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Electrogenic, K<latex>$^+$</latex>-Dependent Chloride Transport in Locust Hindgut

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Electrogenic, K^+-dependent chloride transport in locust hindgut

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Potassium chloride is the major salt recycled in most insect excretory systems. Ion and water reabsorption occur in the rectum by active transport of Cl^- and largely passive movement of K^+. Both these processes are stimulated several fold by a neuropeptide hormone acting via cyclic AMP (cAMP). This Cl^- transport process was investigated by using intracellular ion-sensitive microelectrodes, radiotracer flux measurements, voltage clamping, ion substitutions and inhibitors. The mucosal entry step for Cl^- is energy-requiring and highly selective, and is stimulated directly by cAMP and luminal K^+. Under some experimental conditions, measured electrochemical potentials for cations across the mucosal membrane are too small to drive Cl^- entry by NaCl or KCl cotransport mechanisms; moreover, net $^{36}Cl^-$ flux is independent of the apical Na^+ potential. Similarly no evidence for a HCO_3^--Cl^- exchange was obtained. We conclude that Cl^- transport in locust gut is different from mechanisms currently proposed for vertebrate tissues.

Review of KCl transport across insect epithelia

Many insect epithelia are unusual in that they secrete or reabsorb KCl-rich fluids of low Na^+ content (Maddrell 1978; Harvey 1982). Such secretion is found in Malpighian tubules, lepidopteran midgut, and integument during moulting: reabsorption occurs in locust midgut and rectum, reabsorptive segments of Malpighian tubules and salivary glands. For example, a K^+ pump in the luminal membrane of Malpighian tubules of insects (e.g. desert locust) drives secretion of an isosmotic primary urine (140 mM K^+, 20 mM Na^+, 90 mM Cl^-) with a high K^+:Na^+ ratio compared with that of the haemolymph (12 mM K^+, 103 mM Na^+, 106 mM Cl^-). This fluid passes into the hindgut, where regulation of haemolymph and urine composition is achieved by selective reabsorption of water, ions (largely KCl) and metabolites primarily in the rectum (reviewed by Phillips 1981). We describe in this paper an unusual mechanism of KCl reabsorption in the locust rectum, and this may also be the basis for KCl reabsorption in other insect epithelia (see for example, Cooper *et al.* 1980). A review of earlier observations on locust recta is first in order (see Phillips 1980, 1981; Hanrahan & Phillips 1982).

The main anion, Cl^-, is actively reabsorbed from the rectal lumen against concentration differences of up to 20-fold and electrical potential differences (V_t) of 30 mV *in situ*. That this anion transport is electrogenic and is the source of the transepithelial potential difference has been confirmed *in vitro* by ion-substitutions and measurements of $^{36}Cl^-$ fluxes under short circuit conditions (Williams *et al.* 1978). In contrast, there is initially a large concentration difference between mucosal (M) and serosal (S) sides (140:12 mM K^+) and an electropotential difference (p.d.) favouring mostly passive reabsorption of K^+; i.e. by electrical coupling to Cl^- transport. In confirmation, when stimulated recta are bathed bilaterally in a high Na^+ (110 mM) low K^+ (8 mM) saline *in vitro*, net $^{42}K^+$ and $^{36}Cl^-$ fluxes are equal (4.5 μequiv cm^{-2} h^{-1},

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M→S) under open circuit conditions, but the net active flux of $^{36}Cl^-$ ($J^{net}_{Cl^-}$) is ten times greater than the small net $^{42}K^+$ flux ($J^{net}_{K^+}$) under short-circuit conditions (Hanrahan & Phillips 1982). KCl is absorbed several times faster than NaCl from ligated recta *in situ* (Phillips 1964), confirming the preference of this epithelium for K^+ over Na^+ as a counter-ion for Cl^- transport.

Since levels of Na^+ in fluid entering the rectal lumen are so low, net active Na^+ absorption is quantitatively much less important and occurs apparently by means of a typical serosal Na–K–ATPase. The dissipative entry of Na^+ into rectal cells across the M border is probably used largely to drive cotransport of amino acids (Phillips 1980). Together these observations suggest that there is probably not adequate Na^+ available to drive Cl^- absorption by a Na^+-coupled cotransport process.

Salt reabsorption from ligated locust recta is regulated *in situ* in response to salt depletion or loading (Phillips 1964). We have more recently used short-circuited recta *in vitro* to demonstrate and purify a new neuropeptide hormone (CTSH; relative molecular mass 8000) from the corpus cardiacum and haemolymph of locusts that stimulates active Cl^- absorption ($\Delta J^{Cl^-}_{net}$) severalfold and consequently also the short-circuit current (ΔI_{sc}) and p.d. This hormone increases cAMP levels in rectal tissue by 2.5-fold, and exogenous cAMP (1 mM) completely mimics the actions of a maximum dose of CTSH (reviewed by Phillips 1981; Phillips *et al.* 1980, 1981). Under all experimental conditions studied so far, $\Delta J^{Cl^-}_{net}$ equals ΔI_{sc} after stimulation. The rectal anion pump is highly selective for Cl^-: of nine anions substituted for Cl^-, only Br^- partly sustains ΔI_{sc} caused by cAMP at 50 % of control values (Hanrahan 1982). Under short-circuited conditions, cAMP does not affect $^{22}Na^+$ fluxes (Spring & Phillips 1980) but does increase both unidirectional fluxes of $^{42}K^+$ by fourfold, indicating an increase in K^+ permeability (P_{K^+}) (Hanrahan 1982). Conductance studies indicate that this increase in P_{K^+} occurs largely at the M border. Very high luminal K^+ levels (more than 100 mM) exert an inhibitory effect both on active Cl^- transport and also on P_{K^+}, thereby opposing the actions of cAMP. Flat-sheet cable analysis and ion substitutions reveal large K^+ and Cl^- conductance increases in the M and S borders respectively after stimulation. In the presence of cAMP, only 5 % of transepithelial current flows by paracellular routes, indicating that locust rectum is a tight epithelium with low electrical resistance (100–200 Ω cm²) (Hanrahan 1982; Hanrahan & Phillips 1982; Hanrahan *et al.* 1982*b*).

In summary, electrogenic, K^+-dependent Cl^- transport is the predominant active mechanism in locust rectum and possibly in other insect reabsorptive epithelia. Two types of Cl^- transport are now widely recognized in vertebrate epithelia: Na^+-coupled systems where Cl^- entry into epithelial cells involves cotransport with Na^+ (or functionally equivalent parallel Na^+–H^+ and Cl^-–OH^- exchanges), and bicarbonate-coupled systems where Cl^- entry occurs in exchange for cell HCO_3^- (Frizzell *et al.* 1979; Field, this symposium; reviewed by Hanrahan & Phillips 1982). We present evidence in the remainder of this paper that Cl^- transport in locust rectum does not occur by either of these methods. We also examine the mechanism of K^+-stimulation of rectal Cl^- transport.

Methods

Locust recta were mounted as flat sheets in modified Ussing chambers at 22–24 °C as described by Williams *et al.* (1978) and short-circuited with compensation for series resistance of the saline (Hanrahan 1982). The control saline resembled locust haemolymph: 114 mM Cl^-, 110 mM Na^+, 10 mM K^+, 10 HCO_3^-, 10 Mg^{2+}, 5 Ca^{2+}, 10 mM glucose. Eleven amino acids were

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included at their natural levels because proline, which is actively absorbed from the rectal lumen, is the major metabolic substrate in this tissue (Chamberlin & Phillips 1982). Na^+-free or HCO_3^--free saline were prepared by substituting choline or *N*-methyl-D-glucamine. K_2SO_4 was replaced by an osmotically equivalent amount of sucrose in K^+-free saline. Transepithelial $^{36}Cl^-$ fluxes and I_{sc} were measured simultaneously over long periods after equilibrating recta in the appropriate saline bubbled with 95% O_2 + 5% CO_2 or 100% O_2 (HCO_3^-/CO_2-free saline) for 4 h to ensure steady-state conditions. Chloride transport and I_{sc} were stimulated by adding 1 mM cAMP to the S side.

Intracellular measurements were made by using double-barrelled ion-selective microelectrodes (less than 1 μm tip diameter) similar to those of Fujimoto & Kubota (1976). The following liquid ion-exchangers were used: Orion 92-17102 (Cl^-), Corning 477317 (K^+) and acid monensin in Corning 477317 (Na^+). Reference barrels were filled with either 1 M Na acetate (K^+, Cl^- electrodes) or 0.5 KCl (Na^+ electrodes). Electrodes had average slopes of 52–56 mV per decade of ion activity. Interference from other ions was assessed or corrected for as described by Hanrahan & Phillips (1982), who also outline criteria used for successful cell impalement. A modified Ussing chamber permitted intracellular measurements on recta with their cuticle removed, under both open-circuited and short-circuited conditions and with independent perfusion of M and S sides (Hanrahan *et al.* 1982*a*). Apical (M) and basal (S) membrane potentials (V_a and V_b respectively), differential intracellular ion-selective electrode potentials (V_{Cl^-}, V_{K^+}, V_{Na^+}), transepithelial potentials (V_t) and the voltage-divider ratio (ratio of voltage deflexions, $\Delta V_a/\Delta V_b$, during transepithelial current pulses) were all recorded by using a multichannel recorder.

Consideration of NaCl cotransport models

NaCl cotransport models (Frizzell *et al.* 1979) predict that Cl^- transport should stop when (*a*) external Na^+ is absent, or (*b*) serosal Na^+ transport is inhibited with ouabain, or (*c*) a NaCl (or Na^+, K^+, 2 Cl^- (Geck *et al* 1980)) co-entry step at the M border is inhibited with furosemide. (*d*) The rate of Cl^- transport should depend on the magnitude of the mucosal electrochemical gradient favouring Na^+ entry, and (*e*) a stoichiometric relation might be expected between this dissipative Na^+ gradient and the one opposing Cl^- entry at the M border (see, for example, Duffey *et al.* 1978). (*f*) Finally, a 1:1 stoichiometry of $^{22}Na^+$ and $^{36}Cl^-$ influxes across the M membrane is implied because this process is generally electroneutral (e.g. rabbit ileum (Nellans *et al.* 1973)). We have tested all these predictions.

A large Cl^--dependent I_{sc} (more than 10 μequiv cm^{-2} h^{-1}) persisted during the first 75 min after exposing stimulated recta bilaterally to nominally Na^+-free saline (figure 1). The measured external Na^+ remained well below 200 μM during these experiments and in several instances did not exceed 1 μM. Even after repeated changes of Na^+-free saline over a 4 h period, cAMP still caused a four-fold increase in $J_{net}^{Cl^-}$ and I_{sc} (figure 2). However, these increases were somewhat smaller (not significant; $P > 0.1$) because amino acid absorption is Na^+-dependent and therefore prolonged exposure to Na^+-free saline inhibits rectal metabolism. For example, a halving of I_{sc} is produced simply by incubating recta in control saline (110 mM Na) lacking proline. Exposing stimulated recta bilaterally to 1 mM ouabain or furosemide for 1–2 h does not change the Cl^--dependent I_{sc} (table 1).

We measured intracellular Na^+ and Cl^- activities and mucosal potential (V_a) when V_t

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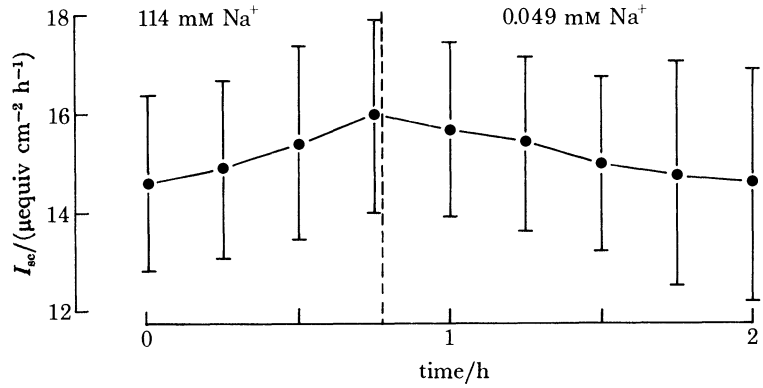

FIGURE 1. Chloride-dependent I_{sc} across cAMP-stimulated locust recta when control saline (114 mM Na^+) was replaced bilaterally with nominally Na^+-free saline (replaced repeatedly with fresh Na^+-free saline). Over 90 % of this I_{sc} could be abolished within 2 min by substituting gluconate for all Cl^- in the saline.

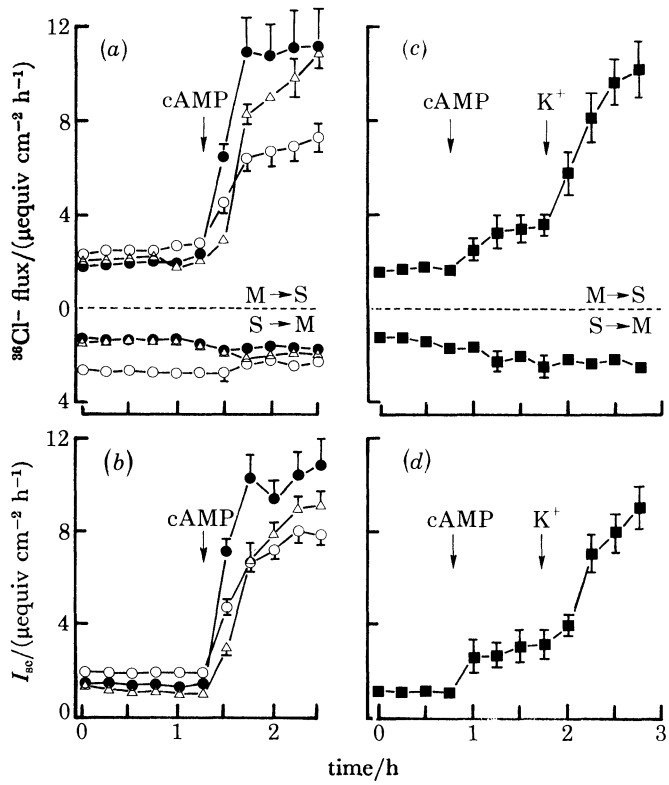

FIGURE 2. The effect of adding 1 mM cAMP on unidirectional $^{36}Cl^-$ fluxes (*a*) and I_{sc} (*b*) after pre-incubating short-circuited locust recta for 4 h in normal (●), Na^+-free (○) or HCO_3^-/CO_2-free (△) salines. The mucosal (M) to serosal (S) flux increased severalfold in all cases, while back fluxes (S→M) were unchanged. Stimulation of forward flux and I_{sc} ((*c*) and (*d*) respectively) were reduced by 70 % in K-free saline, but both parameters were subsequently restored to control levels when 10 mM K^+ was added bilaterally. The increase in I_{sc} was accounted for, within experimental error, by net Cl^- flux (M→S) under all conditions. (From Hanrahan & Phillips (1982).)

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TABLE 1. EFFECT OF INHIBITORS AFTER 1–2 h EXPOSURE ON Cl^-–DEPENDENT I_{sc} ACROSS cAMP-STIMULATED RECTA

(Means ± s.e.m.; from Hanrahan & Phillips (1982).)

inhibitor (n) (1 mM)	I_{sc}/(μequiv cm^{-2} h^{-1}) control	I_{sc}/(μequiv cm^{-2} h^{-1}) inhibitor	percentage inhibition
ouabain (12)	10.7 ± 1.0	10.0 ± 1.0	0
furosemide (8)	8.4 ± 0.8	8.1 ± 1.0	0
acetazolamide (4)	9.1 ± 1.0	8.6 ± 0.9	0
SITS	5.6 ± 0.5	5.6 ± 0.5	0
azide (5)	7.5 ± 1.1	0.29 ± 0.1*	96

*, $P < 0.001$.

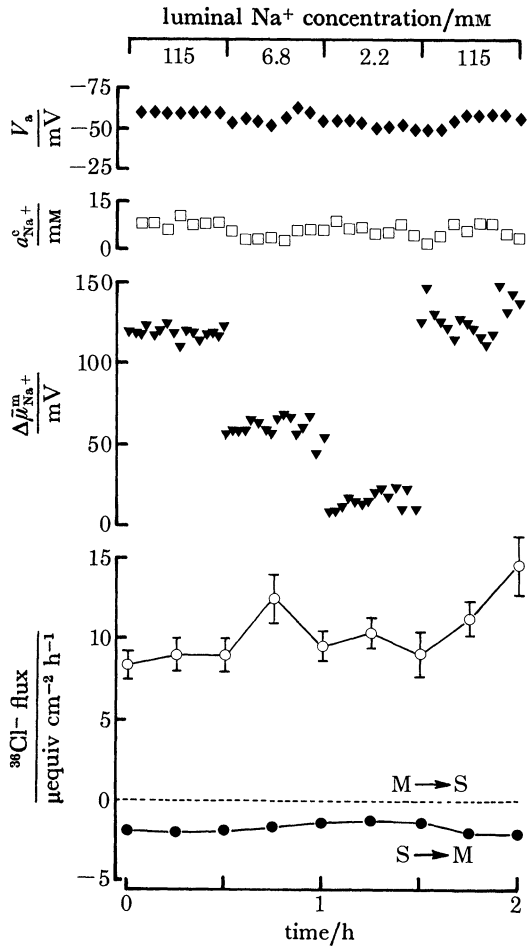

FIGURE 3. Relation between electrochemical potential difference for Na^+ ($\Delta\bar{\mu}^m_{Na+}$) across the mucosal membrane and steady-state unidirectional $^{36}Cl^-$ fluxes across cAMP-stimulated locust recta, when transepithelial p.d. was clamped at 0 mV. Na^+ in normal saline (bathing both sides initially) was replaced stepwise on the lumen side with *N*-methyl-D-glucamine, and luminal Na^+ levels were measured. Recta were repeatedly impaled with double-barrelled microelectrodes to measure mucosal membrane potential (V_a) and intracellular Na^+ activity (a^c_{Na+}), corrected for measured K^+ activity under these conditions. Values of $\Delta\bar{\mu}^m_{Na+}$ were calculated from these data. A typical experiment is shown where each point (◆, □) represents one cell. $^{36}Cl^-$ fluxes (± s.e. where larger than symbol) were measured in identical experiments and were independent of the inward sodium gradient across the mucosal membrane. (From Hanrahan & Phillips (1982).)

was clamped at 0 mV and luminal Na^+ was reduced stepwise and then returned to control levels, all in the presence of cAMP. The net electrochemical difference for Na^+ across the M border ($\Delta\bar{\mu}^{m}_{Na^+}$) was calculated from these data. Chloride fluxes were measured in identical experiments. As shown in figure 3, active $J^{Cl^-}_{net}$ did not change significantly when $\Delta\bar{\mu}^{m}_{Na^+}$ was decreased from 118 ± 0.9 mV (control) to 16 ± 1.3 mV (2.2 mM luminal Na^+). The $\Delta\bar{\mu}^{m}_{Na^+}$ favouring Na^+ entry and the corresponding gradient ($-\Delta\bar{\mu}^{m}_{Cl^-}$) opposing Cl^- entry into the

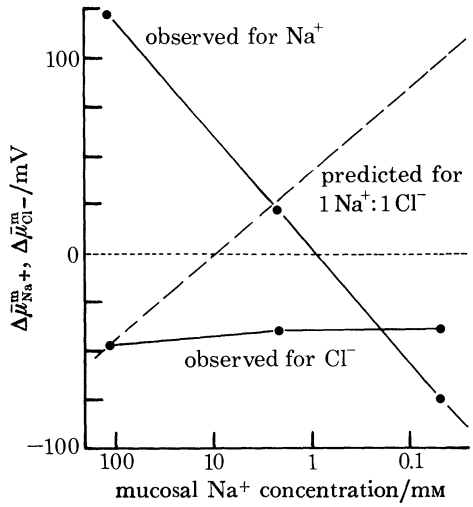

FIGURE 4. Relation between net electrochemical potential differences for Na^+ ($\Delta\bar{\mu}^{m}_{Na^+}$) and Cl^- ($\Delta\bar{\mu}^{m}_{Cl^-}$) across the mucosal border of cAMP-stimulated recta with V_t clamped at 0 mV. A positive sign indicates dissipative and a negative sign indicates energy-requiring steps for net ion absorption into the cell. Recta were bathed initially in control saline bilaterally and then mucosal Na^+ levels were reduced stepwise: values are for new steady-state conditions. The dashed line shows the predicted relation for 1:1 coupling of uphill Cl^- entry to downhill Na^+ entry into the cell. The observed $\Delta\bar{\mu}^{m}_{Cl^-}$ opposing Cl^- entry does not change as a function of either $\Delta\bar{\mu}^{m}_{Na^+}$ or mucosal Na levels, or when $\Delta\bar{\mu}^{m}_{Na^+}$ favours a net flux of Na^+ from cell to mucosa (i.e. below 1 mM Na^+; ± s.e. is included within thickness of points), (From Hanrahan (1982).).

cell at the M border in these experiments is shown in figure 4. The value of $\Delta\bar{\mu}^{m}_{Na^+}$ declines linearly by 58 mV per decade decrease in luminal Na^+ activity because V_a does not change significantly (i.e. M border $P_{K^+} \gg P_{Na^+}$). When luminal Na^+ falls below 1 mM, $\Delta\bar{\mu}^{m}_{Na^+}$ actually favours the exit of Na^+ from cell to lumen so that there is no longer a $\Delta\bar{\mu}^{m}_{Na^+}$ to drive Cl^- entry. Over this whole range of $\Delta\bar{\mu}^{m}_{Na^+}$, the $-\Delta\bar{\mu}^{m}_{Cl^-}$ opposing Cl^- entry remains relatively constant at 38–50 mV and we have seen that $J^{Cl^-}_{net}$ also does not change (figure 2).

We used everted rectal sacs in control saline to measure initial influxes of $^{22}Na^+$ and $^{36}Cl^-$ across the M border, with corrections made for extracellular space by using [^{3}H]mannitol (Hanrahan & Phillips 1982). Cyclic AMP caused a sixfold increase in $^{36}Cl^-$ influx from 0.8 ± 1.0 to 4.9 ± 1.6 nequiv per milligram tissue wet mass per minute, whereas $^{22}Na^+$ influx (0.8 nequiv mg^{-1} min^{-1}) remained unchanged and small. In summary, we have tested six predictions of NaCl cotransport models and consistently obtained negative or unfavourable results.

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Consideration of HCO_3^-/Cl^- exchange

Microelectrode studies (figure 4) reveal that the mucosal entry step for Cl^- is the active one, while there is a favourable net gradient ($\Delta\bar{\mu}^s_{Cl^-}$ of 23 mV) for passive exit of Cl^- from rectal cells at the S border (Hanrahan & Phillips 1982). Does uphill Cl^- entry occur in exchange for HCO_3^- secreted into the rectal lumen by a mechanism inhibited by SITS (4-acetamide-4′-isothiocyano-stilbene-2,2′-disulphonic acid), as in several vertebrate epithelia (reviewed by Hanrahan & Phillips 1982)? If the required HCO_3^- is derived from respiratory CO_2 and catalyzed by carbonic anhydrase, the fate of H^+ produced by this reaction must also be explained. Removal of exogenous HCO_3^-/CO_2 is thought to reduce intracellular HCO_3^- levels (see, for example, Garcia-Diaz & Armstrong 1980) and therefore might be expected to inhibit HCO_3-dependent Cl^- transport. We tested these predictions on locust recta bathed in nominally HCO_3^-/CO_2-free salines, which were weakly buffered to near neutrality by amino acids.

Removal of exogenous HCO_3^-/CO_2 has no affect on I_{sc} and $J^{Cl^-}_{net}$ across cAMP-stimulated recta (figure 2); therefore any HCO_3^- for a postulated HCO_3^-/Cl^- exchange would have to originate from metabolic CO_2. Chamberlin (1981) in our laboratory measured O_2 consumption of locust recta under virtual short-circuit conditions in the presence of cAMP. Assuming that all metabolic CO_2 could be made available to a mucosal anion exchange mechanism, which is most unlikely, the maximum possible efflux of HCO_3^- would be 3.2 μequiv cm^{-2} h^{-1} or about a third of the measured $J^{Cl^-}_{net}$. Moreover, neither 1 mM SITS or acetazolamide, an inhibitor of carbonic anhydrase, had a significant affect on Cl^--dependent I_{sc} in the presence of cAMP (table 1).

These results are perhaps not surprising because an anion exchange process could not explain why ΔI_{sc} equals $\Delta J^{Cl^-}_{net}$ after stimulation, unless there is an equally large net flux of another ion species to cancel out the electrical contribution of HCO_3^-. There is no obvious ionic candidate. Because the pH of the M salines increases from 7.1 to 7.4 and at a slower rate than $\Delta J^{Cl^-}_{net}$ after stimulation, while the S side also becomes slightly alkaline, the fate of H^+ from any hydration of metabolic CO_2 cannot be explained. This slow alkalization of saline may be due to ammonia released from rectal tissue (Hanrahan 1982).

In summary, there is no reason to believe that the major component of Cl^- transport in locust rectum occurs by exchange for bicarbonate at the M border.

Potassium stimulation of chloride transport

Because K^+ is the major cation that enters and is reabsorbed from the rectal lumen *in situ*, we studied the influence of external K^+ levels on $J^{Cl^-}_{net}$ under short-circuit conditions. In the absence of external K^+, both ΔI_{sc} and $\Delta J^{Cl^-}_{net}$ initiated by cAMP are reduced by 70 % of controls and both these parameters of transport can be fully restored by adding back 10 mM K^+ to the external saline (figure 2). Stepwise additions of K^+ to saline containing 110 mM Cl^- reveals that K^+ stimulation of Cl^--dependent I_{sc} obeys Michaelis–Menten kinetics with an apparent activation constant of 6 mM K^+ (Hanrahan & Phillips 1982). Above 100 mM, K^+ has an increasing inhibitory effect on I_{sc}, which is reversible (figure 5; note that ΔI_{sc} still equals $\Delta J^{Cl^-}_{net}$ at 410 mM K^+).

In another series of experiments with the use of stimulated and short-circuited recta, we studied the influence of cations on the kinetics of $^{36}Cl^-$ fluxes. Both the M→S flux of Cl^-

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($J_{ms}^{Cl^-}$) and I_{sc} increased hyperbolically as Cl^- levels were raised bilaterally from 0 to 114 mM. In contrast, the much smaller back flux of $^{36}Cl^-$ ($J_{sm}^{Cl^-}$) increased linearly with concentration, as expected for passive diffusion. Increasing the external K^+ concentration from 0 to 100 mM increased both the Cl^- concentration for half-maximal Cl^- transport (K_t) by fourfold and the maximum rate of net Cl^- transport ($J_{max}^{Cl^-}$) by more than sixfold (table 2) (Hanrahan &

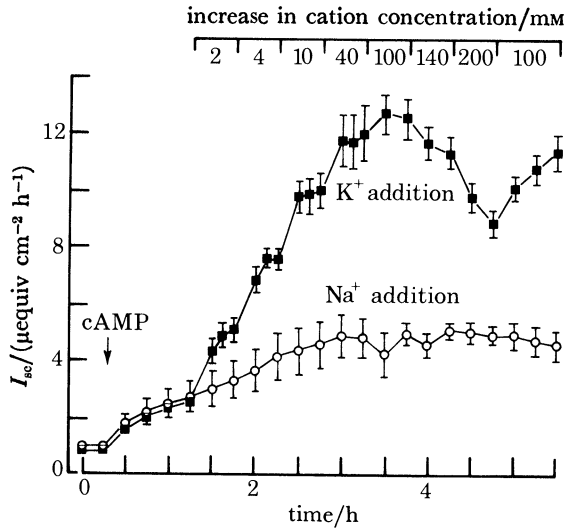

FIGURE 5. After pre-incubation of short-circuited recta for 4 h in K^+-free saline, 1 mM cAMP was added to the serosal side of the recta to stimulate Cl^- transport. K^+ or Na^+ methyl sulphate were then added stepwise to both sides of short-circuited recta. K^+ but not Na^+ caused a large stimulation of Cl^--dependent I_{sc}. The increase in net $^{36}Cl^-$ flux accounted for ΔI_{sc} at both 10 and 140 mM K^+ (Hanrahan 1982). Note the reversible inhibition of I_{sc} above 100 mM K^+. (From Hanrahan & Phillips (1982).)

TABLE 2. INFLUENCE OF EXTERNAL K^+ ON KINETICS OF NET $^{36}Cl^-$ FLUX ACROSS cAMP-STIMULATED, SHORT-CIRCUITED RECTA

(Means ± s.e.m.; from Hanrahan & Phillips (1982).)

	K_t		$J_{max}^{Cl^-}$
[K]/mM	(mM Cl^-)	(n)	μequiv Cl^- cm^{-2}h^{-1}
0	22.7 ± 4.0	(6)	3.5 ± 0.7
10	60.2 ± 8.7	(10)	14.9 ± 1.9
100	99.6 ± 13.4	(7)	23.1 ± 5.3

Phillips 1982). Hill plots of $J_{net}^{Cl^-}$ were linear with slopes near unity, indicating non-cooperative Cl^- interactions (Hanrahan & Phillips 1980*b*). The selectivity sequence for cation activation of Cl^--dependent I_{sc} was 1.0 K^+ > 0.6 Rb^+ > 0.5 Cs^+ > 0.1 NH_4^+ > 0.03 Na^+, which is consistent with a site with moderately weak field strength (Hanrahan 1982). Low external K^+ concentrations are stimulatory only on the luminal side. We shall now consider evidence from microelectrode studies that this cation activation site is located externally at the Cl^- pump on the mucosal membrane (Hanrahan 1982; Hanrahan & Phillips 1982).

Cyclic AMP must stimulate the apical electrogenic Cl^- pump directly because the net electrochemical gradient ($-\Delta\bar{\mu}_{Cl^-}^{m}$) opposing Cl^- entry across the apical membrane is increased

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by 18 mV at the same time that $J_{net}^{Cl^-}$ simultaneously increases by tenfold (figure 6). This $\Delta\bar{\mu}_{Cl^-}^{m}$ value results from increases in both intracellular Cl^- activity (a change of 16 mM) and the V_a (a change of 7 mV) opposing Cl^- entry. K^+ must enhance Cl^- absorption by a direct effect on the mucosal Cl^- pump, rather than by simply electrically short-circuiting the potential (V_a) against which Cl^- transport must work, because placing recta in K-free saline with cAMP causes drastic decreases in both the $-\Delta\bar{\mu}_{Cl^-}^{m}$ opposing Cl^- transport (figure 6) and also $J_{net}^{Cl^-}$

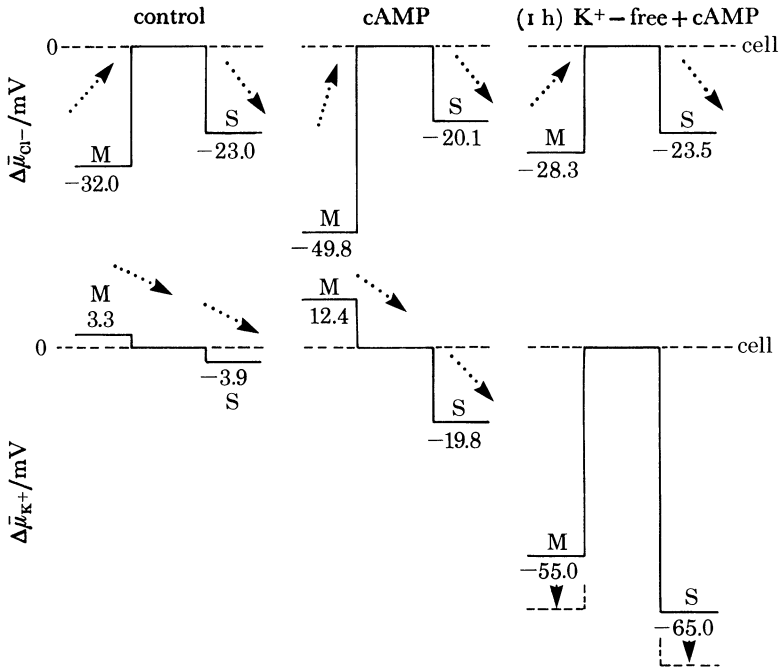

FIGURE 6. Electrical and chemical potential differences across mucosal (M) and serosal (S) cell borders of locust recta were measured with double-barrelled ion-selective microelectrodes under open-circuit conditions in control saline (10 mM K^+) without (control condition) or with (cAMP condition) 1 mM cAMP, or in K^+-free saline with 1 mM cAMP (K^+-free + cAMP condition). From these data (40–97 cells, 5–10 recta) (Hanrahan & Phillips 1982; Hanrahan 1982) the net electrochemical potential differences for Cl^- ($\Delta\bar{\mu}_{Cl^-}$) and K^+ ($\Delta\bar{\mu}_{K^+}$) across M and S borders were calculated relative to the cell (= 0 mV). Arrows indicate direction of open-circuit net $^{36}Cl^-$ and $^{42}K^+$ fluxes in parallel experiments. These fluxes were stimulated by K^+ and cAMP, as in the short-circuit state (shown in figure 2).

(figure 2). Adding 2 mM K^+ to the serosal side of K^+-depleted recta restores intracellular K^+ activity to control levels (60 mM) but does not increase $J_{net}^{Cl^-}$. Conversely, adding 2 mM K^+ only to the mucosal side in similar experiments does not restore intracellular K^+ activity (which remains at 10 mM) but does stimulate $J_{net}^{Cl^-}$ by severalfold (Hanrahan 1982). Together these results provide good evidence that K^+ stimulates at an external site associated with the electrogenic Cl^- pump in the mucosal membrane. This presumably ensures that the Cl^- pump does not expend energy in Cl^- transport when there are not adequate levels of an appropriate counter-ion (i.e. K^+). Likewise, excess absorption of KCl is prevented because very high K levels (i.e. above 100 mM) decrease both active Cl^- transport and mucosal P_{K^+} (Hanrahan 1982; Hanrahan & Phillips 1982).

The stimulatory effects of K^+ on Cl^- transport are reminiscent of enzyme activation.

Chloride entry is apparently not driven by a KCl cotransport process because the mucosal electrochemical difference for K^+ ($\Delta\bar{\mu}^{m}_{K^+}$) is only 25 % of $-\Delta\bar{\mu}^{m}_{Cl^-}$ under open-circuit conditions (figure 6). Moreover, $\Delta\bar{\mu}^{m}_{K^+}$ is less than 4 mV under short-circuit conditions (Hanrahan 1982) into control saline containing cAMP: the corresponding $-\Delta\bar{\mu}^{m}_{Cl^-}$ opposing Cl^- entry is 50 mV. This is also true when V_t is clamped to 0 mV and the M side is bathed with nominally Na^+-free saline. Under these conditions, the combined $\Delta\bar{\mu}^{m}_{Na^+}$ and $\Delta\bar{\mu}^{m}_{K^+}$ is in the wrong direction to drive Cl^- entry. The small $\Delta\bar{\mu}^{m}_{K^+}$ is a consequence of the high permeability of this membrane to K^+. For example, V_a decreases by more than 50 mV per decade increase in mucosal K^+ concentration (Hanrahan 1982).

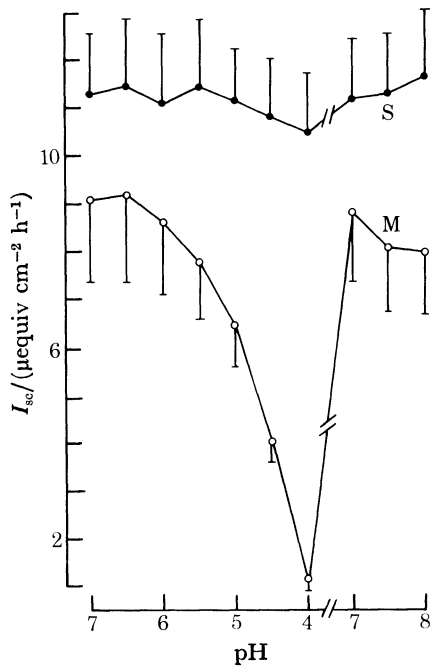

FIGURE 7. The effect of external pH on Cl^- dependent I_{sc} across cAMP-stimulated recta in control saline. Mucosal (M) and serosal (S) pH were varied separately in a stepwise manner fom pH 7.0 to 4.0, and back to 8.0. Saline pH was buffered with 20 mM phosphate and normal amino acids (means ± s.e.m.; n = 6–8).

When the pH of the mucosal saline is varied reversibly from 8.0 to 4.0 (a physiological range), Cl^--dependent I_{sc} only changes below pH 5.5 (figure 7). If we assume typical values for intracellular pH (7.0–7.4), this experiment indicates that uphill entry of Cl^- at the M border cannot be driven by mucosal proton gradients. Having excluded all the obvious potential ion gradients that might drive apical Cl^- transport by a secondary active process, we are left with the hypothesis of a primary Cl^- pump. An anion ATPase that is sensitive to Cl^- has been recently reported in rectal tissue of desert locust (Herrera *et al.* 1978) and dragonfly larvae (Gassner & Komnick 1982), as well as in several vertebrate tissues (reviewed in Hanrahan & Phillips 1982). It remains to be seen if this anion-ATPase is localized in the mucosal membrane of locust rectum and whether it exhibits K^+-dependent kinetics similar to those suggested by flux studies described in this paper. Whether a primary or secondary active process, Cl^- transport in locust rectum apparently does not conform to NaCl cotransport or HCO_3^-–Cl^- countertransport models currently proposed for many vertebrate epithelia.

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