J. exp. Biol. (1975), 63, 321–330
With 6 figures
Printed in Great Britain

CATION DISTRIBUTIONS ACROSS THE LARVAL AND PUPAL MIDGUT OF THE LEPIDOPTERAN, *HYALOPHORA CECROPIA, IN VIVO*

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(*Received* 30 *January* 1975)

SUMMARY

1. The levels of potassium, sodium, magnesium and calcium in leaves, midgut contents, midgut tissue, and blood were analysed in seven developmental stages between feeding, fourth-instar larvae and new pupae of the Cecropia silkworm.

2. Three dramatic changes in cation levels were found: the K level in the contents drops from 284 ± 51 mEquiv./l tissue water in the fifth-instar larva to 51 ± 6 mEquiv./l in the new pupa; the Mg level in the midgut tissue increases from 28 ± 3 mEquiv./l at the time of gut evacuation to 1093 ± 104 mEquiv./l in the new pupa; and the Ca level in the contents drops temporarily from 56 ± 12 mEquiv./l in the feeding fourth instar larva to 17 ± 5 mEquiv./l in the new fifth instar larva. The Na level was never higher than 2·8 ± 0·5 mEquiv./l.

3. The relative levels of the four cations were different for each tissue studied, but each tissue maintained the same relative levels during the developmental stages studied. The sequences are: leaf, Ca > K > Mg > Na; midgut contents, K ⩾ Ca > Mg > Na; midgut tissue, K ⩾ Mg > Ca > Na; and blood, Mg ⩾ K > Ca > Na.

4. There were three large concentration gradients across the midgut; the K level in the midgut contents is approximately 10 times the level in blood; the Mg level in contents is one-half to one-sixth the level in blood; and the Ca level in contents is 3–4 times the level in blood. The K gradient and the Ca gradient are opposed and the Mg gradient is favoured by the electrical gradient across the larval midgut, the contents being 100 mV positive with respect to the blood. The K gradient and the electrical gradient are not present across the pupal midgut while the Mg gradient and the Ca gradient persist.

5. The K gradient is presumably maintained by the midgut K pump, the Mg gradient is aided by the midgut Mg pump, and the Ca gradient suggests that the midgut may possess a Ca pump.

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INTRODUCTION

The ion transport properties of the midgut isolated from the larva of the Cecropia silkworm have been studied for 15 years but the role of the midgut in ion regulation in the living insect is little known. A first step toward an understanding is to examine the *in vivo* levels of sodium, potassium, magnesium, and calcium, in the midgut and surrounding fluids. The cations in blood of Lepidopterous insects have been analysed exhaustively (review by Florkin & Jeuniaux, 1974). Quatrale (1966) measured concentrations of all four cations in paired samples of blood and other tissues during seven developmental stages but of this data only that on blood has been formally published (Jungreis, Jatlow & Wyatt, 1973).

Moreover, it is worth while to re-evaluate Quatrale's findings in the light of our present knowledge about the isolated midgut. Not only potassium but also sodium and the other alkali metal ions are actively transported from blood-side to lumen-side of the isolated midgut (review by Harvey & Zerahn, 1972) and magnesium is actively transported in the opposite direction (Wood, Jungreis & Harvey, 1975). These cation pumps, which were discovered in the isolated midgut, could have a significant role in regulating ion levels *in vivo*.

In addition the electrical potential difference across the midgut could affect the ion levels *in vivo*. Preliminary results from this laboratory show that the midgut contents are about 100 mV positive with respect to the blood *in vivo* in the feeding fifth-instar larva. Moreover, Haskell, Harvey & Clark (1968) have studied the potential difference across freshly isolated midguts and found it to be about 70 mV (lumen-side positive) in feeding fourth-instar larvae, to be 100 mV in feeding fifth-instar larvae, and then to drop abruptly to zero mV just after gut evacuation. It is reasonable to suppose that this same pattern is followed *in vivo*. Considering just the potential difference across the midgut, all of the cations should be present at higher levels in the blood than in the contents in larvae but at the same levels in blood and contents in pupae. That these patterns are not found when a steady state may be presumed suggests that the cation pumps described in the isolated midgut may be functioning in the living insect.

MATERIALS AND METHODS

Hyalophora cecropia (L.) were reared on wild black cherry (*Prunus serotina*) or weeping willow (*Salix babylonika*). Seven developmental stages between the feeding fourth-instar larva and the new pupa were studied (Quatrale, 1966; see also Haskell *et al.* 1968). The late, feeding, fourth-instar larvae (FIV) had been feeding actively for at least 72 h and weighed between 1·2 and 3·1 g. The moulting fourth/fifth-instar larvae (MIV) were within 24 h before the casting of the old skin, had not fed for 55–65 h, and weighed between 1·5 and 5·5 g. The newly moulted, fifth-instar larvae (NV) had cast the old skin 2–15 h previously but had not resumed feeding, which begins at 18 h, and weighed between 1·2 and 3·1 g. The mature, feeding, fifth-instar larvae (FV) had been feeding for about 10 days and weighed between 10·3 and 18·2 g. The fifth-instar larvae which have just evacuated their gut contents (GE) had not fed for 0·5–10 h and were wandering; their entire alimentary canals were empty; and they weighed between 5·2 and 8·3 g. The prepupae (PP) were about 3½ days after gu

evacuation, were green in colour and barrel-shaped, and were within 24 h after the prolegs had retracted; their midguts were collapsed; they had more fat body than the larvae but less than the pupae; and they weighed between 3·1 and 7·9 g. The new pupae (NP) were within 15 h after the casting of the larval skin and they weighed 2·4–5·5 g.

Samples of leaves, midgut contents, mudgut tissue and blood were obtained as follows. The leaves on which the insect had been feeding were cut into small pieces (approximately 5 × 10 mm). The midgut contents were removed with forceps or with a glass tube. They were a green semi-solid mass of minute pieces of leaves (1 × 1 mm) in the feeding fourth-instar larva, a brown semi-solid mass in the moulting fourth, a reddish brown fluid with brownish-green solids in the new fifth, a green semi-solid mass in the feeding fifth-instar larva, absent after gut evacuation and in the prepupa, and a bluish black liquid in the pupa. The midgut tissue was freed of fat body and contents and washed three times for 10–15 sec in ice cold 260 mOsm/l sucrose in the larval stages, 340 mOsm/l sucrose in the prepupa, and 680 mOsm/l sucrose in the new pupa, reflecting the increasing osmolarity of the blood during these stages. The cation levels in the midgut tissue need to be corrected for adhering sucrose solution. These corrections have not been made because the exact amount of adhering sucrose solution is unknown, although Quatrale (1966) gives a value of about 15–20 % using ^{35}S-O_4. The blood sample was collected as drops from an incision at the base of the first abdominal leg in the larval stages and prepupa and from an incision in the front portion of the head in the new pupa. The cells were not removed from the blood samples and the cation levels reported are those for whole blood. However, there is little difference between whole blood (Figs. 1–5) and blood plasma (Jungreis *et al.*, 1973; Jungreis & Tojo, 1973).

The samples were prepared for analysis by slight modifications of the techniques reviewed by Thiers (1957). The samples were placed on pre-tared platinum crucibles and their wet weights were determined as rapidly as possible. The samples were then dried to constant weight at 105 °C to yield the percentage water (Fig. 1). They were pre-ashed and charred by gradually heating to 335 ± 15 °C and then combusted to a white ash at 450 °C in a muffle furnace. Ashing times varied from about 10 h for larval blood to as much as 48 h for leaves. Samples exhibiting black carbon after 48 h of ashing were treated with 12 N-NHO_3 and if necessary 6 N-HCl to produce complete ashing (Thiers, 1957). Ashed samples were dissolved in 0·5–4·0 ml of 6 N-HCl, depending on the amount of sample present and were stored. Each of the samples, after appropriate dilution with distilled water, was analysed for potassium, sodium, magnesium, and calcium with an Atomic Absorption Spectrophotometer (Perkin-Elmer, Model 214). The cation levels were expressed as mEquiv. cation per litre of tissue water (molal). The word 'level' is used to denote the amount of cation per volume of water rather than the word 'concentration' because some of the amounts per volume exceeded the solubility products of any known salts. The means and corresponding standard errors were calculated (N ranged from 7 to 12) and all differences discussed were significant at greater than the 5 % confidence level.

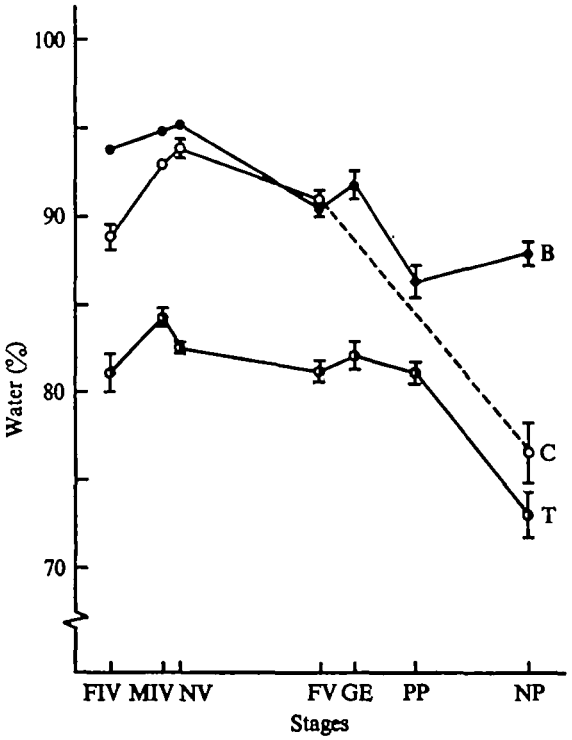

Fig. 1. The changes in percentage water ({wet weight − dry weight}/wet weight) in midgut contents (C; ♀; mean ± 1 standard error of the mean), midgut tissue (T; ◑) and blood (B; ●) during seven developmental stages (FIV = feeding, fourth-instar larva; MIV = moulting, fourth-instar larva; NV = new, fifth-instar larva; FV = mature, feeding, fifth-instar larva; GE = fifth-instar larva which has just evacuated its midgut contents; PP = prepupa, and NP = new pupa). In Figs. 1–5 these seven stages are plotted in units of real time in days, with the time from FIV to NP being 24 days at 25 °C.

RESULTS

Changes in the percentage of water in the midgut contents, midgut tissue, and blood during development are shown in Fig. 1. The percentage water drops sharply in the midgut contents and tissue from larva to pupa but drifts down slowly in the blood. Changes in the K level in these same three tissues during development are shown in Fig. 2. The K level is very high in the contents during the larval stages but drops sharply to the same level as that of blood in the pupa. The K level in midgut tissue is slightly higher in pupa than larva and that in blood is virtually constant during development. Changes in the Na level during development are shown in Fig. 3. The Na level is very low and virtually constant in all three tissues except for a slight increase in the pupal midgut tissue and in the blood of feeding, fifth-instar larvae. Changes in the Mg level during development are shown in Fig. 4. The Mg level is low in larval midgut contents and even lower in pupal contents, increases dramatically in the midgut tissue after gut evacuation (up to 1093 mEquiv./l in the new pupa), and drifts down slowly in blood. Changes in the Ca level during development are shown in Fig. 5. The Ca level in the contents, which is high in feeding larval stages and pupa, drops temporarily nearly to the blood level in the moulting, fourth-instar larva and the new

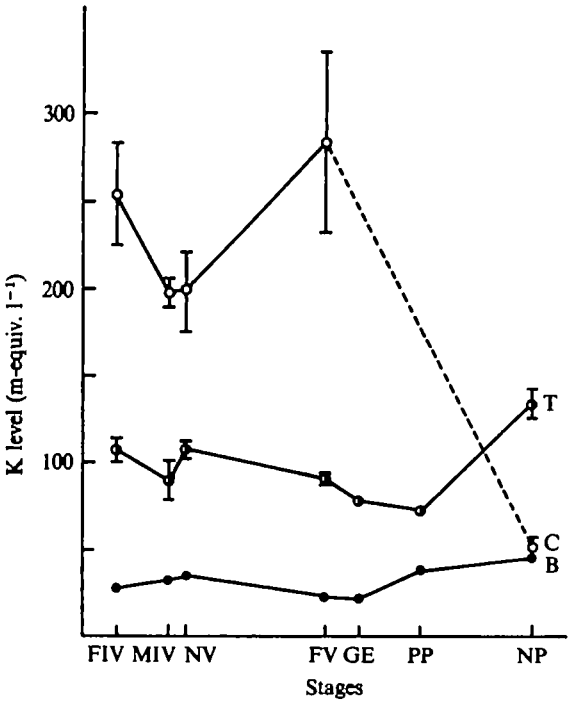

Fig. 2. The changes in K level (mEquiv. per litre of tissue water; molal) in midgut contents, midgut tissue and blood during the same seven development. Symbols as in Fig. 1.

fifth-instar larva, but is virtually constant in midgut tissue and blood during development.

The relative levels of the four cations are different for each tissue studied, but each tissue maintains the same relative levels during the stages studied. The levels of the four cations in the feeding, fifth-instar larva and in the new pupa are shown in Fig. 6. The levels at other larval stages are virtually the same as those in the feeding, fifth-instar larva; and those in the prepupa are virtually the same as those in the new pupa. The ion levels in the leaf are included for comparison. The sequences are as follows: leaf, Ca > K > Mg > Na; midgut contents, K ⩾ Ca > Mg > Na; midgut tissue, K ⩾ Mg > Ca > Na; and blood, Mg ⩾ K > Ca > Na. The only exception is the sequence for the midgut tissue of prepupa and new pupa where Mg ⩾ K > Ca > Na because the Mg level increases dramatically in these two stages (Fig. 4).

There are large concentration gradients across the midgut for three of the four ions studied (Fig. 6). The K level in the midgut contents is about 10 times the level in the blood during the larval stages but it drops to the blood level in the pupa. The tissue K level is intermediate between contents and blood in the larva but higher than either in the pupa. The Mg level in the contents varies from about one-half to about one-sixth of the level in the blood with the tissue being intermediate (except in the pupal midgut as noted above). The Ca level in the contents is 3–4 times that in the blood except that in the moulting fourth instar and new fifth instar larva the Ca level in contents drops to that in blood. The Ca level in tissue is lower than that either in contents or blood throughout development. The possible origin of these concentration gradients is discussed below.

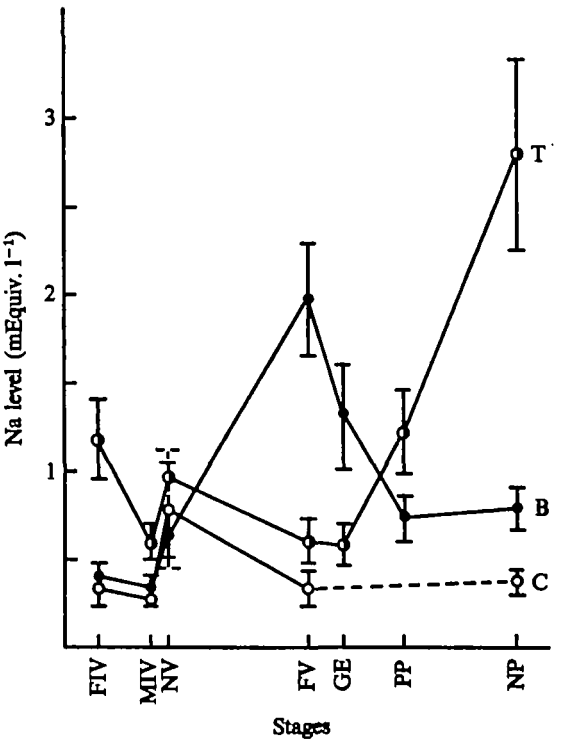

Fig. 3. The changes in Na level in midgut contents, midgut tissue, and blood during development (note expanded scale on ordinate). Symbols as in Fig. 1.

DISCUSSION

Water. The percentage water values are not meaningful unless the osmotic pressure of the tissues or the molecular weight of their solutes are known. However, the percentage water can be used to convert the results, which are expressed as milliEquivalents per litre of tissue water (molal) to milliEquivalents per litre of tissue (molar). Moreover, it is known that the osmotic pressure of the blood increases from 260 ± 4 mOsm/l in the larva (Harvey & Nedergaard, 1964) to 680 mOsm/l in the pupa (Michejda & Thiers 1963) which partially accounts for the increased ion levels in the pupal blood and midgut tissue. Furthermore, in the larva, the K in the midgut contents is of a sufficiently high concentration that it alone could cause the osmolarity of the contents to exceed that of the blood. Presumably the osmolarity of the contents is close to that of blood and therefore some of the K in the contents cannot be active osmotically: the activity of K in the contents is not given by the K 'levels' reported here. A possible explanation is that some of the K is contained inside chunks of undigested leaves. Obviously more information on the osmotic pressure of midgut contents is needed.

Potassium. The level of K is very high in leaf. In the larva it is also high in the midgut contents, but very low in blood; whereas in the pupa the level of K is the same in midgut contents as in blood. The K gradient across the wall of the larval midgut is opposed by an electrical gradient of about 100 mV, whereas there is no electrical gradient in the pupa. The K gradient in the larva and its loss in the pupa can be accounted for by

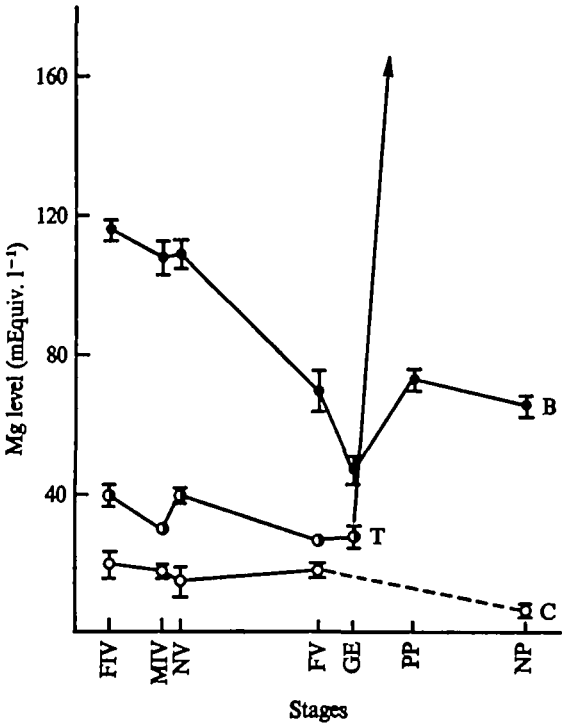

Fig. 4. The changes in Mg level in midgut contents, midgut tissue, and blood during development. Symbols as in Fig. 1. The Mg level in midgut tissue in the prepupa is 368 ± 57 mEquiv. l^{-1} and in the new pupa is 1093 ± 104 mEquiv. l^{-1}.

the K pump, in the midgut, if the pump operates *in vivo* as it does *in vitro*: midguts isolated from larvae actively transport K from blood-side to lumen-side (Harvey & Nedergaard, 1964), but this pump is lost around the time of gut evacuation (Haskell *et al.* 1968). Since the K pump is electrogenic it would also account for the potential difference across the larval midgut and for its absence across the pupal midgut. Showing that the midgut K pump operates *in vivo* is not easy. An example of the questions involved is that the blood-side of the isolated midgut must be stirred very rapidly (Wood, 1972) whereas blood movements *in vivo* are very slow (Harvey, 1957). The role of the midgut K-pump *in vivo* is being studied with potassium selective microelectrodes (Blankemeyer, Wood & Harvey, 1976).

Sodium. The important feature of Na in this study is that its level is very low in all tissues at all developmental stages studied (range 0·3–2·8 mEquiv./l). The Na levels found here are similar to those reported by Michejda & Thiers (1963), but are lower than those reported elsewhere. It is important to note that the techniques used in the present investigation were developed to minimize contamination of the samples and are, therefore, likely to be reliable. It should be pointed out that Na is transported by the isolated midgut but only when the K concentration is very low and when Ca is absent (Harvey & Zerahn, 1971; Harvey & Wood, 1972). Since K and Ca are present in high concentrations and Na in very low concentrations *in vivo*, it is unlikely that any Na is actively transported by the midgut *in vivo*.

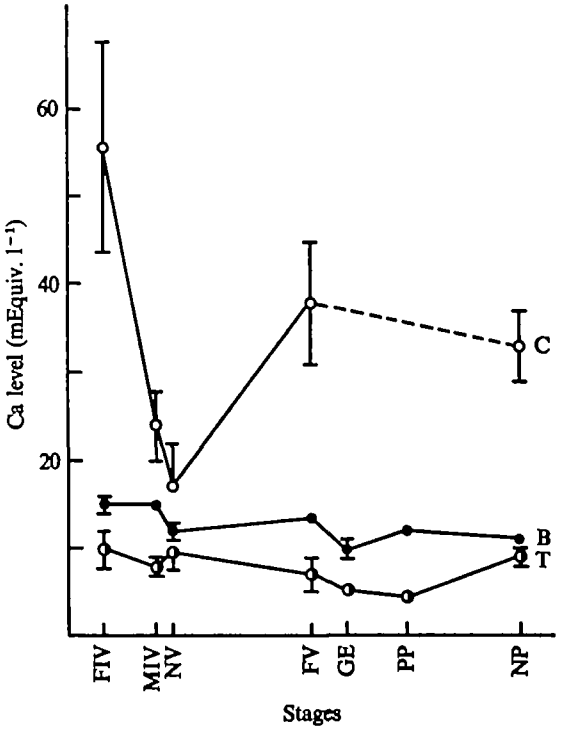

Fig. 5. The changes in Ca level in midgut contents, midgut tissue and blood during development. Symbols as in Fig. 1.

Magnesium. The Mg level is high in leaves, low in midgut contents, intermediate in midgut tissue and high in blood. The persistence of the Mg gradient across the wall of the midgut is aided in the larva by the potential difference across the wall *in vivo*. However, it was recently found that Mg is actively transported from lumen-side to blood-side across the isolated larval midgut (Wood, Jungreis & Harvey, 1975), and this transport presumably accounts for the distribution of Mg in the living larva. The Mg gradient across the midgut is also found in the pupa – when there is no potential difference across the midgut – suggesting that Mg may be transported by the pupal midgut. If indeed Mg were transported by the pupal midgut then the transport would be accomplished by the pupal columnar cells since the columnar and goblet cells present in the larval midgut disappear after gut evacuation (Yamazaki, Haskell & Harvey, unpublished observations). Finally, Mg is extremely concentrated in the midgut of the prepupa and new pupa (368 and 1093 mEquiv./l respectively). Phosphate, derived from haemolymph α-glycerophosphate, is present in amounts stoichiometric with magnesium in the pupal midgut epithelium (Jungreis, Daily & Hereth, 1975). Presumably the magnesium is derived from the blood, since the magnesium in the blood, and the blood volume, are decreasing coincidently as the magnesium in the midgut tissue is increasing. Apparently the particles observed in light micrographs of Cecropia pupal midgut (Brynes & Harvey, unpublished observations) are magnesium phosphate deposits rather than calcium deposits as reported in the *Bombyx mori* midgut (Waku & Sumimoto, 1971).

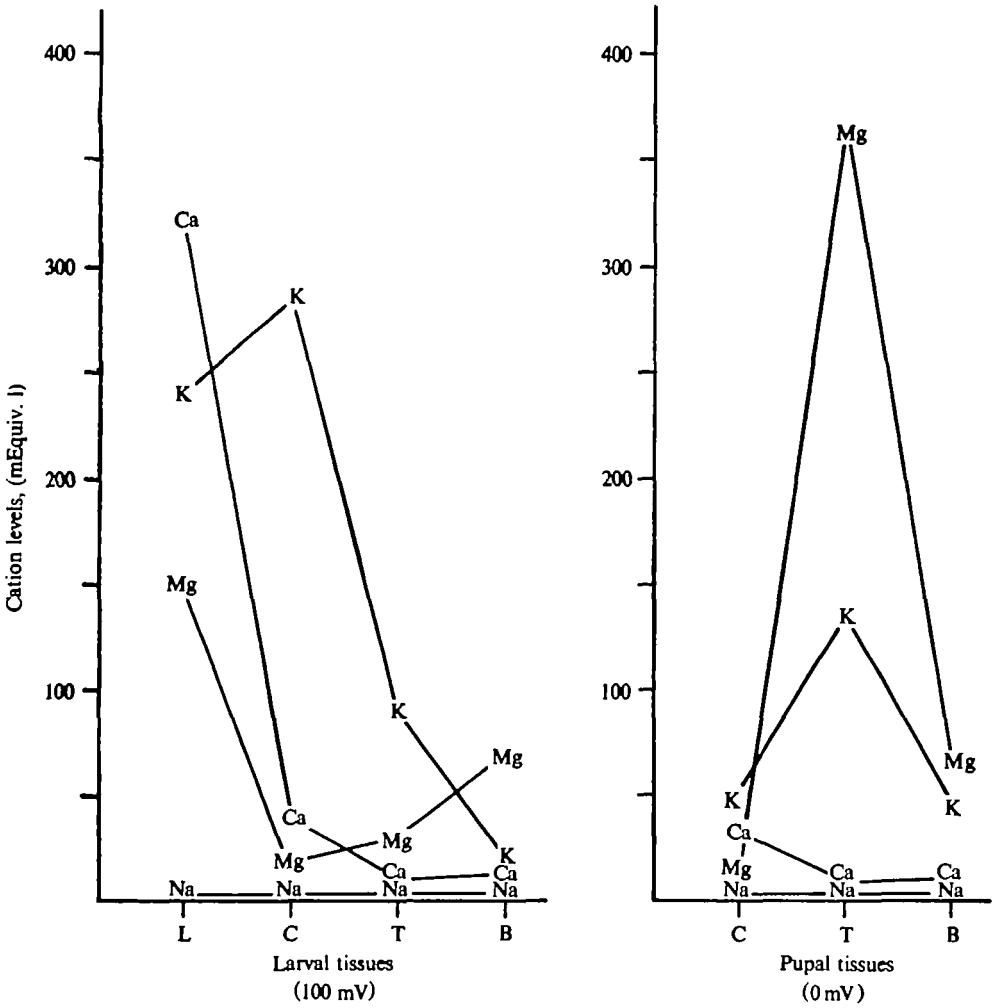

Fig. 6. The relative levels of K, Na, Mg and Ca in leaves, midgut contents, midgut tissue and blood in mature, feeding fifth-instar larvae and new pupae. L = leaves, other symbols as in Fig. 1. The relative levels found in the mature, feeding fifth-instar larvae are typical of the larval stages when the midgut contents are 100 mV positive with respect to the blood; and the levels found in the new pupa are typical of the pupal stages when the midgut contents are at the same potential as the blood. Ca and K are the major cations in leaves; K is the major cation in the midgut contents and larval midgut tissue; and Mg is the major cation in blood and pupal midgut tissue.

Calcium. Ca is the major inorganic cation in leaves, but its level is very low in midgut contents, tissue and blood, and it is very high in faecal pellets (178–1360 mEquiv./l; Quatrale, 1966). The way in which Ca moves through the insect is unknown. The Ca level is higher in the midgut contents than in the blood, a distribution which is opposed by the electrical gradient across the midgut *in vivo* in the larva. It is tempting to suggest that Ca is actively transported by the midgut from blood-side to lumen-side, but the activities of Ca in contents and blood are not known so the speculation is premature. Moreover, if Ca were to be transported by the midgut then the Ca pump would have to be shut off during the moult from fourth to fifth instar since the Ca gradient across

the midgut disappears at this stage. In spite of its importance in cell biology (e.g. Rasmussen, Goodman & Tenenhouse, 1972) Ca is the least studied and least understood cation of the lepidopteran midgut.

This research was supported in part by research grants (AI-04291 and AI-09503) from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

REFERENCES

BLANKEMEYER, J. T., WOOD, J. L. & HARVEY, W. R. (1976). Role of midgut active potassium transport in regulating haemolymph concentration of Cecropia midgut. (In preparation.)

FLORKIN, M. & JENIAUX, C. (1974). Hemolymph composition. In *The Physiology of Insecta*, vol. 5, 2nd ed. (ed. M. Rockstein), pp. 255–307. New York: Academic Press.

HARVEY, W. R. (1957). Metabolic studies on the Cecropia silkworm with special reference to the pupal diapause. Ph.D. Thesis, Harvard University, Cambridge, Masachusetts.

HARVEY, W. R. & NEDERGAARD, S. (1964). Sodium-independent active transport of potassium in the isolated midgut of the Cecropia silkworm. *Proc. natn. Acad. Sci. U.S.A.* **51**, 757–65.

HARVEY, W. R. & WOOD, J. L. (1972). Cellular pools involved in active K-transport across the isolated Cecropia midgut. In *Role of Membranes in Secretory Processes* (ed. L. Bolis, R. D. Keynes and W. Wilbrandt), pp. 310–31. Amsterdam: North Holland.

HARVEY, W. R. & ZERAHN, K. (1971). Active transport of sodium by the isolated midgut of *Hyalophora cecropia*. *J. exp. Biol.* **54**, 269–274.

HARVEY, W. R. & ZERAHN, K. (1972). Active transport of potassium and other alkali metals by the isolated midgut of the silkworm. *Current Topics in Membranes and Transport*, vol. 3 (ed. F. Bronner and A. Kleinzeller), pp. 377–409. New York: Academic Press.

HASKELL, J. A., HARVEY, W. R. & CLARK, R. M. (1968). Active transport by the Cecropia midgut. V. Loss of potassium transport during larval-pupal transformation. *J. exp. Biol.* **48**, 25–37.

JUNGREIS, A. M., DAILY, J. C. & HERETH, M. L. (1975). α-Glycerol phosphatase and glycerol kinase activity in developing silkmoth tissues. *Am. J. Physiol.* (In the Press.)

JUNGREIS, A. M., JATLOW, P. & WYATT, G. R. (1973). Inorganic ion composition of haemolymph of the Cecropia silkmoth: changes with diet and ontogeny. *J. Insect Physiol.* **19**, 225–33.

JUNGREIS, A. M. & TOJO, S. (1973). Potassium and uric acid content in tissues of the silkmoth *Hyalophora cecropia*. *Am. J. Physiol.* **224**, 21–26.

MICHEJDA, J. W. & THIERS, R. E. (1963). Ionic regulation in the development of males of a silkworm: *Hyalophora cecropia*. *Proc. 16th Int. Cong. Zool.*, Washington, D.C., **2**, 39.

QUATRALE, R. P. (1966). Cation concentration changes during development of the silkworm, *Hyalophora cecropia*. Ph.D. Thesis, University of Massachusetts, Amherst, Massachusetts.

RASMUSSEN, H., GOODMAN, D. B. P. & TENENHOUSE, A. (1972). The role of cyclic AMP and calcium in cell activation. *Critical Reviews in Biochemistry* **1**, 95–148.

THIERS, R. E. (1957). Contamination in trace element analysis and its control. In *Methods of Biochemical Analysis* vol. 5 (ed. D. Glick), pp. 273–335. New York: John Wiley and Sons.

WAKU, Y. & SUMIMOTO, K. (1971). Metamorphosis of midgut epithelial cells in the silkworm (*Bombyx mori* L.) with special regard to the calcium salt deposits in the cytoplasm. I. Light microscopy. *Tissue & Cell* **3**, 127–36.

WOOD, J. L. (1972). Some aspects of active potassium transport by the midgut of the silkworm, *Antheraea pernyi*. Ph.D. Thesis, Cambridge University, England.

WOOD, J. L., JUNGREIS, A. M. & HARVEY, W. R. (1975). Active transport of magnesium across the isolated midgut of *Hyalophora cecropia*. *J. exp. Biol.* **63**, 313–320.

NOTE ADDED IN PROOF

Wood and Harvey (1976, in preparation) have just demonstrated that calcium is actively transported from lumen-side to blood-side across the isolated midgut, a disection opposite to that suggested by the *in vivo* distribution of calcium.