J. exp. Biol. **143**, 101–114 (1989)
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MICROELECTRODE EVIDENCE FOR THE ELECTRICAL ISOLATION OF GOBLET CELL CAVITIES IN *MANDUCA SEXTA* MIDDLE MIDGUT

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Accepted 25 August 1988

Summary

The middle midgut of *Manduca sexta* is known to transport K^+ actively from the basal to the apical side, and is thought to be involved in the generation of an extremely high pH in the midgut lumen.

An experimental configuration is described which allows visually guided microelectrode impalements to be made of any compartment in the midgut tissue, with simultaneous verification by fluorescent dye ionophoresis in the live tissue. In this way, many impalements can be performed and verified on a single gut.

The potential differences between impalement sites and the apical compartment were plotted against transepithelial potential difference (TEP) for three sites: goblet cell cytoplasm, columnar cell cytoplasm and goblet cell cavities. In all three cases, the potentials recorded varied significantly and linearly with gross TEP.

Goblet and columnar cell cytoplasm impalements resembled each other both in size and direction of potential, being always negative relative to the lumen. This implied electrical coupling was confirmed by the observation that the two cell types were linked by gap junctions.

Goblet cavity impalements were characterized by a positive potential relative to the lumen. The goblet cavities were isolated from the lumen by a high resistance, and no leakage of Lucifer Yellow or 6-carboxyfluorescein markers through the apical valve was detected.

Using sensible estimates for midgut intracellular pH and *in vivo* TEP, it was shown that the potential difference across the goblet cavity apical membrane *in vivo* would agree closely with the Nernst potential for protons across the membrane, in agreement with a model for generation of high pH.

Introduction

The electrogenic potassium transport in lepidopteran midgut is amongst the most vigorous known, being capable of generating open-circuit voltages in excess of 150 mV, and short-circuit currents in excess of 1 mA cm^{-2} (Harvey *et al.* 1983; Dow, 1986). The apical membrane of the specialized goblet cells lining the midgut

Key words: *Manduca sexta*, electrogenic K^+-ATPase, midgut goblet cell, high pH, alkalinization, Lucifer Yellow microelectrodes.

is believed to be the site for the K^+-ATPase; evidence for this has been obtained from X-ray microanalysis (Dow *et al.* 1984), from microelectrode studies (Moffett *et al.* 1982; Moffett & Koch, 1988*a*,*b*), and from biochemical analyses of the plasma membrane (Wieczorek *et al.* 1986).

The function of the K^+ pump, however, remains controversial. The pump clearly plays a major role in the regulation of K^+ levels in blood, midgut tissue and midgut lumen (Dow & Harvey, 1988). However, a primary role in K^+ excretion is unlikely on the grounds that high blood [K^+], goblet cells and midgut K^+-ATPases are not universal among phytophagous insects, that the larval lepidopteran Malpighian tubules may *rescue* potassium from the rectum (Dow & Harvey, 1988), and that the evolution of a goblet cavity would reduce the efficiency with which the apical K^+ pump could excrete K^+ (Dow, 1984*b*, 1986). The potassium electrochemical gradient established by the pump is also critical in driving uptake of nutrients (Nedergaard, 1977; Giordana *et al.* 1982, 1985). Neither of these processes, however, would benefit from the evolution of a goblet cavity, which could only increase the cost at which K^+ could be moved. It has recently been suggested that the potassium transport also maintains indirectly the extremely high gut pH widespread among lepidopteran larvae (Dow, 1984*b*). Such a high pH might have many advantages; the control of pathogenic microorganisms, the acceleration of digestive hydrolysis, and an increase in digestibility of tannin-rich food (Dow, 1984*b*).

This paper tests the model for generation of high pH (Dow, 1984*b*), and suggests that the goblet cavity is an electrically isolated compartment, very highly polarized by electrogenic K^+ transport, and thus extremely unattractive to protons, which distribute passively according to the Nernst equation, rendering the goblet cavities extremely alkaline. The model predicts that the goblet cavity is not isopotential with the midgut lumen, and that the goblet cell apical membrane is accordingly more highly polarized than would be estimated from measurements of transepithelial potential differences and intracellular resting potentials, and that the degree of polarization of the goblet cavity apical membrane is related linearly to the maximum pH attainable in the midgut lumen. Recently, microelectrode impalements have confirmed that goblet cavities in the posterior midgut, a region in which the goblet cavities are thought not to play a prominent role in gut alkalinization (Dow, 1984*b*), are not isopotential with the midgut lumen (Moffett & Koch, 1988*b*). Using a technique which, for the first time, allows microelectrodes to be guided into specific goblet cavities, this paper shows these predictions to be true for the middle midgut, a region which *is* believed to be involved in the alkalinization of the gut lumen (Dow, 1984*b*). Additionally, the apical valve of the goblet cavities is shown to restrict the diffusion of the fluorescent dyes Lucifer Yellow and 6-carboxyfluorescein, a result previously unexpected (Moffett & Koch, 1988*b*). This is confirmed by electrical measurements, which show that the cavity is an extremely high-resistance compartment. The results are discussed in the context of the Dow (1984*b*) model, and estimates given for the likely extent of polarization of the goblet cavity apical membrane *in situ*. These estimates agree

closely with the transmembrane Nernst potential calculated for protons. Some of this work has been presented elsewhere (Dow, 1984*a*, 1986).

Materials and methods

Rearing

Manduca sexta larvae were obtained from the Zoology Department at Cambridge University and fed an artificial diet. Some larvae were raised from eggs provided by Dr Stuart Reynolds (Bath). All were reared at 25°C in ambient humidity with a photoperiod of 16 h:8 h L:D.

Tissue

Feeding fifth-instar larvae, weighing between 4 and 6 g, were used in all experiments. After chilling the animals on crushed ice for 30 min, the midguts were removed as flat sheets (Cioffi & Harvey, 1981). The middle midgut was used in all experiments because (i) although it has not been as thoroughly studied as the posterior midgut, it is believed to be involved in generating the high pH found in the alimentary canal (Dow, 1984*b*) and (ii) it is the least folded region of the midgut (Cioffi, 1979) and therefore the most transparent for microscopy, especially the area directly above the longitudinal muscles (see Fig. 2).

Perfusion

The flat midgut sheet was mounted, haemolymph-side down, on a perfusion chamber made from Sylgard 184 elastomer (Dow Corning). Polythene tubing was cast into the chamber and connected to gravity sources of oxygenated *Manduca* saline, which independently perfused the basal and apical sides of the tissue (Fig. 1). Perfusate was removed with a vacuum line. *Manduca* saline contained (in mmol l^{-1}): K^+, 32; Cl^-, 36; Mg^{2+}, 1; Ca^{2+}, 1; Tris-HCl, 5; sucrose, 200; pH 8·3.

Transepithelial recording of potential difference

Midgut transepithelial electrical potential differences (TEPs) were measured with a pair of Ag/AgCl wires on either side of the epithelium, connected to a Beckman 3050 voltmeter and/or a chart recorder. Potentials typically varied between 20 and 120 mV during an experiment.

Microelectrode impalements

Microelectrodes were pulled from borosilicate glass (Clark GC100TF-10) using a Campden Instruments microelectrode puller. Electrodes were back-filled with Lucifer Yellow CH (5 mg ml^{-1} in 1% LiCl) to yield final resistances of approximately 100 MΩ. Microelectrodes were inserted into holders containing Ag/AgCl half-cells, and fitted to an electronic Leitz micromanipulator. The amplifier system was made in the Zoology Department at Cambridge, with facilities for bridge balancing, current injection and resistance measurement. It

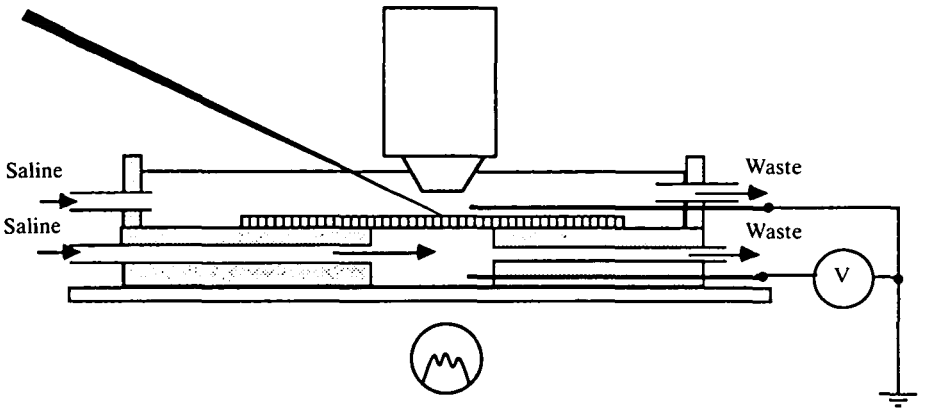

Fig. 1. The experimental apparatus used for impalements. Midguts were pinned onto a microscope slide coated with clear Sylgard elastomer, and viewed by phase-contrast, Nomarski or epifluorescence optics. The tissue was perfused with oxygenated saline, and impalements were made with conventional microelectrodes, filled with Lucifer Yellow CH. Potentials were recorded relative to the apical side of the epithelium. Transepithelial potential differences were measured simultaneously *via* a pair of Ag/AgCl wire electrodes on either side of the epithelium. V, voltmeter for measuring transepithelial potential difference.

was connected to a Gould 20 MHz digital storage oscilloscope (OS 1420) which displayed the resting potentials of impalement sites. All potentials were measured relative to the apical side.

The tissue was viewed under phase-contrast or epifluorescence optics on a Leitz Ortholux microscope, through a 25× phase-contrast, water-immersion objective. This configuration allowed the live epithelium to be viewed in considerable detail (Fig. 2), and the electrode to be advanced into identified, preselected sites.

Dye fills

Once a stable resting potential had been recorded during an impalement, a hyperpolarizing current was passed for between 5 s and 1 min to mark the impalement site with Lucifer Yellow. The injection site could readily be identified by its morphology without fixation or other intervention, allowing recordings to be made from many sites in a single preparation.

To determine whether the observed slow loss of fluorescence from a goblet cavity was due to a gradual photobleaching of Lucifer Yellow within an intact cell, or whether the impalement site was 'leaky', allowing the dye to escape, a short experiment was conducted wherein a time course of bleaching of fluorescence was quantified. Continuous bleaching was compared with bleaching at intervals.

Results

Experimental procedure

Using visual guidance, it proved very easy to obtain good impalements of goble

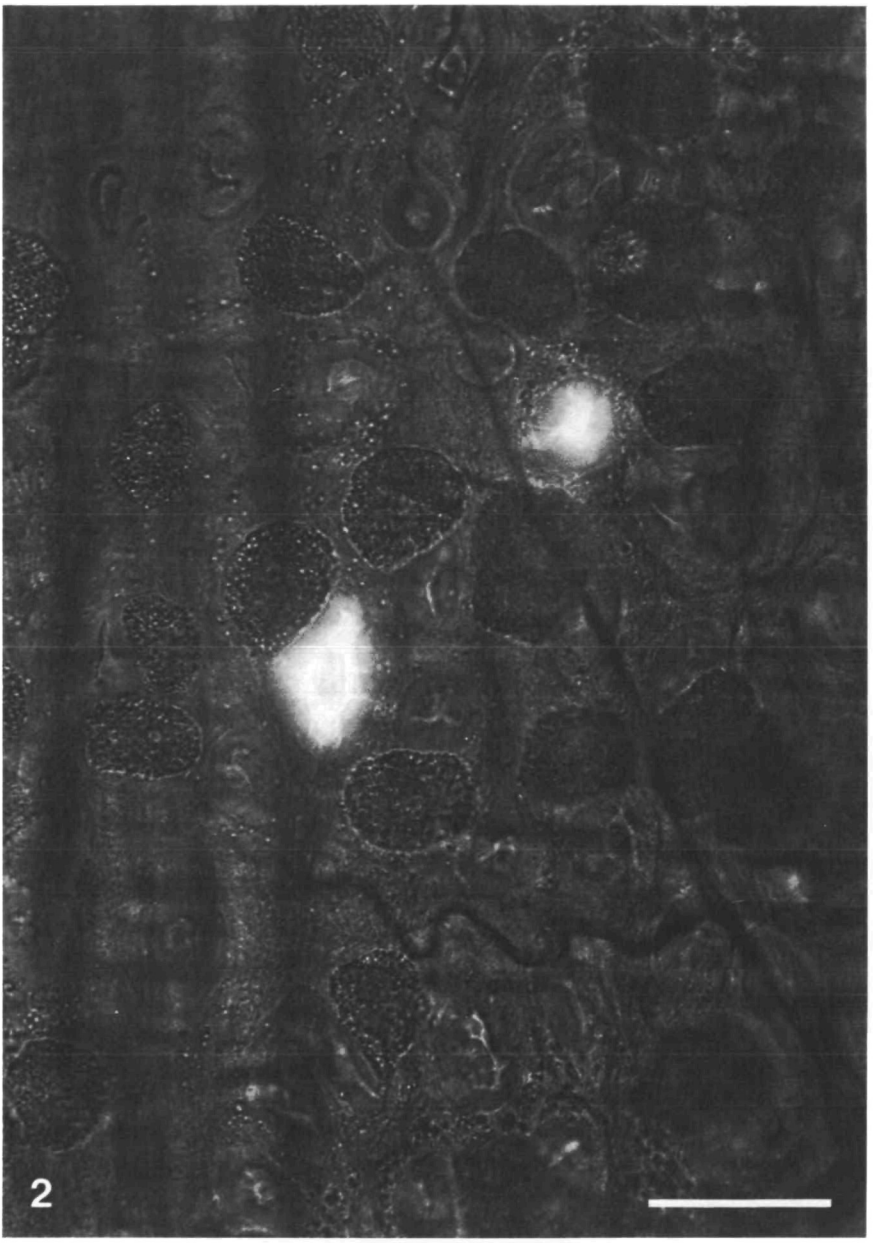

Fig. 2. Light micrograph of *Manduca* middle midgut, illuminated simultaneously by phase contrast and epifluorescence, showing two goblet cavities successfully filled with Lucifer Yellow. Scale bar represents 50 μm. Below the plane of focus can be seen longitudinal muscles running vertically along the left side; circular muscles underlie the folds to the right. A tracheum enters from the lower right. Columnar cells are characterized in phase contrast by a granular apical side, sometimes forming blebs, as seen here (Cioffi, 1980), and a uniform cytoplasm. Goblet cells are characterized by a doughnut shape of cytoplasm, with a conspicuous, jagged, microvillate central cavity.

and columnar cells, and reasonably easy to impale goblet cavities. The site of impalement was verified readily after Lucifer Yellow filling, by comparing phase and epifluorescence views of the impalement site. It was noted that dye injected into either goblet or columnar cell cytoplasm spread rapidly to neighbouring cells, confirming that middle midgut cells are normally linked by gap junctions under open-circuit conditions (Moffett *et al.* 1982; Thomas & May, 1984). However, impalements with potentials and resistances consistent with basal extracellular space (Moffett *et al.* 1982) showed no accumulation of dye, even when viewed during ionophoresis.

In contrast, dye leakage was not observed from any of the goblet cavities. No loss of fluorescence, other than that due to photobleaching, was detected up to 30 min after making fills. This result confirmed resistance measurements relative to the apical side of the epithelium, in which the resistance of cavities was found to be very high ($34 \pm 8\,M\Omega$, $N = 11$). In contrast, the resistances of the cytoplasm of goblet cells ($6 \pm 2\,M\Omega$, $N = 10$) and columnar cells ($4 \pm 1\,M\Omega$, $N = 18$) were low, in good agreement with the average of $6{\cdot}2\,M\Omega$ obtained previously in *Spodoptera* midgut (Thomas & May, 1984).

Lucifer Yellow is known to be precipitated by high potassium levels (Warner & Bate, 1987), and so it could be argued that the lack of dispersion of Lucifer Yellow could arise from immobilization, rather than the tightness of the apical valve. This is unlikely to be the case, as the potassium activity of goblet cavities is no higher than that of cytoplasm (Moffett & Koch, 1988*b*), a level which does not immobilize Lucifer Yellow (Warner & Bate, 1987). However, the possibility was tested by filling impalement sites with 6-carboxyfluorescein, a molecule of slightly lower relative molecular mass (376 *vs* 457), but known not to suffer from problems of precipitation (Warner & Bate, 1987). It was found that the same pattern of dye accumulation was obtained as for Lucifer Yellow. The dye filled all three compartments successfully, but photobleached much more rapidly than Lucifer Yellow. It diffused from goblet or columnar cytoplasm within 1 min, but did not diffuse out of goblet cavities in 20 min.

The potentials of each compartment impaled, relative to the apical side of the tissue, are shown in Fig. 3. As the TEP measured varied widely among guts, and among impalements, the potentials are presented as functions of TEP. It can be seen that, for all three sites, the measured potentials correlate significantly, and approximately linearly, with gross TEP.

Extrapolation to in vivo *conditions*

The TEP across midgut *in situ* is generally agreed to be approximately 150 mV (Harvey *et al.* 1986; Dow, 1986). Given the clear relationship between polarization of the three impalement sites and TEP, it was possible to calculate *in situ* values by extrapolation. These are presented in Table 1, expressed relative to the apical side, as measured, and relative to the basal side, for comparison with previously published results (Blankemeyer & Harvey, 1978; Moffett *et al.* 1982; Moffett & Koch, 1988*a*,*b*; Thomas & May, 1984).

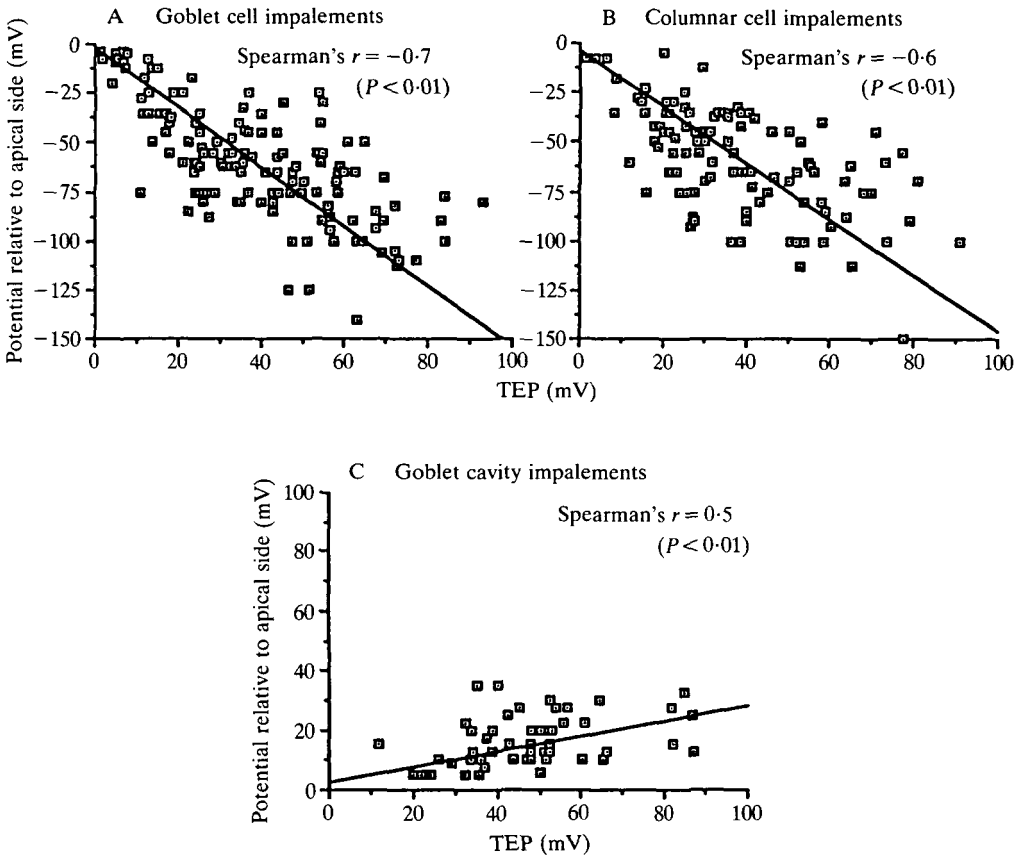

Fig. 3. Scattergrams of potential differences recorded in three sites in *Manduca* middle midgut, relative to the apical lumen, as a function of gross transepithelial potential difference (TEP). (A) Goblet cell cytoplasm (135 impalements), (B) columnar cell cytoplasm (106 impalements), (C) goblet cell cavities (53 impalements). Lines were fitted by eye; all impalements were confirmed by dye fills. Spearman's r values are shown.

Table 1. *Estimated electrical potentials of three* Manduca *middle midgut compartments relative to basal and apical sides* in vivo

Compartment	Estimated PD (mV) Relative to basal side	Relative to apical side
Goblet cytoplasm	−72	−222
Columnar cytoplasm	−80	−230
Goblet cavity	+197	+47

Table 2. *Estimated electrochemical potentials for potassium movements between* Manduca *middle midgut compartments* in vivo

Compartments	Estimated electrochemical PD for potassium (kJ mol^{-1}) Component: Chemical	Component: Electrical	Total electrochemical
Transport pathway *via* goblet cells			
Goblet cytoplasm relative to blood	+4·3	−6·9	−2·7
Goblet cavity relative to cytoplasm	−0·03	+26·0	+25·9
Apical lumen relative to goblet cavity	−0·3	−4·5	−4·8
Absorptive pathway *via* columnar cells			
Columnar cytoplasm relative to apical lumen	+0·3	−22·2	−21·9
Blood relative to columnar cytoplasm	−4·3	+7·7	+3·5
Recycling pathway from columnar to goblet cells			
Goblet cytoplasm relative to columnar cytoplasm	0	+0·7	+0·7

Calculation of electrochemical gradients

Electrochemical gradients for potassium between various midgut compartments were calculated using the following values: electrical potentials, as in Table 1; potassium activities in blood (25 mmol l^{-1}) and middle midgut contents (65 mmol l^{-1}) from Dow & Harvey (1988); and potassium activities of goblet and columnar cells (95 mmol l^{-1}) and goblet cavities (94 mmol l^{-1}) of posterior midgut from Moffett & Koch (1988*b*). The values are presented in Table 2, with chemical and electrical components of the electrochemical gradient shown separately.

All the values used in the calculation, except intracellular and cavity potassium activities, relate to middle midgut. However, the use of posterior midgut data in the calculation is justified on the following grounds: (i) the ionic environment bathing both blood and lumen sides of both middle and posterior midguts is similar (Dow & Harvey, 1988), (ii) potassium transport in the three midgut regions is similar in terms of open-circuit voltage, short-circuit current and flux ratios (Cioffi & Harvey, 1981), (iii) the potentials recorded in goblet cavities of middle midgut (this study) are similar to those published for posterior midgut by Moffett & Koch (1988*b*) and (iv) most significantly, the calculations shown in Table 2 are extremely insensitive to variations in potassium activity values, as the electrical term dominates the electrochemical PD in all cases where significant electrochemical PDs are obtained. However, these assumptions should be borne in mind when interpreting the calculations.

Calculation of Nernst potential difference for protons

The Nernst PD for protons across the goblet cavity was calculated from the maximum pH observed in midgut contents in *Manduca* (11·3 in middle midgut: Dow, 1984*b*), and an estimate of pH 7 for cytoplasmic pH. This value is justified b

the finding that intracellular pH is regulated close to pH 7 in all animal cells measured to date (Roos & Boron, 1981).

Energetics of transport

The electrochemical PD for potassium across the pump site (the goblet cavity membrane) represents the minimum work required for transport, given 100% efficiency in the pump. This allows certain estimates of pump energetics to be made (see Harvey & Zerahn, 1972; Harvey *et al.* 1981, for examples). Data from this paper, and from Moffett & Koch (1988*b*), allow these estimates to be made with greater precision than was previously possible.

The stoichiometry of the pump can be estimated by comparison of the electrochemical PD for potassium (26 kJ mol^{-1}) with generally accepted values for the free energy of ATP hydrolysis (50–55 kJ mol^{-1}), yielding a K^+:ATP ratio of 2:1.

The minimum energy expended on the pump by a 5-g larva can be calculated by multiplying the electrochemical PD (26 kJ mol^{-1}) by the estimated transport rate across the entire midgut (150 μmol h^{-1}: based on Cioffi & Harvey, 1981), yielding an expenditure of 3·9 J h^{-1}, or around 3·9/52 000 = 75 μmol ATP h^{-1}. This can be compared with the larva's gross metabolic product, calculated from the oxygen consumption of a 5-g larva at 25°C (2·85 ml h^{-1}: Reynolds & Nottingham, 1985), and a P:O ratio of 3:1 (Mandel *et al.* 1980), yielding a total ATP production rate of 832 μmol ATP $larva^{-1}$ h^{-1}. The minimum fraction of the larva's total ATP budget occupied by the K^+ pump would then be 9%.

In saline containing 32 mmol l^{-1} K^+, which resembles blood, the K^+:O ratio measured by Harvey & Zerahn (1972) in isolated *Cecropia* midgut was 1·23:1. Assuming a P:O ratio of 3:1, and that the true pump stoichiometry is 2:1, then the K^+ pump uses $1{\cdot}23\times100/(2\times3) = 21\%$ of total midgut ATP production.

Discussion

Experimental procedure

Use of the microscope configuration described here allowed easy impalement and verification of three midgut recording sites. The observation that the polarization of all three compartments correlates with TEP stresses the importance of the scatterplot approach employed in Fig. 3 for open-circuit data. Meaningful values for potentials in midgut compartments can be obtained only by standardizing to *in vivo* conditions.

Testing the model

The model for generation of high pH (Dow, 1984*a*,*b*) predicts that the PD across the apical membrane of the goblet cavity determines the distribution of protons between the goblet cavity lumen and the goblet cytoplasm according to the Nernst distribution. Furthermore, it predicts both that the function of the complex apical valve of the cavity is to isolate electrically the apical membrane, so that it is not

isopotential with the apical lumen, and that it is the potential across the goblet cavity membrane, rather than the gross TEP, which determines the maximum pH that can be generated by the midgut.

Evidence to support these predictions is now available. The goblet cavity is electrically isolated from the apical lumen, as measured by (i) its consistently positive potential relative to the apical side; (ii) its uniformly high resistance; and (iii) its tightness to Lucifer Yellow and 6-carboxyfluorescein. Additionally, it is possible to compare the polarization of the goblet cavity apical membrane with the calculated Nernst PD for protons across the membrane, by extrapolation to *in vivo* conditions. The expected polarization of the goblet cavity apical membrane *in vivo* would be +269 mV. The maximum pH measured in *Manduca sexta* midgut is 11·3 in the middle midgut (Dow, 1984*b*). The Nernst potential for protons across the goblet cavity apical membrane would then be 254 mV. This is in excellent agreement with the calculated electrical PD for the membrane. Put another way, the PD estimated across the goblet cell membrane would account for a luminal pH of up to $7+269/58 = 11{\cdot}6$.

The microelectrode technique for estimation of goblet cavity polarization almost certainly underestimates true values, as potentials recorded on impalement of high-resistance compartments, such as the goblet cavity, will be reduced by damage artefacts; the sealing of highly structured membranes around the electrode is likely to be poor. It might thus be more appropriate to consider only the steepest slope through the points obtained in the goblet cavity impalements of Fig. 3.

Potassium activity gradients in vivo

There are believed to be two main pathways for potassium movement across the midgut (Dow, 1986). In the pump pathway, potassium enters from the basal surface of the goblet (and perhaps columnar) cells, and is pumped out through the apical goblet cavity. In the leak, or absorptive, pathway potassium enters the apical side of the columnar cells together with nutrients, and leaves at the basal surface, returning to the blood. Additionally, there is thought to be recruitment of columnar cell K^+ into the pump pathway *via* gap junctional communication between the cells. The data from Table 2 are abstracted into diagrammatic form in Fig. 4, which shows the directions of hypothesized potassium fluxes across the midgut, together with the associated energy steps, in the three pathways.

These data suggest that, in the middle midgut of the living insect, basal potassium entry is energetically favourable, and so there is no need to invoke transbasal active transport (although this may occur in posterior midgut: Moffett & Koch, 1988*a*). The major energy step is across the goblet cell apical membrane, where the K^+-ATPase is located. Exit of K^+ from the goblet cavity is favoured by a small electrochemical gradient into the midgut lumen: this can be considered as the energy 'overhead' imposed on the potassium pump by the presence of the apical valve.

Potassium entry across the columnar cell apical microvilli in the absorptive

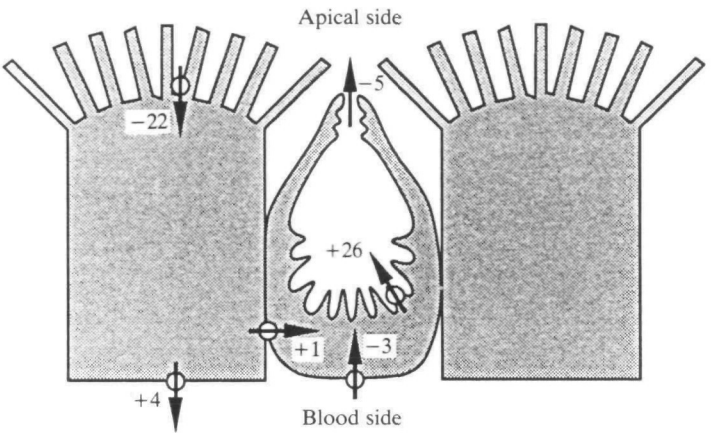

Fig. 4. Diagram of the major routes for potassium movement within *Manduca* midgut. Arrows denote the hypothesized directions of potassium movements between compartments in the pump, absorptive and shunt pathways described in the text. Figures denote estimates for the electrochemical potential difference for potassium ions (in $kJ\,mol^{-1}$) for movement between various midgut compartments in the direction of the arrows, *in vivo*. See text for a description of the assumptions underlying these data.

pathway is strongly favoured, although the electrical gradient is far larger than the chemical gradient. These data are particularly relevant to studies of amino acid uptake, which is believed to be mediated by the electrochemical PD for K^+ across the columnar cell brush border *via* a cotransport mechanism. Our estimate for this PD ($-22\,kJ\,mol^{-1}$) is somewhat larger than that of $-15\,kJ\,mol^{-1}$ in *Bombyx mori* made by Giordana *et al.* (1982). In our data, a lower chemical component is more than offset by a larger electrical component. However, the two estimates are broadly compatible.

It has often been suggested that the K^+ transport pool for the goblet cell ATPase is drawn from both goblet and columnar cells (see Dow, 1986), and microelectrode data (Moffett *et al.* 1982; this paper) implying communication between the cell types support this view. Indeed, Fig. 4 reveals that the electrochemical PD between goblet and columnar cytoplasm is virtually zero. In this context, it is interesting to note that the energetic gradient favouring K^+ entry from the apical side ($-22\,kJ\,mol^{-1}$) greatly exceeds the gradient favouring K^+ entry from the basal side ($4\,kJ\,mol^{-1}$). Rather than a recruitment of blood-side potassium to the goblet cell *via* the columnar cell basal membrane, as was historically argued in the light of the K^+ excretory hypothesis (Harvey *et al.* 1983), the dominant K^+ movement through the columnar cells may be a recycling of K^+ to the goblet cells *via* the columnar apical membrane. This idea is consistent with flux data obtained from Bt-intoxicated midgut tissue (Harvey & Wolfersberger, 1979).

Energetics of transport

It is significant that the estimate for the work needed to pump potassium across

this membrane (26 kJ mol^{-1}) is almost exactly half that of the generally accepted value for the free energy of ATP hydrolysis in cells (50–55 kJ mol^{-1}). Clearly, these data suggest a stoichiometry of $2K^+$:1ATP for the pump. Such a value has long been favoured, although the approximations by which it was reached previously were less direct (Harvey *et al.* 1981). However, it should be noted that, in the middle midgut, mitochondria actually insert into the goblet microvilli (Cioffi, 1979), drastically shortening the diffusion path for ATP and metabolites, and so the free energy of ATP hydrolysis may be markedly higher than the norm. This could have the effect of shifting the stoichiometry towards an alternative value of 3:1.

It is remarkable that this pump uses so large a fraction of the larva's gross metabolic product. Given that the total ATP production rate calculated takes no account of 'housekeeping' metabolism, it is clear that the fraction of the larva's disposable metabolic income which is expended on the midgut K^+-ATPase may be considerably higher than we have calculated. This calculation, though still in a preliminary form, bears out the assertion of Dow (1986) that midgut K^+ transport is an expensive process, and must produce tangible benefits. Such benefits might include not only the regulation of blood potassium levels (Harvey *et al.* 1983), and the absorption of nutrients (Giordana *et al.* 1982), but also the alkalinization of the midgut lumen with further attendant benefits (Dow, 1984*a*,*b*).

Comparing middle and posterior midgut

Now that microelectrode data are available for both posterior midgut (Moffett & Koch, 1988*a*,*b*), which is *not* thought to alkalinize the midgut lumen, and for middle midgut (this paper), which *is* thought to do so, it is instructive to compare them. The potentials recorded for the goblet cavity relative to the apical lumen are comparable with those of Moffett & Koch (1988*b*) in short-circuited posterior midgut. This similarity implies that the ability of the potassium pump to polarize the goblet cavity apical membrane is not the only prerequisite for the creation of an alkaline fluid. If the potassium-transporting activities are generally similar in middle and posterior midgut (Cioffi & Harvey, 1981), then where might the necessary differences lie? The goblet cells in the two regions differ morphologically (Cioffi, 1979); the goblet cells in the anterior and middle midguts have a vastly reduced cytoplasm, and their microvilli all contain mitochondria, in contrast with the goblet cells of the posterior midgut. Similarly, carbonic anhydrase, an enzyme associated with acid–base regulation in many tissues, is associated with the goblet cell apical membranes only of cells in the anterior and middle midguts; in the posterior midgut, it associates with the columnar cell apical membrane (Ridgway & Moffett, 1986). Another possibility is that the H^+ permeability of the goblet cell apical membrane in posterior midgut may be lower than that in middle and anterior midgut; in that case, although an electrical gradient for H^+ equilibration may exist, the opportunity may be reduced.

Whatever the details of the scheme for the generation of high pH that may emerge, certain salient points arise from these data: the electrical isolation of

midgut cavities precludes their role primarily as K^+ excretory cells; and the electrical, rather than the chemical, work performed by the goblet cell K^+ pump dominates its impact on midgut nutrient absorption and high-pH-generation models. Much interesting work needs to be performed to explain the roles of midgut potassium pumping, an extraordinarily energy-intensive process, in ionic homeostasis, nutrient absorption and gut alkalinization.

Part of this work was completed at the University of Cambridge. JATD is most grateful to Dr Simon Maddrell and Dr Michael Bate for the use of their laboratory facilities, and to St Catharine's College, for their support in the form of a research fellowship. Later work on this topic was supported by SERC grant GR/E 07890 and by the General Fund of the University of Glasgow. The authors are most grateful to Miss Susan Kitson for technical assistance.

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