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ACID–BASE TRANSPORT AND CONTROL IN LOCUST HINDGUT: ARTEFACTS CAUSED BY SHORT-CIRCUIT CURRENT

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Summary

1. The commonly used method of passing short-circuit current (I_{sc}) across insect epithelia through Ag–AgCl electrodes, without the use of salt bridges, leads to significant OH^- production at the cathode (lumen side) when high currents are applied.

2. The alkalization of the lumen previously reported when cyclic AMP was added to short-circuited locust hindgut is a result of this phenomenon rather than cyclic-AMP-mediated stimulation of acid–base transport in the hindgut.

3. When salt bridges are used to pass short-circuit current across locust hindgut, acid secretion (J_H) into the lumen equals alkaline movement (J_{OH}) to the haemocoel side, and J_H is similar under both open- and short-circuit conditions. J_H is similar (1.5 μequiv cm^{-2} h^{-1}) in recta and ilea.

4. Addition of cyclic AMP inhibits J_H across the rectum by 42–66 %, but has no effect on the ileum when salt bridges are used.

5. Electrical parameters (I_{sc}, V_t, R_t) reflecting hindgut Cl^- transport (J_{Cl}) before and after stimulation with cyclic AMP are the same whether or not salt bridges are used. We found no evidence of any coupling between J_{Cl} and J_H/J_{OH}.

Introduction

Two of the most intensively studied epithelial transport systems in insects are lepidopteran midgut (reviewed by Harvey, 1982; Harvey and Zerahn, 1972) and locust hindgut (reviewed by Phillips *et al.* 1986). In both cases, progress has resulted from the ability to study these epithelia *in vitro* under short-circuit conditions. We wish to report that the standard short-circuit current methods used in most previous studies of these insect epithelia, namely the methods of Harvey *et al.* (1967), Zerahn (1970), Wood (1972; Wood and Moreton, 1978; modified by Williams *et al.* 1978, and Hanrahan *et al.* 1984) and Mandel *et al.* (1980), are not

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suitable for studies of acid–base transport, which is a major activity of all these membranes.

We were led to reconsider our short-circuit methods because of paradoxical results on acid–base transfer across locust hindgut under open-circuit, as compared to short-circuit, conditions. Locust ileum and rectum both actively secrete H^+ (J_H) into the lumen against large pH (2 units) and electrical differences under open-circuit conditions *in vitro* and also *in situ* (Thomson *et al.* 1988*a*,*b*; Irvine *et al.* 1988). This occurs against electrochemical potential differences of up to 100 mV at the apical membrane. In support of these observations, we report in this paper that, when large electrochemical differences exist, addition of cyclic AMP to the haemocoel substantially reduces rectal J_H but luminal alkalization is never observed. In contrast, Irvine *et al.* (1988) reported that the addition of cyclic AMP caused a dramatic shift from acid secretion (J_H of 0.4 μequiv cm^{-2} h^{-1}) to alkaline secretion at high rates (J_{OH} of 8 μequiv cm^{-2} h^{-1}) across locust ileum under short-circuit conditions. In preliminary studies, Hanrahan (1982) and Hanrahan and Phillips (1982) observed a similar but smaller alkalization of the rectal lumen (J_{OH}=39 % of Cl^- influx) under the same stimulated short-circuit current conditions.

The question therefore arises as to whether short-circuiting modifies cell behaviour in locust hindgut (e.g. by unmasking an apical Cl^-/OH^- exchanger as suggested by Irvine *et al.* 1988), or whether luminal alkalization is a methodological artefact. We have found the latter to be true: when either Ag or Ag–AgCl current-passing electrodes are placed directly in the bathing saline of Ussing chambers, without the intervention of salt bridges, significant amounts of OH^- are produced at the cathode (i.e. the lumen side of all these insect epithelia) at the naturally high I_{sc} values observed across locust hindgut. It is worth recalling that even larger short-circuit currents (I_{sc}) in the same direction (i.e. anions to haemocoel, or cations to lumen) occur across lepidopteran midguts, the contents of which are very alkaline *in situ* (Dow, 1986). Electrode configurations that lack salt bridges have been used in most studies of lepidopteran midgut (but not all; e.g. Moffett, 1980) and locust hindgut. Fortunately, the failure to use salt bridges with current-passing electrodes does not influence other transport or electrical parameters (e.g. Cl^--dependent I_{sc}) previously reported for locust hindgut. Finally we report correct values for J_H obtained with salt bridges under short-circuit conditions in locust ileum and rectum, both before and after adding cyclic AMP.

Materials and methods

Adult female desert locusts (*Schistocerca gregaria* Forskål) 14–22 days beyond their final moult were used for all experiments. They were maintained at 28°C and 60 % relative humidity on a 12 h:12 h light:dark cycle, and fed daily on fresh lettuce and a dried mixture of bran, alfalfa and powdered milk.

Isolated recta or ilea were mounted as flat sheets in miniaturized versions of the Ussing-style chambers described by Williams *et al.* (1978) (similar to those of

Wood, 1972) with 2.0 rather than 5.0 ml of solution per chamber (Thomson, 1990). Saline was constantly circulated and oxygenated in both chambers by means of a gas lift pump, which maintained constant gas tension and circulation regardless of perfusion flow rates. Provision was made for the gravity-fed perfusion (4–5 ml min^{-1}) of each chamber. Typically, recta or ilea were brought to steady-state conditions (defined by stable I_{sc}; after approximately 2 h) under bilateral perfusion, and then perfusion was stopped unilaterally (but mixing was continued) during the experimental period when acid–base transfer was measured.

Rates of luminal acidification (J_H) and contraluminal alkalization (J_{OH}) were determined using a pH-stat technique (PHM 84 research pH meter, TTT 80 titrator, ABU 80 autoburette; Radiometer, Copenhagen, Denmark). J_H and J_{OH} were calculated as the rate of titrant addition (0.01 mol l^{-1} NaOH and 0.01 mol l^{-1} HNO_3, respectively) required to maintain the initial pH.

Transepithelial potential (V_t), short-circuit current (I_{sc}) and calculated resistance (R_t) were determined as described by Hanrahan *et al.* (1984). Briefly, V_t was measured between 3 mol l^{-1} KCl agar bridges located in the two chambers close to the epithelium. Short-circuit current was applied with a dual-channel automatic voltage clamp which allowed for compensation of saline resistance (see Hanrahan *et al.* 1984, for circuit description) through flat-sheet Ag or Ag–AgCl electrodes (0.29 cm^2 area) at the ends of each chamber. In other experiments these electrodes were replaced by salt bridges leading to separate containers for AgCl electrodes. These three arrangements are referred to as the Ag, Ag–AgCl and salt-bridge electrode configurations, respectively. V_t and I_{sc} were recorded on dual-channel strip-chart recorders (1242: Soltec, Sun Valley, CA).

The composition of the experimental saline, which lacked phosphate and bicarbonate, was otherwise based on that of locust haemolymph as described by Chamberlin and Phillips (1982) and Hanrahan *et al.* (1984). Salines were buffered with 2 mmol l^{-1} Mops (pK_a=7.20 at 20 °C) and vigorously aerated with 100 % O_2 for at least 2 h prior to use. This protocol maintained CO_2/HCO_3^- at the trace levels necessary for precise estimation of J_H and J_{OH} (Thomson, 1990). The pH electrodes were calibrated with Radiometer precision buffer solutions (pH±0.005) and the saline was manually titrated to pH 7.00 prior to experiments at 23±1 °C. All values reported are means±standard errors. Statistical significance was determined using paired or non-paired *t*-tests. Differences were considered statistically significant if $P<0.05$.

Results

We previously reported (Thomson *et al.* 1988*a*,*b*) that, under open-circuit conditions in Ussing chambers, unstimulated recta maintain a steady J_H of 1.54 μequiv cm^{-2} h^{-1} for at least 8 h and that this secretion is completely and quickly abolished by 1 mmol l^{-1} KCN. This *in vitro* rate compares favourably with *in situ* estimates of J_H. Short-circuiting (Ag electrode configuration) reduced J_H significantly to 0.93 μequiv cm^{-2} h^{-1}. This was somewhat puzzling since the

treatment should have very slightly enhanced J_H, given that the small opposing V_t of 6–8 mV was abolished. In the present study, when we used the salt-bridge configuration to pass current, we observed a J_H under short-circuit of 1.51 ± 0.10 μequiv $cm^{-2} h^{-1}$ ($N=6$), which is not significantly different from open-circuit results, suggesting an experimental artefact in earlier short-circuit experiments.

We then investigated preliminary reports by Hanrahan (1982) that cyclic AMP initiated alkalization on the lumen side of short-circuited recta. When Hanrahan's (1982) conditions were duplicated exactly (apart from chamber size) high rates of luminal alkalization were observed after adding 1 mmol l^{-1} cyclic AMP to the haemocoel side (Fig. 1). This J_{OH} was approximately 80 % of the simultaneous I_{sc} and rates of alkalization were virtually identical to the net Cl^- fluxes (J_{Cl}) reported by Hanrahan (1982) under similar conditions. (We reconfirmed that stimulated

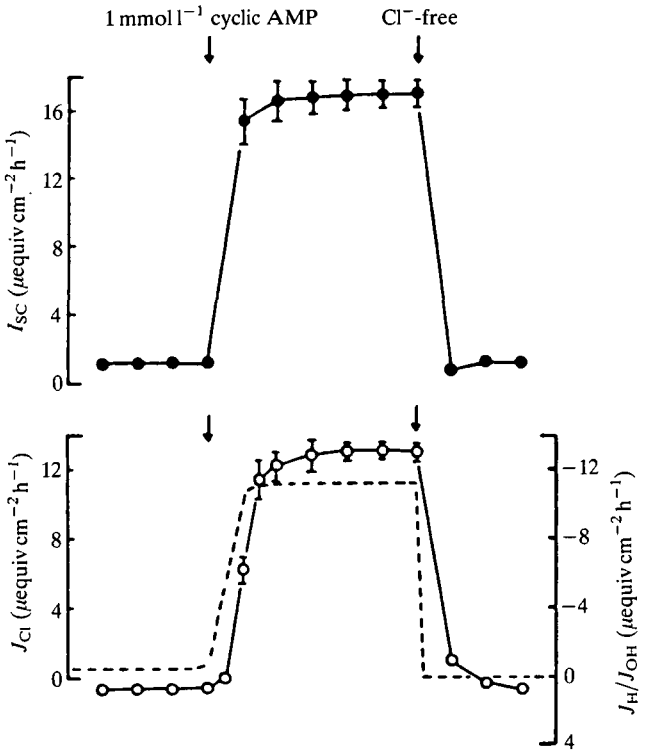

Fig. 1. Effect of contraluminal cyclic AMP and bilateral Cl^- substitution on luminal acid–base transport (J_H/J_{OH}; ○), transepithelial net Cl^- flux (J_{Cl}; dashed line) and short-circuit current (I_{sc}; ●). Positive values of J_{Cl} indicate Cl^- movement from lumen to haemocoel (Cl^- fluxes were taken from Hanrahan, 1982). Positive values of J_H/J_{OH} indicate luminal acidification, whereas negative values indicate luminal alkalization. Positive values of I_{sc} indicate net cation movement into or net anion movement out of the lumen. Salines were CO_2/HCO_3^--free. Contraluminal pH was maintained at 7.00 by continuous perfusion; luminal pH was maintained at 7.00 by pH-stat. Values are means+S.E.; $N=6$.

$I_{sc}=J_{Cl}$; data not shown.) Moreover, when luminal Cl^- was removed (gluconate substitution) bilaterally from the bath, ΔI_{sc}, ΔJ_{Cl} and J_{OH} were all abolished (Fig. 1). Other perturbations that inhibited stimulated J_{Cl} [e.g. bilateral 1 mmol l^{-1} KCN, or 1 mmol l^{-1} DPC (*N*-phenylanthranilic acid) on the haemocoel side] also inhibited J_{OH} (data not shown).

Superficially these data appeared to provide very convincing evidence for a Cl^--dependent alkalization mechanism (e.g. Cl^-/OH^- or Cl^-/HCO_3^- exchange), as suggested earlier for locust ileum, where similar results were observed (Irvine *et al.* 1988). However, this interpretation (i.e. an apical neutral anion exchanger) implies that active Cl^- transport cannot account for the observed stimulated I_{sc}, contrary to considerable earlier evidence (reviewed by Phillips *et al.* 1986).

Upon closer inspection this interpretation proved to be false. If base equivalents were being transported transepithelially, the increase in luminal pH should be accompanied by a concomitant decrease in haemocoel pH (i.e. under steady-state conditions, the change in H^+/OH^- activity on both sides of the epithelium should be of similar magnitude but opposite direction). When the above experiments were repeated, significant changes in rates of contraluminal alkalization were observed, but they were several orders of magnitude less than the rate of acidification observed in the lumen. There was never acid movement to the haemocoel side under any conditions (Table 1). This suggested that hydroxyl equivalents might have been added to the lumen from an external source rather than from the epithelium itself. To test this, Ussing chambers were set up as above without recta and 80 μA of current (a typical current under cyclic-AMP-stimulated conditions) was passed across the current-sending electrodes. As predicted, the luminal bathing saline alkalized at the same rate whether a tissue was present or

Table 1. *Effect of cyclic AMP on luminal and contraluminal acid–base transport (J_H/J_{OH}) under I_{sc} conditions when silver electrodes are used to pass the short-circuit current*

	J_H/J_{OH}		
	Luminal	Contraluminal	I_{sc} (μequiv cm^{-2} h^{-1})
Control	0.93±0.09[a]	1.47±0.10[b]	1.25±0.27[c]
+1 mmol l^{-1} cyclic AMP	−13.7±0.91[a]	0.98±0.11[b]	16.40±1.20[c]

Luminal J_H/J_{OH}, positive values indicate net acid addition to the lumen; negative values indicate net alkali addition to the lumen.

Contraluminal J_H/J_{OH}, positive values indicate net alkali addition to the contraluminal bath.

I_{sc}, short-circuit current (positive values indicate net cation movement into or net anion movement out of the lumen).

Recta were bathed bilaterally with phosphate- and CO_2/HCO_3^--free salines; pH 7.00.

Values are means±S.E.; $N=6$.

[a,c] Values with common symbols are significantly different by paired *t*-test ($P<0.001$).

[b] Values with common symbols are significantly different by paired *t*-test ($P<0.03$).

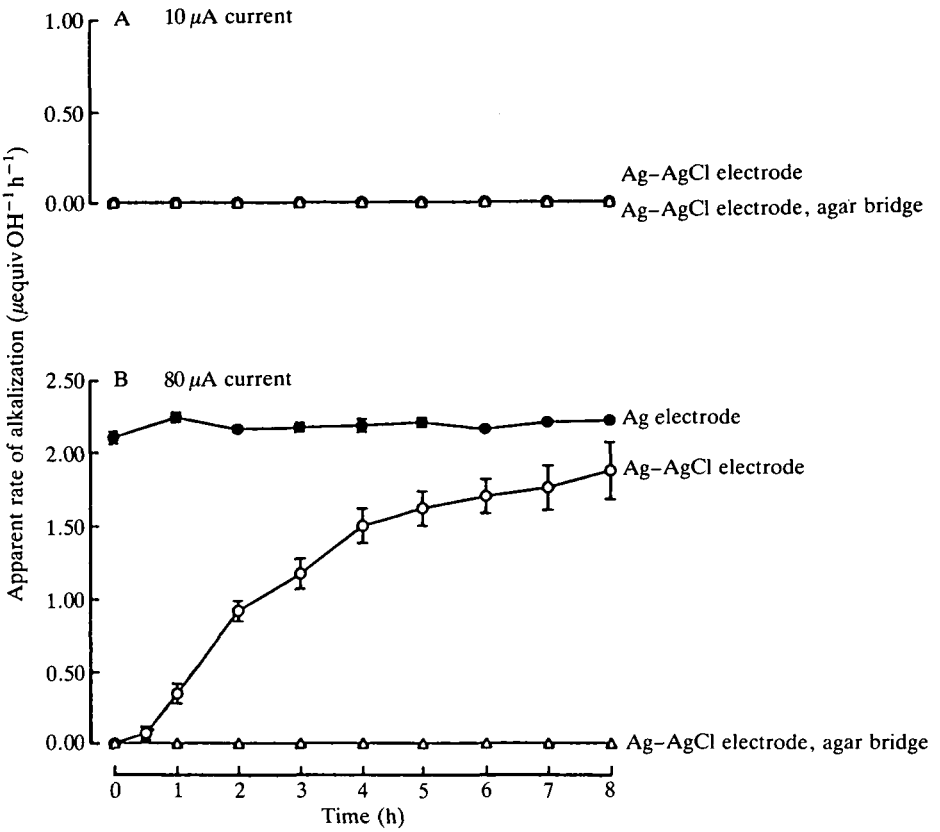

Fig. 2. Effect of electrode configuration and quantity of current passed on apparent rates of luminal alkalization. (A,B) 10 or 80 μA of current passed across the current-sending electrodes, respectively. Experimental chambers were set up without recta and filled with the standard CO_2/HCO_3^--free saline used throughout this study. Rates of alkalization were measured by pH-stat (saline pH maintained at 7.00). Values are means±S.E. (where larger than symbol); N=6.

not (Fig. 2B, Ag electrode configuration, filled circles). Therefore, the luminal alkalization observed by Hanrahan (1982) for the rectum and by Irvine *et al.* (1988) for the ileum under short-circuit conditions was clearly an experimental artefact.

The source of the alkalization was the pair of silver electrodes used to pass the short-circuit current. When a simple Ag cathode is used, bubbles of H_2 gas are formed at the electrode surface and the solution surrounding it alkalizes in proportion to the amount of current passed:

$$2H_2O + 2e^- \rightarrow H_2(g) + 2OH^-(aq).$$

At the anode (haemocoel side), Cl^- from the bath combines with Ag to form AgCl:

The combined effect of these reaction sequences is the replacement of bath Cl^- by an equal number of OH^-, thus explaining the similarity between rates of Cl^- flux, I_{sc} and alkalization. The alkalization appeared to be a cellular event modulated by cyclic AMP simply because cyclic AMP stimulated active electrogenic Cl^- transport, which in turn was accompanied by a concomitant increase in the amount of current passed between the Ag electrodes (and hence the amount of OH^- formed luminally).

This is not a new or novel observation. The problems associated with passing current across bare silver electrodes have been known to electrophysiologists for decades. Under most circumstances, problems with unwanted products of electrolysis can be eliminated by coating the silver electrode with AgCl (either electrolytically or by dipping it in molten AgCl). If Ag–AgCl electrodes are used, the reaction at the anode is the same as above, but Cl^- is electrolytically released at the cathode from the AgCl and Ag is deposited on the electrode. Theoretically, with Ag–AgCl electrodes, the quantity of Cl^- removed from the bath at the anode will be exactly matched by the quantity of Cl^- (rather than OH^-) released at the cathode. In practice, however, we found this not to be the case.

The problem with Ag–AgCl electrodes is that the formation of unwanted electrolytic by-products (e.g. OH^- at the cathode) is directly related to the quality and quantity of the AgCl coating on the electrode. Intuitively this is obvious, but empirically the quality of the coating is generally very difficult to judge, or to control completely, and it changes with time during usage. We found that protection from electrolytic by-products, as judged by OH^- production, varied significantly amongst Ag–AgCl electrodes that appeared visually very similar and which should have been very well coated with AgCl (data not shown). Moreover, the rate of pH change varied significantly (and often dramatically) with the intensity and length of time for which the current was passed (Fig. 2B; Ag–AgCl electrodes, open circles). Although freshly prepared Ag–AgCl electrodes are adequate for long periods at low current levels (10 μA; similar to unstimulated, steady-state levels for locust hindgut; Fig. 2A), the degree of protection from OH^- production is very limited and of very short duration at the high currents (80 μA, or 400 $\mu A\,cm^{-2}$) typical of stimulated locust hindgut. The I_{sc} is even greater for unstimulated lepidopteran midgut. Moreover, short-term control experiments without epithelia present in the Ussing chambers may not reveal the problem of OH^- production, which only appeared after 0.5 h. Clearly Ag–AgCl electrodes by themselves are not suitable for passing large amounts of current in weakly buffered bathing solutions where exogenously induced changes in bath pH cannot be tolerated. Adequate protection can only be obtained by agar bridges (Fig. 2B; triangles). Unfortunately, salt bridges have not been used in most previous studies of insect epithelia.

We found that electrode by-products do not influence the other transport parameters (i.e. other than J_H/J_{OH}) that we reported previously for locust rectum and ileum. For example, when we repeated Hanrahan's (1982) and Hanrahan and Phillips' (1984) experiments on short-circuited locust rectum using agar bridges,

Table 2. *Effect of current-passing electrode configuration on* I_{sc}, V_t *and* R_t *when locust recta are stimulated with cyclic AMP under* CO_2/HCO_3^-*-free conditions*

	I_{sc} (μequiv cm^{-2} h^{-1})	V_t (mV)	R_t (Ωcm^2)
Simple Ag electrodes, control saline	1.25±0.27[a]	6.3±1.1[c]	188.1±15.2[e]
+1 mmol l^{-1} contraluminal cyclic AMP	17.95±1.31[b]	41.5±2.9[d]	86.2±20.8[f]
Ag–AgCl electrodes: agar bridges, control saline	1.30±0.21[a]	6.9±1.3[c]	198.0±14.0[e]
+1 mmol l^{-1} contraluminal cyclic AMP	16.10±1.69[b]	43.0±2.1[d]	99.6±18.6[f]

I_{sc}, short-circuit current (a positive value indicates cation movement into or anion movement out of the lumen).

V_t, transepithelial potential (lumen relative to haemocoel); V_t measured at end of each treatment period.

R_t, transepithelial resistance (calculated from V_t and I_{sc} by Ohm's law).

Bath pH was maintained at 7.00 by bilateral perfusion (8 ml min^{-1}).

Values are means±S.E.; N=6 for each electrode configuration.

[a,b,c,d,e,f] Values with common symbols are not significantly different by Student's t-test (P>0.40).

the measured V_t, I_{sc} (electrogenic Cl^- transport) and R_t were completely unaffected by the change in electrode configuration, both before and after stimulation with cyclic AMP (Table 2). This was expected, because Hanrahan used Ag electrodes only after he had observed no difference from preliminary experiments using Ag–AgCl electrodes (J. W. Hanrahan, unpublished data). Ag–AgCl electrodes were used previously in our laboratory (e.g. Williams *et al.* 1978). Moreover, with the standard well-buffered saline used in previous experiments on locust hindgut (5 % CO_2, 10 mmol l^{-1} HCO_3^-) we found that saline pH changes by 0.1 unit, at most, after several hours at high current densities, when Ag electrodes are used to pass current (Fig. 3). Cl^- transport, for example, is not affected by changes in saline pH between 6.0 and 8.0 (Hanrahan and Phillips, 1982). In summary, we conclude that previous results for insect hindgut obtained using Ag or Ag–AgCl current-passing electrodes are valid unless they involved determinations of acid–base transfer, or unless mechanisms very sensitive to slight changes in luminal pH were studied.

Finally, we have re-investigated acid–base transfer across locust rectum and ileum under short-circuit conditions using the salt-bridge configuration at a bilateral pH of 7.00. Under CO_2/HCO_3^--free conditions, the acidification of the rectal lumen equals the alkalization of the haemocoel side under both open- and short-circuit conditions and these rates are not significantly changed by short-circuiting (Table 3). Moreover, under both open- and short-circuit conditions, cyclic AMP caused a significant reduction in J_H of 66 % and 42 %, respectively (Table 4). This suggests that rectal J_H is probably under hormonal control *in situ*. In contrast, 5 mmol l^{-1} cyclic AMP did not change locust ileal J_H (Table 5), which is similar in rate to unstimulated rectal J_H. N. Audsley (personal communication) in our laboratory has found that extracts of both corpora cardiaca and ventral

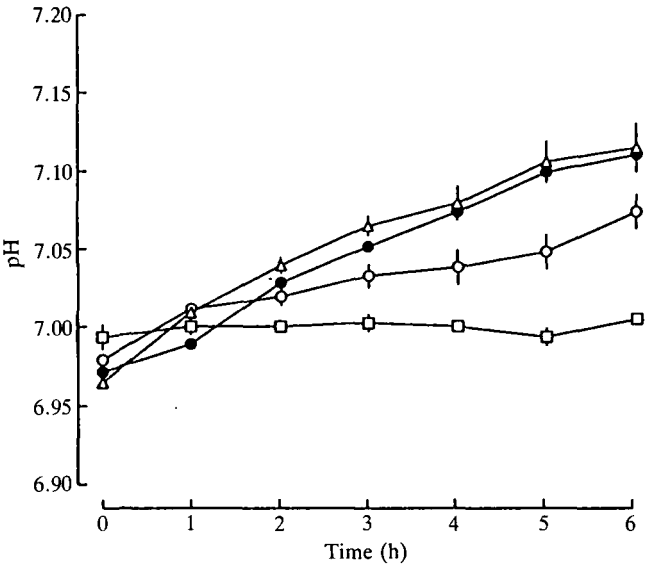

Fig. 3. Change in saline pH with time when Ussing chambers contained 2 ml of complex locust saline (with HCO_3^- present and bubbled with 5% CO_2/95% O_2) without any epithelium present: Ag electrode current-passing configuration in open (i.e. control; □) and short-circuit (80 μA applied; △) conditions; Ag–AgCl electrode configuration with applied current of 80 μA when electrodes were prepared by electrolytic coating for 5 min (●) or 20 min (○).

Table 3. *Relative rates of luminal acidification (J_H) and contraluminal alkalization (J_{OH}) by locust recta when salt bridges are used*

	J_H (μequiv cm^{-2} h^{-1})	J_{OH} (μequiv cm^{-2} h^{-1})	V_t (mV)	I_{sc} (μequiv cm^{-2} h^{-1})
Open-circuit conditions	1.47±0.08[a]	1.44±0.10[a]	6.1±1.1	–
I_{sc} conditions	1.55±0.10[b]	1.57±0.11[b]	7.3±1.4*	1.32±0.22

V_t, transepithelial potential (luminal bath relative to contraluminal bath).

I_{sc}, short-circuit current (positive values indicate net cation movement into or net anion movement out of the lumen).

Recta were bathed bilaterally with phosphate- and CO_2/HCO_3^--free salines; pH 7.00.

* Measured at end of experiment.

Values are means±S.E.; N=6.

[a,b] Values marked with common symbols are not significantly different by paired t-test (P>0.60).

abdominal ganglia 4–7 completely inhibit ileal J_H. This implies that ileal acid secretion is also under hormonal control, but that a second-messenger system other than cyclic AMP is probably involved. The nature of the putative control system *in situ* remains to be investigated.

Discussion

Both lepidopteran midgut (Dow, 1984) and locust hindgut (Thomson *et al.* 1988*a*) maintain large pH differences by transport of acid–base equivalents. Short-circuited preparations provide a means of quantitatively measuring and characterizing active transport. It is perhaps fortunate that these acid–base transfer

Table 4. *Effect of contraluminal cyclic AMP on rectal acid secretion (J_H) under open- and short-circuit current conditions*

	J_H (μequiv cm^{-2} h^{-1})	V_t (mV)	I_{sc} (μequiv cm^{-2} h^{-1})
Control (open-circuit)	1.83±0.15	12.2±1.8	–
+1 mmol l^{-1} contraluminal cyclic AMP*	0.63±0.10[a]	43.7±2.6[a]	–
Control (I_{sc})	1.40±0.14	8.9±1.3†	1.38±0.24
+1 mmol l^{-1} contraluminal cyclic AMP*	0.81±0.11[a]	46.7±2.9†,[a]	17.95±1.31[a]

J_H, rate of rectal acidification; V_t, transepithelial potential (lumen relative to haemocoel); I_{sc}, short-circuit current (a positive value indicates cation movement into or anion movement out of the lumen).

All tissues were brought to steady state in the standard CO_2/HCO_3^--free saline (see Materials and methods). Bilateral pH was maintained at 7.00 by contraluminal perfusion and luminal pH-stat. Control values of J_H, V_t and I_{sc} were determined on each tissue immediately before contraluminal cyclic AMP addition.

* Measurements made 1 h after cyclic AMP addition (the approximate time required to attain steady state, as defined by a stable I_{sc} or V_t).

† Measured at end of each treatment period.

Values are mean±S.E.; N=6 for each treatment.

[a] Values significantly different from respective controls by paired t-test (P<0.001).

Table 5. *Effect of contraluminal cyclic AMP on ileal acid secretion (J_H) under open- and short-circuit current conditions*

	J_H (μequiv cm^{-2} h^{-1})	V_t (mV)	I_{sc} (μequiv cm^{-2} h^{-1})
Control (open-circuit)	1.61±0.19	3.2±1.4	–
+5 mmol l^{-1} contraluminal cyclic AMP*	1.49±0.15	22.1±3.9[a]	–
Control (I_{sc})	1.56±0.13	2.6±1.7†	0.25±0.05
+5 mmol l^{-1} contraluminal cyclic AMP*	1.58±0.22	25.8±2.8†,[a]	10.19±0.45[a]

J_H, rate of rectal acidification; V_t, transepithelial potential (lumen relative to haemocoel); I_{sc}, short-circuit current (a positive value indicates cation movement into or anion movement out of the lumen). All tissues were brought to steady state in the standard CO_2/HCO_3^--free saline (see Materials and methods). Bilateral pH was maintained at 7.00 by contraluminal perfusion and pH-stat. Control values of J_H, V_t and I_{sc} were determined on each tissue immediately before contraluminal cyclic AMP addition.

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† Measured at end of each treatment period.

Values are mean±S.E.; N=6 for each treatment.

[a] Values significantly different from respective controls by paired t-test (P<0.05).

processes have been relatively little studied by this method, because we have shown that the procedure commonly used in the past of omitting salt bridges for current-passing electrodes leads to serious errors. Based on our recent experience, Chamberlin (1990) used agar bridges during measurements of alkaline secretion by *Manduca sexta* midgut under short-circuited conditions (see also Dow and O'Donnell, 1990). However, there is no reason to believe that the production of reaction products at current-passing electrodes in the absence of salt bridges necessarily affects previous estimates of other transport processes in these tissues, at least when well-buffered solutions were used so that saline pH did not change significantly.

When salt bridges are used, estimates of acid secretion by locust ileum or rectum under short-circuit conditions are similar to those in the open-circuit state and *in situ* (Thomson, 1990). This J_H is accompanied by equal movement of base equivalents to the haemocoel side. Contrary to earlier reports (Irvine *et al.* 1988), alkalization of the lumen is never observed, even after Cl^- transport is stimulated 10-fold to about $10\,\mu equiv\,cm^{-2}\,h^{-1}$ by addition of cyclic AMP. Cyclic AMP addition merely reduces rectal J_H. The cyclic-AMP-induced change in J_H is an order of magnitude lower than the concomitant increase in J_{Cl}, indicating that H^+ and Cl^- fluxes are not coupled. In support of this view, complete bilateral Cl^- replacement does not affect rectal J_H (Thomson, 1990; Phillips *et al.* 1986). Moreover, stimulated I_{sc} is independent of external pH over a wide range from pH 6.0 to pH 8.0 (Hanrahan and Phillips, 1982). Hanrahan and Phillips (1984) excluded Cl^- transport by exchange for HCO_3^- at the apical border of locust rectum. Our observation that, in the absence of CO_2/HCO_3^- in the saline, stimulated I_{sc} is not accompanied by any movement of base equivalents into the lumen removes the previous nagging possibility that some component of Cl^- transport might occur by OH^-/Cl^- exchange in locust ileum and rectum. In summary, the results described in this paper greatly strengthen the conclusions of Hanrahan and Phillips (1984) concerning the nature of the Cl^- pump in locust hindgut and make it much less likely that this anion transport could be driven secondarily by primary active proton secretion and recycling (i.e. by HCl co-transport).

Changes in luminal pH of the hindgut *in situ*, in response to acid injection into the haemolymph and to starvation, previously suggested that acid secretion in locust hindgut *in situ* is controlled so as to aid in whole-body pH regulation (Thomson *et al.* 1988*a*). Thomson *et al.* (1988*a*) showed that this control of rectal J_H is not mediated directly through changes in haemocoel pH. More recently, Thomson (1990) has also shown that this control is not mediated directly by changes in haemolymph P_{CO_2} or $[HCO_3^-]$. He has concluded that a significant fraction of the acid–base transport observed in the rectum must be under hormonal control. In support of this hypothesis, we found that a common second messenger of neuropeptide hormones, namely cyclic AMP, inhibits rectal acid secretion *in vitro* (Table 4). Similarly, extracts of both corpus cardiacum and ventral ganglia have been found to reduce greatly the normally basic pH and

bicarbonate levels in absorbate from locust ileal sacs (Lechleitner *et al.* 1989), while N. Audsley (personal communication) recently found that the same locust glandular extracts inhibit acid secretion by locust ileum in Ussing chambers. These studies from our laboratory provide the first evidence that acid–base transport in locust hindgut is normally under hormonal control.

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