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KCl TRANSPORT ACROSS AN INSECT EPITHELIUM: CHARACTERIZATION OF K-STIMULATED Cl ABSORPTION AND ACTIVE K TRANSPORT

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SUMMARY

The kinetics of ^{36}Cl fluxes across cAMP-stimulated, short-circuited locust rectum were studied. Raising external K^+ from 0 to 100 mM increased both K_t and V_{max} for net Cl transport (J^{Cl}_{net}) by four- to six-fold. Hill plots of J^{Cl}_{net} indicated non-cooperative Cl interactions. The sequence for cation stimulation of J^{Cl}_{net} was $K > Rb > Cs > Na > NH_4$. Low levels of K were stimulatory only when added to the mucosal side. Cyclic AMP (cAMP) caused a small active absorption of K, although this was minor compared to the four-fold increase in transepithelial K diffusion (P_K). Neither cAMP stimulation of J^K_{net} nor of P_K was sensitive to Cl removal, suggesting that K-stimulated Cl absorption and K transport are not mediated by the same co-transport mechanism. Potassium is the counter-ion for electrogenic Cl transport because J^K_{net} was less than 10% of the J^{Cl}_{net} during cAMP exposure under I_{sc} conditions, but J^K_{net} equalled J^{Cl}_{net} at open-circuit.

INTRODUCTION

The rectum of the desert locust *Schistocerca gregaria* actively absorbs water and solutes from the lumen of the hindgut (Phillips, 1964*a,b*). Most of the fluid reaching the rectal lumen originates at the Malpighian tubules, which secrete an isosmotic 'primary urine' which typically contains much higher levels of K (140 mM) and considerably less Na (20–47 mM) than are present in the haemolymph (Phillips, 1964*a,b,c*, 1981; Hanrahan, 1982).

We found evidence that Cl is actively transported across this epithelium by an unusual, K-stimulated mechanism (Hanrahan & Phillips, 1982). The purpose of the present work is to examine the relationship between K and Cl transport in more detail; specifically, (i) we use steady-state tracer fluxes to establish whether K alters the maximal rate of transport (J^{Cl}_{max}), the apparent affinity of the transport mechanism for Cl (K^{Cl}_t), or both; (ii) we quantify the K requirements of Cl transport and determine the sidedness and cation selectivity of the stimulation and (iii) we test whether the active components of net Cl and and K absorptions are interdependent by measuring

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the effects of cAMP and Cl omission on transepithelial ^{42}K fluxes. The results indicate that only low concentrations of K are required, that stimulation involves increases in K_t^{Cl} and J_{max}^{Cl}, and that K acts only from the mucosal side. There is a small active component of K absorption, but most K transport is passive and electrically coupled to Cl absorption. Some of these conclusions have been reported in preliminary form as symposia proceedings (Hanrahan & Phillips, 1980*a*,*b*, 1982, 1983).

MATERIALS AND METHODS

Adult female locusts, *Schistocerca gregaria*, were obtained from a colony maintained at U.B.C. The dissection and chambers were those of Williams, Phillips, Prince & Meredith (1978). The rectum was cut longitudinally to produce a sheet and attached across a collar-shaped opening with fine tungsten pins and a rubber 'O'-ring. Five ml of solution were placed in both half-chambers and stirred with 95 % O_2/5 % CO_2 when HCO_3 was present, or with 100 % O_2 without HCO_3. Normal saline contained (mequiv l^{-1}): 110 Na, 10 K, 20 Mg, 10 Ca, 110 Cl, 10 HCO_3, 10 glucose and 100 sucrose. Nominally Cl-free saline was prepared by replacing all Cl with methylsulphate. K-free saline was prepared by isosmolal replacement of K with sucrose. All salines contained the following amino acids as substrates (mmol l^{-1}): 2·9 alanine, 1·0 arginine, 1·3 asparagine, 5·0 glutamine, 11·4 glycine, 1·4 histidine, 1·4 lysine, 13·1 proline, 1·5 serine, 1·9 tryosine, 1·8 valine. Saline pH was 7·2 in HCO_3-containing salines and 7·0–7·4 under HCO_3-free conditions. Experiments were performed at 22 °C.

The voltage clamp used in these studies has been described in detail elsewhere (Hanrahan, 1982; Hanrahan, Meredith, Phillips & Brandys, 1983). Short-circuit current (I_{sc}) and transepithelial potential (V_t) were recorded on a strip chart recorder. Corrections were made automatically for asymmetries between the voltage-sensing KCl agar bridges and for resistance of the bathing saline by the method of Rothe, Quay & Armstrong (1969). These corrections were especially important during large increases in [NaCl] and [K methylsulphate].

Kinetics of transepithelial ^{36}Cl fluxes

Tissues were equilibrated in normal saline under I_{sc} conditions for 3 h, and then the external medium was replaced bilaterally with Cl-free saline (methylsulphate substitution). I_{sc} was near zero under these conditions. Approximately 0·5 h later, the chambers were rinsed three times with fresh Cl-free saline and cAMP was added to the serosal side to a final concentration of 1 mM. Small aliquots of 2 M-NaCl were then added to both sides in order to raise [Cl] stepwise from 0 to 2, 4, 10, 40 and 114 mM. ^{36}Cl fluxes were measured during each step as described previously (Williams *et al.* 1978; Hanrahan, Phillips & Steeves, 1983). ^{36}Cl (New England Nuclear, carrier-free, 5·9 mCi g^{-1} Cl) was added as $H^{36}Cl$ to the 'hot' side in amounts that were too small to alter [Cl] significantly. One ml samples were taken from the 'cold' side at 15-min intervals and replaced with fresh saline. Samples were placed in 10 ml of scintillation fluid (ACS Amersham Corp., Oakville, Ontario) and were counted at constant quench using a scintillation counter (Isocap, Nuclear Chicago). Appropriate corrections were made for dilution during sampling. No correction was necessary for tracer backflux

ıce ^{36}Cl activity on the cold side reached only 0·5 % of that on the hot side.

Cyclic AMP was present when measuring transepithelial flux kinetics in order to stimulate active Cl transport: cAMP was equally effective in stimulating Cl transport when added immediately after dissection or after equilibration *in vitro* for several hours in normal saline. After switching to nominally Cl-free solution (cAMP still present), J_{ms}^{Cl} attained a new steady-state value 15 min after each step increase in [Cl]. Data from the second flux period were used in all calculations. The dependence of J_{ms}^{Cl} on [Cl] was determined in salines containing 0, 10 and 100 mM-K in separate experiments: [K] was adjusted with K methylsulphate.

Measurement of K-dependence of Cl transport

The increase in I_{sc} was used as a measure of J_{net}^{Cl} while adding K-methylsulphate bilaterally. The equivalence of I_{sc} and J_{net}^{Cl} during cAMP-stimulation has been established over the range 0–140 mM-K (see Table 2 and Fig. 1). In other words, elevating [K] does not cause electrogenic transport of ions other than Cl.

After mounting and short-circuiting the tissues, the chambers were rinsed repeatedly in K-free saline for 3–4 h. Cyclic AMP was added to the serosal side, causing I_{sc} to increase to a new steady-state level within 1 h. Aliquots of K-methylsulphate were then added to both sides at 0·5 h intervals to yield sequential concentrations of 0, 2, 4, 10, 40, 100, 140 and 200 mM-K. An identical protocol was followed during control experiments, except that Na-methylsulphate was added. Transepithelial potential and resistance were measured at 15 min intervals.

Transepithelial ^{42}K fluxes

^{42}K (New England Nuclear Corp., 0·13–0·15 Ci g^{-1}) was added as ^{42}KCl to normal saline or as $^{42}K_2CO_3$ to Cl-free saline. Samples were taken at 15 min intervals and counted using an automatic gamma counter (1085, Nuclear Chicago). Initial radioactivity of the labelled side served as a reference in order to correct for tracer decay during experiments (total experimental and counting time = 11 h).

Calculations and statistics

In order to compare the instantaneous I_{sc} with tracer fluxes measured at intervals, I_{sc} recordings were integrated using a planimeter (model L30M, Lasico, Los Angeles, California). Values are means ± standard errors unless stated otherwise; N = number of recta. Where appropriate, significant difference were determined using paired or unpaired t-tests.

Unless otherwise stated, net fluxes were calculated as the difference between mean unidirectional fluxes on different preparations, and standard deviation of net flux (S_{net}) was calculated from the variances of the unidirectional fluxes (S_{ms}, S_{sm}) by

$$S_{net} = \sqrt{\frac{S_{ms}^2}{n_{ms}} + \frac{S_{sm}^2}{n_{sm}}}.$$

As a test of significance of net flux, we used the cautious method of showing non-overlap of confidence limits for forward and back fluxes.

When kinetics of J_{ms}^{Cl} were studied on individual rectal preparations, J_{net}^{Cl} was

estimated by subtracting the mean backflux (J_{sm}^{Cl}) for other preparations at the same [Cl]. This was justified because J_{sm}^{Cl} was very much smaller and very much less variable than J_{ms}^{Cl} (i.e. J_{sm}^{Cl} contributed on average only 5%, maximum 17%, of the total variance in net flux). Calculating the kinetic parameters, K_t and J_{max}, for Cl transport on individual rectal preparations in this way (i.e. ignoring the small variance in J_{sm}) gave an indication of kinetic variation that could not have been obtained from a single Woolf plot using J_{net}^{Cl} calculated from mean J_{ms}^{Cl} and J_{sm}^{Cl} values.

RESULTS

Kinetics of Cl absorption

I_{sc} and J_{ms}^{Cl} increased hyperbolically as [Cl] was elevated on both sides of the rectum (Fig. 1). In contrast, J_{sm}^{Cl} increased linearly and showed no evidence of saturation. As expected, J_{net}^{Cl} and I_{sc} were nearly identical at all Cl concentrations.

Fig. 2 shows that forward fluxes of ^{36}Cl were significantly lower in K-free saline ($P << 0{\cdot}01$) and higher in 100 mM-K saline ($P << 0{\cdot}01$) when compared to normal saline (10 mM-K). Since J_{sm}^{Cl} is identical when tissues are bathed in saline containing 1 or 140 mM-K (Hanrahan, 1982) and increases linearly with chloride concentration

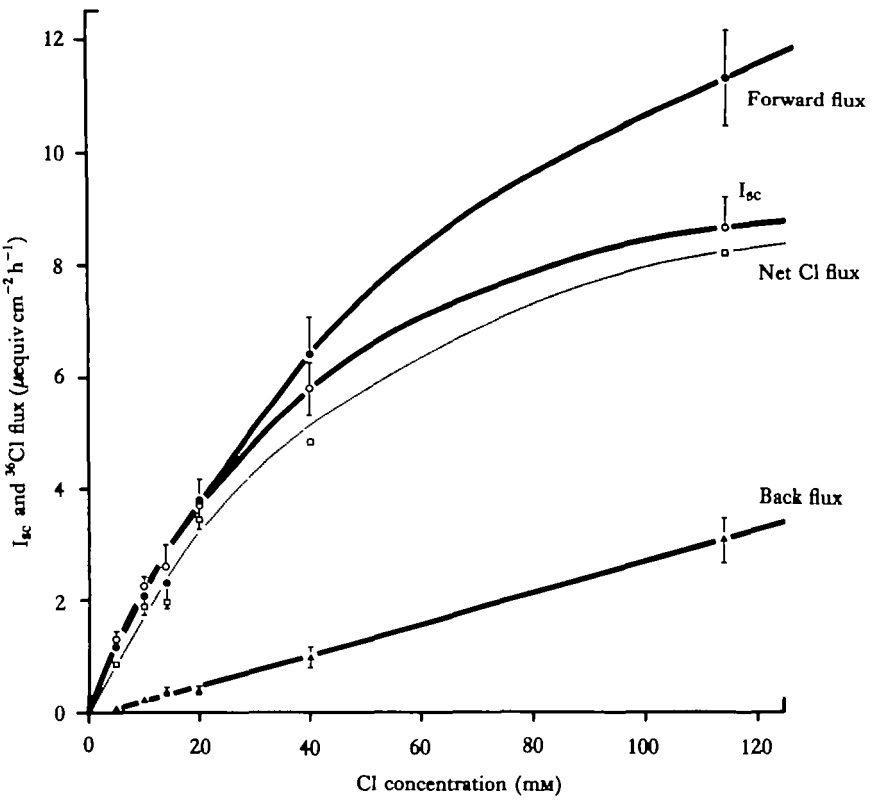


Fig. 1. The dependence of Cl fluxes and I_{sc} on [Cl] with cAMP present. Unidirectional Cl fluxes from (●) mucosa to serosa and (▲) serosa to mucosa, (□) calculated net Cl flux and (○) short-circuit current. Means ± S.E.; $N = 10$.

$r = 0{\cdot}998$; Fig. 1), J^{Cl}_{sm} presumably occurs by passive diffusion. J^{Cl}_{net} was calculated as $J^{Cl}_{ms} - J^{Cl}_{sm}$ at each [K] using the J^{Cl}_{sm} measured in normal saline. Data from individual preparations were fitted by linear regression to the Michealis-Menten equation using the Woolf transformation (Haldane, 1957), because this method is considered to be least sensitive to measurement errors (Blünck & Mommsen, 1978). Weighting procedures were not applied because the type of error (absolute *vs* relative) was unknown. Woolf plots were linear, as indicated in Fig. 3. For ten preparations used to calculate K_t and J^{Cl}_{max} at 10 mM-K, $98{\cdot}2 \pm 0{\cdot}65$ % of the variation in $[Cl]/J^{Cl}_{net}$ was attributable to this linear relationship with [Cl].

As shown in Table 1, both K_t and J^{Cl}_{net} increased significantly ($P < 0{\cdot}001$) as the [K] was raised from 0 to 100 mM. Thus the stimulation of Cl absorption by K did not result from a simple increase in affinity of the transporter for Cl.

Cooperative interactions between Cl-binding sites have been demonstrated for transporting epithelia of prawn intestine (Ahearn, 1978) and the mosquito posterior rectum (Bradley & Phillips, 1977; Phillips, Bradley & Maddrell, 1978). Fig. 4 shows that Hill plots of mean J^{Cl}_{net} had slopes near one at all concentrations of K (e.g. 1·09 at 0 mM, 0·91 at 10 mM, and 0·99 at 100 mM-K). Using the slope as a measure of the Hill constant or number of interacting sites (Segel, 1975), these results suggest that

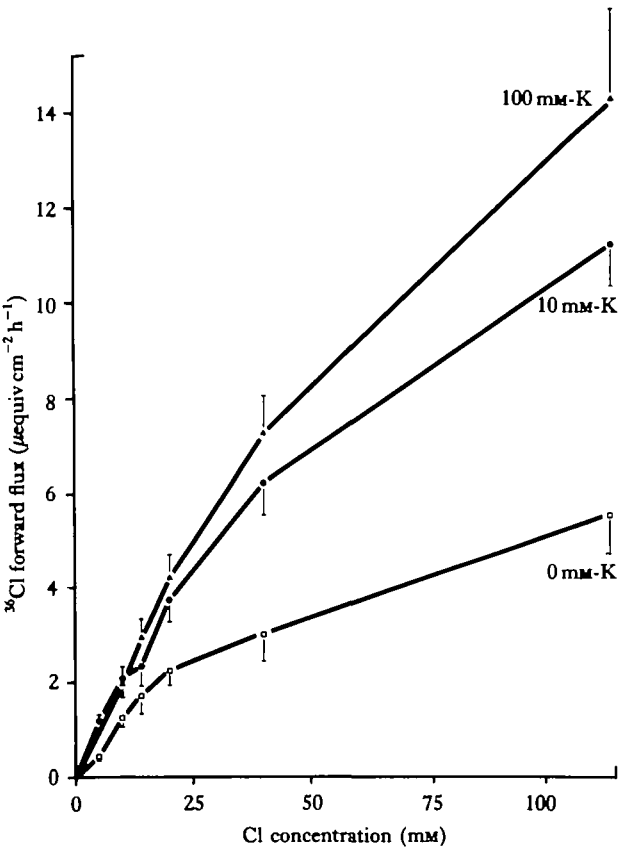


Fig. 2. The influence of external [K] on the relationship between J^{Cl}_{ms} and [Cl] of the saline. Tissues were stimulated by 1 mM-cAMP on the serosal side under I_{sc} conditions. Bilateral [K] was adjusted from 0 mM to 10 mM or 100 mM by adding K-methylsulphate. Means ± S.E.; $N = 6$–10.

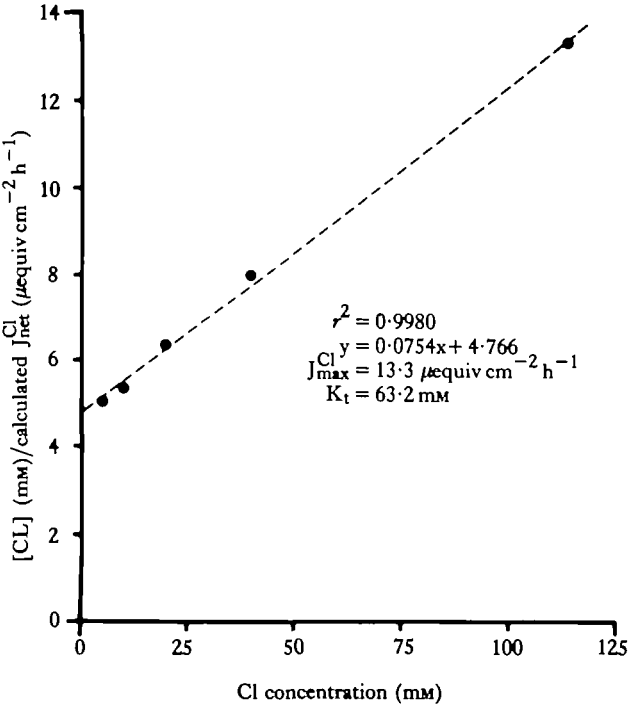


Fig. 3. Representative plot of data used in calculating kinetic constants. J_{net}^{Cl} was obtained at each [Cl] by subtracting mean J_{sm}^{Cl} (six recta) from individually measured forward fluxes (J_{ms}^{Cl}) measured under I_{sc} condition. Data were fitted by standard linear regression to the Michaelis-Menten equation using the Woolf transformation $[Cl]/J_{net}^{Cl} = K_t/J_{max}^{Cl} + [Cl]/J_{max}^{Cl}$.

Table 1. *Effects of external K on the kinetics of steady-state ^{36}Cl fluxes across cAMP-stimulated recta under I_{sc} conditions*

K concentration (mM)	K_t [Cl] (mM)	J_{max}^{Cl} (μequiv cm^{-2} h^{-1})	(*N*)
0	22·7 ± 4·0	3·5 ± 0·7	6
10	60·2 ± 8·7	14·9 ± 1·9	10
100	99·6 ± 13·4	23·1 ± 5·3	7

Means ± S.E.; calculated using Woolf transformation as described in text.

the rate-limiting step in transrectal Cl absorption does not involve cooperative interactions between Cl-binding sites.

I_{sc} and ^{36}Cl fluxes in 'high K' saline

All previous studies of transport across insect hindgut have employed high-Na, low-K salines, even though the rectum normally contains a K-rich, low-Na fluid secreted by the Malpighian tubules. Therefore, transepithelial ^{36}Cl fluxes were measured in saline containing 140 mM-K (i) to determine if I_{sc} still equals J_{net}^{Cl}, as it does in normal saline, (ii) to measure the possible effects of high [K] on J_{sm}^{Cl} and (iii) to examine whether responsiveness to cAMP is altered by this physiological level of K.

Both J_{ms}^{Cl} and I_{sc} increased during exposure to 1 mM-cAMP in high K saline (Tab

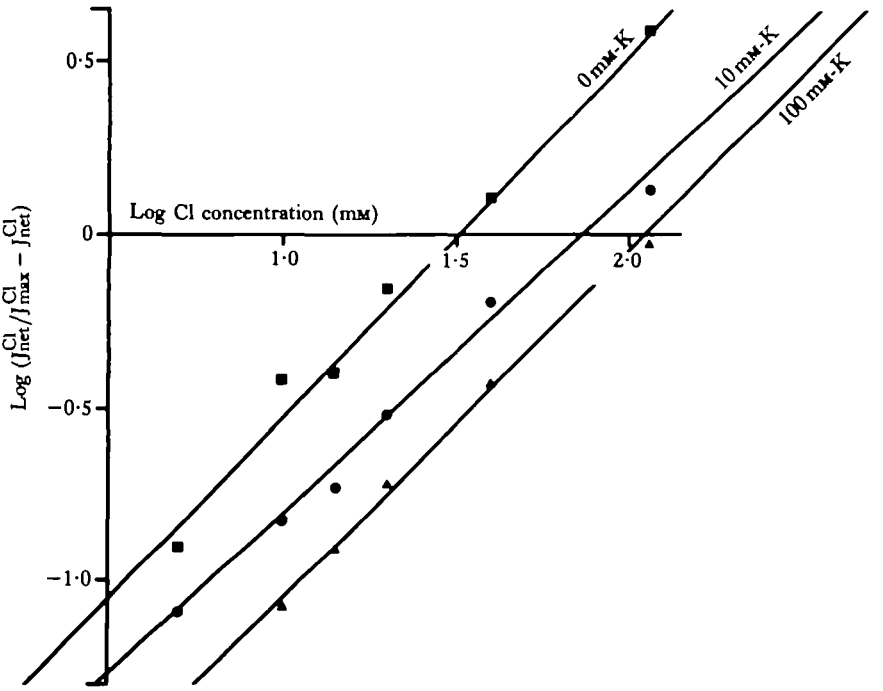


Fig. 4. Hill plots of J_{net}^{Cl} at external [K] = 0, 10, 100 mM. Net fluxes were calculated under I_{sc} conditions in the presence of 1 mM-cAMP on the serosal side, and plotted according to the Hill equation: $\log [J_{net}^{Cl}/(J_{max}^{Cl} - J_{net}^{Cl})] = n\log[Cl] - \log K_t$. Symbols are mean values calculated as described in text.

2). Surprisingly, R_t was 40 Ωcm^2 *higher* during cAMP stimulation in high-K saline (140 mM) than in normal saline (10 mM-K) during cAMP stimulation (Hanrahan, 1982; $P << 0{\cdot}01$). As discussed in a later section, this high resistance is explained by the fact that K permeability of the epithelium varies inversely with saline [K].

It is of interest to compare J_{sm}^{Cl} in high-K saline with J_{sm}^{Cl} in normal saline. From Fig. 1, $J_{sm}^{Cl} = 1{\cdot}3$ μequiv $cm^{-2} h^{-1}$ at [Cl] = 50 mM, [K] = 10 mM, in close agreement with J_{sm}^{Cl} observed when [K] = 140 mM and [Cl] = 50 mM (0·9–1·3 μequiv $cm^{-2} h^{-1}$; Table 2). Chloride permeability (P_{Cl}) is apparently not affected by [K], at least over this range. In summary, after stimulation ΔI_{sc} equals J_{net}^{Cl} when external [K] = 0 mM (Hanrahan, 1982), 10 mM (Fig. 1) or 140 mM (Table 2). Active Cl transport is the major electrogenic process in stimulated locust rectum under all conditions studied.

Apparent K activation constant (K_a) of Cl transport

Since ΔI_{sc} is a good measure of active Cl transport regardless of saline [K], the apparent K_a of K stimulation was estimated from the effects of K addition on ΔI_{sc} in the presence of cAMP.

Fig. 5A shows that addition of cAMP alone caused I_{sc} to increase from 0·86 μequiv $cm^{-2} h^{-1}$ to 1·57 μequiv $cm^{-2} h^{-1}$, in close agreement with J_{net}^{Cl} obtained in K-free saline. When external [K] was then elevated from 0 mM to 100 mM by stepwise

Table 2. *Effects of cAMP on ^{36}Cl fluxes and electrical parameters in high-K (140 mM) saline*

	I_{sc} (μequiv cm^{-2} h^{-1})	J^{Cl}_{ms} (μequiv cm^{-2} h^{-1})	J^{Cl}_{sm} (μequiv cm^{-2} h^{-1})	J^{Cl}_{net} (μequiv cm^{-2} h^{-1})	V_t (mV)	R_t (Ωcm^2)
Control (unstimulated)	2·59 ± 0·36	1·55 ± 0·20	0·85 ± 0·07	0·70 ± 0·21	14·00 ± 2·08	206·5 ± 7·60
1 mM-cAMP (90 min)	8·51 ± 0·96	9·82 ± 0·85	0·93 ± 0·19	8·89 ± 0·87	31·80 ± 3·10	150·50 ± 12·40
Δ	5·92 ± 0·76	8·27 ± 0·87	0·083 ± 0·24	8·19	15·31 ± 3·09	56·04 ± 34·30
P	<<0·01	<<0·01	>>0·2	<0·05	<<0·01	<<0·01

All values are mean ± S.E., except J_{net}, which is the calculated difference between mean J_{ms} and J_{sm} ± the standard deviation of this value (see Methods).
Paired *t*-tests were used to obtain *P* values for all Δ values except ΔJ_{net}, where significance of Δ was inferred by setting confidence limits around J_{net} before and after cAMP addition.
$N = 12$ recta for I_{sc}, V_t; $N = 6$ recta for J^{Cl}_{ms}, J^{Cl}_{sm}.

dition of KCH_3SO_4 to both sides, I_{sc} increased similarly to a maximum rate of 12·68 μequiv $cm^{-2} h^{-1}$. Above 100 mM-K, I_{sc} decreased reversibly (Fig. 5A). V_t increased from 7·2 to 16·8 mV when 1 mM-cAMP was added to K-free saline (data not shown). R_t remained constant when [K] was raised from 10 to 200 mM (Fig. 5B), a surprising result considering the normally high K permeability of this epithelium. To control for possible artefacts which might result from increases in osmotic pressure

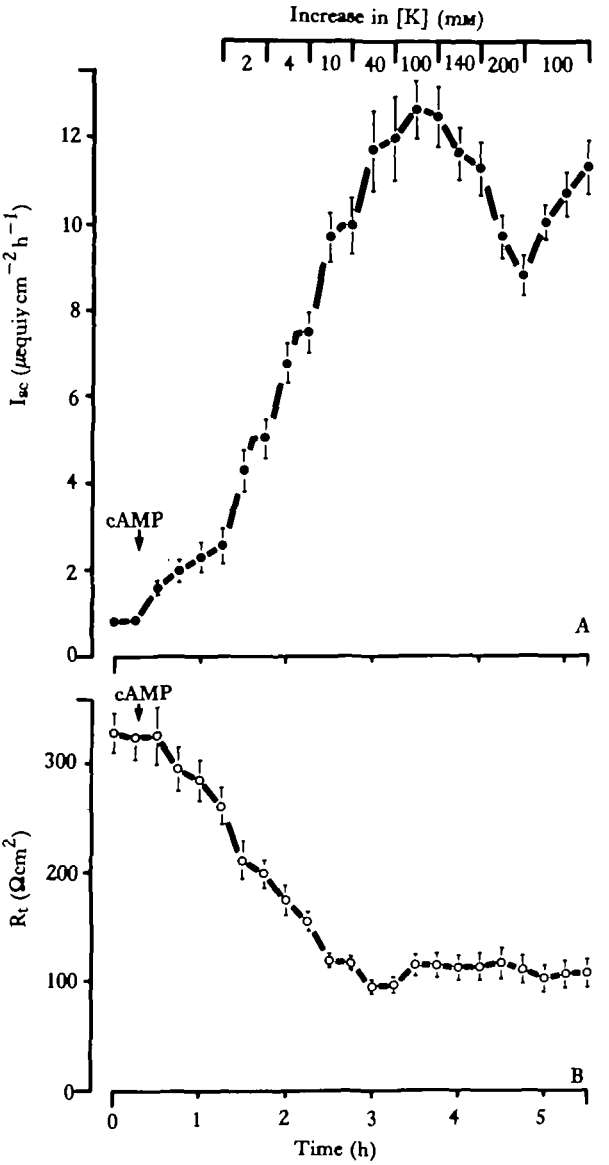
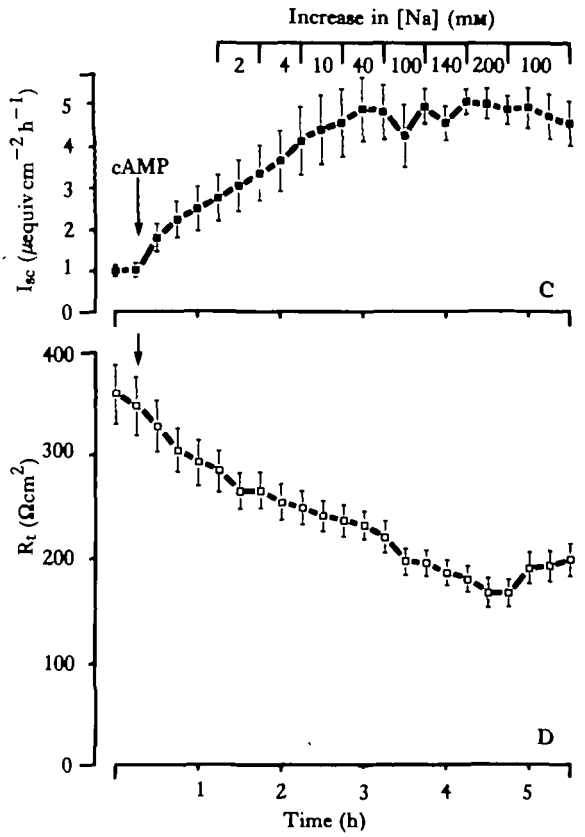


Fig. 5. Effects of stepwise bilateral K additions on (A) I_{sc} and (B) R_t under I_{sc} conditions. Recta were equilibrated in K-free saline for 4 h, then 1 mM-cAMP was added to the serosal side at the arrows. After 1 h exposure to cAMP, K-methylsulphate was added to both sides to give the final concentrations shown. The effects of stepwise bilateral Na additions on (C) I_{sc} and (D) R_t were determined as described for (A) and (B) except that Na-methylsulphate was added to both sides instead of K-methylsulphate. Means ± S.E.; $N = 9$–10 (K addition), $N = 6$ (Na addition).

and ionic strength, Na-methylsulphate was added in parallel experiments under identical conditions (Fig. 5C,D). Na addition did not produce large step-like increases in I_{sc} (Fig. 5C). Also, high [Na] (>100 mM) did not inhibit I_{sc}, in marked contrast to the effects of elevated K levels. R_t declined in a predictable manner when saline [Na] was increased above 10 mM (Fig. 5D), in contrast to the relatively constant R_t observed following K addition over the same concentration range (Fig. 5B).

The difference between mean I_{sc} obtained during Na and K additions was used as a measure of K-dependent Cl transport, since ΔI_{sc} equals J^{Cl}_{net} at all K concentrations. Fig. 6 shows a Woolf plot of the K-dependent I_{sc} stimulation. A linear relationship was obtained between ([K]/K-dependent I_{sc}) *vs* [K] when [K] was greater than 2 mM ($r^2 = 0{\cdot}9984$). The K_a was 3·2 mM-K and the maximum K-dependent I_{sc} was 7·8 μequiv cm^{-2} h^{-1}

The effect of 1 mM-cAMP on I_{sc} was also measured in two tissues when choline was the only monovalent cation added to the saline. After 3 h in the absence of both Na and K, cAMP increased I_{sc} by 4·0 and 4·5 μequiv cm^{-2} h^{-1}, identical to values observed with 200 mM-Na present. Stimulations of this magnitude were also observed when both Na and K were replaced by tetramethyl ammonium (TMA). Cyclic AMP caused I_{sc} to increase from 1·33 to 2·62 μequiv cm^{-2} h^{-1} and from 0·91 to 2·66 μequiv cm^{-2} h^{-1} in two preparations exposed to choline saline lacking Na, K, Ca



Figs 5C & D. For legend see p. 209.

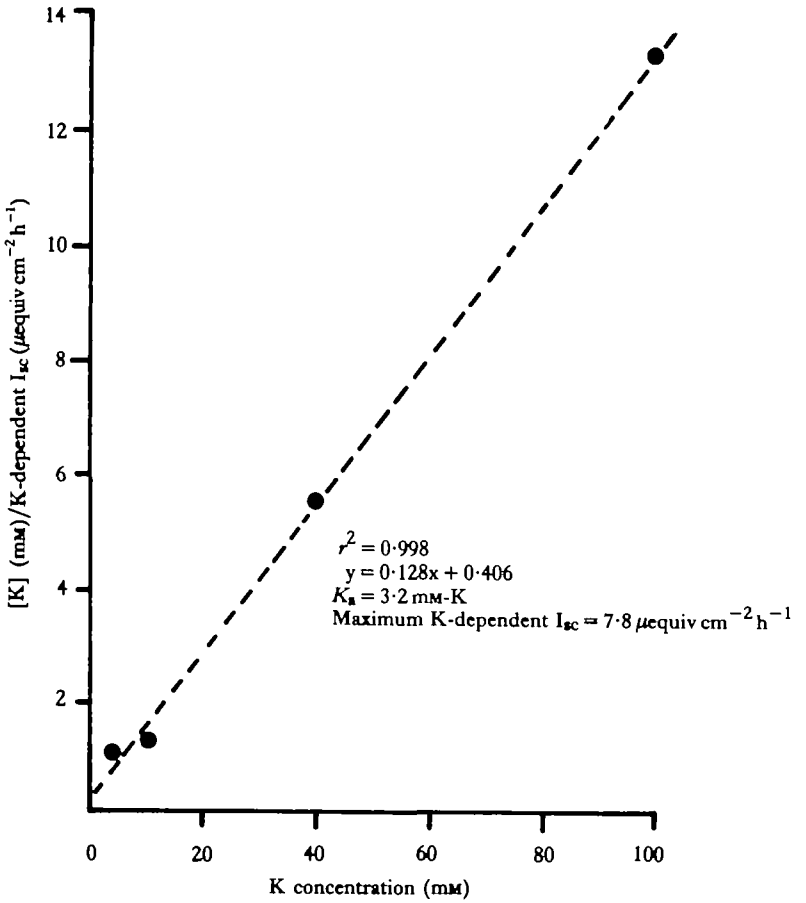


Fig. 6. Woolf plot of the relationship between external [K] and K-stimulated I_{sc}. Recta were exposed to 1-mM-cAMP on the serosal side throughout the experiment. External [Cl] was constant at 114 mM. The difference between I_{sc} in Fig. 5A and Fig. 5C was used to estimate K-stimulated Cl transport. The [K] producing half-stimulation of I_{sc} was 3·2 mM.

and Mg. This suggests that the K-independent component of cAMP-stimulated ΔI_{sc} is either independent of these cations, or choline and TMA can substitute for them. In the next section we examine the relative ability of other cations to stimulate Cl transport.

Selectivity of K stimulation of Cl transport

Tissues were equilibrated for 2–4 h under I_{sc} conditions in K-free saline and then exposed to 1 mM-cAMP. After 2–3 h, various test cations were added bilaterally to a final concentration of 40 mM. Fig. 7 shows the selectivity sequence which was estimated by comparing the cation-stimulated I_{sc} after 1 h. Arranged in order of decreasing potency, the sequence was: 1·0 K > 0·58 Rb > 0·49 Cs > 0·08 NH_4 (and 0·2 Na from Fig. 5). The series K > Rb > Cs > Na is sequence I of Eisenman (1961), corresponding to a selectivity site having moderately weak field strength. This contrasts with the high selectivity of the Cl site described previously (Hanrahan & Phillips, 1980*b*).

Sidedness of K stimulation

I_{sc} was measured during stepwise addition of K-methylsulphate to either the mucosal or serosal side to determine whether K 'activation' of Cl transport occurred specifically at one side of the epithelium. Recta were equilibrated under I_{sc} conditions in K-free saline for 3–4 h and then exposed to 1 mM-cAMP. After I_{sc} reached a new steady-state, aliquots of K-methylsulphate were added to the mucosal or serosal side (final concentration; 2–10 mM). Only low [K] was used in order to minimize the K diffusion current caused by a transepithelial K gradient, and to reduce contamination of the K-free side. To estimate K diffusion current, 'I_{sc}' was recorded during asymmetrical K additions when Cl transport was abolished by (i) adding 1 mM-azide to normal saline and stirring with N_2, and (ii) by replacing Cl with methylsulphate. Corrections for K diffusion ranged from 0–21 % in the presence of a 10 mM: 0 mM (mucosa: serosa) gradient. The mean K diffusion currents measured in this way were subtracted from the I_{sc} measured in unpoisoned tissues in order to calculate true Cl-dependent I_{sc} with K gradients present.

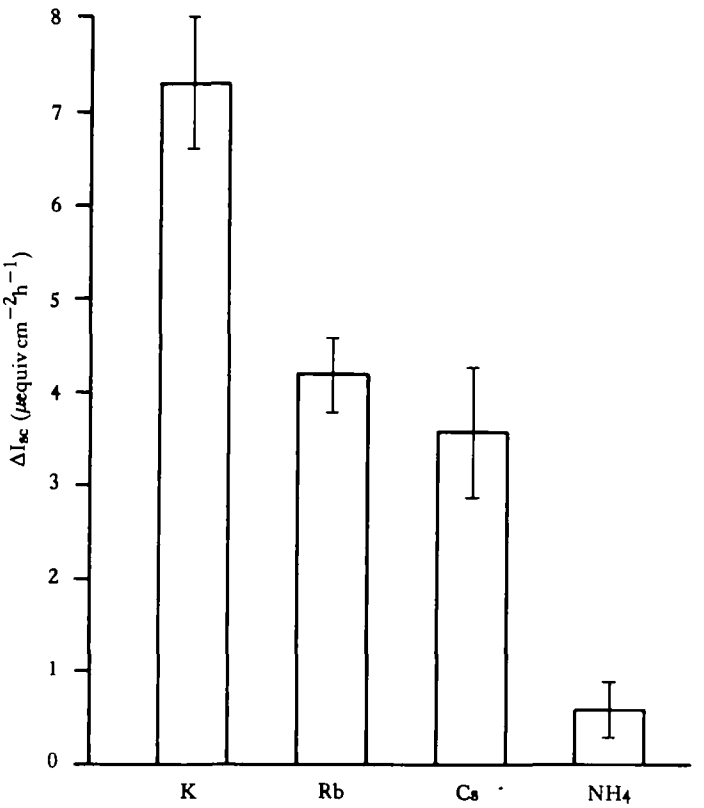


Fig. 7. Selectivity of cation stimulation of active Cl transport. Recta were equilibrated in K-free saline and exposed to 1 mM-cAMP on the serosal side for 1 h. After a steady-state was obtained, one of the above cations was added as sulphate salt to give a final test cation concentration of 40 mM. I_{sc} was measured after 90 min exposure. ($\bar{x} \pm$ S.E.; N = 5–6.)

Fig. 8 shows the effects of adding K-methylsulphate to one side of the epithelium. After corrections, I_{sc} attributable to active Cl transport increased from 1·55 to 6·85 μequiv cm^{-2} h^{-1} when 10 mM-K was added to the mucosa. In contrast, I_{sc} was not changed significantly by serosal addition of K ($P >> 0{\cdot}2$), suggesting that the K activation site is accessible only from the mucosal side.

Transepithelial ^{42}K fluxes under I_{sc} conditions

Normal saline

Active K transport has been reported across locust rectum *in vivo* (Phillips, 1964*b*,*c*) and *in vitro* (Williams *et al.* 1978); however, the relative magnitudes of active and passive components are not known nor are the ionic requirements of K absorption. Considering the dependence of active Cl transport on external K, it was

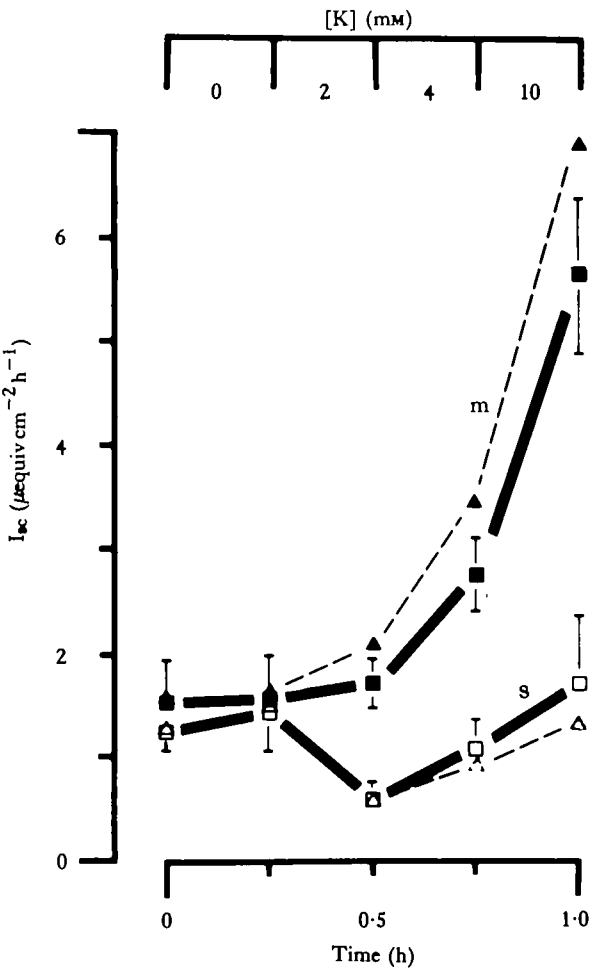


Fig. 8. Effect of adding K-methylsulphate stepwise to one side only on Cl-dependent I_{sc}. After equilibrating recta for 3 h under I_{sc} conditions in K-free saline, 1 mM-cAMP was added to the serosal side and I_{sc} was monitored. Aliquots of K-methylsulphate were then added to the mucosal (m) *or* serosal (s) chamber to give the concentrations indicated. Values were corrected for K diffusional current by subtracting the apparent ΔI_{sc} produced by asymmetrical K addition in azide/N_2 saline as described in the text. (Observed: ■,□; corrected ▲,△.) Means ± S.E.; $N = 7$.

of some interest to study the properties of active K transport, particularly the effe of cAMP, and to test the possibility that there are reciprocal ionic requirements for K and Cl absorption.

Fig. 9 shows the effects of 1 mM-cAMP on (a) unidirectional fluxes of ^{42}K and (b) J^K_{net} across recta bathed in normal saline (114 mM-Cl, 10 mM-K). J^K_{ms} and J^K_{sm} increased from about 0·35 μequiv cm^{-2}h^{-1} initially to 2·08 and 1·56 μequiv cm^{-2}h^{-1} respectively after adding cAMP. A small but significant J^K_{net} was observed after 45 min of cAMP stimulation (0·63 ± 0·26; $P < 0·05$) but it was less than 7% of the J^{Cl}_{net} measured under these conditions.

The four-fold stimulation of *both* J^K_{ms} and J^K_{sm} suggests that cAMP increases the

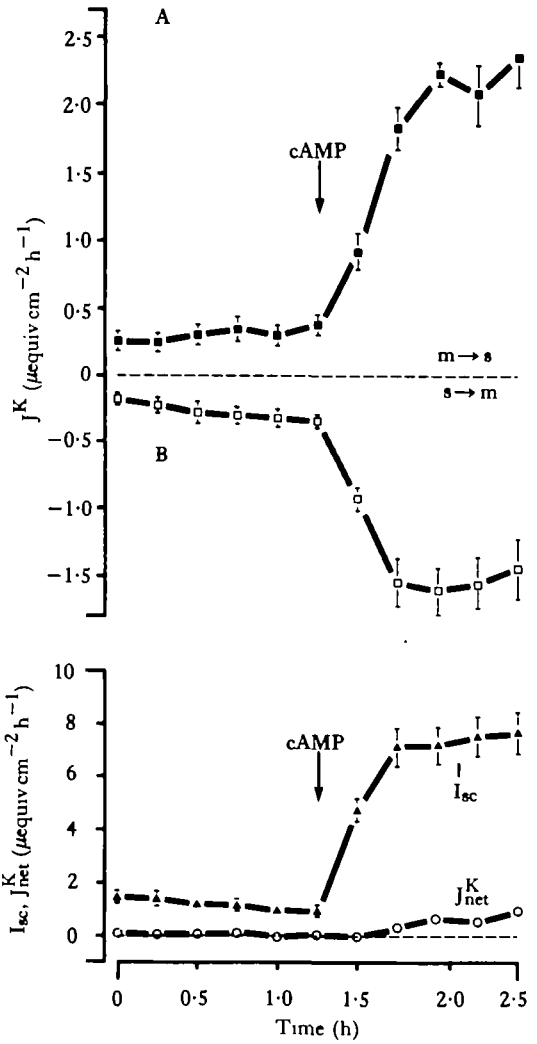


Fig. 9. Effects of cAMP on I_{sc} and K fluxes measured under I_{sc} conditions in normal saline (10 mM-K). (A) ■, unidirectional ^{42}K flux from mucosa to serosa (m→s) and □ in the reverse direction (s→m); (B) ○, J^K_{net} and ▲, I_{sc}. Tissues were pre-equilibrated for 4 h under I_{sc} conditions. Means ± S.E.; $N = 6$, J^K_{ms}, J^K_{sm}; $N = 12$, I_{sc}; S.E. for J^K_{net} is smaller than symbols (value in text).

parent transepithelial K permeability ($P_K = J^K_{sm}/[K]$) from $0{\cdot}98 \pm 0{\cdot}14$ to $4{\cdot}0 \pm 0{\cdot}62 \times 10^{-5}$ cm s^{-1} ($\bar{x} \pm$ S.E., $N = 6$). This method of calculating permeability may lead to some error because it assumes that the tissue acts as a single barrier (Schultz & Frizzell, 1976) whereas ^{42}K must penetrate two membrane barriers because the locust rectum is a tight epithelium (Hanrahan *et al.* 1982). This is discussed in a later section; however, the stimulations of *both* unidirectional fluxes do indicate that P_K increases during cAMP stimulation.

In summary, cAMP has two important effects on transepithelial K movements under I_{sc} conditions: (i) it produces a large (four-fold) increase in transepithelial K diffusion, and (ii) it induces a small active net K absorption.

Cl-free saline

Table 3A shows the effects of 1 mM-cAMP on ^{42}K fluxes in Cl-free saline. After 1 h, both unidirectional fluxes increased by about 400 %. The significance of J^K_{net} was marginal, using a conservative statistical criterion. When Cl was restored to normal levels on both sides (114 mM), there was no change in J^K_{sm} or J^K_{ms} (data not shown; $P > 0{\cdot}2$, paired t-test), although I_{sc} increased six-fold to values typical of cAMP stimulation in normal saline ($11{\cdot}4 \pm 0{\cdot}74$ μequiv cm^{-2} h^{-1}; $N = 12$).

These results show that neither the increased K permeability nor the small J^K_{net} produced by cAMP is affected by omitting Cl from the saline. In view of this independence, it seems unlikely that there is strict chemical coupling between Cl and K movements at either the apical or basal membrane. This conclusion is further supported by the finding that J^K_{net} is less than 8 % of J^{Cl}_{net} under I_{sc} conditions (Fig. 9) and also by the previous observation that 35 % of J^{Cl}_{net} is cation-independent.

^{42}K fluxes in high-K saline

The apical membrane of this epithelium is usually bathed in K-rich (140 mM) Malpighian tubule fluid *in vivo*. The small J^K_{net} observed under I_{sc} conditions during cAMP stimulation (Fig. 9B) might result from the low concentration of K in the saline. Table 3B shows the effects of 1 mM-cAMP on ^{42}K fluxes under I_{sc} conditions when recta were bathed bilaterally in high-K saline (140 mM-K, 50 mM-Cl). Unidirectional and net ^{42}K fluxes were similar to those measured in normal saline containing only 10 mM-K (i.e. Fig. 9). As before, J^K_{net} increased to $0{\cdot}66$ μequiv cm^{-2} h^{-1} during cAMP exposure although this was not significant as judged by the overlap of 90 % confidence intervals. Although J^K_{sm} increased steadily over the course of the experiment, addition of 1 mM-cAMP did not increase J^K_{sm} ($P >> 0{\cdot}2$, paired t-test), in marked contrast to the stimulation observed in normal saline.

Several characteristics of K transfer should be noted. First, J^K_{net} was 0·6–0·8 μequiv cm^{-2} h^{-1} under I_{sc} conditions whether saline [K] was 10 or 140 mM. This indicates that active K transport must saturate at low external levels of K (<10 mM). Second, P_K decreases several-fold at high [K] because J^K_{sm} does not increase proportionally when [K] is raised to high levels. This is shown by comparing J^K_{sm} in Fig. 9 and Table 3B. P_K is three- to four-fold higher when tissues are bathed in normal saline (10 mM-K, 114 mM-Cl) than in 'high-K' saline (140 mM-K, 50 mM-Cl). Although high-K saline contains less Cl than does normal saline, lower [Cl] could not explain e reduced P_K under high-K conditions, because P_K in Cl-free saline (with 10 mM-K)

Table 3. *Effects of cAMP on ^{42}K fluxes and electrical parameters under I_{sc} conditions*

	I_{sc} (μequiv cm^{-2} h^{-1})	J^K_{ms} (μequiv cm^{-2} h^{-1})	J^K_{sm} (μequiv cm^{-2} h^{-1})	J^K_{net} (μequiv cm^{-2} h^{-1})	V_t (mV)	R_t (Ωcm^2)
*A Cl-free saline (10 mM-K)						
Control (unstimulated)	1·92 ± 0·19	0·45 ± 0·13	0·31 ± 0·06	0·14 ± 0·15	13·73 ± 1·14	285·1 ± 14·0
cAMP (60 min)	1·95 ± 0·25	1·91 ± 0·24	1·25 ± 0·19	0·66 ± 0·30	8·05 ± 0·91	161·3 ± 5·8
Δ	0·03 ± 0·18	1·46 ± 0·14	0·94 ± 0·14	0·52	5·74 ± 0·59	123·8 ± 13·15
P	>0·2	<0·01	<0·01	0·05<P<0·1	<<0·01	<<0·01
B High-KCl saline (140 mM-K)						
Control (unstimulated)	2·93 ± 0·30	1·37 ± 0·21	1·36 ± 0·22	0·01 ± 0·30	15·93 ± 1·61	246·6 ± 14·12
cAMP (60 min)	8·70 ± 0·68	2·79 ± 0·38	2·13 ± 0·29	0·66 ± 0·48	33·06 ± 1·58	138·0 ± 10·45
Δ	5·77 ± 0·58	1·42 ± 0·28	0·77 ± 0·37	0·65	17·13 ± 1·67	116·6 ± 10·27
P	<<0·01	<0·01	>0·2	>0·1	<<0·01	<<0·01

*See comparable note in Table 2.
$N = 12$ for I_{sc}, V_t, R_t; $N = 6$ for J^K_{ms}, J^K_{sm}.

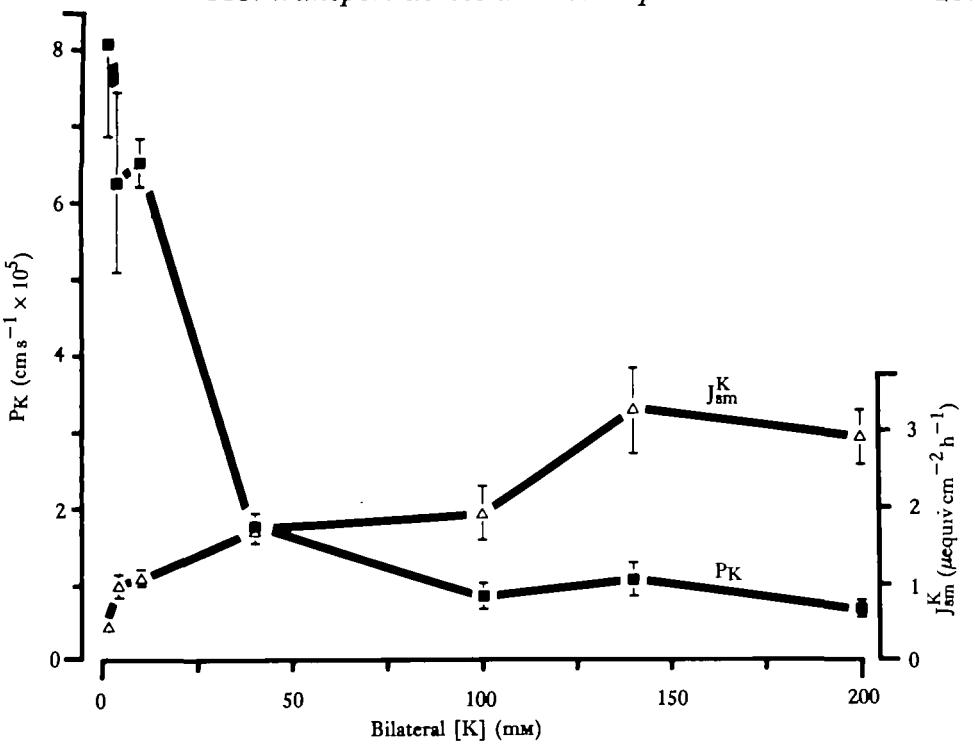


Fig. 10. K backflux measured under I_{sc} conditions and apparent transepithelial K permeability (P_K) as a function of bilateral K concentration. Tissues were equilibrated in K-free saline and exposed to 1 mM-cAMP on the serosal side. J^K_{ms} was measured during two 15-min intervals; however, only the second flux period at each [K] was used in calculations. [K] was elevated by adding K-methylsulphate bilaterally under I_{sc} conditions to give the concentrations shown on the abscissa. P_K was calculated as described in text. Means ± S.E.; $N = 6$.

is also four-fold higher than in high-K saline. Finally, cAMP does not increase P_K by four-fold in tissues bathed with high-K saline, in marked contrast to those bathed in normal or Cl-free salines containing only 10 mM-K. The simplest explanation for these results and the finding that R_t does not decrease when [K] is increased from 10 mM to 200 mM (Fig. 5B), is that K permeability declines at high external K concentrations.

Fig. 10 shows the concentration dependence of P_K. K-methylsulphate was added bilaterally to give concentrations between 2 and 200 mM. J^K_{sm} was measured during two 15-min flux intervals at each [K] and data from the second period were used in calculations of P_K. P_K declined from 8×10^{-5} to 1×10^{-5} cm s^{-1} when K concentration of the saline was increased from 2 mM to >100 mM.

Transepithelial ^{42}K fluxes under open-circuit conditions

Chloride absorption across locust rectum is electrogenic and must, under open-circuit conditions, be matched by a similar flow of cations from mucosa to serosa or a flow of anions in the opposite direction. To determine whether K diffuses transepithelially to maintain electroneutrality during Cl transport, open-circuit ^{42}K fluxes were measured sequentially under three different conditions; initially in normal saline

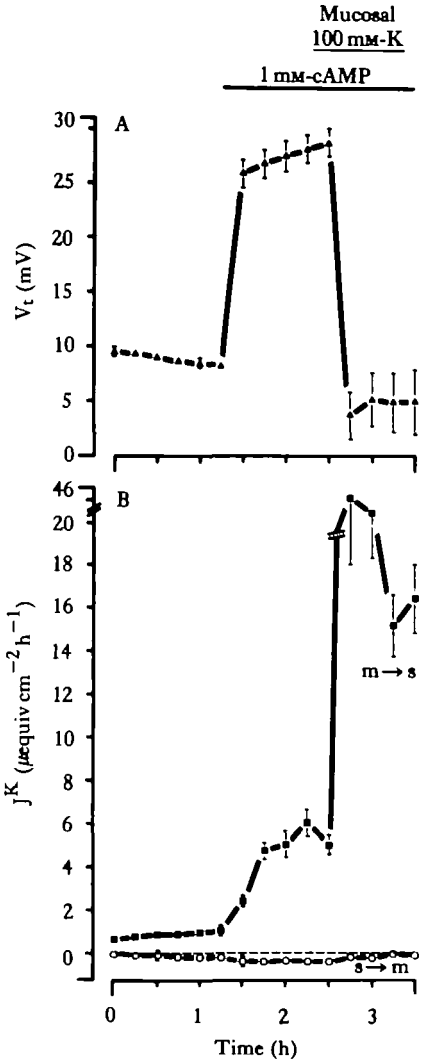


Fig. 11. Effects of sequential addition of 1 mM-cAMP to the serosal side and mucosal addition of KCH_3SO_4 on (A) V_t and (B) ^{42}K fluxes under open-circuit conditions. Recta were initially bathed in normal saline (10 mM-K, 110 mM-Na, 110 mM-Cl). Time zero was preceded by 4 h equilibration under open-circuit conditions. Means ± S.E.; $N = 16$, V_t; $N = 8$, J^K_{ms}, J^K_{sm}

(i.e. under control conditions), then during cAMP-stimulation (10 mM-K bilaterally), and finally, when mucosal [K] was raised to 100 mM in the presence of 1 mM-cAMP to mimic normal *in vivo* K gradients (10:1) across locust rectum.

V_t ranged between 8–10 mV in normal saline (10 mM-K) before adding cAMP (Fig. 11A), in agreement with the previous results. Both forward and back fluxes of ^{42}K were less than 1 μequiv cm^{-2} h^{-1} (Fig. 11B). Serosal addition of cAMP (1 mM) increased V_t from 8 to 28 mV, enhanced J^K_{ms} by 500%, and produced a small but significant increase in J^K_{sm} ($P < 0{\cdot}01$). The resulting J^K_{net} ranged from 4·5 to 5·0 μequiv cm^{-2} h^{-1}. It is noteworthy that J^{Cl}_{net} equalled J^K_{net} at open-circuit both before and after cAMP addition. This result indicates that K is the main counter-ion during active Cl transport even when the [Na] is 11-fold higher than [K] in the external saline (Hanrahan & Phillips, 1983).

The ratio of unidirectional ^{42}K fluxes at open-circuit is higher in normal saline than

[redacted]t predicted from the Ussing flux ratio equation. Under control conditions (normal saline, no cAMP) the ^{42}K flux ratios were between 4 and 6, as compared to a predicted ratio of 1·4. It is unlikely that this discrepancy could result from active transport since no J^{K}_{net} was observed under I_{sc} conditions in the absence of cAMP (Fig. 10). An alternative explanation for high flux ratios is that transmural K movements are not independent. The flux ratio increased further after addition of cAMP (Fig. 11), which is consistent with the appearance of small J^{K}_{net} under I_{sc} conditions (Fig. 9). However, a step increase in mucosal [K] from 10 to 100 mM elevated the steady-state flux ratio to >100: 1, rather than the predicted value of 10·4. This larger discrepancy is probably not due to enhanced active transport, since J^{K}_{net} is similar whether [K] is 10 mM or 140 mM, but it is again consistent with non-independence between transmural ^{42}K fluxes, as has been shown in the basolateral membrane of the turtle colon (Kirk & Dawson, 1981). Increasing luminal [K] to 140 mM in the presence of cAMP to mimic the situation *in vivo* (Fig. 11) led to a large increase in J^{K}_{net} and corresponding dramatic decrease in the V_t opposing Cl transport.

DISCUSSION

The results in this paper suggest that K is required on the mucosal side of locust rectum for Cl to be actively transported at a maximal rate. Since this dependence is seen under I_{sc} conditions and I_{sc} equals J^{Cl}_{net}, K apparently does not stimulate transepithelial Cl absorption simply by acting as a counter-ion. Furthermore, J^{K}_{ms}, J^{K}_{sm} and J^{K}_{net} are much smaller than J^{Cl}_{net} under I_{sc} conditions and are Cl-independent. We conclude that 80 % of net K absorption under open-circuit conditions is electrically coupled to transepithelial Cl absorption (Hanrahan & Phillips, 1983). This would constitute a feedback loop because the concentration of potassium in the lumen would indirectly control passive K reabsorption by modulating the rate of electrogenic Cl transport.

Effects of K on active Cl transport

The relationship between steady-state J^{Cl}_{net} across locust rectum and external [Cl] is satisfactorily described by the Michaelis-Menten equation. K addition increases both K_t and J^{Cl}_{max}, but do these changes directly reflect the properties of the Cl 'pump'? The active step for transepithelial Cl absorption has been localized at the apical membrane using Cl-sensitive microelectrodes under identical conditions to those during ^{36}Cl fluxes (Hanrahan & Phillips, 1980*b*, 1983). The net electrochemical gradient opposing Cl entry varies directly with changes in the rate of Cl transport following K addition, whereas the gradient favouring Cl exit across the basal membrane remains constant. These observations imply that the active entry step is rate-limiting and that steady-state flux kinetics will be largely determined by the apical membrane 'pump'.

Localized electrical coupling across the apical membrane might be the basis for the K-dependence of Cl transport; i.e. K might depolarize the apical membrane thereby reducing the electrochemical potential against which the Cl pump must work, and such a mechanism might not be obvious from measurements of transepithelial fluxes. [redacted]owever, in order to explain the 10-fold difference between J^{Cl}_{net} and J^{K}_{net} under I_{sc}

conditions, tight electrical coupling during Cl entry across the apical membrane would require a very large active return of K from cell to mucosal side (i.e. recycling), because the net electrochemical gradient for K across the apical membrane as measured using ion-sensitive microelectrodes is 0 mV under I_{sc} conditions (Hanrahan, 1982). K secretion has not been observed across this tissue under the wide variety of conditions investigated.

KCl co-entry at a rate equal to the rate of K-dependent Cl transport would contribute significantly to P_K. The observation that P_K is not changed by Cl removal argues against chemical coupling between potassium and chloride. Moreover, when equivalent electromotive forces (e.m.f.) across the apical (E_a) and basal (E_b) membranes were calculated under open-circuit conditions using membrane potentials and resistances obtained by flat-sheet cable analysis (Hanrahan, 1982), E_a and E_b were −55·7 and −52·5 mV before adding cAMP, and −67·9 and −39·9 mV after adding cAMP (cell negative). This increase in apical membrane e.m.f. could be explained by an electrogenic Cl pump, but not by a model which involves parallel electroneutral KCl co-entry and K back-diffusion to the mucosal side, because the measured K gradient would generate an e.m.f. of 12·4 mV in the wrong direction under those conditions.

The simplest model for K stimulation of active Cl transport, consistent with all our data, is one in which K enhances active Cl entry in a manner analogous with enzyme activation. This model might also apply to other insect epithelia where K-stimulated Cl transport has been reported (Cooper, Eaton & Jungreis, 1980).

The K-insensitive component of cAMP-stimulated J^{Cl}_{net} observed in Fig. 5C may result from a single population of Cl pump sites functioning at a low rate under K-free conditions and capable of a graded response to K. Alternatively, two populations of Cl pump sites may exist; one which operates without K and another which is only functional when [K] is elevated.

Passive K transport

J^{K}_{net} is largely passive under open-circuit conditions and electrically coupled to active Cl transport. In support of this view, J^{K}_{net} is only 8 % of J^{Cl}_{net} when locust rectum is short-circuited whereas J^{K}_{net} equals J^{Cl}_{net} under open-circuit conditions. K acts as the counter-ion for electrogenic Cl transport even when much higher concentrations of Na are present in the mucosal solution (114 mM-Na *vs* 10 mM-K). The predominance of K as the counter-ion is ensured *in vivo* because (i) natural K levels (140 mM) are much higher than Na levels (20–40 mM) in the rectal lumen, and (ii) cAMP (which mediates the actions of CTSH) elevates P_K by about 400 %. In contrast, P_{Na} is unaffected by cAMP (Spring & Phillips, 1980).

When K was added to the mucosal side under open-circuit conditions to mimic the *in vivo* K gradient, we expected the mucosal side to become negative with respect to the serosal side (despite the stimulatory effect of K on active Cl transport), because epithelial K conductance is normally high. However, no reversal of V_t was observed when mucosal [K] was elevated to 140 mM. Also, R_t did not change when [K] was elevated from 10 to 200 mM. Both these observations are explained if P_K declined as [K] was increased. This decline in permeability was confirmed by measuring ^{42}K backflux as a function of bilateral [K] under I_{sc} conditions. The exact mechanism

[redacted]s concentration-dependence has not been studied in detail, but it may be analogous to the inverse relationship between mucosal [Na] and the rate of Na entry at the apical membrane reported in frog skin [Biber & Curran, 1970; Fuchs, Hviid Larsen & Lindemann, 1977; Moreno *et al.* 1973; Rick *et al.* 1975; Rotunno, Vilallongs, Fernandez & Cereijido, 1970; Van Driessche & Lindemann, 1979).

Calculating P_K from J^K_{sm} under I_{sc} conditions might result in an over-estimate of transepithelial permeability because it assumes that the epithelium is a single barrier to tracers when in fact P_K depends on membrane potentials and intracellular and extracellular K activities (Schultz & Frizzell, 1976). Intracellular potential has been measured under I_{sc} conditions as a function of external potassium concentration (Hanrahan, 1982). Using equation 14 of Schultz & Frizzell (1976), we calculated that 40% of the apparent decline in K permeability is due to membrane depolarizations and changes in intracellular K activity, but the remaining 60% of the decline in P_K must be due to a real reduction in K permeability. Also, errors due to the simplifying assumptions used in calculating P_K do not explain why cAMP stimulates J^K_{sm} by four-fold in normal saline (10 mM-K; Fig. 9) but not in high-K saline (140 mM-K; Table 3).

Hormonal regulation of salt reabsorption in locust rectum appears to be highly efficient because electrogenic Cl transport and counter-ion permeability (P_K) are stimulated simultaneously. What advantages might arise from a K-inhibitable P_K? When the hindgut contains unmodified (high-K) Malpighian tubule fluid, a K-sensitive P_K would prevent V_t from reversing to negative values and drawing Na from the haemolymph into the gut lumen. The maximum transepithelial electrochemical gradient for Na developed across the rectum *in situ* is smaller than for Cl or K (Phillips, 1964*b,c*), and active Na transport during cAMP stimulation is weak compared to Cl absorption (20%; Spring & Phillips, 1980; Williams *et al.* 1978). Reducing the loss of Na in this manner may be important for an insect feeding on fresh plant matter that is low in Na (14 mM) compared to K (114 mM-lettuce; Hanrahan, 1982). Finally, salt-loaded locusts can produce a strongly hypertonic urine in order to conserve body water (Phillips, 1964*a,b,c*). A decline in potassium permeability might prevent excess K reabsorption under these conditions.

Properties of active K transport

Net flux of ^{42}K from mucosa-to-serosa was measured under I_{sc} conditions during cAMP stimulation. The presence of a small active absorption of K is consistent with earlier findings that K is maintained far below electrochemical equilibrium in recta of salt-depleted (hydrated) locusts (Phillips, 1964*b,c*). In the present study, there was no net flux of K until cAMP was added. This differs from the very low rate of K absorption observed by Williams *et al.* (1978) using a different saline (Berridge, 1966) and a voltage clamp which did not correct for series resistance. We did not measure the K_t of active K absorption in this study; however, it is presumably less than 10 mM-K since J^K_{net} was identical when the bathing saline contained either 10 or 140 mM-K. This high-affinity, low capacity system for K absorption could be responsible for reducing [K] in the rectal fluid to the low levels reported in salt-depleted locusts (0·5 mM; Phillips, 1964*c*). In summary, K is absorbed transepithelially by electrical [redacted]upling to Cl transport under open-circuit conditions and also by an active system

which transports at a low rate but with a high affinity for K. The fact that there was no reduction in the cAMP-stimulated J^K_{net} when Cl was omitted from the saline supports the notion that KCl co-transport is not involved in transepithelial K absorption.

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