjected locusts (Table 3). The above ammonia levels are about four times those that we measured in locust haemolymph (1.1±0.26 mmol l^{-1}, N=12).

Discussion

This study provides the first comprehensive measurements of the major acid–base variables for Malpighian tubule secretion in a terrestrial insect *in situ*. In control locusts starved for 1 day, total CO_2 and HCO_3^- levels are similar in haemolymph and tubular fluids at 8–10 and 5–7 mmol l^{-1}, respectively. In contrast, calculated P_{CO_2} levels (7.3 kPa) are nearly three times higher in tubular fluid than haemolymph. Tubule fluid at pH 6.63 is 0.5 units more acid than haemolymph in the control group. This may be the normal situation if a proton pump drives K^+ and hence fluid secretion. However, there is an alternative possibility: locusts may experience an acidotic state within 1 day of starvation. In support of this idea, J. F. Harrison, C. J. H. Wong and J. E. Phillips (in preparation) found that the pH of faecal pellets from similarly starved locusts averaged 4.62, compared to 6.2 in feeding animals. Thus, control locusts used in our study may already have been responding to a natural acid load prior to acid injection, and this may have diminished the response to our experimental acidotic challenge. Nevertheless, our results permit an estimate of the maximum capacity of the full complement of locust tubules to eliminate excess acid equivalents.

Using measured J_v (initial average of 10 μl h^{-1}; Fig. 1), a median value for the measured pH difference between haemolymph and tubular fluid under the three conditions studied (0.6 pH units), and the measured highest buffer values for

tubular fluid (15 mequiv l^{-1} pH unit^{-1}) for NaCl-injected locusts (Table 1), the estimated maximum rate of excess acid removal by locust Malpighian tubules (J_H) is 0.09 μmol h^{-1}. This value could be considerably less if more conservative values are used in these calculations. While tubule fluid was initially much more acid after HCl injection (Table 1), so was the haemolymph. It is, therefore, not possible to conclude that tubular acid secretion capability was stimulated by HCl injection, especially given the heterogeneity in haemolymph pH observed between the neck and abdomen (Fig. 3). Clearly mechanisms and possible control of tubular acid secretion will require *in vitro* studies, where the composition of the fluid bathing isolated tubules can be precisely controlled. Nevertheless, our *in vivo* studies do provide the necessary groundwork for such future studies.

Given that 10 μmol of acid was injected into locusts, the Malpighian tubules clearly do not have the capacity (at a maximum J_H of 0.09 μmol h^{-1}) to return haemolymph pH to normal values within a 6 h period, as observed by J. F. Harrison, C. J. H. Wong and J. E. Phillips (in preparation) in a parallel study on uncannulated animals. The potential regulatory capacity of the hindgut is many times greater. Both locust ileum and rectum actively secrete acid *in vivo* at 1.5 μmol h^{-1} cm^{-2} in the absence of a pH difference (Thomson *et al.* 1988*a*,*b*, 1991). Correcting for surface area of these hindgut segments, the locust rectum (0.62 cm^2) and ilea (0.4 cm^2) can still secrete H^+ at 0.6 and 0.3 μmol h^{-1}, respectively, against a gradient (0.6 pH units) comparable to the maximum developed by the tubule epithelium. Clearly all three segments of the locust excretory system (tubules, ileum and rectum) contribute to acid excretion, with H^+ secretory capacity increasing and luminal pH decreasing posteriorly. A fall in luminal pH during passage through the hindgut has been observed by Thomson *et al.* (1988*a*) and J. F. Harrison, C. J. H. Wong and J. E. Phillips (in preparation).

Haemolymph pH measured either from the neck or abdomen did not recover substantially to control values even after 6 h (Fig. 3). This lack of recovery of haemolymph pH in the cannulated animals suggests that interruption of tubule fluid reabsorption in the midgut and hindgut may prevent bicarbonate reabsorption necessary for haemolymph pH recovery. In a parallel study using uncannulated desert locusts, haemolymph pH measured at the neck recovered to control values within 6 h of a similar acid injection, and this was due to a rise in haemolymph [HCO_3^-] (J. F. Harrison, C. J. H. Wong and J. E. Phillips, in preparation). Moreover, cannulation and continued starvation eventually lead, after 6–9 h, to a more acid pH in tubular fluid and haemolymph, regardless of experimental treatment (Fig. 2).

Calculations of J_H presented above using buffer values and pH differences do not evaluate the additional acid removal that may be associated with total ammonia excretion. We did not observe increased tubular ammonium secretion attributable specifically to HCl injection; however, using the typical total ammonium concentration in tubular fluid of 5.5 mmol l^{-1} (Table 3), the maximum rate of ammonium secretion (J_{Amm}), assuming a J_v of 10 μl h^{-1}, is 0.06 μmol h^{-1}. Thus, J_{Amm} could potentially increase net acid secretion by locust tubules to a

maximum of 0.15 μmol h^{-1}, assuming that all secreted ammonium trapped H^+ from the haemolymph. Again, the locust hindgut is a much more important site for potential acid removal in the form of ammonia. Oxidation of amino acids by the locust rectum supports a J_{Amm} to the lumen of 0.4 μmol h^{-1} (Thomson *et al.* 1988*b*: corrected for surface area). However, the locust ileum is by far the major source of excreted ammonia, with an unstimulated J_{Amm} of 0.6 μmol h^{-1}, and this can be stimulated to 1.4 μmol h^{-1} by adding 5 mmol l^{-1} cyclic AMP (Lechleitner, 1988; Audsley, 1991).

A surprising observation is that ammonia concentrations equal or exceed those of urate in locust tubular fluid (Table 3). As a result of the additional and much larger J_{Amm} in locust hindgut, the final excreta in locusts would be expected to contain ammonia rather than urate as the predominant nitrogenous end-product, contrary to previous dogma that urate is the major end-product in locusts (reviewed by Cochran, 1975). (There is no evidence for urate production or secretion by locust hindgut.) In a companion study (Harrison *et al.* 1990; J. F. Harrison, C. J. H. Wong and J. E. Phillips, in preparation), we confirm this prediction. The faecal concentrations of ammonia and urate are 270 and 68 mmol kg^{-1} H_2O, respectively, in locusts starved for a day, when care is taken to prevent ammonia loss on exposure of excreta to air. A comparison of these faecal concentrations with those of Malpighian tubule fluid (Table 3) provides interesting new information. Assuming no urate secretion in hindgut, the 15-fold increase in urate levels during passage through the hindgut suggests that about 95 % of tubular fluid is reabsorbed in the hindgut, in agreement with previous estimates (reviewed by Phillips, 1981). The increase in ammonia-to-urate ratio from near 1:1 at the tubules (Table 3) to about 4:1 in the faeces, confirms that the hindgut contributes over 70 % of total ammonia excreted.

Finally, we have assessed the contribution of individual potential buffers to the total buffering capacity of locust Malpighian tubule fluid (Table 4). Using average measured values of pH and solute concentrations in tubule fluid, and pK values from the literature (Robinson and Stokes, 1959), phosphate (3.2 mequiv l^{-1} pH

Table 4. *Estimated buffer contribution (β) of measured solutes in Malpighian tubule fluid of HCl-injected* Schistocerca gregaria *at 20°C, pH 6.71 and constant* P_{CO_2}

	β (mequiv l^{-1} pH unit^{-1})
Bicarbonate	9.90
Phosphate	3.16
Urate	0.84
Total	13.9

Values of β were calculated according to Heisler (1986), using pK values from Robinson and Stokes (1959) and measured solute concentrations in tubule fluid post injection (Table 3).

unit^{-1}) is the major non-bicarbonate buffer in Malpighian tubule fluid, while urate (0.84 mequiv l^{-1} pH unit^{-1}) plays a minor role. The contribution of phosphate to non-bicarbonate buffer value after NaCl injection is 8.4 mequiv l^{-1} pH unit^{-1}, which accounts for the increase in non-bicarbonate buffer value shown in Table 1. However, bicarbonate ion is overall the major buffer (9.9 mequiv l^{-1} pH unit^{-1}) in tubule fluid, accounting for more than 50 % of total buffering capacity (Table 4). Only about 40 % of non-bicarbonate buffer value is represented by total inorganic phosphate and urate. Despite the variability in the tubule fluid concentrations, there must be some additional unmeasured ionic species that account for the remaining 20–30 % of the total buffering capacity of tubule fluid.

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