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COMPARISON OF POTASSIUM TRANSPORT IN THREE STRUCTURALLY DISTINCT REGIONS OF THE INSECT MIDGUT

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SUMMARY

1. Active potassium transport across the isolated midgut of the Tobacco Hornworm larva, *Manduca sexta*, was studied by measuring the short circuit current (I_{sc}) and unidirectional 42-potassium fluxes.

2. The midgut is composed of structurally distinct anterior, middle and posterior regions, all of which are shown to transport potassium, so that by comparing and contrasting their structural and functional properties new information on the mechanism of midgut potassium transport was obtained.

3. It has previously been shown that the potassium pump is located on the apical membrane of the goblet cell. In the anterior and middle regions of the midgut the goblet cell has a large cavity and mitochondria are closely associated with the apical membrane while in the posterior midgut the goblet cavity is much smaller, and mitochondria are not associated with the apical membrane. However, the apical membrane particles which have been implicated in active potassium transport in a number of other insect epithelia are present in all three regions. This observation suggests that the particles are a structural requirement for active transport, and that close association between mitochondria and the transporting membrane is not essential.

4. Comparison of the kinetic influx pool size and the differences in the I_{sc} decay profiles between the three midgut regions suggest that part of the influx pool is a transported pool located in the goblet cavity.

5. A new model to explain the driving force for potassium transport in the midgut is proposed, in which the rate of potassium transport controls the entrance of potassium into the cell, rather than the opposite, currently accepted view.

INTRODUCTION

Isolated midgut from the Tobacco Hornworm larva, *Manduca sexta*, actively transports potassium from the blood side to the lumen side. This tissue has been used in a number of studies as a model for transporting epithelia in insects (reviews by Harvey, 1981; Wolfersberger *et al.* 1981). The midgut epithelium is composed of two main cell types, goblet and columnar cells, arranged as a single layer. Recently it was shown that the midgut can be divided into distinct anterior, middle and posterior regions on the basis of differences in cell structure and in the pattern of folding of the epithelial sheet along the length of the midgut (Cioffi, 1979), as illustrated in Fig. 1. In this paper it will be shown that while each region of the

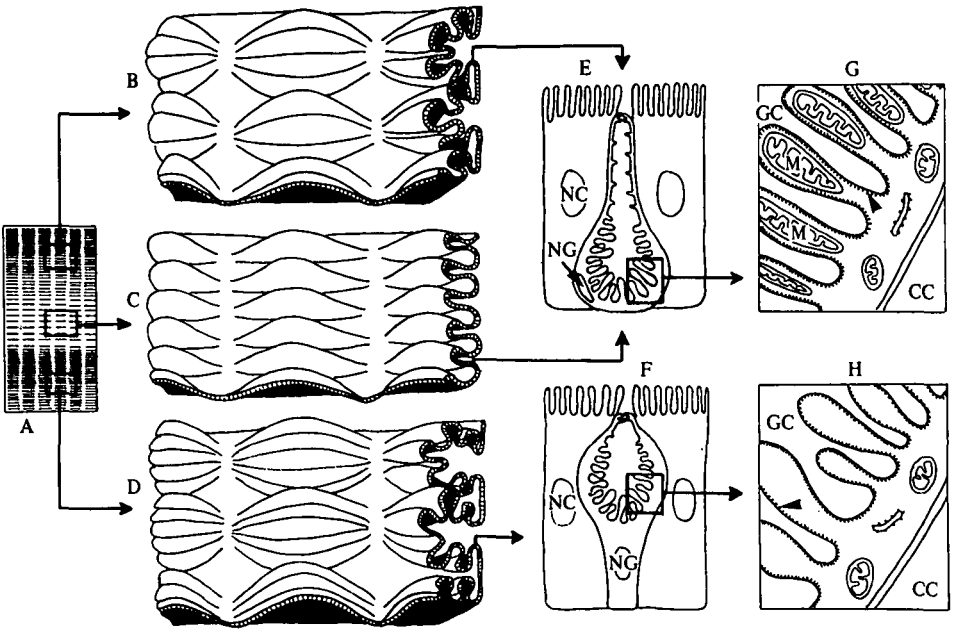

Fig. 1. Diagrammatic representation of the structural differences between the anterior, middle and posterior regions of the midgut. (A) shows an isolated midgut opened longitudinally to form a flat sheet. There are six corrugated or folded strips of tissue running parallel to each other along the length of the midgut, and separated from each other by thin areas where the tissue is not folded. The folding pattern, as seen from the blood side, is illustrated in (B) to (D). In the middle region (C) the folding pattern is simple compared to the superfolded epithelium present in the anterior (B) and posterior (D) regions of the midgut. In the anterior and middle regions the goblet cell has a large basal cavity formed by invagination of the apical membrane, which opens into the midgut lumen via a long neck closed by a valve-like structure (E). The apical membrane is thrown into projections which extend into the cavity, and each projection contains an elongated mitochondrion (G). Small particles (arrows) believed to be involved in potassium transport are present on the cytoplasmic side of the apical membrane. In the posterior region of the midgut, the goblet cavity occupies only the apical half of the cell (F) and opens through a valve directly into the midgut lumen. The cavity is separated from the blood by a long stalk of cytoplasm. Particles (arrow) are present on the cytoplasmic side of the apical membrane (H) although mitochondria are not present in the apical membrane projections. NC, nucleus of columnar cell; NG, nucleus of goblet cell; CC, columnar cell; GC, goblet cavity; M, mitochondrion.

midgut is able to carry out active potassium transport, differences in the rate of transport and in a number of transport related phenomena are observed. This adds a new dimension of interest to the midgut as a model for transporting epithelia.

In *in vitro* preparations of *M. sexta* midgut, potassium transport, as measured by the short circuit current (I_{sc}), has been reported as showing a double exponential decay time-course in which there is an initial rapid decay in the I_{sc} of about 60% during the first 30 min followed by a pseudo steady state characterized by a more gradual decay (Blankemeyer, 1976). A double exponential decay profile has also been observed in midgut preparations from *Hyalophora cecropia* (Harvey & Nedergaard, 1964) and *Antheraea pernyi* (Wood, 1972). For each of these species it has been demonstrated that the net flux measured by ^{42}K or ^{86}Rb tracers is in close agreement

with the I_{sc} in the pseudo steady state (Harvey & Nedergaard, 1964; Harvey, Haskell & Zerahn, 1967; Harvey & Wolfersberger, 1980; Wood, 1972).

A variety of evidence has been presented to show that the potassium pump in the Lepidopteran larval midgut is located on the apical membrane of the goblet cell as follows. (*a*) *Ultrastructural.* The first description of the structure of the midgut was provided for *H. cecropia* (Anderson & Harvey, 1966). A similar structure is found in the midguts of *A. pernyi* (Wood, 1972), *Ephestia kuhniella* (Smith *et al.* 1969), *Bombyx mori* (Akai, 1969) and the anterior and middle regions of the midgut in *M. sexta* (Cioffi, 1979). The apical membrane of the goblet cell is invaginated to form a large cavity, and is thrown into numerous projections which extend into the goblet cavity. Each of these projections contains an elongated mitochondrion which is closely associated with small (10–15 nm) particles studding the cytoplasmic side of the apical membrane. These particles may be part of the potassium transport mechanism, the mitochondria being present as a potential energy source for active transport (Mandel *et al.* 1980*a*, *b*). Similar particles in close association with mitochondria have been implicated in active transport in a number of other insect epithelia (Gupta & Berridge, 1966; Berridge & Oschman, 1969; Oschman & Wall, 1969; Noirot & Noirot-Timothée, 1970; Harvey, 1980). (*b*) *Kinetic influx pool size.* The potassium pool has been shown to be small under open circuit conditions, and large in diet fed larvae under short circuit conditions, when the goblet and columnar cells are coupled (Blankemeyer, 1976). Since the cytoplasmic volume of the goblet cell is small compared to that of the columnar cell, this result suggests that the influx pool is in the goblet cell cytoplasm under open circuit conditions, but that the columnar cell cytoplasm can be included as part of the influx pool when the cells are coupled. (*c*) *Microelectrode impalements.* These studies have shown that the apical membrane of one type of midgut cell, identified by statistical means as the goblet cell, has an electrical resistance which increases when the pump is stopped in anoxia (Blankemeyer, 1976; Blankemeyer & Harvey, 1978), thus further implicating the goblet cell apical membrane as the site of active potassium transport.

In the present paper, comparison of the structural and functional differences between the anterior, middle and posterior regions of the midgut of *M. sexta* provides a new method for studying midgut potassium transport. In all the experiments carried out previously, the region of the gut being investigated was not known, or at least was not specified. In *M. sexta* the degree of epithelial folding is greater in the anterior and posterior regions of the midgut as compared to the middle region. In the anterior and middle regions, the goblet cavity occupies more than 90% of the goblet cell volume, as opposed to about 50% in the posterior region, and there are no mitochondria associated with the apical membrane particles in the posterior region goblet cells (see Fig. 1).

MATERIALS AND METHODS

M. sexta larvae were purchased from Carolina Biological Supply Co., and reared on the artificial diet supplied. Feeding fifth instar larvae, weighing between 4 and 6 g, were used in these experiments, since insects weighing over 6 g were found to be less

stable due to the approaching larval–pupal moult. Each larva was immobilized in crushed ice for 20 min, then the head and last two segments of the larva were cut off and the body wall opened by cutting along the dorsal midline. The edges of the body wall were pulled back and the tracheae were cut as close as possible to the midgut, which could then be removed from the larva. A longitudinal cut was made along the ventral midline to open the midgut into a flat sheet, and the peritrophic membrane and gut contents were removed with forceps. The anterior, middle or posterior region of the midgut was isolated and mounted as a flat sheet in the chamber described by Wood and Moreton (1979). Each half of the chamber was washed for 30 s with oxygenated saline containing 32 mM-KCl, 1 mM-$CaCl_2$, 1 mM-$MgCl_2$, 5 mM-Tris HCl and 166 mM-sucrose at pH 8·3. The tissue was then maintained under short circuit conditions and the I_{sc} recorded throughout the experiment. The flux measurements were begun after the tissue had been on the chamber for 30 min and had therefore entered the pseudo steady state characterized by a slow decay rate. ^{42}K (New England Nuclear) was added to one side of the chamber and 1 ml samples were removed at regular intervals from the opposite side of the chamber and replaced with fresh bathing solution. A 0·1 ml standard was taken from the hot side and diluted to 1 ml with bathing solution. The samples were counted in a Packard Tri-Carb liquid scintillation counter using the Cerenkov effect. In each experiment either an influx (blood side to lumen side) or an efflux (lumen side to blood side) was measured. It was considered inadvisable to carry out more than one experiment on each piece of tissue, since the passive flux has a tendency to increase as a result of tissue damage which occurs when the tissue has been in the chamber for long periods of time. A total of 18 experiments were carried out consisting of three influx and three efflux measurements for each region of the midgut. At the end of each experiment the chamber was drained and the tissue covering the 0·5 cm^2 aperture was cut out. The tissue was washed for 10 s, blotted dry and then weighed. The influx and efflux were calculated, and the kinetic influx pool size was determined using the method described by Wood and Harvey (1975).

RESULTS

The following parameters were measured or calculated for each midgut region:

1. Tissue wet weight per unit area.
2. The time course of the I_{sc}.
3. The specific rate of potassium transport (I_{sc} and K flux per cm^2 and per mg wet weight).
4. The flux ratio (influx/efflux).
5. The kinetic influx pool size.

Each region of the midgut was able to actively transport potassium, but differences in all of these parameters were found between the three regions.

Table 1. *The wet weight of 0·5 cm² of tissue from different regions of the midgut*

(Mean ± 1 S.E.M. (n = 6))

Region of midgut	Wet wt. (mg)
Anterior	34 ± 4
Middle	26 ± 4
Posterior	56 ± 4

Tissue wet weight

The mean wet weights obtained for 0·5 cm² of midgut tissue removed from the chamber at the end of each experiment are shown in Table 1. Per unit area, the thickly folded posterior region is about twice as heavy as the thin middle region, while the anterior region is intermediate between these two.

Time course of the I_{sc}

For each region of the midgut the I_{sc} decays as a double exponential function of time, with a rapid initial decay followed by a slower decay phase. The mean I_{sc} decay profiles are shown in Figs. 2 and 3. The half time of each decay phase and the percentage of the initial I_{sc} which decays during each period were calculated from these curves and are shown in Table 2. Variations in the half time of the rapid exponential and the percentage of the initial I_{sc} which decays during this period were found between the anterior, middle and posterior regions of the midgut. The rapid decay phase for the anterior and posterior regions has a half time of 14 min compared to 7 min for the middle region. However, in the anterior and middle regions this decay represents 72% and 71% of the initial I_{sc} compared to only 38% for the posterior region. The half time of the slow decay phase is similar for all three regions.

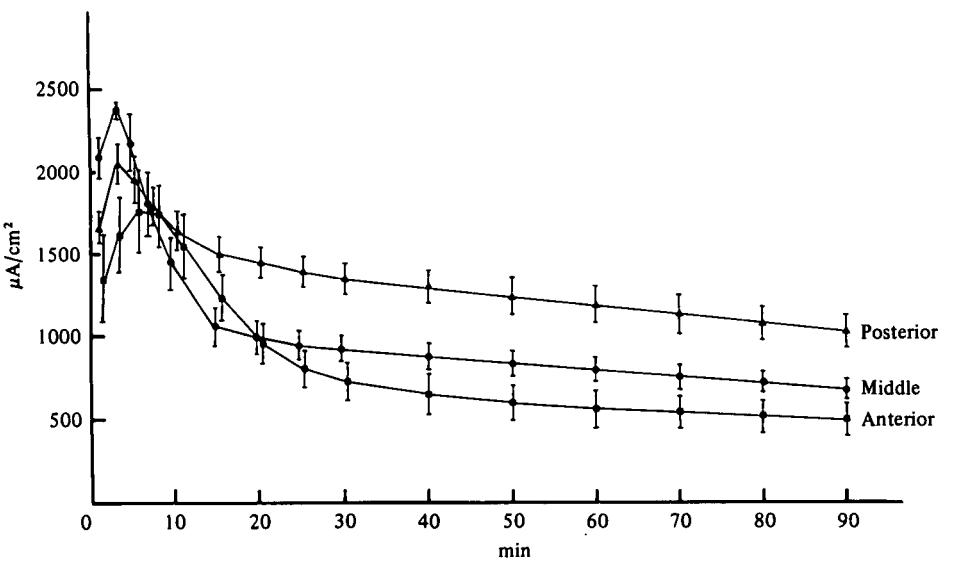

Fig. 2. The short circuit current plotted as a function of tissue area for each midgut region. (Bars represent S.E., n = 6.)

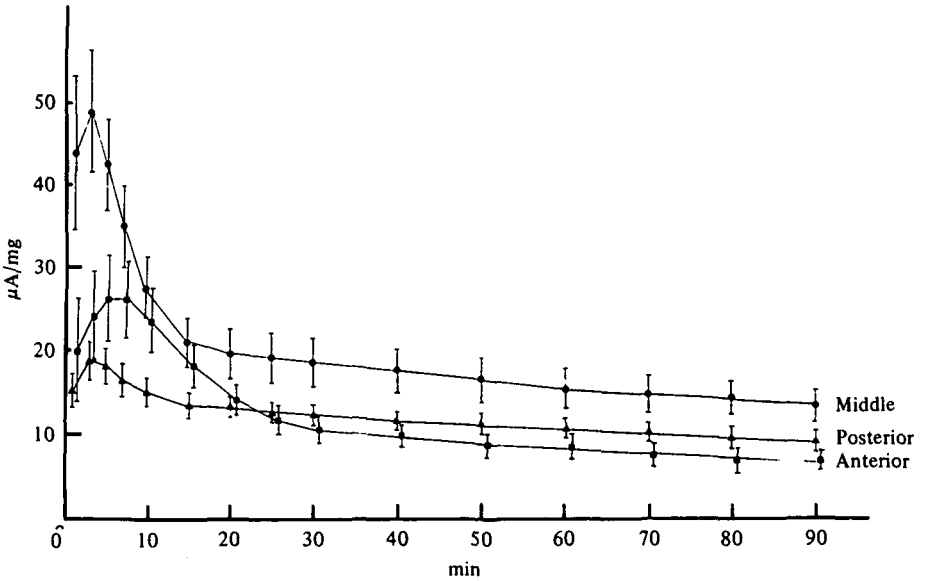

Fig. 3. The short circuit current plotted as a function of tissue wet weight for each midgut region. (Bars represent S.E., $n = 6$.)

Table 2. *The half times for each exponential of the mean I_{sc} decay curves shown in Fig. 2, and the percentage of the initial I_{sc} which decays during each phase*

	Rapid decay phase		Slow decay phase	
Region of midgut	Half time (min)	Initial I_{sc} (%)	Half time (min)	Initial I_{sc} (%)
Anterior	14	72	180	28
Middle	7	71	174	29
Posterior	14	38	151	62

Specific rate of potassium transport

The I_{sc} decay profiles illustrated in Fig. 2 show that when the I_{sc} is plotted as a function of tissue area, the posterior region has the highest transport rate and the anterior region the lowest. The number of goblet cells relative to columnar cells is similar in all three regions. However, because of the thick folding of the epithelium in the posterior region, and to a lesser extent in the anterior region, there are more cells per unit area in these two regions than in the middle. Therefore, to get a better impression of the net transport per cell, the I_{sc} can be expressed as a function of tissue wet weight, with the result that cells in the middle region now have the highest transport rate (Fig. 3). To show that the I_{sc} is a valid measure of potassium transport under these experimental conditions, the net potassium flux (influx–efflux) was calculated for each region of the midgut (Table 3). The data obtained from a typical influx experiment are shown in Fig. 4. It can be seen that while the I_{sc} curve is smooth, the data points on the influx curve are considerably scattered. These fluctuations were observed in every experiment, both influx and efflux, and have als

Table 3. *Relationship between the I_{sc} and the net K flux for each region of the midgut*

(All values are presented ±S.E.M. (n = 3))

Region of midgut	Influx		Efflux		Net flux		I_{sc}	
	μA/cm²	μA/mg	μA/cm²	μA/mg	μA/cm²	μA/mg	μA/cm²	μA/mg
Anterior	561±92	7·1±0·4	57±8	0·9±0·2	504±92	6·2±0·5	484±127	6·1±1·2
Middle	1044±155	18·7±4·0	61±13	1·3±0·4	983±156	17·4±4·0	907±76	16·9±4·8
Posterior	1156±123	10·0±0·8	32±9	0·3±0·1	1124±123	9·7±0·8	1159±102	10·0±0·7

The mean I_{sc} values were obtained from the influx experiments using the I_{sc} at 30 min after addition of ^{42}K. The mean influx and efflux values were obtained from the average flux calculated for the interval of 20–40 min after addition of ^{42}K. The isotope was added 30 min after the tissue was mounted on the chamber.

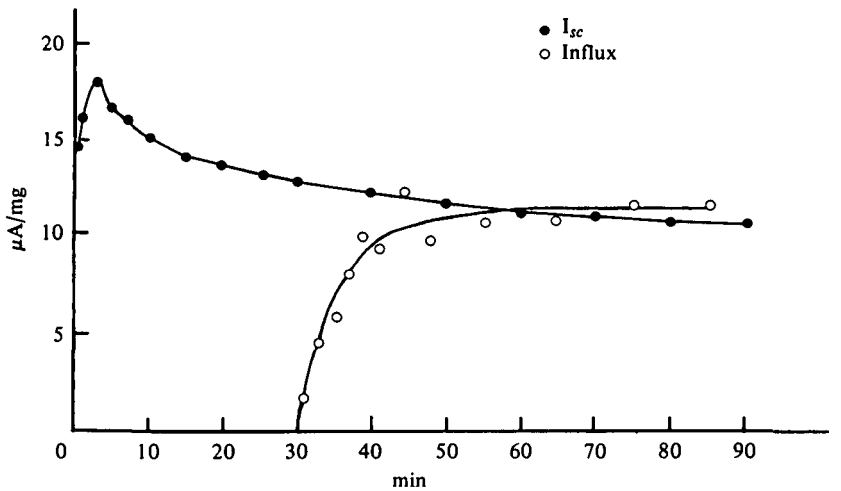

Fig. 4. Data from a typical influx experiment for the posterior region of the midgut.

been noticed by other workers (J. L. Wood, M. G. Wolfersberger, personal communication). Since similar fluctuations in the I_{sc} decay profile are not observed, this problem has previously been ignored, and assumed to be due to experimental error. In a control experiment, isolated basement membrane from which the cells had been digested off was prepared by mounting tissue on the chamber in the usual way, then adding the gut contents of a freshly dissected larva to the lumen side bathing solution. After 30–60 min the chamber was drained, the cells carefully scraped off to leave the basement membrane and muscle layer, and the chamber refilled. Influx and efflux measurements were made across these basement membrane preparations, but no fluctuations were observed during these experiments. This showed that the large scatter of the data points on the flux curves for midgut tissue are not due to inadequacies in the experimental technique such as insufficient stirring, or to sampling error, but are a property of the midgut cells under these experimental conditions. This point will be discussed in more detail later, but because of the scatter of the data points the fluxes are shown in Table 3 as an average value over the interval of 20–40 min after addition of the isotope while the I_{sc} is shown at 30 min after addition of the isotope (i.e. 60 min after the start of the experiment).

Table 4. *The flux ratio for each region of the midgut*

(Data presented ±S.E.M. (n = 3))

Region of midgut	Flux ratio (influx/efflux)
Anterior	13·4±4·7
Middle	14·0±4·3
Posterior	43·2±8·4

Table 5. *The 75 % mixing time (t_{75}) and the kinetic influx pool size for each midgut region*

(Data presented ±S.E.M. (n = 3))

		Kinetic influx pool size	
Region of midgut	t_{75} min	μ-equiv/cm²	μ-equiv/mg
Anterior	6·7±0·4	1·8±0·3	0·024±0·003
Middle	4·8±0·6	2·4±0·5	0·044±0·006
Posterior	7·3±0·3	3·8±0·5	0·034±0·003

Flux ratio

The flux ratio was calculated for each midgut region (Table 4) using the efflux experiments, in which both the efflux and the I_{sc} were measured. Since the I_{sc} is a good measure of the net potassium flux (Table 3), the influx in each experiment was assumed to be equal to the I_{sc} plus the efflux. In the posterior region of the midgut the flux ratio is about three times greater than that of the anterior or middle region.

Kinetic influx pool size

The kinetic influx pool size for each midgut region (Table 5) was calculated according to Wood and Harvey (1975, 1979) from the equation:

$$S = \frac{\mathcal{J}\infty}{\alpha}$$

where S is the pool size, $\mathcal{J}\infty$ is the steady state influx and α is the time-constant for mixing. The pool size is related to the 75 % mixing time by:

$$S = \frac{\mathcal{J}\infty\, t_{75}}{\ln 4}.$$

The mixing time is faster for the thin middle region than the thicker anterior and posterior regions (Table 5). The pool size per cm² of tissue is largest for the posterior region and smallest for the anterior region, but the pool size per mg wet weight of tissue is largest for the middle region, while the anterior region still has the smallest pool. In this respect, the size of the kinetic influx pool seems to follow the same pattern as the rate of potassium transport (see Figs. 2 and 3 and Table 3).

DISCUSSION

The observation that all three midgut regions are able to actively transport potassium can be correlated with structural differences between anterior and posterior type goblet cells. Blankemeyer and Harvey (1977, 1978) suggested that the route of potassium transport is through the goblet cells, involving passive diffusion across the basal membrane into a pool in the goblet cell cytoplasm. The active step would occur at the goblet cell apical membrane, using energy provided by the mitochondria associated with this membrane, so that potassium is transported into the goblet cavity and eventually released into the midgut lumen. According to this model, the posterior region of the midgut should be unable to transport potassium as well as the other midgut regions. The goblet cavity is separated from the blood by a long stalk of cytoplasm through which the potassium must diffuse, and there are no mitochondria associated with the goblet cell apical membrane projections, although the particles which have been implicated in active transport in a number of other insect epithelia are present. The results presented in this study show that the posterior region of the midgut is able to transport potassium very well, suggesting that close association between mitochondria and the transporting membrane is not essential. This is not meant to imply that the transport does not require mitochondrial ATP, but it does suggest that diffusion of ATP, ADP and Pi between mitochondria and plasma membrane is fast enough to meet transport requirements. A similar situation is found in the potassium transporting salivary glands in *Calliphora erythrocephala* (Oschman & Berridge, 1970) although in this case direct measurement of active potassium transport by flux measurements in short circuited tissue is lacking. In salivary gland cells, the apical membrane is invaginated to form canaliculi, which may penetrate almost to the base of the cell. The entire surface of this membrane is thrown into folds or leaflets, the cytoplasmic sides of which are studded with small particles similar to those found on the goblet cell apical membrane, and while mitochondria are present in the cells, there is no particularly close association between mitochondria and the particles. It has frequently been suggested that these particles are the unit of active transport in insects (review by Harvey, 1980) and there is already some evidence to support this idea. Using homogenates of posterior midgut, fractions were prepared by ultracentrifugation and examined by electron microscopy. The goblet cell apical membrane can be recognized by the particles present on this membrane, and a fraction enriched in goblet cell apical membrane has been prepared (M. G. Wolfersberger and M. Cioffi, unpublished data). This fraction contains a potassium modulated ATPase, the specific activity of which is 6- to 7-fold greater than in the crude homogenate (Wolfersberger, 1979). Therefore from the evidence available at this time, it seems that the presence of particles on the transporting membrane is an essential requirement for active potassium transport in insects, and that while ATP is necessary as an energy source (Mandel, 1980*a*, *b*), a close association between mitochondria and the transporting membrane is not essential. In the anterior and middle regions of the midgut, where this close association does occur, it is possible that this organization is not required for potassium transport but is necessary for a process which does not occur in the posterior

region of the midgut. One explanation is that the mitochondria may be involved in maintaining the high alkalinity of the midgut contents in the anterior and middle regions, which have an average pH of about 10, as compared to 8·5 in the posterior region (see review by Harvey, 1980).

As far as analysis of the differences in various transport properties between the three midgut regions is concerned, the midgut can be considered as being composed of three different epithelia: a super-folded epithelium with large goblet cell cavities (anterior), a simply folded epithelium with large goblet cell cavities (middle) and a super-folded epithelium with small goblet cell cavities (posterior) (see Fig. 1). A simplistic approach would be to assume that the parameters which are similar in the anterior and posterior regions but not the middle region are related to the folding pattern, while the parameters similar in the anterior and middle regions but not the posterior region are related to the type of goblet cell present. Thus the observation that the middle region of the midgut has the highest rate of potassium transport per mg of tissue (Figs. 2, 3 and Table 3) could be explained by the folding pattern of the midgut epithelium, since the simple folding in the middle region would allow more efficient mixing of potassium between folds than would the superfolding in the anterior and posterior regions. The more efficient mixing would in turn allow quicker replenishment of potassium in the 'input' unstirred layer and more rapid release from the 'output' unstirred layer, thereby allowing a higher rate of transport per cell in the middle region. However, the posterior region of the midgut has a flux ratio about three times greater than the anterior and middle regions (Table 4), primarily because the efflux in the posterior region is very low. The low efflux in the posterior region is not due to the thicker tissue being less sensitive to mechanical damage and therefore less leaky, since this should also be true for the anterior region. Thus it would seem that the low efflux in the posterior region is probably due to the different type of goblet cell found in this part of the midgut.

Analysis of the differences in the I_{sc} decay profile and kinetic influx pool size between isolated anterior, middle and posterior midgut tissue allow certain hypotheses to be put forward concerning the mechanism of midgut potassium transport and the location of the kinetic influx pool. Wood (1972) suggested that the initial rapid decay phase of the I_{sc} does not represent a decrease in the ability of the midgut to transport potassium, but is due to loss of potassium from the tissue after it is mounted in the chamber. Zerahn (1975) found that there was no change in tissue potassium concentration during the course of an experiment, but since he apparently allowed the tissue to equilibrate for about 15 min before starting to take measurements, the rapid decay phase could have been missed. In leaf-fed *H. cecropia*, the potassium concentration of the blood is 32 μequiv/g, that of the tissue is about 100 μequiv/g, while that of the gut contents is as high as 280 μequiv/g (Harvey *et al.* 1975). It is known that midgut tissue can be loaded with potassium from the blood side but not from the lumen side (Harvey & Zerahn, 1969), and since the midgut lacks a sodium-potassium pump (Jungreis & Vaughan, 1977), it is difficult to explain why the concentration of potassium in the tissue is so much higher than the blood. In addition, no satisfactory explanation has so far been put forward as to how potassium leaves the goblet cavity *in vivo*. One possibility is that a high

ncentration of potassium is maintained in the goblet cavity, allowing diffusion to take place from the cavity to the midgut lumen, and also accounting for the high tissue potassium concentration. Therefore, when the midgut is removed from the larva and placed in 32 mM-KCl, potassium stored in the goblet cavity would be lost as the tissue adjusts to its new environment. The results shown in Table 2 are consistent with this model. The half time of the rapid decay phase is long in the thickly folded anterior and posterior regions of the midgut and short in the thin middle region. Such a result would be predicted if potassium were being lost from the tissue, since this process would take place more slowly from a thick piece of tissue than a thin one. However, this should make no difference to the percentage of tissue potassium which is lost, which should depend on the relative size of the pool from which potassium is being lost. From Table 2 it can be seen that the percentage of the initial I_{sc} which decays during the rapid decay phase is large for the anterior and middle regions of the midgut, both of which have the anterior type goblet cells, and small for the posterior region. In the anterior region, the goblet cavity is about twice the size of the cavity in the posterior type cells, while the goblet cell cytoplasm occupies a larger proportion of the goblet cell volume in the posterior region. Therefore the differences in cell structure and I_{sc} decay profiles between the three midgut regions are consistent with the suggestion that the rapid decay phase of the I_{sc} is due to potassium previously transported into the goblet cavity *in vivo* being released into the bathing solution. At the present time it is not clear how loss of tissue potassium would give rise to a short circuit current, but a potential difference between the cavity and the bathing solution would certainly occur if potassium has a higher mobility than its neutralizing ion.

One of the implications of the discussion so far is that there is a transported pool of potassium in the goblet cavity, and this idea is supported by a comparison of influx kinetics between the three midgut regions. It has been shown previously that in short circuited midguts from diet fed larvae, 75% of the influx pool is in the columnar cells and 25% is in the goblet cells (Blankemeyer, 1976). Using the results described in this paper, three main lines of argument will be presented to show that most of the goblet cell pool is not intracellular, as previously believed (Blankemeyer, 1976; Blankemeyer & Harvey, 1977, 1978; Wood & Harvey, 1975, 1979), but is a transported pool located in the goblet cavity. (1) The 75% mixing time is fast for the thin middle region and slow for the thicker anterior and posterior regions (Table 5), a result which would be expected whatever the location of the influx pool. The pool size per cm^2 of tissue should show the same pattern if the pool is intracellular, in that the thicker tissue should have the largest pool. However, while anterior and middle midgut have the same type of goblet cells, the thicker anterior region has a considerably smaller pool size than the thin middle region, making a cytoplasmic location for both goblet and columnar cell pools unlikely. (2) When the pool size is calculated per mg wet weight of tissue (which is a closer estimation of pool size per cell), the middle region of the midgut has the largest kinetic influx pool. However, the posterior region goblet cells have a larger proportion of cytoplasm than the middle region goblet cells, again making a cytoplasmic location unlikely. (3) Finally, if the size of the influx pool (Table 5) is compared with the rate of potassium transport

for each midgut region (Figs. 2 and 3 and Table 3) it can be seen that the differences between the three regions follow the same pattern, so that the pool size increases with the rate of transport. For the pool size to increase, either its volume or its concentration must increase. It is unlikely that the pool volume could depend on the transport rate, but if the concentration of potassium in the pool is to change then at least part of the pool must be after the pump. We can exclude the possibility that this pool is in the unstirred layer, because then the thin middle region would be expected to have a smaller pool than the anterior region. Therefore the evidence suggests that at least part of the kinetic influx pool is a transported pool located in the goblet cavity. Incidentally, this hypothesis explains Blankemeyer's (1976) finding that when guts from leaf-fed larvae (in which the goblet and columnar cells are never electrically coupled) are short circuited, the size of the kinetic influx pool increases. This result could not be explained in terms of a cytoplasmic pool, but since short circuiting allows the transport rate to increase by eliminating the transepithelial potential difference, an increase in the influx pool size could now be predicted.

The existence of a large potassium pool in the goblet cavity may also help to explain the peculiar fluctuations observed during influx and efflux measurements in the absence of similar fluctuations in the I_{sc} decay profile. While the tissue is on the chamber it can be seen to contract periodically, a phenomenon which has been observed by other workers (J. L. Wood, M. G. Wolfersberger, personal communications). These contractions may be forcing out the contents of the goblet cavity, releasing KCl into the bathing solution, which would cause a sudden increase in the potassium concentration of the lumen side bathing solution but would not register as a current. The potassium concentration of the next sample would then be too low as the cavity refills. Similarly, sudden changes in concentration in the goblet cavity would cause fluctuations in the samples taken for efflux measurements.

If it is true that most of the goblet cell pool is located in the cavity and not the cytoplasm, as the evidence now suggests, then a new model can be proposed relating to the driving force for potassium transport across the midgut epithelium. Rather than passive diffusion into a large cytoplasmic pool from which potassium would be transported into the goblet cavity, it now seems more likely that potassium enters the cell by a form of electrostatic coupling, with potassium ions moving into the cell to replace those transported, thus maintaining a constant cellular potassium concentration. Therefore the entrance of potassium into the cell would be controlled by two factors: the rate of transport and the concentration of potassium in the blood side bathing solution. The main difference between this and the previous model is that rather than the 'input' to the pump controlling the 'output' it now seems more probable that the 'output' controls the 'input'.

In summary, comparison of the transport properties of the three midgut regions in relation to their structural differences has allowed some new ideas to emerge on the mechanism of potassium transport which were not deducible from the information previously available. In addition, the comparative approach has suggested a number of new experiments on this subject plus a method for testing hypotheses using one region of the midgut as a control for the others.

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