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ACTIVE TRANSPORT OF POTASSIUM BY THE CECROPIA MIDGUT

VI. MICROELECTRODE POTENTIAL PROFILE

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INTRODUCTION

Potential differences can only appear across barriers with high electrical resistance. Therefore it should be possible to locate the potential produced by an electrogenic pump across such high resistance barriers by using intracellular microelectrodes. An opportunity to locate such a pump is afforded by the electrogenic active transport of K^+ across the midgut of *Hyalophora cecropia* (Harvey, Haskell & Nedergaard, 1968).

The structure of the midgut encourages the attempt to locate the electrogenic pump. Two types of cell, columnar cells (65 μ tall) and goblet cells (35 μ tall) are arranged in a single layer one cell thick to make up the epithelium. Its basal surface is covered by a basement lamina (10 μ thick), surrounded by an inner sheet of circular muscles and outer bundles of longitudinal muscles, and supplied with tracheoles (Anderson & Harvey, 1966). Presumably the midgut potential is generated by the epithelial cells because they form the only continuous cellular layer in the midgut. The first step in locating the potassium pump is to determine where within the epithelial cells the electrogenic potential appears.

METHODS

Midgut preparation

Midguts were removed from mature fifth-instar larvae of *Hyalophora cecropia* (L.) using the techniques of Nedergaard & Harvey (1968). A gut was tied in place with nylon threads in one of the two types of chamber illustrated in Fig. 1. The tissue was mounted either as a cylinder across the 1 cm. gap between two opposing sections of glass tubing (Fig. 1 *B*) or as a flat sheet across the 1 cm. diameter opening of a glass vessel (Fig. 1 C). The midgut tissue separated two bathing solutions: one in the outer compartment which normally bathed the blood-side of the midgut and one in the inner compartment which bathed the lumen surface.

The standard bathing solution (32-S) contained 32 mM-K^+ plus other ions (Harvey & Nedergaard, 1964). Its composition and that of test solutions in which the potassium concentration was changed are listed in Table 1. High oxygen tensions were normally maintained by circulating the blood-side solution with oxygen. In some experiments the oxygen tension was reduced to about 1 % of atmospheric pressure by using nitrogen to circulate this solution.

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Table 1. *Composition of bathing solutions (mM)*

	2-$KHCO_3$	2-S	32-S	73·5-S	95-S
KCl	0	0	30	71·5	93
$KHCO_3$	2	2	2	2	2
NaCl	0	30	0	0	0
$CaCl_2$	0	5	5	5	5
$MgCl_2$	0	5	5	5	5
Sucrose	256	166	166	83	40

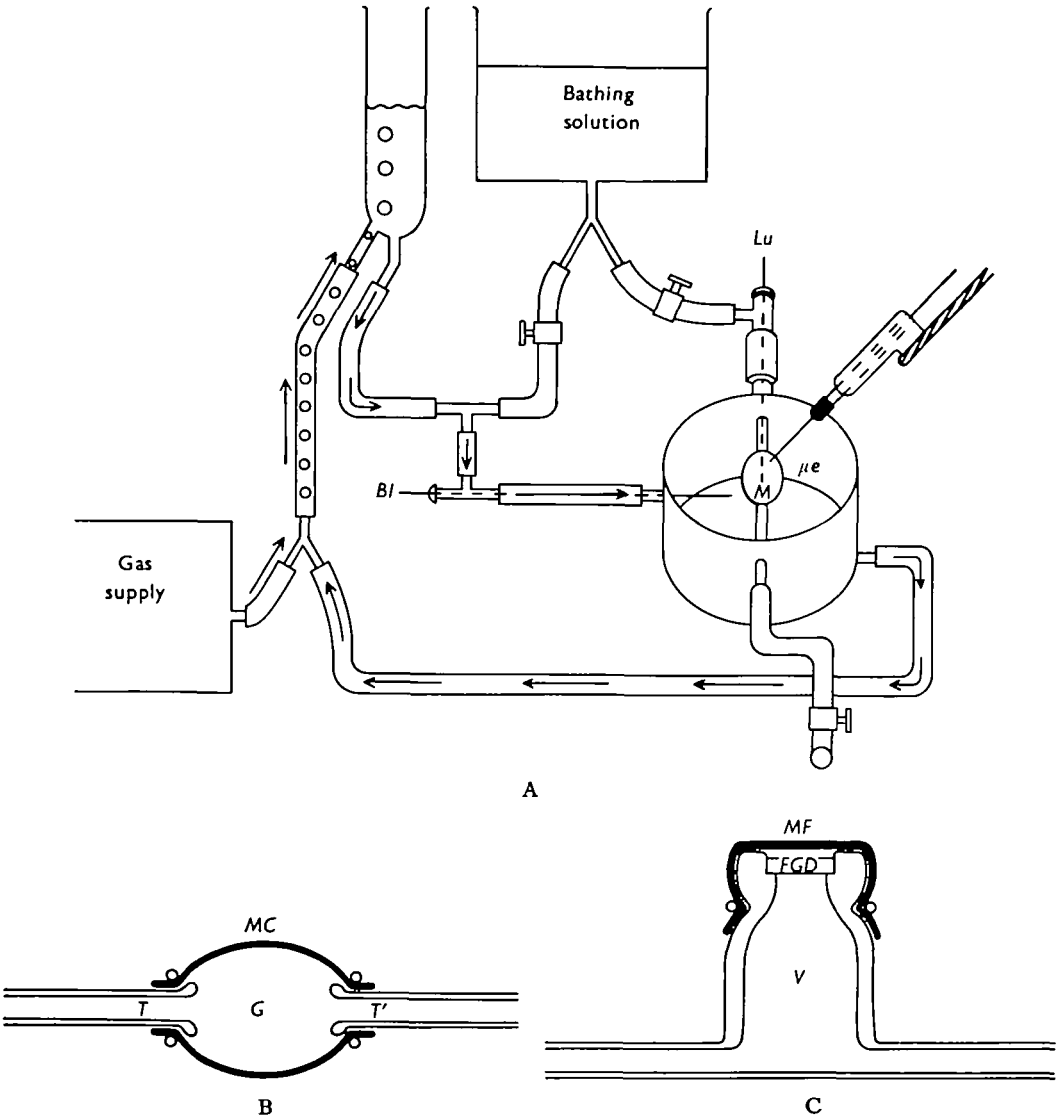

Fig. 1. Midgut chamber. A, Provisions for perfusing the isolated gut and positioning electrodes. *μe.*, Microelectrode; *Bl*, blood-side 'reference' electrode; *Lu*, lumen-side external electrode; *M*, midgut. B, The midgut is mounted as a cylinder (*MC*) across the 1 cm. gap (*G*) between two opposing sections of glass tubing (*T* and *T'*). C, The midgut is mounted as a flat sheet (*MF*) across the 1 cm. diameter opening of a cylindrical glass vessel (*V*) above a fritted glass disc (*FGD*).

After the midgut had been secured in the chamber both compartments were filled with the standard solution. The oxygen supply was turned on to oxygenate and circulate the blood-side solution. After all air bubbles had been removed from the inner compartment, the initial midgut potential was measured. Only midguts with initial potentials greater than 100 mV. were used for experiments. The midgut was allowed to equilibrate in oxygenated 32-S for $\frac{1}{2}$ hr. before the type of gas or the ionic composition of the solution was changed.

Microelectrodes and potential measurements

Glass micropipettes with a medium taper and a tip diameter less than 1 μ as measured with phase optics were pulled from 0·7–1·0 mm. o.d. Kimax glass tubing on a vertical puller designed after Alexander & Nastuk (1953). The microelectrodes were filled immediately with filtered 3 M-KCl by boiling under reduced pressure for approximately 45 min. (Gestland, Howland, Lettvin & Pitts, 1959) and were stored at 4° C. for use within 24 hr.

Two bridges, filled with a 3% agar solution of the corresponding bathing saline, were connected to calomel half-cells to form the external electrodes. The microelectrode was connected by a 3 M-KCl agar bridge to a third calomel half-cell. The potential difference was measured either by a Keithley 601 Electrometer and 370 Recorder or by a Grass P 6 Preamplifier and a Tektronix 502 Dual-Beam Oscilloscope. With the preamplifier–oscilloscope arrangement the potential difference was measured by a null-balance voltage potentiometer on the P 6 Preamplifier.

The midgut potential was measured between the blood side and the lumen reference electrodes (ψ_{lu}-ψ_{bl}). Before each impalement a microelectrode was selected for a tip resistance in saturated KCl between 1 and 15 MΩ as measured by an ohmmeter. The microelectrode was held in a Lucite chuck mounted on a Narishige manipulator. The microelectrode was accepted if it had a tip potential (ψ_{μ_0}-ψ_{bl}) less than 10 mV. measured in 32-S. An impalement was rejected if the tip resistance changed more than 10% or if the tip potential changed more than 2 mV. during the profile. The midgut was normally impaled from the blood-side. Potential profiles were measured between the microelectrode and the blood-side reference bridge (ψ_{μ}-ψ_{bl}) and all potentials were corrected for tip potential becoming (ψ_{μ}-ψ_{μ_0}). All potential plateaus in the profiles were stable and could be recorded for as long as 5 min.

RESULTS

Profiles in oxygenated standard solution

A tracing of a typical potential profile produced during the impalement of a midgut bathed on both sides with oxygenated 32-S is presented in Fig. 2A. The midgut was mounted as a flat sheet and was impaled from the blood-side. As the microelectrode was advanced, the recorded potential dropped to −3 mV. (ψ_{μ}-ψ_{μ_0}). During further advancement two more negative plateaus of −10 and −25 mV. were recorded. Finally, with further advancement the potential jumped to a large positive value of +105 mV. which was equal to the midgut potential. The microelectrode was advanced until it was clearly visible in the lumen with no further change in recorded potential.

All fifty-five of the potential profiles in oxygenated 32-S were similar to Fig. 2A in

that in each case negative plateaus were followed by a single large positive step. Moreover, when the midgut was impaled from the lumen surface the shape of the profile was the reverse of that just described. In three impalements from the lumen side the first change in potential was the immediate loss of the midgut potential and the appearance of a negative potential between the microelectrode and either external electrode. Upon further advancement of the microelectrode several negative potentials were recorded and finally the tip potential was recorded. Furthermore, when the microelectrode was carefully retracted from the lumen after an impalement from the

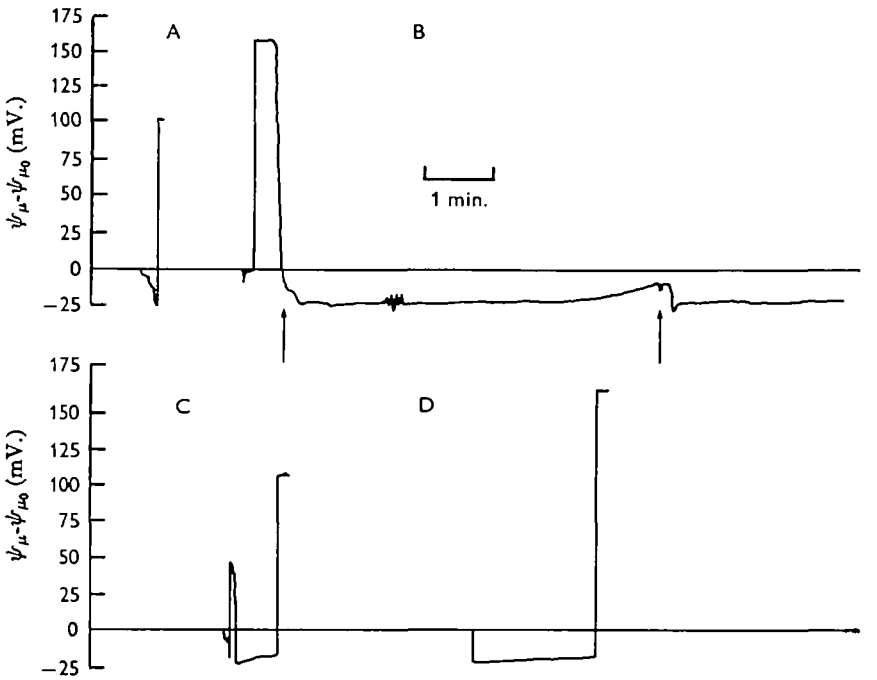

Fig. 2. Potential profiles (ψ_μ-ψ_{μ_0}) recorded when the midgut was bathed in oxygenated 32-S. A, Typical profile of impalement from blood-side, showing three small negative plateaus and a single large positive step to the full midgut potential. B, Profile recorded when the microelectrode was inserted into the lumen, missing the negative plateaus and recording the full midgut potential. The microelectrode was then retracted slowly (arrow) and a negative plateau was recorded for 5 min. At the next arrow the microelectrode was advanced about 25 μ and a negative potential was recorded for an additional 2 min. C, Profile illustrating a rarely observed transient smaller positive potential superimposed on an otherwise 'typical' profile. D, Profile showing a sustained negative plateau followed by the largest positive step recorded (+187 mV.). (Note that the 'positive step' in Fig. 3B during retraction was +183 mV.)

blood side, the same type of profile just reported for the impalement from the lumen surface was recorded. Such a retraction profile is traced in the last part of Fig. 2B. In this impalement, as the microelectrode was advanced from the blood side, the stable negative plateaus were 'missed'. However, when the microelectrode was withdrawn, a negative potential of −23 mV. was recorded for 5 min. When the potential became less negative, the microelectrode was advanced about 25 μ and a negative potential of −22 mV. was recorded for an additional 2 min.

One important exception to these otherwise similar profiles was the occasional

appearance of small positive plateaus prior to the large positive step (7 of 55 impalements). However, in all but two cases the first potential recorded was negative, and in all but two cases the large positive jump was from a negative potential. Usually the small positive plateaus were superimposed on otherwise 'typical' profiles (Fig. 2C).

The relationship between the potential profile and the location of the potentials in the midgut tissue is not immediately clear. Attempts to locate the position of the negative and positive potentials directly by the iontophoretic deposition of lithium carmine using the techniques of Whittembury (1963) were not successful. The distance of excursion of the microelectrode is not a reliable index of the tip position within the midgut tissue because the midgut was not rigidly mounted and it moved when the microelectrode was advanced. When the midgut was rigidly supported by fritted glass, the potential profiles were distorted and the microelectrodes invariably broke. Moreover, distance would not be a reliable index of microelectrode position even if the midgut did not move during the impalement because the probability is great that the tip would travel obliquely through several cells of the highly folded epithelium. The location of the potential steps in the profile will be considered in the discussion.

So that the results can be quantified, two expressions will be defined. The first expression, the maximum negativity, refers to the greatest negative potential in each profile (ψ_μ-ψ_{μ_0}). The average maximum negativity for the fifty-five impalements in oxygenated 32-S was $27 \pm 1{\cdot}2$ mV. (S.E.M.). There was no correlation between the maximum negativity of each impalement and the corresponding midgut potential ($r = -0{\cdot}24$, $P > 0{\cdot}08$). Moreover there was no correlation between the maximum negativity and the tip potential ($r = 0{\cdot}10$, $P > 0{\cdot}20$).

The second expression, the positive step, refers to the positive jump in the electrometer profiles. However, when using the oscilloscope technique of null-balancing the potential difference it was not always possible to measure the negative plateau prior to a positive jump because of the suddenness with which the positive step occurred. An approximation of the size of this step is given by adding the maximum negativity to the midgut potential. This calculation is valid because in the twelve impalements recorded by the Keithley Electrometer, eight recordings jumped from the maximum negativity to the midgut potential. The average 'positive jump' for the fifty-five profiles in oxygenated 32-S was $+125 \pm 3{\cdot}5$ mV. (S.E.M.). The largest positive step recorded was $+187$ mV. (Fig. 2D).

Effects of low oxygen tension on the profile

The reduction in the oxygen tension as a result of the introduction of nitrogen affects only the positive step. The negative potentials of the profile remain unaltered during the time that the midgut potential dropped due to oxygen deprivation (Fig. 3A). The midgut was bathed in oxygenated 32-S, and the midgut potential was $+124$ mV. The microelectrode was advanced from the blood side until a negative potential of -22 mV. was recorded. At this point nitrogen replaced oxygen as the stirring gas. While the midgut potential dropped 110 mV. to $+14$ mV., the microelectrode remained *in situ* and the potential recorded varied by only 1 mV. When the microelectrode had penetrated into the lumen, the midgut potential was recorded. The loss of the midgut potential during oxygen deprivation is reversible, making it possible to

conduct experiments both during the addition of nitrogen and the subsequent re-addition of oxygen. The results of an experiment in which oxygen was reintroduced are shown in Fig. 3 B. As the midgut potential increased by 97 mV., the negativity again remained unchanged. Eight experiments of this type were attempted, five with the addition of N_2 and three with the re-addition of O_2. In six of these eight attempts the negativity remained unaltered, whereas in two cases the negativity fluctuated over a range of 30 mV. as the midgut potential changed by 100 mV. Twelve impalements of midguts whose transepithelial potential had been virtually abolished by oxygen deprivation confirmed that the only change in the profile was a corresponding drop in the large positive step. The maximum negativity for the twelve impalements was $-23 \pm 3{\cdot}2$ mV. (S.E.M.), and the average 'positive' jump was $+33 \pm 2{\cdot}7$ mV. (S.E.M.).

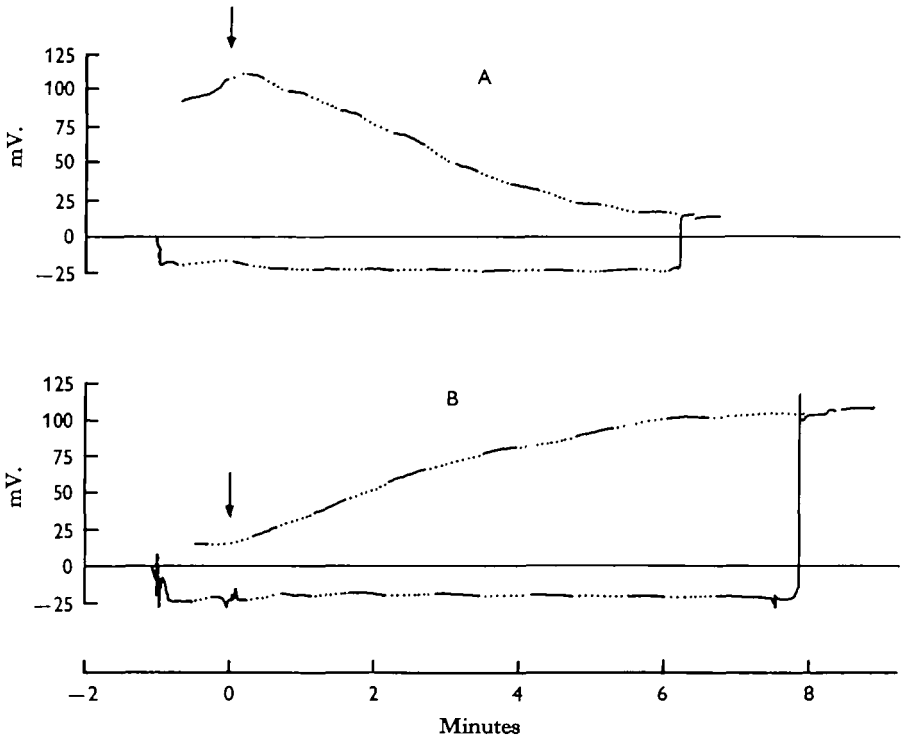

Fig. 3. Effects of oxygen tension on the maximum negativity and the positive step. A, The midgut was bathed in oxygenated 32-S and the midgut potential was +124 mV. as recorded in the upper trace using external electrodes. The microelectrode was 'advanced' to a potential of −22 mV. Nitrogen replaced oxygen as the stirring gas (arrow) and the midgut potential (upper trace) fell 110 mV. to +14 mV. while the negativity (lower trace) varied by only 1 mV. B, Similar to A except that the midgut potential had already been lowered by nitrogen gas. Oxygen was reintroduced (arrow) and the midgut potential (upper trace) increased by 97 mV. while the negativity (lower trace) again remained unchanged.

Effects of changes in the concentration of potassium in the bathing solution

Harvey & Nedergaard (1964) found that changes in the concentration of potassium in the blood-side solution lead to changes in the midgut potential (e.g. the midgut potential drops 40 mV. when the blood-side potassium concentration is decreased

tenfold by substituting sodium for part of the potassium). To determine whether the change in the midgut potential was due to a change in the profile negativity or to a change in the positive jump, the potassium concentration in the bathing solutions was altered. The major effect of changes in the concentration of potassium on the blood side is a change in the maximum negativity, although some change in the positive jump is observed. Figure 4 shows the profile obtained after the blood side bathing solution had been changed from oxygenated 32-S to oxygenated 2-S. In 2-S the midgut potential was +60 mV.; the maximum negativity was −75 mV.; and the positive step was + 135 mV. A profile in oxygenated 32-S taken just before the solution change showed a midgut potential of +120 mV., a maximum negativity of −25 mV.

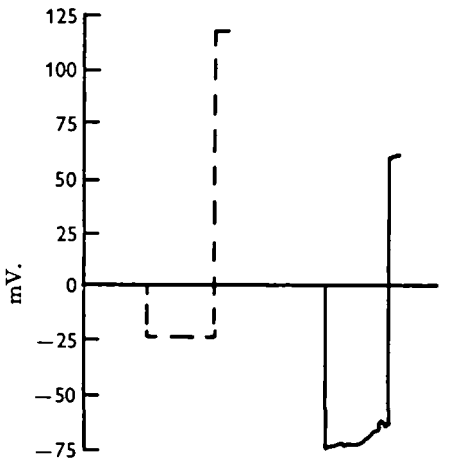

Fig. 4. Effects of potassium concentration on the maximum negativity and the positive step. The dotted profile shows the potential trace taken with the midgut bathed in oxygenated 32-S. The blood-side solution was changed to oxygenated 2-S and the solid trace was recorded. The midgut potential changed from +120 to +60 mV.; the negativity changed from −25 to −75 mV.; however, the positive step changed only 10 mV. from +145 to +135 mV. Therefore, 83 % of the change in midgut potential is due to a change in the maximum negativity.

and a positive step of +145 mV. Therefore, 83 % of the 60 mV. change in the midgut potential was due to the change in the maximum negativity (Fig. 4). Two more experiments in 2-S and seven in 2 mM-$KHCO_3$ yielded similar profiles. Five impalements of midguts bathed in 73·5-S and thirteen in 90-S show a decrease in the maximum negativity with correspondingly smaller changes in the positive step.

DISCUSSION

The potential profiles recorded when the midgut was impaled from the blood side consisted invariably of one or more steps to a maximum negative plateau followed by a single positive step to the full midgut potential (Fig. 2A, C, D). Not only were the negative and positive steps separated spatially, but their magnitudes were affected differently both by oxygen deprivation and by the external potassium concentration.

The maximum negativity was unaffected by oxygen deprivation (Fig. 3) but increased as the potassium concentration on the blood side was decreased (Fig. 4). These

results argue that the negative potentials are passive equilibrium potentials and that potassium is the principal ion involved. However, either some anion must also be involved in an unspecific way or alternatively the potassium concentration in the cell must change, because the plot of maximum negativity against potassium concentration has a slope less than 59 mV.

The positive step on the other hand was but little affected by external potassium concentration (Fig. 4). Like the short-circuit current and the active potassium flux (Harvey, Haskell & Zerahn, 1967), the positive step was greatly reduced by oxygen deprivation (Fig. 3). These results argue that the positive step is caused directly by the electrogenic potassium pump. However, at least part of the positive step has a passive origin because the step is never completely abolished by oxygen deprivation.

The discrete and independent nature of the negative and positive steps argues that they arise across two separate parallel barriers. Furthermore, because the barrier potentials add algebraically to yield the midgut potential, the two barriers are electrically in series. This interpretation implies that the midgut operates electrically as a single compartment separated by two barriers from the internal and external bathing solutions, and implies that the midgut potential is the sum of the potassium equilibrium potential across the blood-side barrier and the electrogenic pump potential across the lumen-side barrier.

It is reasonable to assume that the profile negativity must be located in the epithelial cells themselves. To account for the electrical properties of the midgut the negative potential must develop across a continuous barrier that is electrically in series with a second barrier. The tracheal and muscle cells do not form a continuous barrier. The only other continuous layer in the midgut besides the epithelial cells is the basement lamina. However, this acellular lamina can probably be eliminated as the site of the negative steps because, although it probably contains fixed negative charges, there is no evidence that a potential difference could arise across it. The epithelial cells remain as the only structure that satisfies the electrical requirements for the negative plateaus.

Establishing that the epithelial cells are the site of the profile negativity has important implications regarding the electrical configuration of the midgut. The epithelial cells are thereby identified as the 'midgut' compartment which is separated by two barriers from the two bathing solutions. Since the epithelium is only one cell layer thick, it implies that the physiological membranes of the epithelial cells are divided into blood-side and lumen-side components. Unless there is some intermediate barrier, the 'basal' cell membranes of the epithelial cells are the blood-side barrier and the 'apical' cell membranes are the lumen-side barrier.

A structural basis for dividing the plasma membranes of the epithelial cells into discrete regions may be provided by the zonulae occludentes which join all the epithelial cells together (Anderson & Harvey, 1966). Loewenstein and co-workers (Kanno & Loewenstein, 1964; Loewenstein, 1966) have shown in a number of epithelial tissues joined by zonulae occludentes that the resistance of the junctional membranes is much smaller than the resistance of the non-junctional membranes. If the electrical coupling reflects the structure of the midgut, then the entire epithelium would behave electrically like a low-resistance compartment bounded by two high resistance membranes in series. The zonulae occludentes would electrically separate the basal and apical membranes from each other, and at the same time couple all the

apical plasma membranes together and all the basal plasma membranes together. The potassium equilibrium potential would appear across the coupled basal plasma membranes, and the electrogenic pump potential would appear across the coupled apical plasma membranes. Such an arrangement would account for the monotonous similarity of the impalement profiles even though several possible types of pathway for microelectrode travel are suggested by the structure of the gut. Moreover, a pathway from epithelium to goblet cavity and back to epithelium before entering the lumen would account for small positive steps like the one seen in Fig. 2C.

Although the electrogenic pump has been located across the apical plasma membrane, the exact location of the actual pump sites within the epithelial cells cannot be detected by using microelectrodes. Regardless of where the actual pumps are, whether they are directly in the plasma membrane or in some structure electrically coupled to it, the pump potential would still appear uniformly across the apical plasma membrane. A list of possible attached structures would include the zonulae occludentes, the spike-like units on the plasma membrane lining the goblet cavities, endoplasmic reticulum, and microtubules. However, no direct attachment of either endoplasmic reticulum or microtubules to the plasma membrane has yet been resolved in the Cecropia midgut. On the basis of present evidence the most conservative interpretation of these results is that the electrogenic potassium pump in the Cecropia midgut is located in or on the apical plasma membrane of the epithelial cells.

SUMMARY

1. The potential profile recorded as a microelectrode is advanced from the blood side through the isolated midgut of *Hyalophora cecropia* consists of negative plateaus followed by a large positive step to the full midgut potential.

2. Oxygen deprivation diminishes both the positive step and the midgut potential; the negative plateaus are not affected.

3. Changes in the potassium concentration in the blood-side solution affect both the negative plateaus and the midgut potential; the large positive step remains about the same.

4. From these results it is concluded that the positive step is probably produced by the electrogenic potassium pump and that the negative steps are due to a potassium equilibrium potential.

5. The discrete and independent nature of the negative and positive potentials argues that there are two barriers separating a 'midgut' compartment from the two bathing solutions.

6. It is inferred that the epithelial cells are the site of the profile negativity and therefore that they constitute the 'midgut' compartment. This interpretation implies that the potassium equilibrium potential appears across the basal cell membranes and that electrogenic pump potential appears across the apical plasma membranes of the epithelial cells.

7. The most conservative interpretation of these results is that the electrogenic potassium pump is located somewhere in or on the apical plasma membrane of the epithelial cells.

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