J. exp. Biol. **165**, 85–96 (1992)
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RECOVERY FROM ACUTE HAEMOLYMPH ACIDOSIS IN UNFED LOCUSTS

I. ACID TRANSFER TO THE ALIMENTARY LUMEN IS THE DOMINANT MECHANISM

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Accepted 28 November 1991

Summary

Organismal homeostasis requires regulation of extracellular acid–base status; however, the mechanisms by which insects regulate haemolymph pH are poorly known. We evaluated the recovery of desert locusts *Schistocerca gregaria* Forskål from acute acid loads, initiated by HCl injections into the haemolymph (0.5 pH unit decrease). Haemolymph pH, P_{CO_2} and $[HCO_3^-]$ recovered in 8–24 h, providing the first unequivocal evidence that insects regulate extracellular pH. There were no changes in the concentrations of the primary haemolymph buffer compounds (protein, inorganic phosphate) during recovery. Within 1 h, the tracheal system effectively eliminated the carbon dioxide derived from bicarbonate buffering. During the remainder of the recovery, haemolymph P_{CO_2} was similar to control values; there was no respiratory compensation for decreased haemolymph pH. Approximately 75 % of the acid equivalents removed from the haemolymph during the recovery process were transferred to the lumens of the crop and midgut. Transfer of acid equivalents to the alimentary lumen provides unfed locusts with a mechanism of haemolymph pH regulation that does not compromise intracellular acid–base status or increase ventilatory water loss.

Introduction

Regulation of acid–base status is one of the most critical aspects of organismal homeostasis. The ability of insects to preserve haemolymph acid–base status and the mechanisms by which such regulation may be achieved are poorly understood (Phillips *et al.* 1986). In this study we examine the capacity of the desert locust *Schistocerca gregaria* to recover from acute haemolymph acidosis (HCl injections) and quantify the contribution of some of the possible mechanisms responsible for this recovery.

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Key words: acid–base regulation, locust, alimentary lumen, *Schistocerca gregaria*.

In the terrestrial animals that have been studied, recovery from extracellular acid–base disturbances may occur by a number of mechanisms, including ventilatory adjustment of P_{CO_2}, mobilization of blood buffer compounds, excretion of acid equivalents from the animal by epithelial transport and transfer of acid equivalents to another body compartment (Truchot, 1987). Increases in ventilatory carbon dioxide excretion aid in restoring haemolymph acid–base status after exposure to elevations in environmental or endogenous P_{CO_2} in locusts (Harrison, 1989*a*; Harrison *et al.* 1991). However, haemolymph acid–base and ventilatory responses to metabolic acid loads have not been studied in insects. Metabolic acid loads may be produced in animals during a variety of circumstances, including fasting, ketosis or in association with anaerobic energy metabolism (Masoro and Siegel, 1977). In cockroaches, ventilation increases when the pH of saline irrigating the ventral nerve cord is decreased at constant P_{CO_2} (Snyder *et al.* 1980). The results of Snyder *et al.* suggest that insects have the capacity to reduce haemolymph P_{CO_2} in response to depressed haemolymph pH.

Transfer of acid–base equivalents out of the haemolymph allows organisms to regulate pH and $[HCO_3^-]$ at constant P_{CO_2}. In terrestrial insects, acid–base equivalents may be transferred out of the animal by renal excretion. Alternatively, acid–base equivalents may be transferred from the haemolymph into the intracellular space or the alimentary lumen. The pH of the alimentary lumen of locusts differs from that of haemolymph throughout most of its length (Bodine, 1925; Swingle, 1931; Thomson *et al.* 1988*a*). Because the alimentary lumen in locusts contains a large fraction of body water and bicarbonate (Harrison, 1989*b*), this compartment may have considerable physiological capacity for temporary storage of acid–base equivalents. Transport of acid–base equivalents across renal or alimentary epithelia has been demonstrated in a variety of insects (Ramsay, 1956; Irvine *et al.* 1988; Thomson *et al.* 1988*a*,*b*; Lechleitner *et al.* 1989; Chamberlin, 1990; Dow and O'Donnell, 1990; Stagg *et al.* 1991). However, it is not known whether regulatory adjustments of acid–base transport by the excretory or alimentary epithelia occur during haemolymph pH regulation. In this study, we test for transfer of acid equivalents to the alimentary lumen in response to haemolymph acidosis. In a companion paper (Harrison and Phillips, 1992), we examine the role of renal acid and nitrogen excretion in the recovery from HCl injections.

Materials and methods

General techniques

Locusts (*Schistocerca gregaria*) were obtained from a colony maintained as described previously (Thomson *et al.* 1988*a*). Adult female locusts, 14–25 days past their final moult, were used in all experiments. Animals were fed on lettuce *ad libitum* for 2 h at 35 °C, and then kept solitarily in cardboard cartons with access only to distilled water-soaked cotton for 20–24 h at 21 °C (±0.5 °C). Locusts were then injected with 25 μl of 400 mmol l^{-1} HCl or NaCl using a 100 μl Unimetrics

syringe *via* the fourth or fifth abdominal intersegmental membrane. Bleeding from the injection site was generally minimal. Animals were then returned to their individual cartons.

Owing to limitations in sample sizes, not all variables were measured for each animal, and all samples were terminal. Therefore, effects of HCl injections were evaluated by comparing values for HCl- and NaCl-injected animals under similar conditions. Variables measured concurrently from single animals are described below. For each experimental series, half of the locusts were injected with HCl and half with NaCl.

Haemolymph analyses

The pH and total carbon dioxide content (C_{CO_2}, mmol l^{-1}) of haemolymph were measured 15 min, 1 h, 4 h, 8 h and 24 h after HCl injections and 15 min, 1 h and 24 h after NaCl injections. Haemolymph was sampled as described previously, and both pH and C_{CO_2} were measured on single animals (Harrison, 1988). A 3 μl haemolymph sample was transferred *via* a PE-10 cannula to a polyethylene constriction vessel joined to a PE-10 KCl–agar bridge (3 mol l^{-1} KCl, 3 % agar). Haemolymph pH was measured with a glass microelectrode and a Keithley model 616 electrometer as described previously (Harrison *et al.* 1990). Preliminary trials indicated that if pH was measured within 5 min, loss of CO_2 from the vessel was not detectable. C_{CO_2} was measured on 10 μl samples by gas chromatography as previously described (Harrison *et al.* 1990). Haemolymph P_{CO_2} (kPa) and [HCO_3^-] (mmol l^{-1}) were calculated using carbonic acid dissociation constants and solubility coefficients for locust haemolymph at 21 °C (α=0.3318 mmol l^{-1} kPa^{-1}, pK=6.235; Harrison, 1988) according to the following equations:

$$P_{CO_2} = C_{CO_2}/[\alpha(10^{pH-pK} + 1)],$$

$$[HCO_3^-] = C_{CO_2} - P_{CO_2} \times \alpha.$$

Haemolymph protein and inorganic phosphate concentrations were measured in a second group of animals using unclotted samples injected directly into 5 % trichloroacetic acid (TCA, Harrison *et al.* 1990).

Acid–base status of the alimentary lumen

Luminal pH was measured prior to injections and 24 h after HCl or NaCl injections in a third group of animals. The pH of the various sections of the alimentary lumen was measured using a glass microelectrode inserted through a small tear (<1 mm) in the epithelium after rapid (<5 min) dissection to expose the gut. The reference electrode used was a PE-10 KCl–agar bridge placed in the lumen, in series with a calomel electrode. The potential difference between the reference and pH electrodes was measured with the Keithley electrometer.

We measured the total ammonia concentration (Amm_{tot}) and C_{CO_2} of the contents of alimentary lumen 24 h after HCl or NaCl injections in a fourth group of animals. Luminal contents were removed with forceps after rapid (<5 min) dissection. C_{CO_2} was measured immediately by gas chromatography. Samples of

gut contents used for measurement of Amm_{tot} were immediately placed in 19 times their volume of 5 % TCA to prevent loss of NH_3 and frozen. This solution was later diluted 1500 times with distilled water, mixed and centrifuged (5 min, 1500 *g*). The supernatant was analyzed for Amm_{tot} using an assay modified from Kun and Kearney (1974). The masses of the luminal contents of the crop and midgut were determined 24 h after injections on a fifth group of animals from the difference between the mass of intact segments and the mass of segments with luminal contents removed by washing with isosmotic saline.

Non-bicarbonate buffer values of the crop and midgut contents were measured 24 h after NaCl or HCl injections using the fifth group of animals. Samples were diluted 1000 times with 100 mmol l^{-1} KCl, to approximate the KCl-rich fluids found in these compartments (Dow, 1981). Carbon dioxide was removed by adding HCl to lower solution pH below 4, followed by stirring for 2 h. Samples were then alkalized by the addition of KOH (Radiometer TTT80 titrator and model ABU80 Autoburette), while pH was measured with a Radiometer GK2321C pH electrode. We calculated nonbicarbonate buffer value (mequiv kg^{-1} pH $unit^{-1}$) as the quantity of base equivalents required to titrate 1 kg of bicarbonate-free sample by 1 pH unit. Buffer values were measured over the range of pH corresponding to the difference in the pH of HCl- and NaCl-injected animals (crops 5.3–6.0; midguts 6.2–7.0). Buffer values were corrected for the buffering due to the dilution saline by subtracting the buffer values for 100 mmol l^{-1} KCl solutions.

Calculations

The phrase 'addition of 1 μequiv of acid to a compartment' indicates that the compartment has been acidified in a manner equivalent to the addition of 1 μequiv of HCl. The conditions of this study do not permit discrimination between acid equivalent transfer out of a compartment and base equivalent addition to the compartment.

Acid equivalent transfer into the luminal compartments of the alimentary tract was calculated as:

$$\{\Delta\text{pHa} \times \beta + \Delta[HCO_3^-]_a\}M .$$

The symbols ΔpHa and $\Delta[HCO_3^-]_a$ indicate the average difference in pH and bicarbonate values of the compartment between NaCl- and HCl-injected animals. β is the non-bicarbonate buffer value of the compartment, averaged between NaCl- and HCl-injected animals. M is the mass of the luminal contents of the compartment (kg). $[HCO_3^-]$ was calculated from pH and C_{CO_2} as described for haemolymph.

Statistics

Data were tested for normality (Kolmogorov's test) and heteroscedasticity (Fmax test). Data satisfying assumptions of analysis of variance (ANOVA) were tested for significance with ANOVA or t-tests. *Post-hoc* contrasts for multiple

comparisons were performed using the Bonferroni procedure to maintain the overall level for statistical significance of $P<0.05$ (Wilkinson, 1987).

Results

Haemolymph acid–base status

Fifteen minutes after acid injections, haemolymph $[H^+]$ was increased by 10.3×10^{-8} mmol l^{-1}, while haemolymph C_{CO_2} was approximately halved (Fig. 1). Recovery to values statistically equivalent to resting occurred in 8 h ($[H^+]$) or 24 h (C_{CO_2}). Injection of 10 μmol of NaCl into the haemolymph had no effect on haemolymph $[H^+]$ or C_{CO_2} (*post-hoc* contrasts, $P>0.05$), indicating that the injection procedure did not affect haemolymph acid–base status (Fig. 1).

Acid injection had no effect on haemolymph protein or inorganic phosphate concentrations (Table 1). As these compounds account for a large fraction of the haemolymph non-bicarbonate buffer value in *S. gregaria* (Harrison *et al.* 1990), we constructed a Davenport diagram of haemolymph acid–base status after acid injection assuming that the haemolymph non-bicarbonate buffer value also remained constant throughout the experimental period. The Davenport diagram (Fig. 2) illustrates that recovery of acid–base status occurs primarily by movement along the CO_2 isobar, indicating that recovery occurs predominantly by non-respiratory mechanisms.

Fifteen minutes after acid injection, calculated haemolymph P_{CO_2} was signifi-

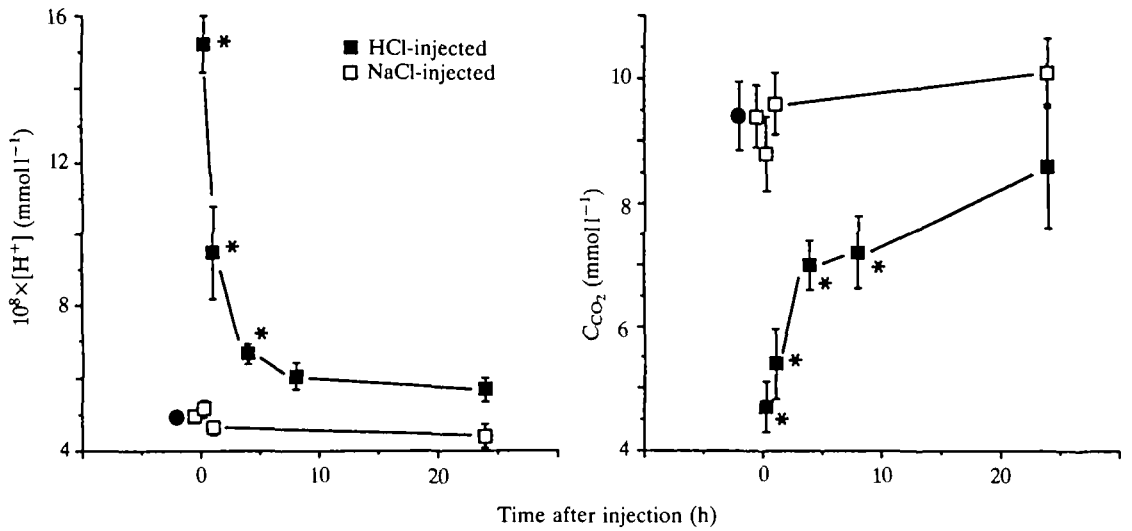

Fig. 1. Changes in haemolymph $[H^+]$ and C_{CO_2} as a function of time after HCl injection (filled squares) or NaCl injection (open squares). Circles indicate pre-injection values. Asterisks indicate that values differed significantly from pre-injection values (*post-hoc* contrasts, $P<0.05$). Means and standard errors, $N=8$–10.

Table 1. *Injection of 10 μmol of HCl into the haemolymph of* Schistocerca gregaria *had no effect on the concentrations of protein or inorganic phosphate in the haemolymph (ANOVA, P>0.05, N=8–9 at each)*

	Protein ($mg\,l^{-1}$)		Phosphate ($mmol\,l^{-1}$)	
	Mean	S.E.	Mean	S.E.
Pre-injection	54.8	1.80	3.5	0.44
0.25 h	56.8	4.23	3.5	0.67
8 h	57.0	3.95	3.3	0.34
24 h	55.3	1.70	4.0	0.39

Times indicate hours after injection.

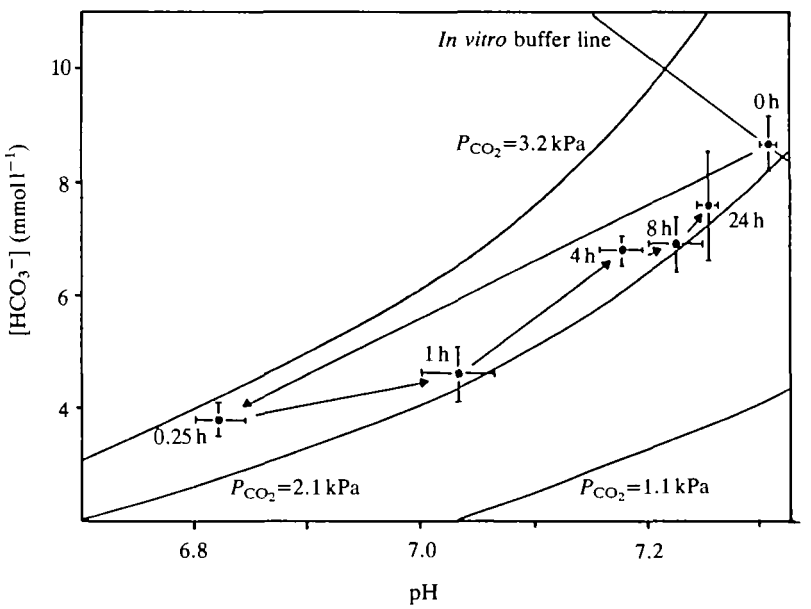

Fig. 2. Davenport diagram indicating the changes in calculated P_{CO_2} and $[HCO_3^-]$ with time after HCl injection. Times next to symbols indicate hours after acid injection. Means and standard errors are presented, N=8–10. The non-bicarbonate buffer line of 14.3 mmol l^{-1} pH unit^{-1} is plotted through the values for uninjected animals (Harrison *et al.* 1990).

cantly elevated (Fig. 2; *post-hoc* contrast, F=6.21, P<0.05); however, from 1 to 24 h after injection, haemolymph P_{CO_2} values did not differ significantly from control values (Fig. 2; *post-hoc* contrasts, P>0.05). Calculated haemolymph $[HCO_3^-]$ returned to values equivalent to controls in 24 h (Fig. 2; *post-hoc* contrasts).

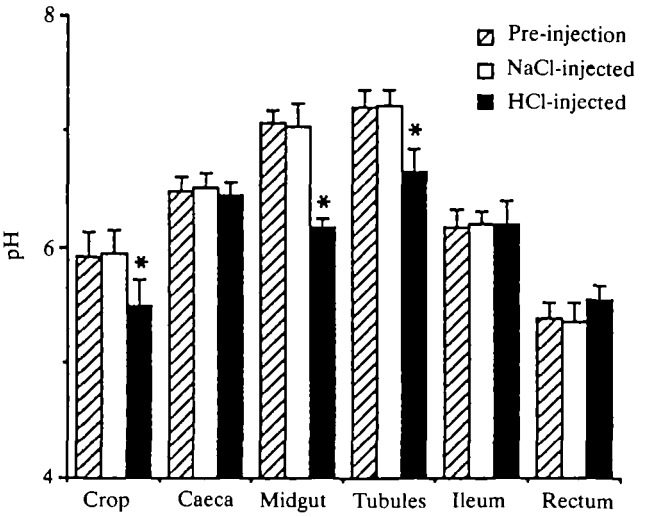

Fig. 3. The pH of the various sections of the locust alimentary lumen before injections and 24 h after NaCl or HCl injections into the haemocoel. Acid injection significantly reduced the luminal pH in the crop, in the midgut and at the point of entry of the Malpighian tubules (asterisks). Means and standard errors, N=11–12.

Transfer of net acid to the alimentary lumen

Twenty-four hours after HCl injections, gut luminal pH values were significantly lower than pre-injection values in the crop, midgut and at the point of Malpighian tubule entry (Fig. 3, t-tests, $P<0.05$). NaCl injection had no effect on the luminal pH of the alimentary canal (Fig. 3, t-tests, $P>0.05$). Midgut and crop luminal pH values were significantly lower after HCl injections than after NaCl injections (Table 2, t-tests, midgut: $t=4.01$, $P<0.001$; crop: $t=2.29$, $P<0.05$).

In the midgut, C_{CO_2} values were significantly lower after HCl injection than after NaCl injection (Table 2, t-test, $t=2.41$, $P<0.05$). In the crop, C_{CO_2} values were low and similar after HCl or NaCl injection (Table 2, t-test, $P>0.05$). NaCl- and HCl-injected animals did not differ in their luminal Amm_{tot} values (Fig. 4), indicating that effects of NH_4^+ transfer to the alimentary lumen on acid elimination from the haemolymph need not be considered. Using compartment buffer values and volumes (Table 2), we estimated that 50% of the injected acid load was transferred to the lumen of the midgut and 24% into the lumen of the crop.

Discussion

This study provides the first unequivocal evidence that insects regulate haemolymph acid–base status. After an injection of HCl that reduced haemolymph pH by 0.5 pH units and haemolymph $[HCO_3^-]$ by 50%, locusts completely recovered haemolymph pH, P_{CO_2} and $[HCO_3^-]$ within 24 h. The dominant

Table 2. *Variables used for calculation of acid equivalent transfer to the lumens of the midgut and crop after injection of 10 μmol of HCl into the haemolymph of* Schistocerca gregaria

	Midgut		Crop	
	Mean	S.E. (*N*)	Mean	S.E. (*N*)
pH				
HCl-injected	6.17	0.093 (12)	5.33	0.188 (11)
NaCl-injected	7.04	0.195 (12)	6.00	0.209 (11)
C_{CO_2} (mmol l^{-1})				
HCl-injected	6.0	0.79 (8)	0.7	0.32 (6)
NaCl-injected	10.6	1.01 (8)	1.4	0.38 (8)
[HCO_3^-] (mmol l^{-1})				
HCl-injected	2.8	0.85 (8)	0.1	0.39 (6)
NaCl-injected	9.2	1.22 (8)	0.5	0.44 (8)
Buffer value (mequiv kg^{-1} pH $unit^{-1}$)	58.9	6.09 (5)	56.0	8.51 (4)
Mass of contents (mg)	86.7	8.61 (7)	64.8	11.05 (16)
Acid transferred to lumen (μequiv)	5.0		2.4	

Values given correspond to those measured 24 h after injections (see text for details of calculations).

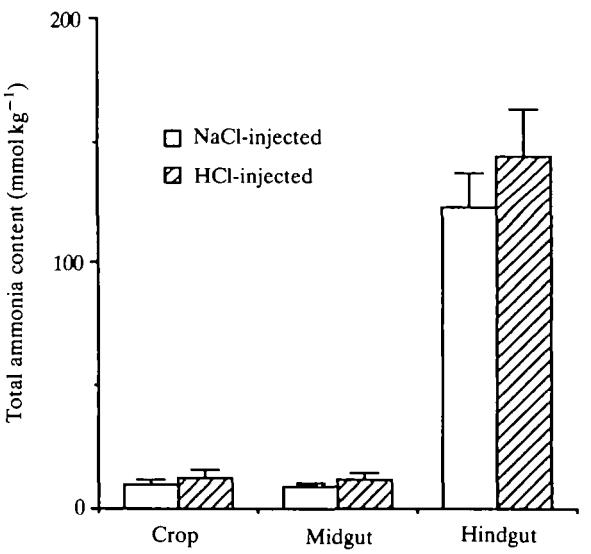

Fig. 4. The total ammonia content in the lumens of the crop, midgut and hindgut (ileum and rectum combined) did not differ 24 h after NaCl or HCl injection (*t*-tests, $P>0.05$). Means and standard errors, $N=8$–11.

mechanism by which this recovery occurred was transfer of net acid equivalents out of the haemolymph into the alimentary lumen.

Initially, approximately 50 % of the injected HCl was buffered by bicarbonate and approximately 50 % by non-bicarbonate buffers (calculated as described in Harrison *et al.* 1990). The elevated haemolymph P_{CO_2} 15 min after the HCl injection suggests that initially the tracheal system did not release all of the carbon dioxide derived from bicarbonate buffering. However, from 1 to 24 h after the HCl injections, haemolymph P_{CO_2} did not differ from that in control animals. The tracheal system effectively eliminated all of the carbon dioxide produced from bicarbonate buffering, but we found no evidence for respiratory compensation (reduced P_{CO_2}) for reduced haemolymph pH during long-term recovery (1–24 h).

Haemolymph levels of protein and inorganic phosphate did not vary after HCl injections. It is possible that concentrations of unmeasured buffer compounds (organic phosphates or citrate; Harrison *et al.* 1990) increased after HCl injections. However, since protein and inorganic phosphate account for 85 % of the non-bicarbonate buffer value in the haemolymph of quiescent *S. gregaria* (Harrison *et al.* 1990), it seems likely that an increased haemolymph non-bicarbonate buffer value was not a mechanism of blood pH regulation.

If metabolic consumption of protons within the haemolymph is small, recovery of haemolymph acid–base status at constant P_{CO_2} and haemolymph buffer value must occur by transfer of acid equivalents out of the haemolymph. Supporting this assumption, the cell fraction (and presumably the metabolic capacity) of locust haemolymph is small (<2 %, J. F. Harrison, unpublished data). We estimate that 75 % of the acid equivalents removed from the haemolymph during the recovery period are transferred to the lumens of the midgut and crop (Table 2). A further 15 % of the acid equivalents are excreted as ammonium (Harrison and Phillips, 1992). The midgut is a quantitatively more important sink for acid equivalents than is the crop, as indicated by its larger volume and its greater change in pH and calculated $[HCO_3^-]$. There was no evidence for a decreased pH in the lumen of the hindgut after HCl injections (Fig. 3). The unchanging luminal pH within the hindgut after acid injection is consistent with the finding that the pH of faecal pellets does not change after acid injection (Harrison and Phillips, 1992).

The decrease in pH of the alimentary lumen at the point of Malpighian tubule entry suggests that a portion of the acid transfer into the midgut may be mediated by the Malpighian tubules. Some of the fluid secreted by the Malpighian tubules is known to flow forward into the midgut in locusts, particularly in unfed animals (Dow, 1981). Recently, we have found that the pH of secreted Malpighian tubule fluid decreases during haemolymph acidosis when tubules are cannulated *in vivo* (Stagg *et al.* 1991). However, acid secretion by the Malpighian tubules can only account for at most 20 % of the acid equivalents transferred to the lumen of the midgut (Stagg *et al.* 1991). Therefore, the majority of the acid equivalents transferred from haemolymph to midgut lumen must be transported by the midgut epithelia and/or transferred from the crop lumen. In larval lepidopteran midgut,

base equivalent transfer to the lumen has been well-demonstrated (Chamberlin, 1990; Dow and O'Donnell, 1990). However, acid–base transport by the locust midgut has not been studied.

Presumably, the acid transfer to the lumen of the crop is mediated by the salivary glands, as the crop epithelium is relatively impermeable to ions and insect salivary glands are known to secrete ions, enzymes and various metabolites (Miles, 1967; Oschman and Berridge, 1970; Bay, 1978). In the only insect in which the acid–base status of salivary fluid has been examined, the saturniid moth *Antheraea peryi*, the secreted fluid is alkaline and high in bicarbonate (Kafatos, 1968). The low pH of the locust crop indicates that the situation must be quite different in *S. gregaria*.

Why is acid equivalent transfer into the fore- and midgut the dominant mechanism of haemolymph acid–base regulation in unfed locusts? Unfed locusts have been shown to retain food within the alimentary canal, improving fractional digestion and absorption and reducing the excretion rate of urinary pellets (Baines *et al.* 1973). In addition, in unfed locusts, the pH of the hindgut lumen is already near the minimum luminal pH sustainable by the hindgut epithelia *in vitro* (Thomson *et al.* 1988*b*). Together, these effects limit the capacity of unfed locusts to increase acid excretion in urinary pellets (Harrison and Phillips, 1992). Therefore, non-respiratory recovery from haemolymph acid loads requires transfer of acid equivalents either to the intracellular space or to some other storage compartment within the animal. Transfer of acid equivalents to the alimentary lumen provides unfed locusts with a temporary mechanism of haemolymph pH regulation which does not compromise intracellular acid–base status or require increases in ventilation that would increase water loss. The acid equivalents stored within the alimentary lumen are presumably excreted later, when locusts feed and produce frequent urinary pellets. This hypothesis has not yet been tested.

While transfers of acid equivalents to the fore- and midgut are important mechanisms of haemolymph pH regulation after HCl injection, it is not yet known whether locusts actually utilize such mechanisms under natural conditions. Unfed locusts excrete acid equivalents, perhaps derived from oxidation of sulphur- and phosphate-containing proteins (Harrison and Phillips, 1992). Potentially, acid equivalent transfer to the fore- and midgut may function to remove non-volatile metabolic acid loads during long-term fasting (Masoro and Siegel, 1977) when faecal production is very low. While changes in midgut pH may reduce the activity of digestive enzymes (reviewed by Applebaum, 1985), digestion and absorption is virtually complete within 6 h after a meal in locusts (Baines *et al.* 1973). After digestion and absorption have been completed, suspending regulation of the pH in the alimentary lumen may be inconsequential.

This work was partially supported by NATO and Killam PDF's to J.F.H., an NSERC undergraduate research award to C.J.H.W. and an NSERC operating grant to J.E.P. J. Fewell made helpful comments on the manuscript.

References

APPLEBAUM, S. W. (1985). Biochemistry of digestion. In *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, vol. 4 (ed. G. A. Kerkut and L. I. Gilbert), pp. 279–311. Oxford: Pergamon Press.

BAINES, D. M., BERNAYS, E. A. AND LEATHER, E. M. (1973). Movement of food through the gut of fifth-instar males of *Locusta migratoria migratorioides* (R & F). *Acrida* **2**, 319–332.

BAY, C. M. H. (1978). The secretion and action of the digestive enzymes of the salivary glands of the blowfly, *Calliphora*. *J. Insect Physiol.* **24**, 141–149.

BODINE, J. H. (1925). Physiology of the Orthoptera. Hydrogen ion concentration of the blood and alimentary tract of certain Orthoptera. *Biol. Bull. mar. biol. Lab., Woods Hole* **48**, 79–82.

CHAMBERLIN, M. E. (1990). Luminal alkalinization by the isolated midgut of the tobacco hornworm (*Manduca sexta*). *J. exp. Biol.* **150**, 467–471.

DOW, J. A. T. (1981). Countercurrent flows, water movements and nutrient absorption in the locust midgut. *J. Insect Physiol.* **27**, 579–585.

DOW, J. A. T. AND O'DONNELL, M. J. (1990). Reversible alkalinization by *Manduca sexta* midgut. *J. exp. Biol.* **150**, 247–256.

HARRISON, J. M. (1988). Temperature effects on haemolymph acid–base status *in vivo* and *in vitro* in the two-striped grasshopper *Melanoplus bivittatus*. *J. exp. Biol.* **140**, 421–435.

HARRISON, J. F. (1989*a*). Ventilatory frequency and haemolymph acid–base status during short-term hypercapnia in the locust, *Schistocerca nitens*. *J. Insect Physiol.* **35**, 809–814.

HARRISON, J. M. (1989*b*). Temperature effects on intra- and extracellular acid–base status in the American locust, *Schistocerca nitens*. *J. comp. Physiol.* B **158**, 763–770.

HARRISON, J. F. AND PHILLIPS, J. E. (1992). Recovery from acute haemolymph acidosis in unfed locusts. II. Role of ammonium and titratible acid excretion. *J. exp. Biol.* **165**, 97–110.

HARRISON, J. F., PHILLIPS, J. E. AND GLEESON, T. T. (1991). Activity physiology of the two-striped grasshopper, *Melanoplus bivittatus*; gas exchange, hemolymph acid–base status, lactate production, and the effect of temperature. *Physiol. Zool.* **64**, 451–472.

HARRISON, J. F., WONG, C. J. H. AND PHILLIPS, J. E. (1990). Haemolymph buffering in the locust, *Schistocerca gregaria*. *J. exp. Biol.* **154**, 573–579.

IRVINE, B., AUDSLEY, N., LECHLEITNER, R., MEREDITH, J., THOMSON, B. AND PHILLIPS, J. (1988). Transport properties of locust ileum *in vitro*: effects of cyclic AMP. *J. exp. Biol.* **137**, 361–385.

KAFATOS, F. C. (1968). The labial gland: a salt-secreting organ of saturniid moths. *J. exp. Biol.* **48**, 435–453.

KUN, E. AND KEARNEY, E. B. (1974). Ammonia. In *Methods of Enzymatic Analysis*, vol. 4 (ed. H. U. Bergmeyer), pp. 1802–1806. New York: Academic Press.

LECHLEITNER, R. A., AUDSLEY, N. AND PHILLIPS, J. E. (1989). Composition of fluid transported by locust ileum: influence of natural stimulants and luminal ion ratios. *Can. J. Zool.* **67**, 2662–2668.

MASORO, E. J. AND SIEGEL, P. D. (1977). *Acid–base Regulation: Its Physiology, Pathophysiology and the Interpretation of Blood–gas Analysis*. Philadelphia: W. B. Saunders.

MILES, P. W. (1967). Studies of the salivary physiology of plant-bugs: transport from haemolymph to saliva. *J. Insect Physiol.* **13**, 1787–1801.

OSCHMAN, J. L. AND BERRIDGE, M. J. (1970). Structural and functional aspects of salivary fluid secretion in *Calliphora*. *Tissue and Cell* **2**, 281–310.

PHILLIPS, J. E., HANRAHAN, J., CHAMBERLIN, M. AND THOMSON, B. (1986). Mechanisms and control of reabsorption in insect hindgut. *Adv. Insect Physiol.* **19**, 330–422.

RAMSAY, J. A. (1956). Excretion by the Malpighian tubules of the stick insect, *Dixippus morosus* (Orthoptera, Phasmidae): calcium, magnesium, chloride, phosphate and hydrogen ions. *J. exp. Biol.* **33**, 697–707.

SNYDER, G. K., UNGERMAN, G. AND BREED, M. (1980). Effects of hypoxia, hypercapnia, and pH on ventilation rate in *Nauphoeta cinerea*. *J. Insect Physiol.* **26**, 699–702.

STAGG, A. P., HARRISON, J. F. AND PHILLIPS, J. E. (1991). Acid–base parameters in Malpighian tubule secretion and response to acidosis. *J. exp. Biol.* **159**, 433–447.

SWINGLE, M. C. (1931). Hydrogen ion concentration within the digestive tract of certain insects. *Ann. ent. Soc. Am.* **24**, 489–495.

THOMSON, R. B., SPEIGHT, J. D. AND PHILLIPS, J. E. (1988*a*). Rectal acid secretion in the desert locust, *Schistocerca gregaria*. *J. Insect Physiol.* **34**, 829–837.

THOMSON, R. B., THOMSON, J. M. AND PHILLIPS, J. E. (1988*b*). NH_4^+ transport in acid-secreting insect epithelium. *Am. J. Physiol.* **254**, R348–R356.

TRUCHOT, J. P. (1987). *Comparative Aspects of Extracellular Acid–base Balance*. Berlin, New York: Springer-Verlag.

WILKINSON, L. (1987). *SYSTAT: The System for Statistics*. Evanston, Illinois: SYSTAT, Inc.