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GLUCOSE TRANSPORT BY THE *IN VITRO* PERFUSED MIDGUT OF THE BLUE CRAB, *CALLINECTES SAPIDUS*

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SUMMARY

1. The midgut of *Callinectes sapidus* is capable of net transmural glucose absorption.

2. The mucosal glucose influx by the midgut has a sodium-dependent, saturable component and a sodium-independent, non-saturable counterpart.

3. The unidirectional mucosal to serosal flux and the mucosal influx of glucose are depressed by metabolic inhibitors, the presence of mucosal phlorizin or serosal ouabain.

4. The low rate of net transmural glucose flux and the kinetic characteristics of mucosal influx suggest that the midgut does not play an important role in total nutrient absorption.

5. Thin layer chromatographic analysis shows that most of the glucose appears as phosphorylated forms upon entering the midgut, suggesting that the efflux of free glucose across the serosal border requires an active mechanism.

INTRODUCTION

Nutrient absorption is an important function of the gut of animals. The mechanisms of nutrient absorption in mammalian intestine have been extensively investigated (see Code, 1968 for a review). These studies led to the formulation of the well-known Na^+-gradient hypothesis (see Schultz & Curran, 1970; Crane, 1977; Schultz, 1977 for reviews). Relatively little is known concerning the nutrient absorptive processes in the gut of invertebrates. In crustaceans, the foregut and hindgut, lined with a cuticle, are generally considered to be of little importance in nutrient uptake. The principal site of absorption is believed not to be in the tubular midgut but in the hepatopancreas (see Gibson & Barker, 1979; Dall & Moriarty, 1983 for recent reviews). During the last decade, a number of reports on nutrient transport mechanisms in the crustacean midgut have appeared (Ahearn, 1974, 1976; Ahearn & Maginniss, 1977; Brick & Ahearn, 1978; see Ahearn, 1982 for a review). These studies showed that the crustacean midgut exhibits many of the basic

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transport characteristics of mammalian intestine, such as Na^+-dependency and saturation behaviour. Kinetic evidence, however, indicated that the midgut does not play a major role in nutrient uptake. There have been no other nutrient transport studies on crustacean midgut.

The present study deals with glucose transport across the midgut of the common blue crab, *Callinectes sapidus*. The results indicate that characteristics of transmural transport and mucosal influx of glucose by this tissue are similar to those exhibited by the midgut of the freshwater shrimp, *Macrobachium rosenbergii* (Ahearn & Maginnis, 1977; Ahearn, 1982). Thus, the present work supports the above suggestion that the role of the crustacean midgut in nutrient absorption is limited, implying that other organs, such as the hepatopancreas, assume this function.

Preliminary results from this investigation have been reported previously (Chu, 1983).

MATERIALS AND METHODS

Animals

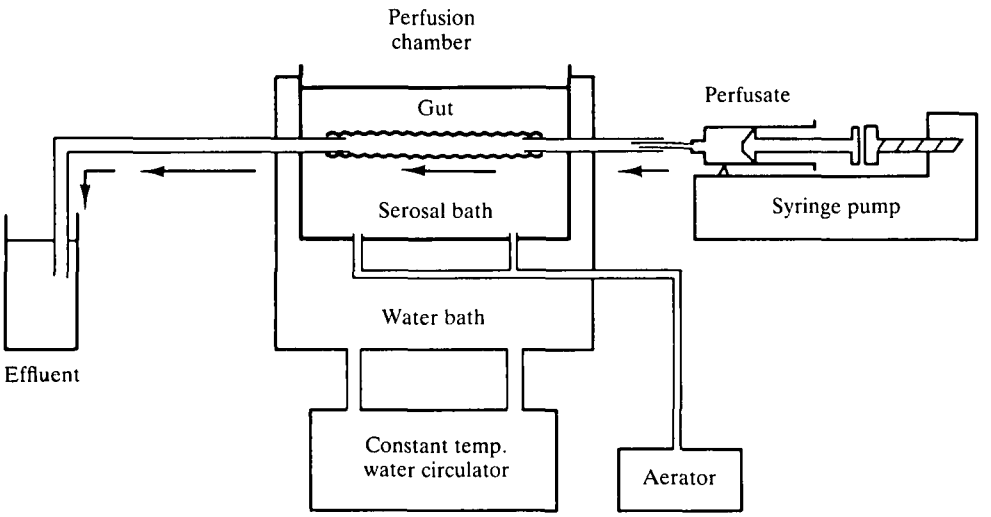
Blue crabs were purchased from Gulf Specimen Co., Inc., Panacea, Florida and were maintained in running sea water at 20 ± 1 °C for 7–10 days before experimentation. Each animal was kept in an individual cage and fed daily with 4–5 freeze-dried krill. All animals used in the present study were intermoult individuals, usually with a carapace width of greater than 10 cm and a body weight of greater than 100 g.

Physiological salines

The composition of physiological saline used in this study was based on analyses of osmotic pressure and ionic concentrations of haemolymph of the animal (Chu, 1984). The saline contained the following (in $mmol\,l^{-1}$): NaCl, 460; KCl, 11; $CaCl_2$, 13; $MgSO_4.7H_2O$, 13; mannitol, 50; and Hepes buffer, 5; with an osmolality of 960 $mosmol\,kg^{-1}$. It also included various concentrations of glucose. The saline was titrated to a pH of 7·5 with a few drops of 1 mol l^{-1} NaOH solution. Sodium-free saline was prepared by substituting sodium chloride with an osmotically equivalent amount of recrystallized choline chloride. The saline was adjusted to the same pH by adding KOH solution. All chemicals used were reagent grade or purer.

Determination of transmural glucose flux

Transmural glucose transport across the midgut was studied using an *in vitro* perfusion technique. The midgut excised from the animal was flushed with physiological saline and then secured with cotton threads to two catheters, which were blunt hypodermic needles (18 gauge) in a perfusion chamber (Fig. 1). The tubular midgut mounted in the chamber was usually between 1·5 and 2·5 cm long. The midgut was perfused with physiological saline using a syringe pump (Harvard Apparatus). The serosal medium, with a volume of 5 ml, was maintained at 20 ± 1 °C and continuously oxygenated and stirred by bubbling with water-saturated air throughout the



experimental period. Unidirectional transmural glucose fluxes were determined in separate pieces of tissues with [6-^{3}H]-D-glucose as the tracer. About 15 min elapsed from the animal being killed to the start of a flux experiment, which usually lasted for 90–150 min. Preliminary experiments showed that transmural glucose fluxes were not affected by variation of perfusion rate from 50–150 μl min^{-1}. All experiments reported here were done at a perfusion rate of 100 μl min^{-1}. Tritiated activity was measured with a Beckman LS-100C liquid scintillation counter using Aquasol as the scintillator.

To measure unidirectional mucosal to serosal flux, the tracer was added to the perfusing saline and 0·1-ml samples of the serosal medium were taken at 10-min intervals. The volume in the bath was maintained constant by replacing each sample with an equal volume of saline. The appearance of radioactivity in the bath was linear with time after an initial equilibration period of 15 min and remained constant for 2 h. The flux was computed from the slope of the linear regression line of the increase in tritiated activity with time.

To measure unidirectional serosal to mucosal flux, tritiated glucose was placed in the bath and equal volumes of the effluent were collected every 5 or 10 min. Tritiated activity of the effluent stabilized after the first 20 min. The flux was calculated from the mean activity of five or more samples after this initial period of equilibration.

In some experiments determining unidirectional mucosal to serosal flux, two samples of serosal medium were taken. One was counted as described and the other was first evaporated to dryness, then redissolved in the same volume of saline and counted. This procedure was used to estimate the flux of non-volatile forms of glucose through the midgut, by eliminating any tritiated water which may have resulted from metabolism by the tissue.

In a series of experiments, change of unidirectional mucosal to serosal glucose flux across the midgut with mucosal glucose concentration was investigated by perfusing

the gut sequentially with salines of increasing glucose concentration and equal specific radioactivity. The glucose concentrations used were 0·5, 1·0, 2·5 and 5·0 mmol l^{-1}. Glucose concentration in the serosal medium was maintained constant at 5·0 mmol l^{-1}. In another series of experiments, variation of unidirectional serosal to mucosal flux with serosal glucose concentration was studied by exposing the tissue sequentially to salines of the above glucose concentrations in the serosal bath and keeping the concentration in the perfusing medium at 5·0 mmol l^{-1}.

To investigate the effects of inhibitors and hexoses on unidirectional mucosal to serosal transport, the flux was determined in control conditions for 50 min and then the compound of interest was added to the perfusate or the serosal medium. After 10 min of incubation, sampling was resumed as before for another 50 min. The effect of Na^+ was studied in a similar manner, by determining flux across midgut perfused with sodium-free saline before or after the control flux period.

In some experiments, unidirectional transmural glucose fluxes across the midgut were measured simultaneously by double labelling, with [6-^{3}H]-D-glucose and [^{14}C(U)]-D-glucose added to the perfusate and bath, respectively. Radioactivity of samples from the mucosal and serosal media was corrected for quenching with automated external standardization to obtain disintegrations per min. Spillover of ^{14}C activity to the ^{3}H window could then be corrected accordingly and the fluxes were computed as before.

All fluxes were expressed in nmol of glucose per cm^2 tissue per hour, by considering the tubular midgut as a cylinder.

Determination of mucosal glucose uptake

The *in vitro* perfusion technique was also adapted to determine mucosal uptake of glucose by the midgut. After mounting the midgut in the perfusion chamber, it was perfused with glucose-free saline for 3 min and then with saline containing glucose together with [6-^{3}H]-D-glucose as the tracer of glucose uptake and [1,2-^{14}C]-polyethylene glycol (M_r 4000) as a marker for extracellular space. The length of the perfusion time varied from 1·5 to 60 min. At the end of the perfusion period, the serosal medium was immediately replaced with ice-cold saline and the gut was flushed with the same solution. The mounted midgut was then cut between the catheters, blotted gently, weighed, and placed in a scintillation vial. The tissue was moistened with 0·1 ml distilled water and solubilized in 0·5 ml Protosol at 40°C overnight. After cooling, 3·4 ml of distilled water and 10 ml of Aquasol were added. Radioactivity was determined with a Beckman LS-100C liquid scintillation counter. Activity of perfusing saline and tissue samples was corrected for quenching with automated external standardization to obtain disintegrations per min. After corrections for the spillover of ^{14}C activity to the ^{3}H window and extracellular tritiated activity, the amount of glucose taken up was computed and expressed as nmol of glucose per cm^2 of tissue per min, by considering the midgut as a cylinder. Preliminary experiments showed that there was no significant change in the rate of glucose uptake among tissues perfused for 3 min at 50, 100 or 150 μl min^{-1}. All

experiments reported here were done at a perfusion rate of $100\,\mu l\,min^{-1}$. Radiochemicals, Protosol and Aquasol were obtained from New England Nuclear.

The effect of luminal Na^+ on mucosal glucose uptake was investigated by perfusing the midgut with radioactively labelled sodium-free saline. The effects of various inhibitors were studied by incubating the midgut in saline containing the inhibitor for 15 min before mucosal uptake determination.

Thin layer chromatography and enzyme treatment

In a few experiments determining mucosal to serosal flux, the chemical state of tritiated activity in the serosal medium was investigated with one-dimensional, ascending thin layer chromatography. As a high salt content interfered with chromatographic development, the salts were first precipitated in 95 % ice-cold ethanol and spun down in a clinical centrifuge. The supernatant was concentrated by mild heating and the treatment was repeated three times. The ethanol extract was then spotted on a microcrystalline cellulose plate ('Avicel', Anatech, Inc.). The plate was developed in pyridine/ethyl acetate/acetic acid/water (5:5:1:3) at room temperature (Wolfrom, Patin & de Lederkremer, 1965). The solvent was allowed to ascend for 10 cm and the plate was then air-dried. Along a single separation path, segments of $0{\cdot}5\times1{\cdot}5$ cm were scraped from the plate, each placed in a scintillation vial, and broken up in 4 ml of distilled water with sonication. Then, 10 ml of Aquasol was added for scintillation counting. A standard of [^{3}H]glucose was analysed together with the samples and the relative band speed (R_F) of unlabelled glucose was also resolved by spraying with aniline diphenylamine reagent (Sigma).

The chemical state of tritiated glucose absorbed by the midgut was investigated with the same technique. The tissues, after uptake experiments, were extracted in 2 ml of 70 % ethanol for 24 h. Preliminary experiments showed that more than 95 % of total radioactivity was extracted within this period. The chromatographic procedure was the same as before. In addition to labelled and unlabelled glucose standards, the R_F of glucose-6-phosphate standard (Sigma) was also determined.

The presence of phosphorylated glucose compounds was further investigated in a few experiments as follows. Ethanol extract from a single tissue was divided into two portions for analysis. Both were evaporated to dryness and redissolved in an equal volume of pH 9 Tris buffer. One sample was treated with alkaline phosphatase (Sigma) for 60 min at 37°C. The other portion, acting as control, was processed identically except that the phosphatase was denatured by heat treatment at 100°C for 60 min beforehand. The two samples were then evaporated to dryness again, redissolved in 70 % ethanol, and analysed by chromatographic procedures as before.

Statistical analysis

Mean values of flux were compared using paired or unpaired Student's t-test. In unpaired tests, if the variance ratio indicated unequal variances, a modified t-test was used instead (Bailey, 1959). Significant difference between mean values was assumed at a probability level of 0·05.

RESULTS

Transmural glucose fluxes

Results of unidirectional transmural glucose fluxes are shown in Table 1. With separate pieces of isolated midgut and 5 mmol l^{-1} glucose on both sides of the tissue, the mucosal to serosal flux, J_{ms}, is significantly greater than the flux in the reverse direction, J_{sm}. The difference between the two gives a significant net flux, J_{net}, of 40 nmol $cm^{-2} h^{-1}$. This finding of a net glucose absorption across the midgut is supported by simultaneous determination of the two fluxes by double labelling experiments carried out in identical glucose concentrations. These experiments resulted in a net flux not significantly different from the one determined above. In experiments where the glucose concentration in the luminal medium was lowered to 2·5 mmol l^{-1} and that of the serosal medium maintained at 5·0 mmol l^{-1}, a net mucosal to serosal glucose flux was still observed. The net flux was not significantly different from that determined with 5·0 mmol l^{-1} glucose on either side of the gut ($P = 0{\cdot}084$). This finding indicates that net glucose absorption can take place against a concentration gradient and that a luminal glucose concentration of 2·5 mmol l^{-1} is probably large enough to saturate the transport mechanism (see next section).

Transmural glucose fluxes as a function of glucose concentration

The fluxes J_{ms} and J_{sm} were hyperbolic functions of mucosal and serosal glucose concentrations, respectively (Fig. 2). J_{ms} significantly exceeded J_{sm} throughout the range of glucose concentration tested (0·5–5·0 mmol l^{-1}). The difference between J_{ms} and J_{sm} gave the net flux, J_{net}, which appeared to approach saturation above a luminal glucose concentration of 2 mmol l^{-1}, suggesting that at least one saturable process is involved in net transmural glucose absorption.

Table 1. *Transmural glucose fluxes across the isolated midgut of* Callinectes sapidus

Mucosal [glucose] (mmol l^{-1})	Transmural glucose fluxes (nmol $cm^{-2} h^{-1}$) J_{ms}	J_{sm}	J_{net}
	Independent determinations		
5·0	55·7 ± 5·1 (9)	15·7 ± 2·7 (6)	40·0 ± 5·8
	Simultaneous determinations		
5·0	62·7 ± 11·2 (3)	18·1 ± 4·2 (3)	44·6 ± 9·6 (3)
2·5	58·5 ± 8·9 (3)	32·6 ± 10·2 (3)	25·9 ± 5·7 (3)

Values are means ± S.E.M. (no. of experiments).

In independent experiments, unidirectional fluxes from mucosal to serosal (J_{ms}) and from serosal to mucosal sides (J_{sm}) were determined in separate pieces of tissues. In simultaneous experiments, they were determined by double labelling. In each case, glucose concentration in the mucosal medium was as indicated, while that of the serosal medium was maintained at 5 mmol l^{-1}.

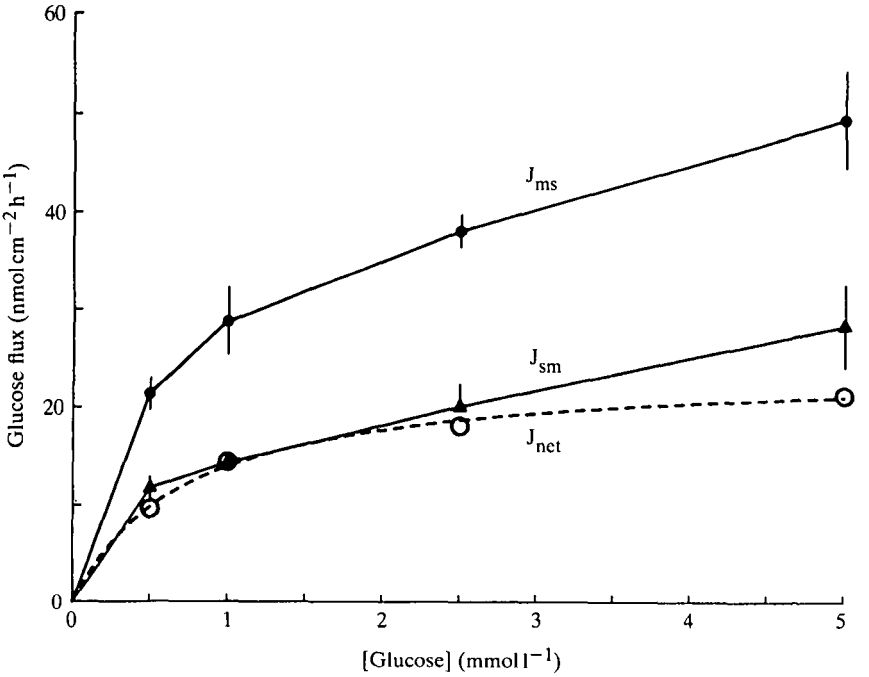


Fig. 2. Variation of transmural glucose fluxes across the isolated midgut of *Callinectes sapidus* with glucose concentration. Data points of unidirectional fluxes from mucosal to serosal, J_{ms} (closed circles), and from serosal to mucosal, J_{sm} (closed triangles), are the means of four experiments, and the bars represent ±1 S.E.M. In some cases, half of the bar is omitted for clarity. The difference between the two fluxes gives the net flux, J_{net} (open circles), the trend of which is shown by the dashed line.

Inhibition of unidirectional mucosal to serosal glucose flux

Unidirectional mucosal to serosal glucose flux across the isolated midgut decreased significantly upon the addition of potassium cyanide or sodium azide, both inhibitors of oxidative metabolism, to the serosal medium (Fig. 3A). The flux was also depressed by iodoacetic acid, an inhibitor of the glycolytic pathway.

Removal of Na^+ from the luminal medium also inhibited mucosal to serosal flux (Fig. 3B). Ouabain (0·1 mmol l^{-1}), a specific inhibitor of the ubiquitous Na^+,K^+-ATPase, reduced J_{ms} when administered to the serosal but not the mucosal medium. In contrast to ouabain, phlorizin (0·01 mmol l^{-1}), a potent inhibitor of glucose transport in many animal tissues, depressed J_{ms} when added to the mucosal medium (Fig. 3C). At a higher concentration (0·1 mmol l^{-1}), phlorizin administered to the serosal medium also reduced J_{ms} significantly. Yet the degree of inhibition was much less than that observed when the same concentration of phlorizin was added to the perfusing medium.

The effects of three hexoses on J_{ms} of glucose are shown in Fig. 3D. In the presence of equimolar D-galactose in the luminal medium, the unidirectional flux of 5·0 mmol l^{-1} glucose was reduced by 30%. The same amount of L-glucose and D-fructose had no apparent effect on J_{ms}.

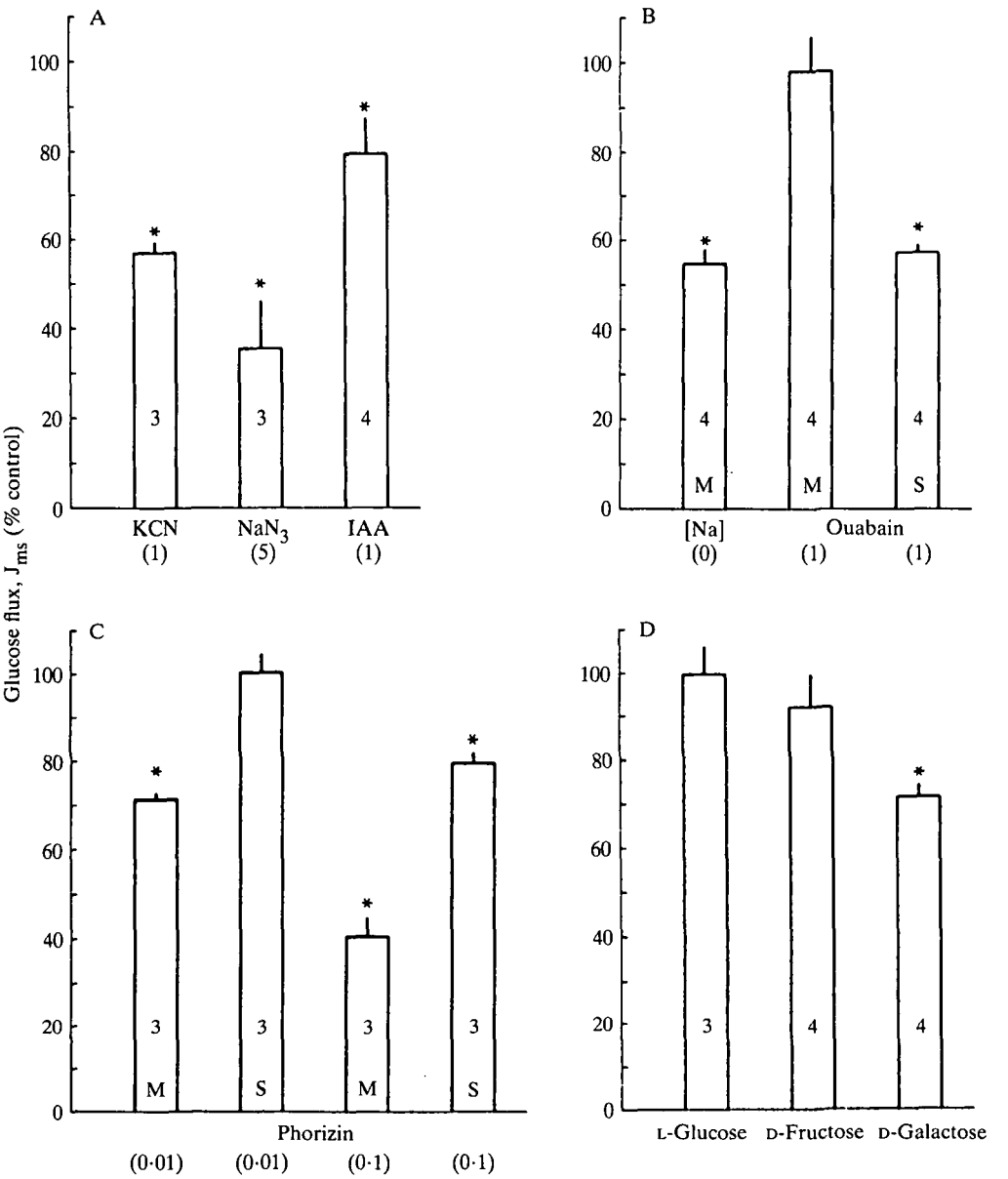


Fig. 3. (A) Effects of the metabolic inhibitors, potassium cyanide, sodium azide and iodoacetic acid (IAA), on unidirectional mucosal to serosal glucose flux across the isolated midgut of *Callinectes sapidus*. Results are expressed as mean percentage of initial flux (i.e. before treatment) and the vertical lines represent 1 S.E.M. Numbers of experiments are indicated. Asterisks indicate significant differences from the control glucose flux. Concentrations are given in mmol l^{-1} in parentheses. (B) Effects of Na^+ and ouabain. The letters M or S indicate whether the treatment was administered in the mucosal or serosal medium, respectively. (C) Effects of phlorizin. (D) Effects of the hexoses, L-glucose, D-fructose and D-galactose. All hexoses were administered to the mucosal medium at 5 mmol l^{-1}.

Mucosal glucose uptake as a function of time

The uptake of 1 mmol l^{-1} glucose by the midgut between 1·5 and 60 min is shown in Fig. 4A. The midgut accumulated glucose rapidly with time and the uptake gradually levelled off. During this period, the apparent extracellular space (expressed as percentage of tissue wet weight) estimated by the uptake of [^{14}C]polyethylene glycol also increased (Fig. 4B). The initial rapid increase apparently represents entry of the marker into the extracellular space. The slow increase after

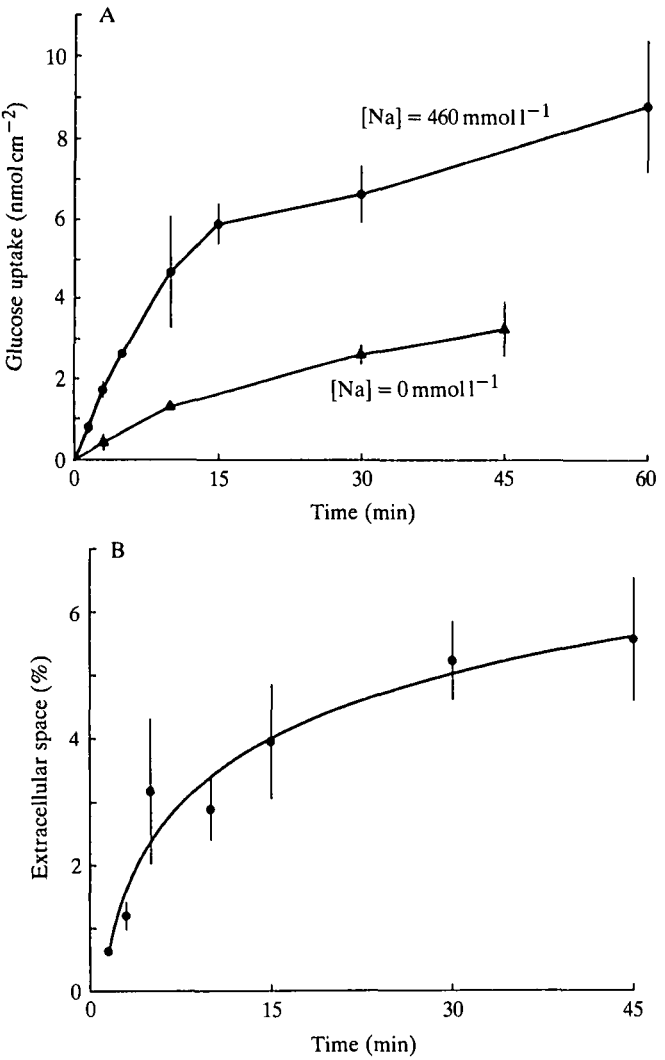


Fig. 4. (A) Time course of mucosal glucose uptake (mmol l^{-1} glucose) by the isolated midgut of *Callinectes sapidus* in the presence (460 mmol l^{-1}; closed circles) and absence (closed triangles) of Na^+. Data points are the means of 3–6 experiments and the bars represent ±1 S.E.M. If no bars are shown, they are within the regions of the symbol. (B) Extracellular space of the isolated midgut of *Callinectes sapidus* as a function of time. Symbols as in (A).

this initial period is probably due to the gradual penetration of the marker into the cells through diffusion and mass transfer processes. Even over prolonged perfusion periods, the correction for apparent extracellular space represents only a small fraction (less than 10%) of total glucose uptake. All data on mucosal uptake reported were corrected for the extracellular space determined simultaneously in the same tissue. With sodium-free saline as the perfusate, the uptake was markedly reduced (Fig. 4A). After 30 min of exposure, the glucose uptake in the absence of Na^+ made up less than 40% of the total uptake.

The glucose uptake measured in these experiments represents the sum of several processes including mucosal influx, metabolism, fluxes on the serosal border and backflux into the lumen. During prolonged perfusion, these processes apparently approached steady state as indicated by the near plateau for glucose uptake. To minimize the contribution of processes other than mucosal influx, an appropriate perfusion time should be selected within the linear phase of mucosal uptake. In the presence or absence of Na^+, the uptake was linear for the first 10 min. Over this perfusion period, the intercepts of the two linear regression lines are not significantly different from zero. Thus, a standard incubation time of 3 min was chosen to determine initial rate of uptake in subsequent experiments. This uptake rate is referred to as the mucosal glucose influx.

Glucose influx as a function of glucose concentration

To investigate the kinetics of glucose influx, the midgut was perfused with salines containing 0·05–1·5 mmol l^{-1} glucose. Mucosal influx showed a biphasic relationship to glucose concentration: from 0·2 to 1·5 mmol l^{-1} glucose the influx was linear with glucose concentration and below 0·2 mmol l^{-1} glucose it was curvilinear (Fig. 5A). Upon removal of Na^+ from the luminal medium, glucose influx was essentially a linear function of external glucose concentration, with a vertical intercept that is not significantly different from zero. This finding indicates that glucose influx in the absence of luminal Na^+ is through a diffusive pathway, although the possibility of a Na^+-independent, saturable glucose carrier with a low affinity for the hexose cannot be ruled out. The apparent diffusion constant, K_d, of this Na^+-independent glucose entry, given by the slope of the linear regression line, has a value of $8{\cdot}3\times10^{-2}$ nmol $cm^{-2}\,min^{-1}\,mmol^{-1}$ glucose. The product of this constant multiplied by the glucose concentration was subtracted from the total influx at each concentration studied, to give the Na^+-dependent mucosal glucose influx. The Na^+-dependent influx of glucose, J, followed saturation kinetics described by the Michaelis–Menten equation:

$$J = \frac{J_{max}[G]}{K_m + [G]}, \tag{1}$$

where [G] is the luminal glucose concentration, J_{max} is the maximal flux, K_m is the half-saturation constant. The K_m and J_{max} values were computed by the single reciprocal plot of J *vs* J/[G] (Fig. 5B). The corresponding values are

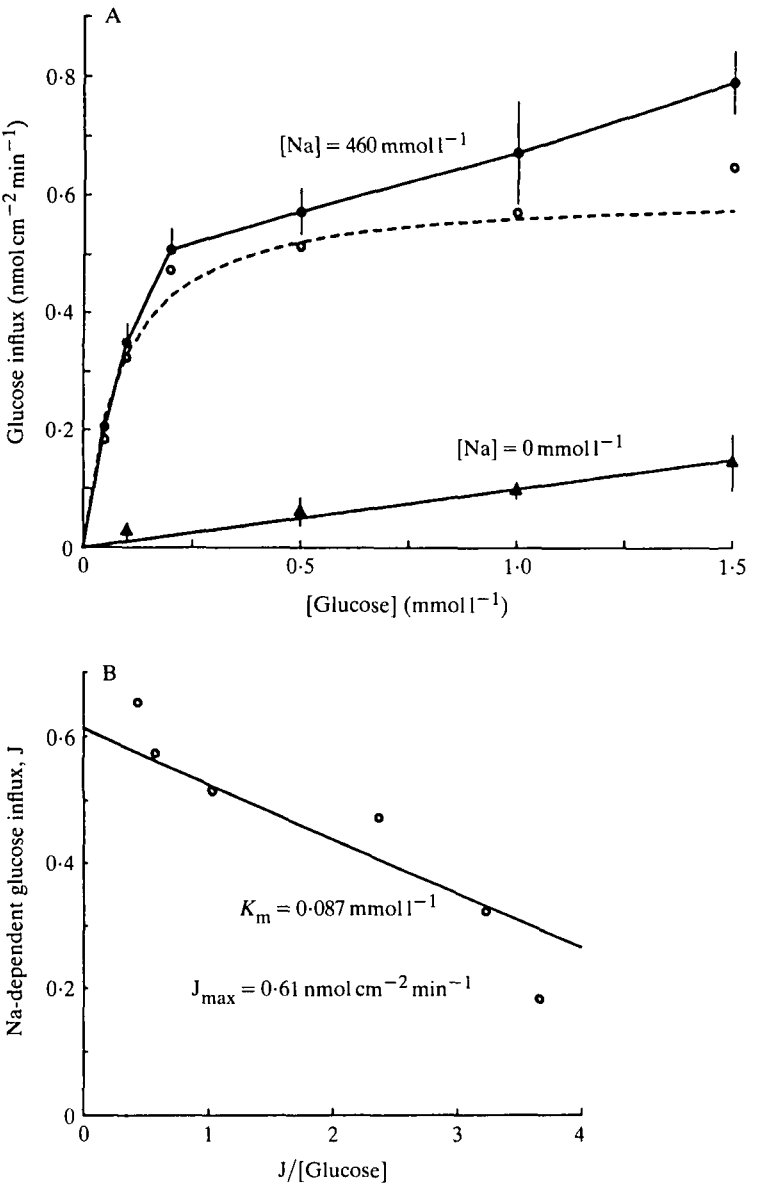


Fig. 5. (A) Mucosal glucose influx as a function of glucose concentration in the presence (460 mmol l^{-1}; closed circles) and absence (closed triangles) of Na^+. Data points are the means of 4–6 experiments, and bars represent ±1 S.E.M. In some cases, half of the bar is omitted for clarity. If no bars are shown, they are within the region of the symbol. The difference between the fluxes in the two conditions gives Na^+-dependent glucose influx (open circles). The dashed line representing this uptake was computed from the Michaelis–Menten equation using the kinetic constants derived from the single reciprocal plot illustrated in B. (B) Single reciprocal plot of Na^+-dependent glucose influx J vs J/[glucose]. Each point is the mean of 5–6 determinations at the same glucose concentration. The linear regression line was computed from all determinations ($N = 34$; $r^2 = 0{\cdot}43$). K_m was derived from the slope, and J_{max} from the vertical intercept of the regression line.

$0{\cdot}087 \pm 0{\cdot}019\,\text{mmol}\,l^{-1}$ and $0{\cdot}61 \pm 0{\cdot}04\,\text{nmol}\,cm^{-2}\,min^{-1}$. These results indicate that Na^+-dependent glucose influx is a carrier-mediated process.

Thus, the total glucose influx, J_{tot}, can be represented by the following relationship:

$$J_{tot} = \frac{J_{max}[G]}{K_m + [G]} + K_d[G]. \quad (2)$$

The first term is a Na^+-dependent, saturable component and the second term a Na^+-independent, non-saturable counterpart.

Inhibition of mucosal glucose influx

The effects of metabolic inhibitors on influx of $1\,\text{mmol}\,l^{-1}$ glucose by the midgut are shown in Fig. 6. Both sodium azide and iodoacetic acid reduced glucose influx, suggesting that it is dependent on energy resources derived from both aerobic and anaerobic pathways. As already shown, removal of Na^+ from the luminal solution significantly decreased glucose influx. However, when the midgut was pre-incubated in saline with azide, the glucose influx determined in sodium-free saline was not

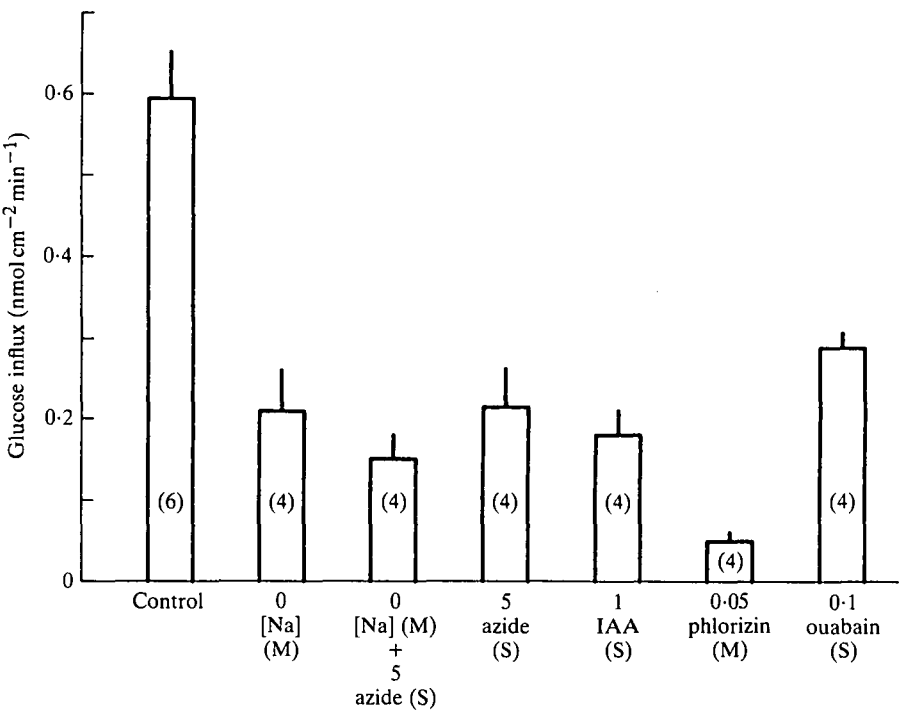


Fig. 6. Effects of inhibitors and Na^+ on the mucosal glucose influx by the isolated midgut of *Callinectes sapidus*. Results are expressed as mean values of (*N*) experiments, and vertical lines represent 1 S.E.M. M or S within parentheses indicates whether the treatment was administered on the mucosal or serosal medium, respectively. Inhibitor concentrations are given in $\text{mmol}\,l^{-1}$. Abbreviations: azide, sodium azide; IAA, iodoacetic acid.

significantly different from influxes determined in either treatment. This result indicates that Na^+-independent glucose influx does not utilize energy derived from oxidative metabolism and thus provides additional evidence that this route of glucose tranport is a diffusive pathway into the cells.

Influx of glucose by the midgut was markedly reduced by the luminal presence of phlorizin ($0{\cdot}05\,mmol\,l^{-1}$). Ouabain, when added to the serosal medium, also inhibited glucose influx, indicating that the functioning of Na^+,K^+-ATPase is required for mucosal entry of glucose.

Thin layer chromatography and enzyme treatment

Thin layer chromatographic analysis of the serosal medium from J_{ms} experiments resulted in a single peak consisting of more than 70% of the total tritiated activity, with an R_F indistinguishable from the R_F of 0·48 exhibited by the glucose standard. In experiments to determine J_{ms} where the samples from the serosal medium were evaporated to dryness before counting, a reduction of 10–20% of tritiated activity resulted when compared to unprocessed samples. This finding indicates that, as a result of metabolism, part of the tritiated activity in the serosal medium is in the form of volatile tritiated water. However, it was noted that the drying procedure also led to a decrease of about 10% in the activity of the parent perfusing solution. Thus, the reduction in activity in the serosal samples might partially be due to an artifact. This was confirmed in experiments using the non-metabolizable 2-[^{3}H(G)]-deoxy-D-glucose (New England Nuclear) as the tracer, in which a decrease of tritiated activity was also evident after drying. Thus, the actual reduction of tritiated activity due to loss of metabolic water was 10% or less. Despite this artifact, chromatographic analysis of the serosal medium indicates that at least 60% of tritiated activity translocated through the midgut is in the form of free glucose.

Thin layer chromatographic analysis of ethanol extracts from midgut exposed to $0{\cdot}1\,mmol\,l^{-1}$ glucose for 3 min illustrated that less than 10% of the intracellular tritiated activity was in the form of glucose (Fig. 7A). The major peak had an R_F indistinguishable from that of glucose-6-phosphate ($R_F = 0{\cdot}12$). An increase of either luminal glucose concentration to $1{\cdot}0\,mmol\,l^{-1}$ and/or the exposure period up to 30 min did not alter this distribution pattern of tritiated activity (Fig. 7A,B). These results suggest that most of the glucose absorbed in the midgut epithelium is probably in the phosphorylated form(s). Experiments with alkaline phosphatase provided additional evidence for this interpretation. As shown in Fig. 7C, treating the midgut extract with the enzyme before chromatographic analysis resulted in two peaks of tritiated activity, one coinciding with the peak exhibited by the control tissues and the other residing in the glucose region.

DISCUSSION

Characteristics of glucose transport by the midgut

The present study illustrates that the isolated midgut of the blue crab, *Callinectes sapidus*, is capable of net transmural glucose absorption. Glucose enters the mucosal

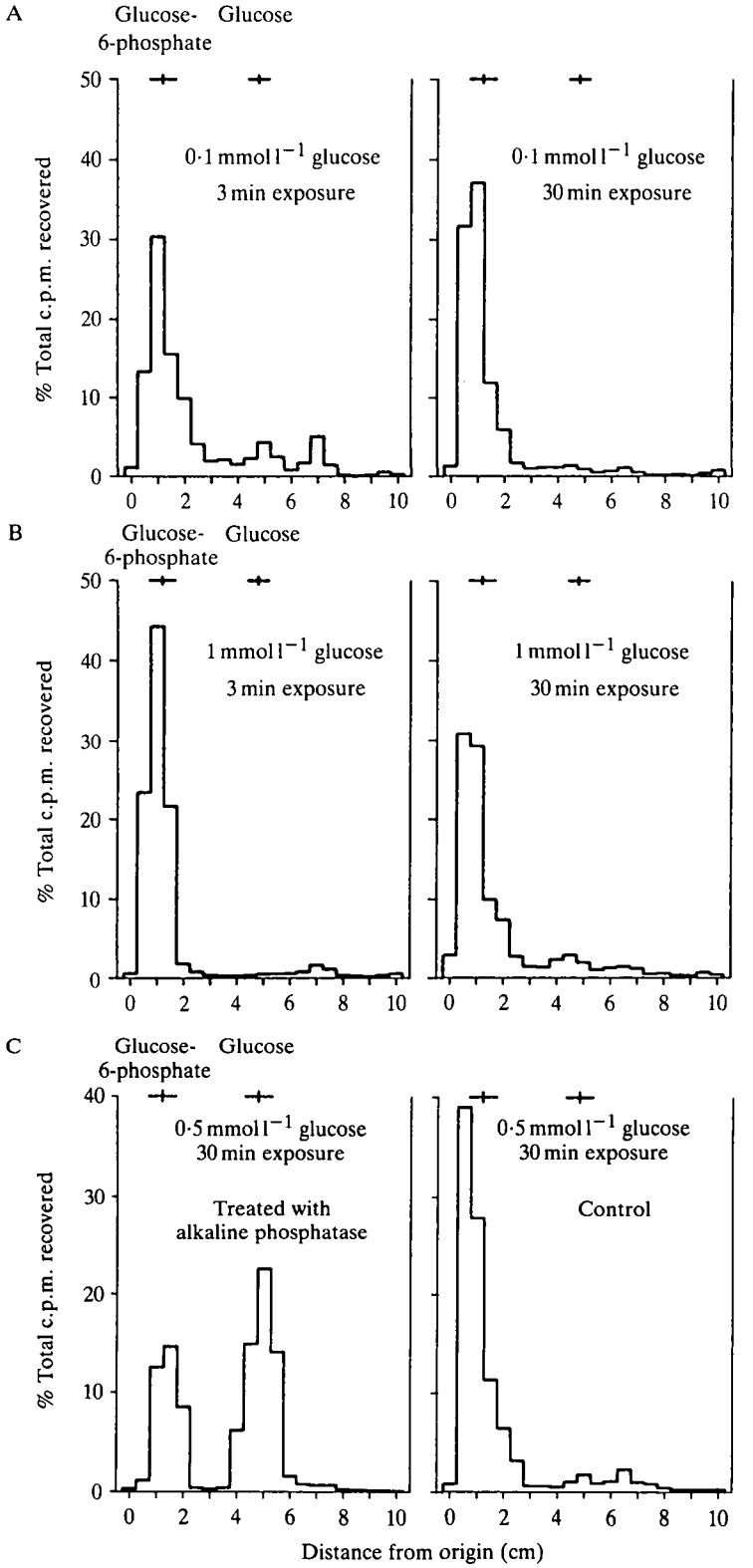

A
Glucose-6-phosphate
Glucose
0·1 mmol l⁻¹ glucose
3 min exposure
0·1 mmol l⁻¹ glucose
30 min exposure
B
1 mmol l⁻¹ glucose
3 min exposure
1 mmol l⁻¹ glucose
30 min exposure
C
0·5 mmol l⁻¹ glucose
30 min exposure
Treated with alkaline phosphatase
0·5 mmol l⁻¹ glucose
30 min exposure
Control
% Total c.p.m. recovered
Distance from origin (cm)

Fig. 7

border of the midgut *via* two routes: (1) a Na^+-dependent, saturable mechanism and (2) a Na^+-independent, non-saturable pathway that probably represents diffusion into the cells. The Na^+-dependency of the glucose transport process is in common with most active nutrient transport mechanisms across epithelia. Most work in this area tends to support the Na^+-gradient hypothesis proposed by Crane (1965). According to this hypothesis, entry of nutrients into the cells is coupled to downhill movement of Na^+. The low intracellular Na^+ concentration is maintained by active extrusion of Na^+ by Na^+,K^+-ATPase on the serosal border of the epithelium. This model can readily account for the inhibitory effects of metabolic inhibitors and ouabain on transmural glucose flux as well as mucosal influx reported here. Ouabain, through its inhibitory effect on Na^+,K^+-ATPase, would probably increase the intracellular Na^+ concentration. Given a smaller Na^+ gradient, mucosal entry of glucose is reduced, and so is the transmural flux. Inhibition of metabolism with potassium cyanide, sodium azide and iodoacetic acid depresses glucose transport, probably due in large part to lack of ATP for phosphorylation of Na^+,K^+-ATPase.

The unidirectional mucosal to serosal glucose flux across the midgut of *Callinectes sapidus* is sensitive to phlorizin, a characteristic of the active glucose transport processes well established in mammalian intestine and kidney tubule (Lotspeich, 1960). Phlorizin is most effective when applied to the mucosal medium, suggesting that the midgut of *C. sapidus*, like the above mammalian tissues, possesses a phlorizin-sensitive Na^+/glucose cotransporter at its apical membrane. The inhibitory effect of phlorizin on mucosal glucose influx strengthens this suggestion. The reduction of transmural glucose flux by $0{\cdot}1\ mmol\ l^{-1}$ phlorizin administered to the serosal bath may be an indirect consequence of the inhibitory effect of a high concentration of phlorizin on the activity of various enzymes (see Crane, 1960 for a review), including the Na^+,K^+-ATPase (Britten & Blank, 1969; Robinson, 1969). Other possibilities such as the presence of a moderately phlorizin-sensitive glucose transport system in the serosal border or the diffusion of this compound through the paracellular pathway and subsequent inhibition at the apical membrane, cannot be excluded.

Unidirectional mucosal to serosal glucose flux across the midgut of *Callinectes sapidus* was decreased by the presence of D-galactose, but not D-fructose and L-glucose. Na^+-dependent mucosal glucose influx inhibited by galactose was observed in the midgut of the freshwater shrimp, *Macrobrachium rosenbergii* (Ahearn & Maginniss, 1977). This inhibitory effect may be due to the interaction of galactose with the glucose binding site, implying that the two hexoses share a common

Fig. 7. (A) Radiochromatogram of ethanol extracts of midgut of *Callinectes sapidus* from mucosal uptake experiments using $0{\cdot}1\ mmol\ l^{-1}$ glucose. Each chromatogram is the mean of duplicate experiments. At the top of the figure, the R_F and range of glucose ($R_F = 0{\cdot}48$) standard are represented by vertical and horizontal lines, respectively. The same parameters of glucose-6-phosphate ($R_F = 0{\cdot}12$) are also shown. (B) Radiochromatogram of ethanol extracts from mucosal uptake experiments using $1{\cdot}0\ mmol\ l^{-1}$ glucose. (C) Radiochromatogram of ethanol extracts from mucosal glucose uptake experiments using $0{\cdot}5\ mmol\ l^{-1}$ glucose: effect of alkaline phosphatase. The ethanol extracts were pre-treated with alkaline phosphatase or the heat-treated enzyme (control).

transport system. A common transport mechanism for D-glucose and galactose are found in the intestine and proximal tubule in the kidney of mammals (see Crane, 1960; Kinne, 1976 for reviews). However, since the nature of the competition of galactose with glucose transport was not investigated in the midgut of *C. sapidus* nor *M. rosenbergii*, the notion of a common transport mechanism for the two hexoses in the crustacean gut has not been verified. Other mechanisms, such as sharing a limited energy resource for uptake between the two hexoses or a change of transmembrane potential induced by galactose, could also lead to a decrease in mucosal to serosal glucose flux.

Mechanisms of glucose translocation across the midgut

The net transmural glucose flux across the midgut bilaterally incubated in 5 mmol l^{-1} glucose is about 40 nmol $cm^{-2} h^{-1}$. The total mucosal glucose influx in the same substrate concentration, as calculated from equation 2, is 60 nmol $cm^{-2} h^{-1}$. Thus, more than 50% of the glucose entering the mucosal border of the midgut epithelium is subsequently translocated to the serosal medium. Most of this net glucose delivery is in the form of free glucose. Most glucose accumulated by the midgut, however, is in the form of phosphorylated compound(s), probably representing various stages of metabolic degradation. The same observation has been made in the midgut of the freshwater shrimp, *Macrobrachium rosenbergii* (Ahearn & Maginniss, 1977). This phenomenon is in contrast to glucose transport in the mammalian intestine. In the latter system, free glucose is accumulated to a high level inside the epithelium so that the serosal exit of glucose can simply be explained by a diffusive pathway (see Crane, 1968 for a review). Since experimental studies indicate that the intracellular glucose concentration is low in the crustacean midgut, an alternative mechanism to account for the translocation of glucose in the serosal border is necessary.

Ahearn & Maginniss (1977) proposed two hypotheses for the transmural transport of glucose through the midgut of *M. rosenbergii*. The first model suggests that the two steps of the glucose transport process in the luminal and basolateral membranes are linked to phosphorylation and dephosphorylation, respectively. The phosphorylation–dephosphorylation hypothesis, originally proposed to account for active glucose absorption in mammalian intestine, was abandoned as a result of more recent evidence (see Crane, 1960 for a review). Phosphorylation-linked sugar transport, however, has been demonstrated in bacteria (Simoni *et al.* 1967; Kaback, 1968) and yeast (van Steveninck, 1968, 1969, 1970, 1972; Jaspers & van Steveninck, 1975). The present finding of the accumulation of phosphorylated compounds in crustacean midgut cannot be taken as conclusive evidence for this hypothesis since many cells increase the concentration of metabolic intermediates on the supply of nutrients.

The second model proposes that part of the free glucose that passes through the luminal membrane is rapidly metabolized and the rest is actively translocated across the basolateral membrane *via* a high-affinity glucose carrier. The presence of a serosal sugar pump has been suggested in the rat intestine (Esposito, Faelli & Capraro, 1973). The results of the present study are compatible with both proposed

models and do not favour either. Studies on glucose fluxes at the serosal border are necessary to clarify the glucose efflux mechanism.

Role of the midgut in nutrient absorption

The magnitude of the net glucose absorption across the midgut of *Macrobrachium rosenbergii* is $103{\cdot}9 \pm 23{\cdot}1$ nmol $cm^{-2} h^{-1}$ (1 mmol l^{-1} glucose; Wyban, Ahearn & Maginniss, 1980). Noting that this net flux is lower than nutrient fluxes determined in mammalian intestine, Ahearn (1982) asserted that the crustacean midgut delivers less quantity of nutrients to the blood per unit surface area than does mammalian intestine, and accordingly, the midgut plays a minor role in total nutrient absorption. The net glucose flux determined in the midgut of *Callinectes sapidus* ($40{\cdot}0 \pm 5{\cdot}8$ nmol $cm^{-2} h^{-1}$; 5 mmol l^{-1} glucose) is about half of that in *M. rosenbergii*. Thus, this finding supports the above notion that the crustacean midgut is insignificant in total nutrient absorption. Additional evidence is provided by the kinetic analysis of mucosal glucose influx.

The kinetic constants, K_m and J_{max}, of the Na^+-dependent glucose influx by the midgut of *Callinectes sapidus* are in the same range as those determined in the same tissue of *M. rosenbergii* (Ahearn & Maginniss, 1977). High-affinity transport systems with low maximal transport rates for amino acids are also present in the midgut of the same species (Brick & Ahearn, 1978) and the marine shrimp, *Penaeus marginatus* (Ahearn, 1974, 1976). Ahearn (1982) pointed out that the half-saturation constants of nutrient transport mechanisms in the crustacean midgut are one or two orders of magnitude lower than those in the mammalian intestine. In addition, the maximal transport rates in the midgut are markedly lower. He suggested that the low J_{max} limits the nutrient absorptive role of the midgut and the high-affinity nature may be physiologically advantageous to the animal. It is widely believed that the hepatopancreas of crustaceans is the major site of nutrient absorption (see Gibson & Barker, 1979; Dall & Moriarty, 1983 for recent reviews). Presumably, nutrient levels in the lumen of the tubular midgut would be low. Under these conditions, a high-affinity glucose transport system is desirable for more complete uptake of the sugar. The findings of the present study support such a salvage absorptive function of the midgut and thus provide indirect evidence for the importance of the hepatopancreas in nutrient uptake. This hypothesis is supported by a study using cell suspension techniques which showed that kinetic properties of alanine transport by the hepatopancreas of the American lobster, *Homarus americanus*, are comparable to those reported in mammalian intestine (Ahearn, Monkton, Henry & Botfield, 1983). With a low luminal nutrient concentration, nutrients taken up by the midgut may be largely utilized for generating energy to support other physiological functions that have been suggested for this tissue, such that net transmural nutrient absorption is minimal. These proposed functions include secretion of the peritrophic membrane (Forster, 1953; Georgi, 1969; Johnson, 1980) and fluid absorption during ecdysis (Mykles & Ahearn, 1978; Mykles, 1980).

The hypothesis that the midgut does not play a significant role in total nutrient absorption rests upon the comparison of kinetic studies on the gut of different animal

species. It should be emphasized that the kinetic constants determined in these studies represent estimates made under different experimental conditions, and thus are subject to variations. For example, kinetic constants of glucose uptake by mammalian intestine are dependent on luminal Na^+ concentration (Crane, Forstner & Eichnolz, 1965; Bihler, 1969; Hopfer, 1977). Further, the extent of the unstirred layer, which tends to vary in different *in vitro* preparations, also affects the values estimated for the half-saturation constant (Wilson & Dietschy, 1974; Thomson & Dietschy, 1980). Therefore, the differences in experimental techniques adopted make the comparison of kinetic constants difficult. Accordingly, hypotheses based on comparison of kinetic data from different laboratories remain speculative. Recently, there have been kinetic studies on gut transport processes of different species under identical experimental conditions (Thomson, Hotke & Weinstein, 1982; Ferraris & Ahearn, 1983; Karasov, Solberg & Diamond, 1983). This relatively new approach to comparative gastrointestinal physiology would be rewarding in elucidating the importance of the crustacean midgut in nutrient absorption.

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