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ION TRANSPORT ACROSS THE MIDGUT OF THE TOBACCO HORNWORM (*MANDUCA SEXTA*)

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Summary

1. The transport of K^+, Na^+ and Cl^- across the three morphologically distinct regions of the tobacco hornworm midgut was measured under open-circuit and short-circuit conditions. Using a saline which contained physiological levels of haemolymph ions, amino acids and sugars, it was shown that all three sections actively secrete K^+ and Cl^- and absorb Na^+.

2. The anterior section maintained the highest short-circuit current (I_{sc}), transepithelial potential difference (PD) and net K^+ secretion. The middle section had the lowest I_{sc}, PD and K^+ secretion, but absorbed Na^+ at the greatest rate. The posterior section had the greatest rate of Cl^- secretion.

3. Omission of K^+ depressed the I_{sc}. Subsequent addition of K^+ stimulated the I_{sc} to control levels in the middle and posterior sections, but not in the anterior section. Omission of Cl^- or Na^+ also inhibited the I_{sc}. Reintroduction of Cl^- had no stimulatory effect and, although reintroduction of Na^+ stimulated the I_{sc}, control levels were not attained.

4. Unlike the results reported in previous studies, the net K^+ transport exceeded the I_{sc} in all three midgut sections. The deficit in I_{sc} was not made up by the transport of Na^+ and Cl^-. The results are discussed with respect to proposed models of ion transport across this epithelium.

Introduction

The physiology of the larval lepidopteran midgut has been studied in a variety of species such as *Manduca sexta*, *Bombyx mori*, *Hyalophora cecropia*, *Philosamia cynthia* and *Spodoptera littoralis*. Of these, *Manduca sexta* has received extensive attention as a model for the study of epithelial ion transport because it is easily raised in the laboratory on an artificial diet and the midgut of this large insect engages in vigorous rates of K^+ secretion (Dow, 1986). In addition, this species is widely used to study the hormonal control of development (Bollenbacher, 1988), feeding behaviour (Timmins *et al.* 1988) and energy metabolism (Gies *et al.* 1988),

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and thus there is a great deal of information on the biology of this organism. Despite the interest in ion transport across the tobacco hornworm midgut (see reviews by Harvey *et al.* 1981, 1983; Harvey, 1980; Blankemeyer and Harvey, 1977; Wolfersberger *et al.* 1982; Dow, 1986), there have been surprisingly few studies of solute fluxes across this epithelium. Except for a recent report of Cl^- transport by Chao *et al.* (1989), only the transepithelial transport of K^+, under short-circuit conditions, has been measured (Cioffi and Harvey, 1981; Wolfersberger and Giangiacomo, 1983; Moffett *et al.* 1983; Moffett and Koch, 1985). The transepithelial transport of other solutes has not been measured in this species.

The tobacco hornworm midgut is composed of three morphologically distinct regions (Cioffi, 1979) which transport K^+ at different rates (Cioffi and Harvey, 1981). Recent studies (Chamberlin, 1990) indicate that the rate of luminal alkalinization also differs among the midgut regions. These studies clearly indicate that the midgut is divided into functional as well as morphological regions, yet there have been no other studies examining regional ion transport. In this communication the transepithelial transport of K^+, Na^+ and Cl^- was measured in the three midgut sections using a saline which contains physiological levels of haemolymph amino acids, sugars and ions. This saline maintains midgut function for several hours (Chamberlin, 1989) and more closely reflects haemolymph composition than the saline used in previous transport studies (see Cioffi and Harvey, 1981). Ion fluxes were monitored under short-circuit conditions to determine if the ions are actively transported. These fluxes were compared to those obtained under open-circuit conditions to determine if the large transepithelial potential, which would be present *in vivo*, affects the rate of ion transport.

Materials and methods

Animals

Tobacco hornworms were grown from eggs or larvae purchased from Carolina Biological Supply Company (Burlington, North Carolina). The larvae were maintained at 28 °C on a 16 h: 8 h light:dark cycle and fed an artificial diet (Carolina Biological Supply Co.). Fifth-stage larvae weighing 3–5.3 g were used in all experiments.

Measurement of transepithelial ion fluxes

Larvae were decapitated and a dorsal incision made to expose the midgut. The anterior, middle or posterior midgut was then cleaned of adhering tracheae and Malpighian tubules, opened, removed from the animal, and stretched over the collar of a modified Ussing chamber (Hanrahan *et al.* 1984). The opening (0.159 cm^2) of the collar was backed with fine nylon screen to support the tissue and the midgut was secured to the collar with a cotton thread. Each half-chamber was filled with 5 ml of control saline (Table 1) and vigorously bubbled with 100 % oxygen.

The PD was measured with calomel electrodes connected to KCl–agar bridges

Table 1. *Composition of salines used in this study*

	Control	K^+-free	Na^+-free	Cl^--free
Na_2HPO_4	6.0	6.0		6.0
K_2HPO_4			6.0	
$MgCl_2$	5.0	5.0	5.0	
$MgSO_4$				5.0
$CaCl_2$	1.0	1.0	1.0	
$CaSO_4$				1.0
KOH	5.8		5.8	5.8
N-methylglucamine		18.0	12.0	
Potassium citrate	7.7		7.7	7.7
Citric acid		7.7		
Sodium succinate	2.8	2.8		2.8
Succinic acid			2.8	
Glucose	2.0	2.0	2.0	2.0
Trehalose	27.0	27.0	27.0	27.0
Glutamine	9.4	9.4	9.4	9.4
Serine	8.9	8.9	8.9	8.9
Proline	7.4	7.4	7.4	7.4
Glycine	12.8	12.8	12.8	12.8
Histidine	9.7	9.7	9.7	9.7
Malic acid	5.6	5.6	5.6	5.6
Threonine	4.6	4.6	4.6	4.6
Alanine	3.6	3.6	3.6	3.6
Polyethylene glycol (M_r 400)	140.0	140.0	140.0	140.0

All values are in mmol l^{-1}.

which were inserted into the saline on either side of the midgut. PD (lumen positive) and I_{sc}, with compensation for saline resistance, was measured with a custom-made voltage clamp (Duke University Physiology Department Technical Shop) and monitored on Soltec recorders. When measuring ion fluxes under short-circuit conditions, the current clamp was turned off briefly during sampling and the PD noted. The specific epithelial resistance was calculated from the I_{sc} and this potential.

Unidirectional K^+, Na^+ and Cl^- fluxes were measured under open-circuit and short-circuit conditions. The tissues were allowed to equilibrate in the chambers for approximately 90 min prior to the measurement of isotopic fluxes. ^{42}K (119–244 mmol l^{-1} KCl, 1.53–3.43 mCi ml^{-1}), ^{22}Na (0.015–0.018 mmol l^{-1}, carrier-free, 1000 μCi ml^{-1}) and ^{36}Cl (1.2 mol l^{-1} HCl, 650 μCi ml^{-1}) were purchased from Dupont/New England Nuclear (Wilmington, Delaware). Unidirectional isotopic fluxes were measured by adding stock ^{42}K, ^{22}Na or ^{36}Cl to one half-chamber (referred to as side I). After 15 min, a 1 ml sample was taken from the other half-chamber (side II) and the volume replaced with 1 ml of isotope-free saline. This sampling procedure was repeated every 15 min for a total of 90 min. A 50 μl sample from side I was diluted with 950 μl of saline and counted to determine

the specific activity of side I. ^{22}Na and ^{42}K were counted on a Beckman 5500 gamma counter and samples containing ^{36}Cl were added to 10 ml of scintillation fluid (scintiverse Bio-HP) and counted on a Beckman LS 8000 scintillation counter.

The results of the flux studies indicated that all three measured ions were actively transported by the midgut tissue. To determine if deletion of these ions would affect the I_{sc}, another set of experiments was performed in which the tissues were mounted in control, K^+-free, Na^+-free or Cl^--free saline (compositions in Table 1) and the I_{sc} monitored after 77–103 min. The I_{sc} of ion-depleted midguts was compared to that of tissues that had been bathed continuously in control saline for 90 min (control in Table 3). Ions were reintroduced bilaterally to ion-depleted midguts in the form of a methylsulphate or *N*-methyl-D-glucamine salt to the final concentration found in control saline. After 15 min the I_{sc} was again noted.

Calculations and statistics

The unidirectional flux for each 15-min period was calculated as described by Hanrahan *et al.* (1984). The radioactivity on side II never exceeded 0.5 % of that of side I and therefore no correction for backflux was necessary. The I_{sc} declined slightly with time and therefore a comparison between tracer fluxes with the I_{sc} measured at the time of sampling would underestimate the I_{sc} over the entire flux period. A comparison of I_{sc} with tracer fluxes was made by integrating the I_{sc} chart recordings with the use of a Hewlett Packard digitizing pad and calculating the area using a computer program. All values are presented as means±standard errors with N indicating the number of midguts. Statistical analyses were conducted using a Student's *t*-test. $P \leq 0.05$ was considered to represent a significant difference.

Throughout this paper I_{sc} and ion fluxes are reported in units of $\mu equiv\,cm^{-2}\,h^{-1}$. The midgut epithelium, however, varies in thickness along its length and the amount of cellular material per unit area will be different for the three midgut regions. Surface area can be converted to mass by using the factors reported by Cioffi and Harvey (1981; anterior: $68\,mg\,cm^{-2}$; middle: $52\,mg\,cm^{-2}$; posterior: $112\,mg\,cm^{-2}$).

Results

Fig. 1 shows the time course of I_{sc} and PD for the three midgut sections. Note that there is an initial rise then a fall in these measurements. During the 90-min experimental period, however, the I_{sc} and PD declined only slightly (see Figs 2–7). The resistances were 200.2±7.7, 226.5±15.6 and 216.6±9.2 Ωcm^2 (N=43) for the anterior, middle and posterior sections, respectively. Steady-state fluxes were achieved 30 min after addition of isotope, indicating that, at most, 30 min was necessary for isotopic mixing in the extracellular and intracellular ion pool. Net K^+ and Cl^- secretion and net Na^+ absorption occurred in all three midgut sections under open- and short-circuit conditions (Figs 2–7). These net

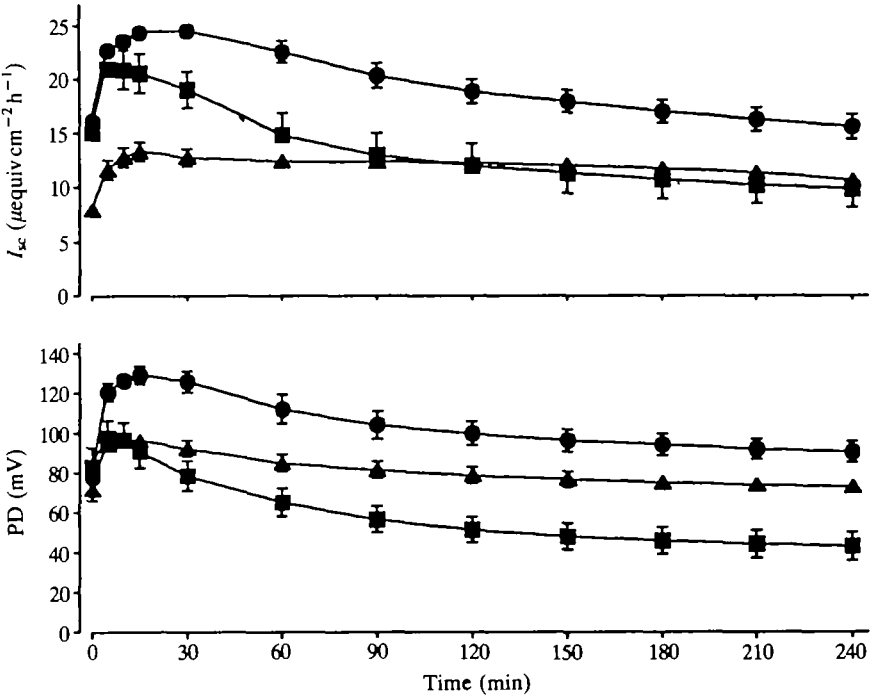

Fig. 1. Changes in short-circuit current and potential difference (PD) with time for the three sections of the tobacco hornworm midgut: (●) anterior section, (■) middle section, (▲) posterior section.

rates of ion transport remained constant throughout the entire 90 min experimental period.

Unidirectional fluxes measured between 30 and 45 min after isotope addition are summarized in Table 2. The flux ratios of K^+, Na^+ and Cl^- were all greater than 1 under short-circuit conditions, indicating active transport. Under open-circuit conditions there were no regional differences in the K^+ flux from haemolymph to lumen ($J^K_{H\rightarrow L}$) or lumen to haemolymph ($J^K_{L\rightarrow H}$). In contrast, under short-circuit conditions, the anterior section had a significantly greater $J^K_{H\rightarrow L}$ and significantly smaller $J^K_{L\rightarrow H}$ than the other sections. The middle section had the greatest $J^{Na}_{L\rightarrow H}$ under open-circuit conditions, but there were no regional differences in $J^{Na}_{H\rightarrow L}$ when the tissue was short-circuited. There was a significant increase in $J^{Na}_{H\rightarrow L}$ of the anterior section when this flux was measured under short-circuit conditions and this flux was significantly greater than that measured in the middle and posterior sections. The $J^{Cl}_{H\rightarrow L}$ of the posterior midgut was significantly higher than that of the other sections under short-circuit conditions, but only greater than that of the anterior section under open-circuit conditions.

Omission of K^+ caused a marked drop in I_{sc} in all midgut sections (Table 3). Reintroduction of K^+ stimulated the I_{sc} in all regions, but control levels of I_{sc} were regained only in the middle and posterior sections. The omission of Na^+ and Cl^-

2. *Unidirectional fluxes in different midgut regions under open-circuit and short-circuit conditions*

	Anterior		Middle		Posterior	
	$J_{H\rightarrow L}$	$J_{L\rightarrow H}$	$J_{H\rightarrow L}$	$J_{L\rightarrow H}$	$J_{H\rightarrow L}$	
litions						
	30.97±4.59	6.43±2.07	23.42±2.52	6.90±1.47	22.26±3.82	4.
	(6)	(6)	(6)	(6)	(6)	
	0.04±0.02*	3.77±0.46†	0.03±0.02	5.31±0.43*†	0.08±0.03	3.
	(8)	(8)	(8)	(8)	(8)	
	3.47±0.32*†	0.86±0.09†	3.75±0.58	0.56±0.10‡	5.10±0.28	1.
	(8)	(8)	(8)	(8)	(8)	
litions						
	37.03±1.81†‡	1.28±0.27†‡	22.41±0.99	4.49±0.98	23.28±4.77	2.
	(6)	(5)	(6)	(5)	(6)	
	0.17±0.05†‡	2.77±0.25	0.04±0.03	3.67±0.56	0.05±0.02	3.
	(8)	(8)	(8)	(8)	(8)	
	2.37±0.33‡	1.31±0.13†	2.67±0.29‡	0.76±0.08	4.09±0.47	1.
	(8)	(8)	(8)	(8)	(8)	

$cm^{-2}h^{-1}$) are presented as mean±S.E. with N indicated in parentheses.
nificant difference from values obtained under short-circuit conditions.
e values that are significantly different from those obtained for the middle and posterior sections, respectively.

Table 3. *Effects of ion substitution and reintroduction on the* I_{sc} *across the different regions of* Manduca sexta *midgut*

	Anterior	Middle	Posterior
Control	20.30±1.13 (6)	12.99±2.03 (6)	12.52±0.60 (6)
K^+-free	0.88±0.42 (5)*	−0.13±0.34 (5)*	1.24±0.44 (5)*
$+K^+$	12.77±1.71 (5)*†	8.29±0.87 (5)†	10.01±1.12 (5)†
Na^+-free	13.56±1.78 (4)*	5.00±0.67 (3)*	7.14±0.90 (4)*
$+Na^+$	14.39±1.98 (4)*†	5.45±1.17 (3)*†	9.01±0.96 (4)*†
Cl^--free	7.99±0.95 (4)*	3.78±0.30 (4)*	9.17±0.93 (4)*
$+Cl^-$	7.26±0.83 (4)*	3.25±0.26 (4)*	8.63±0.95 (4)*

The I_{sc} (μequiv cm^{-2} h^{-1}) is expressed as mean±S.E. with N indicated in parentheses.
* denotes a significant difference from the control.
† indicates a significant stimulation by the reintroduction of the ion.

Table 4. *Comparison of integrated short-circuit current and net* K^+, Na^+ *and* Cl^- *fluxes*

Midgut section	I_{sc}	Net K^+ flux	Net Na^+ flux	Net Cl^- flux
Anterior	17.5	35.8	−2.6	−1.1
Middle	7.7	17.9	−3.6	−1.9
Posterior	11.7	20.8	−3.0	−2.7

All values are expressed as μequiv cm^{-2} h^{-1} (means±S.E., N=8).
The I_{sc} was determined by integration (see Materials and methods) and the net flux is the difference between unidirectional fluxes measured 45 min after isotope addition.
The negative sign indicates that the flux opposes the I_{sc}.

also caused a depression of the I_{sc}. Reintroduction of Na^+ significantly stimulated the I_{sc}, but control values were not reached. Reintroduction of Cl^- had no effect.

A comparison of integrated I_{sc} with isotope fluxes is shown in Table 4. Note that the I_{sc} is not equivalent to the sum of net K^+, Na^+ and Cl^- fluxes. The disparities between net ion transport and the I_{sc} for the anterior, middle and posterior sections were 14.6, 4.7 and 3.4 μequiv cm^{-2} h^{-1}, respectively.

Discussion

Previous measurements of transepithelial ion transport across tobacco hornworm midgut involved bathing the tissue with a simple saline which contained K^+ as the only monovalent cation and sucrose as the only exogenous metabolic substrate (see Cioffi and Harvey, 1981; Crawford and Harvey, 1988). In this saline, the I_{sc} would rise and then fall in a biphasic fashion. The results presented in this study indicate that midguts bathed in a more complex saline also display an initial

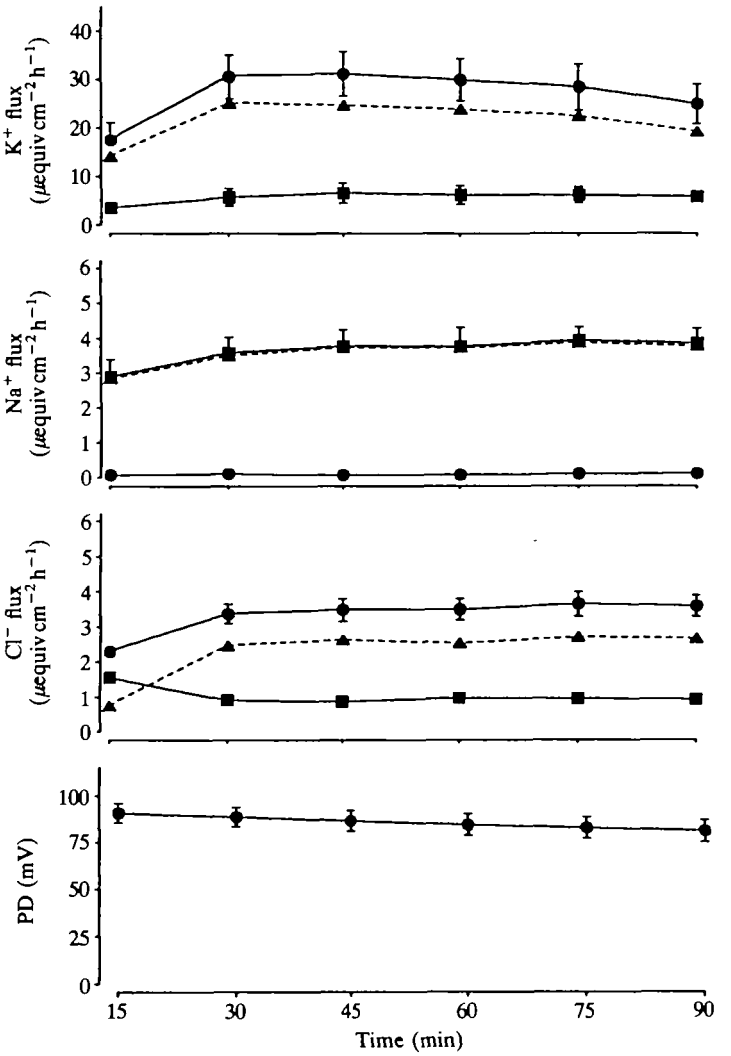

Fig. 2. Open-circuit ion fluxes and PD in the anterior section of the midgut: (■) unidirectional flux from lumen to haemolymph, N=6–8; (●) unidirectional flux from haemolymph to lumen, N=6–8; (▲) net flux. Isotope was added at time 0 and PD was measured at the time of sampling. Error bars have been omitted if smaller than the symbol.

rise and subsequent fall in PD and I_{sc}. After approximately 90 min, however, the rates of PD and I_{sc} decline were less than 2 % h^{-1} (calculated from values in Figs 2–4) and 0.5 % h^{-1} (calculated from values in Figs 5–7), respectively. These rates are far slower than the 'pseudo steady-state' decline of 20 % h^{-1} reported by Crawford and Harvey (1988). These results, along with those of Chamberlin (1989), indicate that the midgut requires the presence of metabolic substrates and/or other ions to sustain *in vitro* performance.

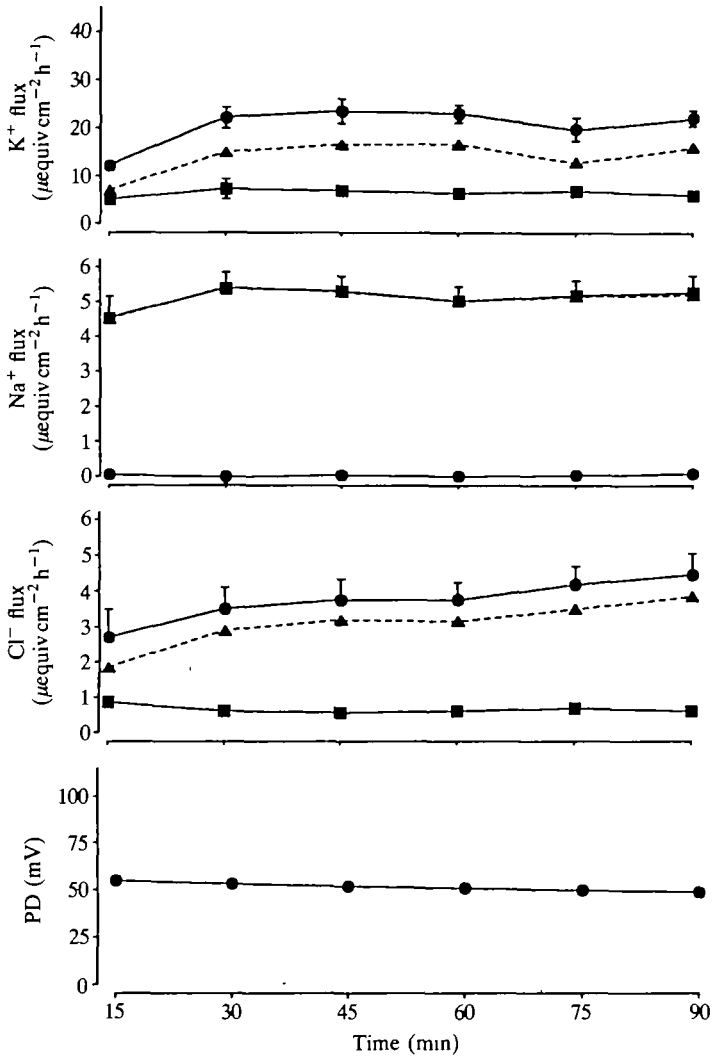

Fig. 3. Open-circuit ion fluxes and PD in the middle section of the midgut: (■) unidirectional flux from lumen to haemolymph, N=6–8; (●) unidirectional flux from haemolymph to lumen, N=6–8; (▲) net flux. See Fig. 2 for other details.

Researchers, using the simple saline described above, have reported the I_{sc} to be as low as 243 μA cm^{-2} (9.1 μequiv cm^{-2} h^{-1}; Mandel *et al.* 1980) and as high as 1159 μA cm^{-2} (43.2 μequiv cm^{-2} h^{-1}, Cioffi and Harvey, 1981). Similarly, resistances have been reported from 78.3 Ωcm^2 (Moffett, 1980) to 230 Ωcm^2 (Harvey and Wolfersberger, 1979). These disparities may arise from the use of different-sized larvae and reflect the changing properties of the midgut epithelium as larval growth proceeds. In this study the larvae were generally smaller and the saline more complex than those used in previous studies and therefore quantitative comparisons between this and previous studies should be made judiciously.

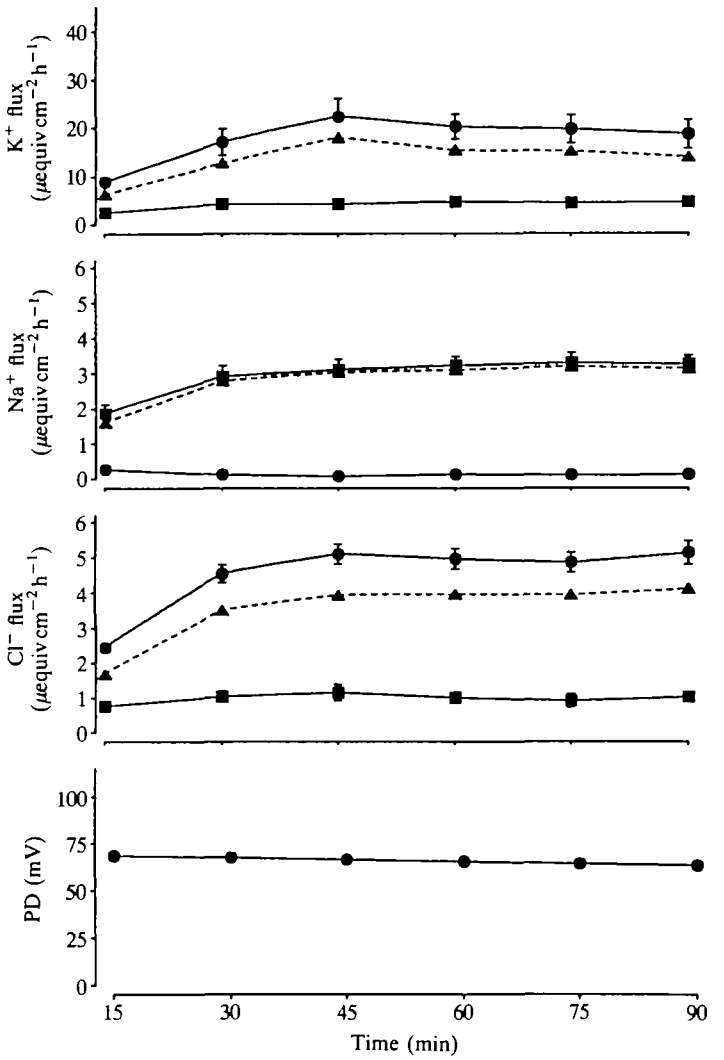

Fig. 4. Open circuit ion fluxes and PD in the posterior section of the midgut: (■) unidirectional flux from lumen to haemolymph, N=6–8; (●) unidirectional flux from haemolymph to lumen, N=6–8; (▲) net flux. See Fig. 2 for other details.

The midgut of the tobacco hornworm is composed of three distinct regions which differ in the gross epithelial folding pattern as well as in the structure of the columnar and goblet cells (Cioffi, 1979). This structural heterogeneity is reflected in regional differences in I_{sc}, PD and rates of ion transport. The anterior section consistently had the highest I_{sc} and PD, while the middle section had the lowest. Whereas there were no regional differences in K^+ transport under open-circuit conditions, under short-circuit conditions the rate of K^+ secretion was greatest in the anterior section. This contrasts with the findings of Cioffi and Harvey (1981), in which the anterior section was shown to have the lowest rate of K^+ secretion and

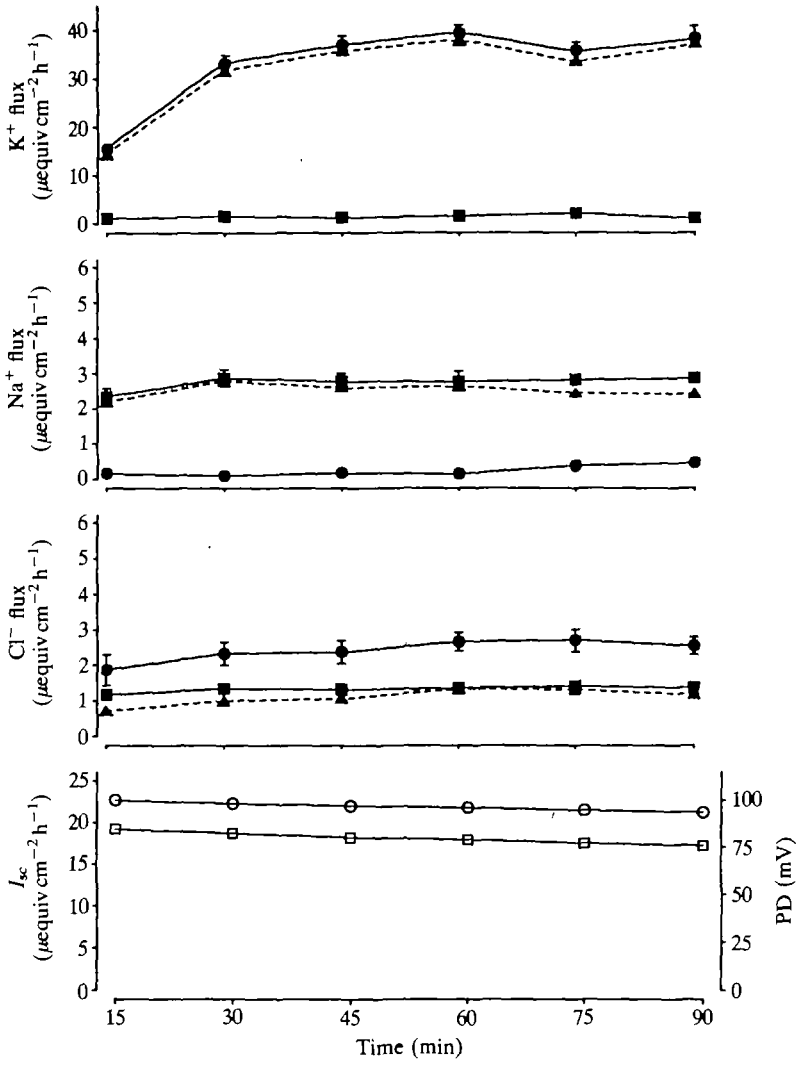

Fig. 5. Ion fluxes under short-circuit conditions in the anterior section of the midgut: (■) unidirectional flux from lumen to haemolymph, N=5–8; (●) unidirectional flux from haemolymph to lumen, N=6–8; (▲) net flux. Isotope was added at time 0, I_{sc} (□, μequiv cm^{-2} h^{-1}) was measured at the end of each 15-min flux period, and the PD (○) was determined when the I_{sc} was briefly turned off during sampling. Error bars have been omitted if smaller than the symbol.

I_{sc}. The $J^{K}_{L\rightarrow H}$ in the posterior region increased significantly under open-circuit conditions, indicating that this process is energized, to some extent, by the PD.

In previous studies the net K^+ flux was virtually equivalent to the I_{sc}. In this study the K^+ flux exceeded the I_{sc} in all three midgut sections. Omission of K^+ produced a large fall in I_{sc}, indicating that K^+ transport is electrogenic, although allosteric effects of K^+ on other transporters cannot be discounted. Reintroduc-

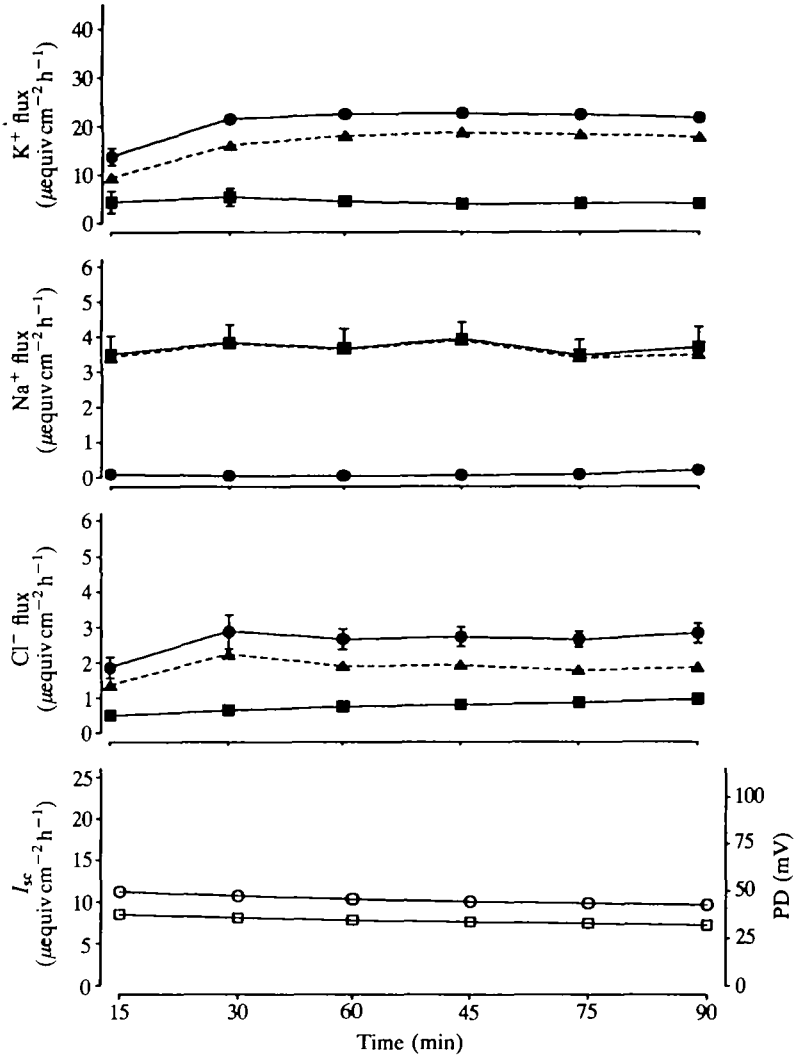

Fig. 6. Ion fluxes under short-circuit conditions in the middle section of the midgut: (■) unidirectional flux from lumen to haemolymph, N=5–8; (●) unidirectional flux from haemolymph to lumen, N=6–8; (▲) net flux. Other symbols and experimental details are as in Fig. 5.

tion of K^+ stimulated the I_{sc}, although, in the anterior section, the control values of I_{sc} were not achieved by this manoeuvre. This may indicate that ion substitution can cause non-specific damage to the epithelium, such as perturbation in cell volume regulation or metabolic impairment.

The mechanism of electrogenic K^+ secretion across lepidopteran midgut is not well understood. Several workers have proposed that electrogenic K^+ secretion occurs *via* an apically located K^+-ATPase (reviewed by Dow, 1986). The evidence for this model is based upon measurements of the K^+ electrochemical gradient

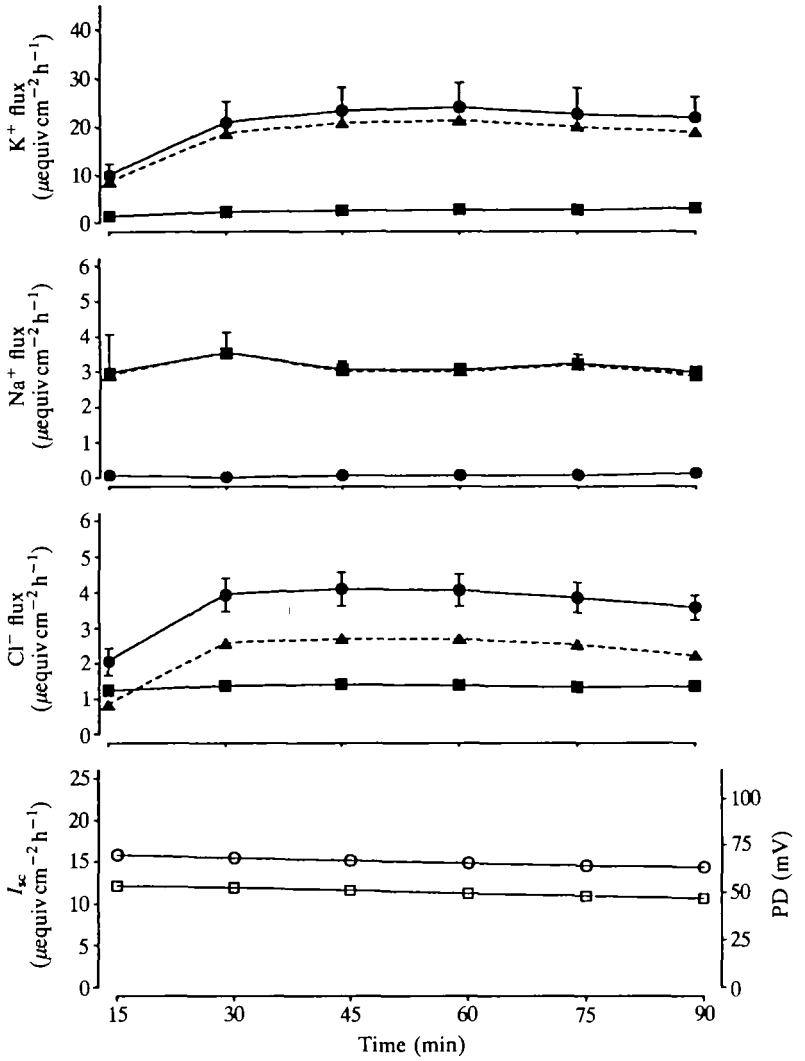

Fig. 7. Ion fluxes under short-circuit conditions in the posterior section of the midgut: (■) unidirectional flux from lumen to haemolymph, N=8; (●) unidirectional flux from haemolymph to lumen, N=8; (▲) net flux. Other symbols and experimental details are as in Fig. 5.

across the apical membranes and the isolation of the K^+-stimulated ATPase from goblet cell membranes (Wieczorek *et al.* 1986). Recently, however, evidence has been put forward to support a different model of K^+ secretion. Schweikl *et al.* (1989) and Wieczorek *et al.* (1989) have shown that the midgut cells have a H^+-ATPase which secretes hydrogen into the goblet cell lumen. The H^+ is then exchanged for K^+. The function of the H^+-ATPase is not to acidify the lumen, but to generate a large electrical gradient across the apical membrane which will drive H^+ absorption and K^+ secretion *via* the H^+/K^+ exchanger. Assuming an

intracellular pH of 7.2, under the conditions of previous studies (saline pH=8.3; see Cioffi and Harvey, 1981) only the electrical component of the electrochemical gradient would drive the H^+/K^+ exchanger. Therefore, the current generator (the H^+-ATPase) would determine the rate of K^+ secretion. In this study, the saline was adjusted to haemolymph pH of 6.7. This pH is lower than the assumed intracellular pH and thus there would be a chemical as well as an electrical driving force for the H^+/K^+ exchanger. Under these conditions, the rate of K^+ secretion *via* the H^+/K^+ exchanger may exceed that of the electrogenic H^+-ATPase.

In the study by Wieczorek *et al.* (1989) the presence of anions stimulates apical membrane H^+ secretion, but dissipates the membrane potential. Thus, an anion conductance must exist on the apical membrane. In support of this view, Cl^- is secreted by all regions of the midgut. Omission of Cl^- causes a significant drop in I_{sc}. This may be due to inhibition of apical anion conductance and therefore limit the secretion of H^+ by the apical H^+ pump. Alternatively, the removal of Cl^- may inhibit entry of K^+ *via* basal KCl cotransporters (Zeiske and Schroder, 1988) and thus suppress the I_{sc}. It should be noted, however, that introduction of Cl^- to tissue bathed in Cl^--free saline did not stimulate I_{sc}. This may indicate non-specific damage caused by long-term Cl^- deletion, as has been described in renal epithelia (Chamberlin *et al.* 1984).

Recently Chao *et al.* (1989) have demonstrated Cl^- absorption across the posterior midgut of *M. sexta*. This observation contrasts with the observations made in the present study. The conditions of the two studies differ in the composition of saline and size of larvae used and these differences may account for the conflicting observations. Interestingly, Chao *et al.* (1989) postulate that there may be more than one transepithelial Cl^- transport system and speculate that Cl^- secretion may also occur. The conditions of the present study appear to have been appropriate for revealing this secretory process. This secretion of Cl^- may account for the higher luminal Cl^- concentration in the posterior *versus* anterior sections of the midgut observed *in vivo* (Dow *et al.* 1984).

Dow *et al.* (1984) failed to detect intracellular levels of Na^+ in the midgut of *M. sexta*, yet there is Na^+ in the midgut contents and haemolymph. Since there is a favourable electrochemical gradient for Na^+ entry from either the haemolymph or lumen (see Moffett and Koch, 1988*a*,*b*; Dow *et al.* 1984) and since it is unlikely that the membranes are absolutely impermeable to Na^+, it would seem plausible that Na^+ is transported by the midgut. This hypothesis was confirmed in this study in which all three midgut sections actively transported Na^+. The $J^{Na}_{H\rightarrow L}$ was negligible, indicating that Na^+ permeability was low and edge damage was minimal. Sodium may be cotransported across the apical membranes with amino acids since it has been shown that Na^+ gradients can energize amino acid transport in membrane vesicles isolated from the midgut of *M. sexta* (Hennigan and Wolfersberger, 1989) as well as that of another caterpillar, *Philosamia cynthia* (Hanozet *et al.* 1984). It is not clear, however, what mediates Na^+ efflux, although a Na^+ pump seems unlikely since efforts to isolate a Na^+/K^+-ATPase from this tissue have been unsuccessful (Jungreis and Vaughan, 1977).

It is clear that Na^+ transport by *M. sexta* midgut differs from that of *Hyalophora cecropia* midgut. In *H. cecropia* net Na^+ secretion has been demonstrated and complete replacement of K^+ with Na^+ can maintain a substantial I_{sc} (Zerahn, 1971). This is clearly not the case for *M. sexta*. Moffett (1980) showed that complete substitution of K^+ with Na^+ results in a negligible I_{sc}. In this study, the removal of K^+ led to a precipitous fall in I_{sc}, although 17.6 mmol l^{-1} Na^+ was present. Although there is net absorption of Na^+ by *M. sexta* midgut, omission of

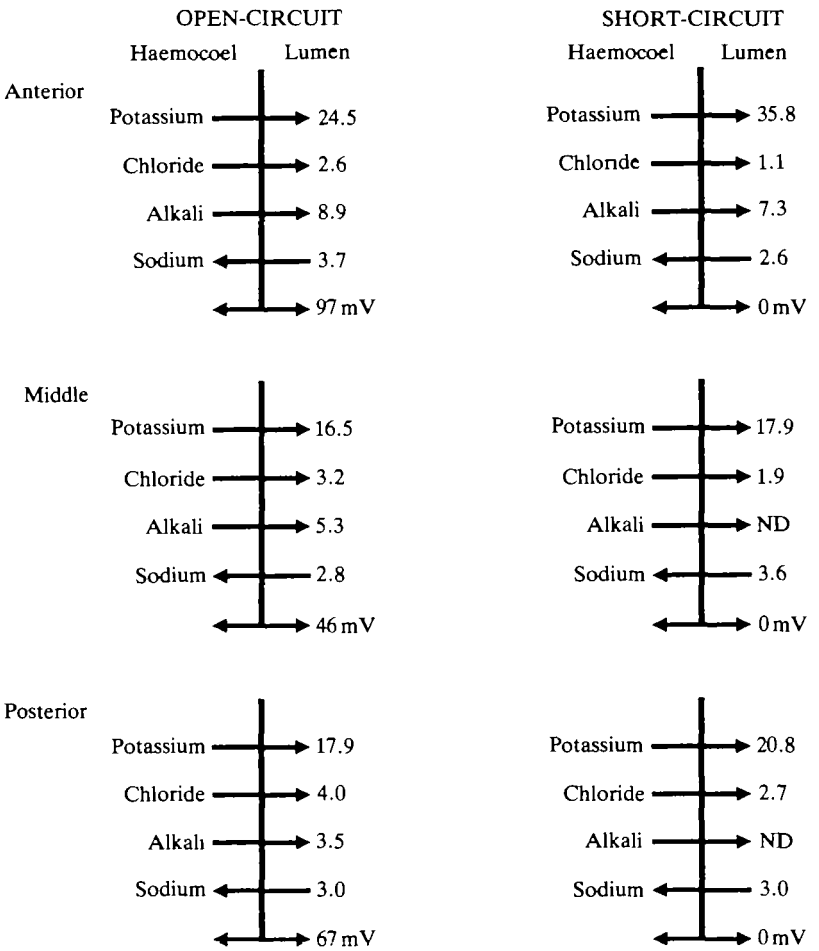

Fig. 8. A summary of net transepithelial fluxes across the different regions of the tobacco hornworm midgut under open-circuit and short-circuit conditions. The fluxes are expressed in units of μequiv cm^{-2} h^{-1}. The transepithelial potential is expressed with reference to the haemolymph side of the tissue. Alkali fluxes are those reported in Chamberlin (1990). ND, not determined.

Na^+ from the saline inhibited the I_{sc}. This may reflect metabolic inhibition due to impaired substrate uptake. Alternatively, the presence of Na^+ may stimulate K^+ entry and I_{sc}, as has been suggested by Thomas and May (1984).

Under open-circuit conditions, net K^+ secretion is not balanced by Na^+ absorption and Cl^- secretion (see Fig. 8). Charge neutrality may be kept by secretion of anions such as HCO_3^-, phosphate, citrate or succinate. In addition, absorption of cations such as basic amino acids, H^+, Mg^{2+} or Ca^{2+} could maintain charge balance. The presence of carbonic anhydrase in midgut tissue (Ridgway and Moffett, 1986), high luminal pH *in vivo* (Dow, 1984) and luminal alkalinization *in vitro* (Chamberlin, 1990) indicate that HCO_3^- secretion or H^+ absorption is plausible. The possibility of polyvalent or organic cation absorption is strengthened by the observations that these processes occur in other lepidopteran larvae (Hanozet *et al.* 1989; Wood and Harvey, 1976; Wood *et al.* 1975). The midgut of *M. sexta* actively secretes anionic dyes (Nijhout, 1975) and therefore secretion of polyvalent organic anions present in the haemolymph (e.g. succinate, citrate) is conceivable. Elucidation of these transport processes in this species awaits further experimentation.

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