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Voltage-Current Relation and K^+ Transport in Tobacco Hornworm (*Manduca sexta*) Midgut

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Summary. Voltage-current curves for the isolated midgut of the tobacco hornworm were determined by transient and steady voltage clamping over the range of 200 to −200 mV. Over this range the transient method yields a linear relation while the steady method usually yields a curve consisting of two lines of differing slope which intersect at zero voltage. The difference between the results of the methods is due to a slow decline in total conductance which accompanies steady voltage clamping.

Holding the midgut at short circuit increases the total conductance of the tissue in a manner consistent with increasing shunt conductance; this effect was seen in both diet-reared and leaf-reared animals.

When potassium transport is inhibited by substitution of choline or sodium for potassium in bathing solution the total conductance decreases and the voltage-current curve intersects the normal curve in the hyperpolarizing region. Applying a simple equivalent circuit analysis to the results from partial or total potassium replacement suggests that the electromotive force of the potassium transport system is of the order of 140–190 mV. The conductance decrease during inhibition of potassium transport by transient anoxia is of similar magnitude, suggesting that a major effect of metabolic inhibition is to decrease the active conductance of the potassium transport pathway.

The midgut of larval *lepidopteran* insects transports K^+ from blood to lumen by an electrogenic process (Harvey, Haskell & Nedergaard, 1968) which amounts for the majority of the short-circuit current (I_{sc}) under standard conditions. The biochemical nature of the electrogenic pump is largely unknown, although a K^+ ionophore may be involved (Jungreis & Blondin, 1977). The energetic coupling between metabolism and active K^+ transport does not follow the pattern of sodium transport in amphibian epithelia (*cf.* Vieira, Caplan & Essig, 1972*a*, *b*) in that, while the pump is oxygen-dependent, the rate of oxygen consumption is independent of the rate of K^+ transport or the electrical gradient faced by the tissue (Harvey, Haskell & Zerahn, 1967). The midgut thus presents a challenge to the universality of models of active epithelial ion transport derived from studies of sodium-transporting tissues.

Blankemeyer and Harvey (1977, 1978) have begun to formulate equivalent circuit models for the midgut which take into account intracellular and extracellular conductance pathways of midgut epithelium. Of particular consequence for such models is their finding that the electrogenic K^+ pump is apparently confined to the apical membrane of the goblet cells, one of three cell types represented in the epithelium. This finding implies that, while there may be several distinct passive pathways for ion permeation through the midgut, all of the K^+ current passes through the apical membrane of goblet cells. The present studies show that a simple equivalent circuit model can be used to characterize the behavior of the active route as it responds to metabolic and kinetic effects on transport rate and to voltage clamping.

Materials and Methods

Larvae of *Manduca sexta* were reared at 26 °C under a 7-hr-dark/17-hr-light cycle. Diet-reared larvae were fed the artificial diet of Yamamoto (1969) as modified by Bell (*unpublished*). Leaf-reared larvae were raised on tobacco plants.

Midguts were removed from cold-anesthetized fifth-instar larvae weighing from 8–12 g. They were mounted in a chamber patterned after that of Wood and Moreton (1978) in which the midgut is mounted as a flat sheet covering a circular aperture 0.22 cm^2 in area. The midgut was centered axially between two voltage bridges filled with 3 M KCl agar and connected to calomel electrodes. The voltage electrodes used in this study were paired to

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have a voltage asymmetry of less than 0.3 mV. Current was applied to the tissue via 3 M KCl agar bridges connected to heavily chloridized silver electrodes. The bathing solution on each side was vigorously oxygenated and stirred with 100% oxygen. Hypoxia was induced by bubbling with 100% nitrogen. A difference between our methods and those advanced by Wood and Moreton (1978) is that in the present study solution resistance was determined at the beginning of each experiment by passing current without the tissue in place. The values of solution resistance obtained in this way were applied to the voltage clamp by an automatic circuit in the subsequent experiment with the tissue in place, and were also used in computation of the tissue resistance.

The voltage clamp used in the present study is capable of clamping the transepithelial voltage either chronically or during brief periods of preselected duration and frequency. During intervals between such pulses the transepithelial voltage may either be allowed to resume its spontaneous value, or it may be held at the short-circuit value. Voltage/current (V/I) curves determined by the method of clamping at each voltage point for periods of greater than a minute will be referred to as steady curves; curves determined by pulses to voltage points between which the midgut remains at its spontaneous potential or at short-circuit will be referred to as pulse curves. Voltage and current were recorded by a Hewlett-Packard 17500 A two-channel recorder. Where rapid response was needed, as in the pulse curves, a Gould Brush 220 recorder was used.

The basic bathing solution for the midgut is "32 KS" (Zerahn, 1971) which contains (in mmol/liter): sucrose, 166; KCl, 30; $KHCO_3$, 2; $CaCl_2$, 5; $MgCl_2$, 5; Tris HCl, 5; pH = 8.0. This bathing solution approximates the ionic composition of larval *lepidopteran* blood; the sucrose substitutes for the trehalose and amino acids which account for a substantial fraction of the blood osmotic pressure (Jungreis, Jatlow & Wyatt, 1973; Florkin & Jeuniaux, 1974). In some experiments NaCl was substituted for the KCl and $NaHCO_3$ (designated 32 Na). In other experiments K^+ concentrations spanning the range 2 meq/liter to 70 meq/liter were desired. Because the midgut can transport Na^+ if the Na^+ concentration is high, freshly recrystallized choline chloride replaced potassium chloride in these experiments. All solutions contained 166 mmol/liter sucrose, irrespective of their ionic composition, since this nonpenetrating solute is necessary for maintaining effective isotonicity (Moffett, 1979).

Results

Response of the Midgut to Voltage Clamping

Figure 1 *A* shows voltage and current record for an episode of steady voltage clamping at an electrical potential opposite to the lumen + potential. As first shown by Wood (1972) and Wood and Moreton (1978), the onset of voltage clamping causes an unstable current which rapidly declines within the first 100 msec and slowly declines thereafter. The slow component of the decline may require several minutes for equilibration. This current pattern is seen for both hyperpolarizing and depolarizing voltages. Accordingly, where steady clamping is indicated, the current values are those measured after complete equilibration. For pulse clamping, the object was to measure V/I after the rapid decline, part of which occurs during the response time of the clamp circuit, but before the slow decline had become significant. Hence, for pulse clamping, pulse durations of 0.3 sec were used and the current values given were those measured at the end of the pulse. Figure 1 *B* shows an example of such a pulse of voltage clamping.

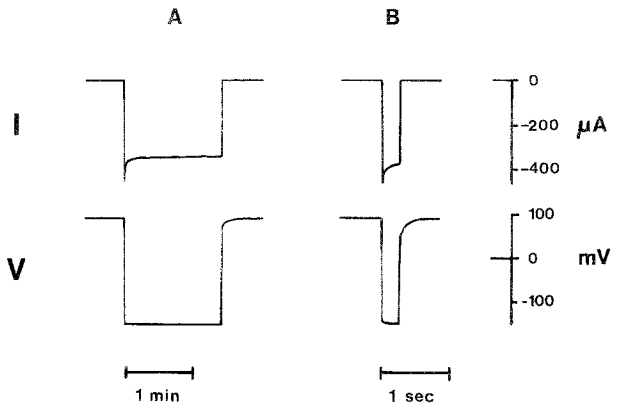

Fig. 1. (*A*) Current (*I*) and voltage (*V*) records for an episode of voltage clamping from open circuit to a less lumen + potential. Clamping was continued until *I* equilibrated. (*B*): An 0.3-sec pulse of clamping as used in generating pulse curves. The *I* value taken from such pulses was that at the end of the pulse. Note the time scale in *B* is different from that in *A*

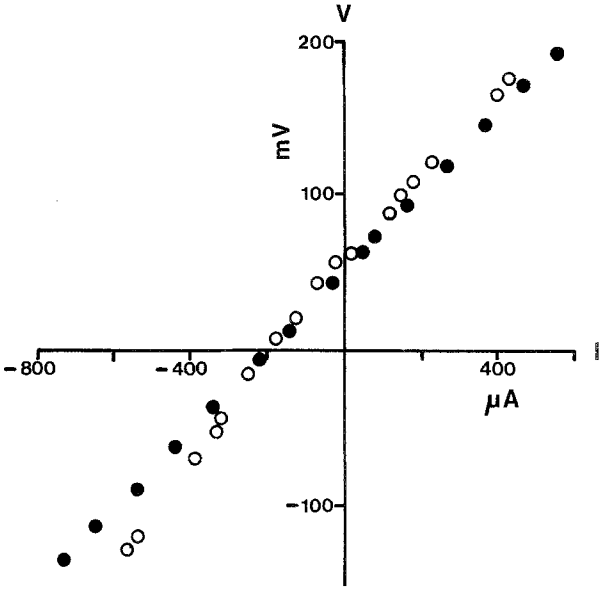

Fig. 2. Pulse (filled circles) and steady (open circles) V/I curves taken sequentially from the same tissue. In this and subsequent V/I plots, the polarity of V is with respect to the lumen side

Current-Voltage Relationship of Midgut

Figure 2 shows pulse and steady V/I curves for a single midgut. Typically the steady curve shows a

slight break at zero voltage, while the pulse curve approximates a straight line. Wood and Moreton (1978) measured similar steady V/I curves but attributed the curvature to an artifact resulting from the decay of the short-circuit current during the series of measurements. In the present study hyperpolarizing and depolarizing points of the curves were collected in alternation, so that a steady decay of the short-circuit current cannot account for the curvature. Since such breaks in linearity were not observed in pulse V/I curves, the apparent rectification seen in the steady curves must reflect changes in tissue resistance which accompany steady clamping (i.e., changes occurring during the slow component of current decline), and might reflect voltage-induced changes in the ionic milieu of the tissue resulting from the transport number effect (Barry & Hope, 1969*a*, *b*; Wedner & Diamond, 1969), or changes in the ionic composition of the cytoplasm.

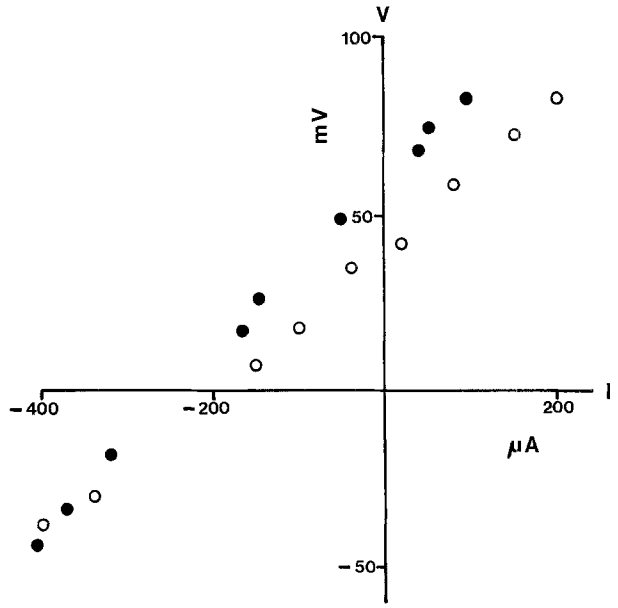

Fig. 3. Pulse V/I curves from open circuit (filled circles) and from short circuit (open circles). The short-circuit curve shown was made 5 min after open-circuit curve; a subsequent open-circuit curve (not shown) was similar to the initial one shown. The curves shown are from a midgut from a diet-reared animal

Effects of Short-Circuiting the Midgut

In these experiments the V/I relationship was determined by the pulse method; during the 10-sec interim between pulses and the tissue, either was allowed to return to its spontaneous potential or was held at short circuit. In most cases the curve determined at short circuit was bracketed in time between two open-circuit curves to compensate for effect of processes associated with the slow decay of I_{sc}. Five minutes were allowed for equilibration between short-circuit and open-circuit curve determinations. Slopes and intercepts of the V/I curves were determined by linear regression. A typical experiment is shown in Fig. 3. Note that the effect of short circuiting is to rotate the curve to a lower slope with the intersection of the open circuit and short-circuit curves in the vicinity of the point corresponding to I_{sc}. Of four experiments with die-treated larvae, all showed a decrease in the slope of the V/I plot with short circuiting; of these differences, one was significant at the 0.1 level, the rest at the 0.01 level or better. The mean difference in slope corresponded to a difference in resistivity of $8.6 \pm 3.9\ \Omega\ \text{cm}^2$ (SE). Upon obtaining this result, I hypothesized that this difference might be due to formation of intercellular electrical connections, a process reported by Blankemeyer and Harvey (1977) to occur under short-circuit conditions in diet-reared larvae but not in leaf-reared larvae. However, in five experiments with leaf larvae, all showed decrease in V/I slope; of these, four were significant at the 0.01 level or better. The mean difference was $3.3 \pm 0.7\ \Omega\ \text{cm}^2$ (SE); the mean open-circuit resistivity was $78.3 \pm 14.7\ \Omega\ \text{cm}^2$ (SE). This finding suggests that the

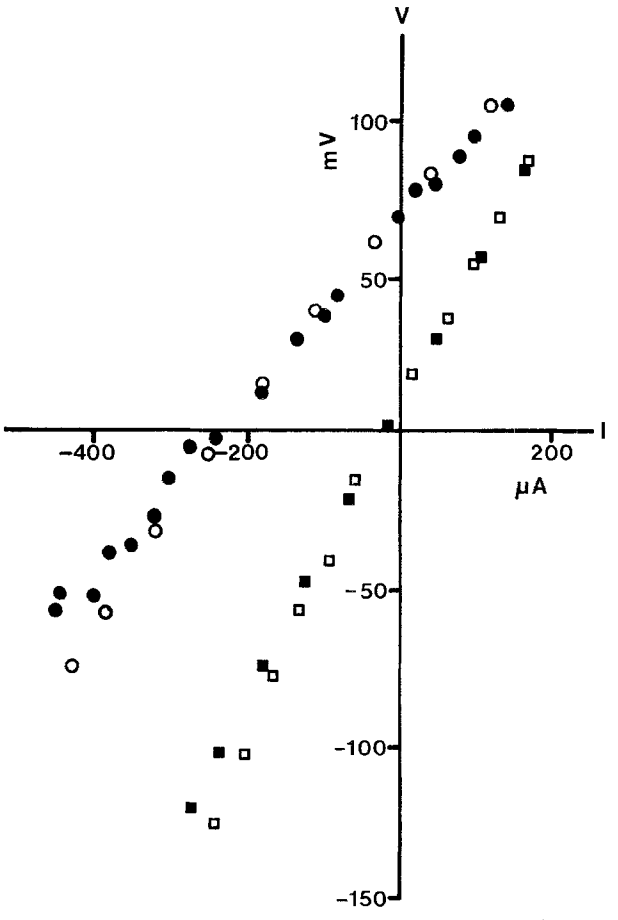

Fig. 4. Steady (open symbols) and pulse (filled symbols) V/I curves in 32 KS (circles) and in 32 Na (squares)

difference in resistivity between open-circuited and short-circuited diet tissues is not solely due to the formation of intercellular current pathways under short-circuit conditions, but does not rule out a contribution of such processes to the resistivity of short-circuited midgut.

Effects of Potassium Concentration

Figure 4 shows steady and pulse V/I curves for a single midgut as measured under normal conditions and after several minutes in bathing solution in which potassium was entirely replaced by sodium. Note that the effect of abolishing potassium transport by this method is to rotate the V/I plot around a point of intersection of the two curves in the hyperpolarizing region. A similar effect is seen if choline is substituted for potassium.

To more closely examine the relationship between tissue conductance and potassium transport, tissue resistance was measured by one or several pulses of voltage clamping during brief exposures to solutions containing either 2, 10, 32, 50, or 70 meq/liter potassium, choline being substituted at the lower potassium concentrations to maintain isosmoticity and approximate electrical conductivity. For convenience in graphing and analysis, resistivity is converted to conductivity (K_t). In these experiments K_t and I_{sc} are those measured as soon as a stable I_{sc} was established after each solution change. This equilibration never required more than 4 min and generally required about 2 min. A similar method was used by Harvey and Zerahn (1972) to establish the relationship between I_{sc} and potassium concentration. The pooled results of eight experiments, plotted as K_t/I_{sc} (Fig. 5), suggests that K_t bears an approximately linear relation to I_{sc} over almost the entire range in which potassium concentration changes can modulate I_{sc}.

Effect of Hypoxia

With the onset of N_2 hypoxia the I_{sc} and open-circuit potential of midgut begin to decrease within 10–20 sec. The I_{sc} typically reaches 50% of its prehypoxic value in 2 min or less, and is entirely abolished within 5–10 min. Recovery upon restoration of O_2 is even more rapid and is essentially complete unless the I_{sc} has been allowed to remain at zero for more than several minutes. Figure 6 shows normoxic and hypoxic V/I curves for a typical experiment. As in the sodium substitution experiments (Fig. 4), the normoxic and hypoxic curves intersect in the hyperpolarizing region.

The effect of hypoxia on the relation K_t/I_{sc} was examined by measuring K_t by repeated pulses during the onset of and recovery from N_2 hypoxia. In these experiments I_{sc} was not allowed to fall to very low values and a single cycle of hypoxia and recovery lasted about 7 min, with recovery typically being within a few percent of the initial I_{sc}. Figure 7 shows

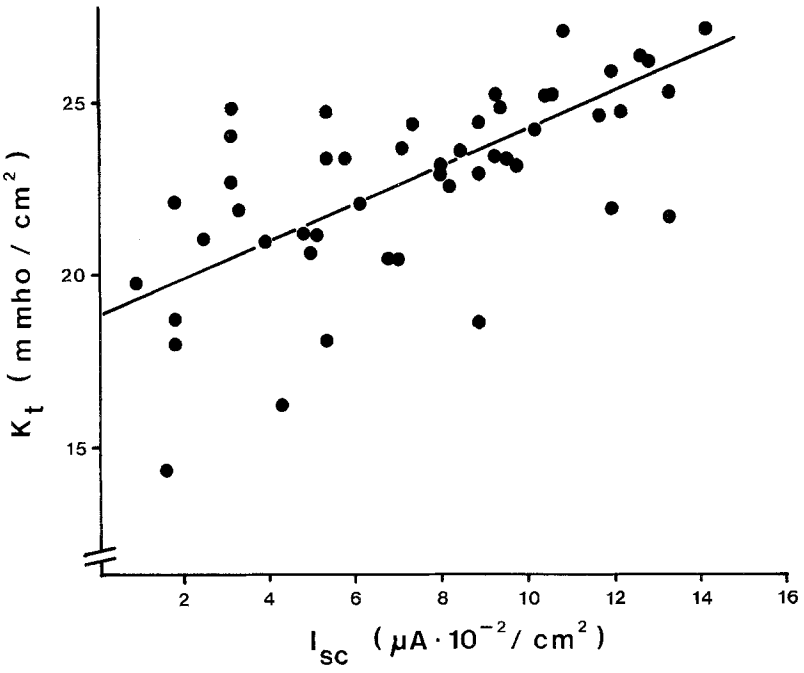

Fig. 5. Tissue conductance (K_t) as a function of short-circuit current (I_{sc}). Both K_t and I_{sc} in this figure and in Fig. 7 are scaled for nominal tissue surface area. Points are pooled from eight experiments in which each tissue was briefly exposed to a series of bathing solution potassium concentrations as described in the text. The slope of the regression line is 5.26 μmho/μA

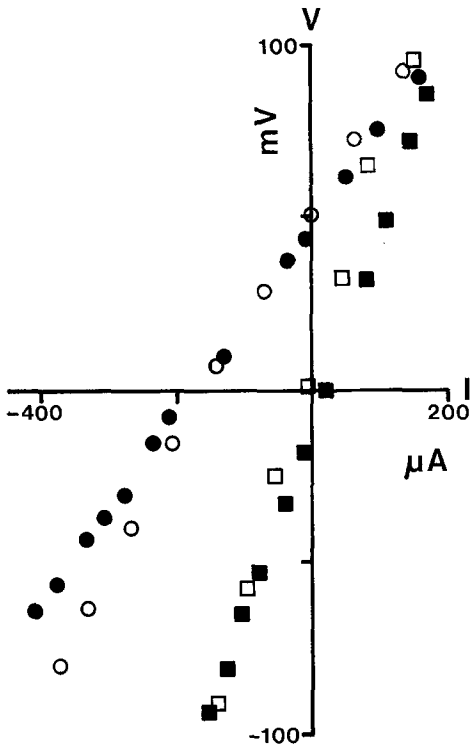

Fig. 6. Pulse (filled symbols) and steady (open symbols V/I curves in normoxia (circles) and after I_{sc} had been abolished by several minutes of N_2 bubbling (squares)

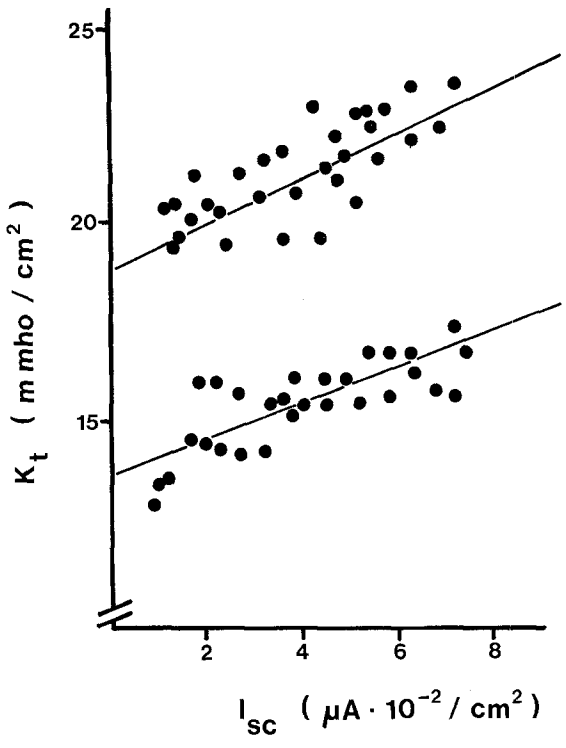

Fig. 7. K_t as function of I_{sc} as I_{sc} falls during transient N_2 hypoxia. The upper group of points was pooled from three experiments in which the bathing solution was 32 KS+38 mM choline chloride. The slope of the regression line is 5.39 μmho/μA. The lower group of points was pooled from two experiments in which 32 KS was the bathing solution. The slope of the regression line is 4.69 μmho/μA

pooled points from five experiments. K_t begins to decrease synchronously with I_{sc} at the onset of hypoxia and decreases linearly with it until oxygen is restored. Typically K_t follows a similar ascending route during recovery. In three of the experiments the bathing solution contained 32 meq K^+/liter and 38 meq choline/liter (i.e., it was the same as the 32 meq K^+/liter solution used in the experiments shown in Fig. 5). In two experiments 32 KS was the bathing solution. The difference in the intercepts with the vertical axis for the two groups is due to the difference in bathing solution resistivity between the two groups. This effect is treated in the discussion.

Discussion

Although the conductance of the midgut epithelium is high in comparison to so-called high-impedance tissues, it is possible to distinguish a conductance element which responds rapidly and reversibly to changes in transport rate; such conductance changes seem likely to reflect the behavior of the active pathway for potassium.

Yonath and Civan (1971) have shown that the behavior of a simple epithelial equivalent circuit consisting of an active conductance K_a, a parallel leak conductance K_l and an ion-specific electromotive force E having a short-circuit current I_{sc} can be described by trajectories in the plane K/I_{sc} which are diagnostic for changes in single elements of the circuit. According to this formalism the V/I relationship of the circuit is given by

$$V=(I+E\cdot K_a)/K_t=(I+E\cdot K_a)/(K_a+K_l)$$

where K_t represents net tissue conductance. It follows from this relation that where $V=E$, I is independent of K_a. Agents which solely alter K_a will generate V/I curves which rotate around the point $V=E$, $I=E\cdot K_l$. Agents which alter only K_l generate V/I curves which rotate around the point $V=0$, $I=I_{sc}$. Agents which alter only E generate parallel V/I curves which pass through differing I_{sc}. Agents which affect more than one of the circuit elements may generate more complicated trajectories; nevertheless, it is important to note that the curves generated by inhibitors whose effect is unknown can help to confirm hypotheses about the action of such agents.

The effect of short circuiting the midgut (Fig. 3) is to reduce the slope of the V/I relation in a way

suggestive of an increase in K_l. In the midgut epithelium this "leak" conductance may comprise several anatomically separate passive pathways. It also includes contributions from active pathways other than that for potassium, for example those for Ca^{++} transport (Wood & Harvey, 1976) and Mg^{++} transport (Wood, Jungreis & Harvey, 1975). In principle the residual conductance when K^+ transport is abolished corresponds to the K_l of Yonath and Civan (1971) as their model is applied to the midgut. The apparent change in K_l seen with shortcircuiting could thus arise from changes in active transport of Ca^{++} (Wood & Harvey, 1976), Mg^{++} (Wood, Jungreis & Harvey, 1975), or possibly to ionic readjustments which change cell volume or morphology in such a way as to increase paracellular conductance. As pointed out earlier, opening of intercellular current pathways might also have this effect.

Much attention has been devoted to the electromotive force (E) of ion pumps since this element of equivalent circuits also figures in thermodynamic analyses of the transport process (*c.f.* Hong & Essig, 1976). The behavior of E may thus provide information about metabolic coupling to the transport process. As used in these studies, both sodium and choline seem to satisfy the criteria of nontransportability, nonpenetration, and inertness set out by Ussing and Windhager (1964) for probes of K_a (*see* Harvey, Haskell & Nedergaard, 1968; Zerahn, 1971). Two methods of estimating the electromotive force of the potassium transport system (E_K) are available from the present results: the intersection of V/I plots in 32KS and 32Na, and the slope of the relation K_t/I_{sc}, since the latter slope is equivalent to $1/E$ for processes which affect solely K_a (Yonath & Civan, 1971). Using the former method (*see* Fig. 4), the mean value of E_K for four experiments was 141 ± 18 mV (SD). The latter method seems superior because it depends on values of K_t measured under conditions in which net transport is always occurring, in contrast to the former method in which one value of K_t is determined under conditions of no net transport. For the eight experiments pooled in Fig. 5, the inverse of the slope of the regression line gives an E_K of 190 mV. Although this value is higher than that given by the intersections of V/I plots in the sodium substitution experiments, there is cause to believe that it is a slight underestimate of the E_K. Changes in bathing solution conductance will change K_l in the presence of paracellular shunts in which ions behave as if in free solution. This effect occurs in midgut (*see* Fig. 7). When sodium or choline are substituted mole-for-mole for potassium in the bathing solution, the differences in equivalent conductance between potassium and the substitute ions result in reduction of electrical conductivity. This effect amounts to approximately 10% over the range of potassium concentrations used in the experiments of Fig. 5; it is less for the sodium substitution experiments. A difference in solution conductivity of this magnitude will cause a difference in K_t of about $0.1 \times 10^{-3}\ \Omega^{-1}$, as determined from the data in Fig. 7. Correcting for this difference would increase the value of E_K calculated from Fig. 5 by approximately 30 mV.

At the level of simplification of the Yonath and Civan (1971) model, it is implicit that the individual circuit elements are actually composites; thus, for example, K_a contains a contribution from both the apical and basal membranes of the transporting cells, and E is an overall electromotive force applied to the transported cation which contains a contribution from the pump and from electrochemical gradients along pathways in series with the pump. Some recent models have sought to differentiate between the overall E and K_a and those specifically due to the pump (e.g., Schultz, Frizzell & Nellans, 1977; Lewis, Wills & Eaton, 1978). While at present such a complete circuit model for the midgut could not be constructed with directly determined values, some evidence suggests that the active potassium path is dominated by the properties of the apical membrane. Blankemeyer and Harvey (1977) found that the resistance ratio of apical to basal cell membranes of the presumptive transporting cells was of the order of 4, with the transbasal potential being very small by comparison with the transapical potential. Other studies with conventional intracellular electrodes (Wood, Farrand & Harvey, 1969) and K^+-specific microelectrodes (D.F. Moffett, *unpublished*) suggest that the K^+ distribution across this membrane is close to electrical equilibrium. These findings strongly suggest that the basal membrane of the transporting cells makes little contribution to the net E_K and K_a.

Values of E_K estimated from the present results are somewhat greater than corresponding values determined for sodium-transporting tissues; for example E_{Na} of toad urinary bladder is of the order of 105–158 mV (Yonath & Civan, 1971; Saito, Lief & Essig, 1974; Chen & Walser, 1975; Hong & Essig, 1976); for rabbit urinary bladder, 116 mV (Lewis, Wills & Eaton, 1978); for frog skin, about 130 mV, (Helman & Fisher, 1977); for rabbit colon, 117 mV (Schultz, Frizzell & Nellans, 1977). In contrast, the proton electrogenic pump of *Neurospora* plasma membrane has a reversal potential of about 400 mV, close to the maximum free energy available from ATP hydrolysis (Gradmann et al., 1978).

In some sodium-transporting tissues with relatively small K_l, the V/I plot shows a transition point in the hyperpolarizing region, corresponding to E,

beyond which the slope abruptly increases (Civan, 1970; Helman & Fisher, 1977). No such transition point was noted in the present study, even in experiments in which the transepithelial potential was clamped at values up to 400 mV. While this finding would at first seem to conflict with evidence that the V/I relation of electrogenic pumps is nonlinear (Gradmann et al., 1978), it can be accounted for by the presence of a substantial K_l. This is the case in the midgut as shown in Fig. 5 where extrapolation of the relation K_t/I_{sc} to zero I_{sc} shows that K_l accounts for much of K_t under normal conditions. As noted by Helman and Fisher (1977), this would tend to mask the effect of pump rectification on the V/I plot.

The slopes of the K_t/I_{sc} plots during hypoxic transients and during transient changes in I_{sc} due to changes in potassium concentration are similar (*compare* Figs. 5 and 7). This finding argues that a major, and perhaps the sole, effect of metabolic inhibition is to reduce K_a. Such a result has precedence in the finding of Hong and Essig (1976) that 2-deoxy-D-glucose inhibits sodium transport in toad bladder primarily as a result of its effect on K_a. Blankemeyer and Harvey (1978) have shown that hypoxia greatly increases the apical resistance of goblet cells, suggesting that the change in K_a occurs specifically at the site of K^+ transport.

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