IDENTIFICATION OF CELL TYPES CORRESPONDING TO MICROELECTRODE IMPALEMENTS IN INSECT MIDGUT

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IDENTIFICATION OF CELL TYPES CORRESPONDING TO MICROELECTRODE IMPALEMENTS IN INSECT MIDGUT

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ABSTRACT

The chamber-mounted midgut of Manduca sexta larvae actively transports potassium ions from the hemolymph-side to the lumen-side. The midgut epithelium is composed of two major cell types, goblet and columnar cells. Microelectrode impalements previously performed on the midgut demonstrated two impalement types based on their distinct electrical potentials: the Low Potential Difference cell (LPD) and the High Potential Difference cell (HPD) (Blankemeyer and Harvey, 1978). The LPD and HPD impalement types have yet to be directly linked with the two histological cell types. The LPD impalement type has been shown to be the site of active transport due to resistance measurement changes seen in oxygen and anoxia, whereas the HPD impalement type has been shown to be not involved in active transport (Blankemeyer and Harvey, 1978). Using iontophoretic injection of fluorescent dye into LPD and HPD impalement types, we have located the dye-filled cells with the fluorescent microscope, and directly linked the goblet cell with the LPD impalement type and the columnar cell with the HPD impalement type. Thus, for the first time in this polymorphic tissue, the impalement type responsible for active ion transport, the LPD type, has been directly identified as the goblet cell.

Key Words: active transport, Lucifer Yellow CH, microelectrode, potassium, insect, midgut.

INTRODUCTION

Active transport of ions across epithelia is widespread throughout virtually all animals and plants. The identification of the cell type actually responsible for transepithelial active transport has been a difficult problem in polymorphic epithelia. The larvae of the insect, Manduca sexta (Tobacco hornworm), feed on leaves of solanaceous plants that have potassium concentrations much higher than the hemolymph (Quatrale, 1966). One of the structures which actively transports potassium, in vitro, is the insect midgut, which is composed of two major cell types and actively transports potassium from the hemolymph to the lumen.

MORPHOLOGY

The first detailed study of the structure of an insect midgut was performed by Anderson and Harvey (1966) on Hyalophora cecropia (L.) larvae. More recently Cioffi (1978) carefully studied the hornworm midgut.

The midgut of fifth-instar larvae is composed of two major cell types, goblet and columnar cells, and a third type, regenerative cells. These cells form a one-cell thick, highly folded epithelium that rests on a basement lamina and circular and longitudinal muscles.

Goblet cells

The goblet cell is usually flanked by columnar cells and has a basally located nucleus. It has a large rounded basal end which tapers to form an apical neck. The apical membrane invaginates, forming a large cavity which apparently communicates with the midgut lumen via

a valve-like structure (Anderson and Harvey, 1966). The nucleus is juxtaposed to the cavity. The goblet cell is about 50um tall and 30-40um in diameter in the region of the cavity. The ring of cytoplasm surrounding the cavity is usually less than 3um wide and varies in extent along the midgut (Cioffi, 1978).

Columnar cells

The columnar cell is about 65um tall and has a 25um basal diameter and is characterized by long, thin microvilli on the lumenal border and a central nucleus. A columnar cell is often found on either side of each goblet cell, as previously mentioned, and the apical ends of the columnar cells extend beyond the apical end of the goblet cell. Infoldings of the basal membrane penetrate the cytoplasm to the level of the nucleus.

ACTIVE TRANSPORT

Fifth instar Lepidopteran larvae (Cecropia) normally feed on leaves with a potassium content of over 239mM potassium (Quatrale, 1966). The midgut contents are also high in potassium content (Harvey et al., 1975); however, the hemolymph potassium concentration is much lower, approximately 23mM, and midgut tissue is about 90mM potassium (Jungreis et al., 1973). Harvey and Nedergaard (1964) found that the chamber-mounted midgut actively transports potassium from hemolymph to lumen in solutions without a requirement for sodium. The chamber-mounted midgut typically has a potential difference of 100mV between the hemolymph-side and lumen-side bathing solutions (positive from lumen to hemolymph) (Blankemeyer, 1981).

Impalement of the midgut with microelectrodes (from the hemolymph-side) reveals the two step profile as would be expected from a one-cell-thick epithelium. Impalement from the hemolymph side reveals a negative 25mV (with respect to the hemolymph solution) membrane potential, the HPD (High Potential Difference), and a potential near zero, the LPD (Blankemeyer and Harvey, 1978).

There are no known specific pump inhibitors, albeit that anoxia rapidly reduces the potential and short-circuit current to zero. The increase in resistance accompanying anoxia was used to identify the impalement type involved in active transport, the LPD (Blankemeyer and Harvey, 1978). Although circumstantial evidence (Blankemeyer and Harvey, 1978) suggests that the LPD is the goblet cell and the HPD is the columnar cell, no direct confirmation was made before this study.

MATERIALS and METHODS

The midguts used in these experiments were from fifth instar larvae of the tobacco hornworm Manduca sexta. Larvae were raised on an artificial diet (Carolina Biological), modified from Yamamoto (1968). The excised midguts (see Blankemeyer, 1981) were mounted in a perfusion chamber which allows access to one side of the epithelium. The mounted midgut was perfused with an oxygenated solution containing 32mM KCl, 5mM Tris, 1mM CaCl2, 1mM MgCl2 and 166mM sucrose. This solution has a pH of 8.3 and was used at room temperature.

MICROELECTRODES

Microelectrodes were made by pulling Omega Dot GC-120-2 glass (A-M Systems) on an ISA M-1 microelectrode puller (Industrial Sciences Associates). These double-barrelled microelectrodes were examined with a microscope for a sharp taper and approximately a 0.5 micron tip diameter. One side of the microelectrode was backfilled with 250mM KCl. The other side was backfilled just beyond the shank with 3% Lucifer Yellow CH and then diluted by backfilling with 0.1% LiCl (Stewart, 1978).

ELECTRONICS

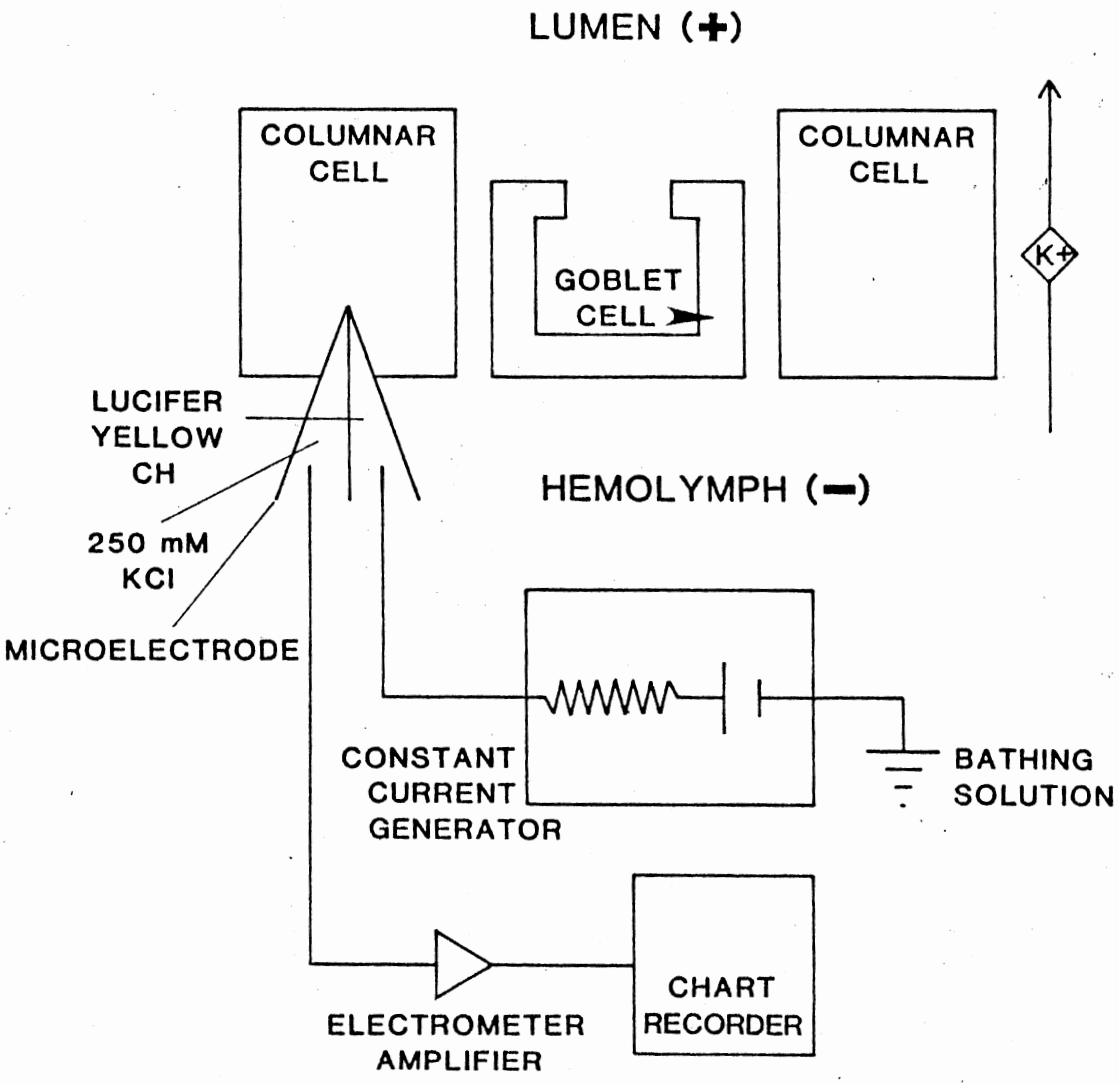
The electrode signals were acquired and amplified with a 3431J electrometer amplifier (Burr-Brown). The microelectrode was mounted on a Narishige micromanipulator with a Haer hydraulic drive. Impalement of a cell (see Fig. 1) was recorded on a Linear chart recorder as a deflection due to the change in potential. When HPD types were investigated, only those impalements with a potential of -16mV or more

negative were used. When LPD types were investigated, only those impalements with a potential of +5mV to -5mV were used. Only one impalement type was studied in each tissue and the number of impalements per tissue ranged from 2-8. Before dye injection, the potential of the impaled cell was allowed to stabilize (10-30sec). Cells were injected with dye by applying hyperpolarizing current pulses to the distal end of the dye-filled barrel of the microelectrode. Cells were pulsed 125 times. Pulse duration was 250msec at a typical hyperpolarizing current of 2.4nA.

TISSUE PREPARATION

The tissue was removed from the chamber and fixed in an aqueous solution of 4% formaldehyde in 0.1M Na2P04 (pH 8.3) for 4-24 hours at room temperature. The tissue was then bound to a specimen block with O.C.T. compound (Lab-Tek) and 24 to 40 micron serial sections were cut on a cryomicrotome (American Optical). The sections were placed on slides and viewed on a Nikon Labophot microscope equipped with UV epi-illumination as well as bright field illumination. Photographs were taken through the microscope with a Nikon 35mm camera on Kodacolor II film with an ASA rating of 100 and on Ilford ASA 400 film. Exposure time ranged from 10 to 40 seconds.

Figure 1. Schematic Representation of Columnar and Goblet Cells, Illustrating an Impalement of a Columnar Cell with a Double-barrelled Microelectrode. One barrel is backfilled with 250mM KCl and detects the potential change upon impalement of the cell. The signal is amplified and recorded. The other barrel of the microelectrode is backfilled with Lucifer Yellow CH dye. Current pulses which drive the dye into the cell are generated by a constant current source.


LUMEN (+)
COLUMNAR CELL
GOBLET CELL
COLUMNAR CELL
K+
LUCIFER YELLOW CH
250 mM KCl
MICROELECTRODE
HEMOLYMPH (−)
CONSTANT CURRENT GENERATOR
BATHING SOLUTION
ELECTROMETER AMPLIFIER
CHART RECORDER

RESULTS

A profile of HPD and LPD impalements is shown in Fig. 2. The microelectrode was usually advanced toward the tissue from the hemolymph side. The potential is zero until a cell is impaled. The first impalement shows a -22mV potential, an HPD impalement, and the second impalement shows a +3mV potential, an LPD impalement.

Serial sections of the dye-injected midgut were examined with UV epi-illumination at 125X for evidence of flourescence. Often, flecks of dye not associated with any cell were found on top of the tissue sections. However, cells containing fluorescent dye were recovered in all but two experiments. Table 1 shows a summary of the recoveries of identifiable cells, that is, cells which we could clearly determine as goblet or columnar cells. Recovery of dye-injected cells was high (approximately 75%) and, of those recovered, about 50% were clearly identifiable. In order for a cell to be identified, the plane of section had to be such that the presence or absence of an intracellular cavity could be definitively established.

LPD Impalement Type

Recovery of dye-injected goblet cells from LPD impalements provides direct evidence that the LPD impalement type is the goblet cell. Figure 3 shows a photomicrograph of a typical recovered cell within a tissue section after an LPD impalement and dye-injection. The photomicrograph was taken using transepithelial (bright field) illumination and UV epi-illumination at 500x. A narrow ring of cytoplasm contains the fluorescent dye and surrounds a distinct cavity, which is large and rounded at the basal end and tapers to form an apical neck.

Figure 2. HPD and LPD Impalement Profiles. A tracing from a chart record shows the zero potential of the microelectrode tip in the hemolymph bathing solution. The microelectrode was advanced toward the midgut tissue from the hemolymph side. The first deflection is -22mV (with respect to the hemolymph bathing solution), an HPD impalement. The microelectrode is retracted, leaving the cell, and the potential returns to zero. Impalement at a different site on the tissue shows a deflection of +3mV, an LPD impalement. Again, retraction of the microelectrode returns the potential to zero.

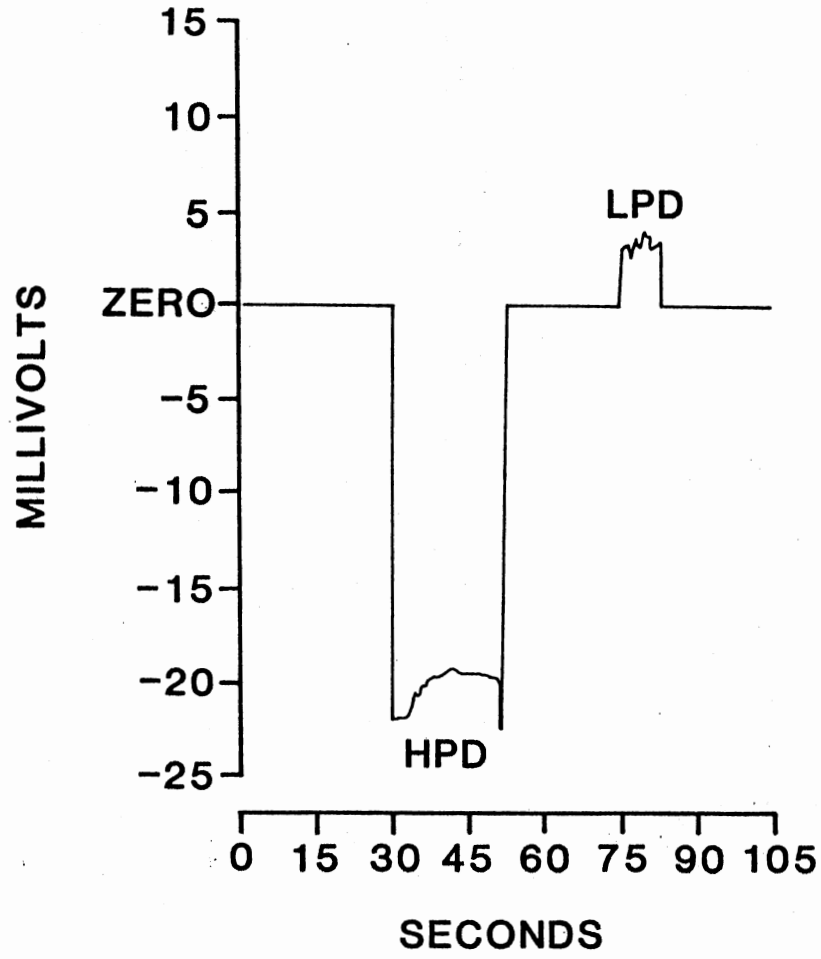

15
10
5
ZERO
-5
-10
-15
-20
-25
MILLIVOLTS
LPD
HPD
0 15 30 45 60 75 90 105
SECONDS

Table I: SUMMARY OF DYE RECOVERY EXPERIMENTS. The two major impalement types in the midgut are the HPD (High Potential Difference) and the LPD (Low Potential Difference) types. The HPD type has a potential of -16mV to -35mV and the LPD type has a potential of +5mV to -5mV. In each tissue, only one impalement type, HPD or LPD, was impaled. The number of impalements per tissue ranged from 2 to 8. Only those cells that were clearly identifiable were counted. All identifiable recoveries from LPD impalements were goblet cells and all identifiable recoveries from HPD impalements were columnar cells.

IMPALEMENT TYPE	POTENTIAL (mV)	NUMBER OF IMPALEMENTS	NUMBER OF RECOVERIES	CELL TYPE OF RECOVERIES
HPD	-16 to -35	39	16	Columnar
LPD	+5 to -5	30	11	Goblet

HPD Impalement Type

Recovery of dye-injected columnar cells from HPD impalements provides direct evidence that the HPD impalement type is the columnar cell. Figure 4 shows a photomicrograph of a typical recovered cell within a tissue section after an HPD impalement and dye-injection. The photomicrograph was taken using transepithelial (bright field) illumination and UV epi-illumination at 250x. The cytoplasm containing the fluorescent dye is much more extensive than in Figure 3, the goblet cell, and no cavity is present.

Figure 3. Photomicrograph of a Dye-injected LPD (+5mV to -5mV) Cell Type. The photomicrograph was taken under transepithelial illumination and ultraviolet epi-ilumination. Goblet cells are characterised by a narrow ring of cytoplasm encircling a large central cavity and a tapering apical neck. Lucifer Yellow CH dye was pulsed into the cytoplasm of the goblet cell as evidenced by the photomicrograph. x500.

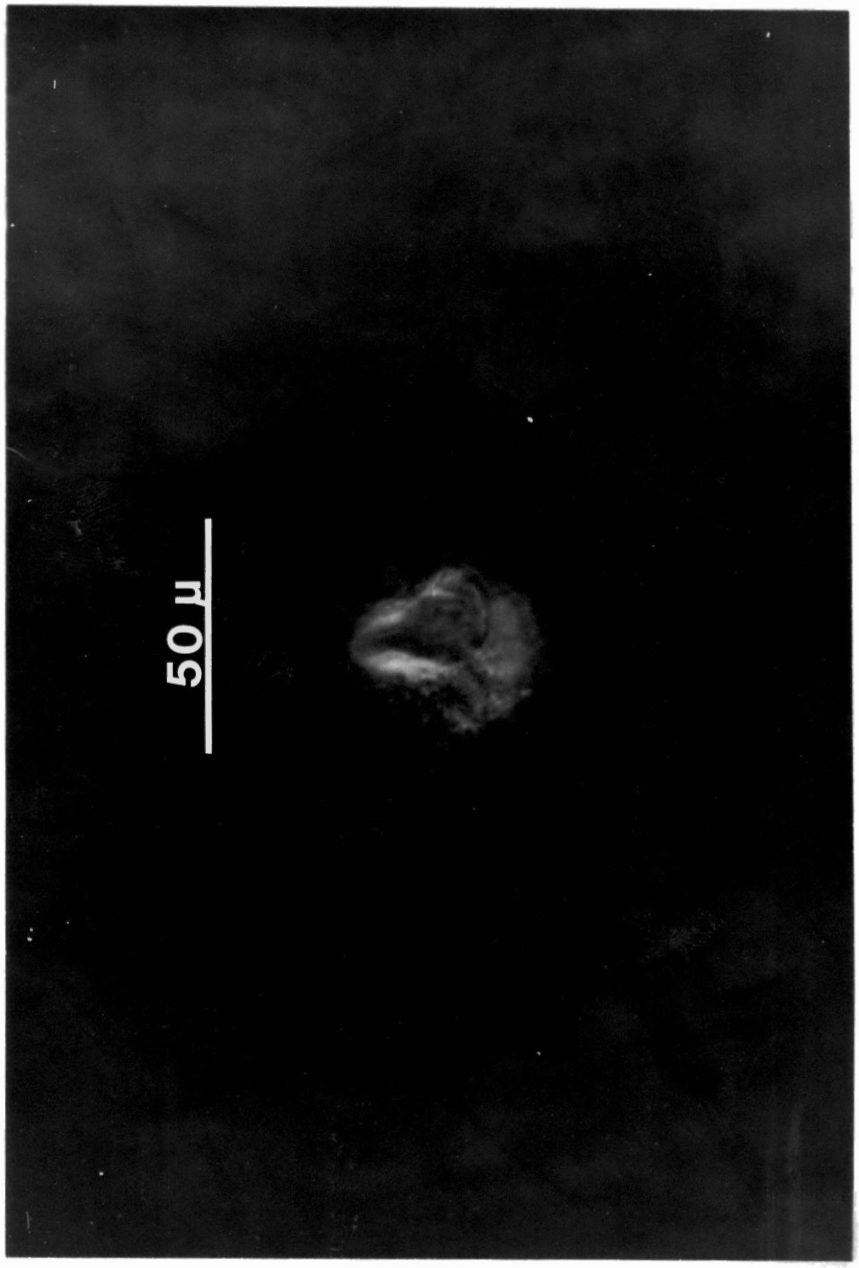
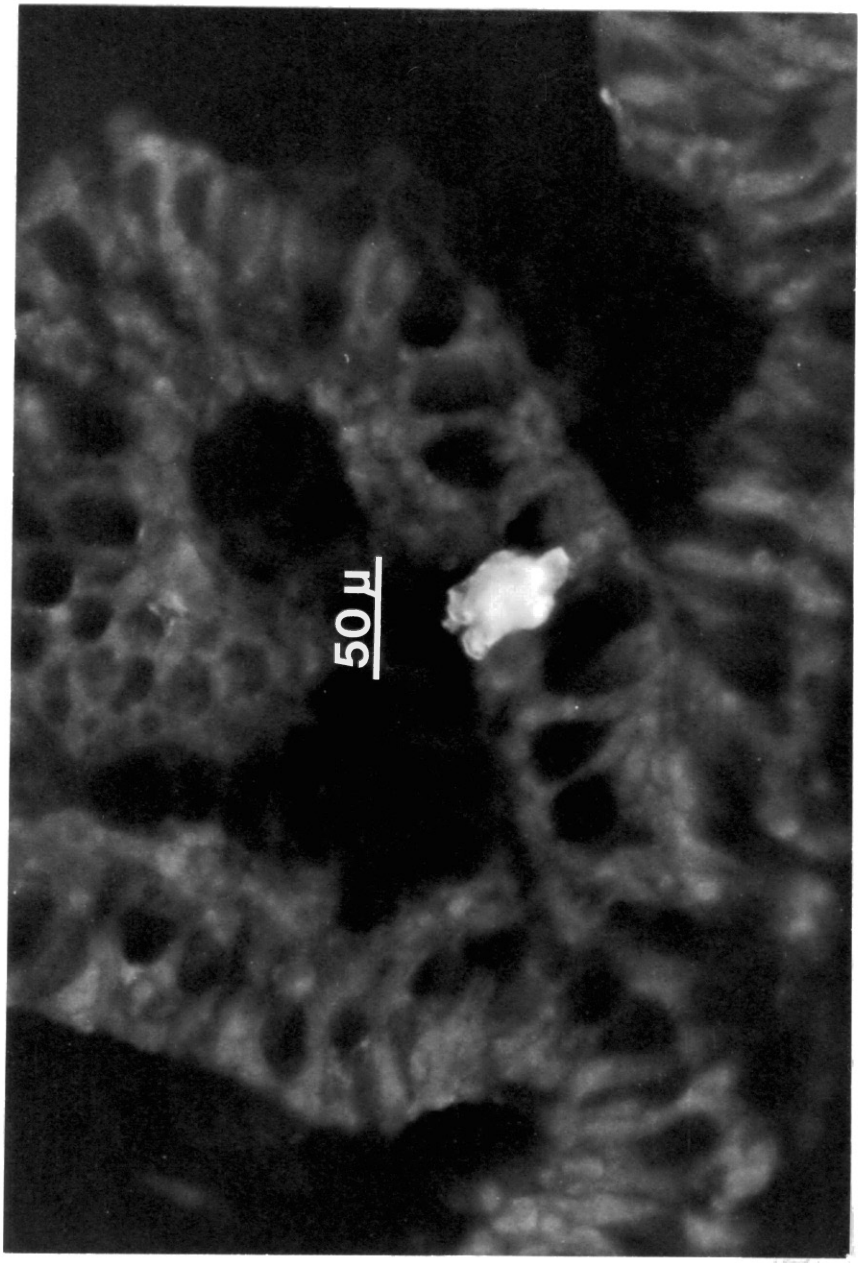

50 μ

Figure 4. Photomicrograph of a Dye-injected HPD (-16mV to -35mV) Cell Type. The photomicrograph was taken under transepithelial illumination and ultraviolet epi-ilumination. Columnar cells are characterised as being over twice as tall as wide and extend to the apical surface of the epithelium. The columnar cell membrane encloses a continous cytoplasmic mass, that is, there is no cavity present. The columnar cell is flanked on either side by goblet cavities. Lucifer Yellow CH dye was pulsed into the cytoplasm of the columnar cell as evidenced by the photomicrograph. x250.


50 μ

DISCUSSION

My impalements of the HPD (High Potential Difference) and LPD (Low Potential Difference) types yielded profiles (Fig. 2) very similar to those of Blankemeyer and Harvey (1978). The LPD impalement type has been previously identified as the one involved in transepithelial potassium active transport (Blankemeyer and Harvey, 1978). Dye recovery from the HPD and LPD impalements provides direct evidence (see Fig. 3 and 4) that the HPD impalement type is the columnar cell and the LPD impalement type is the goblet cell.

Supporting evidence also indicates that the LPD impalement type is actually the goblet cell. First, LPD types were encountered about half as frequently as HPD types. Blankemeyer and Harvey (1978) showed the same frequency in their impalements. This corresponds to the observation by Anderson and Harvey (1966) on the silkworm (Hyalophora cecropia), another Lepidopteran insect, that goblet cells are only about 50% as common as columnar cells. Second, once an LPD type was impaled, a very slight advancement or retraction of the micromanipulator would result in displacement of the microelectrode from the cell. This conclusion is supported by the observation by Anderson and Harvey (1966) as well as Cioffi (1978) that the goblet cell is composed of a very narrow (usually less than 3uM) ring of cytoplasm.

The columnar cell is the most numerous cell type of the midgut epithelium according to Anderson and Harvey (1966) and Cioffi (1978) and also has a much greater volume of cytoplasm, being nearly 65uM tall and 25uM in diameter with no cavity. HPD impalement types were much more frequently encountered than LPD types as Blankemeyer and Harvey (1978) also found. Likewise, once an HPD type was impaled, the

microelectrode could be advanced or retracted a much greater distance than with an LPD impalement type without displacement, providing supporting evidence that the HPD impalement type is the morphological columnar cell.

One problem with the dye recovery experiments was the presence of dye flecks on several tissue sections. These extracellular flecks of dye were often found in tissue sections and were quite frequently adjacent to a dye-injected cell. I feel this is, in part, due to a sectioning artifact in which dye-injected cells were cut and some of the dye was pulled out of the cells by the microtome. Also, any slight movement of the tissue or microelectrode during pulsing can dislodge it from the cell resulting in extracellular dye expulsion.

Approximately 75% of all impalements resulted in recovery of dye-injected cells. About 50% of these recovered cells could be identified to cell type. The plane of section of the dye-injected cells determined whether or not I could clearly identify their histological cell type. Of the identifiable cells, no columnar cells were recovered from LPD impalements and no goblet cells were recovered from HPD impalements. I feel the evidence clearly indicates that the columnar cell is the HPD impalement type and the goblet cell is the LPD impalement type. Thus the goblet cell is responsible for transepithelial active transport of potassium in insect midgut.

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