J. exp. Biol. 108, 293–304 (1984)
Printed in Great Britain © The Company of Biologists Limited 1984

ACTIVE POTASSIUM ION TRANSPORT ACROSS THE CATERPILLAR MIDGUT

II. INTRACELLULAR MICROELECTRODE STUDIES

BY M. V. THOMAS AND T. E. MAY

Shell Research Limited, Sittingbourne Research Centre, Sittingbourne, ME9 8AG, U.K.

Accepted 16 June 1983

SUMMARY

Intracellular microelectrodes were used to record from individual cells in midguts isolated from *Spodoptera littoralis* caterpillars. Recorded potentials, referenced to the basal (haemolymph) surface, showed a bimodal distribution, with maxima in the ranges 0 to −10 and −30 to −40 mV. In experiments where the fluorescent dye Lucifer Yellow CH was iontophoresed from the recording microelectrode, fluorescence was associated with single cells only for membrane potentials more negative than −25 mV. Examination of tissue sections showed these cells to be of both columnar and goblet types, in an approximate 2:1 ratio. This conclusion conflicts with that of a previous study on other caterpillar species, in which it was concluded that the goblet cells had basal membrane potentials of only a few mV. Attempts to discriminate between the two cell types by resistance measurements were unsuccessful. The resistance values obtained were substantially higher than those in the previous study, although they are consistent with those predicted from the overall tissue resistance. The major electrical effect of potassium ion transport inhibition by 1 m-KCN was on the apical membrane, supporting the view that the potassium pump is located there. The major initial effect of potassium ion removal was on the basal membrane, which is as expected if this membrane is permeable primarily to potassium. Our inability to discriminate between goblet and columnar cells by any electrical criterion suggests that both cell types may be able to transport potassium.

INTRODUCTION

Studies of transepithelial active ion transport are complicated by the fact that the ions must pass across at least two membranes in series and different processes are almost certainly involved at the basal and apical sites. In particular, only one of the two processes needs to be energy-dependent and only passive (albeit specific) mechanisms may be required at the other membrane. The classical models for transepithelial ion transport (see Keynes, 1969 for a review) of which Koefoed-Johnson & Ussing's model for the frog skin is probably the most well known (Koefoed-Johnson & Ussing, 1958) are all of this type. Such models have provided a very useful framework for the

Key words: Potassium transport, transepithelial ion transport, caterpillar midgut.

interpretation of experimental results, but detailed tests of them, involving the use of intracellular microelectrodes to distinguish between electrical events at the basal and apical membranes, have been made difficult by the complex cellular architecture of many epithelia.

In many respects the caterpillar midgut epithelium appears to be an ideal model system for such detailed studies, as it has a very active potassium transporting system (Harvey & Nedergaard, 1964; Harvey & Zerahn, 1969, 1972) and unlike many vertebrate epithelia it consists of only a single layer of cells, so the 'two membrane' model is more likely to be a reasonable approximation in this case. A complicating factor, however, is that two distinct cell types are present (Anderson & Harvey, 1966). From their morphology, these are named goblet and columnar cells, with the goblet cells corresponding to about one-third of the tissue, both by number and area. It has been suggested on ultrastructural grounds that potassium ion transport occurs primarily *via* the goblet cells (Anderson & Harvey, 1966), and microelectrode experiments by Blankemeyer & Harvey (1978) apparently allowed the two cell types to be distinguished by electrical criteria alone. The basis for this discrimination was that both for *Hyalophora cecropia* and *Manduca sexta* midguts, microelectrode impalements from the haemolymph (basal) side of the tissue gave a frequency distribution with two major peaks, having modes at -28 and -9 mV in the case of *M. sexta*. Since the former group was several times more numerous than the latter, these authors postulated that the 'high potential difference' and 'low potential difference' recordings arose from columnar and goblet cell impalements respectively. Attempts to confirm this hypothesis for *M. sexta* by dye (Niagara Sky Blue) ionophoresis and subsequent tissue sectioning were unsuccessful, although examination of whole mounts apparently suggested that dye injected into 'high potential difference' cells appeared in the columnar cells.

These authors also made a series of cell resistance measurements under normal and anoxic conditions, and concluded that the greatest anoxia-induced resistance change occurred at the apical membranes of the 'low potential difference' cells. They therefore suggested that the active potassium ion transport system was located on these membranes, i.e. on the apical membranes of the goblet cells.

In the accompanying paper (Thomas & May, 1983), we found that the effectiveness of a variety of substances in inhibiting potassium transport in *Spodoptera littoralis* midguts was greater when they were applied on the basal (haemolymph) than when applied on the apical (lumen) side of the tissue. As discussed there, this type of observation does not necessarily provide firm evidence for the location of the active transport system. It did, however, draw to our attention the possibility that potassium transport could well be inhibited by the effects on the basal membranes (e.g. reduction of potassium permeability) even if the active transport system is confined to the apical membranes.

We intended to use intracellular microelectrodes to discriminate between basal and apical membrane effects of potassium transport inhibitors on the *S. littoralis* midgut preparations. Blankemeyer & Harvey (1978) had not investigated this species, so we felt it prudent to begin by confirming their observations on this tissue. We also attempted to identify impaled cells by ionophoresis of the fluorescent dye Lucifer Yellow CH (Stewart, 1978). The results of our experiments, reported here, suggest that Blankemeyer & Harvey's conclusions do not apply to this tissue.

MATERIALS AND METHODS

All materials and methods, in respect of experimental material, bath design, electrical recording and saline composition were exactly as outlined in the accompanying paper (Thomas & May, 1983), apart from the additional facilities and procedures necessary for intracellular microelectrode recording, dye injection and tissue preparation for microscopy. *S. littoralis* caterpillars, reared on spinach or cabbage leaves, were used for all experiments.

The high-impedance amplifier used for the microelectrode measurements was based on a previously published design (Thomas, 1977), with the addition of a standard bridge circuit to allow current to be passed through the electrode.

Cell potentials were always measured with reference to the haemolymph side of the tissue. In some experiments we wished to measure microelectrode potentials under voltage-clamp conditions. Unfortunately, the three-electrode method used to compensate the transepithelial potential measurements for voltage drops in the bath (Thomas & May, 1983, Fig. 2) cannot provide proper compensation for the microelectrode measurements, since the distance between VA and the microelectrode will be only half that between VA and VB. Although an appropriately modified three-electrode compensation system could have been used, the simpler solution of using an independent reference electrode for the microelectrode measurements was adopted. The reference electrode, which was of the same construction as the other bath electrodes, was sited about 1 mm away from the tissue, over which distance the voltage error was expected to be acceptably small.

The microelectrodes were pulled from thin-walled filament glass (Clark Electromedical Supplies, Reading, UK) and were normally filled with 3 M-KCl. Their resistances were typically 10–15 MΩ. In some experiments, the electrodes instead contained the intracellular marker dye Lucifer Yellow CH (Stewart, 1978) as a 3 % solution in 1 M-LiCl. The dye was ionophoresed into the impaled cells by 0·5 Hz hyperpolarizing current pulses of 5–10 nA amplitude. Ionophoresis was normally continued for up to 20 min, but was interrupted after a shorter time if the cell membrane potential showed a significant (>30 %) decrease. The preparations were then fixed, embedded in glycol methacrylate, sectioned and viewed in a u.v. fluorescence microscope, following the procedures described by Stewart (1978). This method allowed morphological identification of the type of cell impaled (i.e. goblet or columnar), independently of the electrical criteria proposed by Blankemeyer & Harvey (1978).

RESULTS

Microelectrode impalements and dye ionophoresis

Microelectrodes were advanced into the tissue (from the haemolymph side) until a clear potential step was seen, and the steady value of this step (normally measured after about 1 min) was recorded. A frequency distribution of the recorded potentials was compiled following the procedure used by Blankemeyer & Harvey (1978, Fig. 3) and it was based on a similarly large number of observations (242 as opposed to 227). Our results are shown in Fig. 1.

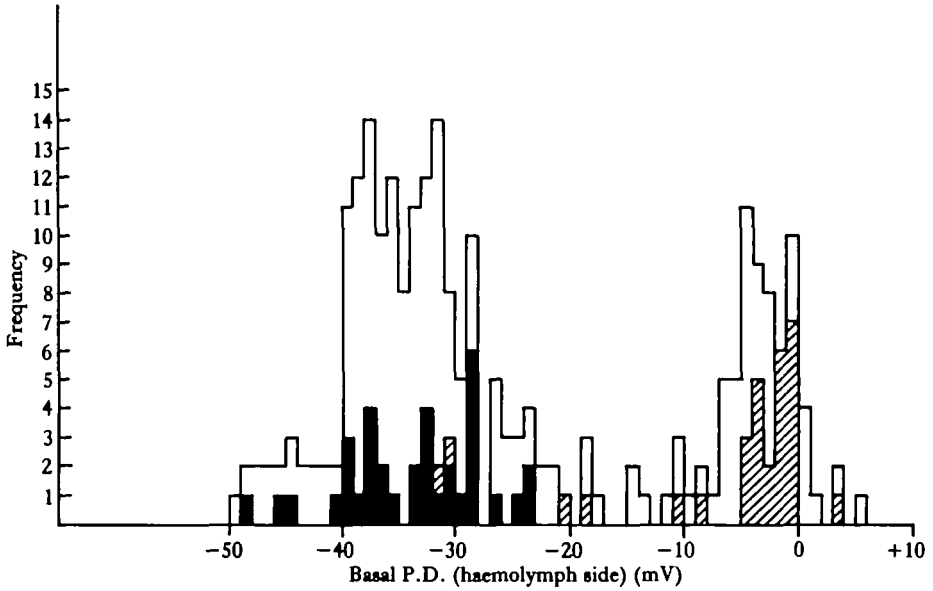
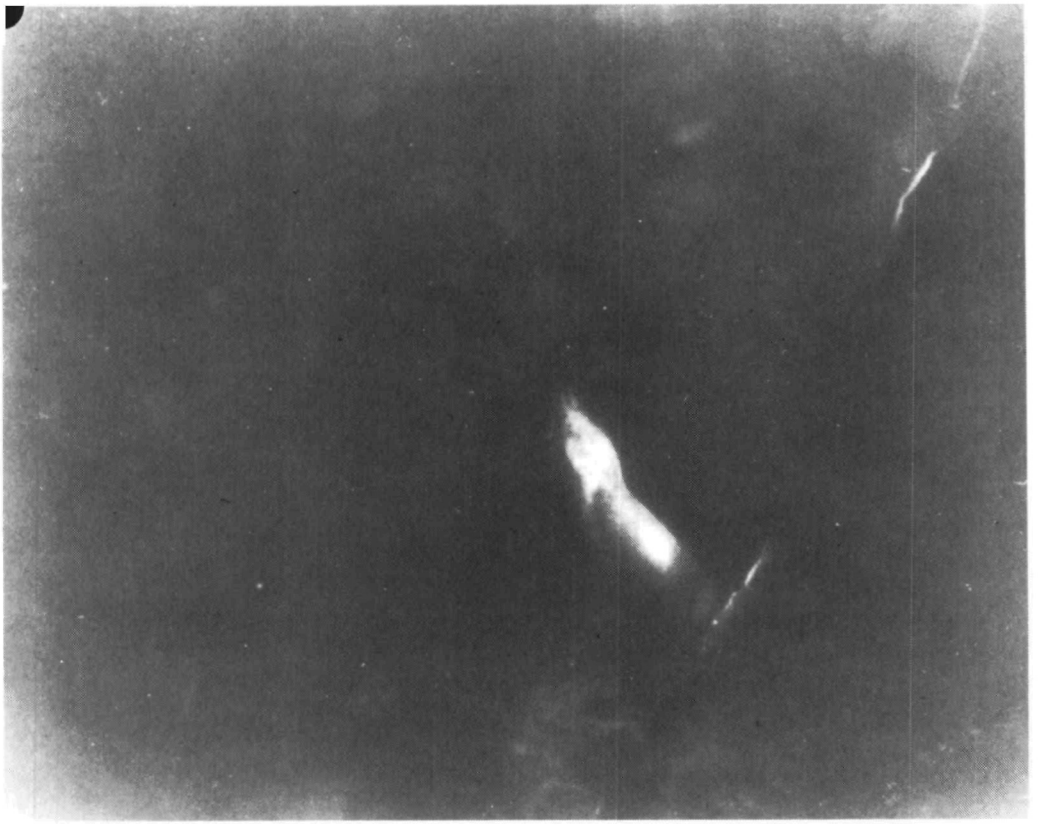


Fig. 1. Frequency distribution of 242 microelectrode impalements of *Spodoptera littoralis* midgut epithelium as a function of the potential recorded. Shaded results are from impalements for which ionophoresis of Lucifer Yellow CH was attempted; dark shading indicates those impalements for which fluorescence associated with a single cell could subsequently be observed in a whole mount of the tissue.

These results are very similar to those of Blankemeyer & Harvey, with broad maxima between 0 and −10 and −30 and −40 mV. In only two respects do they differ slightly. First, the separation between the maxima is much clearer in our experiments. Blankemeyer & Harvey obtained a third, subsidiary, peak at −18 mV in *M. sexta* midguts, which they tentatively attributed to interstitial (replacement) cells, but no such peak was observed in their experiments on *H. cecropia*. Second, the largest potentials that we recorded were some 10 mV greater (−50 mV) than in their experiments (−40 mV). Clearly, neither difference is likely to be of major significance.

However, the dye injection experiments suggested a different interpretation from that postulated by Blankemeyer & Harvey. Of the 242 impalements, ionophoresis of Lucifer Yellow CH was carried out in 65 cases, and these results are indicated by the shaded portions of Fig. 1. Heavy shading indicates those attempts (35) that resulted in clearly localized fluorescence when the whole tissue was observed in a u.v. fluorescence microscope. Cross-hatching indicates those attempts (30) in which the fluorescence was not clearly localized; in these cases the fluorescence was more diffuse and rapidly became fainter as the dye dispersed through the tissue.

Fig. 2. Fluorescence observed from two different cells in *Spodoptera littoralis* midguts, following ionophoretic injection of Lucifer Yellow CH through the recording microelectrode and tissue fixation and sectioning described in the text. In Fig. 2A the fluorescence distribution is as expected for a columnar cell, whereas in Fig. 2B it is as expected for a goblet cell. Both cells had resting potentials of about −30 mV. In each case the photograph is printed in both positive (above) and negative (below) form, to show the greatest overall detail. Magnification × 500.



2A

Intracellular measurements in voltage-clamped midguts

The previous measurements were all made on midguts maintained under open-circuit conditions, in which they generated transepithelial potentials of some 80 mV, lumen side positive (Thomas & May, 1983). For a membrane potential of −30 mV on the haemolymph side of the epithelium, the membrane potential on the lumen side would thus be −110 mV. If the transepithelial potential is forced to change, then this change will be distributed across the two membranes according to their relative resistances. For an apical (lumen-side) resistance R_A and a basal (haemolymph-side) resistance R_B, the fraction, F, of any voltage change imposed across the tissue that is recorded by an intracellular microelectrode with reference to the haemolymph side will be

$$F = \frac{R_B}{R_A + R_B}$$

The results can equally well be expressed in terms of R_A/R_B, the apical-to-basal resistance ratio, as done in analogous experiments by Blankemeyer & Harvey (1978), but we prefer to give them as the fractional voltage change, F, as that is what is actually measured.

The value of F was determined by measuring the change in microelectrode potential when the midgut was short-circuited, and dividing it by the transepithelial potential. The short-circuit current, I_{sc}, and the tissue resistance (obtained from the slope of the tissue current-voltage relation) were also measured. The results are shown in Table 1. There is a clear correlation between F and the microelectrode potential, as can be seen from Fig. 3, which also shows data obtained under normal conditions in experiments designed to investigate the effects of potassium transport inhibitors (see Table 2).

Table 1. *Results of microelectrode recordings from* Spodoptera littoralis *midguts referenced to the haemolymph (basal) surface*

	Transepithelial potential (mV)	Micro-electrode potential (mV)	I_{sc} (μA)	Tissue resistance (KΩ)	F
Preparation 1	73	−51	32	2·28	0·45
	69	−20	28	2·46	0·29
	66	−50	28	2·31	0·44
	63	−4	24	2·63	0·29
Preparation 2	91	−8	47	1·94	0·47
	89	−36	47	1·89	0·40
	90	−24	46	1·96	0·36
	89	−4	47	1·89	0·28
	89	−4	47	1·89	0·25
	89	−40	46	1·93	0·40
	88	−8	46	1·91	0·26
	88	−41	46	1·91	0·39
	89	−46	45	1·98	0·39
	90	+90	45	2·0	0·84

F is the fraction of the change in transepithelial potential that is recorded by the microelectrode when the midgut is short-circuited. The nominal tissue area is 0·03 cm^2.

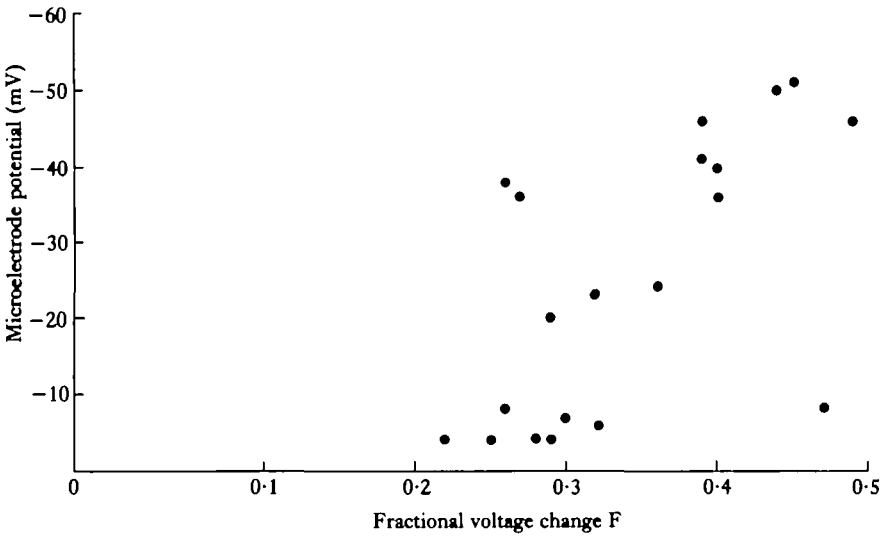


Fig. 3. Microelectrode potentials recorded from *Spodoptera littoralis* midguts, referenced to the haemolymph (basal) surface, as a function of F, the fraction of the change in transepithelial potential that is recorded by the microelectrode when the midgut is short-circuited.

No formal attempt has been made to segregate these results into 'high potential difference' and 'low potential difference' impalements, but it is clear that F is about 0·25 for 'low potential difference' impalements and 0·4 for 'high potential difference' ones. This corresponds to apical-to-basal resistance ratios of about 3 and 1·5 respectively, which are significantly different from those obtained by Blankemeyer & Harvey (1978) on *M. sexta*. There, ratios of 4·3 and 23·5 were obtained.

As a general check on the validity of these measurements, in one experiment the microelectrode was advanced completely through the tissue (final result in Table 1). It recorded the complete transepithelial potential under open-circuit conditions and a fractional voltage change, F, of 0·84 under short-circuit conditions. Considering the errors that are inherent in voltage-clamp measurements of the midgut epithelium, on account of the relatively low tissue resistance (Wood & Moreton, 1978), the small departure from unity for F is not unexpected.

Many more experiments than those shown in Table 1 and Fig. 3 would be necessary to obtain firm evidence for two populations of 'high potential difference' cells with different F values, and this possible method was not pursued further.

Thus we were unable to discriminate between goblet and columnar cell impalements by any electrical criterion. The only possible method of cell identification would have been by dye injection and tissue sectioning after the necessary experimental measurements had been made, a procedure which would have been far too difficult for routine use.

Effects of potassium ion transport inhibition on microelectrode measurements

In spite of the difficulties in interpreting the microelectrode results, a few experiments of this type were considered worthwhile. They may also give additional

Table 2. *Effects of KCN- and potassium-free salines on the electrical properties of* Spodoptera littoralis *midgut tissue*

	Experiment number	Transepithelial potential (mV)	Micro-electrode potential (mV)	I_{sc} (μA)	Tissue resistance (KΩ)	F
1 mM-KCN	1	98	−44	54	1·81	0·49
		16	−33	6	2·67	0·28
	2	99	−7	51	1·94	0·30
		27	−10	10	2·7	0·19
Zero K^+	1	58	−38	21	2·76	0·26
		30	−63	9	3·33	0·33
	2	63	−36	24·5	2·57	0·27
		33	−60	10·5	3·14	0·36
	3	95	−23	48·5	1·96	0·32
		49	−27	20	2·45	0·29
	4	96	−6	50	1·91	0·28
		48	−3	19	2·53	0·32
	5	79	−4	40	1·98	0·22
		32	−7	11·5	2·78	0·19

For each experiment, the values immediately before (above) and 1–2 min after (below) changeover to the test solution are shown. Solutions were changed on both sides of the tissue.

information that would assist in interpreting the microelectrode measurements made under normal conditions.

Two methods of inhibiting potassium transport were employed. These were exposure of both sides of the tissue to 1 mM-potassium cyanide, and replacement of potassium citrate in both bathing media by isotonic sucrose (see Thomas & May, 1983). The results are shown in Table 2.

In both cyanide experiments, exposure to cyanide produced a large fall both in transepithelial potential and in I_{sc}. There was also an approximately 50% rise in tissue resistance. The first microelectrode result is clearly a 'high potential difference' one, and the potential decreased by only 13 mV on exposure to cyanide, from −46 to −33 mV. Therefore the lumen-side membrane potential must have changed from −144 mV to −49 mV. Thus, the major effect of cyanide is apparently on the apical membrane of this cell. There was also a substantial decline in F, representing an increase in the apical-to-basal resistance ratio R_A/R_B. Since there was a rise in overall tissue resistance, the decline in F was more likely to be the result of a rise in R_A than a fall in R_B (we did not attempt to measure individual cell resistances in these experiments). This too suggests an effect at the apical membrane.

The second impalement was clearly a 'low potential difference' one, and apart from the correspondingly lower value of F, the result was not very different. However, it is consistent with the hypothesis advanced above that 'low potential difference' impalements are those of damaged cells, and this will be discussed further below.

The results of the potassium removal experiments suggest a rather different effect on the tissue. As for cyanide, there was a decline in both transepithelial potential and I_{sc}, and a rise in overall tissue resistance. Since these measurements had to be made

rather quickly, exposure to potassium-free saline was not prolonged, and accordingly its effects are probably somewhat underestimated in these experiments (see Thomas & May, 1983).

The first two results represent 'high potential difference' impalements. Although the transepithelial potential declined by 28 and 30 mV respectively, the microelectrode (basal membrane) potentials *increased* by 25 and 24 mV. This means that the calculated potentials across the apical membrane hardly changed (from −96 to −93 mV and from −99 mV to −93 mV respectively). In both experiments there was also a small increase in F, which roughly paralleled the increase in tissue resistance, tentatively suggesting that the change in F was primarily due to an increase in R_B. These experiments thus suggest that the major short-term effect of potassium removal is at the basal membrane.

The last two results represent 'low potential difference' impalements. As expected from our interpretation of these impalements, there was very little change in the membrane potential, or in F, when the midgut was perfused with potassium-free saline. The other result was for an intermediate microelectrode potential value (−23 mV) and it appears to resemble more closely those from the two 'low potential difference' than those from the two 'high potential difference' cells.

DISCUSSION

Many of our experimental results are similar to those obtained by Blankemeyer & Harvey (1978) on *H. cecropia* and *M. sexta*. They are also in full agreement with the earlier study by Wood, Farrand & Harvey (1969) on *H. cecropia*. Our interpretation of these results is, however, radically different from that of Blankemeyer & Harvey. Since the present experiments were performed on a different species, we cannot strictly claim to have disproved their interpretation. In view of the similarity of *S. littoralis* midguts to those from *H. cecropia* and *M. sexta*, with regard to both ultrastructure (F. A. Williamson & T. E. May, in preparation) and electrical properties (Thomas & May, 1983), we would, however, be surprised if the findings of the present study applied to this tissue alone.

Cell identification

The major difference is our interpretation of the distribution pattern of microelectrode potentials, shown in Fig. 1. The results of the dye ionophoresis experiments (Fig. 2) showed unequivocally that the 'high potential difference' impalements were from both columnar *and* goblet cells, the successful identifications being in the ratio 7:4. While it would be unwise to take this ratio too literally, in view of the relatively small number of cells identified, the predominance of columnar cells is expected from the larger relative contribution that they make to the total midgut area in this species (F. A. Williamson & T. E. May, in preparation), just as in *H. cecropia* (Anderson & Harvey, 1966).

The results obtained with Lucifer Yellow CH on other preparations (Stewart, 1978) suggest that this substance can cross low-resistance intercellular junctions, so would be expected to diffuse throughout the tissue if the cells were electrically

connected. Since we were able to inject single goblet and columnar cells successfully, it appears unlikely that the cells in the midgut epithelium are electrically coupled under normal conditions. This conclusion is in agreement with that of Blankemeyer & Harvey (1978), who were able to demonstrate coupling by electrical means under normal conditions.

Our inability to ionophorese Lucifer Yellow CH into single cells, following 'low potential difference' impalements, suggests these impalements correspond either to a separate electrically connected network of cells (which to us appears unlikely) or to effectively extracellular microelectrode tip locations. This second possibility includes impalements of damaged cells, which we believe to be the most probable interpretation, although there may also be others. In support of this interpretation, during the first few seconds of a 'low potential difference' impalement the potential was often seen to decline from an initially more negative value, and it is interesting that Blankemeyer & Harvey (1978) observed a similar effect (their Fig. 2). This is exactly the type of response expected from a cell that had been damaged by impalement.

Resistance measurements

The tissue resistance of *S. littoralis* midgut tissue is around 70 Ωcm^2 (Thomas & May, 1983, see also Table 2). This is close to that obtained for *H. cecropia* midguts, measured with a similar bath and recording system (Wood & Moreton, 1978), for which resistances of around 100 Ωcm^2 were obtained soon after midgut isolation from the insect.

The area occupied by a single cell (whether goblet or columnar) in the epithelium is of the order of 10 $\mu m \times 10\,\mu m$, i.e. $10^{-6} cm^2$ (F. A. Williamson & T. E. May, in preparation), which again is similar to *H. cecropia* (Anderson & Harvey, 1966). Neglecting intercellular current pathways, and assuming all cells to be of equal resistance and not electrically coupled, the resistance per cell is thus expected to be of the order of $10^8\,\Omega$. In fact this may be an underestimate, since the epithelium is somewhat folded, so it will in fact contain a proportionately larger number of cell.

A resistance measured in this way will be the sum of the apical and basal resistances, R_A and R_B, since they are electrically in series when the tissue measurements are made. In contrast, the resistance measured by an intracellular microelectrode will equal the parallel resistance of R_A and R_B, i.e. $R_A R_B/(R_A + R_B)$, since current passed through the microelectrode will be able to leave the cell through either membrane. The mean apical-to-basal resistance ratio for a 'high potential difference' impalement is about 1·5 (see Results section), which for $R_A + R_B = 10^8\,\Omega$ would give a measurement of 24 MΩ with a microelectrode.

The mean resistance that we obtained by the microelectrode technique, of 6·8 MΩ, is thus *not* excessively high by this criterion, even though it is considerably higher than Blankemeyer & Harvey's 1978 estimate of $2{\cdot}3 \times 10^5\,\Omega$ for corresponding impalements in *M. sexta*. These authors also found a much higher apical-to-basal resistance ratio of 23·5 for their 'high potential difference' impalements, which might partly account for the discrepancy, since for that ratio a cell resistance of only 4 MΩ would be predicted. However, we can offer no firm explanation as to why the two estimates of the apical-to-basal resistance ratio should differ so widely.

Effects of potassium ion transport inhibition

Our finding that the electrical effects of KCN are primarily at the apical (luminal) membrane is in agreement with the proposed location of the potassium pump, for which the primary evidence is ultrastructural (Anderson & Harvey, 1966). It also agrees with the effects of anoxia on microelectrode measurements in *H. cecropia* (Wood *et al.* 1969), where most of the potential change was found to occur at the apical membrane.

The effects of potassium-free saline, which are primarily on the basal (haemolymph) membrane, are also in agreement with the results of that study. The hyperpolarization observed at the basal membrane when the extracellular potassium concentration is reduced suggests that this membrane behaves at least partially as a potassium electrode, and also that the potassium concentration gradient across the membrane increases over at least the first few minutes after external potassium removal. Whether this reflects the existence of additional processes that act to conserve tissue potassium content under such conditions, or whether it is merely a transient phenomenon that occurs until the potassium pump has depleted the intracellular potassium content, is not clear. Radioisotope techniques would probably be required to obtain a definite answer, but this area of research is complicated by the conflicting results so far obtained from radioisotope flux measurements by different workers (e.g. Harvey & Zerahn, 1969; Zerahn, 1975; Wood & Harvey, 1975). Blankemeyer (1976) has proposed an explanation for the discrepancy, but further discussion of it is beyond the scope of this paper.

Further interpretation of all our results is made difficult by our inability to discriminate between goblet and columnar cells by any electrical criterion. Future studies may perhaps bring to light some differences between the electrical properties of these cells, but if there are any differences, they do not seem great enough to allow reliable cell identification by this means.

If their properties are indeed similar, this naturally suggests that the columnar cells may also be able to transport potassium. The major evidence for potassium transport *via* the goblet cells is ultrastructural and this route was initially suggested by Anderson & Harvey (1966) for *H. cecropia* on that basis, the major evidence being the larger number of mitochondria and smaller amount of rough endoplasmic reticulum in the goblet cells. The same applies in *S. littoralis* midguts (F. A. Williamson & T. E. May, in preparation), and so a similar interpretation here might seem attractive. However, the electrical measurements are simply not in agreement with it, and suggest that this very interesting problem should be carefully re-examined. Its satisfactory resolution must await the development of cytochemical techniques that will allow the potassium transport system to be localized at the membrane level.

REFERENCES

ANDERSON, E. & HARVEY, W. R. (1966). Active transport by the Cecropia midgut. II. Fine structure of the midgut epithelium. *J. Cell Biol.* **31**, 107–134.

BLANKEMEYER, J. T. (1976). The route of active potassium ion transport in the midgut of *Hyalophora cecropia* and *Manduca sexta*. PhD. thesis, Temple University.

BLANKEMEYER, J. T. & HARVEY, W. R. (1978). Identification of active cell in potassium transporting epithelium. *J. exp. Biol.* **77**, 1–13.

HARVEY, W. R. & NEDERGAARD, S. (1964). Sodium-independent active transport of potassium in the isolated midgut of the Cecropia silkworm. *Proc. natl Acad. Sci. U.S.A.* **51**, 757–765.

HARVEY, W. R. & ZERAHN, K. (1969). Kinetics and route of active K-transport in the isolated midgut of *Hyalophora cecropia*. *J. exp. Biol.* **50**, 297–306.

HARVEY, W. R. & ZERAHN, K. (1972). Active transport of potassium and other alkali metals by the isolated midgut of the silkworm. In *Current Topics in Membranes and Transport*, Vol. 3, (eds F. Bronner & A. Kleinzeller), pp. 367–410. New York: Academic Press.

KEYNES, R. D. (1969). From frog skin to sheep rumen: a survey of transport of salts and water across multicellular structures. *Q. Rev. Biophys.* **2**, 177–281.

KOEFOED-JOHNSON, V. & USSING, H. H. (1958). The nature of the frog skin potential. *Acta physiol. scand.* **42**, 298–308.

STEWART, W. W. (1978). Functional connections between cells as revealed by dye-coupling with a highly fluorescent naphthalimide tracer. *Cell* **14**, 741–759.

THOMAS, M. V. (1977). A microelectrode amplifier with an improved method of input capacitance neutralisation. *Med. and biol. Eng. and Comput.* **15**, 450–454.

THOMAS, M. V. & MAY, T. E. (1983). Active potassium ion transport across the caterpillar midgut. I. Tissue electrical properties and potassium ion transport inhibition. *J. exp. Biol.* **108**, 273–291.

WOOD, J. L., FARRAND, P. S. & HARVEY, W. R. (1969). Active transport of potassium by the Cecropia midgut. VI. Microelectrode potential profile. *J. exp. Biol.* **50**, 169–178.

WOOD, J. L. & HARVEY, W. R. (1975). Active transport of potassium by the Cecropia midgut; tracer kinetic theory and transport pool rise. *J. exp. Biol.* **63**, 301–311.

WOOD, J. L. & MORETON, R. B. (1978). Refinements in the short-circuit technique and its application to active potassium transport across the Cecropia midgut. *J. exp. Biol.* **77**, 123–140.

ZERAHN, K. (1975). Potassium exchange between bathing solution and midgut of *Hyalophora cecropia* and time delay for potassium flux through the midgut. *J. exp. Biol.* **63**, 295–300.