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STUDIES ON LOCUST RECTUM

II. IDENTIFICATION OF SPECIFIC ION TRANSPORT PROCESSES REGULATED BY CORPORA CARDIACA AND CYCLIC-AMP

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SUMMARY

1. The unidirectional fluxes of $^{36}Cl^-$ and $^{22}Na^+$ across short-circuited locust recta bathed in a simple NaCl saline were followed with time. Unidirectional fluxes and net flux of $^{22}Na^+$ to the haemocoel side all remained constant for at least 4 h and were unaffected by either corpora cardiaca homogenate (CC) or cAMP.

2. Both CC and cAMP stimulated influx and net flux of $^{36}Cl^-$ to the haemocoel side. Over the whole time course of the experiment, i.e. both before and after stimulation, net Cl^- flux approximately equalled the short-circuit current (I_{sc}).

3. Neither CC nor cAMP caused substantial stimulation of I_{sc} or transepithelial electropotential difference (PD) if all Cl^- in the bathing saline was replaced by either sulphate or nitrate or acetate.

4. Acetate saline sustains I_{sc}, PD and transepithalial resistance (R) at higher levels than does simple Cl-saline.

5. Experiments with Cl-free, SO_4-salines suggest that alternate electrogenic transport processes can be slowly turned on when Cl^- is absent, provided a complex saline which contains several organic constituents, or simple acetate saline, is present.

INTRODUCTION

There is a factor present in the corpora cardiaca (CC) of *Schistocerca gregaria* which causes large changes in active ion transport across rectal epithelia as indicated by a 2- to 3-fold increase in the short-circuit current (I_{sc}) and by a smaller increase in transepithelial electropotential difference (PD). This factor apparently acts by first elevating intracellular levels of cAMP (Spring & Phillips, 1980*a*). The question remains as to which specific ion transport process is affected by the CC factor and cAMP.

Williams *et al.* (1978) showed that under steady-state conditions there is a large net flux of both Na^+ and Cl^- from the lumen side of short-circuited, unstimulated recta. These two net fluxes are of nearly equal magnitude but, being of opposite

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electrical charge, they do not make a substantial net contribution to the total I_{sc}. Moreover, steady-state I_{sc} does not change when chloride in the bathing media is replaced by nitrate or sulphate. Williams *et al.* suggest that the balance of the I_{sc} is due to H^+ secretion to the lumen or anion absorption (HCO_3^-, organic anions, PO_4^{3-}) to the haemocoel.

There was reason to believe that CC and cAMP act specifically by increasing electrogenic Cl^- transport. The initial rapid fall in I_{sc} following removal of recta from locusts is due to a decline in active Cl^- uptake, whereas Na^+ transport is not similarly affected (Williams *et al.* 1978), and these stimulants restore I_{sc} and PD to the original high levels (Spring & Phillips, 1980*a*). However, inhibition of Na^+ absorption, or stimulation of HCO_3^-, PO_4^{-3} or organic anion uptake would have the same effect on I_{sc}. In the present paper we consider some of these possibilities by studying the effect of cAMP and CC on fluxes of $^{22}Na^+$ and $^{36}Cl^-$, and on the I_{sc} of recta bathed in various Cl-free salines.

MATERIALS AND METHODS

The experimental animals and the method used to measure short-circuit current (I_{sc}), transepithelial potential difference (PD) and resistance (R) across *in vitro* recta of *Schistocerca gregaria* were identical to those reported by Spring & Phillips (1980*a*).

Measurement of ion fluxes

Unidirectional fluxes of $^{36}Cl^-$ and $^{22}Na^+$ were measured under short-circuit conditions as described by Williams *et al.* (1978). Isotopes were obtained from New England Nuclear, Inc., in the following forms: a 0·41 M-$Na^{36}Cl$ solution at pH 7·0 (5·8 mCi.g^{-1}) and 24 mCi.ml^{-1} of $^{22}NaCl$ in H_2O at pH 4·5 (carrier free). Isotopes were added to side 1, and at 20 min intervals 2·0 ml aliquots of bathing solution were removed from side 2 to determine the amount of isotope which had crossed the membrane. The solution removed during sampling was replaced with an equal volume of unlabelled saline. Unidirectional fluxes over each 20 min period were calculated as described by Williams *et al.* (1978).

Fluxes in opposite directions were measured on different preparations concurrently with I_{sc} and PD. Average I_{sc} and PD values were very similar for influx and efflux studies and were consequently pooled. Net flux was calculated as the difference between the two mean unidirectional fluxes. Variances for the net fluxes were calculated using the formula for common variance of unequal sample sizes (Larkin, 1976).

Ninety minutes after the initiation of short-circuiting, a maximum dose of stimulant in saline (0·1 pr CC or 0·3 mM cAMP) was added to the haemolymph side of the preparation. During experiments in which samples were removed from the haemolymph side, no attempt was made to replace the stimulant lost through sampling, so that the concentration of stimulant in the bathing saline fell stepwise with time after the first 20 min (see Fig. 2).

Cl-free salines

A number of simple Cl-free salines were prepared by replacing all of the Cl^- in simple saline with SO_4^{2-}, or NO_3^-, or acetate. These are subsequently referred to as

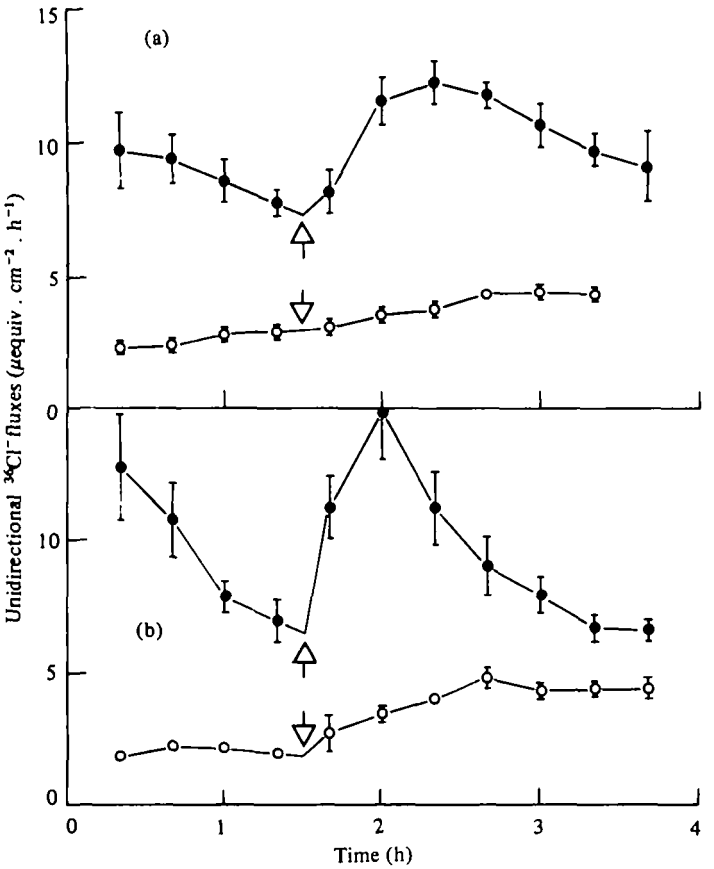

Fig. 1. Unidirectional Cl^- fluxes with time for short-circuited recta bathed in simple Cl-saline (mean ± S.E.M.). ●, Influx (L → H); ○, backflux (H → L). (*a*) 0·1 pr CC added at arrows. (*b*) 0·3 mM final concentration cAMP added at arrows.

NO_3-saline, SO_4-saline-1 (equivalent solution), SO_4-saline-2 (equimolar solution), and acetate salines respectively (Table 1). All these salines are of similar osmotic concentration, except for SO_4-saline-2 which is distinctly hyperosmotic, and SO_4-saline-1 which is slightly hypo-osmotic to the others. Complex SO_4-saline was prepared according to Williams *et al.* (1978).

To study the effect of anion substitution on the response of steady-state I_{sc} to stimulation, preparations were initially bathed in one of the Cl-free salines for 1 h. The bathing saline, which now contained any Cl^- lost from the tissue, was completely replaced with four changes of the same saline before experiments were begun. Subsequent changes from one saline to another also involved four complete changes of the saline in each chamber.

Wood & Moreton (1978) have discussed the error in estimation of I_{sc} when membrane resistance is low relative to that of the bathing saline. The specific resistance of various salines used in the present study are shown in Table 1. The actual resistance of the bathing saline between the PD recording electrodes was measured and was on average less than 5% of membrane resistance. The resulting underestimation of

Table 1. *Composition of salines used in this study*

(All of the simple salines contained 10 mM glucose, 3 mM L-glutamate, 2·5 mM-$MgSO_4$, 1 mM-KH_2PO_4, 24 mM-$NaHCO_3$. The major salts in each saline differed as indicated below.

Type of simple saline	Major salts (mM)	Specific resistance of saline (20 °C) (Ω.cm)
Cl-saline (normal saline)	185 NaCl, 11 KCl, 3 $CaCl_2$	48·8
NO_3-saline	185 $NaNO_3$, 11 KNO_3, 3 $Ca(NO_3)_2$	51·4
Acetate-saline	185 Na acetate, 11 K acetate, 3 Ca (acetate)$_2$	71·5
SO_4-saline-1	93 Na_2SO_4, 5·5 K_2SO_4, 3 $CaSO_4$	59·5
SO_4-saline-2	185 Na_2SO_4, 11 K_2SO_4, 3 $CaSO_4$	34·8
Complex SO_4-saline*	12·3 Na_2SO_4, 4·3 K_2SO_4, 2 $CaSO_4$	174·0

* Complex saline also contained (mM): 10·5 $NaHCO_3$, 13 $MgSO_4$, 7·4 disodium succinate, 1·87 trisodium citrate, 12·8 malic acid, 16·6 glucose, 5·56 maltose, 128·3 sucrose, 2·67 glycine, 4·61 proline, 2·64 glutamine, 12·3 glutamic acid, 30 mg/l penicillin and 100 mg/l streptomycin sulphate. The pH was adjusted to 7·00 with NaOH, and total osmotic concentration was 317 m-osmol.

NOTE. The value for glucose concentration in complex salines reported by Williams *et al.* (1978) is in error. It should be 16·6 rather than 1·6 mM.

true I_{sc} and the small residual membrane PD under apparent short-circuit conditions are not sufficient to change conclusions drawn in this paper.

The two stimulants, CC homogenate and cAMP, were prepared as stock solutions in SO_4-saline-2. All Cl-free salines contained some SO_4^{2-} and the small volume of stimulant solution added (50 μl) did not substantially change the composition of the bathing media.

RESULTS

Effects of CC homogenate on Na^+ and Cl^- Fluxes

The I_{sc} and PD across unstimulated recta bathed in simple saline have already been reported (Spring & Phillips, 1980*a*). The mean values with time are shown by the dashed lines in Figs. 2 and 4. During steady-state (1·5–4 h), unstimulated recta consistently show a slight decline in the fluxes of $^{36}Cl^-$, $^{22}Na^+$, and $^{42}K^+$, i.e. large spontaneous increases in these fluxes do not occur (Williams *et al.* 1978).

The effect of stimulation by CC homogenate on unidirectional Cl^- fluxes across voltage-clamped recta is shown in Fig. 1(*a*). As previously observed by Williams *et al.* (1978), there was an initial decline in net influx (L → H) (lumen → haemocoel) but no change in backflux (H → L) over the first 90 min. When CC homogenate was then added to the haemocoel side, there was a rapid increase in the influx of Cl^- (L → H) but no corresponding change in backflux (H → L). The magnitude of the response can be better appreciated from Fig. 2(*b*), which compares net Cl^- flux and I_{sc}. The net Cl^- flux equals or exceeds the I_{sc} and closely parallels the increase in I_{sc} following the addition of CC homogenate.

CC homogenate has no effect on either the unidirectional (Fig. 3) or net fluxes of Na^+ (Fig. 4) across short-circuited recta. The net flux remains relatively constant at 2·1 ± 0·2 μequiv.cm^{-2}.h^{-1} (mean ± SEM; L → H) over the entire course of the experiment.

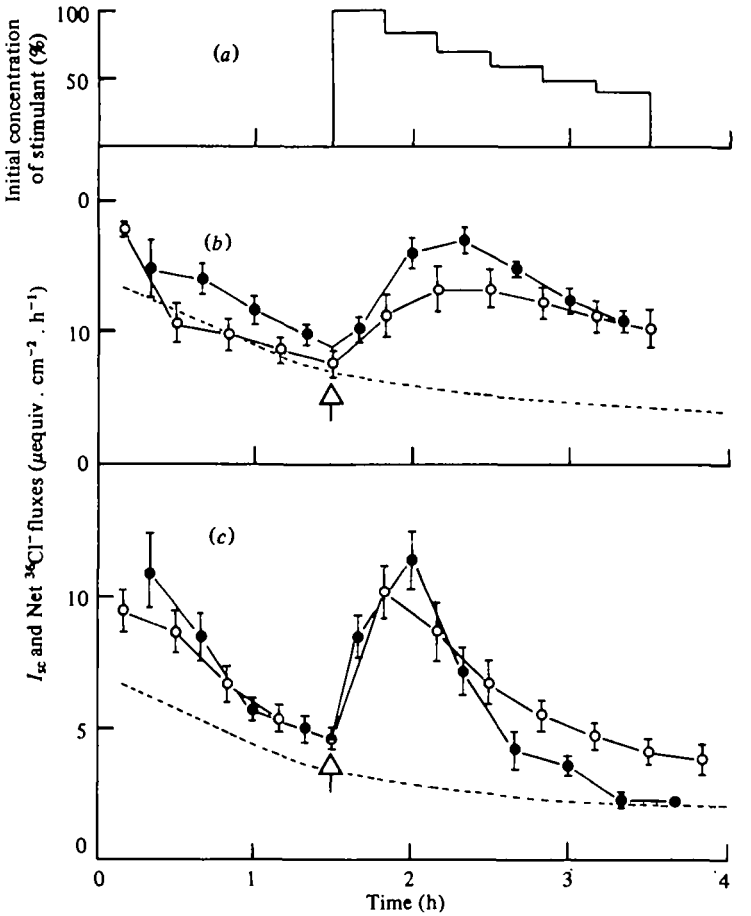

Fig. 2. I_{sc} and net Cl^- fluxes for short-circuited recta bathed in simple Cl-saline (mean ± S.E.M.). ●, Net Cl^- influx (L → H); ○, I_{sc}; dashed line indicates mean I_{sc} for unstimulated recta (from Spring & Phillips, 1980*a*). (*a*) Histogram indicates decreasing concentration of stimulant (expressed as a % of the original dose) with time due to sampling procedure. (*b*) 0·1 pr CC added at arrow. (*c*) 0·3 mM final concentration cAMP added at arrow.

Effects of cAMP on Na^+ and Cl^- Fluxes

The effect of stimulation by cAMP on unidirectional Cl^- fluxes is illustrated in Fig. 1(*b*), and on net Cl^- flux and I_{sc} in Fig. 2(*c*). The results are similar to those described for CC homogenate but with the following differences. There is a small abrupt increase in the backflux (H → L) of Cl^- when the recta are stimulated by cAMP. The increases in both I_{sc} and net Cl^- flux are much more rapid following the addition of cAMP compared to CC homogenate (Fig. 2*b*, *c*). As with CC homogenate, the increase in net Cl^- flux is more than sufficient to account for the entire increase in I_{sc} following stimulation with cAMP.

Cyclic-AMP had no effect on either unidirectional (Fig. 3) or net Na^+ fluxes (Fig. 4). The net flux (3·0 ± 0·2 μequiv.cm^{-2}.h^{-1}) remained relatively constant over the course of the experiment, and was slightly lower than the value (4·4 μequiv.cm^{-2}.h^{-1}) reported by Williams *et al.* (1978), who used a complex saline.

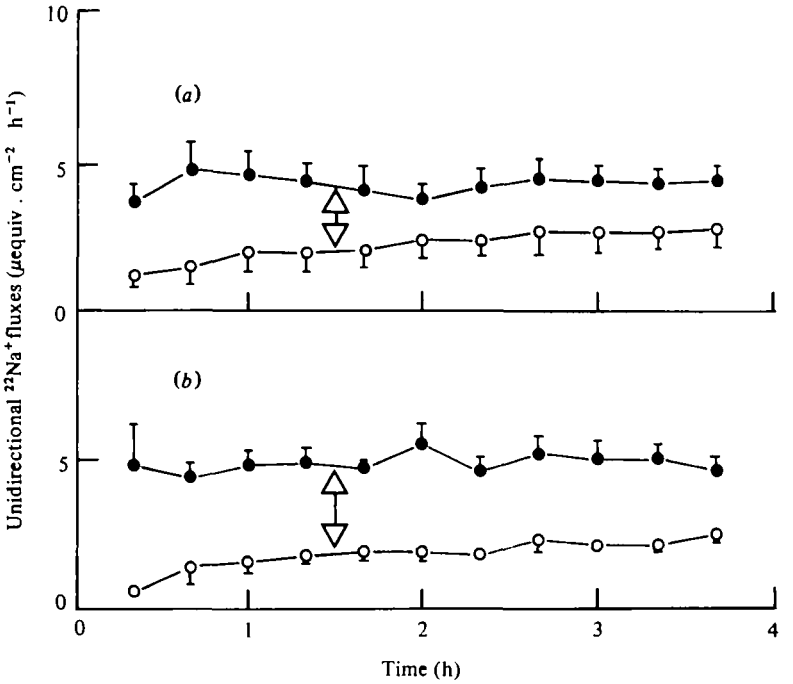

Fig. 3. Unidirectional Na^+ fluxes with time for short-circuited recta bathed in simple Cl-saline (mean ± S.E.M.). ●, Influx (L → H); ○, backflux (H → L). (*a*) 0·1 pr CC added at arrows. (*b*) 0·3 mM final concentration cAMP added at arrows.

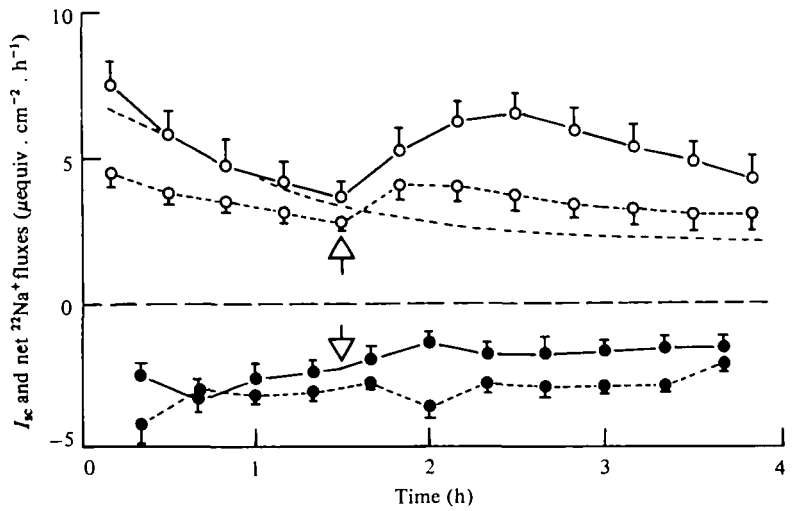

Fig. 4. I_{sc} and net Na^+ fluxes for short-circuited recta bathed in simple Cl-saline (mean ± S.E.M.). ●, Net Na^+ influx (L → H); ○, I_{sc}; ——, 0·1 pr CC added at arrows; – –, 0·3 mM final concentration cAMP added at arrows; dashed line without points indicates mean I_{sc} for unstimulated recta (from Spring & Phillips, 1980*a*).

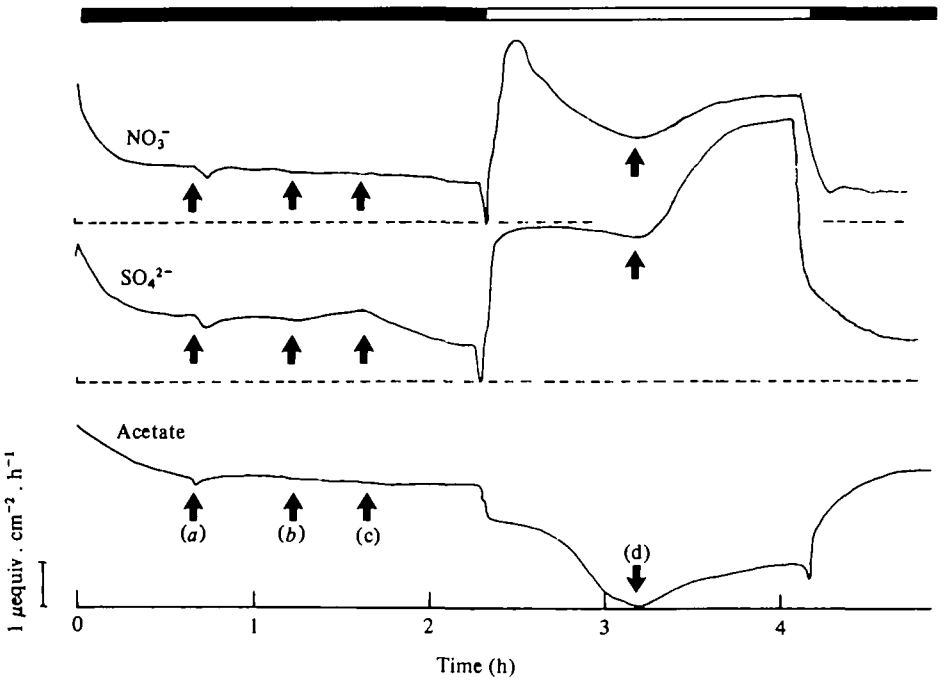

Fig. 5. Individual traces of I_{sc} with time for recta bathed in simple NO_3-saline, SO_4-saline-2, or acetate saline. Solid bar indicates time period during which recta were bathed in Cl-free saline; open bar indicates simple Cl-saline present. (*a*) Initial change of Cl-free saline to fresh Cl-free saline. (*b*) 0·3 mM final concentration cAMP added at arrow. (*c*) 0·1 pr CC added at arrow. (*d*) 0·3 mM final concentration cAMP added at arrow when normal Cl-saline was present.

Effects of anion substitutions on rectal response to CC or cAMP

The flux experiments just described do not alone provide conclusive proof that increased Cl^- absorption accounts for all or part of the ΔI_{sc}, in spite of the close quantitative correlation shown in Fig. 2. For example, rectal transport of Cl^- might be coupled to that of other ions (e.g. Na^+, K^+, HCO_3^-) and hence might be electrically neutral, either wholly or in part. This is the case for many vertebrate epithelia and also the gut of freshwater prawn (Ahearn, 1978). If this were true, other anion transport processes which are apparently present in the locust rectum (Williams *et al.* 1978), or H^+ secretion, might be responsible for the increase in I_{sc} following stimulation. This possibility was investigated using Cl-free salines (Fig. 5 and Table 2).

Recta bathed in either hyperosmotic SO_4-saline-2 or isosmotic NO_3-salines respond in similar ways. The initial I_{sc} and PD are low, and the decline over the first 1·5 h is greatly reduced compared to recta in normal Cl-saline (see Fig. 1 for comparison). Addition of CC or cAMP has little or no effect on either I_{sc} or PD. The I_{sc} increases very rapidly when these Cl-free salines are replaced with normal Cl-saline and the preparations are once again responsive to stimulation by CC homogenate or cAMP, albeit to a lesser degree. The steady-state I_{sc} and PD for preparations bathed a second time with either SO_4-saline or NO_3-saline were very low. In summary, I_{sc} and PD

Table 2. *Average values* (± S.E.M., n = 4–8) *for all recta treated as shown in Fig.* 5, *indicating changes in* I_{sc} *and PD due to various stimuli for short-circuited recta bathed in four different simple salines, identified by major anion*

(I_{sc} expressed as mean ± S.E.M. Only mean PD values are shown (in parentheses). In all cases I_{sc} indicates L → H movement of anions, and H side was always negative to L side under open-circuit conditions.

Sequential events	I_{sc} (μequiv.cm^{-2}.h^{-1}) and PD (mV) Initial bathing saline: Chloride	Sulphate	Nitrate	Acetate
(*a*) Initial I_{sc} (PD) at 10 min post-dissection	6·7 ± 0·3 (24)	1·8 ± 0·3 (17)	2·0 ± 0·6 (14)	4·3 ± 0·7 (30)
(*b*) Steady-state I_{sc} (PD) t = 2 h	2·9 ± 0·3 (15)	1·5 ± 0·2 (9)	0·8 ± 0·5 (5)	3·5 ± 0·7 (27)
(*c*) ΔI_{sc} (ΔPD) following addition of 0·1 pr CC	+3·6 ± 0·4 (+10)	−0·7 ± 0·1 (−4)	−0·1 ± 0·1 (0)	0 (0)
(*d*) ΔI_{sc} (ΔPD) following addition of 0·3 mM cAMP	+3·4 ± 0·5 (+6)	+0·7 ± 0·1 (+0·5)	0 (0)	0 (0)
(*e*) ΔI_{sc} (ΔPD) when placed in simple chloride saline	—	+2·2 ± 0·3 (+11)	+3·6 ± 0·8 (+13)	−2·7 ± 0·2 (−25)
(*f*) Time (min) for half of change in I_{sc} to occur during (*e*)	—	8·5 ± 2·3	8·5 ± 2·6	17 ± 4
(*g*) ΔI_{sc} (ΔPD) when returned from chloride saline to chloride-free saline	—	−4·3 ± 0·6 (−12)	−2·5 ± 0·4 (−10)	+2·6 ± 0·2 (+13)
(*h*) Time (min) for half of change in I_{sc} to occur during (g)	—	5·5 ± 0·5	3·8 ± 0·8	23 ± 4
(*i*) Final (4·5 h) steady-state I_{sc} (PD) in original saline	2·1*	0·4 ± 0·2 (2)	0·8 ± 0·1 (2)	3·7 ± 0·6 (13)

* Spring & Phillips (1980*a*).

only increase substantially following the addition of either CC or cAMP if Cl^- is present in the saline.

The substitution of acetate for Cl^- causes a qualitatively different response from that produced by NO_3- or SO_4-salines (Fig. 5, Table 2). The initial I_{sc} for recta in acetate saline decreases only very slightly with time, with the result that the steady-state I_{sc} and PD are very much higher than for any of the other salines, including normal Cl-saline. The addition of CC homogenate or cAMP has no effect on the I_{sc} or PD. Substitution of Cl^- for acetate causes a relatively slow *decrease* in I_{sc} and PD of unstimulated recta to unusually low values. Replacing the Cl-saline with acetate saline again causes I_{sc} and PD to increase rapidly to the original levels for acetate. In summary, acetate saline causes a much larger I_{sc} and PD across unstimulated recta than does normal Cl-saline, but there is no response to CC or cAMP when this organic acid is the major anion.

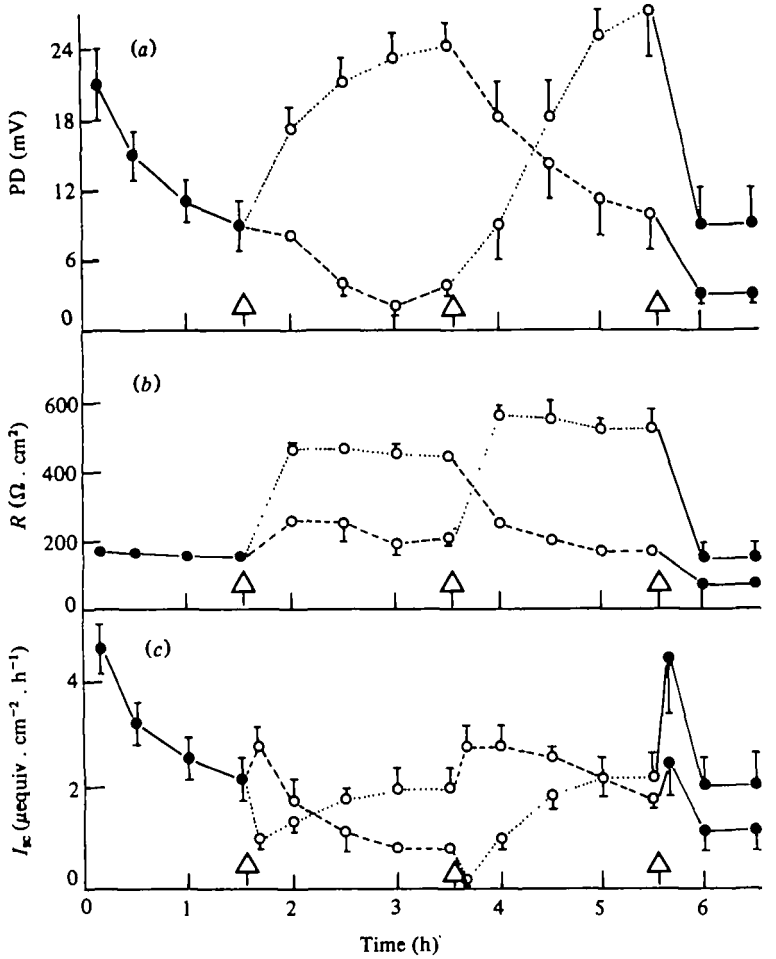

Fig. 6. Electrical parameters (PD, R, I_{sc}) with time for voltage-clamped recta bathed in simple Cl-saline, and simple and complex SO_4-salines (mean ± S.E.M.). ——, Preparations bathed in simple Cl-saline. – – –, Preparations bathed in simple SO_4-saline-1. · · · ·, Preparations bathed in complex SO_4-saline. Arrows indicate time of saline change.

Evidence for alternate electrogenic processes

Williams *et al.* (1978) reported that their complex Cl-free salines supported steady state I_{sc} across unstimulated recta equally well as their complex Cl-saline. We observed, however, that simple NO_3- or SO_4-salines (Table 2) would support a steady-state I_{sc} and PD less than half that across preparations bathed in simple Cl-saline. To determine whether the discrepancies between our observations and those of Williams *et al.* were due to the use of simple rather than complex salines, we exposed individual recta sequentially to simple Cl-saline, simple SO_4-saline-1, and complex SO_4-saline in different orders (Fig. 6).

An unexpected observation is that the order of substitution is important. Prepara-

tions transferred from Cl-saline into simple SO_4-saline-1 exhibited a slow decrease in I_{sc} and PD to half their original values, while R (transepithelial DC resistance) increased about 30%. When simple SO_4-saline-1 was replaced by complex SO_4-saline, I_{sc} showed an initial transient decline to near zero, then rose slowly over the subsequent 90 min, stabilizing at the same level (2·1 μequiv.cm^{-2}.h^{-1}) as for steady-state preparations in Cl-saline. Concurrently PD and R increased to 2–3 times their values in Cl-saline. When these preparations were again placed in Cl-saline, all three electrical parameters returned to the original levels for Cl-saline.

For preparations treated in the reverse order (Fig. 6), the initial transfer from simple Cl- to complex SO_4-saline caused the PD and R to increase 2- to 3-fold. I_{sc} showed a transient decrease, then stabilized at the value for steady-state preparations in simple Cl-saline. This previous exposure to complex SO_4-saline somehow permitted recta subsequently to sustain an abnormally high I_{sc} for some time when simple SO_4-saline-1 was added. However, returning such preparations to simple Cl-saline caused I_{sc}, PD and R to decline to very low levels.

In summary, complex SO_4-saline and Cl-saline sustain the steady-state unstimulated I_{sc} across locust recta equally well, as first reported by Williams *et al.* (1978). The different response to anion substitutions between the results in Fig. 5 and experiments by Williams *et al.* (1978) is clearly due to the use of simple rather than complex sulphate saline. It follows that alternate electrogenic transport processes can be gradually turned on when Cl^- is absent, but only when a component of complex saline (as yet unidentified) is present.

DISCUSSION

The results clearly demonstrate that cAMP and CC homogenate increase rectal I_{sc} and PD in the same way, by stimulating electrogenic transport of Cl^- from the lumen. The evidence for this conclusion may be summarized as follows. Firstly, if Cl^- is not present in the bathing saline, then neither I_{sc} nor PD change substantially when cAMP or CC is added (Fig. 5). Clearly these two stimulants do not affect other electrogenic transport processes (H^+/HCO_3^- or phosphate or organic anions) which have been postulated for the locust rectum (Williams *et al.* 1978). Our experiments do not eliminate possible stimulation of these transport processes if they are electrically neutral.

Secondly, the increase in net Cl^- flux follows stimulation with both CC and cAMP approximately equals the increase in I_{sc}, at least within the limits of experimental error (Fig. 2). It follows that this additional component of Cl^- transport cannot be coupled to a co-transport of Na^+ or counter-transport of HCO_3^-, even if the overall transport process is electrogenic (i.e. the coupling ratio is not 1:1); otherwise net Cl^- flux should have greatly exceeded ΔI_{sc}. Most cases of active Cl^- transport across vertebrate epithelia are currently thought to involve one of these coupled mechanisms (Frizzel, Field, & Schultz, 1979). Other evidence supports a lack of such coupling in the locust rectum. Acetazolamide, an inhibitor of H^+/HCO_3^- transport across other epithelia, does not inhibit the stimulation of I_{sc} across locust rectum (Spring & Phillips, 1980*a*). Moreover, no increase in Na^+ transport (influx or net influx; Figs. 3, 4) accompanies stimulation of Cl^- transport. However, a coupled process in which

additional Na^+ is recycled within the rectal epithelium would have gone undetected in our experiments.

Under open circuit conditions *in vivo*, one might expect increased passive absorption of cations to accompany stimulation of Cl^- transport, due to the larger PD across the rectal wall. It might therefore be advantageous if CC or cAMP also increased cation permeability of this epithelium. Such a dual action of a single stimulant on both cation and anion movements has been reported for Malpighian tubules of *Rhodnius* (Maddrell, 1971) and salivary glands of *Calliphora* (Berridge, 1977). The failure of either cAMP or CC to alter unidirectional fluxes of $^{22}Na^+$ (Fig. 3) suggests that this is not the case for locust rectum. It remains to be seen whether the absorption of K^+, the predominant cation in the rectal lumen, and water is controlled by the purified CC factor or cAMP.

Spring (1979) and Hanrahan (1978) have summarized preliminary observations concerning the possible physiological role of the Cl^- transport stimulating factor which is present in CC homogenate and also in the haemolymph of locusts (see Spring & Phillips, 1980*b*). It seems to promote retention of chloride in the rectum of locusts fed a dilute, low-chloride diet (e.g. lettuce). It has proportionately less effect on water reabsorption, so that haemolymph Cl^- levels are raised. This is consistent with earlier *in vivo* studies of Phillips (1964*a–c*).

The different patterns observed when complex and simple SO_4-salines are substituted for Cl-saline (Fig. 6) suggest that there is an alternative transport mechanism which can be slowly turned on within unstimulated recta after external Cl^- is removed. This would explain the initial drop and subsequent complete recovery of I_{sc} when preparations are transferred from Cl- to complex SO_4-saline. This time lag may reflect the slow rate of diffusion into rectal tissue of a substance present in complex salines, or the time for intracellular regulatory events to occur. Such a lag would also explain why simple SO_4-saline will temporarily sustain I_{sc} much better if recta are previously exposed to complex SO_4-saline for some time. Similarly, long-term exposure to acetate saline decreases the ability of recta to transport Cl^- subsequently, as indicated by greatly reduced I_{sc} in Cl-saline (Fig. 5). Yet such recta respond exceedingly well if re-exposed to acetate. This is again consistent with the idea of a slow switch to other transport processes capable of generating I_{sc}. T. Baumeister in our laboratory has demonstrated that electrogenic transport of [^{14}C]acetate largely accounts for the enhanced I_{sc} and PD across recta bathed in simple acetate saline (Table 2); however, this system may normally transport other organic substances. This is under investigation.

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