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THE SOURCE OF SHORT-CIRCUIT CURRENT ACROSS LOCUST RECTUM

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SUMMARY

1. Recta of desert locusts were mounted as flat sheets in 'Ussing-type' chambers and various parameters of tissue viability were monitored. The trans-epithelial resistance, the electropotential difference, the short-circuit current (I_{sc}), and unidirectional fluxes of $^{22}Na^+$, $^{36}Cl^-$ and $^{42}K^+$ all remained relatively constant during the 3rd and 4th h.

2. The direction of the I_{sc} indicated a net transport of either anions to the haemocoel, or cations to the lumen side. This current was abolished by KCN and was sensitive to temperature (Q_{10} = 2·4).

3. There was a rapid decline in I_{sc} over the first 2 h, which could be abolished by substituting NO_3^- or SO_4^{2-} for Cl^- in the bathing medium, indicating that this fall in current is due to a decline in the rate of Cl^- transport. Measurements of $^{36}Cl^-$ fluxes under short-circuit conditions confirm this interpretation.

4. In the steady-state (3rd and 4th h), however, the same anion substitutions had no effect on I_{sc}. Concurrent flux measurements indicated that net Na^+ and K^+ transport to the haemocoel side equals or slightly exceeds that of Cl^- in the same direction. Consequently all of the I_{sc} must be due to unidentified ion transport processes. Transport of H^+ to the lumen, or HCO_3^- and organic anions to the haemocoel side, is proposed.

INTRODUCTION

Selective reabsorption in the rectum is ultimately responsible for the regulation of haemolymph composition in the desert locust, *Schistocerca gregaria*, and possibly most terrestrial insects (reviewed by Phillips, 1977*a*). Most interest has centred on the capacity of this organ to produce hyperosmotic urine by the transfer of a hyposmotic absorbate from the lumen to the haemocoel side. Since it has recently been shown that steady-state fluid transport in the locust rectum can be driven by the absorption of any one of Na^+, K^+, or Cl^- ions (Phillips, 1977*a*, *b*), the properties and organization of ion transport processes in this epithelium are of renewed interest. The study of ion transfer processes across several other epithelia has been greatly advanced by

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application of short-circuit current and tracer flux methods (Ussing & Zerahn, 1951) under the defined conditions which are possible *in vitro*. The application of these methods to the insect rectum has been restricted because previous *in vitro* preparations of this organ exhibited rapid loss of most activity within an hour of extirpation (reviewed by Goh & Phillips, 1978). Most earlier studies were carried out during this period. We have found that activity during this initial period partly reflects a transient state when the rectal tissue swells dramatically and internal concentrations change following transfer from an hyperosmotic environment on the lumen side *in vivo* to isosmotic fluid *in vitro*. However, vigorous oxygenation of the lumen side will subsequently sustain water and solute absorption by everted rectal sacs at near steady rates for several hours (Goh & Phillips, 1978). We were thus encouraged to develop the short-circuited preparation described in the present paper. A preliminary report of this work has appeared in abstract form (Phillips, Williams, Spring & Prince, 1977).

MATERIALS AND METHODS

Animals were adult female *Schistocerca gregaria*, one to three months past their final moult, and fed upon lettuce and a mixture of dried grass, bran, yeast and powdered milk. The colony was maintained at 28 °C and 50% relative humidity under a photoperiod cycle of 16 h light and 8 h dark.

To study ion transport using the short-circuit current method, recta were carefully dissected free of trachael connexions, slit lengthwise using iridectomy scissors inserted through the anus, and removed by transverse cuts at the two ends. The resulting square of tissue measured about 0·8 × 0·8 cm.

The 'Ussing-type' chambers used in this study (Fig. 1 *a*, *b*) were modified from those described by Wood (1972), and consisted of male and female half cells, held together in a vice-like frame. The circular male orifice over which the sheet of rectal tissue was mounted, had an internal diameter of 0·5 cm (i.e. a surface area of 0·196 cm^2). To attach the membrane, the male half cell was laid on end so that the orifice faced upward and was filled with saline solution. Watchmakers forceps were used to impale the peripheral areas of the rectal tissue and cuticle (lumen side up) on eight fine metal pins which were permanently mounted radially around the outer side of the cylinder about 1 mm from the end. A snug-fitting rubber O-ring was then placed around the lip of the male orifice above the circle of pins to seal the rectal membrane in place. The effectiveness of the seal was checked by tipping the chamber to the normal upright position and noting whether saline escaped from the chamber. The male and female chambers were then clamped together in the vice. The rectal tissue did not touch the walls of the much larger female orifice; instead, the latter chamber was sealed by means of an outer O-ring mounted in a groove in the face of the chamber. The amount of saline was then adjusted so that both chambers contained 7 ml.

Fluid in each chamber was circulated by a gas-lift pump so as to deliver a flow of saline against the surface of the rectal wall, from the large reservoirs. Rapid and turbulent mixing, especially near the rectal membrane, was indicated by dye injection. Uniform colour was observed within a few seconds. The gas mixture contained 95% O_2 and 5% CO_2, and was bubbled through water before being introduced into the chambers to minimize evaporation of the bathing solution during long experiments. Loose fitting lids on the reservoirs prevented loss of fluid by splattering.

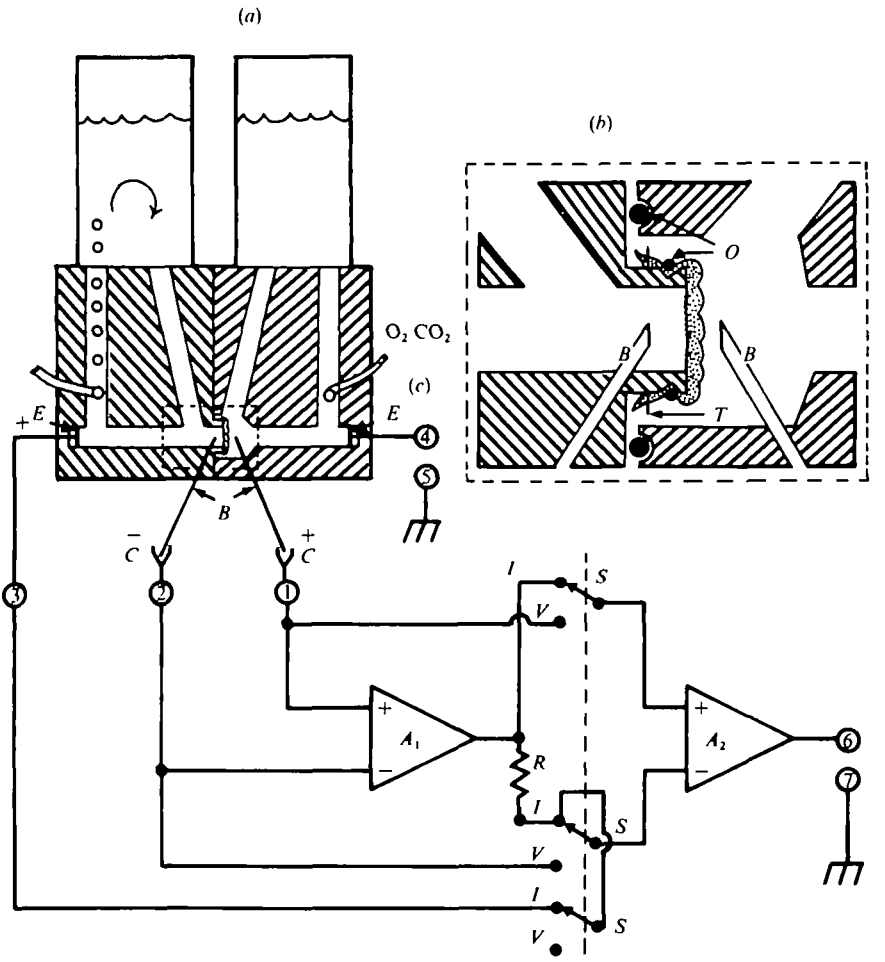

Fig. 1. The method used to short-circuit locust recta. (*a*) A side-view of the chambers used to mount locust recta. Arrow indicates direction of swirling produced by gas-lift pumps. (*b*) An enlargement of the area enclosed by dotted lines in Fig. 1 *a*. The stipled area indicates rectal tissue; *T*, tungsten pins; *O*, *O*-rings. (c) A block diagram of the circuitry used in this study as it is connected to the rectal chambers (Fig. 1 *a*). A_1 and A_2 are FET instrumentation amplifiers (Teledyne Philbrick: Dedham, Mass.) set at 2000× and 1× gain respectively. Short-circuit current is applied across the rectum by means of two silver plate electrodes (*E*) connected at ③ and ④. It is read as a voltage drop across a 10 k Ω resistor (*R*) with the gang switch (*S*) in the current (*I*) position (as shown). The PD across the rectum is read by means of 2 calomel electrodes (*C*) connected at ① and ② to 1·5 M-KCl agar bridges (*B*) with the gang switch in the voltage (*V*) position. Short-circuit current or potential difference are recorded graphically on a 'Fisher Recordall' strip chart recorder attached at ⑥. A 'Keithley model 602' electrometer can be attached across ① and ② to check and calibrate I_{sc} and PD measurements.

Most experiments were performed at a room temperature of 22 ± 1 °C. However, to determine the effect of temperature on I_{sc}, cooling coils were introduced into the chamber reservoirs and cooling fluid was circulated through these coils using a 'Haake model FE' constant temperature water-circulating pump. Temperature in both reservoirs was monitored with standard thermometers.

To change the saline during the course of experiments, a large volume (about 250 ml) was added through the top of each reservoir while maintaining the solution

level at a fixed point with a suction pump. Such changes took 1–2 min and were 99·9% effective, as measured by $^{36}Cl^-$ dilution.

The 'normal' physiological saline (Cl-saline) used in all experiments was modified from that of Berridge (1966): 24·5 mM-NaCl, 10·5 mM-$NaHCO_3$, 8·5 mM-KCl, 2 mM-$CaCl_2$, 13 mM-$MgCl_2$, 7·4 mM-disodium succinate, 1·87 mM-trisodium citrate, 12·8 mM-malic acid, 1·6 mM-glucose, 5·56 mM-maltose, 79·8 mM-sucrose, 2·67 mM-glycine, 4·61 mM-proline, 2·64 mM-glutamine, 12·3 mM-glutamic acid, 30 mg/l penicillin, and 100 mg/l streptomycin sulphate. The pH was adjusted to 7·00 with NaOH. The measured osmotic concentration of this saline was 317 m-osmol. Bubbling with the gas mixture used in this study lowered the pH of this bathing medium. The measured pH during experiments remained between 6·74 (1 h) and 6·70 (5–6 h), while differences in pH between the bathing media in the two chambers did not exceed 0·04 units.

Several other physiological salines were used in which Cl^- was completely replaced by either SO_4^{2-}, or NO_3^-, or acetate. These are subsequently referred to as SO_4-saline, NO_4-saline and acetate saline respectively. They were otherwise identical to Cl-saline except that sucrose levels in the SO_4-saline were increased to 128·3 mM to maintain an osmotic concentration of 317 m-osmol. In all experiments, identical solutions were placed on both sides of the rectum.

The Cl^- content of the tissue was measured by quickly blotting recta on 'Kleenex', and placing them in 1 ml of distilled water in capped polythene vials. After 24 h, when the cells had fully lysed, the Cl^- concentration of the resulting solution was determined with a 'Radiometer CMT 10' chloride titrator.

To measure I_{sc} we initially used the 3 electrode method of Wood (1972) and Wood & Moreton (1978) which corrects for voltage drop through the bathing solutions in the two chambers (described by Williams, 1975). When we found that the resistance of the bathing media between the PD recording electrodes was less than one percent of that across the rectal wall, we changed in later experiments to the two electrode system described in Fig. 1 *a*, *c*, which proved more convenient and reliable. The electrical resistance of the membrane in the open-circuit condition was determined by applying current (0–300 μA cm^{-2}) of known magnitude briefly (10 s) across the rectal wall and recording the change in trans-epithelial PD. The membrane resistance in the short-circuit condition was estimated from the short-circuit current and the open-circuit PD, which was measured at intervals by briefly stopping the short-circuit current for 5 s. In both cases, resistances were calculated from the current and the measured Δ PD using Ohm's law.

Unidirectional fluxes of $^{36}Cl^-$, $^{22}Na^+$, $^{35}SO_4^{2-}$ and $^{42}K^+$ were measured under short-circuit conditions. Isotopes were obtained from New England Nuclear Inc. in the following forms (specific activity): a 0·73 M-$Na^{36}Cl$ solution at pH 7·0 (4 mCi gm^{-1}), 2·3 mCi ml^{-1} of $^{22}NaCl$ in H_2O at pH 4·5 (carrier free), 6·3 mM-$Na_2{}^{35}SO_4$ solution (800 mCi m-mol^{-1}), and a 0·5 M-^{42}KCl solution (150 mCi gm^{-1}). When addition of isotope to one side of the membrane changed ion concentrations of the bathing media significantly (e.g. $^{36}Cl^-$ experiments), an unlabelled but otherwise equivalent solution was added in the same amount on the other side so that gradients of ion concentration or pH were not established across the rectal wall. Isotope was added to one side and 1-μl aliquots were removed to determine activity on this side (1) both initially and

after 4 h. At 15 min intervals, an aliquot of bathing solution (either 0·5 or 3·0 ml) was removed from the opposite side (2) to determine the amount of the isotope which had crossed the membrane. The fluid removed from this side was replaced with the appropriate volume of unlabelled saline. Activity on side 2 did not rise above 5–10% of that on side 1 during any of these experiments; therefore, back flux of isotope to side 1 was not significant. Under these conditions it was possible to estimate the unidirectional flux over each 15 min interval using the following equation (adapted from Shaw, 1955):

$$J_{1\rightarrow 2} = \frac{a_2 . V . c}{a_1 . T . A},$$

where $J_{1\rightarrow 2}$ is the unidirectional flux in mol cm^{-2} h^{-1}, a_1 represents the concentration of radioactivity (counts min^{-1} ml^{-1}) on side 1, a_2 is the increase in concentration of radioactivity on side 2 (in the same units) over the 15 min interval, V is the volume of fluid on side 2 in ml, C is the concentration of the unlabelled ion in the saline solution (mol ml^{-1}), T is the time interval over which the flux occurred (i.e. 0·25 h), and A is the area of the membrane (i.e. 0·196 cm^2).

The $^{36}Cl^-$, $^{22}Na^+$ and $^{42}K^+$ activities in solutions were measured by placing aliquots on stainless steel planchets, evaporating to dryness under a heat lamp and counting with a 'Nuclear Chicago Model 470' gas-flow detector and automatic sample changer. Appropriate corrections were made for radioactive decay and self-absorption, where these were significant. The activity of $^{35}SO_4^{2-}$ was measured as previously described by Maddrell & Phillips (1975).

RESULTS

Viability of preparation: electrical parameters

The long-term viability of the *in vitro* preparation, as indicated by various electrical parameters, is shown in Fig. 2. The PD across the rectal wall (lumen positive) under open-circuit conditions declines slowly from an initial value of 26·2 ± 3·9 mV to 15·6 ± 3·1 mV after 6 h (Fig. 2*a*). The PD averages 12·5 mV after 18 h. This is in good agreement with values observed *in vivo* (Phillips, 1964*b*) and for everted rectal sacs (Goh & Phillips, 1978). The PD was also routinely measured at the beginning and the end of short-circuit experiments. Under these conditions the value fell from 36·5 ± 1·4 to 16·3 ± 1·4 (± S.E., $n > 21$) over the 4 h experimental period. The trans-epithelial electrical resistance under both open-circuit and short-circuit conditions (200–280 Ω cm^{-2}) does not change significantly over the first 6 h (Fig. 2*b*). The mean I_{sc} is initially high (209 μA cm^{-2}, or 7·8 μequiv cm^{-2} h^{-1}) but drops rapidly to a value of 3 μequiv cm^{-2} h^{-1} within 2 h and thereafter falls only slightly over the next 2 h (Fig. 2*c*). While most experiments were terminated at 4 h, preparations maintained a reasonably stable I_{sc} of about 2 μequiv cm^{-2} h^{-1} (50 μA cm^{-2}) for at least 8 h (Fig. 3*a*). These measurements indicate that transport activity remains relatively constant during the 3rd and 4th h, which is subsequently referred to as the steady-state period. Inclusion of lactalbumin hydrolysate (8 g l^{-1}) and yeast extract (4 g l^{-1}) in the saline caused a significant increase of 20% in I_{sc} over the whole experimental period (Williams, 1975) but these constituents were omitted in the present studies because they made it difficult to prepare Cl-free salines.

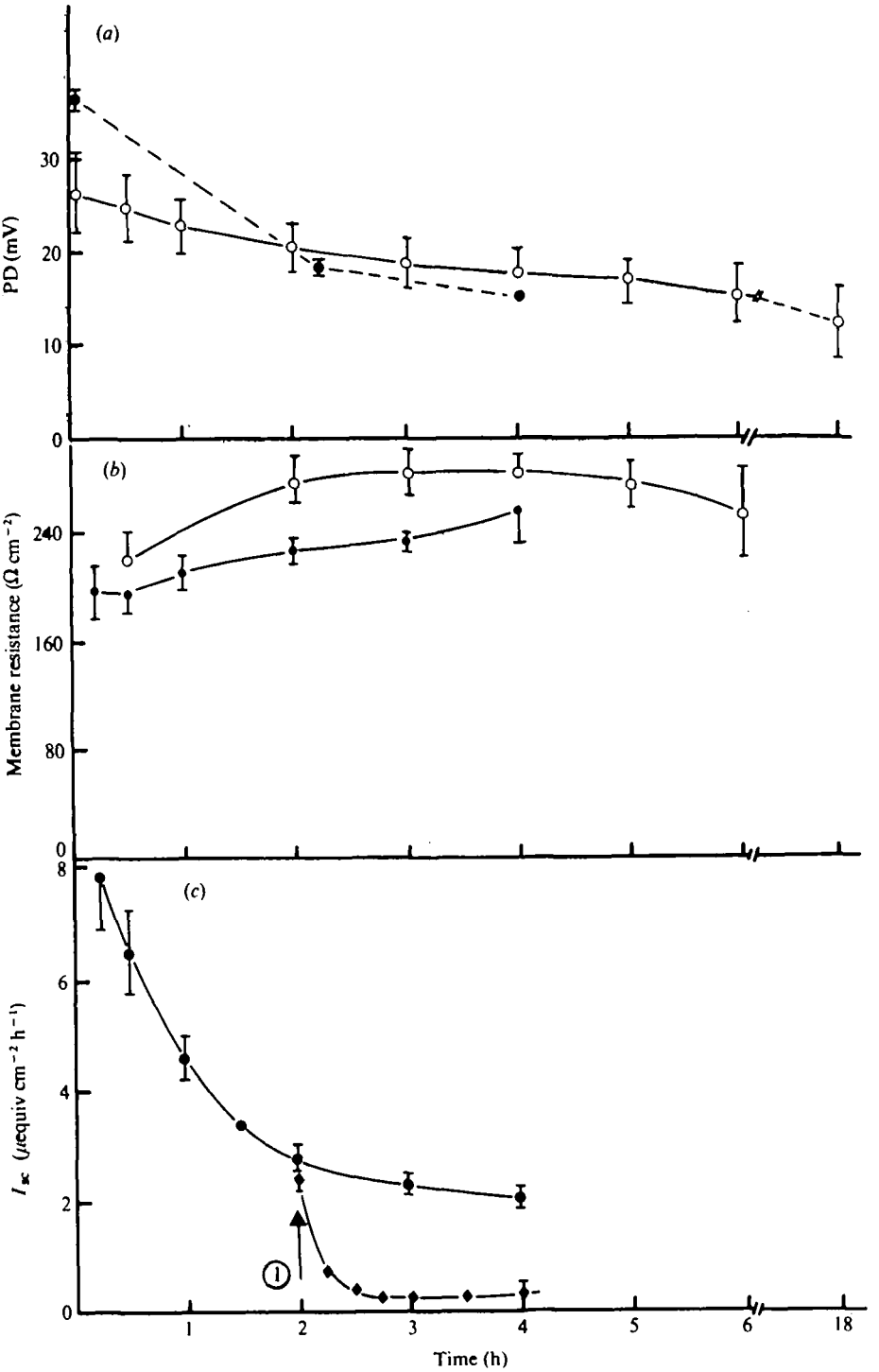

Fig. 2. Viability of rectal preparations bathed in Cl-saline, as indicated by electrical parameters. (*a*) Trans-epithelial PD (lumen positive; mean ±S.E., where larger than symbol): ○, open-circuit conditions (n = 4–9); ●, short-circuit conditions (n = 21–63). (*b*) Trans-epithelial d.c. resistance (mean ±S.E.): ○, open-circuit conditions (n = 14–16); ●, short-circuit conditions (n = 3–20). (*c*) Short-circuit current (mean ±S.E., where larger than symbol) without KCN (●, n = 11), and with KCN added at ① (◆, n = 4).

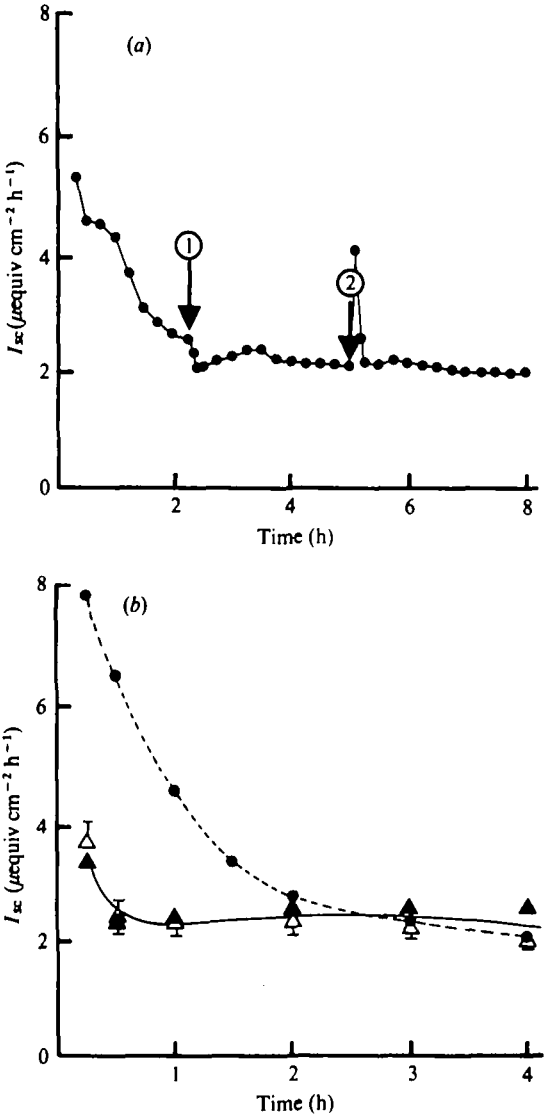

Fig. 3. The effect of anion substitutions in the bathing saline on short-circuit current across recta. (*a*) The change in short-circuit current in a typical preparation when the bathing saline is changed from normal Cl-saline to SO_4-saline at ① and back to Cl-saline at ②. (*b*) The short-circuit current (mean ±S.E., where larger than symbol) across recta bathed in Cl-saline (●, from Fig. 2*c*), compared with recta bathed in SO_4-saline (▲, n = 10) and NO_3-saline (△, n = 6).

We considered the possibility that the initial decline in I_{sc} was associated with re-arrangement of ionic gradients and transport activities during adjustment of the tissue to short-circuit conditions, rather than a consequence of removing recta from the animal. To test this hypothesis, some preparations were left in the open-circuit state in the chambers for 2 h before I_{sc} was applied. These preparations did not show a large initial fall in I_{sc}, but rather exhibited the same I_{sc} during the next 2 h period as did preparations which had been short-circuited from the time of extirpation

(Williams, 1975). Clearly the initial decline in I_{sc} is a consequence of removing the rectum from the locust (see Discussion).

The metabolic dependence of the I_{sc} was confirmed by several observations. Anoxia severely reduced I_{sc}, and 1 mM-KCN in normal, oxygenated Cl-saline caused a rapid loss of more than 85% of the I_{sc} during the steady-state phase (Fig. 2*c*). Clearly the ion transport processes causing the current are largely dependent on aerobic respiration. Individual preparations were subjected to abrupt 5–15 °C changes in temperature over a range of 10 to 35 °C. The I_{sc} at 10 °C was less than 30% of the normal value at 25 °C. The average Q_{10} for the I_{sc} over the whole temperature range was 2·4 (Williams, 1975).

Source of the I_{sc}: anion substitutions

The direction of the I_{sc} shown in Fig. 2*c* indicates a net transport of either anions to the haemocoel side or cations to the lumen side. Since previous studies (Phillips, 1964*a*, *b*; Phillips, 1977*a*, *b*; Goh, 1971) indicate that active absorption of chloride, sodium and potassium ions occurs from the lumen, we initially considered the possibility that transport of the anion is in excess of the two cations. This excess Cl^- transport might then be the principal source of the steady I_{sc} during the 3rd and 4th h. If this interpretation is correct, then substitution of other anions for chloride in the bathing medium might not only abolish, but actually reverse the directions of the I_{sc}. When the medium bathing single preparations was changed from normal saline to SO_4-saline (Cl-free) and then back to normal saline, the I_{sc} did not alter significantly (Fig. 3*a*). When preparations were continuously bathed on both sides in the same Cl-free medium (either NO_3-saline, or SO_4-saline, or acetate saline) for 4 h, the initial rapid fall of I_{sc} over the first 2 h disappeared, indicating that this decline in current was probably due to the loss of some Cl^- transport activity (Fig. 3*b*). The I_{sc} observed in the presence of NO_3- and SO_4-salines was constant from 0·5 to 4 h and was similar in size and direction to that observed in the presence of normal Cl-saline over the 3rd and 4th h (Fig. 3*b*). During the latter period, the PD across the rectal wall in SO_4-saline was 11–20 mV (haemocoel negative) which is very similar to the value observed when Cl^- is present. More surprisingly, acetate saline maintained the I_{sc} at levels well above the normal (Williams, 1975), and caused a 28–76% increase in PD. We will report in more detail on this stimulatory effect of acetate in a later paper.

We considered the possibility that there was sufficient residual chloride in the rectal tissue during the 3rd and 4th h to sustain transport of this anion when recta were bathed in Cl-free media. The chloride content of freshly dissected recta was measured and found to be only $1 \pm 0{\cdot}04$ μmol rectum^{-1} (mean ± S.E.) which is only a small fraction of the total net charge transported over the 4 h experimental period, as indicated by the I_{sc}. In the presence of SO_4-saline, the chloride content of recta fell 90% within the first h and did not change thereafter. Tissue chloride clearly does not sustain transport of this anion during the steady-state period. Another possibility is that traces of chloride in reagents and from the KCl-agar electrodes might maintain chloride transport if the K_m of the latter process is exceptionally low. The initial chloride concentration in the SO_4-saline was found to be below the detection level of 0·5 mM. During typical short-circuit experiments with SO_4-saline, the measured

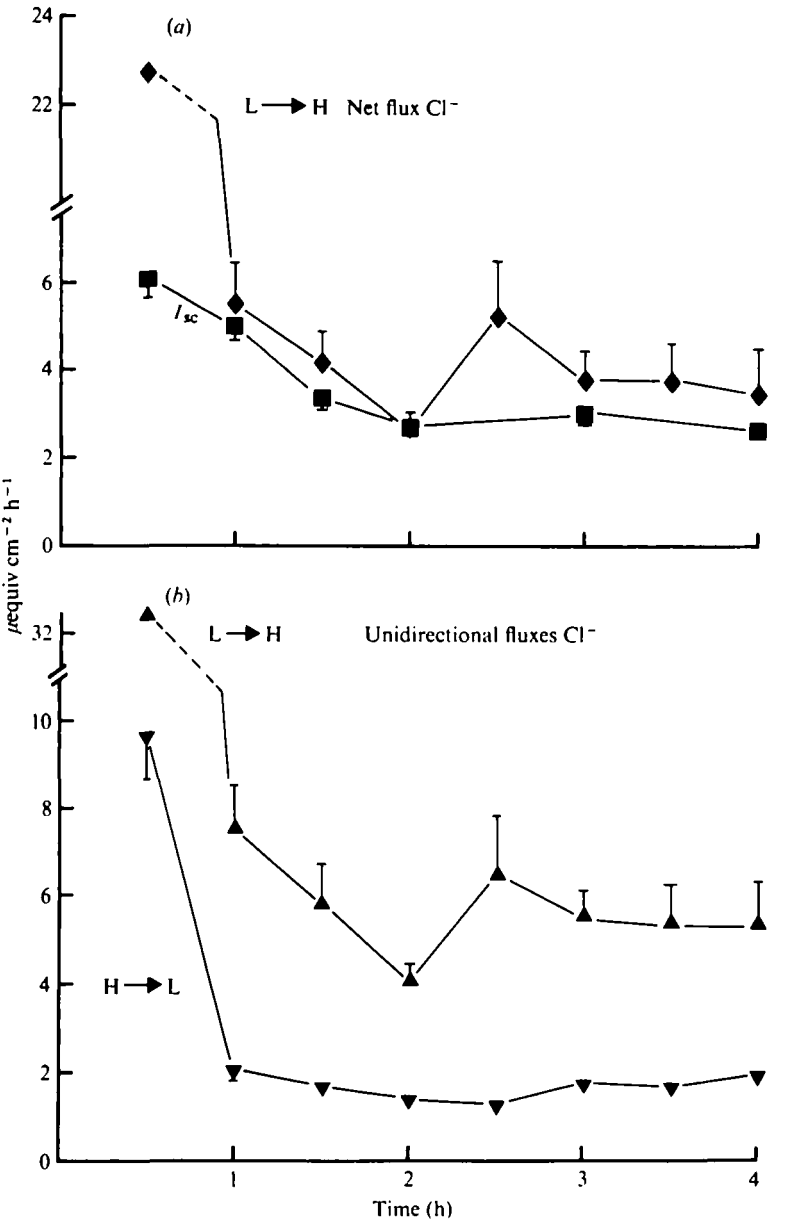

Fig. 4. The unidirectional and net flux rates of Cl^- across recta bathed in normal Cl-saline under short-circuit conditions (mean ± S.E., where larger than symbol; $n = 8$–10). (*a*) The net flux rate (◆) calculated from the difference in unidirectional fluxes is compared with the I_{sc} (■) measured simultaneously during these experiments ($n = 16$–20). A positive sign of the *Y* axis indicates net movement of negative charges from lumen to haemocoel side. (*b*) The unidirectional flux rates, lumen to haemocoel (▲, L → H) and haemocoel to lumen (▼, H → L).

level of chloride in the bathing medium increased to 1–2 mM at most after 5 h, if the medium was not changed. The flux of $^{36}Cl^-$ from lumen to haemocoel side was subsequently measured when the bathing medium contained 2·5 mM-Cl^- and was found to average only 0·3 μequiv cm^{-2} h^{-1}, or less than 10% of the influx in the presence of normal Cl-saline (Fig. 4). In summary, the I_{sc} observed during the 3rd and 4th h remains unchanged when there is no significant transport of chloride ions. Since absorption of Na^+ and K^+ is in the wrong direction to explain the I_{sc}, clearly other ion transport processes must give rise to the net current. Evidence that H^+ secretion to the lumen side or that HCO_3^- absorption might be responsible is considered in the Discussion.

Source of the I_{sc}: radio-isotopic flux studies

The short-circuit experiments outlined above provide no direct evidence that Cl^-, Na^+, and K^+ ions are actively transported by the present *in vitro* preparation. Since transport of all these monovalent ions is indicated by previous studies both *in vivo* and with other *in vitro* preparations of this organ (Phillips, 1964*a*, *b*; Goh, 1971; Phillips, 1977*a*, *b*) it is important to determine whether these transport processes also occur under short-circuit conditions. We therefore studied unidirectional fluxes of $^{22}Na^+$, $^{36}Cl^-$ and $^{42}K^+$.

All experiments were conducted with normal Cl-saline on both sides of the rectal wall and under short-circuit conditions. The I_{sc} and either the influx or efflux were followed simultaneously with time over the first 4 h after removal of recta from locusts. Since average I_{sc} values during influx and efflux studies on different preparations were very similar, these values are pooled in Figs. 4–6, and compared to the net flux. The average flux values during the 3rd and 4th h are summarized in Table 1. Unidirectional fluxes of $^{36}Cl^-$ are initially very high (Fig. 4*b*), as is the resulting net flux to the haemocoel side (Fig. 4*a*). This initial rapid fall in $^{36}Cl^-$ net flux closely parallels the decline in I_{sc} during the first h, as predicted from anion substitution studies described above (Fig. 3*b*). Cl^- fluxes remain constant during the 3rd and 4th h, indicating the presence of active chloride transport of more than sufficient magnitude to account for the steady-state I_{sc}. However, chloride transport is exceeded slightly by active Na^+ transport in the same direction (i.e. from lumen to haemocoel side, Fig. 5*a*), so that the net contribution of these two ions toward the I_{sc} is small and in the wrong direction (Table 1).

Unlike the fluxes of $^{36}Cl^-$, those of $^{22}Na^+$ are not significantly different over the whole 4 h experimental period (Fig. 5*b*). As predicted by studies with everted sacs (Phillips, 1977*a*, *b*; Goh & Phillips, 1978) the net flux of K^+ to the haemocoel side under I_{sc} conditions is very small because the bathing medium contains only 8·5 mM-K^+ (Fig. 6*a*). Like the fluxes of $^{22}Na^+$, those of $^{42}K^+$ are relatively constant over the 1st to 4th h (Fig. 6*b*). The steady-state (i.e. 3rd and 4th h) ratios of influx to efflux under short-circuit conditions are approximately 3:1, 4:1 and 5:3 for $^{36}Cl^-$, $^{22}Na^+$ and $^{42}K^+$ respectively (from Table 1).

These experiments provide the most rigorous proof to date that the locust rectum is capable of actively transporting chloride, sodium and potassium ions. This conclusion agrees with the findings of other types of experiments on intact locusts and with everted rectal sacs (Phillips, 1964*a*, *b*; Goh, 1971; Phillips, 1977*a*, *b*; Goh &

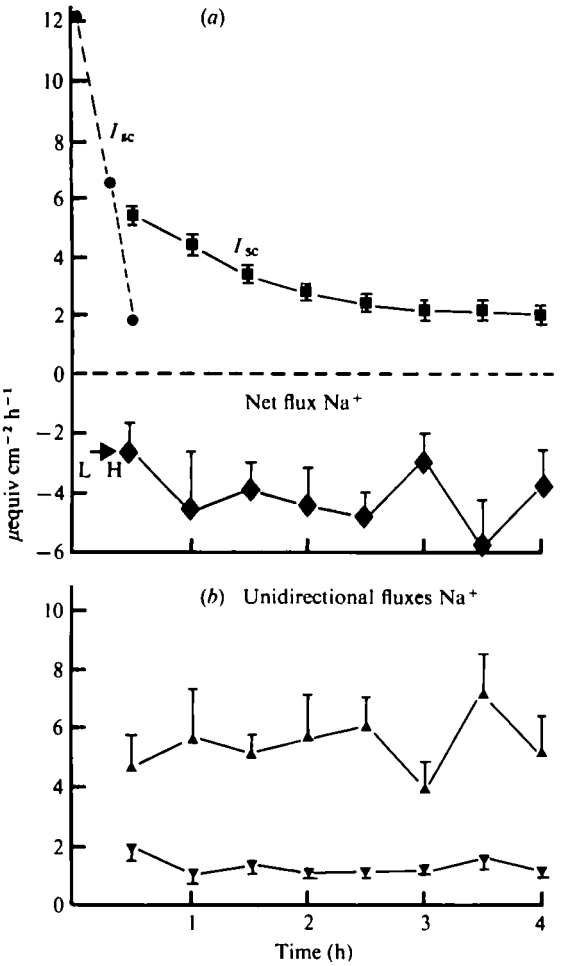

Fig. 5. The unidirectional and net flux rates of Na^+ across recta bathed in Cl-saline under short-circuit conditions (mean ± S.E., where larger than symbol; n = 8–10). (*a*) The net flux rate (◆) calculated from the difference in unidirectional flux rates is compared with the I_{sc} (■) measured simultaneously during these experiments (n = 16–20). The broken line (●) indicates the average I_{sc} exhibited by the rectal preparations used by Herrera *et al.* (1976). A positive sign of the Y axis indicates net movement of negative charges to haemocoel side. (*b*) The unidirectional flux rates, lumen to haemocoel (▲, L → H) and haemocoel to lumen (▼, H → L).

Phillips, 1978). The flux studies also indicate that the initial rapid decline of I_{sc} over the first 1–2 h is largely due to a fall in Cl^- transport activity, while the transport of Na^+ and K^+, as well as other unidentified transport processes (possibly H^+ secretion, or HCO_3^- and organic anion reabsorption) are relatively constant over the first 4 h. Clearly the unidentified ion transport processes must equal those of Cl^- or Na^+ in magnitude and must account for all of the net I_{sc} during the steady-state phase (Table 1).

Table 1. *A summary of the average flux rates of monovalent ions across the rectal wall under short-circuit conditions during the 3rd and 4th h; means* ± S.E. *for* 8–10 *preparations* (40–50 *observations*)

(H, haemocoel; L, lumen. A negative sign of net flux and I_{sc} values indicates movement of negative charges to haemocoel or positive charges to lumen. Differences in influx and efflux values for each ion are highly significant ($P \leq 0.01$).)

Ion	Saline concentration (mM)	Unidirectional fluxes (μequiv cm^{-2} h^{-1})	Net flux (μequiv cm^{-2} h^{-1})
Cl^-	80*	5·4 ± 0·4 L → H 1·6 ± 0·1 H → L	−3·8 ± 0·4 L → H
Na^+	70	5·7 ± 0·5 L → H 1·3 ± 0·7 H → L	+4·4 ± 0·5 L → H
K^+	8·5	0·5 ± 0·04 L → H 0·3 ± 0·02 H → L	+0·2 ± 0·05 L → H
A. Expected I_{sc} calculated from net fluxes ($Na^+ + K^+ + Cl^-$)			0·8 (−0·2 to +1·7)†
B. Observed I_{sc} during flux studies (54 preparations)			−2·4 ± 0·1
C. Unknown ion transport processes (B-A)			−3·1 (−2·1 to −4·2)†
D. Proposed ion transport processes:			
H^+			−1·4‡ H → L
HCO_3^-			−1·4 L → H

* Cl^- concentration of Berridge Ringer + $^{36}Cl^-$ solution.
† Maximum and minimum estimates using S.E.
‡ Measured rate of acidification of rectal lumen contents *in vivo* (Speight, 1968).

DISCUSSION

The success of the short-circuited preparation used in this study can be judged by comparing the observed transfer rates with those *in vivo* (Phillips, 1964*b*) and with everted rectal sacs (Goh, 1971; Phillips, 1977*a*, *b*; Goh & Phillips, 1978). The steady-state rates of Na^+ and Cl^- transport under short-circuit conditions (Table 1) are 2 to 3 times greater than the net absorption rates previously reported (reviewed by Goh & Philips, 1978). Since the latter values are the consequence of active transport less the net back diffusion which occurs under open-circuit conditions, exact comparisons are difficult. Moreover, it is not clear whether the external or internal diameter of the orifice wall holding the rectum (Fig. 1*b*) best describes the effective surface area of membrane exposed to I_{sc} in the present study. Because of the small size of this opening, the larger estimate of effective surface area is 56% greater than the minimum value which we have used; therefore, in calculating fluxes and I_{sc} *per cm*^{2}, we may have overestimated the true rates. Despite the problem of exact comparisons between various types of preparation, the short-circuited preparations used in this study (during 3rd and 4th h) clearly retain remarkably well those ion transport activities which we have previously identified. The significance of the much higher initial I_{sc} and Cl^- transport compared to those during the steady-state period is discussed later.

The net transport of Na^+, Cl^-, and K^+ which occurs to the haemocoel side of locust recta under short-circuit conditions during the steady-state phase cannot entirely account for the I_{sc} (Table 1). The rectum must transport some other anion to the haemocoel side or cation to the lumen side at a rate of 2 to 4 μequiv cm^{-2} h^{-1}. We

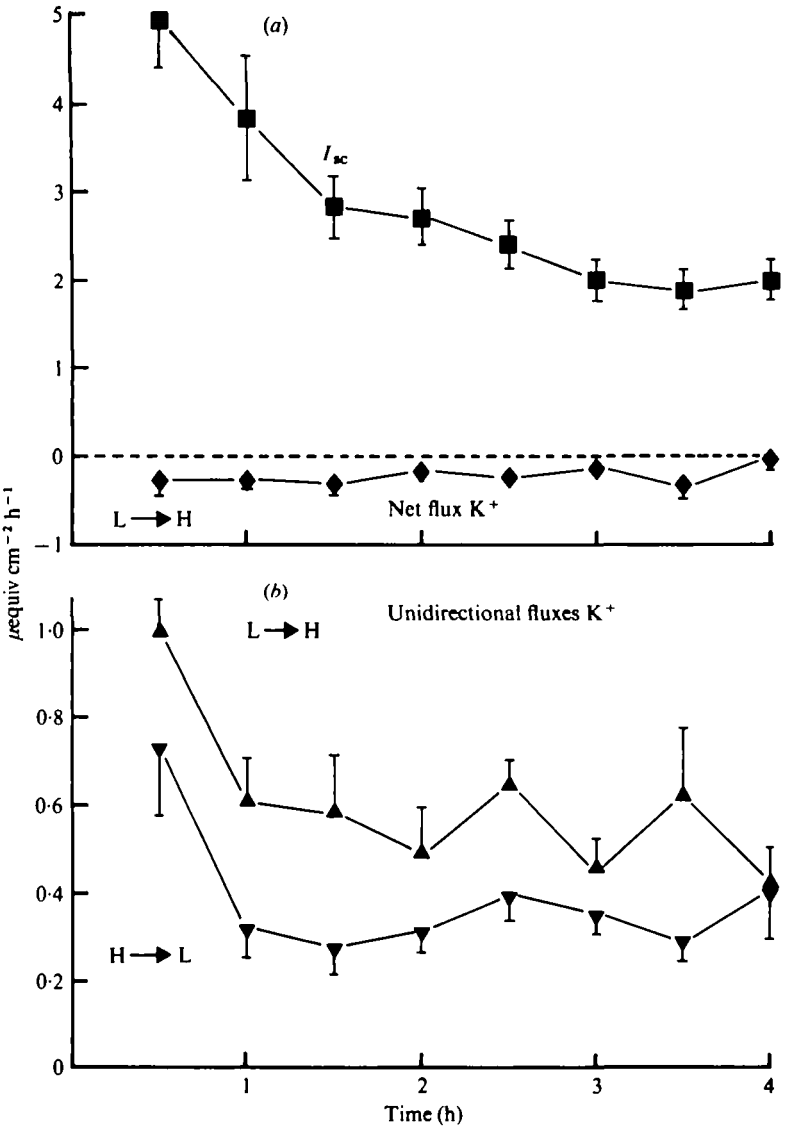

Fig. 6. The unidirectional and net flux rates of K^+ across recta bathed in Cl-saline under short-circuit conditions (mean ±S.E., where larger than symbol; n = 8–10). (*a*) The net flux rate (◆) calculated from the difference in unidirectional fluxes is compared with the I_{sc} (■) measured simultaneously during these experiments (n = 16–20). A positive sign of the Y axis indicates net movement of negative charges from lumen to haemocoel side. (*b*) The unidirectional flux rates, lumen to haemocoel (▲, L → H) and haemocoel to lumen (▼, H → L).

suggest that HCO_3^- and H^+ might be the respective anion and cation, for the following reasons. Fluid entering the locust rectum *in vivo* is quickly acidified to a pH of 5·0–6·5 (Phillips, 1961, 1965) and this occurs against a large electro-chemical potential difference. Speight (1968) injected various buffer solutions into lumina of ligated recta *in situ* and from the initial rate of pH decline obtained a *minimum* estimate

for H^+ secretion of 1·4 μequiv cm^{-2} h^{-1}. If transport of a bicarbonate ion to the haemocoel accompanies each movement of a hydrogen ion in the opposite direction, then a net I_{sc} of at least 2·8 μequiv cm^{-2} h^{-1} would be generated. There is a 50% excess of cations over chloride in absorbate collected from the haemocoel side of everted rectal sacs (Phillips, 1977*a*, *b*) so that HCO_3^- may indeed be absorbed. Transport of H^+ and HCO_3^- in other systems commonly requires the presence of carbonic anhydrase. This enzyme has been detected in rectal tissue of the desert locust by both biochemical and histochemical methods (J. Hanrahan, personal communication). An inhibitor of this enzyme, 'Diamox' (5×10^{-4} M), causes 25–40% inhibition of the I_{sc} during the steady-state phase (J. Spring, personal communication). In summary, we believe that H^+ and HCO_3^- transport might account for the unknown component of the I_{sc}, either in whole or in part. However, absorption of phosphate ions and organic anions should also be considered, because inorganic monovalent ions account for only half of the solute in intercellular spaces of cockroach recta (Wall, 1971).

The transport of unidentified ions by locust recta does not help to explain why various anion substitutions fail to alter the I_{sc} during the steady-state period. Since this observation presumably reflects the cellular organization of various ion pumps in the rectum, possible explanations are worth considering. Diamond (1962) has proposed that Na^+ and Cl^- absorption by the vertebrate gall bladder are tightly coupled so that the pump is neutral. Thus abolishing Cl^- transport also eliminates Na^+ absorption and so I_{sc} should not change. Since everted rectal sacs of the locust can absorb any one of Na^+, K^+ or Cl^- in the absence of the others (Goh, 1971; Phillips, 1977*a*, *b*), coupled transport of NaCl is unlikely to explain the results in Fig. 3. We also do not favour the suggestion that a general anion pump in the rectal epithelium can transport all of the anions which we have used. The measured L → H flux of $^{35}SO_4^{2-}$ across short-circuited recta bathed in SO_4-saline is less than 0·05 μM cm^{-2} h^{-1} ($n = 3$); i.e. substantial SO_4^{2-} absorption does not occur. A third possibility, which will be tested, is that the chloride pump can transport HCO_3^- to the haemocoel side when Cl^- concentrations are very low; i.e. these two anions may compete for a common pump. A final possibility is that both Na^+ and Cl^- transport processes involved coupled, neutral exchanges for another cation (e.g. H^+ or K^+) and anion (e.g. HCO_3^-) respectively, as has been proposed for gill epithelia of fresh-water teleosts (reviewed by Maetz, 1971). Thus, absence of either Cl^- or Na^+ in bathing media might have little effect on I_{sc}. According to this interpretation, an additional electrogenic component (e.g. H^+ secretion or organic anion absorption) must be postulated to explain the insensitive I_{sc} and PD which persist following anion substitution. Chloride transport cannot involve a rigid anion exchange under all conditions because an electrogenic component of the chloride transport is indicated by the initial decline of net Cl^- flux (Fig. 4*a*), I_{sc}, and PD (Fig. 2) over the first h in present experiments.

An initial rapid fall in transport activity is common to all *in vitro* preparations of insect recta reported to date (reviewed by Goh & Phillips, 1978). This may be a consequence of removing recta from natural hormonal stimulation. Observations by J. Spring *et al.* (1978) in our laboratory support this view. Extracts of corpora cardiaca (CC; 1/20th gland in 7 ml) cause a 2- to 3-fold increase in both I_{sc} and net $^{36}Cl^-$ flux across locust recta during the steady-state phase. Stimulation of $^{36}Cl^-$ influx can alone account for all of the I_{sc} increase and $^{22}Na^+$ fluxes are not

altered significantly. A corresponding increase of 10–15 mV in trans-rectal PD on stimulation suggests that the Cl^- pump has an electrogenic component. The stimulatory agent in CC extracts is heat stable and is mimicked by either $0{\cdot}3 \times 10^{-3}$ M c-AMP or 10^{-2} M-theophylline in the bathing media. Indeed, rectal tissue levels of c-AMP increase 2- to 3-fold following exposure to CC extract. The time course of the decline in I_{sc} following CC treatment is similar to the initial decline shown in Fig. 4*a*. Clearly, the initial fall in I_{sc} and Cl^- transport rate over the first h may be due to removal of recta from natural hormonal stimulation. Since Cl^- transport can drive water absorption (Goh, 1971; Phillips, 1977*a*, *b*), this Cl^- transport stimulating factor from the CC may be the same as the antidiuretic factor which has been reported from the same source (reviewed by Mordue, 1972). We have reason to believe that the trauma of handling and dissecting locusts causes release of such a stimulatory agent. It is also conceivable that initial swelling of rectal tissue *in vitro* (Goh & Phillips, 1978) may trigger c-AMP synthesis as part of a mechanism controlling cell volume. However, it must be emphasized that the stimulatory factor can normally be detected in the haemolymph only under specific physiological conditions; i.e. the rectum *in situ* is apparently not always stimulated by this substance.

When this work was nearing completion, Herrera *et al.* (1976, 1977) published results of experiments using a different short-circuited preparation of the locust rectum. They concluded that electrogenic transport of Cl^- is largely responsible for the I_{sc}; however, their interpretation is based solely on ion substitution experiments and not on concurrent flux studies. Moreover, their studies were restricted to the initial transitory phase (first 40 min), when I_{sc} was falling precipitously (see Fig. 5*a* for comparison). In our study, we have extended observations to the steady-state (i.e. unstimulated) phase, during which anion substitutions yield quite different results. Several differences in methods might explain why transport activity of our preparation is sustained for several h, while that of Herrera *et al.* is apparently not. We used a complex tissue culture medium and vigorously stirred both sides of the rectum with 95% O_2–5% CO_2, which may have sustained transport of H^+, HCO_3^-, or organic anions. Herrera *et al.* used a simple saline and bubbled with pure O_2 on one side only of a *sac* preparation. They report no direct evidence for substantial transport of Na^+, K^+ and other ions (e.g. H^+), which several workers in our laboratory have observed using three different kinds of preparation, including *in situ* recta. However, they do report that the initial I_{sc} is inhibited by KCN and partially by 'Diamox', and that both the initial I_{sc} and PD are Cl^- dependent. We concur with these observations.

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