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Intracellular K^+ Activities and Cell Membrane Potentials in a K^+-Transporting Epithelium, the Midgut of Tobacco Hornworm *(Manduca sexta)*

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Summary. Transbasal electrical potential (V_b) and intraepithelial potassium chemical activity ($(K^+)_i$) were measured in isolated midgut epithelium of tobacco hornworm (*Manduca sexta*) using double-barrelled glass microelectrodes. Values of V_b ranging from +8 to −48 mV (relative to blood side) were recorded. For all sites, $(K^+)_i$ is within a few millivolts of electrochemical equilibrium with the blood side bathing solution. Sites more negative than −20 mV show relatively high sensitivity of V_b to changes in blood side K^+ concentration: 43% of these sites can be marked successfully with iontophoresed Lucifer yellow CH dye and shown to represent epithelial cells of all three types present in the midgut. In about half of successful marks, "dye-coupling" of several adjacent cells is seen. Low potential sites – those with V_b less negative than −20 mV – typically do not show high sensitivity of V_b to changes of external K^+, but rather $(K^+)_i$ rapidly approaches the K^+ activity of blood side bathing solution. These sites can seldom be marked with Lucifer yellow (4% success). The mean $(K^+)_i$ of the high potential sites is 95 ± 29 (SD) mM under standard conditions, a value which is in accord with published values for the whole tissue.

Key Words insect midgut · active K^+ transport · K^+-specific microelectrode · intracellular K^+ · cell membrane potential · Lucifer yellow · dye-coupling

Introduction

The midgut of larval lepidopterans actively transports K^+ from hemolymph ("blood") side to lumen side, countering influx to the hemolymph from the high K^+ plant diet (Harvey & Nedergaard, 1964). Thus the midgut of these insects is an accessory excretory organ as well as an alimentary organ. The ionic composition of the hemolymph is also adapted for phytophagy in that it contains, in comparison to the blood of most animals, relatively high levels of K^+ and low levels of Na^+. Values of 38 meq K^+/liter and <1 meq Na^+/liter have been reported for the hemolymph of the tobacco hornworm (Jungreis, Jatlow & Wyatt, 1973).

Isolated midguts maintain a transepithelial electric potential (V_T) which may be as great as 120 mV (lumen-positive) immediately after mounting. This potential arises directly from electrogenic K^+ transport (Harvey et al., 1968), and the V_T and rate of net K^+ transport decline steadily during the several hours of useful life of the isolated preparation (Harvey & Nedergaard, 1964). Initial studies using intracellular microelectrodes (Wood, Farrand & Harvey, 1969) showed that the greater part of the V_T is due to the apical membrane potential of the single layer of epithelial cells, suggesting that the electrogenic pump is to be found at the luminal border of the epithelium. The midgut epithelium of *Manduca* consists of three cell types: (in order of abundance) columnar cells, goblet cells and regenerative cells. The goblet cells of lepidopteran midgut are not homologous to vertebrate goblet cells and their structure led to the hypothesis that they are the K^+ transporting cells (Anderson & Harvey, 1966).

More recent microelectrode studies (Blankemeyer & Harvey, 1978) have identified three populations of transbasal electrical potentials (V_b). Comparison of the relative abundance of the three populations of V_b values to the relative abundances of the three cell types led these workers to conclude that goblet cells possess the lowest (least negative) values of V_b while regenerative cells have intermediate values and columnar cells have the highest values. The latter studies also reported apparent change in apical membrane resistance during normoxic-hypoxic transitions carried out with the microelectrode in a "low potential cell". These changes were attributed to the decrease in pump-related conductance of the apical membrane expected to occur when the K^+ pump is inhibited

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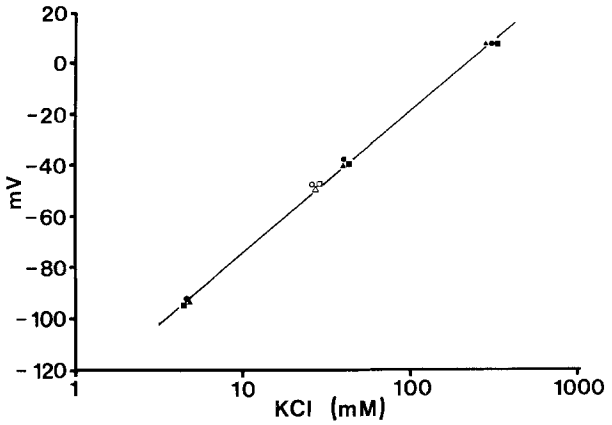

Fig. 1. A typical calibration curve for a K^+ electrode as determined at three separate times during an experiment. The horizontal axis gives chemical activity of K^+ relative to standard solutions of KCl. The filled symbols give K^+ barrel voltages for calibration standards. The open symbols give K^+ barrel voltages for standard 32 KS bathing solution. The straight line was drawn by eye using the calibration points

by anoxia, and were taken as evidence that the apical pump was located specifically in midgut cells with low V_b.

If the low values of V_b reported by Blankemeyer and Harvey (1978) are representative of a population of intact, K^+ transporting cells, the electrochemical gradient faced by K^+ entering across the basal membrane might necessitate a thermodynamically active entry step, assuming that these cells also had the high intracellular chemical activity of K^+ characteristic of animal cells. The present studies measured the electrochemical gradients faced by K^+ ions entering the midgut from the basal side by simultaneous determination of V_b and intraepithelial K^+ activity ($(K^+)_i$). Assuming a transcellular route of transported K^+ (Harvey, 1980; but also *see* Zerahn, 1977), we also sought to determine which values of V_b can be attributed to the basal membrane potential of midgut cells, as determined by simultanous measurement of V_b and injection of Lucifer yellow CH dye.

Materials and Methods

Larvae of *Manduca sexta* were raised on tobacco leaves ("leaf larvae") or on artificial diet (Yamamoto, 1969) ("diet larvae"). In both cases larvae were reared at 25 °C under a 17-hr light, 7-hr dark photoperiod. For electrode penetrations midguts were removed from 5th instar larvae which had been cold-anaesthetized. The morphologically distinct posterior midgut (Cioffi, 1979; Cioffi & Harvey, 1981) was mounted horizontally as a sheet covering a circular aperture which separated two compartments. In early experiments bathing solution on both sides of the midgut was stirred and oxygenated by bubble-lift systems using 100% O_2; in most experiments the two compartments were gravity-perfused with oxygenated bathing solution. The composition of the standard bathing solution (32 KS) is (in mM/liter) KCl 30; $KHCO_3$ 2; $CaCl_2$ 5; $MgCl_2$ 5; Tris HCl 5 (pH = 8.0); sucrose 166. Other bathing solutions are described in the appropriate text. V_T was monitored continuously by means of 3 M KCl agar bridges mounted in the perfusion chambers and connected to calomel electrodes whose output was measured by a Keithley 610 A electrometer. Microelectrode penetrations were made under open-circuit conditions in preparations in which V_T ranged from 100 to 40 mV at the time of penetration.

Double-barrelled glass microelectrodes were prepared from 1.5 mm OD Omega Dot tubing (Glass Co. of America) or from theta tubing (R & D Optical, Spencerville, Mass.). For K^+-specific electrodes, one barrel ("K^+ barrel") was silanized by exposure to 100% dimethyl dichlorosilane vapor for 1–3 min, followed by baking at 100 °C for 1 hr. The tip of the silanized barrel was filled with K^+-specific ion exchange resin (Corning 477317). The latter barrel was then backfilled with 0.50 M KCl. The second barrel ("reference barrel") was filled with 3 M sodium acetate. Useful electrodes had reference barrel resistances of 20–200 MΩ and K^+ barrels whose tip resistance was of the order of 10^{10} Ω and which registered changes in potential of 50–60 mV per decade change in K^+ activity, as described by the Nernst equation; response was logarithmic over the calibration range of 5–500 mM KCl (Fig. 1). In most experiments K^+-specific electrodes were recalibrated after every 2–3 penetrations. Data were discarded when the calibration failed to match previous calibrations. The KCl chemical activity of calibration solutions was taken from standard tables (Shedlovsky & Mac Innes, 1937). Interference by Na^+ in determination of $(K^+)_i$ can be ruled out since Na^+ is present within the tissue in relatively slight quantity (Jungreis et al., 1973) and is nominally absent from bathing solutions. The mean K^+ activity of 32 KS as determined under experimental conditions was 30.6 ± 0.78 (SD) mM ($n = 23$). This value is slightly higher than would be expected for a pure solution of 32 mM KCl; the reason for the higher value is unknown, but a similar finding for lepidopteran saline was reported by Rheuben (1972).

Single-barrelled nonspecific electrodes were made from 1.5 mm OD Omega Dot glass and filled with 3 M KCl. Electrodes with tip resistances of 30–45 MΩ were used.

Electrodes were advanced into the basal or blood side of the tissue by means of remote hydraulic drive (F. Haer & Co.). For experiments with single-barrelled electrodes a WPI M701 amplifier-preamplifier system was used. For experiments using K^+-specific electrodes a Keithley 604 electrometer was used. A Brush 220 pen recorder was used for data recording. In all cases the potentials were measured with reference to a common ground on the blood side. The K^+-specific voltage of the K^+ barrel was computed by subtracting V_b as measured by the reference barrel from the net voltage recorded by the K^+ barrel. When gravity perfusion of bathing solution was used, stable values of V_b and $(K^+)_i$ could be recorded for many minutes in typical penetrations, and it was also routinely possible to change perfusion solutions without disturbing the placement of the electrode.

The ratio of the resistance between electrode tip and blood side bath to the total tissue resistance ("fractional resistance") was measured by passing a transepithelial current pulse sufficient to depolarize V_T by about 20 mV and measuring the resulting deflection of V_b. The microelectrode input resistance was determined by passing a 1-nA current pulse between microelectrode (reference barrel in the case of K^+ electrodes) and the blood side bath and measuring the resulting voltage deflection.

Acceptable penetrations showed crisp transitions of the reference potential trace; data were rejected if V_b changed by more than 2 mV over the period of measurement, if the tip potential of the reference barrel measured after a penetration differed by more than 2 mV from that measured before penetration, or if the microelectrode input resistance changed substantially, as measured during and after penetration.

For iontophoretic marking of impaled cells with the fluorescent dye Lucifer yellow CH, we used double-barrelled electrodes like those used for K^+-specific electrodes. One barrel was filled with 3% Lucifer yellow (Sigma Chemical) in 0.1% LiCl. The other barrel was filled with 3 M sodium acetate and was used for measurement of V_b. The use of double-barrelled electrodes for cell marking duplicates the electrode tip dimensions used for simultaneous measurement of V_b and $(K^+)_i$. Dye iontophoresis was begun after a stable V_b had been recorded for at least 1 min. Hyperpolarizing constant current pulses of 1 nA lasting 0.1 sec at a frequency of 2/sec were passed for periods of up to 20 min; most marking periods lasted 7–12 min. Typically if V_b was stable for the first minute of the period it remained so or declined slightly over the remainder of the period. Marking pulses were terminated immediately if V_b became unstable. For assessment of marking success, sites which were marked for 1 min or more were considered valid attempts.

In most marking experiments several sites were marked. Then the tissue was rapidly removed from the chamber, mounted on a microscope slide, and flattened by applying gentle pressure to the cover slip. In some cases the fresh tissue was examined under a Leitz Ortholux fluorescence microscope. In most cases the tissue was fixed immediately by infiltrating 4% methyl formcel in methanol under the coverslip while flattening was maintained. After a few minutes the methyl formcel was replaced with cold 4% buffered formalin (pH = 7.0). Fixation in the latter was continued for several hours. The fixed tissue was dehydrated in ascending concentrations of ethanol and cleared in methyl salicylate. The whole mount of the cleared tissue was examined for evidence of marked cells. In most cases the cleared tissue was subsequently embedded in paraffin, serial-sectioned at 15 μ and reexamined. This allowed for confirmation of cell type attributions made upon examining the whole mount. Other details of these experiments are given in the appropriate text. Relative numbers of the three midgut cell types were determined by examination of sections prepared as described above and lightly stained with basic fuchsin.

Results

V_b and $(K^+)_i$ Under Standard Conditions

Although the epithelium consists of a single layer of cells, it is highly folded, so that in its course through the epithelium the electrode tip sometimes registers several discrete electrical and chemical transitions before it penetrates the apical border. The transitions might reflect the passage of the electrode through more than one cell, its passage from one intracellular region to another, or its passage to or from intercellular spaces. A representative K^+ and voltage profile containing two such transitions is shown in Fig. 2. Each stable, discrete transition within a profile is denominated a penetration.

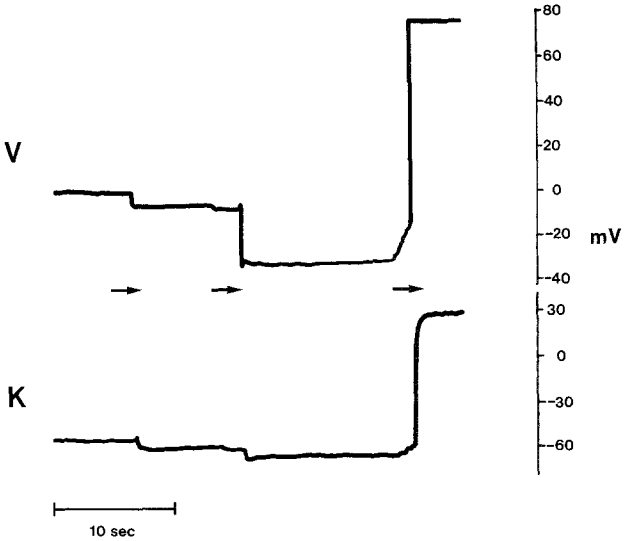

Fig. 2. A typical profile of the midgut as measured by V (reference) and K (K^+-specific) barrels. Penetration proceeds from blood side (left) to lumen side (right). Arrows indicate advances of the electrode; at other times the electrode was stationary. Two electrically and chemically distinct sites (downward deflections of V and K) were encountered in this profile. The upward deflections of V and K mark the entry of the electrode tip into the free solution on the lumen side. Midgut was from a diet-reared animal. The $(K^+)_i$ of the two sites is 38 and 88 mM, respectively

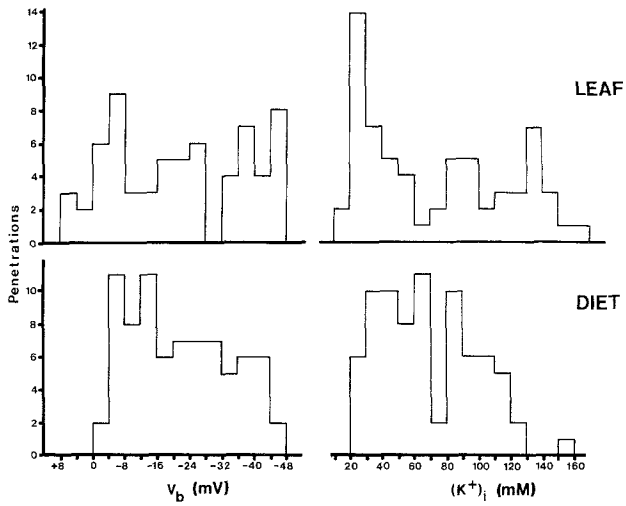

Fig. 3. Histogram of V_b and $(K^+)_i$ recorded from leaf-reared and diet-reared animals using double-barrelled electrodes. Four leaf-reared and four diet-reared animals were used for the experiments

Figure 3 shows histograms of V_b and $(K^+)_i$ recorded from leaf animals and diet animals using double-barrelled electrodes. In agreement with the findings of Blankemeyer and Harvey (1978) it is clear that a wide range of intraepithelial voltages can be recorded, and several modes of V_b and $(K^+)_i$ can be distinguished by examination, al-

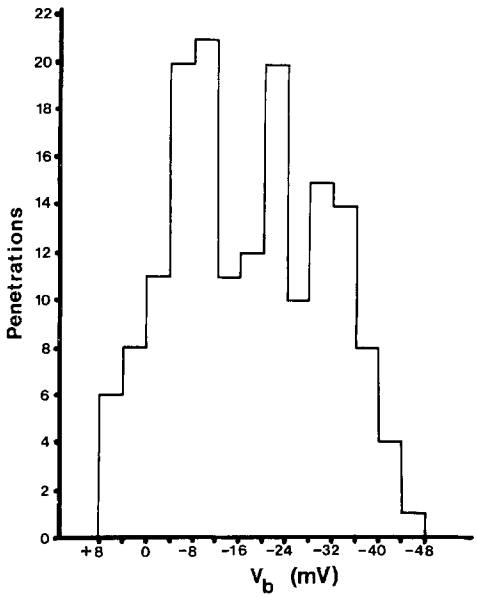

Fig. 4. Histogram of V_b values recorded from midguts of leaf-reared larvae using single-barrelled electrodes. Ten larvae were used for these experiments

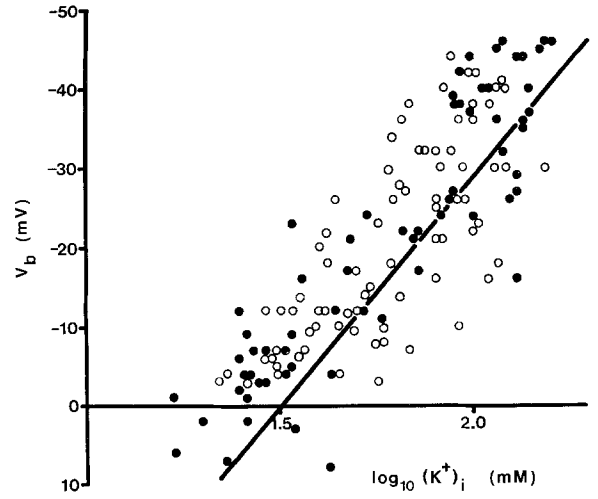

Fig. 5. The relationship between V_b and $(K^+)_i$ in 32 KS bathing solution. Open circles are from diet-reared animals, filled circles from leaf larvae. The solid curve is that of electrochemical equilibrium as calculated from the mean of the measured values of K^+ activity of 32 KS

though there are no accepted statistical methods for distinguishing multiple modes in such data. A matter of concern in measurements of V_b and $(K^+)_i$ is the possibility of damage to cells upon penetration; double-barrelled electrodes might be expected to cause more damage than single-barrelled electrodes of similar tip resistance. Therefore we also made a number of penetrations using single-barrelled electrodes (Fig. 4). Proportions of low, intermediate and high V_b values recorded with single-barrelled electrodes are comparable to those recorded with double-barrelled electrodes, suggesting that the use of double-barrelled electrodes does not in itself bias measurement of intraepithelial voltages.

In Fig. 5, V_b is plotted against $(K^+)_i$ for the data shown in Fig. 3. This plot shows that for both leaf and diet animals there is a direct logarithmic relationship between V_b and $(K^+)_i$ with most points falling close to the line of electrochemical equilibrium. No difference is apparent between leaf and diet animals in this relationship, although the most extreme examples of V_b and $(K^+)_i$ were recorded from leaf animals.

There was a slight tendency for sites with less negative V_b to display lower fractional resistances. For example, the mean fractional resistance of 23 sites with V_b less negative than -10 mV was 0.31 ± 0.19 (SD); for 12 sites with V_b between -10 and -19 mV it was 0.35 ± 0.20 (SD), while for 24 sites with V_b more negative than -19 mV it was 0.44 ± 0.17 (SD).

Effect of Changing External K^+ on V_b and $(K^+)_i$

Experiments to be described in this section were designed to determine the sensitivity of V_b and $(K^+)_i$ to changes in external K^+ concentration. In the first series of experiments single-barrelled electrodes were used. After an acceptable penetration was achieved in 32 KS the perfusion solution was changed to either 2 mM K (obtained by replacing KCl with equivalent amount of choline chloride) or 70 mM K (obtained by adding potassium gluconate). Recording was continued in the new solution until quasiequilibrium was reached, followed by a return to 32 KS. Penetrations in which V_b did not recover to a value similar to the initial value were discarded. The change in V_b resulting from change in external K^+ is expressed in Fig. 6 as the "Nernst slope", the change in V_b for a 10-fold change in external K^+ concentration. The slope may be expected to differ from the ideal as a result of the shunting effect of chloride and as a result of paracellular current pathways, so that these experiments can give only a relative measure of the response of intraepithelial sites to changes in the K^+ gradient between the free solution and that site; however, we might expect that intracellular sites would show at least a moderate degree of K^+ sensitivity and relative stability of $(K^+)_i$, while damaged cells or extracellular sites in communication with the free solution would be expected to show both small changes in V_b and rapid,

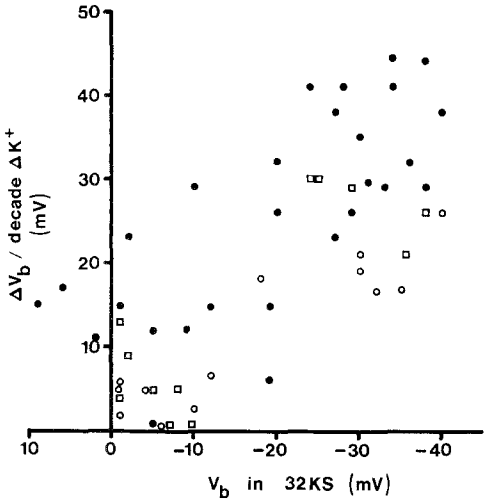

Fig. 6. Effect on V_b of changing bathing solution K^+ concentration. The horizontal axis gives V_b as measured under standard conditions in 32 KS; the vertical axis gives the sensitivity of V_b to a change in external K^+ concentration. The filled circles were determined for changes between 32 KS and 70 mM K solution, the open circles for changes between 32 KS and 2 mM K. These experiments utilized midguts of 4 leaf larvae. For comparison, the open squares show V_b changes from experiments in Fig. 7 (solution changes between 32 KS and 5 mM K)

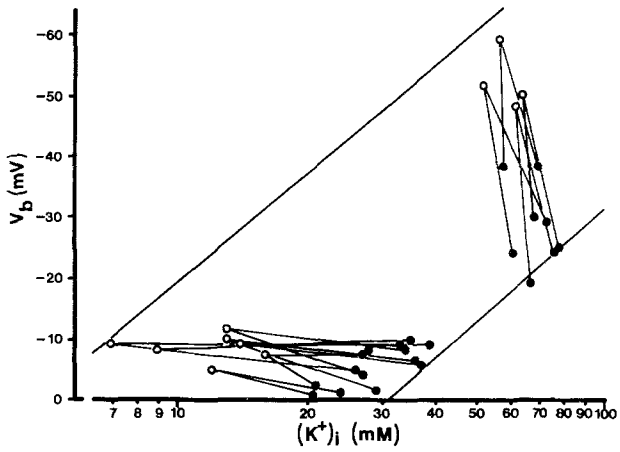

Fig. 7. Simultaneous measurement of changes of V_b and $(K^+)_i$ with solution changes between 32 KS and 5 mM K. For each site, 3 points connected by lines are shown: one open circle giving values after quasiequilibration in 5 mM K and two filled circles showing values recorded under standard conditions before and after the transition. Diagonal lines are lines of electrochemical equilibrium with the two external K^+ activities used in the experiment. These experiments utilized midguts from two diet-reared larvae. Changes of V_b are also plotted in Fig. 6

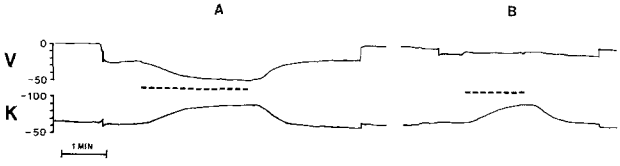

Fig. 8. Reference (V) and K^+ (K) electrode records for two transitions typical of experiments in Fig. 7. Traces A show responses of a high potential site while traces B show responses of a low potential site. The horizontal dashed line between the traces indicates the period of superfusion with 5 mM K solution on both sides of the tissue; at other times the tissue was superfused with 32 KS on both sides. The two transitions shown were recorded sequentially from the same midgut, using the same microelectrode. The more rapid equilibration of K^+ at the low potential site was typical of both of these experiments and of those shown in Fig. 6

large changes in $(K^+)_i$. The experiments with single-barrelled electrodes (Fig. 6) show that the Nernst slope is large and consistent for V_b values more negative than about -20 mV; for less negative values it is less consistent with many very low values.

In a second series of experiments double-barrelled electrodes were used and V_b and $(K^+)_i$ were measured simultaneously during changes between 32 KS and 5 mM K (Fig. 7). These experiments showed that for penetration with V_b more negative than about 20 mV, not only are V_b changes relatively large, but $(K^+)_i$ changes were small and slow. In contrast, for less negative values of V_b, changes in V_b are small and $(K^+)_i$ rapidly approaches the new bathing solution concentration. Typical records for such transitions are shown in Fig. 8.

Iontophoretic Marking of Impaled Cells

In experiments to be described in this section we visualized cells which were sites of low (-2 to -15) and high (more negative than -20 mV) V_b. Iontophoresed Lucifer yellow CH dye is held to mark only intact cells, including both those that are penetrated by the dye-bearing electrode and those "dye-coupled" to the impaled cell (Stewart, 1978). Before each experiment we elected to mark either "high potential" or "low potential" sites. In typical experiments from 4 to 12 sites could be marked. For all "low potential" experiments, we used electrodes from a batch that had already been used successfully to mark "high potential" sites.

Representative marked cells and cell groups are shown in Fig. 9. The Table shows the results of all experiments. To summarize the results, "high potential" sites give a relatively high yield of clearly marked cells, including examples of all three cell types found in the midgut. In many cases, dye-coupling of two or more adjacent cells is seen. In contrast, marking of "low potential" sites gives a low yield of marked cells, giving additional

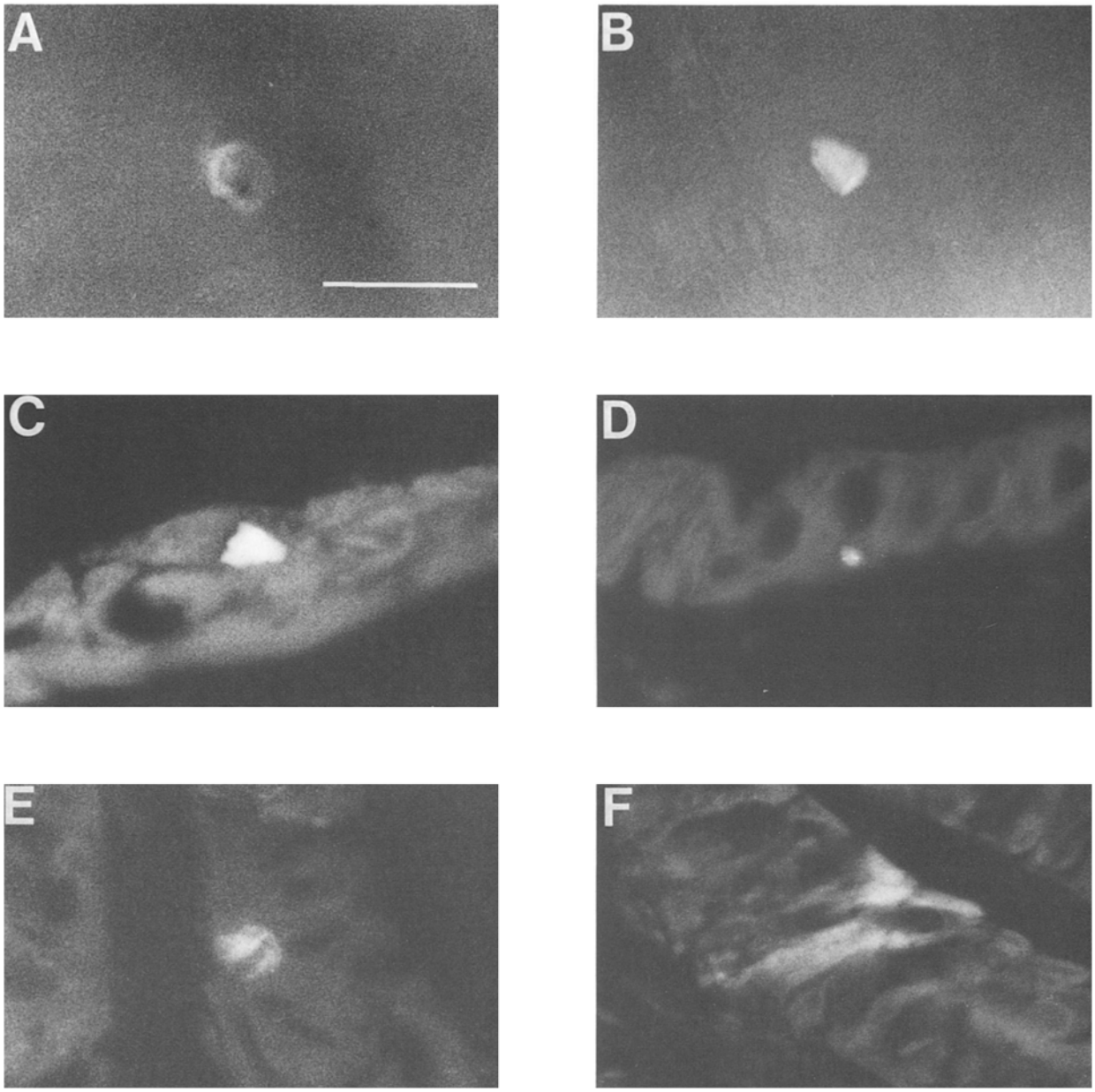

Fig. 9. Cells and cell groups marked with Lucifer yellow as seen with fluorescence microscopy. All examples shown resulted from marking "high potential" sites. The scale bar in *A* is 50 microns – this scale applies to all micrographs in the figure. *A*. Goblet cell seen end-on in a whole-mounted preparation. Focal plane is at the level of the goblet lumen. *B*. Columnar cell seen end-on in a whole-mounted preparation. *C*. Columnar cell seen in section. The plane of the section passes through the apex of a fold in the tissue, so that the cell is seen end-on. *D*. Regenerative cell in section, recognizable by its small size, rounded shape, and basal location in the epithelium. *E*. Goblet cell coupled to two neighboring columnar cells. A complex fold in the epithelium causes plane of section of pass obliquely through the cell group. Part of a columnar cell is seen uppermost, with the goblet lumen in the middle and part of a second columnar cell lowermost. *F*. Dye-coupled cell group including a goblet cell and a columnar cell seen in sagittal section. Portion of a second columnar cell is seen directly above the lumen of the marked goblet cell

support for the hypothesis that most "low potential" sites are extracellular.

The goblet lumen is apparently open to the gut lumen (Anderson & Harvey, 1966) and a microelectrode advanced into the goblet lumen would presumably register the total transepithelial potential. Careful examination of marked goblet cells in whole mount (for example Fig. 9*A*) suggests the dye is in the cytoplasm but not the goblet lumen; lumens of goblet cells seen in section (Fig. 9*E*

Table

V_b (mV)	Solitary cells			Coupled cells		Success
	Col	Regen	Goblet	Col-Goblet	Col-Col	
−20 to −50	15	2	2	5	8	74 penetrations in 12 tissues; 43% success
0 to −20	1	0	1	1	0	68 Penetrations in 8 tissues; 4% success

and *F*) lack dye. We observed no instance in which only the goblet lumen was dye-marked. With the reservation that goblet lumen matrix might be lost during fixation or sectioning, this finding is consistent with cytoplasmic location of the microelectrode tip.

The yield for marking of "high potential" sites is considerably less than the 100% that might be expected if all such sites were intracellular, but it is likely that the method underestimates the number of intracellular sites. First, some cells may be only faintly marked as a result of blocking of the dye electrode early in the iontophoresis period. Faintly marked cells could easily be masked by the native blue-green fluorescence of the midgut tissue. Also, some cells which had been successfully marked might be sloughed before the end of the experiment. Damage to the impaled cell that might occur when the electrode is withdrawn could cause sloughing or dye loss from the cell.

To compare relative numbers of cell types marked with Lucifer yellow to relative numbers of types present in the tissue, we counted cells in stained sections from three diet-reared animals. Results were as follows for 100 cells: 54 columnar: 36 goblet: 10 regenerative.

Discussion

The chemical activity of potassium is typically greater in cytoplasm of animal cells than in their extracellular fluids. For example, in studies using ion-selective microelectrodes, values of 40–120 mM have been reported from vertebrate small intestine (Lee & Armstrong, 1972; Zeuthen & Monge, 1975; White, 1976), 54–68 mM from vertebrate renal proximal tubule (Khuri et al., 1972; Fujimoto et al., 1980), 43–72 mM from vertebrate urinary bladder (DeLong & Civan, 1978; Lewis et al., 1978) and 87 mM from vertebrate gallbladder (Reuss & Weinman, 1979). Reports from insect tissue include 90–147 mM from salivary gland and skeletal muscle (Rheuben, 1972; Palmer & Civan, 1975; Gupta et al., 1978).

The lepidopteran midgut is in many respects the best tissue available for study of the electrogenic potassium transport of insect epithelia, but its complicated geometry, the presence of more than one cell type, and the apparent variable coupling between cells pose particular problems for use of the microelectrode technique. Tissues composed of cells of uniform morphology typically show quite uniform membrane potentials and internal ionic activities when values from damaged cells are excluded (for example, DeLong & Civan, 1978; Reuss & Weinman, 1979). An important question for the present study is whether the great variability of V_b and $(K^+)_i$ values is due to differences in the composition of cytoplasm between different cell types, or to artifacts of penetration failure or penetration damage. In the present studies we used measurements of fractional resistance and microelectrode input resistance to determine that the electrode was in the interior of the tissue, was intact, and was not blocked. However, the major criteria used to validate penetrations were the sharpness of the transition of V_b upon advancement of the electrode, the stability of V_b after penetration, and the stability of the microelectrode tip potential as measured before and after penetration. The use of these simple criteria, which involve no assumptions about the electrical properties of the midgut epithelium or its component cells, was necessary because at the beginning of these studies we did not know enough about the midgut to formulate more sophisticated validation criteria. For example, criteria which depend upon application of an agent known to alter cell membrane properties (Armstrong & Garcia-Diaz, 1981) or the assumption of universal coupling between cells (Reuss & Weinman, 1979) could not be applied to this tissue. We believe that application of stability criteria, together with use of the fractional resistance and microelectrode resistance measurements allowed us to eliminate most cases of artifactual "pre-tip potentials" (Nelson et al., 1978) and most cases of gross damage to high V_b cells. It would not allow us to distinguish readily between extra-

cellular spaces separated from the bathing solution by a significant resistance barrier, and damaged or intact low potential cells. As a result, application of these criteria biases the sample of V_b values in favor of less negative values since, in our experience, more negative values are more likely to be rejected for instability of V_b. This bias may be responsible for the differences in relative numbers of high and low V_b values between the present studies and those of Blankemeyer and Harvey (1978).

Blankemeyer and Harvey (1978) assumed that the chance of randomly penetrating a cell of a given type in the midgut is closely related to the relative abundance of that type in the tissue. While columnar cells are about twice as abundant as goblet cells, other factors, such as cellular geometry and cytoplasmic volume, could affect penetrability. Our results in dye marking studies (Table) show that the chance of penetrating, marking and recovering a goblet cell is somewhat lower than might be expected from the relative number of goblet cells. This difference could arise from factors which affect the chance of successfully penetrating a goblet cell, or to a greater chance of damaging these cells with passage of iontophoretic current or by withdrawal of the microelectrode. By the same token, our failure to dye-mark significant numbers of low V_b cells cannot rule out the existence of such cells. However, the combination of electrical and morphological approaches used in the present studies provides results that agree that most sites more negative than -20 mV are intracellular and include examples of all three cell types; K^+ activity measurements from this more negative subpopulation (mean $(K^+)_i = 95.2 \pm 29.0$ (SD) mM for the 74 points with V_b equal to or more negative than -20 mV, Fig. 5) are in good accord with values of 90–140 meq K^+/liter cell water obtained in gross chemical analysis of midgut tissue (Harvey et al., 1975; Giordana & Sacchi, 1977; Zerahn, 1977). This agreement also argues against the possibility of significant numbers of low K^+ cells. If there are significant numbers of cells which have high $(K^+)_i$ but low V_b, they would have to be so delicate as to be almost always damaged upon penetration (*see* Fig. 5).

Many studies have dealt with the question of the route of transported K^+ through the midgut (for reviews *see* Zerahn, 1977; Harvey, 1980); a major question about the route involves the location of the K^+ transport pool. Considerable evidence now supports the hypothesis that the apical pump is confined to goblet cells and that the transport pool is intracellular (Blankemeyer & Harvey, 1978; Harvey, 1980). However, alkali metal competition studies (Zerahn, 1977) show that the rate of active K^+ transport, while dependent upon blood side K^+ concentration, is relatively independent of the net intracellular K^+ concentration, as measured from whole tissue. The latter studies also showed that the success with which alkali metals compete for transport is not determined solely by their relative abundance in cell water. These findings led Zerahn (1977) to hypothesize that the transport pool is extracellular. Results of the present studies bear on this question. When bathing solution K^+ is reduced from 32 to 5 meq/liter, active K^+ transport is immediately reduced by half (estimated from Harvey & Zerahn, 1972). Potassium activity at high potential sites changes very little; for the 4 sites in Fig. 9 the mean decrease is $16 \pm 3\%$ (SE). In contrast, the K^+ activity of low potential sites follows the bathing solution activity change quite closely (Fig. 9). This is the behavior to be expected of the transport pool if the rate of active K^+ transport is regulated by the K^+ activity of the pool. Unfortunately, the Lucifer yellow method does not provide positive evidence about the location of the electrode tip in most low potential sites. The lack of marked cells suggests that in most cases the tip is extracellular, but the relative under-representation of goblet cells in the high potential sites leaves open the possibility that at least some low potential sites are in goblet cells. This would explain the responsiveness of K^+ transport to changes in blood side K^+ while the much greater nontransport pool of K^+ in columnar cells remains relatively stable. This mechanism would allow for potassium homeostasis in nontransporting cells, but it presumably would not work for goblet cells which are coupled to columnar cells. Also, in this case the goblet cells would have to have some remarkable properties. The apical membrane would have to have both a high permeability to K^+ and a low selectivity for K^+ over Cl^-. The net charge of cytoplasmic proteins would need to be quite small. The enzymes, especially those that serve the pathway of oxidative energy metabolism, would have to be insensitive to changes of intracellular K^+ activity, since O_2 consumption is constant over a wide range of extracellular K^+ concentrations (Harvey et al., 1967; Mandel et al. 1980). Lastly, the substantial metabolic burden of active K^+ transport would fall entirely on cells which comprise less than half of the tissue mass; it is questionable whether the energy metabolism of goblet cells alone can be equal to this task (Harvey et al., 1967).

Since K^+ is close to electrochemical equilibri-

um at most sites, regardless of the value of V_b, it is not necessary to postulate a thermodynamically active uptake of K^+ at the basal border of midgut; in fact, most sites show V_b slightly more negative than would be required for K^+ equilibrium, a displacement which would favor passive entry of K^+. These findings are in accord with the passive entry of K^+ into the transport pool suggested by Wood et al. (1969).

The midgut and other non-neural tissues of *Manduca sexta* larvae lack the (Na^+-K^+)-ATPase associated with K^+ accumulation and volume regulation in animal cells (Jungreis & Vaughan, 1977), and apparently cannot recover from osmotically induced swelling (Moffett, 1979). These findings, together with those of the present studies, suggest that in larval lepidopteran cells cell volume and K^+ distribution between hemolymph and cytoplasm might be determined by a "double-Donnan" equilibrium (Macknight & Leaf, 1977) in which the osmotic pressure of large cytoplasmic solutes is balanced by relatively high levels of amino acids and trehalose in the hemolymph (Florkin & Jeuniaux, 1974).

Passage of intracellular marking dye between adjacent cells has been correlated with electrical coupling between cells mediated by gap junctions (Kanno & Loewenstein, 1964). Gap junctions and septate junctions have been reported to join homologous and heterologous cells in lepidopteran midgut (Flower & Filshie, 1975). It appears probable that dye coupling is a conservative indication of electrical coupling, since the dye molecules are considerably larger than the small ions that would presumably carry electrical current between cells. The present studies suggest that under open-circuit conditions roughly half of the midgut epithelial cells of diet-reared animals are coupled, forming units of 2–5 cells. Goblet cells are frequently included in these units. This coupling, because it appears to involve only small groups of cells, could easily have been missed in the microelectrode studies of Blankemeyer and Harvey (1978). This coupling, including variable proportions of cell types, might account for the variability of the fractional resistance values. Alternatively, it is possible that the hyperpolarizing current pulses used for dye ionotophoresis may have induced coupling under conditions in which it would otherwise not have occurred. Further studies will be necessary to establish the importance of this local coupling for the process of active potassium transport.

The luminal surface of the midgut of leaf-reared larvae is exposed to an environment considerably different from that of diet-reared animals. In particular, the K^+ concentration of leaf tissue is far higher than that of artificial diet (Jungreis et al., 1973). In the studies of Blankemeyer and Harvey (1978), the coupling coefficient approaches 1.0 in diet-reared midgut under short-circuit conditions but is low in leaf larvae under the same conditions. In the present studies performed under open-circuit conditions no important differences were noted between leaf and diet animals in the distribution of values of V_b and $(K^+)_i$ or the sensitivity of V_b to changes of external K^+, suggesting that the basal cell membrane properties are not affected by these conditions of rearing.

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