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

II. THE TRANSAPICAL ELECTROCHEMICAL GRADIENTS

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

The apical surface of the midgut of *Manduca sexta* larvae is composed of the apical membranes of columnar cells, in the form of microvilli, and the apical goblet of goblet cells. Considerable evidence has suggested that the apical electrogenic pump that is responsible for transepithelial K^+ transport is located on the apical membrane of goblet cells. In the present study the transapical potentials and K^+ chemical activity [(K^+)] gradients of columnar and goblet cells of posterior midgut were examined in the short-circuited gut. In some experiments the recording site was localized by ionophoresis of $NiCl_2$ followed immediately by fixation in rubeanic acid.

The (K^+) of goblet cavities was substantially higher than that of the free solution on the gut luminal side (mean value of 94 mmol l^{-1} in standard bathing solution). The goblet cavity was electrically positive to the gut lumen (mean value of 40 mV in standard bathing solution). When the rate of pumping of K^+ into the goblet cavity was decreased by hypoxia or decreased bathing solution $[K^+]$, the electrical potential gradient between cytoplasm and goblet cavity decreased while intracellular (K^+) and goblet cavity (K^+) were relatively stable. These studies provide evidence that a negatively charged goblet matrix is present in goblet cavities. Furthermore, they suggest that it is the voltage-sensitivity of the apical pump to the electrical component of the transapical electrochemical gradient, and not a concentration-dependence of the pump, that exercises the major role in determining the relationship between extracellular (K^+) and net K^+ transport by the isolated gut.

INTRODUCTION

It has been proposed that the goblet cells of lepidopteran midgut possess an apical electrogenic K^+ pump that extrudes K^+ into the goblet cavity and is responsible for active transepithelial K^+ transport (Harvey, Cioffi, Dow & Wolfersberger, 1983). The electrical and chemical nature of the goblet cavity could be expected to have important effects on the functioning of the apical pump.

Key words: *Manduca sexta* midgut, K^+ transport, goblet cell, membrane potential, ion-specific microelectrode, Ni^{2+} precipitation.

The presence of a 'matrix' of acid mucopolysaccharide in the goblet cavities is indicated by histochemical studies (Schultz, Lozano & Cajina-Quezada, 1981) and by X-ray microanalysis (Dow, Gupta, Hall & Harvey, 1984) which shows a high sulphur concentration in the goblets, a percentage dry weight considerably higher than that of the bathing medium, and a concentration of Cl^- in the goblet cavities which is far from sufficient to balance the charge due to K^+.

In the preceding paper (Moffett & Koch, 1988) we presented evidence that columnar and goblet cells form a limited syncytium, and that entry of K^+ into the common pool of K^+ in the cytoplasm of both cell types may be mediated by two mechanisms, at least one of which must be thermodynamically active.

In the present study we have measured the gradients of potential and of K^+ chemical activity between midgut cytoplasm and the goblet cavities, and between goblet cell cavities and the midgut lumen, when the midgut is bathed in high (70 mequiv l^{-1}), standard (32 mequiv l^{-1}) or low (10 mequiv l^{-1}) K^+ concentrations. Combining these results with those reported in Moffett & Koch (1988), we show that the goblet cavities represent a significant energetic barrier for the apical electrogenic pump, and suggest that the kinetics of the transport system with respect to extracellular K^+ chemical activity are determined by the electrical component of the apical electrochemical gradient rather than by the chemical activity of K^+ available on the intracellular side of the transporting membrane.

Furthermore, the stability of the K^+ chemical activity in the goblet cavities in the face of large changes in net K^+ transport induced by changing medium K^+ concentration suggests that the (K^+) of the cavities is mainly determined by the presence of fixed negative charges rather than by the rate at which K^+ is actively brought in.

MATERIALS AND METHODS

Recordings were made from the posterior midgut of fifth instar larvae of *Manduca sexta* as described in the preceding paper (Moffett & Koch, 1988), except that in some experiments double-barrelled electrodes consisting of a reference barrel filled with 3 mol l^{-1} KCl and a barrel filled with 1 mol l^{-1} $NiCl_2$ were used to localize recording sites. Ni^{2+} was ionophoresed into such sites by passing 500 ms, 5 nA bipolar pulses for about 5 min, according to the method described by Quicke & Brace (1979) for intracellular marking of neurones. Within several minutes of the termination of pulses, the tissue was fixed for 5 min in 95 % ethanol saturated with rubeanic acid. This treatment precipitates the Ni^{2+} as an insoluble coloured organometal complex. The site of the mark was determined in whole mounts of the cleared tissue by examination with Nomarski optics.

The composition and designation of bathing solutions and the method of inducing transient N_2 hypoxia were as given in the preceding paper.

RESULTS

Goblet lumen electrical potential (V_g) and potassium activity [$(K^+)_g$] differ from luminal free solution

The potential profile of the short-circuited gut in standard (32KS) bathing solution showed that in the apical region there was frequently a potential several tens of millivolts positive to the free solution on the gut luminal side. The K^+ chemical activity of these sites was appreciably above that of the gut luminal solution. For 24 such sites, the mean $(K^+)_g$ was $94 \pm 3{\cdot}7\,\text{mmol}\,l^{-1}$ (S.E.) and the mean V_g was $40 \pm 3{\cdot}3\,\text{mV}$ (S.E.). Examples of profiles with and without such apical components are shown in Fig. 1. The sites of positive potential were identified as goblet cavities by ionophoresis of Ni^{2+} which was then immobilized by treatment with rubeanic acid (see Materials and Methods) and visualized under Nomarski optics (Fig. 2). A precipitate could be found with delays of up to 15 min between injection and fixation.

The effects of varying bathing solution [K^+]

Reduction of K^+ concentration, over the range from 70 to $10\,\text{mmol}\,l^{-1}$, nearly halved I_{sc}, but lowered $(K^+)_g$ by 21 % and had little effect on V_g (Table 1). The effect on K^+ transport is consistent with results from several previous studies (Moffett, 1979; Moffett & Koch, 1985).

The effect of hypoxia on V_g and $(K^+)_g$

The effect of N_2 hypoxia on V_g and $(K^+)_g$ were examined in two ways. In the first approach, an electrode was inserted into a goblet cavity and after a successful impalement had been established, the superfusate was switched from 32KS

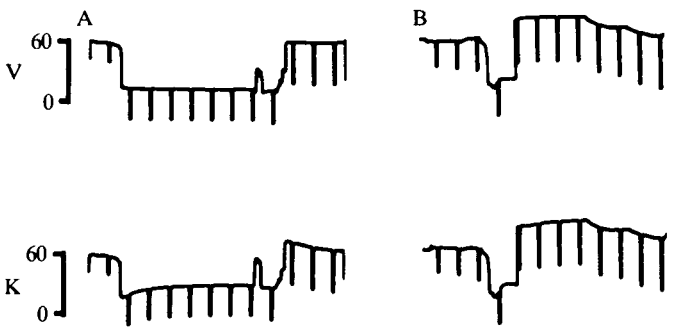


Fig. 1. Voltage and K^+ profiles without (A) and with (B) a positive-going apical component. These examples were recorded from the same tissue using the same electrode pair. The traces labelled V are the output of the reference barrel, those labelled K are the output of the K^+-selective barrel. The arrows show advances of the microelectrode drive. The vertical scale bars show 0 to −60 mV. Downward deflections are negative relative to the haemolymph-side superfusate. The negative-going pulses seen in this record and in Fig. 4 are steps of voltage applied by the voltage clamp for measurement of the net resistance between the haemolymph-side voltage-clamp electrode and the microelectrode tip. The resistance increases as the microelectrode is advanced across the basal membrane, and a further increase is seen as the electrode tip crosses the apical membrane.

Table 1. *Means and standard errors of V_g, $(K^+)_g$ and normalized I_{sc} in 70K, 32K and 10K*

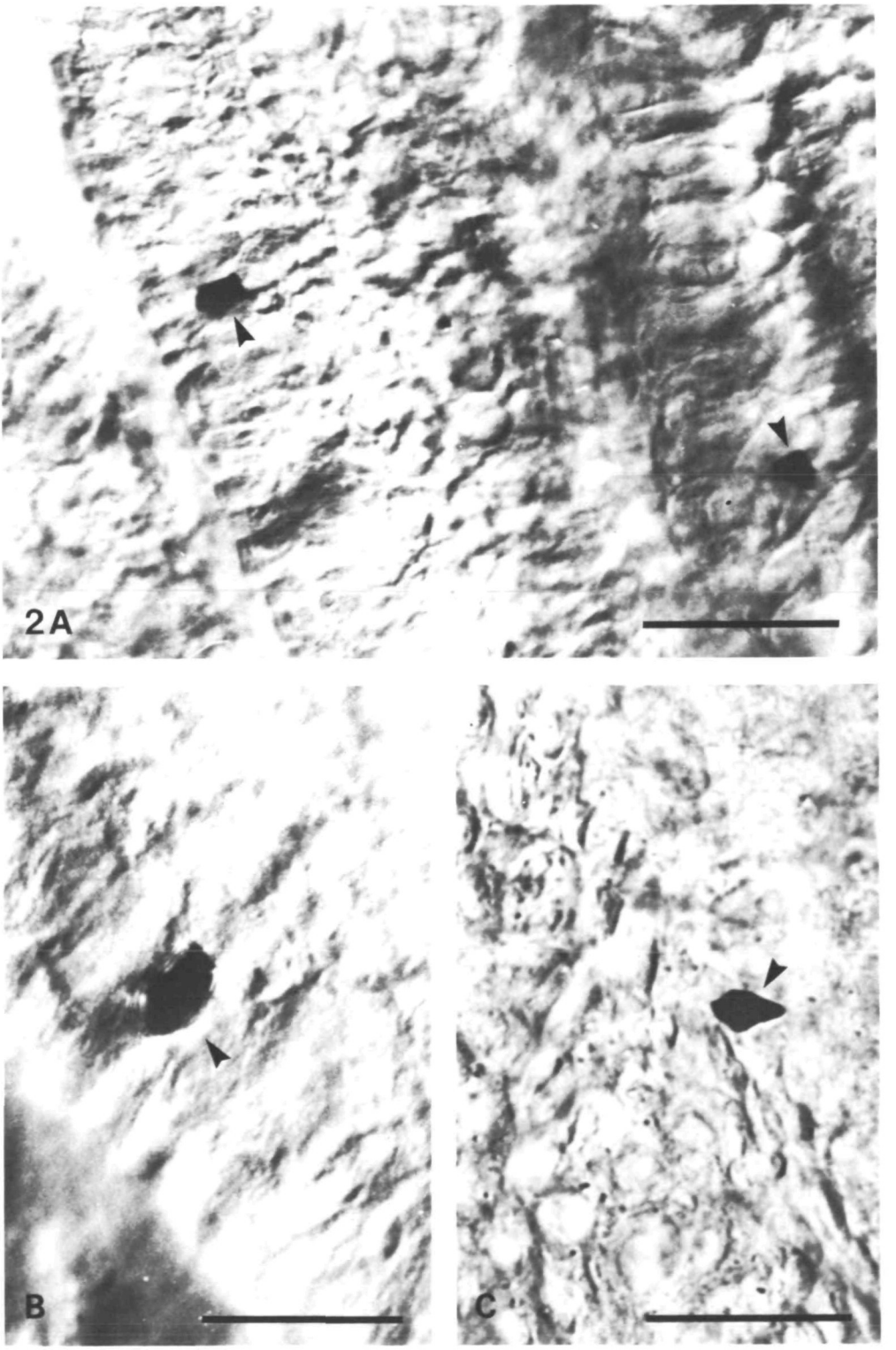
	70K	32K	10K
V_g (mV)	36 ± 16·9	32 ± 17·4	33 ± 19·5
$(K^+)_g$	97 ± 26·4	87 ± 24·3	77 ± 33·4
Normalized I_{sc} (%)	121 ± 1·4	100*	70 ± 2·2

* Mean I_{sc} in 32K = 243 ± 17·7 $\mu A\,cm^{-2}$ (S.E.).
$N = 9$ for 70K and 32K, 6 for 10K.
For details of media see Materials and Methods.

equilibrated with 100 % O_2 to the same solution equilibrated with 100 % N_2. This treatment almost immediately initiated a decrease in I_{sc}. We then monitored V_g and $(K^+)_g$ during a transient hypoxia of several minutes duration which was terminated by restoring oxygenated solution before I_{sc} had fallen to a low value. Normalized V_g and $(K^+)_g$ were plotted against the normalized I_{sc} for 10 such partial transients recorded from four tissues (Fig. 3). A remarkable feature of the partial transients was that while V_g decreased during hypoxia, $(K^+)_g$ was essentially unaffected. It was not possible to obtain recordings of complete transients, because if hypoxia was continued, the impalement was invariably lost when I_{sc} reached 20–30 % of its initial value.

To obtain information about the ultimate values of V_g and $(K^+)_g$ in severe hypoxia, we performed a second type of experiment in which we made a number of profiles in a normoxic tissue, then superfused it with anoxic saline until I_{sc} had fallen to between 30 and 10 % of its initial value. (We are terming this level of inhibition of I_{sc} as 'severe' hypoxia to distinguish it from the briefer transients which typically resulted in much less inhibition of I_{sc}; see also the legends for Figs 3 and 4 and the Discussion.) We then sampled the hypoxic tissue with a similar number of profiles. Oxygenated bathing solution was readmitted and a third series of profiles was made immediately after the recovery of I_{sc}. Table 2 shows the results of such experiments on two tissues. An important result of these experiments is that during severe hypoxia we did not find any sites more positive than 10 mV, but we did find in some profiles sites on the apical side with high (K^+) and potentials between +10 and −20 mV (Table 2). Fig. 4 shows a typical profile containing such a site. Recovery of

Fig. 2. Ni^{2+}-labelled goblet cavities in a cleared whole mount of midgut, as viewed by Nomarski optics. (A) In this tissue, two goblets were labelled (arrowheads). The view is from the haemolymphal side. We see the left one from the side and the right one end-on. Scale bar, 50 μm. (B) An enlarged view of the left-hand cell shown in A. The cell lies in a fold of epithelium with its cytoplasm projecting to the left towards the haemolymphal side of the tissue. Scale bar, 25 μm. (C) An oblique view of a labelled goblet cavity which lies along the side of a fold of epithelium in which the luminal surface of the tissue is turned towards the viewer. The basal cytoplasm of the cell projects to the left and away from the viewer. Scale bar, 25 μm.


2A
B
C

positive potentials occurred within a few minutes after oxygenated saline had been readmitted, apparently with the same time course as the recovery of I_{sc}. Thus V_g is sensitive to hypoxic inhibition of the apical pump, but $(K^+)_g$ is not.

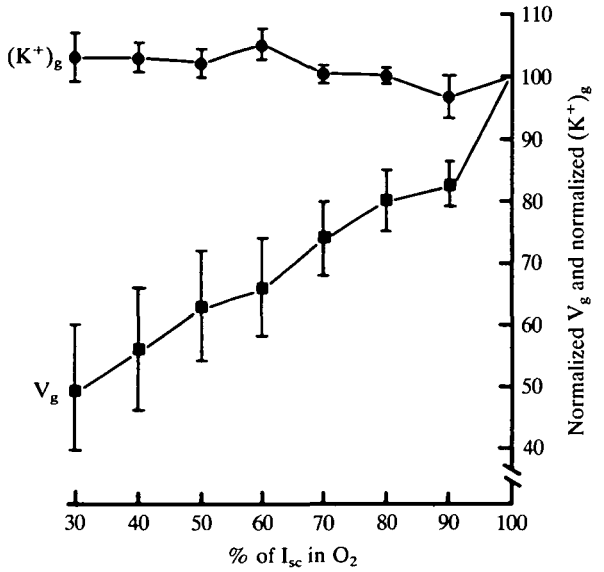


Fig. 3. Changes of V_g (squares) and $(K^+)_g$ (circles) in transients of N_2 hypoxia. V_g and $(K^+)_g$ are normalized to their initial values and plotted against the normalized I_{sc}. This figure represents the results of 10 transients from four tissues. The vertical bars show ±1 S.E. Of the 10 transients, six were carried to the maximum level of inhibition shown (30% of initial I_{sc}). The mean time to attain this level of inhibition was 232 ± 15 s (S.E.M.).

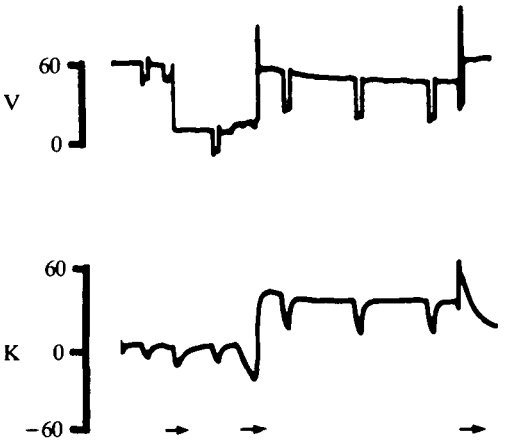


Fig. 4. An example of a transepithelial profile of voltage and K^+ recorded after 8 min of N_2 hypoxia. In this tissue I_{sc} had fallen to 15% of the initial value. Details of the traces are the same as in Fig. 1. The vertical scale bars are 0 to −60 mV for the V trace and +60 to −60 mV for the K trace. The microelectrode was advanced at the arrows.

Table 2. *Means and standard errors of* V_g, $(K^+)_g$ *and normalized* I_{sc} *for eight penetrations in 'severe' hypoxia*

V_g (mV)	$(K^+)_g$ (mmol l^{-1})	% I_{sc}
2 ± 3·6	75 ± 9·2	25 ± 3·4

DISCUSSION

Effects of fixed negative charges on $(K^+)_g$

When the K^+ concentration in the bathing medium is reduced more than six-fold, the concentration in the goblet cavity is diminished by only 21 % (Table 1; Fig. 5) while net K^+ transport falls by almost one-half (see fig. 5 of Moffett & Koch, 1988). This suggests that goblet cavity (K^+) is not determined simply by a dynamic balance between active transport into the goblet cavity and diffusional exit *via* the goblet pore. Instead, the relative constancy of $(K^+)_g$ can be explained by the presence of fixed negative charges in the goblet cavity, for which there is a variety of evidence (see Introduction).

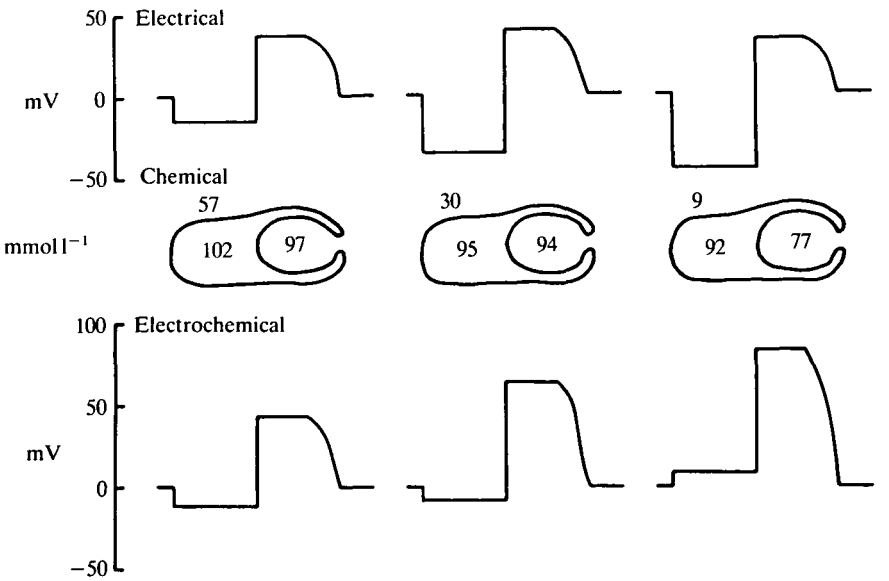


Fig. 5. Idealized electrical profiles (top), K^+ chemical activities (middle) and the resulting K^+ electrochemical gradients (bottom) in the short-circuited midgut epithelium, shown for three bathing solution K^+ chemical activities. The extracellular activity of 57 mmol l^{-1} corresponds to 70K solution, 30 mmol l^{-1} to 32K solution, and 9 mmol l^{-1} to 10K solution. Negative-going electrochemical gradients are favourable for passive K^+ movement, positive-going gradients are thermodynamically uphill. Note that the basal electrochemical gradient becomes positive between 32K and 10K. The net electrochemical gradient that must be overcome by the transport system to move K^+ into the goblet cavity becomes steadily greater as bathing solution K^+ chemical activity is decreased.

In the presence of an anion matrix in the goblet cavity, V_g and $(K^+)_g$ can be expected to be determined by the sum of two processes: diffusion of pumped K^+ between matrix and gut lumen, and a Donnan equilibration of luminal K^+ and Cl^- with goblet cavity fixed charges. At high pump rates, V_g and $(K^+)_g$ are dominated by the diffusional component. The result is a positive V_g and a $(K^+)_g$ greater than that predicted by the Donnan equilibrium. At very low pump rates V_g and $(K^+)_g$ revert to the values determined by the Donnan equilibrium. The result is a negative V_g and a somewhat lower $(K^+)_g$. Furthermore, the value of V_g can be expected to be quite sensitive to the pump rate, while $(K^+)_g$ can be expected to be relatively insensitive, except at very high rates of pumping. These predictions agree with our results from the transient hypoxia experiments in which V_g and $(K^+)_g$ were recorded continuously from a single goblet. In our experiments I_{sc} never reached zero even when superfusion with N_2-equilibrated solutions lasted more than 10 min, but instead became stable at a value between 10 and 30 % of the initial value in 100 % oxygen. Complete anoxia is impossible under our experimental conditions because the N_2-equilibrated superfusate comes into contact with air as it flows across the opening in the chamber that allows for access of microelectrodes to the haemolymphal side of the tissue. The small negative or positive potentials that persisted under these conditions thus could be interpreted as representing an intermediate between the electropositivity resulting when transport rate is high and the equilibrium state that would exist with the electrogenic pump completely stopped.

X-ray microanalysis studies (Dow *et al.* 1984; Gupta, Dow, Hall & Harvey, 1985) show a decrease in K^+ concentration in the goblet cavity with complete inhibition of K^+ transport by anoxia or *Bacillus thuringiensis* endotoxin. These results conflict with our studies in which transport was briefly depressed by hypoxia (Fig. 3). The discrepancy can be explained if matrix material were lost under the conditions of inhibition imposed in the X-ray microanalysis studies. Such loss is indicated by table 1 of Dow *et al.* (1984) which shows that after hypoxia the elemental sulphur concentration of goblet cavity was decreased from 58 ± 7 to 16 ± 2 mmol kg^{-1} wet mass. In the same table, hypoxia is shown to decrease the percentage dry weight of the goblet cavity from 21 ± 3 to 10 ± 2 %. Similar results are seen in table 1 of Gupta *et al.* (1985) in which inhibition of K^+ transport by *Bacillus thuringiensis* toxin is accompanied by a decrease in elemental sulphur from 58 ± 7 to 10 ± 2 mmol kg^{-1} wet mass and of percentage dry weight from 21 ± 3 to $9 \pm 0{\cdot}5$ %. The goblet cavities of toxin-treated tissues were found to be enlarged in comparison with most control cavities when the tissue was viewed by scanning transmission electron microscopy. These results strongly suggest that relatively complete inhibition of K^+ transport is associated with loss of the goblet matrix. We suggest that loss of goblet matrix could occur as a result of the hydrostatic pressure and volume changes that would probably occur in the matrix system as it reverts to Donnan equilibrium from the non-equilibrium condition as pump rate becomes very low. The onset of such effects explains why our electrodes became dislodged from the goblet cavity when pump inhibition became substantial. However, the matrix did not appear to be lost in these brief hypoxic transients, because complete recovery of

the short-circuit current was observed and goblet cavity penetrations similar to those of controls could be obtained within a few minutes after oxygenated saline had been readmitted.

How extracellular K^+ determines the activity of the apical pump

Profiles of potential and of K^+ concentration across the epithelium with different external K^+ concentrations, as found here and in Moffett & Koch (1988), are given in Fig. 5. As extracellular (K^+) is reduced, the driving force for passive basal entry of K^+ is reduced and ultimately reversed, and at the same time the magnitude of the energetic barrier to apical K^+ transport increases. Since both cytoplasmic and goblet (K^+) are relatively resistant to this treatment, it is the electrical components of the basal and apical electrochemical gradients that are mainly responsible for these changes. It is difficult to attribute the approximately 50% decrease of net K^+ transport that takes place between 70KS and 10KS to the 10 mmol l^{-1} (approximately 10%) change in availability of cellular K^+ to the putative apical pump enzyme, particularly since in this concentration range such a change in (K^+) results in a modest stimulation, rather than inhibition, of the isolated enzyme (Wieczorek, Wolfersberger, Cioffi & Harvey, 1986). On theoretical grounds electrogenic pumps can be expected to be voltage-sensitive (see, for example, Gadsby, Kimura & Noma, 1985, and sources cited therein), and it now appears likely that such voltage-sensitivity could account both for the inhibition of apical pumping by basal-side Ba^{2+}, as reported in Moffett & Koch (1985, 1988), and for the effect of bathing solution (K^+) on net K^+ transport *via* the apical pump described in several previous studies (Moffett, 1979; Moffett & Koch, 1983, 1985).

In another study (Moffett, 1980), the net current–voltage relationship of the whole epithelium was found to be linear over the range +200 to −200 mV. This finding is not necessarily inconsistent with the voltage-sensitivity of the apical pump that we hypothesize here. The previous studies showed that complete inhibition of the apical pump by N_2 hypoxia reduced the total tissue conductance by only about 20%. If it is assumed that apical pump-related conductance falls to a very low value when the pump is inhibited, this result suggests that the conductance of the whole tissue is dominated by pathways parallel to the apical membrane of goblet cells; such parallel conductance pathways could include both columnar cells and paracellular shunts. The large parallel conductances, which we have shown (Koch & Moffett, 1987) could easily mask pump-related changes in conductance that would otherwise be expected when the transepithelial potential is changed.

Goblet cells are present throughout the length of the midgut, although their ultrastructure (Cioffi, 1979) and carbonic anhydrase histochemistry (Ridgway & Moffett, 1986) differ among anterior, middle and posterior regions of the gut. Circumstantial evidence associates the goblets with the potassium transport activity of the gut (Cioffi & Harvey, 1981), but Dow (1984) has advanced a hypothesis linking the goblet with active alkalinization of midgut contents. The present studies demonstrate that the goblets are electrically as well as chemically isolated from the midgut lumen. This isolation is a requirement for the hypothesis of a role in

alkalinization, but it also ensures that the apical pump faces a relatively stable chemical environment and is protected from midgut contents.

The preceding paper (Moffett & Koch, 1988) provided evidence that passive entry of K^+ into the midgut cytoplasm could be supplemented by an active process. Because of the effect of intracellular fixed charges discussed in that paper, such a process could not greatly affect intracellular (K^+), and is not needed to maintain intracellular (K^+). However, it could decrease the negativity of the basal potential (Fig. 5 and see fig. 4 of Moffett & Koch, 1988) and thus decrease the electrical component of the energetic barrier that the apical pump must work against under short-circuit conditions.

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