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SHORT COMMUNICATION

TRANSPORT OF SODIUM AND POTASSIUM ACROSS THE ISOLATED MIDGUT OF THE LARVAE OF *TENEBRIO MOLITOR* RELATED TO THE FINE STRUCTURE OF THE EPITHELIUM

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For meal-worms, which are able to survive under extremely dry living conditions, the normal situation is that the K^+ concentration of the haemolymph regulates the process of water conservation via the rectal complex (Grimstone, Mullinger & Ramsay, 1968; Koefoed, 1975). The aim of this paper has been to study the handling of sodium and potassium by the midgut epithelium of the larva of *Tenebrio molitor* and to discover if the transport of these ions is reflected in the structure of the epithelium.

Meal-worms measuring from 1·8 to 1·9 cm were obtained from stock cultures maintained on dry bran. Such meal-worms were considered normal. Their average weight was 0·1 g. Freshly moulted larvae were not used.

A piece of the midgut was emptied and within 2 min mounted in the apparatus described in Fig. 1.

The apparatus was mounted on a stereomicroscope and shielded by a grounded 'Faraday cage'. Five mM Amaranth (Merck. 1248) was added to the solution washing the gut lumen to detect possible leakages.

The Ringer solution (including the Ringer's with Amaranth) contained: Na, 66 m-equiv/l, and K, 36 m-equiv/l (both as chlorides), which corresponds to the concentration of these ions in the haemolymph of 'normal' meal-worms (Ramsay, 1964). The sucrose content of the Ringer was 166 mM. Samples were taken at 5–10 min intervals: from the lumen side 50 μl and from the haemocoel side 150 μl. Each time the 150 μl taken from the haemocoel side were replaced. The specimen was weighed on a Cahn Electrobalance. The total potassium and sodium content of the specimen was measured on a flame photometer (Unicam SP 90 B). The extracellular space was measured by addition of [^{14}C]inulin. All the experiments were done at room temperature.

The midgut is circular in cross-section. The epithelium consists of very tall and narrow, uniform columnar cells plus some regenerating cells. The conspicuous basal lamina consists of many electron-dense particles with some space of lesser electron density between. The basal plasma membranes are highly folded, forming long and

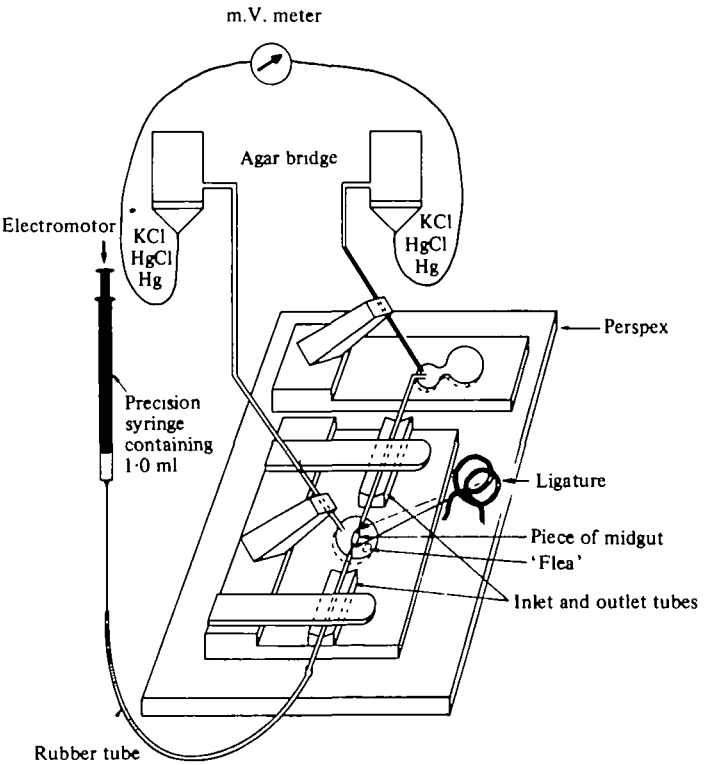

Fig. 1. Midguts with an average volume of 3·43 mm^3 was mounted on two glass tubes in an open chamber of perspex containing the stirred solution bathing the haemocoel side. The size of the bath was 450 μl. The glass tubes had an outer diameter of 1·6 mm. The end of the tube where the gut was mounted was narrower, with an outer diameter of 0·5 mm and rounded. The outlet tube terminated in another open chamber from which samples from the solution bathing the lumen were taken. The lumen solution was pumped through the midgut with a speed of 10 μl/min by a precision syringe containing 1·0 ml. The potential difference was measured by calomel electrodes connected via agar Ringer bridges to the solutions bathing haemocoel side and lumen of the gut.

narrow channels with extremely narrow openings toward the basal lamina. Many of the channels run through most of the cell, having connexions with each other and perhaps with the apical membrane between the microvilli. The cells are joined by desmosomes, septate desmosomes and gap junctions. Mitochondria are associated with the previously mentioned channels, and the cells contain rough endoplasmic reticulum, free ribosomes, Golgi complexes and many lysosomes. This fine structure corresponds to the previously described typical form of midgut epithelium fine structure in more primitive insects with high content of sodium in the haemolymph relative to potassium and with active transport of sodium from lumen to haemocoel side (O'Riordan, 1969).

No significant potential difference across the midgut epithelium was recorded. Measurements of p.d. from ten meal-worms showed an average of 0·4 mV ± 0·2, lumen negative.

No sure signs of survival were found, therefore the total concentration of potassium was measured in pieces of the midgut from the same meal-worm immediately aft

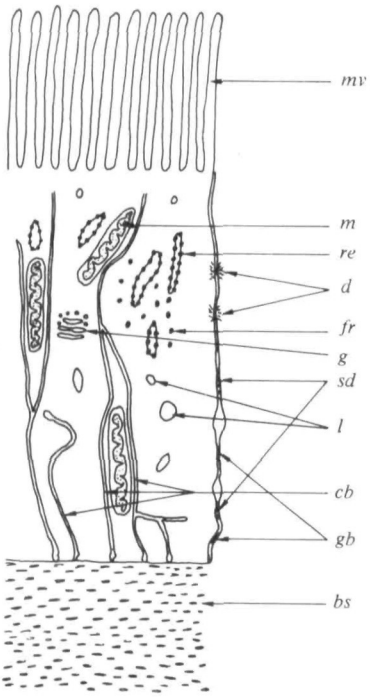

Fig. 2. Diagram of the most important features of the midgut epithelium of the larva of *Tenebrio molitor*. *bs*, Basal lamina, *cb*, channels formed by folding of the basal cell membranes; *d*, desmosomes; *fr*, free ribosomes; *g*, Golgi complex; *gp*, gap junctions; *l*, lysosomes; *m*, mitochondria; *mv*, microvilli of the brush border; *re* rough endoplasmic reticulum; *sd*, septate desmosomes.

dissection and after the midgut piece had been set up in normal bathing solution for 3 h. The figures are listed in Table 1. If the midgut was dead the total concentration of potassium in the empty gut could be expected to approach the concentration of the ions in the bathing solution with time. Table 1 shows that this was only found in one case. This gut (no. 3) must be presumed dead.

The extracellular space, expressed as inulin space in microlitres, was much larger on the haemocoel side (mean value, 0·49 μl/mm^2) than on the lumen side (mean value 0·095 μl/mm^2). The very conspicuous basal lamina (Fig. 2) may explain this difference.

The Na^+ was labelled with ^{22}Na on the haemocoel side and ^{24}Na on the lumen side. The K^+ was labelled with ^{42}K on either the haemocoel side or the lumen side of the gut. Table 2 demonstrates a net flux of Na^+ from lumen to haemocoel side; since the bathing solutions on the two sides were identical, the transport must be considered to be active. There is no significant net transport of potassium over the meal-worm midgut. Twelve measurements of the flux in each direction gave mean values of 0·24 $\pm$ 0·04 μequiv/h/mm^2 for K^+ flux from lumen to haemocoel side and 0·31 $\pm$ 0·04 μ-equiv/h/mm^2 for K^+ flux in the opposite direction. Regarding potassium it was necessary to compare different specimens.

Table 3 shows that the *in vitro* exchange of cellular sodium must be solely from the haemocoel side, and that of cellular potassium was about twice as fast with the haemocoel side as with the lumen side. It is a general trait for the insect midgut that the

Table 1. *Nine midguts from different meal-worms each divided into two parts*

(In those with the suffix (*a*) the total content of potassium was measured immediately after dissection. In those with the suffix (*b*) the same measurements were made after the specimen was kept in normal bathing solution (like the ordinary experimental procedure) for 3 h.)

No. of specimen	(*a*) (μequiv/g wet wt.)	(*b*) (μequiv/g)
1	100	114
2	84	103
3	88	68
4	101	93
5	92	98
6	69	185
7	56	102
8	101	117
9	61	83
Mean value	83 ± 7	112 ± 11
Bathing solution	36	

Table 2. *Mean values of Na^+ flux across isolated midguts of* 11 *meal-worms in ordinary bathing solution*

(The flux was measured after 40 min when steady state was obtained. H, Haemocoel; L, lumen.)

H→L (μequiv/h/mm²)	L→H (μequiv/h/mm²)	$\frac{L \rightarrow H}{H \rightarrow L}$	Net flux (μequiv/h mm²)
0·069 ± 0·012	0·214 ± 0·028	4·16 ± 0·95	0·15 ± 0·03 (L→H)

Table 3. *Mean values of sodium and potassium originating from haemocoel and from lumen in the midgut cells of* 13 *specimens after* 1 *h experiments measured by labelling the bathing solutions with ^{22}Na, ^{24}Na and ^{42}K*

(No corrections for extracellular space was made for the individual data of the table. The investigations with ^{42}K on haemocoel and on lumen were made on different animals. The K^+ content of the extracellular space is 0·036 μequiv/ml and the Na^+ content 66 μequiv/ml.- μequiv/mg mean wet weight was corrected for extracellular space according to the mean value previously mentioned.)

	μequiv/mg wet weight	μequiv/μequiv total content of the ion in the specimen	Mean μequiv/mg weight
		From haemocoel	
K	0·064 ± 0·016	0·274 ± 0·076	0·044
Na	0·057 ± 0·004	0·262 ± 0·030	0·021
		From lumen	
K	0·025 ± 0·006	0·128 ± 0·028	0·022
Na	0·004 ± 0·002	0·026 ± 0·009	negative .

epithelium shows asymmetry with respect to the cellular exchange of both ions and uncharged molecules like amino acids. There is a very rapid cellular exchange with the haemocoel side solution and a slow or non-existing exchange with the lumen solution regardless of the direction of a transport of the ions or molecules. In the midgut of the larvae of *Hyalophora cecropia*, for example, this is found for K^+, which is actively transported over the epithelium from haemocoel side to lumen side of the

t (Harvey & Zerahn, 1969), as well as for some amino acids, which are actively transported in the opposite direction (Nedergaard, 1977).

The experiments were done without Ca^{2+} and Mg^{2+} in the bathing solutions, but the authors did not find that the p.d. was affected by addition of 5 mmol/l of Ca^{2+} and 5 mmol/l of Mg^{2+}, both as chlorides to the bathing solutions. Altering the Na^{+} concentration on either side of the epithelium between 0 and 87 μequiv/ml, and the K^{+} concentration between 0 and 40 μequiv/ml, similarly had no effect on the p.d.

The only cations in the bathing solutions were sodium and potassium, so that the finding that there was neither significant net flux of potassium nor significant potential difference over the epithelium suggests that chloride may be involved in the transport of sodium.

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REFERENCES

GRIMSTONE, A. V., MULLINGER, A. M. & RAMSAY, J. A. (1968). Further studies on the rectal complex of the meal worm *Tenebrio molitor* L. (Coleoptera, Tenebrionidae). *Phil. Trans. R. Soc.* B **253** (788), 343–382.

HARVEY, W. R. & ZERAHN, K. (1969). Kinetics and route of active K-transport in the isolated midgut of *Hyalophora cecropia*. *J. exp. Biol.* **50**, 297–306.

KOEFOED, B. M. (1975). The cryptonephridial system in the meal worm *Tenebrio molitor*: Transport of radioactive potassium, thalium and sodium; a functional and structural study. *Cell. Tiss. Res.* **165**, 63–78

NEDERGAARD, S. (1977). Amino acid exchange between blood and intestinal tissue in the larvae of the silk moth, *Hyalophora cecropia*. Abstracts 11th FEBS Meeting, Copenhagen: B 300.

O'RIORDAN, A. M. (1969). Electrolyte movement in the isolated midgut of the cockroach (*Periplaneta americana* L.). *J. exp. Biol.* **51**, 699–714.

RAMSAY, J. A. (1964). The rectal complex of the meal worm *Tenebrio molitor* L. *Phil. Trans. R. Soc.* B **248**, 279–314.