Extracellular space values and intracellular ionic concentrations in the isolated midgut of Philosamia cynthia and Bombyx mori

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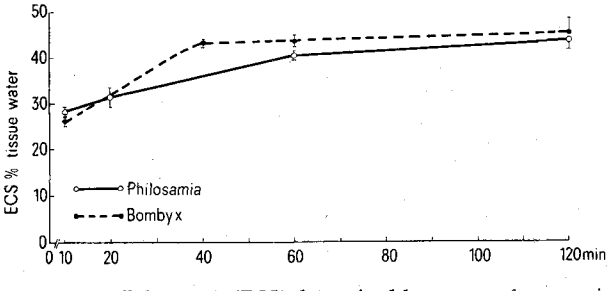
Summary. Total extracellular space values have been determined in the midguts of 2 lepidopteran larvae, Philosamia cynthia and Bombyx mori. The values found are 42% and 45% tissue water respectively. Intracellular concentrations of Na^+, Ca^{++} and Mg^{++} are very low, while K^+ concentration is 197.2 mEq/l cell water in Philosamia and 180.9 mEq/l cell water in Bombyx.

Extracellular space (ECS) evaluation is essential to determine cellular ionic concentrations: while both ECS value and cellular concentrations have been extensively studied in vertebrate tissues, few data are available in the literature about the intestinal tissue of lepidopteran larvae. These insects have an extracellular fluid, the haemolymph, richer in bivalent (Mg^{++}, Ca^{++}) than in monovalent (Na^+, K^+) cations, where the Na/K ratio is lower than 1[1]. Moreover, the midgut of these larvae is known to extrude K ion from the haemolymph to the lumen. The active transport of this ion is electrogenic, i.e. it generates a large transepithelial potential difference with the positive pole in the lumen[2,3]. It has been demonstrated in the isolated midgut of Hyalophora cecropia that K^+ transport is Na independent, ouabain insensitive and strictly oxygen dependent[4,5]. The morphological features of this tissue have been carefully studied[6,7]: the midgut contains in addition to columnar, goblet cells, cytologically different from those of vertebrates, which do not secrete mucus and whose participation in K^+ transport has been suggested. Both kind of cells present deep infoldings in the basal region of the plasma membrane.

Intracellular ionic concentrations in the perfused midguts of Philosamia cynthia and Bombyx mori

	K	Na	Mg	Ca	Cl
Philosamia cynthia (mEq/l cell water)	197.2±8.4 (4)	5.9±1.6 (4)	10.0±1.2 (4)	4.2±1.2 (4)	39.2±5.1 (4)
Bombyx mori (mEq/l cell water)	180.9±6.8 (8)	9.8±1.5 (6)	14.8±3.8 (8)	18.4±1.8 (8)	42.8±3.4 (4)

Number of experiments in parenthesis.



Total extracellular space (ECS) determined by means of sucrose in tube preparations of Philosamia cynthia and Bombyx mori at different periods. Means ± SE. 4 experiments for each point in Philosamia, 5 in Bombyx.

It therefore seems interesting to investigate the extracellular space value and intracellular ionic concentrations in the midgut of these larvae. In this work, labelled sucrose has been used to measure the extracellular volume in the midgut of 2 different larvae of Lepidoptera. The intracellular concentrations of Na^+, K^+, Ca^{++}, Mg^{++} and Cl^- have been calculated.

Materials and methods. Experiments were performed on Philosamia cynthia and Bombyx mori larvae in their last instar. The midgut was excised from the larva, the peritrophic membrane and enclosed intestinal content gently removed, and the tissue mounted as a tube on the perfusion apparatus described elsewhere[8]. The perfusion media had a composition very near to that determined experimentally in the haemolymph (unpublished data) of each larva:

	NaCl	$KHCO_3$	KCl	$MgSO_4$	$CaCl_2$	Sucrose	
Philosamia	4	25	–	74	18	126	mEq/l
Bombyx	1.7	25	21	88	18	80	mEq/l

Sucrose was added to both solutions to reach the osmolarity as determined in the haemolymph (Philosamia: 284.6 ± 9.3 mOsm/l, 3 experiments; Bombyx: 290 ± 2.9 mOsm/l, 3 experiments). The perfusion media were aerated and stirred by bubbling with 95% O_2 and 5% CO_2, pH 7.4. Total extracellular space evaluation was made on tissues incubated for different periods (10, 20, 40, 60 and 120 min) in these perfusion media containing on both sides of the epithelium sucrose ^{14}C (Radiochemical Center, Amersham) 1 μC/ml. After incubation the midgut was removed, blotted on filter paper Whatmann N. 587 E, shattered, placed in a tared tube and rapidly weighed. 1 ml of bi-distilled water was added, the suspension thoroughly mixed, frozen, thawed and resuspended. After centrifuging for 30 min, supernatants were assayed for radioactivity by a liquid scintillation spectrometer (Tri-Carb Packard 3003 series). The sediments were dried overnight and weighed to obtain the

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weight of dry tissue and total tissue water. ECS expressed as % of tissue water was then calculated from the following formula

$$\text{ECS } \% = \frac{\text{Supernatant total cpm}}{\text{Perfusion media cpm/ml} \cdot \text{Tissue water}} \cdot 100$$

In a few experiments, ECS has been determined by means of inulin ^{14}C in isolated midguts of Bombyx mori perfused for 60 min, following the described procedure.

Supernatants were also assayed for Na^+, K^+, Mg^{++}, Ca^{++} and Cl^-. For Na^+, K^+ and Cl^- determinations, supernatants were previously diluted 1:1 with $HClO_4$ 0.6 N and centrifuged to precipitate the proteins. Na^+ and K^+ were determined by means of a flame photometer (Beckman DU-2). Mg^{++} and Ca^{++} were assayed colorimetrically after preparation of samples following Magnesium Merckotest (Art. No. 3338) and Calcium Kit (Clinton) indications. Samples were then read by C.E. 343 Single Sample Spectrometer Cecil Instrument Ltd, Cambridge. Chlorides were determined by means of mercurimetric tritration according to Chloride Merckotest (Art. No. 3311).

Results and discussion. In midguts of Philosamia cynthia mounted as tubes, a stable total ECS value of about 42% tissue water is reached with sucrose after 60 min (figure). Experiments not reported here, performed with sulphate ^{35}S, give after 10 min an ECS value of 40.5 ± 0.7% tissue water (4 experiments): this value remains constant for 60 min, but in the second h it slowly increases with time, presumably because sulphate enters the cells or somehow interferes with the charges of epithelial membranes. Total extracellular space has also been determined with sucrose in Bombyx mori midgut, where a constant ECS value of about 45% is reached after 40 min (figure). In this tissue the inulin ECS after 60 min of incubation is 35.1 ± 0.9% (4 experiments): this lower value can easily be explained by the larger molecular weight of the marker. It is known that sucrose is not a good marker for intestinal tissue of vertebrates since it is metabolized: on the other hand, the constant value found in the midgut of both Lepidoptera suggest that the entity of metabolization, if any, is negligible in these species. Furthermore, Bombyx mori midgut seems to be lacking in α-glucosidase[9].

A very small ECS is reported by Harvey et al.[10] for the midgut of another Lepidopterum, Hyalophora cecropia; this value has been questioned by Zerahn[11]. This author determined on the same larva a sucrose ECS value very similar to those reported in this paper (45–48% tissue water). The particular morphological features of this epithelium, characterized by deep plications and invaginations of the plasma membrane[6] may provide the reason for the large ECS. Moreover it should be emphasized that the midgut of these larvae cannot be scraped, so that the ECS determination is performed on the entire tissue. Besides, the non-epithelial part of the midgut is a minor fraction of the tissue[6]. Large ECS are not unusual in vertebrates intestines too[12,13].

The tissue concentrations of Na^+, K^+, Mg^{++}, Ca^{++} and Cl^- have been determined in midguts perfused for 60 min as tubes. The intracellular values were then calculated, correcting for the proper ECS value (table). Na ion concentration is very low, not very far from that of the perfusion fluid; divalent cations are also present in very small amount, even if Mg concentration is quite high in the extracellular fluids, being in Philosamia the most concentrated cation (74 mEq/l). Potassium concentration, on the contrary, is very high in both animals, being 197.2 ± 8.4 in Philosamia and 180.9 ± 6.8 mEq/l cellular water in Bombyx. These K^+ cellular concentrations are considerably higher than those found in the midgut of Hyalophora by Zerahn who reported a cellular concentration value of 137 ± 8 mEq/l[2].

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Effect of vinblastine on pancreatic enzymes secretion induced by cyclic nucleotide derivatives[1]

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Summary. Vinblastine did not affect the basal secretion of enzymes from the rat pancreas, but it potentiates the secretory response to dibutyryl cyclic AMP. This potentiation is confirmed by the observation of numerous pictures of exocytosis at the apical part of the acinar cell. Dibutyryl cyclic GMP by itself, or associated with vinblastine, failed to modify the spontaneous release of enzymes or the secretion induced by dibutyryl cyclic AMP.

The presence of microtubules in the acinar cell of the exocrine pancreas has been recognized by several authors[2,3]. They have been involved in the secretory response of the pancreas to cholinergics[2] and digestive hormones[3]. Both types of secretagogues enhance the level of cGMP[4] in the acinar cell[5] but fail to affect the level of cAMP[4–6]. Recent evidence suggests that cAMP[7,8] and cGMP[8] may play a role in the regulation of the structure and function of microtubules and tubulin. It is generally admitted that cAMP and its dibutyryl derivative (DbcAMP[4]) stimulate enzyme release in the pancreas of many species[9–12]. However, the effect of DbcGMP[4] is still disputed[5,12,13]. In order to investigate the possible participation of microtubules in the secretory response of the pancreas to both cyclic nucleotide derivatives, we have examined the effects of a mitotic spindle-inhibitor (vinblastine[4,14]) upon both the function and the ultrastructure of the acinar cell.

Materials and methods. Pancreata were taken from $2^1/_2$ months old albino rats fasted for 12 h. They were trimmed from lymphatic ganglia and epiploic fat and cut into 8 to 10 pieces. The incubation medium, enriched by D-glucose