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Metabolite transport by membrane vesicles isolated from the midgut of Philosamia cynthia larvae

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Fisiologia. — *Metabolite transport by membrane vesicles isolated from the midgut of Philosamia cynthia larvae* (*). Nota di GIORGIO M. HANOZET, BARBARA GIORDANA e FRANCA V. SACCHI, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — Sono state preparate vescicole di membrane dall'intestino medio di *Philosamia cynthia* (Lepidotteri) allo stadio larvale, utilizzando una procedura semplificata recentemente messa a punto. Le vescicole sono state utilizzate per studiare il trasporto a livello della membrana di fenilalanina e glucosio, in presenza di gradienti di sali di Na e K. A differenza di quanto osservato con membrane di intestino di mammifero, il gradiente di K, oltre a quello di Na, permette un accumulo contro gradiente dell'amminoacido, mentre il glucosio è poco permeabile attraverso la membrana sia in presenza di Na che di K.

The " sodium gradient hypothesis " proposed by Crane [1, 2] is the most widely accepted model for the absorption of glucose and amino acids in vertebrate intestine: the Na electrochemical gradient across the mucosal border

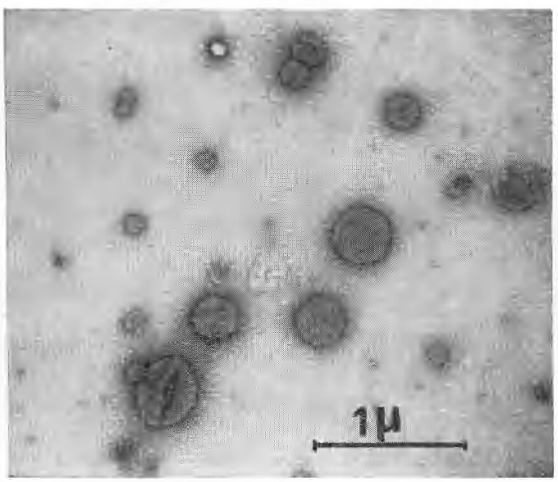

Fig. 1. - Electron micrograph of negatively stained membrane vesicles from *Philosamia cynthia* midgut.

of the enterocyte provides the driving force for the " uphill " transport of these metabolites. Recently this hypothesis received further support from experiments performed with a subcellular system of isolated plasma membrane vesicles [3–5]. On the other hand the phytophagous larvae of Lepidoptera

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have a very low Na concentration in the lumen content, intestine and hemolymph and they show a high K concentration in the lumen [6, 7, 8].

In the present work a preparation of membrane vesicles from the midgut of the silkworm *Philosamia cynthia* has been used to investigate the dependence of metabolites uptake upon Na and K gradients.

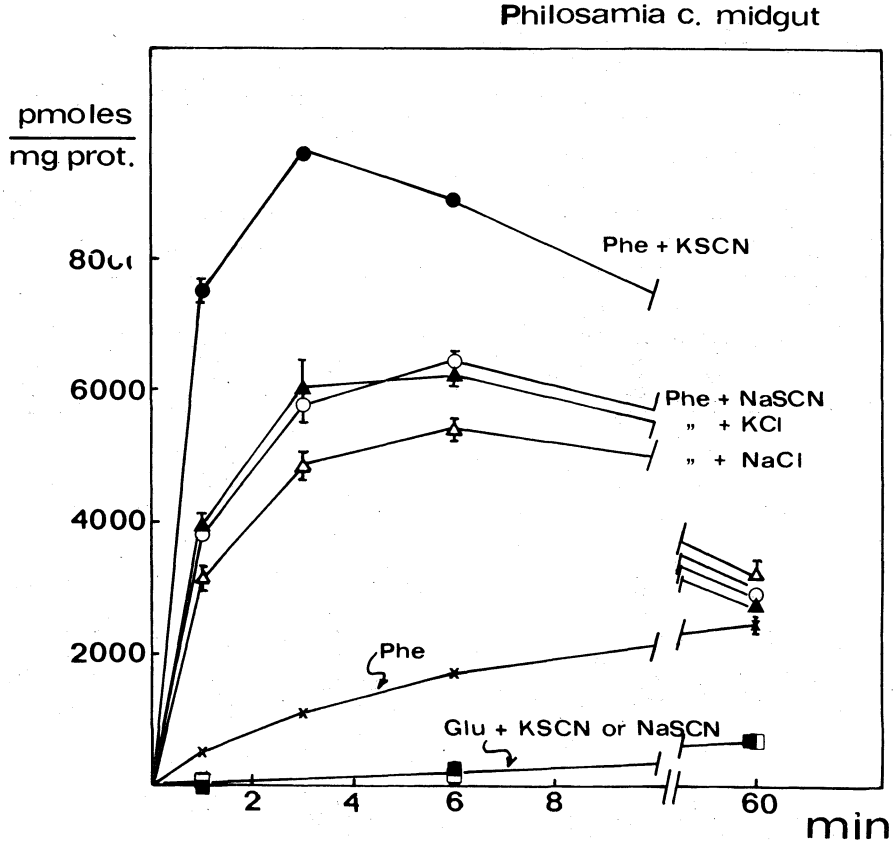

Fig. 2. – Uptake of Phenylalanine (Phe) and D-glucose (Glu) by membrane vesicles from *Philosamia cynthia* midgut. Phenylalanine (1 mM) uptake in the presence of an initial gradient (100 mM outside and 0 inside) of: KSCN (●), NaSCN (○), KCl (▲), NaCl (△). Phenylalanine (1 mM) uptake in the absence of any salt gradient (×). D-glucose (1 mM) uptake in the presence of an initial gradient (100 mM outside and 0 inside) of KSCN (■) or NaSCN (□). The buffer was in all cases 10 mM HEPES-Tris pH 7.5, 100 mM D-mannitol. The bars indicate the SE. When not given, the SE were smaller than the symbols used.

The vesicles were prepared from midguts of *Philosamia cynthia*, in their last larval instar. 1–2 gr of fresh midguts, deprived of the peritrophic membrane and malpighian tubules, were homogenized with a Potter Elvehjem homogenizer in 50 mM mannitol and 2 mM HEPES (N-2-hydroxyethylpiperazine-N'-ethanesulphonic acid)-Tris buffer, pH 7.1. The vesicles were then obtained

according to Kessler *et al.* [4]. The pellets from the second and third centrifugation were resuspended in 100 mM mannitol, 10 mM HEPES-Tris buffer, pH 7.5. The vesicles from rabbit small intestine were prepared following the same procedure from 5 gr fresh scraped mucosa homogenized in a Waring blendor at maximum speed for 2 min. The uptake of metabolites was determined incubating membrane vesicles at room temperature in a mixture of the following composition: 100 mM mannitol, 10 mM HEPES-Tris buffer, pH 7.5, 1 mM labelled substrate, and salt gradients as indicated in the legends of the figures. At selected times samples of 20 µl were withdrawn from the incubation mixture, diluted with 1.5 ml ice-cold 150 mM NaCl + 1 mM HEPES-Tris (stop solution), filtered through a Sartorius filter (SM 11305, 0.6 µm pore size) and rapidly rinsed with 10 ml ice-cold stop solution. Radioactivity readings were then performed by means of a liquid scintillation

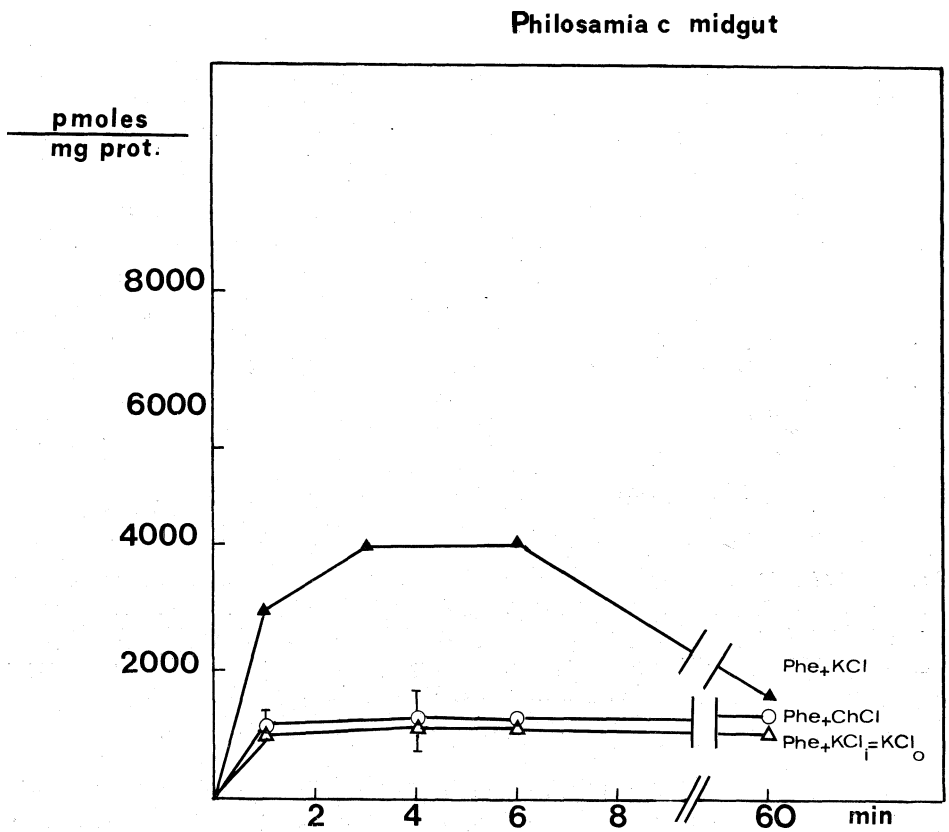

Fig. 3. – Phenylalanine (Phe) uptake by membrane vescicles from *Philosamia cynthia* midgut. Phenylalanine (1 mM) uptake in the presence of an initial gradient (100 mM outside and 0 inside) of KCl (●) and Choline Cl (○). Phenylalanine (1 mM) uptake in the absence of K gradient: (△) KCl (100 mM outside and 100 mM inside). For this purpose the vesicles were preequilibrated for 30 min in 100 mM KCl. The buffer was in all cases 10 mM HEPES-Tris pH 7.5, 100 mM D-mannitol. The bars indicate the SE. When not given, the SE were smaller than the symbols used.

spectrometer (Tri-Carb Packard 3003 series). The radiochemicals, D-(U-^{14}C) glucose, L-(U-^{14}C) phenylalanine and D-(1-^{3}H)-3-O-methylglucose, were purchased from Amersham Radiochemical Centre. For electron microscopy, a negative staining procedure was used: the vesicles (diluted to about 1 mg protein/ml) were directly stained with 1 % uranyl acetate for 30 s. Protein determination was carried out according to Lowry *et al.* [9].

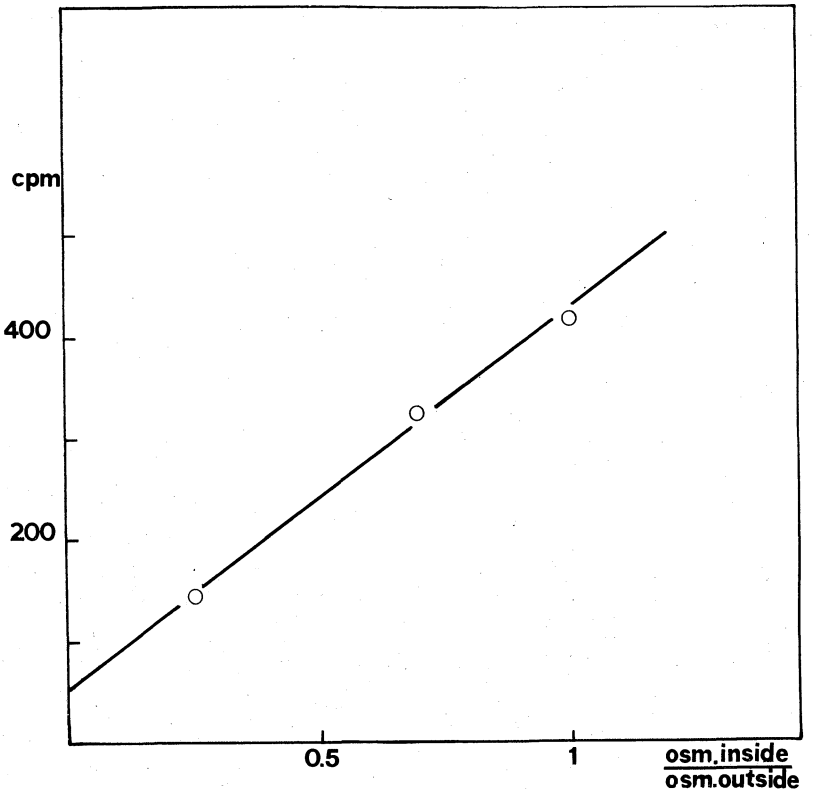

Fig. 4. - Effect of medium osmolarity on phenylalanine uptake by membrane vesicles from *Philosamia cynthia* midgut. The vesicles were prepared in 100 mM D-mannitol, 10 mM HEPES-Tris, pH 7.5 and were incubated for 60 min in a medium of the following composition: 10 mM HEPES-Tris, pH 7.5, 0.5 mM phenylalanine, 20 mM KSCN and varying concentrations of D-mannitol.

A micrograph or the vesicles obtained from the midgut of *Philosamia cynthia* can be seen in Fig. 1: the vesicular size and shape are not very different from those previously observed in preparations from mammalian intestine [4, 10]. L-phenylalanine uptake by these vesicles is K sensitive (Fig. 2). A concentrative transport of phenylalanine in the presence of both sodium and potassium salt gradient is apparent: however the " overshoot " is much more remarkable when a KSCN concentration gradient is provided. SCN^- ion is more permeable than chloride across the plasma membranes and

so it causes a higher polarization, inside negative, across the membrane vesicle [4, 5]. This result supports the hypothesis of a membrane electrical potential involvement in phenylalanine uptake. Without a salt concentration gradient no accumulation takes place, the uptake being only equilibrative.

It can be observed that when equilibrium is attained, the same uptake values are reached, whatever the initial condition. On the contrary D-glucose uptake is extremely low and even after 1 hour of incubation it does not attain the phenylalanine value at equilibrium. With choline chloride initial gradient or in the presence of K^+ but in the absence of K^+ gradient, no transient accumulation of phenylalanine takes place (Fig. 3). The amount of phenylalanine uptake at equilibrium is in inverse proportion to the osmolarity of the medium (Fig. 4): extrapolation to infinite medium osmolarity shows a very small binding value (about 10% of the equilibrium uptake). Therefore the

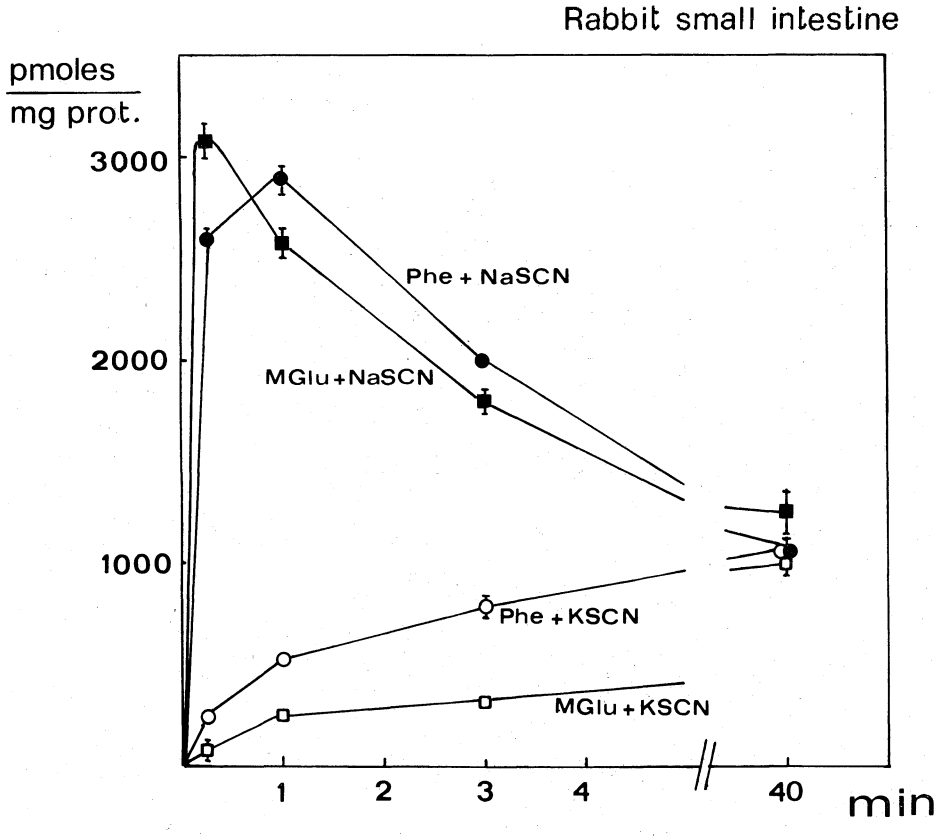

Fig. 5. – Uptake of phenylalanine (Phe) and 3-*O*-methylglucose (Mglu) by brush border membrane vesicles from rabbit small intestine. Phenylalanine (1 mM) uptake in the presence of an initial gradient (100 mM outside and 0 inside) of NaSCN (●), and of KSCN (○). 3-*O*-methylglucose (1 mM) uptake in the presence of an initial gradient (100 mM outside and 0 inside) of NaSCN (■), and of KSCN (□). The buffer was in all cases 10 mM HEPES-Tris pH 7.5, 100 mM D-mannitol. The bars indicate the SE. When not given, the SE were smaller than the symbols used.

observed uptake of phenylalanine could be accounted for almost completely by a transport into an osmotically active space.

By comparison in Fig. 5 the uptake of phenylalanine and 3–*O*–methylglucose by brush border membrane vesicles obtained with the same procedure from rabbit small intestine is shown. The uptake of both compounds shows an " overshoot " in the presence of an initial gradient of NaSCN, whereas with KSCN only a simple equilibrative transport can be observed. It is worth note that the uptake of 3–*O*–methylglucose is very similar to that reported for D-glucose uptake for the same tissue [4].

These experiments suggest that, unlike in mammalian intestine, in *Philosamia cynthia* midgut, whose luminal environment contains K as the main cation, the driving force for the absorption of amino acids could be provided by a K electrochemical gradient.

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