Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Barbara Giordana, Franca Sacchi

Transepithelial electrical parameters in the midgut of three different larvae of Lepidoptera

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **60** (1976), n.2, p. 151–155.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1976_8_60_2_151_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1976.

Fisiologia. — *Transepithelial electrical parameters in the midgut of three different larvae of Lepidoptera.* Nota di BARBARA GIORDANA e FRANCA SACCHI (*), presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — Sono state determinate le concentrazioni di sodio e potassio nell'emolinfa di larve mature di *Philosamia cynthia*, *Macrothylatia rubi* e *Bombyx mori*. Nelle tre specie l'emolinfa ha sempre una concentrazione di Na molto bassa e il rapporto Na/K è largamente inferiore all'unità.

L'intestino medio isolato e perfuso con opportune soluzioni fisiologiche presenta una differenza di potenziale transepiteliale di 60–70 mV con il polo positivo nel lume. La rimozione del potassio dalle soluzioni perfondenti provoca in tutte e tre le specie una drastica caduta del potenziale. In *Philosamia* e *Macrothylatia* inoltre la differenza di potenziale intestinale raggiunge, in assenza di K, apprezzabili valori negativi ($\sim$ 20 mV, lume negativo).

The phytophagous larvae of Lepidoptera have a diet with a high K content. The midgut of these larvae plays a peculiar role in ionic homeostasis. This feature has been well studied in the isolated midgut of *Hyalophora cecropia* which shows a transepithelial electrical potential difference with the positive pole in the lumen [1]. This unusual polarity has been related to an active K secretion into the lumen, due to an electrogenic pump [2] whose characteristics have been well studied [3, 4] and are still open to discussion [5, 6].

This paper refers to preliminary results obtained in the midgut of three different larvae of Lepidoptera: *Philosamia cynthia* (Saturnidae), *Macrothylatia rubi* (Lasiocampidae) and *Bombyx mori* (Bombicidae).

The experiments were performed during September and October on wild larvae of *Philosamia cynthia* and *Macrothylatia rubi* captured in the countryside near Milano. *Bombyx mori* larvae were supplied by " Sezione specializzata per la Bachicoltura ", Padova. *Philosamia cynthia* larvae were reared on fresh leaves of *Ailanthus glandulosa*, *Macrothylatia rubi* on fresh leaves of *Rubus fruticosus* and *Bombyx mori* on *Morus nigra* fresh leaves. All the larvae were used in their last larval instar, when they reached the following weights: 8–9 gr *Philosamia*, 4–6 gr *Macrothylatia* and 4–5 gr *Bombyx*.

To determine the Na and K concentrations in the haemolymph, the larvae were bled by cutting a proleg: the haemolymph was collected in a test tube and diluted 1 : 10 with distilled water. 0.6 N $KClO_4$ was then added to this solution in equal amounts (1 : 1 ratio) to precipitate the proteins. Each sample was centrifuged for 10 minutes, the clear supernatant was removed and assayed for Na and K concentrations by a flame photometer (Beckman DU-2). Na and K concentrations in the lumen content were determined by opening

(*) Istituto di Fisiologia Generale dell'Università di Milano.

(**) Nella seduta del 14 febbraio 1976.

the caterpillars lengthwise, perforating the midgut and collecting the intestinal content into a test tube, centrifuging for 5 minutes to have a solution free from leaf fragments and then going on as for the haemolymph.

The electrical measurements were performed on the midguts isolated and mounted on an apparatus as described by Nedergaard and Harvey [3] with short-circuiting modifications after Harvey, Haskell and Zerahn [7]. The apparatus is represented in fig. 1.

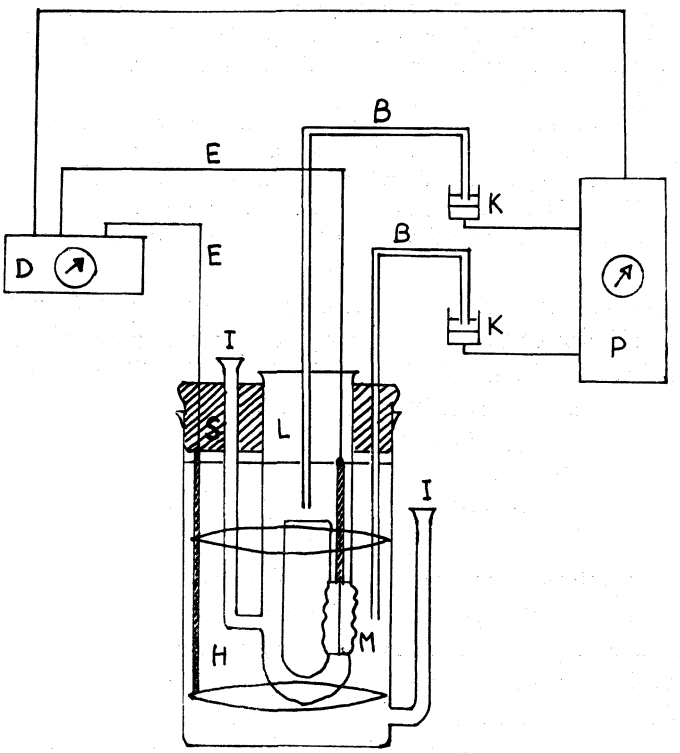

Fig. 1. - Experimental apparatus. M: midgut (different diameters of the tubes are used according to the size of the midgut); L: lumen side chamber; H: haemolymph side chamber; P: potentiometer (type 457 A, Vescovini, Parma); D: short-circuit device (type 406, Vescovini, Parma); I : gas inlets; S: lumen side chamber support; K: kalomel electrodes; B: Agar–KCl bridges; E: Ag-AgCl electrodes (apparatus from Nedergaard S. and Harvey W.R. [3], and from Harvey W. R., Haskell J. A. and Zerahn K. [7]).

In order to isolate the midgut, the head and the portion anterior to the last pair of prolegs, and the one posterior to the third pairs of prolegs were cut away. The peritrophic membrane with the enclosed content was gently removed from the intermediate segment, the tegument was then cut lengthwise and the midgut tightly tied as a tube on the described apparatus. The volume of the bathing solution in the lumen side of the midgut was approximately 5 ml and in the blood side 50 ml. The bathing media on both sides were areated and stirred by bubbling with 95 % O_2 and 5 % CO_2.

On the basis of the determined concentrations of Na and K in the haemolymph and from concentrations of Ca and Mg reported in the literature [8] different standard solutions were prepared for the three species:

	NaCl mM/l	$KHCO_3$ mM/l	KCl mM/l	$CaCl_2$ mM/l	$MgSO_4$ mM/l	Sucrose m/Ml
Philosamia	7.5	25.0	—	6.0	25.5	112.5
Macrothylatia	3.5	25.0	—	12.0	30.0	93.2
Bombyx	1.7	25.0	21.0	12.2	50.0	25.5

The pH of the solutions was 7.4. In the experiments performed in the absence of K, this ion was replaced by equal amounts of sodium. Sucrose was added to all solutions to obtain the proper osmolarity.

The Na and K concentrations found in the haemolymph and lumen content of the three different species are reported in Table I. The haemolymph always shows a very low Na content and the Na/K ratio is below 1, a value very different from that found in Vertebrates and most Insects. This feature has been demonstrated to be a specialization of Lepidoptera [8, 9]. Na concentration in *Bombyx mori* is much lower than the early values reported in the literature [10,8] but in good agreement with those more recently reported [11].

TABLE I.

Na *and* K *concentrations in haemolymph and lumen content. Mean values* ± SE

Number of experiments in parenthesis.

	Philosamia cynthia		*Macrothylatia rubi*		*Bombyx mori*	
	Na	K	Na	K	Na	K
Hemolymph mEq/l Means±SE	2,5±0,1 (5)	24,5±0,3 (5)	3,5±0,2 (7)	25,6±2,7 (6)	1,7±0,05 (6)	46,2±0,9 (6)
Lumen content mEq/l Means ± SE	< 1,0 (2)	212,0 (2)			1,3±0,1 (9)	149,5±2,9 (9)

K concentration values are quite high compared to plasmatic concentration in Vertebrates and other Insects. Na is extremely low also in the lumen content while K reaches very high values so that there is a steep potassium concentration gradient between lumen and haemolymph. These data suggest

that the midgut epithelium should play a role in maintaining low and constant haemolymph K concentration.

The isolated midguts of the three larvae have in the chosen standard solutions high transepithelial electrical potential differences (ΔE), lumen positive: the mean values are reported in Table II.

TABLE II.

Transepithelial electrical potential differences (ΔE). *Mean values* ± SE

Number of experiments in parenthesis.

	Philosamia cynthia	*Macrothylatia rubi*	*Bombyx mori*
ΔE mV Means ± SE	71,6±6.8 (7)	60,0±6,5 (11)	69,1±2,3 (35)

These potentials were measured 15 minutes after isolation. The short-circuit values are very similar in the three larvae, being approximately 240 μA/cm². ΔE variations have been followed in standard conditions for 120 minutes: *Philosamia* seems to be largely the more stable tissue since ΔE declines only slightly during the experimental period. *Bombyx mori* ΔE decreases quite rapidly with time and it reduces to half in 60–70 minutes. *Macrothylatia* ΔE declines in the first 40 minutes up to 2/3 of the initial value, then it maintains nearly constant values. It has been well stated in *Hyalophora cecropia* that the ΔE polarity (lumen positive) is due to a potassium active transport from haemolymph to the lumen [2, 3, 4]; therefore the effect on ΔE of K removal from the solutions bathing lumen and haemolymph sides has been tested in the three species (fig. 2). Under these conditions the ΔE always drops very rapidly thus confirming that K is the most important ion in the genesis of the potential; it is worth noting that the effect is reversible in all the species.

It can be seen (fig. 2) hat in *Philosamia* and in *Macrothylatia* ΔE always reaches appreciably negative values (lumen negative to haemolymph) in K-free medium, a behaviour that has also been observed in *Hyalophora cecropia* [12]. This negative ΔE in K–free medium of the same composition on both sides seems to suggest the active transport of an ion, either a cation moving from the lumen to the haemolymph or an anion moving to the lumen. Therefore the ions involved should be: Na^+, Ca^{++}, Mg^{++}, H^+ and Cl^-, HCO_3^-. Very recently an active Mg absorption has been demonstrated in *Hyalophora cecropia*[13] and this would of course give the reason for the lumen negative potential in K absence. The Na ion seems to be ruled out since it is present in very small amounts both in lumen content and haemolymph; furthermore few experiments performed in *Philosamia* by removing this ion from the perfusion fluids show that the cation does not influence the ΔE values. An H^+ absorption or

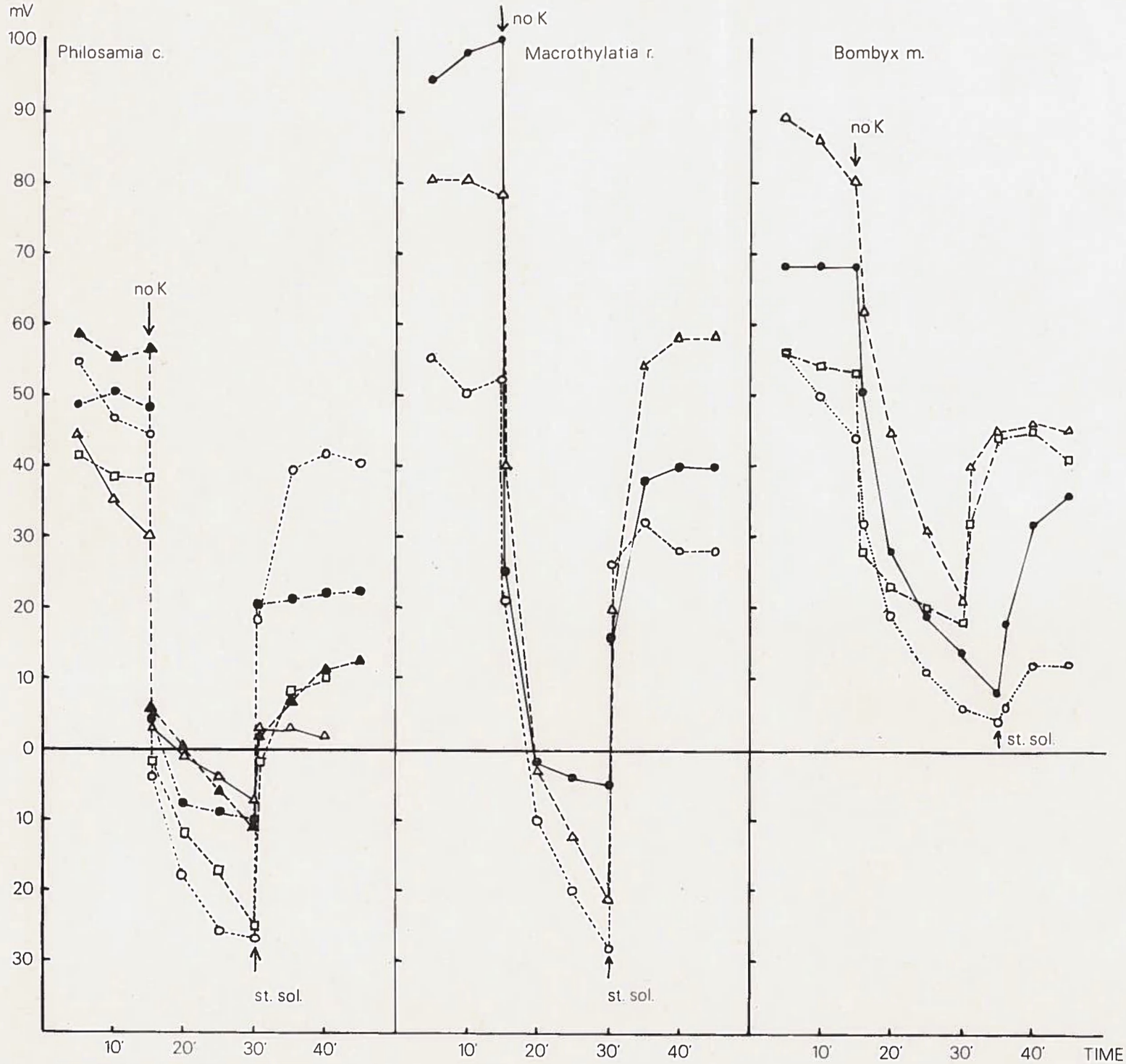

Fig. 2. – Transepithelial electrical potential difference variations when the standard solution is replaced by K-free solution. St. sol.: standard solution; no K : K-free solution.

HCO_3^- secretion should be taken into account since *in vivo* in these silkworms a large pH gradient between the lumen (pH $>$ 9) and the haemolymph (pH $<$ 7) exists.

Bombyx mori midgut behaves in a different way since K removal does not cause a ΔE reversal even when the midgut is exposed to a K–free solution for a longer period (up to 40 minutes).

Acknowledgments. The Authors are very much indebted to prof. V. Capraro for his helpful criticism. The Authors are also grateful to prof. G. Reali, Director of " Istituto Sperimentale per la Zoologia Agraria-Sezione specializzata per la Bachicoltura ", Brusegana, Padova, Italy, for kindy supplying *Bombyx mori* larvae.

REFERENCES

[1] R. W. HARVEY and S. NEDERGAARD (1964) – *Sodium independent active transport of potassium in the isolated midgut of Cecropia silkworm*, « Proc. Natl. Acad. Sci. », *51*, 757–762.

[2] W. R. HARVEY, J. A. HASKELL and S. NEDERGAARD (1968) – *Active transport by the Cecropia midgut.* III: *Midgut potential generated directly by active* K *transport*, « J. exp. Biol. », *48*, 1–12.

[3] S. NEDERGAARD and W. R. HARVEY (1968) – *Active transport by the Cecropia midgut.* IV: *Specificity of the transport mechanism for potassium*, « J. exp. Biol. », *48*, 13–24.

[4] K. ZERAHN (1971) – *Active transport of the alkali metals by the isolated midgut of* Hyalophora cecropia, « Phil. Trans. Roy. Soc. Lond. B. », *262*, 315–321.

[5] W. R. HARVEY and J. L. WOOD (1973) – *The route of cation transport across the Silkworm midgut.* In: *Transport mechanisms in Epithelia*, Munksgaard, Copenhagen, pp. 342–357.

[6] K. ZERAHN (1975) – *Potassium exchange between bathing solution and midgut of* Hyalophora cecropia *and time delay for Potassium flux through the midgut*, « J. exp. Biol. », *63*, 295–300.

[7] W. R. HARVEY, J. A. HASKELL and K. ZERAHN (1967) – *Active transport of potassium and oxygen consumption in the isolated midgut of* Hyalophora cecropia, « J. exp. Biol. », *46*, 235–248.

[8] G. DUCHATEAU, M. FLORKIN and C. LECLERCQ (1953) – *Concentration des bases fixes et types des composition de la base totale de l'hemolimphe des Insectes*, « Arch. Int. Physiol. » *61*, 518–549.

[9] M. FLORKIN and C. JEUNIAUX (1975) – *Haemolymph composition.* In *The physiology of Insecta*, Rockstein, pp. 255–307.

[10] J. M. TOBIAS (1948) – *The high potassium and low sodium in the body fluid and tissues of a phytophagous Insect, the silkworm* Bombyx mori *and the change before pupation*, « J. Cell. Compar. Physiol. », *31*, 143–148.

[11] M. NAOUMOFF and C. JEUNIAUX (1970) – *Modifications de la composante cationique inorganique de l'homlymphe au cours du developpment et des metamorphoses de quelques Lepidopteres*, « Arch. Int. Physiol. Biochim. », *78*, 357–365.

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

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