

# ELECTROPHYSIOLOGY OF $K^+$ TRANSPORT BY MIDGUT EPITHELIUM OF LEPIDOPTERAN INSECT LARVAE

## I. THE TRANSBASAL ELECTROCHEMICAL GRADIENT

BY DAVID F. MOFFETT AND ALAN R. KOCH

*Zoophysiology Program, Department of Zoology, Washington State University, Pullman, WA 99164-4220, USA*

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### SUMMARY

Basal membrane potential ( $V_b$ ) and intracellular  $K^+$  activity  $[(K^+)_i]$  were recorded, using microelectrodes, in isolated, superfused, short-circuited midgut of fifth instar larvae of *Manduca sexta*. The electrochemical gradient across the basal membrane was favourable for  $K^+$  entry as long as  $[K^+]_b$  was  $32 \text{ mequiv l}^{-1}$  or greater. In  $20 \text{ mequiv l}^{-1}$   $K^+$   $V_b$  rose so that in some cells the basal electrochemical gradient was unfavourable for  $K^+$  entry. In  $10 \text{ mequiv l}^{-1}$   $K^+$ , the basal electrochemical gradient of all cells was unfavourable for  $K^+$  entry. This result suggests that an active  $K^+$  pump may augment passive basal  $K^+$  entry.

Addition of  $2 \text{ mmol l}^{-1}$   $Ba^{2+}$  to midgut resulted in substantial hyperpolarization of  $V_b$  accompanied by relatively small changes in  $(K^+)_i$ ; the net effect was to move  $(K^+)_i$  farther away from electrochemical equilibrium with external  $K^+$ .

Identification of recorded cells by ionophoretic injection of Lucifer Yellow showed that both major cell types of the epithelium (goblet and columnar cells) had similar control values of  $V_b$  and  $(K^+)_i$ ; and responded similarly to  $Ba^{2+}$ , suggesting the presence of effective chemical or electrical coupling between the transporting goblet cells and the non-transporting columnar cells.

Hypoxia reduced transepithelial  $K^+$  transport, both in the absence and in the presence of  $Ba^{2+}$ . In the absence of  $Ba^{2+}$ ,  $(K^+)_i$  was within a few millivolts of equilibrium and the effect of hypoxia was small. In the presence of  $Ba^{2+}$ , when  $(K^+)_i$  was far from equilibrium with extracellular  $K^+$ , hypoxia markedly depolarized the basal membrane.

The results are compatible with the suggestion that  $Ba^{2+}$  partially blocks basal  $K^+$  entry, but does not directly affect the apical pump. Hypoxia inhibits the apical pump. Since the active transepithelial transport of  $K^+$  was reduced after  $Ba^{2+}$  treatment even though  $(K^+)_i$  was unchanged, it appears that the activity of the apical pump is primarily controlled by the voltage step across the apical membrane.

**Key words:** *Manduca sexta* midgut,  $K^+$  transport, membrane potential, ion-selective electrode, Lucifer Yellow, barium, short-circuit current.

## INTRODUCTION

The isolated midgut of the tobacco hornworm larva (*Manduca sexta*) actively transports  $K^+$  from basal (haemolymphal) to apical (luminal) side. The preponderance of evidence from morphological studies (Cioffi, 1979), biochemical studies (Harvey, Cioffi & Wolfersberger, 1983) and electron microprobe studies (Dow, Gupta, Hall & Harvey, 1984) suggests that an electrogenic pump located on the apical membrane of goblet cells plays a major role in energizing the  $K^+$  transport and is the common route for  $K^+$  extrusion from the epithelium; columnar cells are thought not to possess this pump, but might still form part of the route of  $K^+$  through the epithelium to the apical pump.

An electrophysiological study (Moffett, Hudson, Moffett & Ridgway, 1982), using ion-selective microelectrodes under open-circuit conditions, has also indicated the presence of active  $K^+$  transport across the apical membrane since, in both columnar and goblet cells bathed in  $32 \text{ mmol l}^{-1} K^+$  saline, the  $K^+$  gradient across the apical membrane is far from electrochemical equilibrium, in a direction unfavourable for  $K^+$  exit. The gradient across the basal membrane is consistent with passive entry at this point. The conductance of such entry would have to be quite high because of the very high pump current, which may be as great as  $700 \mu\text{A cm}^{-2}$  of epithelium.

The  $K^+$  permeation blocker  $Ba^{2+}$  affects the relationship between extracellular  $K^+$  concentration and the rate of net  $K^+$  transport:  $Ba^{2+}$  reduces net  $K^+$  transport when extracellular  $K^+$  is less than about  $50 \text{ mequiv l}^{-1}$ , and stimulates transport at higher external  $K^+$  concentrations (Moffett & Koch, 1985). These results may indicate two parallel processes for  $K^+$  entry, one of which is  $Ba^{2+}$ -sensitive. Noise-analysis studies (Zeiske, Van Driessche & Ziegler, 1986) suggest that the  $Ba^{2+}$ -sensitive process is attributable to  $K^+$  channels similar to those widely found in animal cell membranes.

The present study provides further characterization of the  $K^+$  distribution across the basal membrane of the midgut of *Manduca sexta* larvae, while the subsequent paper (Moffett & Koch, 1988) investigates the apical membrane.

## MATERIALS AND METHODS

Fifth instar larvae of *Manduca sexta* were reared on a synthetic diet as in previous studies (Moffett *et al.* 1982). The animals weighed 6–8 g and thus were in day 2 of the fifth instar according to the schedule of L. M. Riddiford (personal communication) in which ecdysis to the fifth instar occurs during the dark hours of day 0.

Double-barrelled glass microelectrodes were prepared from 2 mm o.d. theta glass (R & D Optical Systems, Inc., Spencerville, MD) according to the methods of Brown & Flaming (1977). Typical electrodes had tip resistances of 50–70 M $\Omega$  measured against the reference barrel. For Lucifer Yellow marking, electrodes were prepared and used as in previous studies (Moffett *et al.* 1982). Potassium electrodes were as in previous studies (Moffett *et al.* 1982) except that one barrel was silanized with pure trimethylchlorosilane vapour according to the method of Charlton, Silverman & Atwood (1981). In most experiments the outputs of the two intracellular electrodes, the voltage difference between them (i.e. the  $K^+$ -specific

component of the K<sup>+</sup> barrel's output) and the short-circuit current were recorded simultaneously using a four-channel recorder.

The posterior midgut was removed from cold-anaesthetized animals. Malpighian tubules were removed and the midgut was mounted in a chamber like that used by Thompson, Suzuki & Schultz (1982) except that thread instead of an O-ring was used to attach the tissue to the Lucite ring, and the nylon support mesh was not used. Mounted tissues were gravity-superfused on both sides with bathing solutions. All bathing solutions contained (in mmol l<sup>-1</sup>) CaCl<sub>2</sub>, 5; MgCl<sub>2</sub>, 5; Tris buffer, 5 (pH 8.0); sucrose, 166. Potassium chloride was added to give K<sup>+</sup> concentrations indicated by the denominations of the solutions; for example, the standard bathing solution contains 32 mequiv l<sup>-1</sup> K<sup>+</sup> and is denominated 32KS. The K<sup>+</sup> chemical activities [(K<sup>+</sup>)<sub>b</sub>] of the solutions were: 10KS, 9 mmol l<sup>-1</sup>; 20KS, 18 mmol l<sup>-1</sup>; 32KS, 30 mmol l<sup>-1</sup>; 70KS, 57 mmol l<sup>-1</sup>. Bathing solutions were gassed with 100% O<sub>2</sub> for normal use or with 100% N<sub>2</sub> to induce hypoxia.

The midguts were maintained under short-circuit conditions with compensation for solution resistance using the voltage-clamp circuit and methods described by Hanrahan, Meredith, Phillips & Brandys (1984). For the experiments in which superfusate composition was changed in the course of the experiment, the efficacy of the compensation was especially important and was frequently checked by advancing the microelectrode very close to the surface of the tissue and running through the sequence of solutions to be used, adjusting the compensation circuit as necessary to keep the solution immediately adjacent to the basal membrane isoelectric for all the solutions to be used.

Previous studies (Moffett *et al.* 1982, confirmed by Thomas & May, 1984) provided evidence that penetrations yielding V<sub>b</sub> at least as negative as -20 mV in standard bathing solution are valid and that less negative values are suspect. Accordingly, -20 mV was taken as a criterion threshold for penetrations used in these studies.

The chord conductance of the basal membrane (G<sub>b</sub>) was estimated as follows. The driving force for K<sup>+</sup> entry was calculated as the difference between the measured basal membrane potential and the equilibrium potential (V<sub>eq</sub>) calculated from the measured intracellular and extracellular K<sup>+</sup> chemical activities. This value was divided into the simultaneously measured short-circuit current (I<sub>sc</sub>).

For identification of cells stained by ionophoresis of Lucifer Yellow, whole mounts of stained tissues fixed and cleared as described previously (Moffett *et al.* 1982) were examined using a Leitz Ortholux fluorescence microscope.

## RESULTS

### *Effect of haemolymph-side [K<sup>+</sup>] on (K<sup>+</sup>)<sub>i</sub> and V<sub>b</sub>*

As external K<sup>+</sup> concentration was varied, there were changes in recorded values of V<sub>b</sub>, (K<sup>+</sup>)<sub>i</sub> and I<sub>sc</sub> (Fig. 1). Typical relationships between V<sub>b</sub> and (K<sup>+</sup>)<sub>i</sub> are shown for individual impalements by the connected data points in Fig. 2. Diagonal lines in

Fig. 2 indicate calculated electrochemical equilibria for different  $K^+$  concentrations on the haemolymphal side. Points above and to the left of their appropriate line show an electrochemical gradient favourable for  $K^+$  entry. Figs 3 and 4 show the aggregate of all points from such experiments without any indication of which points are derived from the same impalement. The effect of reducing the  $[K^+]_i$  of the bathing solution is to hyperpolarize  $V_b$  with, typically, a relatively small change in  $(K^+)_i$ . These experiments showed that the electrochemical gradient across the basal membrane is favourable for net  $K^+$  entry in almost all cases when the extracellular  $[K^+]_o$  is  $20\text{ mmol l}^{-1}$  or greater, but for  $10\text{ mmol l}^{-1}$   $K^+$  the gradient is clearly not favourable for  $K^+$  entry.

Fig. 5 shows the relationship between the mean electrochemical driving force acting on  $K^+$  and the mean  $I_{sc}$  for each of the extracellular  $K^+$  concentrations used in this study. Since the  $I_{sc}$  maintained by the gut at any  $K^+$  concentration declines intrinsically with time, the  $I_{sc}$  values for every penetration were normalized to the value obtained in 32KS during that penetration. We could not distinguish any

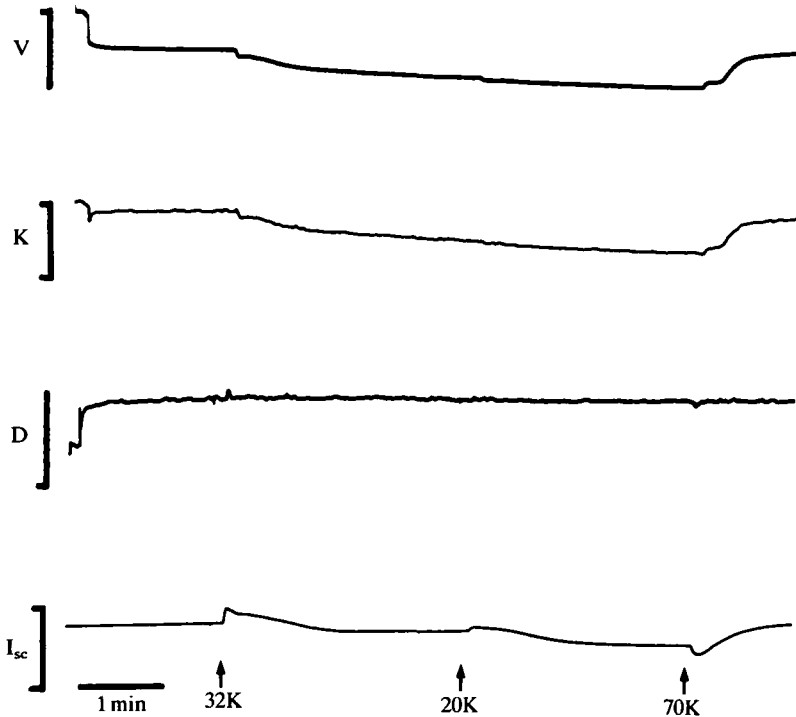


Fig. 1. Changes in  $V_b$ ,  $(K^+)_i$  and  $I_{sc}$  with changes in superfusate  $K^+$  concentration. This figure shows the initial penetration of a cell while the tissue is superfused with 70K, and the effects of changing the superfusate to 32K, to 20K and then back to 70K. V is the output of the reference barrel, K that of the  $K^+$ -selective barrel, D is the difference trace provided by differential amplification of the V and K signals.  $I_{sc}$  is the short-circuit current. The difference trace represents  $(K^+)_i$ ; its relative stability indicates that there is only a small change in  $(K^+)_i$  during this sequence of superfusate  $K^+$  concentrations. The scale bars are: V, 60 mV; K, 60 mV; D,  $150\text{ mmol l}^{-1}$   $K^+$ ;  $I_{sc}$ ,  $500\text{ }\mu\text{A cm}^{-2}$ .

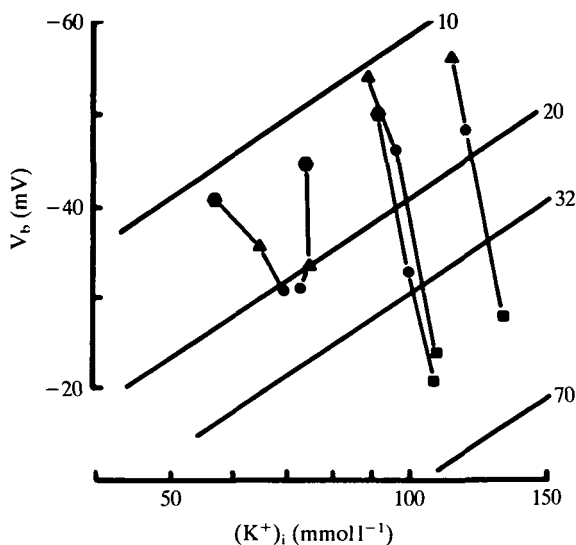


Fig. 2. This figure shows representative changes in  $V_b$  plotted against  $(K^+)_i$  for different superfusate  $K^+$  concentrations. Values recorded from a single cell are connected. Squares, 70K; circles, 32K; triangles, 20K; hexagons, 10K. The diagonal lines are the calculated electrochemical equilibrium lines for the  $K^+$  chemical activities of each of the superfusates.

Table 1. *Effects of  $Ba^{2+}$  and  $Ba^{2+}$  + hypoxia on transbasal electrochemical gradient, current and conductance*

Condition	$(K^+)_i$ ( $\text{mmol l}^{-1}$ )	$V_b$ (mV)	$V_b - V_{eq}$ (mV)	$I_{sc}$ ( $\mu\text{A cm}^{-2}$ )	$G_b$ ( $\text{mS cm}^{-2}$ )
A ( $N = 7$ )					
Control	$90 \pm 4$	$41 \pm 2$	$13 \pm 1$	$404 \pm 35$	$35 \pm 4$
$Ba^{2+}$	$82 \pm 3$	$69 \pm 4$	$43 \pm 3$	$305 \pm 25$	$8 \pm 1$
B ( $N = 8$ )					
Control	$92 \pm 5$	$40 \pm 2$	$11 \pm 1$	$415 \pm 59$	$39 \pm 6$
$Ba^{2+}$	$84 \pm 4$	$70 \pm 6$	$44 \pm 5$	$305 \pm 42$	$7 \pm 1$
$Ba^{2+} + N_2$	$75 \pm 4$	$50 \pm 3$	$27 \pm 2$	$179 \pm 29$	$8 \pm 2$

secular trend in the values of  $V_b$  or the magnitude of the driving force in records of penetrations made over the useful life (approximately 3 h) of isolated midguts.

#### *Hyperpolarization of the basal membrane by $Ba^{2+}$*

Superfusion with  $2 \text{ mmol l}^{-1}$   $BaCl_2$  in 32KS typically hyperpolarized  $V_b$  and reduced  $I_{sc}$ , with little change in  $(K^+)_i$  (Fig. 6). For five experiments,  $Ba^{2+}$  hyperpolarized  $V_b$  by 28 mV, decreased  $(K^+)_i$  by  $8 \text{ mmol l}^{-1}$  and decreased chord conductance to approximately one-quarter of its control value (Table 1A). In all acceptable impalements (see above), voltage responded to  $Ba^{2+}$  with some degree of

hyperpolarization. The change in the relationship between  $V_b$  and  $(K^+)_i$ ; shows that the gradient for  $K^+$  is carried farther away from electrochemical equilibrium in the direction of a larger inward driving force, with most of the change occurring in the voltage component of the electrochemical gradient (Fig. 7).

*Ba<sup>2+</sup> hyperpolarizes both columnar and goblet cells*

Columnar and goblet cells cannot be distinguished by their basal potentials (Moffett *et al.* 1982; Thomas & May, 1984). To investigate whether both major cell types had similar responses to  $Ba^{2+}$ , as suggested by the consistency of the responses, effects were investigated in cells identified by ionophoretic injection of Lucifer Yellow. Both columnar and goblet cells are hyperpolarized by  $Ba^{2+}$  (Table 2).

*Hyperpolarization by Ba<sup>2+</sup> is dependent on oxidative metabolism*

To investigate the effects of anoxia upon the response to  $Ba^{2+}$ , cells impaled in oxygenated 32KS were superfused with 32KS + 2Ba (32KS solution containing  $2 \text{ mmol l}^{-1} \text{ Ba}^{2+}$ ) until hyperpolarization was complete and then the superfusate was switched to 32KS + 2Ba which had been equilibrated with 100%  $N_2$ . Recording was continued until  $I_{sc}$  had fallen to about one-third of its initial value. A representative set of oscillograph traces is shown in Fig. 6 and a plot of all experiments is shown in Fig. 8. These experiments form a subset of the group of experiments summarized in

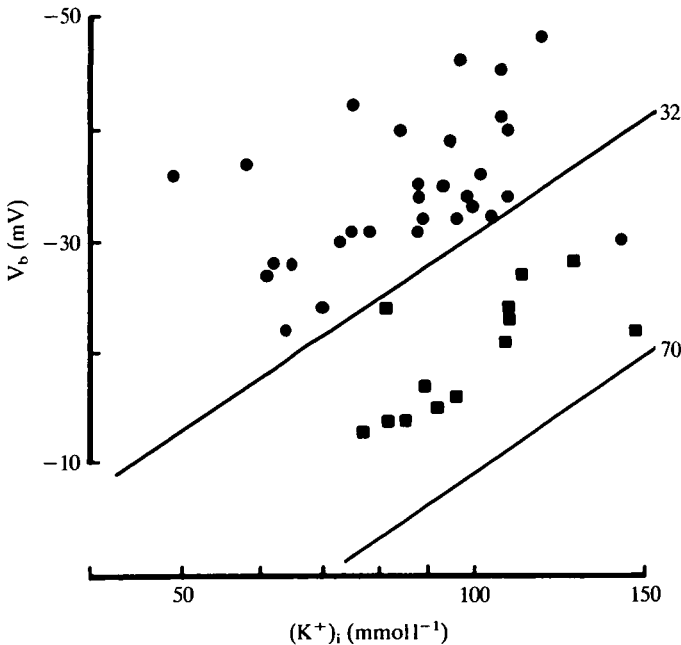


Fig. 3.  $V_b$  and  $(K^+)_i$  in 70K and 32K. This plot is similar to Fig. 2 except that it shows all points without any indication of which pairs of points are recorded from the same cells. Note that all the points in 70K (squares) are above the equilibrium line; in these cells  $(K^+)_i$  is lower than would be expected if it were in equilibrium with the  $K^+$  in the superfusate. With one exception this is also true for the cells in 32K (circles).

Table 2. *Lucifer Yellow labelling of cells that hyperpolarized in the presence of Ba<sup>2+</sup>*

Cell type(s)	No. of cells
Dye-fills single cell	
(1) single columnar	12
(2) single goblet	7
Dye-fills two or more adjacent cells	
(3) columnar-to-columnar	4
(4) columnar-to-goblet	2
Total sites (eight tissues)	25

Table 1A; results of the anoxia experiments are shown in Table 1B. Anoxia in the presence of Ba<sup>2+</sup> rapidly restored V<sub>b</sub> to a value similar to that before the application of Ba<sup>2+</sup>, without a significant change in G<sub>b</sub>. A few control experiments with anoxia in the absence of Ba<sup>2+</sup> showed that it uniformly resulted in small (0–3 mV) depolarizations in V<sub>b</sub> and small increases in (K<sup>+</sup>)<sub>i</sub>; the net effect was to bring intracellular K<sup>+</sup> even closer to electrochemical equilibrium.

DISCUSSION

*Active and passive uptake of K<sup>+</sup>*

When (K<sup>+</sup>)<sub>b</sub> is 9 mmol l<sup>-1</sup>, the net electrochemical driving force is of the wrong polarity to drive passive K<sup>+</sup> uptake (Fig. 5). Under these conditions, active uptake of K<sup>+</sup> must occur across the basal membrane because tissues bathed in solutions

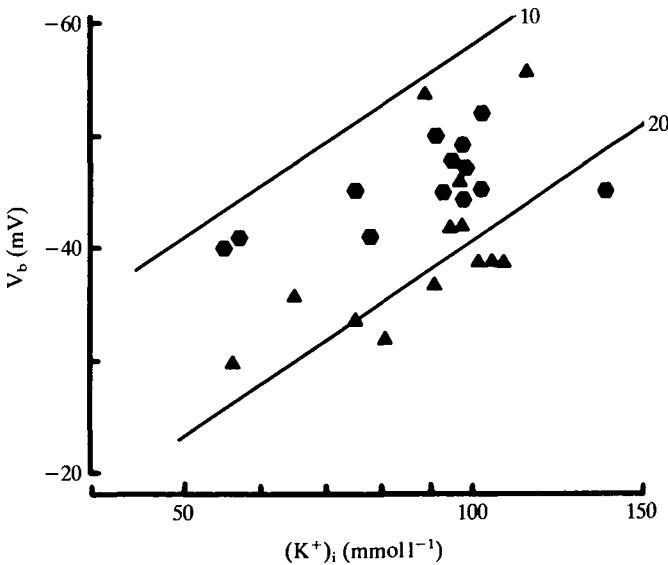


Fig. 4. V<sub>b</sub> and (K<sup>+</sup>)<sub>i</sub> in 20K and 10K. Note that in 20K (triangles) some cells have higher than equilibrium values of (K<sup>+</sup>)<sub>i</sub>, and in 10K (hexagons) this is the case for all cells.

containing even as little as  $4 \text{ mmol l}^{-1} \text{ K}^+$  continue to generate an  $I_{sc}$  (Fig. 5; Moffett & Koch, 1985; Moffett, 1979). It can also be seen (Fig. 5) that the two curves representing the relationship of  $I_{sc}$  and electrochemical driving force to  $(\text{K}^+)_{\text{b}}$  are not congruent. Since all the current must pass through the basal membrane, a non-conductive process must be supplementing passive flow of  $\text{K}^+$  across the basal membrane, and this process must make a relatively large contribution to the total current at low  $(\text{K}^+)_{\text{b}}$  and a smaller one at higher  $(\text{K}^+)_{\text{b}}$  as the driving force for passive entry increases. We reported earlier (Moffett & Koch, 1985) that the relationship between bathing solution  $\text{K}^+$  concentration and  $I_{sc}$  could be resolved into a low-affinity and a high-affinity component. The present results suggest that the active pathway might correspond to the high-affinity component inferred in the previous studies.

The basal  $\text{K}^+$  uptake cannot be mediated by a  $\text{Na}^+/\text{K}^+$  exchange mechanism like the one well-known in other animal cells. First, the bathing solutions used in these studies (and most other midgut studies) are nominally sodium-free. Also, a sodium-

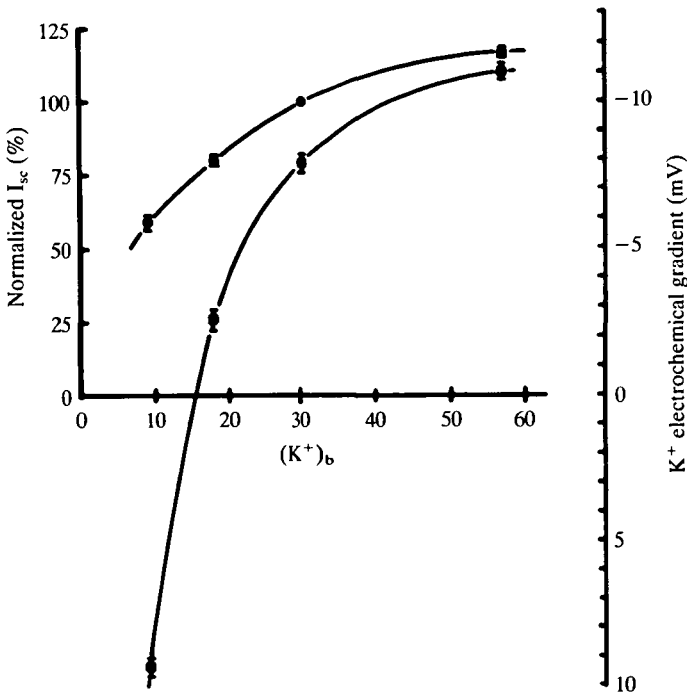


Fig. 5. Normalized  $I_{sc}$  and transbasal  $\text{K}^+$  electrochemical gradient in relation to superfusate  $\text{K}^+$  chemical activity. This figure shows the normalized  $I_{sc}$  (upper curve) and the simultaneously measured  $\text{K}^+$  electrochemical gradient (lower curve) calculated from the data shown in Figs 3 and 4. The mean  $I_{sc}$  at the normalization point is  $235 \pm 22.4 \mu\text{A cm}^{-2}$  (S.E.). For 70K,  $N = 13$ ; for 32K,  $N = 18$ ; for 20K and 10K,  $N = 9$ . Negative values of the electrochemical gradient are favourable for passive  $\text{K}^+$  entry. Superfusate  $\text{K}^+$  concentrations are expressed as their corresponding  $\text{K}^+$  chemical activities as measured by the  $\text{K}^+$ -selective electrodes. The vertical bars show  $\pm 1$  S.E.



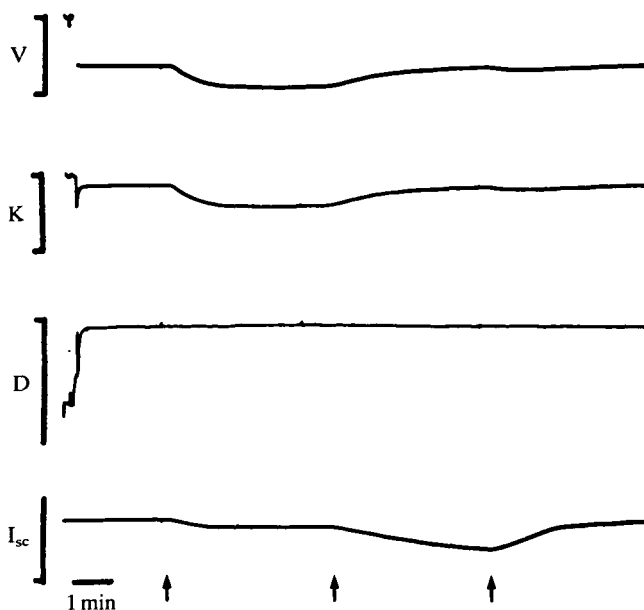


Fig. 6.  $V_b$ ,  $(K^+)_i$  and  $I_{sc}$  changes with  $Ba^{2+}$  and subsequent apical pump inhibition by  $N_2$  hypoxia. The reference electrode trace (V), the  $K^+$  electrode trace (K) and the difference trace (D) show the initial penetration in oxygenated 32K, the change to oxygenated 32K+2 mmol l<sup>-1</sup>  $Ba^{2+}$  at the first arrow, and the change to  $N_2$ -equilibrated 32K+2 mmol l<sup>-1</sup>  $Ba^{2+}$  at the second arrow, followed by recovery in oxygenated 32K. The scale bars are 60 mV for the V and K traces, 100 mmol l<sup>-1</sup>  $K^+$  for the D trace; and 200  $\mu A cm^{-2}$  for the  $I_{sc}$  trace.

stimulated, ouabain-inhibited ATPase activity is not detectable in the midgut (Jungreis & Vaughn, 1977). Furthermore, ouabain is without effect on the short-circuit current (Haskell, Clemmons & Harvey, 1965). Finally, in unpublished experiments, we have found no detectable effect of ouabain (0.1 mmol l<sup>-1</sup>) on  $I_{sc}$ ,  $V_b$  or  $(K^+)_i$ . These measurements were made in 10KS, a condition in which the basal pump must account for all of the  $K^+$  influx.

#### *Effect of $Ba^{2+}$ on the $K^+$ electrochemical gradient*

Addition of 2 mmol l<sup>-1</sup>  $Ba^{2+}$  to the standard bathing solution resulted in a 4- to 5-fold decrease in the chord conductance of the basal membrane (Table 1). This result is consistent with reports from insect rectum (Hanrahan, Wills, Phillips & Lewis, 1986) and other membrane systems (reviewed by Lewis, Hanrahan & Van Driessche, 1984) and with our previous studies (Moffett & Koch, 1985) which showed that under these conditions  $K^+$  uptake is partly blocked.

The hyperpolarization of  $V_b$  induced by  $Ba^{2+}$  can be explained as follows. When  $Ba^{2+}$  partially blocks  $K^+$  permeation, the apical pump begins to draw  $(K^+)_i$  away from electrical equilibrium; that is to say, when the apical pump extrudes a  $K^+$  from the cytoplasm, replacement of the positive charge with a  $K^+$  from the haemolymph-

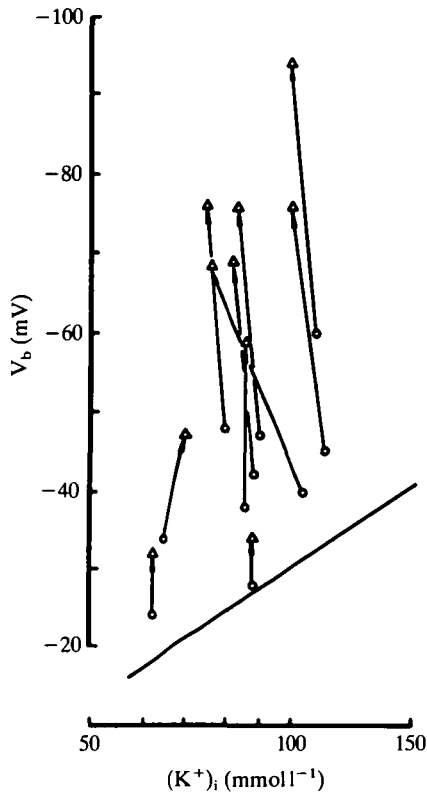


Fig. 7. Hyperpolarization of midgut cells by  $Ba^{2+}$ . Cells were penetrated in 32K (circles); subsequently the superfusate was changed to 32K + 2 mmol l<sup>-1</sup>  $Ba^{2+}$  (triangles). Arrows connect pairs of points collected from the same cell. The diagonal line is the line of electrochemical equilibrium with 32K.

side bathing solution requires a greater driving force. Macroscopic electroneutrality of the cytoplasm must be maintained. The loss of a positive charge from the cytoplasm *via* the apical pump could be made up by entry of a cation from outside, or by generation of one within the cytoplasm, or by loss of an anion from the cytoplasm. Because the activities of  $Ca^{2+}$  and  $Mg^{2+}$  in cytoplasm are much lower than that of  $K^+$ , effectively  $K^+$  is the only cation available in substantial quantity from the bathing solution, and  $Cl^-$  the only anion. Cytoplasmic  $Cl^-$ , as determined by X-ray microanalysis, is reported to be 21 mmol kg<sup>-1</sup> wet mass for goblet cells and 12 mmol kg<sup>-1</sup> wet mass for columnar cells (Dow *et al.* 1984). Appropriate corrections for cytoplasmic water content and concentration/activity ratio would bring the values close to the calculated  $Cl^-$  activity that would result from passive distribution across the basal membrane (12 mmol l<sup>-1</sup> for a typical  $V_b$  of 40 mV). The mean  $(K^+)_i$  in 32KS is  $92 \pm 5$  mmol l<sup>-1</sup> (Moffett *et al.* 1982). Thus if all cytoplasmic  $Cl^-$  were depleted,  $(K^+)_i$  could fall by on average less than 13%. The mean drop in  $(K^+)_i$  actually observed was 9% (calculated from Table 1A). Thus the insensitivity of

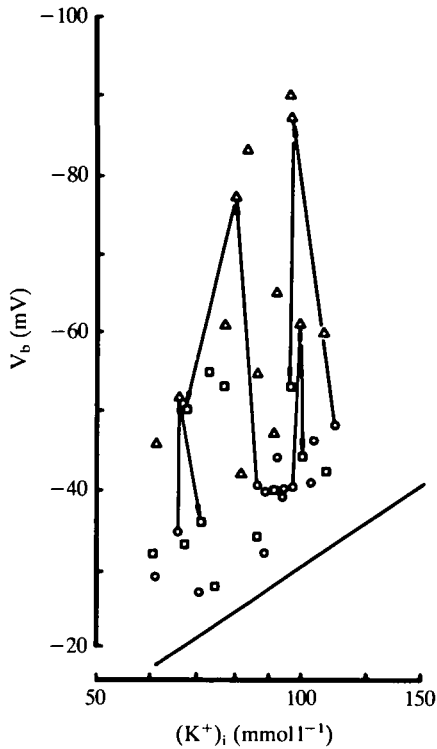


Fig. 8. Effect of Ba<sup>2+</sup> and subsequent apical pump inhibition. Fig. 6 is an example of the experiments summarized in this figure. Cells were penetrated in 32K (circles), then superfused with 32K+2 mmol l<sup>-1</sup> Ba<sup>2+</sup> (triangles), giving responses like those shown in Fig. 7. Finally the superfusate was changed to N<sub>2</sub>-equilibrated 32K+2 mmol l<sup>-1</sup> Ba<sup>2+</sup> (squares), causing a reversion to less negative values of V<sub>b</sub>. The diagonal line is the line of equilibrium with 32K. Points connected by arrows are representative sequences recorded from the same cells.

(K<sup>+</sup>)<sub>o</sub>; to changes in extracellular [K<sup>+</sup>] (Moffett *et al.* 1982; this study) and to Ba<sup>2+</sup> is understandable.

In a report of similar experiments in which Ba<sup>2+</sup> was applied to locust rectum, Hanrahan *et al.* (1986) provided an equivalent circuit analysis of the effect of reducing the K<sup>+</sup> conductance of one membrane in an epithelium in which both sides are dominated by K<sup>+</sup> conductance. Under short-circuit conditions the resistances and electromotive forces of the apical and basal membranes are effectively in parallel. The effect of reducing the conductance of one membrane is to drive the potential of that membrane towards the electromotive force of the other membrane. As we show in the succeeding paper (Moffett & Koch, 1988), the apical pump adds a substantial element of hyperpolarization to the apical potential even under short-circuit conditions. In the conceptual frame of the analysis of Hanrahan *et al.* (1986), when the conductance of the basal membrane of the midgut is reduced by Ba<sup>2+</sup> (Table 1), V<sub>b</sub> is driven towards the large electromotive force of the apical electrogenic pump.

Hypoxia rapidly inhibits the apical electrogenic pump (Mandel, Riddle & Storey, 1980). Evidence that the hyperpolarization caused by  $Ba^{2+}$  is pump-related is provided by the experiments in which  $Ba^{2+}$  was followed by hypoxia (Figs 6, 8; Table 1B). When the pump was inhibited, the  $Ba^{2+}$ -induced hyperpolarization was reduced as the pump electromotive force fell, without a further change in the chord conductance of the basal membrane (Table 1B).

#### *Functional electrical coupling between midgut cells*

The results of both the present and previous studies in this laboratory (Moffett *et al.* 1982) and by other workers (Thomas & May, 1984) confirm that ionophoretically injected Lucifer Yellow is typically confined to the impaled cell or at most it migrates into a few immediately adjacent cells. The result is the same whether the midgut is under open-circuit conditions, as in the experiments of Moffett *et al.* (1982), or under short-circuit conditions, as in the present studies. In this the midgut differs from the locust rectum, in which 'dye-coupling' is extensive and is associated with electrical coupling, presumably *via* intercellular gap junctions (Hanrahan & Phillips, 1984).

If the  $Ba^{2+}$ -induced hyperpolarization is driven by the apical pump, as the  $Ba^{2+}$  plus hypoxia experiments suggest, the findings that this hyperpolarization was seen in every impalement, and is shown by dye ionophoresis to occur in both cell types, are evidence that the cytoplasm of both epithelial cell types are electrically connected to the apical pump. If the apical electrogenic pump is confined to goblet cells, the columnar cells must be electrically coupled to them. The coupling could be mediated by intercellular junctions whose diameter restricts the passage of Lucifer Yellow. This possibility is supported by the predominance of the crystalline form of gap junction in the midgut epithelium (Hakim & Baldwin, 1984), and by reports of electrically conductive but dye-impermeable junctions in insect integumentary epithelium (Blennerhassett & Caveney, 1984). Gap junctional coupling need not be universal: each goblet cell together with its surrounding columnar cells could form a coupled functional unit, as suggested by previous findings (Moffett *et al.* 1982).

#### *Fixed cytoplasmic charges and the basal $K^+$ pump in cytoplasmic $K^+$ homeostasis*

The biological importance of the basal active  $K^+$  uptake is still unclear. Under our experimental conditions most of the energetic burden of transepithelial  $K^+$  transport is borne by the apical pump. The basal pump appears to be unnecessary for intracellular  $K^+$  homeostasis at haemolymphal  $K^+$  concentrations. The apparent lack of necessity for such a pump *in vitro* may be an artefact of the experimental conditions used in this and other such studies.

These studies were carried out in minimal salines designed to facilitate the study of transepithelial  $K^+$  transport. These salines support a gradually declining  $I_{sc}$  for several hours *in vitro*. The decline in  $I_{sc}$  does not necessarily indicate that the tissue itself is unhealthy, since a similar decline in  $I_{sc}$  is seen in tissues bathed in Grace's solution, a relatively complete lepidopteran cell culture medium (our unpublished

observations). Rather, the decline may be due to the washout of stimulatory factors contained in the haemolymph (Wolfersberger & Giangiacomo, 1983).

Nevertheless, the minimal salines differ from lepidopteran haemolymph in several potentially important ways. The pH of the salines (8.0) is more than 1 unit higher than that of haemolymph, amino acids and trehalose in the haemolymph are replaced by sucrose in the salines, and the concentrations of  $Mg^{2+}$  and  $Ca^{2+}$  are both  $5\text{ mmol l}^{-1}$  in the salines compared with values of 50 and  $10\text{ mmol l}^{-1}$ , respectively, in haemolymph (Jungreis, Jatlow & Wyatt, 1973). Also, the haemolymph of *Manduca sexta* contains about  $5\text{--}17\text{ mmol l}^{-1}\text{ Na}^+$  (Dow *et al.* 1984). In a previous communication (Moffett & Koch, 1985) we pointed out a possible reduction of passive entry of  $K^+$  by the  $Ca^{2+}$  concentration of haemolymph. If passive  $K^+$  entry is reduced, the active entry process will become more significant for transepithelial  $K^+$  transport. Also, the greater concentration in haemolymph of permeant cations other than  $K^+$  and the presence of permeant anions other than  $Cl^-$  might well decrease the ability of cytoplasmic fixed negative charges to keep ( $K^+$ ); constant. In this case, the basal pump might play a more important role in homeostasis of ( $K^+$ ); than it appears to do *in vitro*.

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