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ACTIVE TRANSPORT BY THE CECROPIA MIDGUT

V. LOSS OF POTASSIUM TRANSPORT DURING LARVAL-PUPAL TRANSFORMATION

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INTRODUCTION

Few serious attempts have been made to study either alterations of active ion transport during ontogenic development or the developmental consequences of altered ion environments within and around cells (see Kroeger, 1966 and Harvey & Haskell, 1966 for references). Nevertheless, to cite but a few examples of the importance of the ionic mileau for molecular activities associated with development; protein synthesis in cells and cell-free systems from bacteria has a potassium optimum (Lubin & Ennis, 1964); ribosomal structure and activity require magnesium (Tissières & Watson, 1958); and the complexing of DNA with histone depends on ionic strength (Huang, Bonner & Murray, 1964).

The isolated midgut of the silkworm *Hyalophora cecropia* is favourable material for studies of alterations of ion transport during development. Both the oxidative metabolism and the timing of nucleic acid and protein synthesis have been examined in relation to the well-known endocrine system of Cecropia (for references see Krishnakumaran, Berry, Oberlander & Schneiderman, 1967). Active potassium transport, which dominates active ion transport in the larval midgut, has been reasonably well characterized (Harvey & Nedergaard, 1964; Haskell, Clemons, & Harvey, 1965; Anderson & Harvey, 1966; Harvey, Haskell & Zerahn, 1967; Harvey & Haskell, 1968). The cells of the larva do not ordinarily divide but simply become larger as the insect grows. Two principal hormones, ecdysone and juvenile hormone, appear to alter the expression of genes contained within the stable cellular population, although a specific action of neither of these hormones on the midgut has ever been reported. Because synthetic ecdysone (Siddall, Cross & Fried, 1966) and juvenile hormone (see Williams, 1967, have recently become available, a rigorous assessment of their effects on *in vitro* ion transport has become feasible.

Cecropia larvae feed on an unlimited supply of leaves from deciduous trees and at maturity metamorphose to pupae which subsequently enter a prolonged dormant state known as diapause. The lumen of the larval midgut is swamped with water, nutrient, and potassium contained within the leaves. Evidently the midgut tissue has available considerable sources of energy to prevent the entrance of potassium into the blood while nutrients are absorbed from the midgut lumen. By contrast diapausing pupae are not exposed to the high concentrations of potassium from leaves because

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they do not feed. The pupa can live on stored water and nutrients for as long as 3 years and yet have sufficient reserves to develop into a functional adult moth. The oxygen consumption of the larval midgut is among the highest on record for any cell or tissue (Harvey, Haskell & Zerahn, 1967) and far exceeds that of the entire diapausing pupa (Schneiderman & Williams, 1953).

In the present paper we shall present evidence that the active tranport of potassium is curtailed while passive permeability to potassium remains low at the close of the feeding stage in synchrony with the earliest phase in the transformation of the larval epithelium to pupal epithelium. Apparently the curtailment is synchronized with an increase in ecdysone level and a drop in juvenile hormone level but neither synthetic ecdysone nor juvenile hormone appears to have a direct effect on the active potassium transport by midguts isolated from mature feeding larvae.

Table 1. *The time course of development of* Hyalophora cecropia *from the fourth larval instar through pupation at* 27° *C.*

Stage	Day	Description
Feeding fourth-instar larva	−8·0	Newly moulted fourth-instar insect starts to feed
Moulting fourth-instar larva	−3·5	Moulting process begins with retraction of integument from red and yellow tubercles
	−2·0	Insect begins to spin moulting pad
	−0·5	Insect's head is withdrawn from old capsule which is now translucent and grey-green in colour
	−0·25	Outer integument is very wrinkled and soon will be cast
Feeding fifth-instar larva	0	Moulting ends as insect sheds fourth-instar skin and becomes a feeding fifth-instar larva
Evacuation of midgut	10	Gut evacuation occurs (1–2 hard faecal pellets followed by 1–2 large green faecal masses and several ml. of green-yellow fluid). The process takes about 10 min., after which the insect begins to wander
Spinning of cocoon	10·5	Insect begins to spin cocoon
	11	Outer cocoon is finished and tanned
	12·5	Inner cocoon is finished and tanned
	20	Pupation occurs

MATERIALS AND METHODS

Larvae of *Hyalophora cecropia* (L.) were fed on shoots of *Salix babylonica* (weeping willow) or *Prunus serotina* (wild black cherry). Additional larvae reared on cherry were provided by Dr William H. Telfer. Two days prior to use, larvae were brought into a laboratory where the temperature ranged from 25 to 30° C. and the relative humidity ranged from 50 to 65%. A detailed study of external characteristics diagnostic of developmental stage including observations on weight and activity was performed by Mr Fredric Oltsch. From his data and other observations a time-table was constructed covering the period from the feeding fourth-instar larval stage to the end of the pupal moult. The time-course of development is summarized in Table 1. Stages used

in the present study include: feeding fourth-instar larvae, insects moulting from fourth to fifth instar, feeding fifth-instar larvae, insects which have just evacuated the contents of their midguts and insects which are spinning cocoons (see Fig. 1).

Although the midguts of fourth instar larvae are relatively small and the midguts of insects spinning cocoons are delicate, the techniques described by Nedergaard & Harvey (1968) for narcotization of the fifth-instar larvae and dissection and cannulation of its midgut proved satisfactory for all of the stages studied. The electrical potential difference across the isolated midgut wall was measured as described by Nedergaard & Harvey, (1968) and the short-circuit current as described by Harvey, Haskell & Zerahn (1967). Unless otherwise stated, all midguts were perfused with aerated saline containing 30 mM/l. KCl, 2 mM/l. $KHCO_3$, 5 mM/l. $CaCl_2$, 5 mM/l. $MgCl_2$ and 166 mM/l. sucrose.

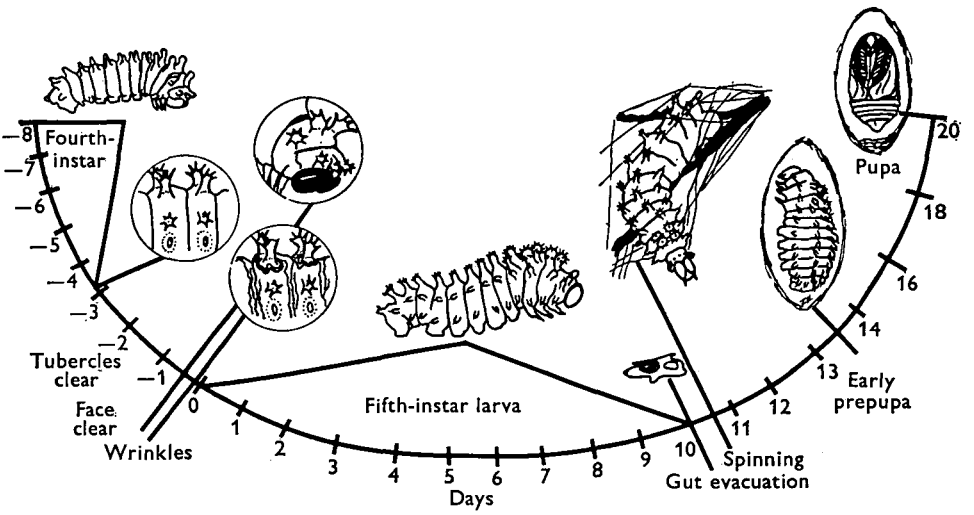

Fig. 1. Time sequence of developmental stages of *Hyalophora cecropia*, fourth larval instar through pupation. Day 0 marks the moult from the fourth to the fifth instar.

RESULTS

Initial maximal potential

The magnitude of the potential generated by the isolated Cecropia midgut can be recorded as early as 1 min. after the gut has been dissected from the insect and perfused in the glass chamber. The potential rises during the first 5–10 min. of perfusion, reaches a maximum and then gradually declines (see Fig. 1: Harvey, Haskell & Nedergaard, 1968). Most of the experiments in this study occupied no more than 5 hr.; however, under identical conditions isolated midguts exhibit sizeable potentials for as long as 24 hr. (Harvey & Nedergaard, 1964). The highest potential measured in the minutes immediately following the isolation of the gut will be referred to as the initial maximal potential.

The magnitude of the initial maximal potential is plotted as a function of the developmental stage of the larva for individuals from the fourth larval instar through the spinning of the cocoon in Fig. 2. The lumen of the isolated gut was always positive

with respect to the blood-side; however, the magnitude of the potential varied significantly with the developmental stage of the donor larva (Table 2). Midguts isolated from feeding fourth-instar larvae yielded an average potential of 68 mV. This value increased rather abruptly to 104 mV. immediately prior to the moult from the fourth to the fifth instar. The value dropped to about 92 mV. during most of the fifth instar. Toward the latter part of the fifth instar, as judged from the increase of the larval weight to over 13 g., the potential dropped slightly but significantly to 74 mV. For less than 1 hr. following the evacuation of the gut contents the potential reached a

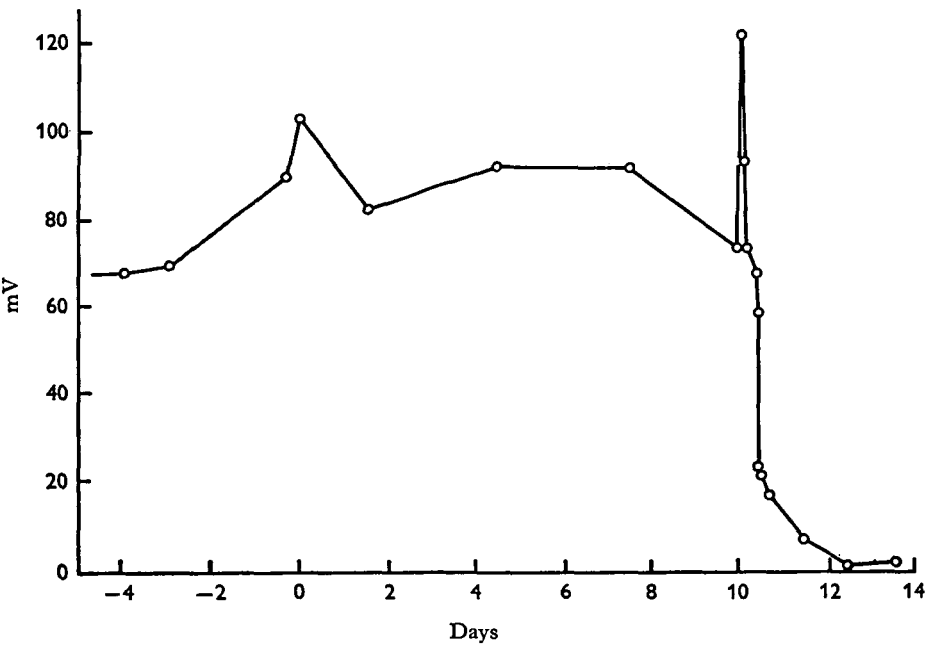

Fig. 2. The initial maximal potential of the isolated midgut of Cecropia during five stages of development. Note potential increases immediately before moult to fifth instar (day −0·25) and immediately after evacuation of the midgut contents (day 10). A permanent loss of potential occurs after gut evacuation.

peak of 124 mV. The potential then fell precipitously during the next 12 hr. and usually was lost completely at the onset of cocoon spinning or shortly thereafter. The potential was not demonstrable at any time during the subsequent prepupal or pupal transformations.

Effects of cation changes on the potential during development

To examine contributions of specific cations to the potential changes just reported, a study of the effects on the potential of changing the concentration of specific cations in the bathing solution was performed at each of the five developmental stages. The concentration of a single cation was changed on either the blood-side, the lumen-side, or on both sides of the midgut by replacing some or all of the experimental cation with an indifferent cation such as choline or sodium. The effect on the potential was expressed in terms of the slope of the potential/log ion concentration curve (Nernst slope).

Table 2. *The initial maximal potential exhibited by the midgut of the Cecropia larva during stages from fourth instar through cocoon spinning*

No. of experiments	Stage	Initial maximal potential ±S.E. (mV.)
32	Fourth instar	68 ± 6·5
12	Moulting fourth (−3·5 days)	69 ± 9·6
10	Moulting fourth (−0·5 days)	90 ± 14
4	Moulting fourth (−0·25 days)	104 ± 12
26	Moulting fourth (all)	83 ± 5·8
4	Fifth instar (1–5 g.)	83 ± 11
22	Fifth instar (5–9 g.)	92 ± 7·9
47	Fifth instar (9–13 g.)	92 ± 2·4
10	Fifth instar (> 13 g.)	74 ± 1·4
83	Fifth instar (all)	90 ± 2·4
8	Evacuated gut (< 1 hr.)	124 ± 3·9
4	Evacuated gut (1–2 hr.)	94 ± 5·4
6	Evacuated gut (3 hr.)	75 ± 9·4
5	Evacuated gut (10 hr.)	68 ± 15·6
1	Evacuated gut (11 hr.)	59
2	Evacuated gut (12 hr.)	23
7	Spinning cocoon (1 hr.)	22 ± 5·9
3	Spinning cocoon (3 hr.)	15 ± 7·4
13	Spinning cocoon (20 hr.)	7·9 ± 3·0
4	Spinning cocoon (44 hr.)	0·5 ± 1·5
7	Spinning cocoon (72 hr.)	2·1 ± 1·0

Potassium. As previously reported, when the potassium concentration in solutions bathing the blood-side, lumen-side, or both sides of the midgut isolated from a mature fifth-instar larva was changed from 32 to 2 mM/l., the average slope of the potential/log ion concentrations curve was +34, −7, and +22 mV., respectively (Harvey, Haskell & Nedergaard, 1968). To confirm the earlier report similar experiments were repeated on ten mature fifth-instar larvae and values of +34, −6 and +20 mV., respectively, were obtained (Table 3 and Fig. 3). In Table 3, the results of alterations in potential following potassium changes in the solutions bathing midguts from the other developmental stages are also recorded. The changes in the potential were always in the same direction as those just described for the mature fifth-instar insects but usually corresponded to a somewhat smaller slope.

Examination of the individual values for mature fifth-instar larvae reveals that the magnitude of the Nernst slope is dependent upon the potential exhibited by the midgut just prior to the change in ion concentration—the higher the prior potential the greater the change in potential when the potassium concentration is lowered. When the potential prior to a decrease in potassium concentration on the blood-side was above 100 mV., the slope approached the theoretical value of 59 mV. (Fig. 4A). Extrapolating the line of least squares in Fig. 4A (blood-side) to zero potential prior to the concentration change yields a slope of +17 mV. A similar relationship holds for changes of potassium concentration on the lumen-side except that even with the largest prior potentials a slope of only −15 mV. is observed and with zero prior potential the slope amounts to but −1 mV.

Table 3. *Effects obtained by changing the concentration of K, Ca, Mg, and Na in the solution bathing either the blood-, lumen-, or both sides of the midgut*

Stage of experimental animal	$\Delta E/\Delta \log [C^+]$ + S.E. (mV)		
	Lumen-side	Blood-side	Both sides
	K: 32 to 2 mM/l.		
Fourth	−4·6±1·4	+16±4·1	+5·9±1·9
Moulting fourth	−11±5·5	+24±5·5	+12±2·5
Fifth	−6·3±2·9	+34±3·9	+20±3·0
Evacuated gut	−4·8±2·2	+33±5·4	+17±4·0
Spinning	−1·8±1·0	+4·8±0·8	+2·6±1·3
	Ca: 5 to 0·5 mM/l.		
Fourth	+7·3±2·5	−4·5±1·4	0·0±2·9
Moulting fourth	+9·8±3·8	+5·6±2·7	+6·8±1·6
Fifth	+14±1·0	−4·5±3·0	+4·0±1·8
Evacuated gut	+12±3·8	−4·2±3·6	+2·3±5·6
Spinning	+1·0±0·4	−0·2±0·4	−0·6±0·4
	ΔE ± S.E. (mV.)		
	Mg: 5 to 0 mM/l.		
Fourth	+0·2±1·2	−2·0±2·1	+4·8±2·6
Moulting fourth	+1·1±1·6	+1·1±1·8	+0·4±0·5
Fifth	+1·1±1·6	+1·3±0·9	+1·3±0·6
Evacuated gut	+2·4±1·8	+3·9±3·0	+0·6±0·9
Spinning	−0·5±1·0	−5·1±2·1	+0·6±1·4
	Na: 0 to 32 mM/l.		
Fourth	−2·9±1·8	−3·4±0·6	−7·0±4·0
Moulting fourth	+0·3±1·3	−2·2±0·8	−2·0±0·5
Fifth	−3·6±0·8	−1·2±1·2	−0·9±2·5
Evacuated gut	+1·5±1·9	+0·7±0·6	−6·3±5·0
Spinning	−0·2±0·7	−0·4±0·4	−0·1±0·4

The relationship between the potential prior to changing the potassium concentration and the Nernst slope in each of the developmental stages is plotted in Fig 4B. Midguts from fifth-instar insects had an average potential of 58 mV. prior to the changing of the potassium concentration on the blood-side and yielded an average slope of +34 mV. Those from fourth-instar insects, with an average prior potential of 21 mV. gave a slope of +16 mV.; preparations from insects moulting from fourth to fifth with an average prior potential of 55 gave a slope of +24 mV.; preparations from insects which had recently evacuated their guts had an average prior potential of 49 mV. and a slope of +33 mV.; and those from spinning insects which had an average prior potential of but 4 mV. yielded a slope of but +5 mV. (Fig. 4B).

Comparison of Fig. 4A and B reveals a difference between the low potentials that sometimes occur in midguts from fifth-instar larvae with the low potentials that are invariable measured at the time of spinning. Whereas a change in potassium concentration on the blood-side of midguts from fifth-instar larvae with a prior potential of zero yields a Nernst slope of +17 mV., a similar concentration change with insects spinning their cocoon and having zero potential yields a Nernst slope

of but + 3·5 mV. Evidently the low potentials in insects involved in spinning are not due to chloride shunting as seems likely in the fifth-instar larvae but to some more disruptive change.

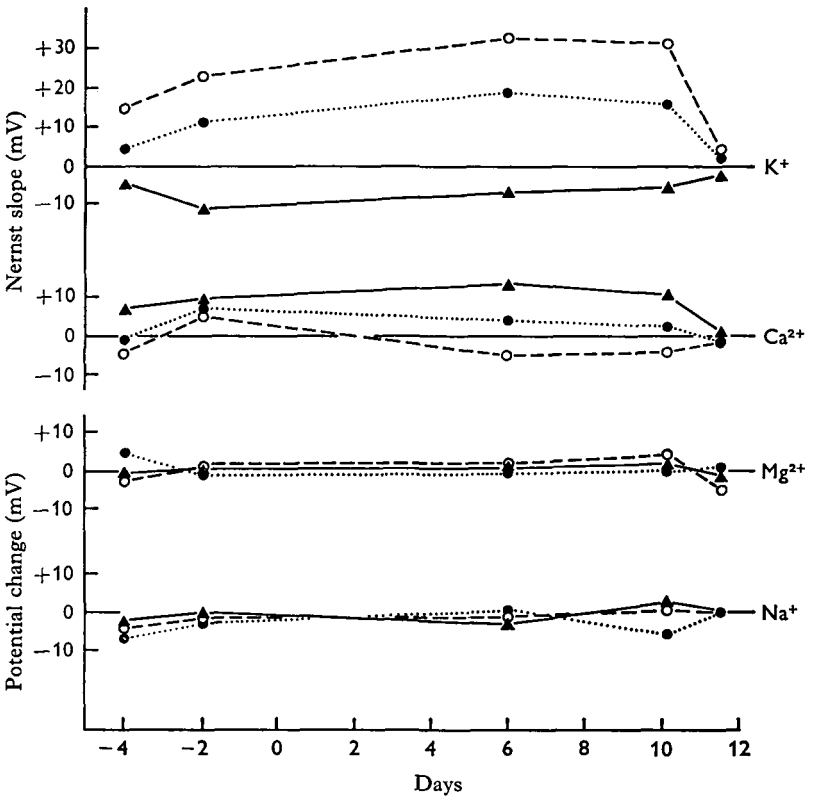

Fig. 3. Nernst slope (mV.) obtained for concentration changes of K or Ca in solutions bathing either the blood-side, lumen-side or both sides of the midgut during five stages of development (−4 days corresponds to feeding fourth instar larva, −2 days to moulting fourth, 6 days to feeding fifth, 10 days to gut evacuation and 11½ days to spinning). Changes in potential (mV) caused by Mg concentration change from 5 to 0 mM/l. and Na addition (32 mM/l.) to solutions on either the blood-side, lumen-side or both sides of the midgut. Closed triangles represent changes on the lumen-side; open circles, changes on the blood-side, and closed circles, changes on both sides.

Magnesium and sodium. The standard solution in which the midgut was bathed at the outset of each experiment contained 5 mM/l. magnesium and no sodium. The replacement of all of the magnesium by sodium rarely changed the potential during any of the stages examined. In order to maintain constant tonicity and total ionic concentration when the sodium concentration was increased above that of the standard solution, a modification of this solution was prepared which contained 32 mM/l. choline chloride in addition to the substances normally present and 64 mM/l. less sucrose. The addition of choline at this concentration was without significant effect. When all of the choline was replaced by sodium the potential occasionally underwent change; however, these changes were not consistently in the same direction. Moreover, there was no consistent pattern of response to sodium as a function of develop-

mental stage. The data for magnesium omission and sodium addition during the five developmental stages are presented in Table 3 and Fig. 3.

Calcium. Changes in calcium concentration in solutions bathing freshly cannulated midguts from mature fifth-instar larvae yielded potential changes in the opposite direction from those predicted by the Nernst equation, i.e. decreases in the concentration of this cation on the negative side of the membrane led to potential increases and on the positive side to potential decreases (Harvey, Haskell & Nedergaard, 1968). The changes in calcium concentration are thought to alter the permeability of the

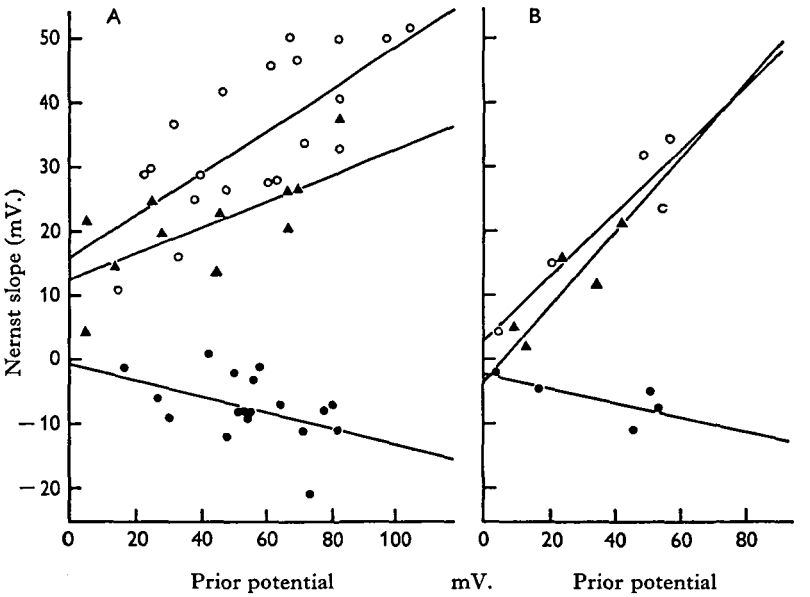

Fig. 4A. Nernst slope obtained for changes in K concentration on the blood-side (open circles), lumen-side (closed circles) and both sides (closed triangles) of the midgut of fifth-instar insects compared to the potential immediately before the change.

Fig. 4B. Average Nernst slope obtained for change in K concentration on the blood-side, lumen-side and both sides of midguts of the five developmental stages of Cecropia compared to the average potential prior to change in each stage.

midgut to other ions. A tenfold drop in calcium on the blood-side increased the potential by approximately 5 mV. giving a Nernst slope of approximately −5 mV. in all cases except in those insects moulting from the fourth to fifth instar and in larvae-spinning cocoons. In moulting animals the reverse effect was noted and in larvae-spinning cocoons there was no effect. A drop in calcium concentration on the lumen-side always lowered the potential and gave positive Nernst slopes and simultaneous lowering of calcium concentrations on both sides gave Nernst slopes intermediate between those for the blood-side and the lumen-side (Table 3 and Fig. 3).

pH *of midgut contents*

Contents of the midguts of freshly dissected larvae were measured by a Beckman Model G pH meter with a Fisher glass electrode (13–639–200). The average pH of midgut contents of seventeen feeding fourth-instar larvae was 8·8; of seventeen individuals moulting from the fourth instar to the fifth instar was 8·5; and of thirty-

four fifth-instar larvae was 9·4. The pH of the blood of fifth-instar insects averaged 6·3. The increase in alkalinity of the midgut contents as the insects increased in weight and advanced in developmental stage is illustrated in Fig. 5. By contrast, pupae chilled for 6 months but showing no visible sign of development contain midgut fluid with a pH of 6·5 and blood with a pH of 6·4.

Hormonal control of K transport?

Because the evacuation of the gut is the first visible sign of the impending metamorphosis and because it is a time when ecdysone titres are rising in *Bombyx mori* (Burdette, 1962; Shaaya & Karlson, 1965) and in *Manduca* (Yamamoto *et al.*, cited

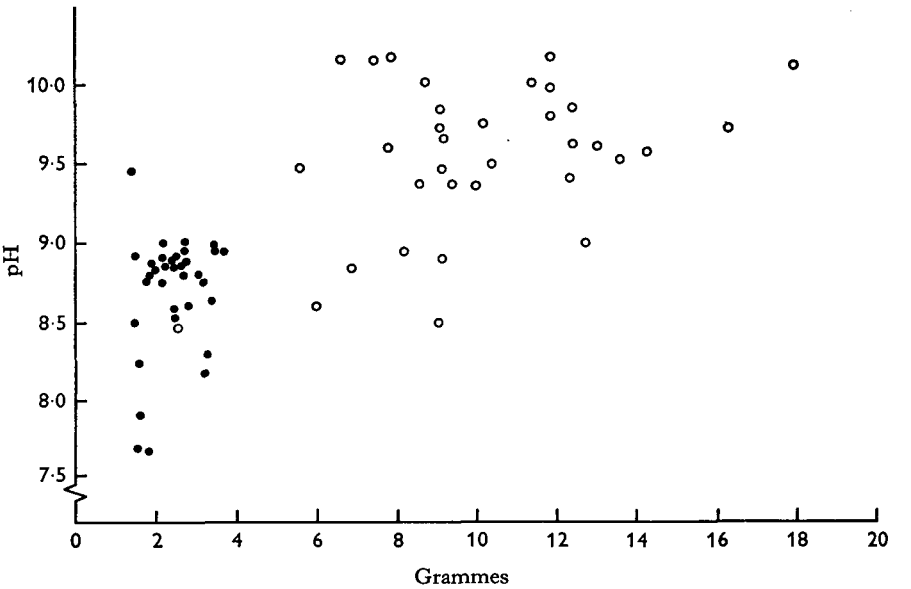

Fig. 5. pH of the contents of the midgut compared to the weight of the larva. Closed circles represent feeding and moulting fourth-instar insects. Open circles represent fifth-instar insects.

in Kaplanis *et al.* 1966), it is attractive to postulate that ecdysone might act directly to inhibit the mechanism for active K-transport thereby leading to the loss of potential in midguts isolated from 'spinning larvae'. Harvey & Nedergaard (1964) have shown that most of the short-circuit current of the midgut is accounted for by the active transport of potassium from blood-side to lumen-side of the isolated midgut; therefore, the measurement of the current is a valid measure of the activity of the potassium pump. Sufficient partially purified, assayed β-ecdysone provided by Dr G. R. Wyatt was available to bathe a single isolated midgut at a concentration above that required to bring about normal development in a brainless diapausing pupa (0·11 mg./ml.). A midgut was perfused in oxygenated saline in the improved apparatus (Harvey, Haskell & Zerahn, 1967). A stable current of 2400 μA. was measured for 5 min. Ecdysone was added with a Krogh syringe and the current was measured for 15 min. Larval blood (1·2 ml.) was then added to the solution bathing the preparation and the current was followed for another 15 min. Neither the addition of ecdysone nor

the subsequent addition of blood had any effect on the current. Finally, the viability and sensitivity of the midgut preparation was tested by stopping the stirring in the blood-side solution for 1 min. The current dropped to 400 μA. and returned to 2000 μA. within 10 sec. after stirring was restored.

When synthetic α-ecdysone (Siddall, Cross & Fried, 1966) became available through the generosity of Dr A. D. Cross, a total of eight preparations were exposed to ecdysone or ecdysone and larval blood under conditions similar to those just described. The results which have been reported elsewhere in detail (Harvey & Haskell, 1967) showed that regardless of the presence or absence of larval blood, synthetic α-ecdysone at a concentration of 2·7 μg./ml. had no effect on the midgut potential or short circuit current in 20 min. A potent synthetic 'juvenile hormone' supplied by Prof. Carroll M. Williams similarly was without effect.

DISCUSSION

A simple explanation for the loss of potential and short-circuit current by the midgut during cocoon spinning would be that either the midgut cells are being sloughed or that their lateral boundaries have separated providing extracellular pathways which completely swamp the active transport of potassium and abolish the transepithelial potential. The delicate appearance of the midgut isolated from insects spinning the cocoon invites this interpretation. However, flux measurements with ^{42}K indicate that after the midgut has lost the transepithelial potential at the time of spinning, its permeability to potassium, instead of increasing as might be expected if the lateral cell boundaries were separating, in fact decreases substantially. The influx of potassium is but 3·4 μ-equiv./hr., which is about 30 times less than the value in the fifth-instar larva, and the outflux is but 2·0, which is slightly less than that in a midgut of similar size isolated from a fifth-instar larva (Harvey & Haskell, 1968). Significantly, histological sections of midguts fixed after measurements which demonstrated that the potential had disappeared reveal the first signs of the transformation to a pupal epithelium (Brynes, unpublished observations). Assurance that the midgut cells retain their integrity at this time is provided by analyses of ionic concentrations of the midgut tissue (Quatrale, 1966). During the time of cocoon spinning, the concentrations of sodium, potassium, and calcium remain unchanged and that of magnesium increases greatly. Subsequently, as the larva pupates, the magnesium concentration increases further and the potassium concentration increases by 50%. Together these observations suggest that the midgut cells remain intact, that their functional surfaces remain tightly opposed to one another and that the loss of potential at the time of spinning is caused by structural changes associated with the turning off of the active potassium-transport mechanism.

Apparently, there is a correlation between the shutdown of the net active transport of potassium across the midgut wall and the titres of juvenile hormone and ecdysone. At the onset of spinning juvenile hormone is presumably decreasing in titre while ecdysone is increasing (Williams, 1961; Burdette, 1962; Yamamoto *et al.*, cited by Kaplanis *et al.* 1966). A simple hypothesis is that ecdysone turns off the potassium pump. That both partially purified and synthetic ecdysone at concentrations high enough to initiate adult development do not quickly influence the short-circuit current

in feeding fifth-instar larvae argues against this hypothesis. However, the early work of Schmidt and Williams, (1953) suggested that the growth factor of "active blood" which induced spermatogenesis in cultured germ cells of *H. cecropia* might be a small molecule tightly conjugated to a protien because the activity was non-dialyzable and somewhat heat labile. More recently Reddy and Wyatt (1967) found no effect of of crystalline synthetic ecdysone from Siddall, *et al.* (1966) on the incorporation of uridine into RNA or leucine into protein in wing epithelia isolated from diapausing purpae of *H. cecropia*. Although stimulation of RNA and protein synthesis occurred when the synthetic hormone was injected into the pupa, blood from hormone-injected pupae was inactive *in vitro*. Furthermore, Clever (1965) has demontrated that following the injection of ecdysone into *Chironomous tentans* larvae, a sequential change of the puffing pattern in the chromosomes of the salivary glands begins in 20 min. and continues for the next 75–100 hr. By contrast when excised glands are incubated in saline containing the same or higher concentration of ecdysone, only the first two puffs of the sequence appear. One interpretation of these results is that ecdysone is activated, perhaps by attachment to protein, in the insect's body. Although the addition of blood to the ecdysone-containing solution had no effect on the isolated midgut, the possibility remains that ecdysone could be activated in some tissue not present in the isolated gut preparation.

Another possibility is that there are several physiologically different ecdysones corresponding to different fractions prepared by the chemists as suggested by Kaplanis *et al.* (1966) and others. In this case the synthetic hormone, which apparently corresponds to α-ecdysone, may be active in stimulating a moult but not active in other processes. A further possibility is that ecdysone may shut off the potassium pump only in the absence of juvenile hormone. Because juvenile hormone is lipid soluble it may be retained by the tissues of the isolated midgut in sufficient quantity to protect the K-pump from the effects of added ecdysone. However, the most likely interpretation of our data is that ecdysone does not affect the midgut potassium pump.

Even if ecdysone and juvenile hormone indeed have no effect on the active transepithelial transport of potassium it would be quite premature to conclude that they have no action on the mechanism which actively transports sodium out of cells and potassium into cells (Kroeger, 1966), although the likelihood that Na-transport plays a significant role in phytophagous insects that contain almost no sodium is slight. Our present knowledge of transepithelial K-transport in the isolated midgut does not justify the conclusion that transport across the epithelium is dependent on transport into or out of the component cells, although the evidence does not allow us to conclude that the two processes are independent either.

Further studies of the chemical and structural differences between the midgut isolated from fifth instar larvae and capable of active potassium transport and the midgut isolated from spinning larvae and lacking this capability may reveal not only the nature of the K-transport system but some developmental consequences of its demise as well.

SUMMARY

1. The electrical potential across the isolated midgut of five developmental stages of the Cecropia silkworm was studied by changing the concentration of single cations

in solutions bathing each side of the midgut. The stages included feeding fourth-instar insects, insects moulting from the fourth to the fifth instar, feeding fifth-instar insects, insects which had evacuated their midguts, and insects spinning cocoons.

2. Average values of the initial maximal potential exhibited by the midgut in solutions containing K, Mg, and Ca but no Na, for the stages mentioned above, were 68, 83, 90, 124, and 2 mV., respectively.

3. In all of the developmental stages studied except the 'spinning larva', reducing the potassium concentration from 32 to 2 mM/l. on the blood-side of the isolated gut lowers the potential, on the lumen-side of the gut raises the potential and on both sides gives an intermediate value.

4. When the potential prior to a decrease in concentration of potassium on the blood-side is over 100 mV., the Nernst slope approaches 59 mV.

5. A tenfold reduction in the concentration of magnesium or the addition of 32 mM/l. sodium to the solutions bathing the isolated gut has no systematic effect on the potential.

6. A tenfold drop in the concentration of calcium in the solutions causes changes in the potential in the opposite direction from those predicted by the Nernst equation.

7. The pH of the midgut contents rises from early fourth instar to late fifth instar. The hydrogen-ion concentration of the blood is about 1000 times more than that of midgut contents in fifth-instar insects.

8. Neither synthetic ecdysone, partially purified natural ecdysone nor juvenile hormone has an effect on the potential or current of the isolated midgut over periods as long as 30 min.

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