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Study on the Genesis of the Transepithelial Electrical Potential Difference in the Midgut of Leucophaea maderae

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Fisiologia. — *Study on the Genesis of the Transepithelial Electrical Potential Difference in the Midgut of* Leucophaea maderae. Nota di V. FRANCA SACCHI e BARBARA GIORDANA (*), presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — L'intestino medio della *Leucophaea maderae*, isolato e perfuso con una opportuna soluzione fisiologica, presenta una differenza di potenziale transepiteliale (dp) variabile, con valori compresi tra 16 mV con polo negativo nel lume e 20 mV con polo positivo nel lume. L'analisi della concentrazione di sodio e potassio a cavallo dell'intestino medio *in vivo* e gli esperimenti condotti *in vitro* sulla dp (effetto della concentrazione del potassio, della ouabaina e dell'ossigenazione) suggeriscono la presenza di due meccanismi di trasporto implicati nella genesi, della dp, identificabili con una estrusione di potassio ioni dall'emolinfa al lume e con un assorbimento di sodio in direzione opposta.

The ionic composition of insect hemolymph varies widely in the different orders. Florkin and Jeuniaux [1], studying a large number of insects, have shown that there are two "extreme" hemolymphatic ionic patterns: one characterized by high Mg and K and low Na and Cl, typical of phytophagous **Lepidopteran larvae**, and a second one, found in more primitive orders (i.e. *Dictyoptera, Odonata*), which present high concentrations of Na and Cl and low concentrations of K and Mg. Excretion in insects is accomplished by the Malpighian tubule-rectum system [2]; however in the larvae of Lepidoptera the midgut also plays a role in the excretion of K ions. It is now well demonstrated [3] that the goblet cells present in the midgut tissue of these larvae are able to transport K ions from the hemolymph to the lumen, the transport being O_2 dependent, Na independent and ouabain insensitive. The K extrusion gives rise to a high lumen positive electrical potential difference (up to 100 mV) across the midgut [4].

In *Periplaneta americana*, which is characterized by a high Na concentration in the hemolymph, sodium is absorbed in the rectal pads [5] and in the midgut [6, 7, 8]. A possible homeostatic role of the midgut in K extrusion has been suggested for these animals by Sauer *et al.* [8]. This hypothesis has been investigated, and this work reports preliminary results obtained on the isolated midgut of *Leucophaea maderae* (*Dictyoptera*).

MATERIALS AND METHOD

Experiments were performed on adult *Leucophaea maderae* reared at 27° and fed ad libitum with bread, lettuce and water. Animals were always anesthesized with ether.

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(**) Nella seduta del 13 maggio 1978.

Na and K determinations. 1) Hemolymph. Hemolymph samples were taken by slipping a capillary between the abdominal tergites into the heart. The hemolymph was conveniently diluted with distilled water and proteins were precipitated with 0.6 N $HClO_4$. After centrifuging for 10 min., the supernatants were assayed for Na and K by means of a flame photometer (Corning flame photometer 430). 2) Intestinal content. Animals were fasted for 48 h before dissection.

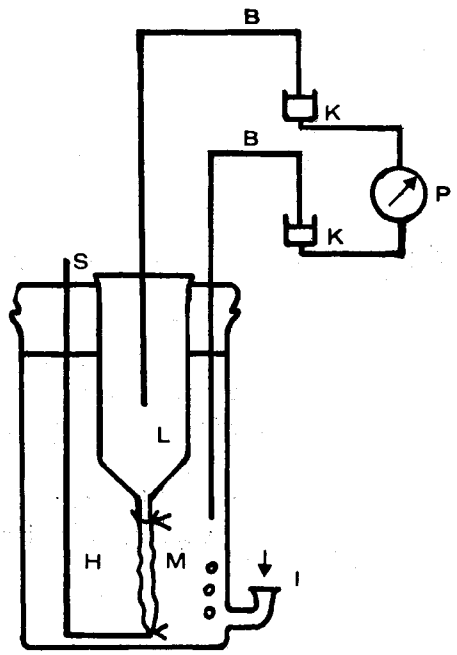

Fig. 1. – Experimental apparatus. M: midgut; L: lumen side chamber; H: hemolymph side chamber; P: Voltmeter; I: gas inlet; S: midgut support; K: Kalomel electrodes; B: agar-KCl bridges.

The dorsal part of the tergites was cut away exposing the intestine; the midgut was excised immediately below the caeca and above the Malpighian tubules. The perithrophic membrane with the enclosed intestinal content was removed, placed in a tared tube and weighed.

Distilled water was then added, the suspension thoroughly mixed by means of a Vortex and centrifuged for 10 mins. Na and K concentrations were then determined. 3) Midgut tissue. After dissecting the animal as previously described, the same portion of the midgut was excised, the lumen content removed, the tissue lightly blotted on filter paper (Whatman n°. 1), squashed and placed in a tared tube. The midguts of three animals were pooled together and weighed.

One ml distilled water was added, the suspension mixed, frozen, thawed, resuspended and centrifuged for 30 mins. The supernatant was diluted 1 : 1 with 0.6 N $HClO_4$, centrifuged for 5 mins and essayed for Na and K. The sediments were dried overnight and weighed to obtain total tissue water.

Transepithelial electrical potential difference (pd) measurements. The isolated midguts were mounted as sacs on the apparatus described in Fig. 1. Salines having an ionic pattern close to that found in the hemolymph were prepared. The experimental salines had the following composition:

	Na^+	K^+	Mg^{2+}	Ca^{2+}	SO_4^{2-}	Cl^-	HPO_4^{2-}	$H_2PO_4^-$	Sucr.	Glu.	
Standard solution .	108.4	12	6	3	6	118	3.2	2	100	30	mM
K-2 solution . . .	108.4	2	6	3	6	118	3.2	2	100	30	mM
No K solution . .	108.4	—	6	3	6	118	3.2	2	100	30	mM

Sucrose was added until the osmolarity determined in the hemolymph (about 400 mOsm) was reached; the pH of salines was 7. The pd was recorded by means of a Keithly 155 microvoltmeter. When the effect on pd of different K concentrations was observed, suitable volumes from a molar KCl solution were added to bathing fluids. Ouabain, when used, was added from a concentrated solution to the bathing fluids to obtain a final concentration of 10^{-3}M.

Solutions were stirred and aerated by bubbling with air only the hemolymphatic side of the tissue, since luminal or hemolymphatic aeration assures the maximum activity of the epithelium.

Results and discussion

Table I shows Na and K distribution across the midgut of *Leucophaea maderae*: sodium concentration is high in the hemolymph and relatively low in the tissue and lumen content, while the reverse is true for potassium. This distribution should be maintained by the activity of the epithelium: to keep the hemolymphatic pattern constant, Na has to be transferred from lumen to hemolymph, while potassium should be extruded. It must be noted that fresh tissue concentrations give only approximate information, since intracellular concentrations can be quite different, according to mucosal and hemolymphatic extracellular space volumes.

The midgut, isolated and perfused with a standard solution, exhibits a transepithelial potentials difference (pd) which, after an equilibration period, remains almost stable for more than two hours. The spontaneous pd, measured 15 minutes after isolation, varies between — 16 mV (lumen negative) and + 20 mV (lumen positive), the distribution being reported in Fig. 2. This variability does not seem to be due to diet or breeding

conditions, but seasonal influences cannot be excluded. In any case, these pd were recorded between December and June. This wide range of pd values is puzzling and quite unusual, since it involves a reversal of polarity.

TABLE I

Na *and* K *concentrations in the hemolymph, lumen content and fresh midgut tissue.*

Means ± S.E. Number of experiments in parenthesis.

	Na	K
Hemolymph . . . . . mEq/l hemolymph	103.2±5.6 (10)	13.6±0.9 (9)
Midgut . . . . . mEq/l tissue water	48.3±2.3 (11)	153.4±3.8 (11)
Lumen content . . . . . mEq/kg wet weight	22.3±3.5 (4)	103.6±8.7 (5)

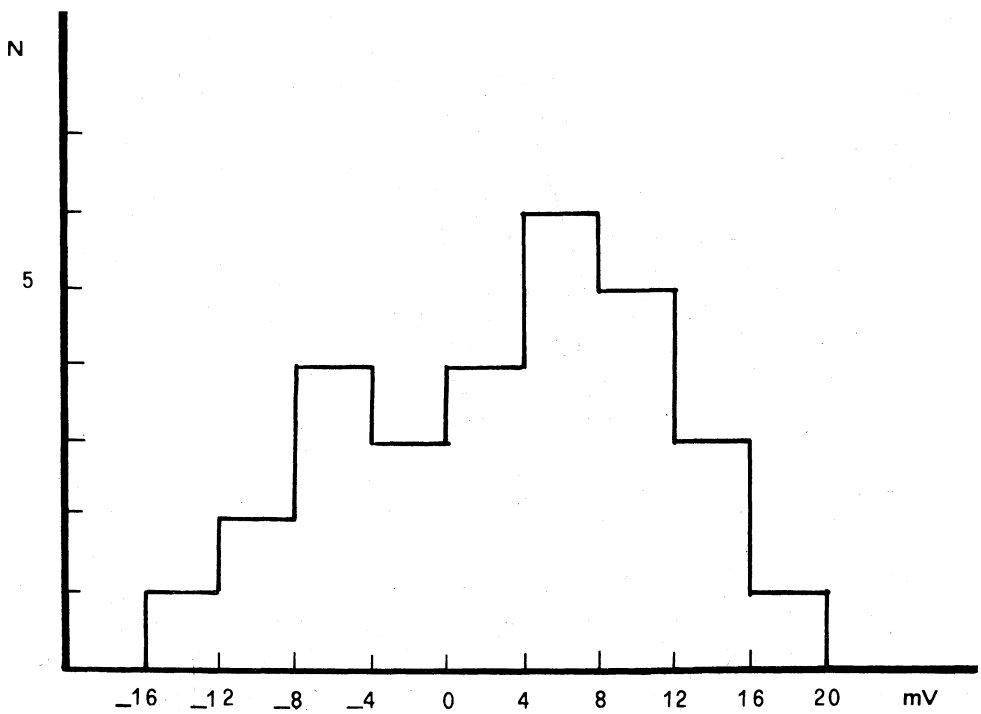

Fig. 2. – Distribution of spontaneous pd values. N: number of experiments. Positive values refer to a positive polarity in the lumen; negative values refer to a negative polarity in the lumen.

Fig. 3 shows that the pd always drops dramatically when the standard solution is replaced with K-free solution, whatever the polarity of the spontaneous potential. Moreover, when the initial pd is lumen positive, a relevant potential of opposite polarity is revealed by K absence. The pd recovers almost completely when K ions are restored. Fig. 4 shows

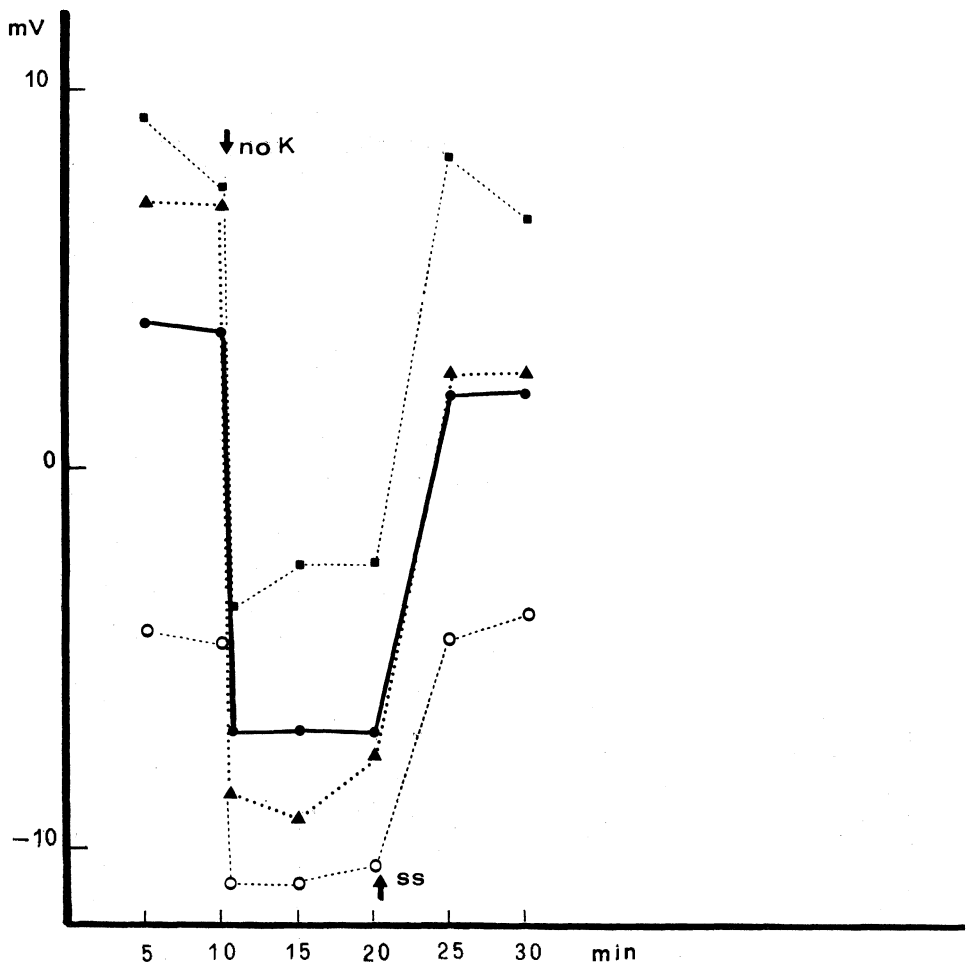

Fig. 3. – Effect on pd of K absence in the perfusion fluids. Typical experiments. Positive and negative values as explained in Fig. 2.

the effect on pd of a progressive increase of K concentration in the bathing fluids: the pd (means of 10 expts) goes from a lumen negative value at K 2 mM to lumen positive values at higher concentrations of K. The effect of the cation on pd seems to involve a saturating process.

Both the drop in pd in K absence and the lumen positive pd rise with increasing K concentrations are consistent with rheogenic transport of K from the hemolymph to the lumen.

The lumen negative pd—spontaneous or evidenced by low K concentrations—and sodium distribution across the midgut (Fig. 2) could be due to an absorption of sodium from lumen to hemolymph This hypothesis is supported by the influence on pd of ouabain, which is a specific inhibitor of Na-K ATPase involved in Na absorption.

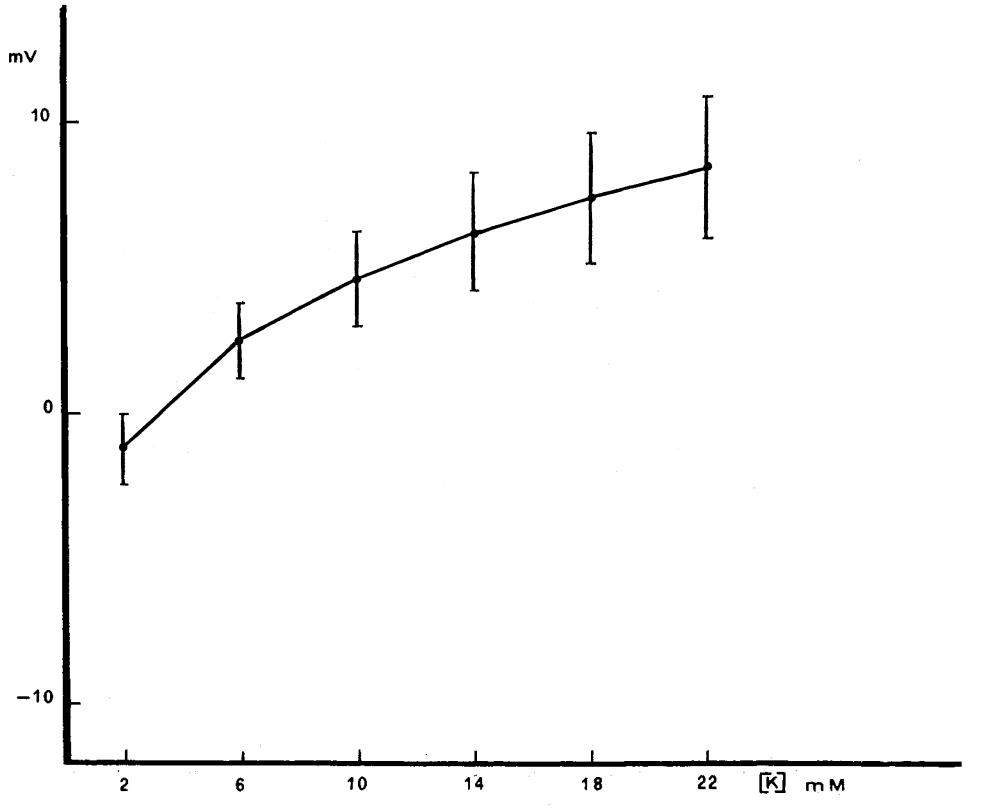

Fig. 4. – Effect on pd of K concentration in the perfusion fluids. Means ± S.E. (10 experiments). Positive and negative values as in Fig. 2.

Fig. 5 shows that the addition of the drug to hemolymphatic solution causes a significant increase in the lumen positive pd while the lumen-negative potential declines towards zero (typical experiments: 13 experiments are consistent with the graphs reported in the fig.). The inhibitor does not affect the pd when added to the luminal solution. These results can be explained by the presence of a sodium absorption which is at least partially ouabain sensitive, and emphasize the presence of two independent transport mechanisms involved in the genesis of transepithelial pd.

Fig. 6 reports the influence on pd of the absence of hemolymphatic aeration when the midgut is perfused with standard solution: the pd drops, reaches lumen-negative values and completely recovers when oxygen is restored. When the tissue is aerated from the luminal side only, the lack of

air causes very similar effects. Fig. 7 shows the same experiment carried out by perfusing the tissue with a solution containing potassium 2 mM: in these experimental conditions the pd is always lumen-negative, since the K extrusion is reduced. In the absence of air, the absolute value of the pd increases noticeably, the effect being reversed. These experiments can be

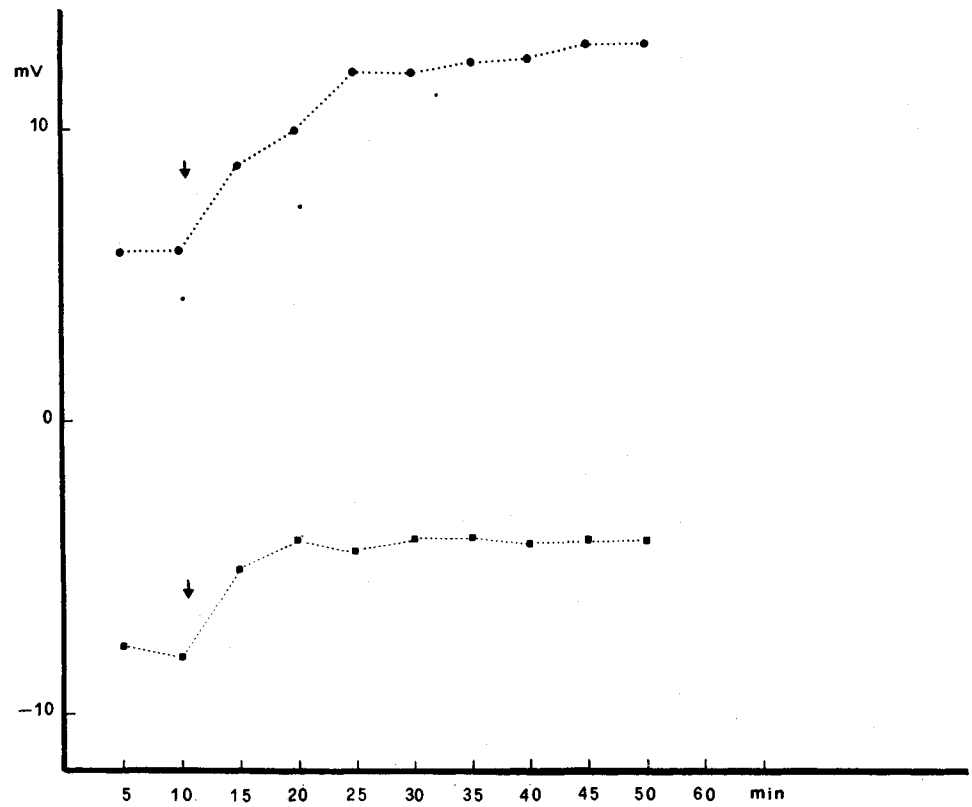

Fig. 5. – Effect on pd of 10^{-3}M ouabain added to the hemolymphatic perfusion fluid. Typical experiments. Positive and negative values as explained in Fig. 2.

explained by a different oxygen sensitivity of the two transport mechanisms: potassium excretion seems to be oxygen dependent, while sodium absorption is scarcely sensitive to aeration. As suggested for the small inhibitory effects of nitrogen on sodium generated pd across the isolated ventriculus of *Periplaneta americana* [6], the lumen negative pd could be maintained by anaerobic metabolism. It should be noted that potassium excretion is not abolished when K is reduced to 2 mM.

The wide range of spontaneous pd found in the midgut can be related to 2 transport mechanisms the activity of which varies according to unidentified physiological conditions of the insect.

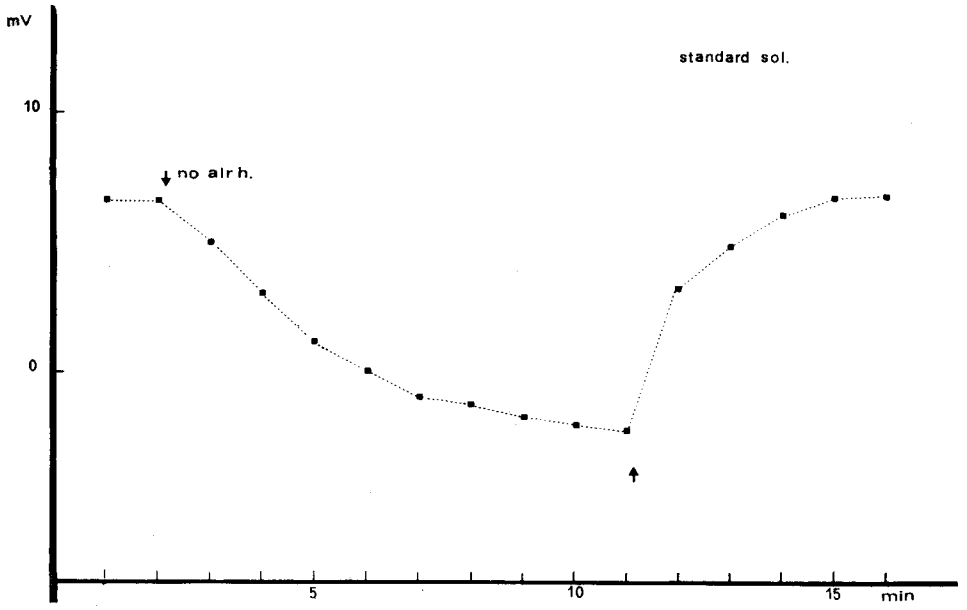

Fig. 6. – Effect on pd of hemolymphatic aeration. ↓ no air h: removal of hemolymphatic aeration; ↑: hemolymphatic aeration restored. Typical experiment. Positive and negative values as explained in Fig. 2.

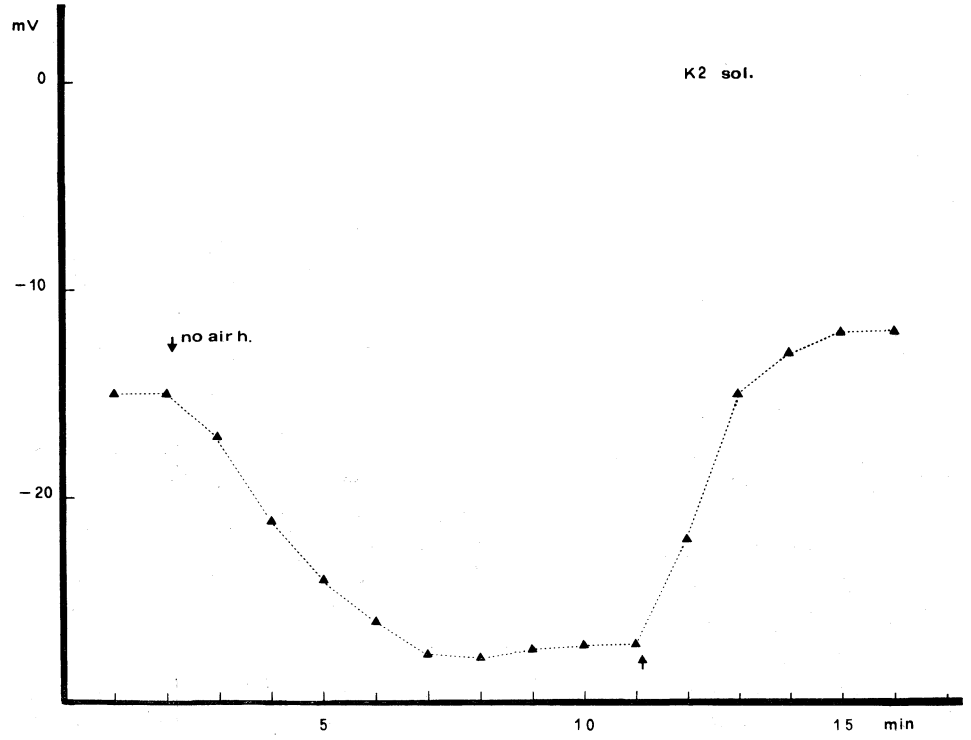

Fig. 7. – Effect on pd of hemolymphatic aeration when the midgut is perfused with K 2 solution. ↓ no air h: removal of hemolymphatic aeration; ↑: hemolymphatic aeration restored. Typical experiment. Positive and negative values as explained in Fig. 2.

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