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RESEARCH ARTICLE

Transepithelial D-glucose and D-fructose transport across the American lobster, *Homarus americanus*, intestine

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SUMMARY

Transepithelial transport of dietary D-glucose and D-fructose was examined in the lobster *Homarus americanus* intestine using D-[^{3}H]glucose and D-[^{3}H]fructose. Lobster intestines were mounted in a perfusion chamber to determine transepithelial mucosal to serosal (MS) and serosal to mucosal (SM) transport mechanisms of glucose and fructose. Both MS glucose and fructose transport, as functions of luminal sugar concentration, increased in a hyperbolic manner, suggesting the presence of mucosal transport proteins. Phloridizin inhibited the MS flux of glucose, but not that of fructose, suggesting the presence of a sodium-dependent (SGLT1)-like glucose co-transporter. Immunohistochemical analysis, using a goat anti-rabbit GLUT5 polyclonal antibody, revealed the localization of a brush border GLUT5-like fructose transport protein. MS fructose transport was decreased in the presence of mucosal phloretin in warm spring/summer animals, but the same effect was not observed in cold autumn/winter animals, suggesting a seasonal regulation of sugar transporters. Mucosal phloretin had no effect on MS glucose transport. Both SM glucose and SM fructose transport were decreased in the presence of increasing concentrations of serosal phloretin, providing evidence for the presence of a shared serosal GLUT2 transport protein for the two sugars. The transport of D-glucose and D-fructose across lobster intestine is similar to sugar uptake in mammalian intestine, suggesting evolutionarily conserved absorption processes for these solutes.

Key words: D-glucose transport, D-fructose transport, transepithelial transport, SGLT1, GLUT2, GLUT5, sodium dependent, co-transport, lobster, *Homarus americanus*, intestine, phloridizin, phloretin.

INTRODUCTION

Dietary sugars are the main source of energy in living organisms and they play an essential role in the proper functioning of organs (Brown, 2000; Wright et al., 2007). Absorption of glucose has been extensively studied in mammals, leading to the finding that two distinctly different types of sugar transport proteins have evolved to transfer this nutrient across membranes of living organisms. These transporters include the SLC5 co-transporter gene family and the SLC2 gene family (Wood and Trayhurn, 2003). The SLC5 co-transporter gene family is a large family of 75 kDa proteins consisting of several sodium-dependent glucose co-transporter (e.g. SGLT) proteins that transport glucose in conjunction with sodium across biological membranes and require sodium to function effectively. In contrast, the SLC2 gene family is a family of 50 kDa sodium-independent glucose transport proteins (e.g. GLUT).

While sugar transporters have been extensively studied in mammals, very little is known about the absorption of sugars in invertebrates. In a study conducted on the midgut of the wasp larva *Aphidius ervi*, apical and basolateral fluxes of D-glucose and D-fructose, immunocytochemistry and western blot analysis were used to determine the characteristics and localization of sugar transport proteins in midgut epithelial plasma membranes (Caccia et al., 2007). The results revealed the localization of SGLT1 and GLUT5 on the apical membrane (Caccia et al., 2007). GLUT2 was shown to be present on the basolateral side of the gut and, in the presence of high concentrations of sugars, GLUT2 was inserted into the apical side of the membrane to maximize uptake of the sugars (Caccia et al., 2007). The findings by these authors are important because they are the only known non-vertebrate eukaryote study that describes in detail the mechanism for carrier-mediated transport of sugars across an invertebrate epithelium, and they also support the evolutionary conservation of sugar transporters in eukaryotic organisms. Additionally, sugar transport in bacteria and parasitic protozoans occurs by members of the major facilitator (MF) superfamily, which are evolutionarily similar to the mammalian sugar transporters (Maiden et al., 1987; Henderson, 1990).

In crustaceans, the hepatopancreas and the intestine play a role in the absorption of dietary glucose (Ahearn et al., 1985; Verri et al., 2001), with the hepatopancreas being the major site of sugar absorption (Ahearn and Maginniss, 1977; Chu, 1986; Sterling et al., 2009). The crustacean hepatopancreas is a large, bilateral organ that is composed of five different cell types: E, F, R, B and M cells (Verri et al., 2001). The cell types differ in structure and, as a result, play different roles in digestive and absorptive functions (Verri et al., 2001). In contrast, the intestine is made up of a single type of epithelial cell and is considered a scavenger organ that absorbs nutrients that were not taken up by the hepatopancreas (Ahearn et al., 1992a; Wright and Ahearn, 1997). The mechanism of glucose transport in purified brush boarder membrane vesicles of hepatopancreatic epithelial cells in shrimp (*Panaeus japonicas*) and lobster (*Homarus americanus*) is carrier mediated *via* an SGLT1-like transport protein (Ahearn et al., 1985; Verri et al., 2001; Sterling et al., 2009).

Apart from the above studies conducted in insects, transepithelial sugar transport mechanisms have not been studied in detail in any free-living eukaryotic invertebrate organism. Although the lobster diet is primarily protein, lobsters often feed on plants like algae, which contain significant amounts of glucose (Conklin, 1995). In the present study, we characterized transepithelial D-glucose and D-fructose transport across the *H. americanus* intestine. We hypothesized that sugar transporters must be highly conserved throughout evolution because D-glucose and D-fructose molecules are the same for all organisms that use them for energy; hence, we expected transepithelial sugar transport in crustaceans to be similar to the transport paradigm proposed in mammals.

MATERIALS AND METHODS

Animals

Live, male lobsters (*H. americanus*, Milne-Edwards 1837), were purchased from a local seller (Fisherman's Dock, Jacksonville, FL, USA). Each lobster weighed approximately 0.5 kg and was kept in a tank containing fresh seawater at 15°C. Animals were fed frozen mussel meat twice a week until needed for an experiment. Each animal was dissected by first severing the ventral nerve cord located under the cephalothorax and abdomen. Using scissors, the carapace and the tail were vertically cut in half, exposing the intestine. The part of the intestine used for each experiment was cut from 1 cm posterior to the stomach to about two-thirds of the length of the tail. This fraction of intestine was composed of midgut tissue only.

Mucosal to serosal transport

Experimental set up

In vitro transmural mucosal to serosal (MS) transport of D-[^{3}H]glucose and D-[^{3}H]fructose was studied using a perfusion apparatus as previously described (Ahearn and Maginniss, 1977). Isolated whole intestine was flushed with physiological saline (410 mmol l^{-1} NaCl, 15 mmol l^{-1} KCl, 5 mmol l^{-1} $CaSO_4$, 10 mmol l^{-1} $MgSO_4$, 5 mmol l^{-1} $NaHCO_3$, 5 mmol l^{-1} Hepes/KOH at pH 7.1) and mounted on an 18–20 gauge needle at both ends of the perfusion apparatus using a surgical thread. The length and diameter of the experimental intestine were measured and the intestinal surface area was calculated using the equation: $A=\pi ld$, where l and d represent the length and diameter of the intestine, respectively. The perfusion bath (serosal medium) was filled with 35 ml of physiological saline. The experimental perfusate (physiological saline plus appropriate experimental treatments) was pumped through the intestine using a peristaltic pump (Instech Laboratories Inc., Plymouth Meeting, PA, USA) at a rate of 0.38 ml min^{-1} [a rate previously shown to provide constant transmural transport in lobster intestine for over 3 h of incubation without added oxygen at 23°C (Conrad and Ahearn, 2005)].

Time course and drug inhibition experiments

Transport time course experiments were conducted by adding experimental concentrations of D-glucose or D-fructose (2.5, 5, 10, 20, 35 and 50 μmol l^{-1}) to different 50 ml tubes (Falcon, Newark, DE, USA) containing physiological saline and 5 μl of D-[^{3}H]glucose or D-[^{3}H]fructose (American Radiolabeled Chemicals Inc., St Louis, MO, USA), respectively. Prior to the start of experimentation, triplicate aliquots of each experimental perfusate (200 μl) were collected from each Falcon tube to determine the total counts of radioactively labeled sugar in each tube, and from the bath to determine the amount of background radioactivity at the beginning of an experiment. Experimental solutions were then perfused through the intestine for a total of 30 min at each concentration of sugar.

For inhibition experiments, the experimental perfusate contained physiological saline, 25 μmol l^{-1} unlabeled D-glucose or D-fructose, 5 μl of D-[^{3}H]glucose or D-[^{3}H]fructose, and either of the inhibitors phloridizin (Pfaltz & Bauer Inc., Waterbury, CT, USA) and phloretin (Acros Organics, Morris Plains, NJ, USA). Both phloridizin and phloretin were dissolved in 100% ethanol. All experimental procedures were carried out at room temperature (23°C). For both time course and inhibition experiments, triplicate aliquots of radioactive samples (200 μl) were collected from the serosal medium after passage across the intestine every 5 min for the duration of each experimental treatment, and placed into 7 ml scintillation vials containing 3 ml of scintillation cocktail. An equal amount of physiological saline was added to the serosal medium in order to maintain a constant volume in the bath.

Serosal to mucosal transport

Time course experiments

The *in vitro* transmural serosal to mucosal (SM) time course of D-[^{3}H]glucose and D-[^{3}H]fructose transport was studied by adding 5 μl of the corresponding radioactively labeled D-glucose or D-fructose and an appropriate amount of unlabeled D-glucose or D-fructose (2.5, 5, 10, 20, 35 and 50 μmol l^{-1}) to the bath. The specific activity of radiolabeled sugars in the bath was determined by collecting triplicate 200 μl samples of solution from the bath. The effects of changes in experimental serosal media on SM transport were measured by collecting the total radioactive perfusate exiting the intestine every 5 min over 30 min intervals into 7 ml scintillation vials containing 3 ml of scintillation cocktail.

Exponential decay analyses

The nature of the hypothesized serosal sugar transporter was determined by perfusing the intestine with carrier-free radioactive sugar alone (no unlabeled sugar added to commercially labeled sugars) for 15 min and then sequentially increasing the concentration of the serosal inhibitor (unlabeled sugar or drug) for the duration of the experiment. Concentrations of unlabeled sugar were 1, 2.5, 5, 10, 25, 50 and 100 μmol l^{-1}, while those of the unlabeled drug, phloretin, were 5, 10, 25, 50, 100, 250 and 500 μmol l^{-1}. Perfusate samples were collected and counted for the amount of radioactivity as described above for SM sugar transport.

Data analyses

Each radioactive experimental sample collected was placed in a 7 ml tube containing 3 ml scintillation cocktail and counted for radioactivity in a Beckman LS6500 scintillation counter. The amount of radioactivity in each tube for both MS and SM experiments was reported in counts per minute (c.p.m.). The mean background count was subtracted from each triplicate sample at each time point. Transmural MS and SM flux rates, expressed in pmol cm^{-2} min^{-1}, were calculated using the perfusate specific activity of each sugar and by taking the sample c.p.m. mean during each time period and applying the respective intestinal surface area. Slopes of the time course data were determined by linear regression analysis and data curve-fitting procedures using Sigma Plot 10.0 software (Systat Software Inc., Point Richmond, CA, USA). The slopes obtained from the time course MS analysis were plotted as a function of sugar concentration and curve fitted to a hyperbolic curve to obtain the Michaelis–Menten kinetic constants:

$$J = J_{max}\,[\text{sugar}] / (K_m + [\text{sugar}])\,, \quad (1)$$

where J is transmural MS sugar transport, J_{max} is apparent maximal MS D-[^{3}H]glucose or D-[^{3}H]fructose transport (pmol cm^{-2} min^{-1}), K_m

is an apparent affinity constant and reflects the relative ease of sugar binding to the rate-limiting transport system ($\mu mol\,l^{-1}$) and [sugar] is the perfusate sugar concentration ($\mu mol\,l^{-1}$).

For MS drug inhibition experiments, bar graphs were obtained by plotting flux rate as a function of experimental treatment, expressed as mean (± s.e.m.) $pmol\,cm^{-2}\,min^{-1}$. Experiments were repeated three times (i.e. three animals per experiment) and pooled for final analysis of treatment effects. The non-parametric Kruskal–Wallis *H*-test was used to determine significant differences between treatments, and treatment means were considered significantly different at $P<0.05$.

For exponential decay inhibitory experiments, the mean c.p.m. of triplicate samples for each concentration was plotted against the concentration of serosal sugar or inhibitor. Curve-fitting analysis using an exponential decay curve including an asymptote was used to determine the percentage of transmural transport of sugar that was carrier mediated. The percentage of carrier-mediated transport was estimated by dividing the *y*-intercept obtained from the curve-fitting report by the mean c.p.m. of sugar transport in the absence of serosal inhibitor.

Histology

Whole lobster intestines were isolated and dissected into small pieces in cross-section. Sections were placed in 10% buffered formalin and stored at room temperature for about 3–4 days. Each section was dehydrated by placing it in a separate vial containing one of several sequential concentrations of aqueous ethyl alcohol (70, 80, 90, 95 and 100%) followed by three 1 h immersions in citrisolv. Tissues were then placed in a 50:50 citrisolv:paraffin mix overnight at 57°C. Intestines were removed from the mix and placed in paraffin at 57°C for 1 h after which each intestinal piece was embedded in cross-section in a plastic block covered with paraffin and was allowed to cool at room temperature overnight. Excess paraffin was trimmed off the face of the blocks and the lobster tissues were cut into ribbons of 7 µm thickness using a microtome and placed into a water bath at 37°C. Each ribbon was positioned on a labeled glass slide and each slide was placed on a slide warmer at 60–65°C for about 30–35 min. After tissues had adhered to the slides, the slides were placed in a slide box and stored at room temperature for processing.

Prior to staining, tissue sections were re-hydrated by placing each tissue slide in different vials containing citrisolv and several concentrations of aqueous ethyl alcohol (citrisolv 1, 2 and 3, 100, 100, 95, 95, 80, 80, 70% ethyl alcohol, in order) for 5 min at each exposure. After exposure to 70% ethyl alcohol, the slides were held in water for 2–3 min before analysis.

Immunohistochemistry

Rehydrated slides were incubated in pre-incubation buffer [Tris-buffered saline (TBS) and 2% normal goat serum] at room temperature for 1 h to block non-specific binding. Next, the slides were washed three times in TBS and then incubated overnight at 4°C in 1:100 rabbit polyclonal primary antibody (Millipore, Temecular, CA, USA) to GLUT5 diluted in pre-incubation buffer. After incubation, the slides were washed 3 times in TBS and then incubated in the dark for 1 h in 1:100 goat anti-rabbit secondary antibody (Millipore) conjugated with FITC diluted in pre-incubation buffer. Vector shield was used to place coverslips on the slides for storage. Pictures of the slides were taken using an Olympus BX60 microscope. Negative controls were not incubated in primary antibody.

RESULTS

D-[^{3}H]Glucose and D-[^{3}H]fructose MS, SM and net transport kinetics

MS and SM transport experiments over a 30 min time course at a variety of D-[^{3}H]glucose and D-[^{3}H]fructose concentrations were performed in triplicate (three animals each) using perfused intestines to determine the kinetic characteristics of the rate-limiting transport systems responsible for transmural transport of both sugars in both directions across the tissue. As shown in Fig. 1A, transmural MS D-[^{3}H]glucose transport at pH 7.0 (bath and lumen) was a hyperbolic function of perfusate D-glucose concentration over the range from 2.5 to 50 $\mu mol\,l^{-1}$. Under these conditions, D-[^{3}H]glucose transmural MS transport displayed the following carrier-mediated kinetic constants: K_m=15.2±3.5 $\mu mol\,l^{-1}$ glucose and J_{max}=37.0±9.1 $pmol\,cm^{-2}\,min^{-1}$.

Transmural SM D-[^{3}H]glucose transport across lobster intestine was a linear function of serosal D-[^{3}H]glucose concentration and showed no indication of saturating over the concentration range from 2.5 to 50 $\mu mol\,l^{-1}$ D-glucose (Fig. 1B). This linear SM transport rate was $0.493\pm0.002\,pmol\,cm^{-2}\,min^{-1}\,(\mu mol\,l^{-1})^{-1}$ D-glucose and represents diffusional transfer across the tissue or transport by a low affinity facilitated carrier system that showed no sign of saturation within the substrate range employed.

Combining the MS and SM transport data from Fig. 1 for 2.5–50 $\mu mol\,l^{-1}$ D-[^{3}H]glucose (i.e. $J_{net}=J_{MS}-J_{SM}$) resulted in the net absorptive flux of glucose across the tissue over this sugar concentration range.

Triplicate transmural MS transport measurements using D-[^{3}H]fructose perfused at concentrations from 2.5 to 50 $\mu mol\,l^{-1}$ were

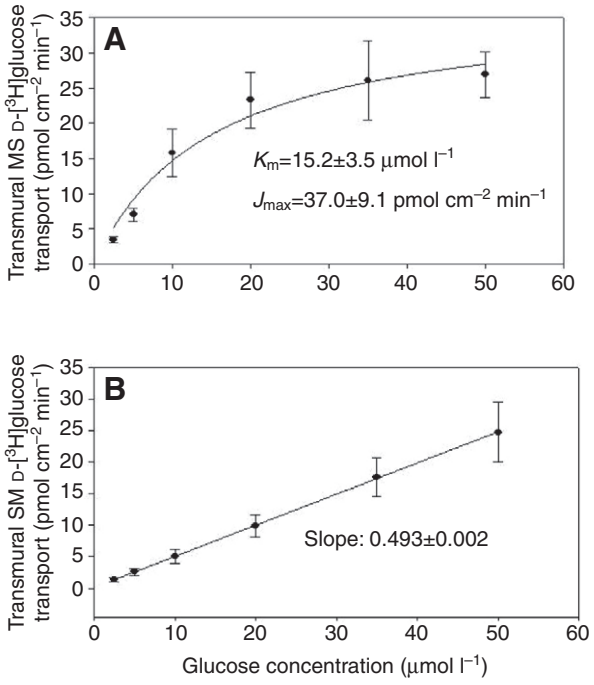

Fig. 1. (A) Effect of increasing D-glucose concentration (2.5, 5, 10, 20, 35 and 50 $\mu mol\,l^{-1}$) on mucosal to serosal (MS) transmural D-[^{3}H]glucose transport. K_m, affinity constant; J_{max}, maximum transport. (B) Effect of increasing D-glucose concentration (2.5, 5, 10, 20, 35 and 50 $\mu mol\,l^{-1}$) on serosal to mucosal (SM) transmural D-[^{3}H]glucose transport. Individual MS and SM glucose transport experiments were conducted during warm spring/summer months. Each data point represents the mean (±s.e.m.) D-[^{3}H]glucose transport at each concentration from N=4 animals.

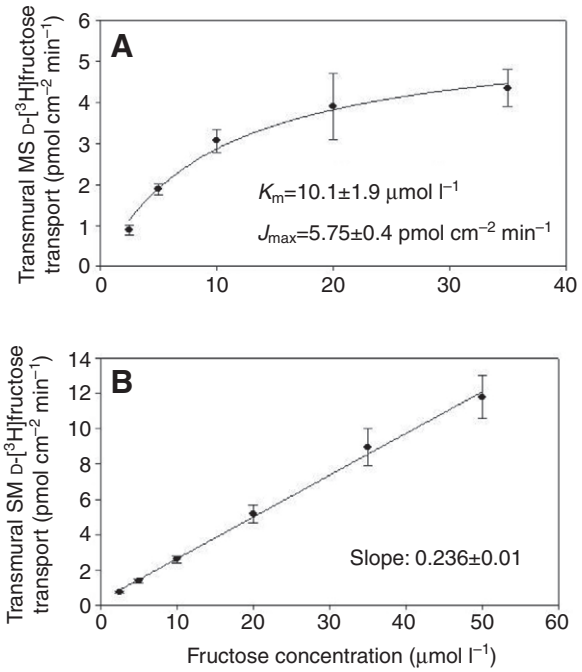

Fig. 2. (A) Effect of increasing D-fructose concentration (2.5, 5, 10, 20, 35 and 50 μmol l^{-1}) on MS transmural D-[^{3}H]fructose transport. Individual experiments were conducted during cold autumn/winter months. (B) Effect of increasing D-fructose concentration (2.5, 5, 10, 20, 35 and 50 μmol l^{-1}) on SM transmural D-[^{3}H]fructose transport. Individual experiments were conducted during cold autumn/winter months. Each data point represents the mean (±s.e.m.) D-[^{3}H]fructose transport at each concentration from N=3 animals.

hyperbolic functions of luminal sugar concentration (Fig. 2A) as found for luminal D-glucose, and also followed Eqn 1. While apparent affinity constants for the two sugars were similar (glucose K_m=15.2±3.5 μmol l^{-1}; fructose K_m=10.1±1.9 μmol l^{-1}), apparent maximal transport velocity for D-fructose was only 15.5% that of D-glucose (glucose J_{max}=37±9.1 pmol cm^{-2} min^{-1}; fructose J_{max}=5.75±0.4 pmol cm^{-2} min^{-1}), implying only a slightly higher apparent binding affinity of intestinal transporters for fructose but considerably smaller quantities of this sugar being transferred across the gut at each luminal concentration than seen for D-glucose.

Transmural SM transport of D-[^{3}H]fructose, like that of D-[^{3}H]glucose, was a linear function of luminal D-fructose concentration over the range 2.5–50 μmol l^{-1} (Fig. 2B), and the rate of transfer [0.236±0.01 pmol cm^{-2} min^{-1} (μmol l^{-1})$^{-1}$] was approximately half that displayed over the same concentration range for D-glucose (Fig. 1B). As with SM D-[^{3}H]glucose transport, SM D-[^{3}H]fructose transport may take place either by diffusion or by a low affinity carrier-mediated system over comparable concentration ranges. Combining MS and SM D-[^{3}H]fructose transport data (i.e. J_{net}=J_{MS}–J_{SM}) resulted in net absorptive transport only at concentrations from 0 to 10 μmol l^{-1}, indicating that only very low concentrations of this sugar were likely delivered to the blood by intestinal transport systems.

Effect of inhibitors on transmural D-[^{3}H]glucose and D-[^{3}H]fructose MS transport

Phloridzin inhibits sugar transport *via* the mucosal sodium-dependent SGLT1-like transport protein. Transmural 25 μmol l^{-1} MS D-[^{3}H]glucose transport was significantly (P<0.05) inhibited in the presence of 100 μmol l^{-1} mucosal phloridzin (Fig. 3A), while 25 μmol l^{-1} MS D-[^{3}H]fructose transport was insensitive to phloridzin (Fig. 3B). Control experiments showed that vehicle (100% ethanol) alone at the concentration used was ineffective on both glucose and fructose transport (Fig. 3A,B). These data suggest that transmural MS D-[^{3}H]glucose transport uses a mucosal SGLT1-like transport protein, but D-[^{3}H]fructose transport does not, and the insensitivity of glucose and fructose to the vehicle indicates that the effects observed on both sugars are true indicators of the effect of phloridzin alone.

Phloretin inhibits sugar transport *via* a GLUT2-like facilitated diffusion transport protein. In the presence of 100 μmol l^{-1} mucosal phloretin, 25 μmol l^{-1} MS D-[^{3}H]glucose transport was unaffected (Fig. 3C) but 25 μmol l^{-1} MS D-[^{3}H]fructose transport was significantly (P<0.05) inhibited under the same conditions (Fig. 3D). Both glucose and fructose were insensitive to 100% ethanol (Fig. 3C,D).

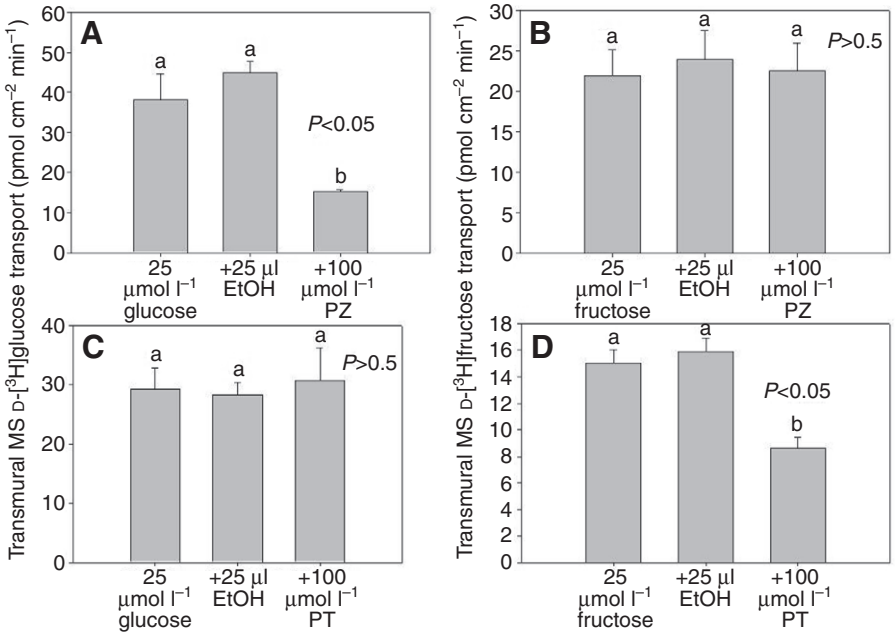

Fig. 3. (A) MS transport of 25 μmol l^{-1} D-[^{3}H]glucose in the presence of 100 μmol l^{-1} luminal phloridzin (PZ). (B) MS transport of 25 μmol l^{-1} D-[^{3}H]fructose in the presence of 100 μmol l^{-1} mucosal phloridzin. (C) MS transport of 25 μmol l^{-1} D-[^{3}H]glucose in the presence of 100 μmol l^{-1} mucosal phloretin (PT). (D) MS transport of 25 μmol l^{-1} D-[^{3}H]fructose in the presence of 100 μmol l^{-1} mucosal phloretin. The individual experiments for MS glucose and fructose transport in the presence of the inhibitors were conducted during warm spring/summer months of the year. Each bar represents the mean (±s.e.m.) obtained from time course data for each treatment from N=3 animals. Different letters indicate significant differences between treatments.

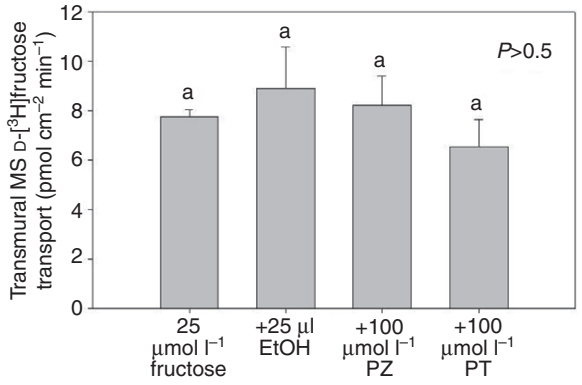

Fig. 4. Seasonal effect of MS transport of 25 µmol l^{-1} D-[^{3}H]fructose in the presence of 100 µmol l^{-1} mucosal phloridizin and 100 µmol l^{-1} mucosal phloretin. Experiments were conducted during cold autumn/winter months of the year. Each bar represents the mean (±s.e.m.) obtained from time course data for each treatment of *N*=4 animals. There was no significant difference between treatments.

Seasonal effect on sugar transport

The influence of mucosal phloretin on MS fructose transport was affected by season. Experiments conducted in the warm spring/summer seasons showed that mucosal phloretin significantly decreased MS fructose transport (Fig. 3D), but the same effect was not observed in cold autumn/winter months (Fig. 4). Also, in Fig. 3D, the rate of fructose transport in animals in warm spring/summer months (Fig. 3D) was about twice that in cold autumn/winter months (Fig. 4). Kinetic results of MS fructose transport were also obtained from animals in autumn/winter months (Fig. 2A). When the transport rate of 25 µmol l^{-1} MS fructose from the kinetic study (Fig. 2A) is compared with 25 µmol l^{-1} MS fructose transport rates obtained in both phloretin experiments (Fig. 3D, Fig. 4), the results show similar transport rates in animals in autumn/winter months (Fig. 2A, Fig. 4) and a much higher fructose transport rate in animals in summer/spring months (Fig. 3D). One of the possible upregulated sugar transporters appears to be a phloretin-sensitive GLUT2-like mucosal fructose carrier system (Fig. 3D, Fig. 4).

Immunohistochemistry

Immunohistochemical analysis using a goat anti-rabbit GLUT5 polyclonal antibody revealed a strong FITC signal from the luminal border of the intestinal epithelium, indicating the brush border localization of a GLUT5-like fructose transport protein (Fig. 5B). No GLUT5-like signal was observed at the basolateral epithelial membrane, as there was no FITC response from this side of the tissue (Fig. 5B), nor was there any non-specific cross-reactivity observed in the negative control (Fig. 5A).

Tentative identification of serosal D-[^{3}H]glucose and D-[^{3}H]fructose transport protein

Increasing serosal unlabeled D-glucose and phloretin concentrations steadily decreased carrier-free SM transmural D-[^{3}H]glucose transport by 70% and 73%, respectively (Fig. 6A,B). Similarly, increasing serosal unlabeled D-fructose and phloretin concentrations exponentially decreased carrier-free SM transmural D-[^{3}H]fructose transport by 52% and 51%, respectively (Fig. 6C,D). These results are an indication of a shared serosal GLUT2-like transporter responsible for a portion of transmural SM transport of both D-[^{3}H]glucose and D-[^{3}H]fructose across the serosal surface of intestinal cells.

DISCUSSION

D-Glucose and D-fructose are major sources of biological fuel for representatives of all life kingdoms on this planet. Because of their importance in numerous biological processes, the mechanisms by which cells are able to obtain these sugars from their environment have been intensively studied from prokaryotes to eukaryotes. Despite the wide diversity of life forms that have been investigated with respect to their membrane sugar transport properties, only two distinctly different types of sugar transport proteins have evolved to transfer these nutrients across membranes of living organisms. The first system couples transmembrane sugar transport to an existing electrochemical gradient such as that for sodium or protons (secondary active transport) and consists of members of the SLC5 co-transporter gene family (SGLT group). The second group of sugar transport proteins is composed of those that act as facilitated diffusion systems transferring sugar molecules across a membrane down a concentration gradient, belonging to the SLC2 gene family (GLUT group). Numerous structurally distinct members of both transporter groups have been reported across cells of both prokaryotes and eukaryotes. Within multicellular organisms, such as mammals and other vertebrates, different members of each transport group may be localized to dissimilar tissues or organs with distinct functions perhaps served by subtle structural differences that may occur among gene family members.

While considerable attention has been directed at defining amino acid structural differences among transporter gene family members, and identifying dissimilarities in tissue or cellular localization of different members throughout a multicellular organism, there has been less work on how members of the SLC5 and SLC2 families are organized as a suite of transporters responsible for delivering sugars across multiple membrane barriers such as occurs in epithelial

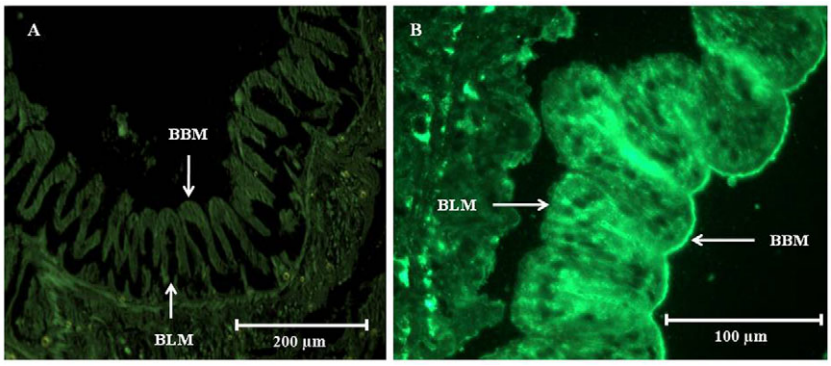

Fig. 5. Cross-sections of lobster intestine and immunofluorescence localization of rabbit polyclonal antibody to GLUT5 transport protein. (A) Negative control of a cross-section of the lobster intestine in the absence of the primary antibody. (B) A positive brush border membrane (BBM) localization of the GLUT5 transport protein on the lobster intestine visualized using FITC-labeled goat anti-rabbit IgG. No basolateral membrane (BLM) localization of GLUT5 transport protein was observed.

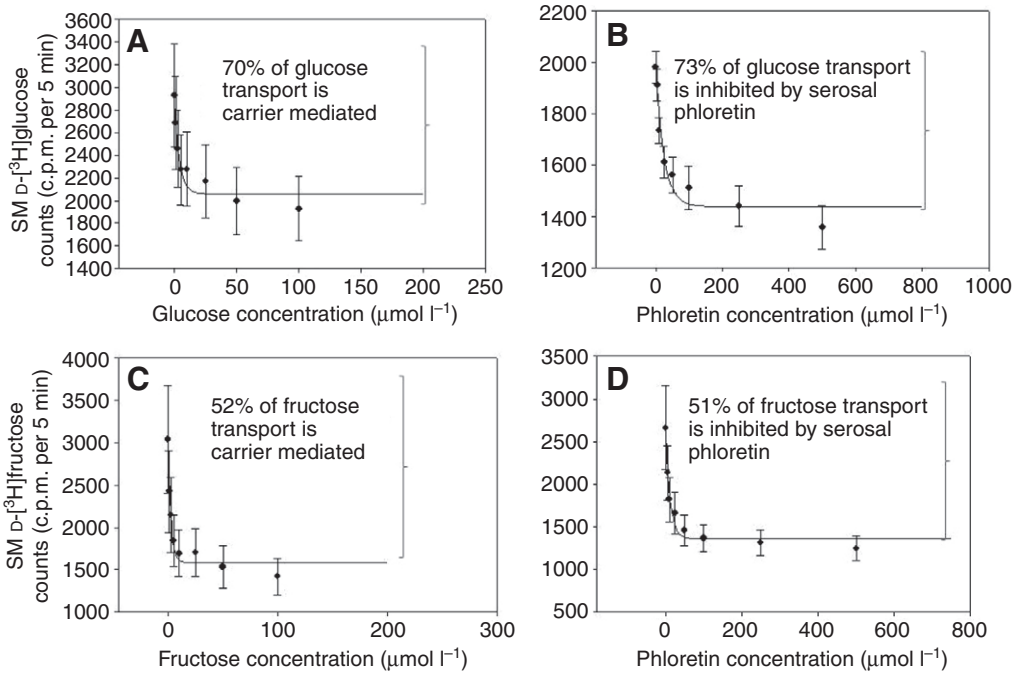

Fig. 6. (A) Effect of increasing serosal glucose (0, 1, 2.5, 5, 10, 25, 50 and 100 μmol l^{-1}) on transmural SM carrier-free D-[^{3}H]glucose transport. (B) Effect of increasing serosal phloretin (0, 5, 10, 25, 50, 100, 250 and 500 μmol l^{-1}) on transmural SM carrier-free D-[^{3}H]glucose transport. (C) Effect of increasing serosal fructose (0, 1, 2.5, 5, 10, 25, 50 and 100 μmol l^{-1}) on transmural SM carrier-free D-[^{3}H]fructose transport. (D) Effect of increasing serosal phloretin (0, 5, 10, 25, 50, 100, 250 and 500 μmol l^{-1}) on transmural SM carrier-free D-[^{3}H]fructose transport. Each data point represents the mean (±s.e.m.) c.p.m. per 5 min of triplicate samples collected at each concentration for N=3 animals.

cells lining gastrointestinal tracts. Epithelial cells possess two distinctly different membranes on either cell pole and, as a result, these different structural aspects of a single cell may have different functions during transcellular transit of molecules such as sugars. During the last 30 years a transmembrane sugar transport paradigm has been developed for mammalian epithelial cells involving members of both SLC5 and SLC2 gene families, which has broad relevance to many vertebrate epithelia. In mammalian intestine, D-glucose is transported across the luminal (brush border) membrane from dietary constituents by the combination of a secondary active sodium-dependent SGLT1 transporter and a facilitated diffusional sodium-independent GLUT2 carrier process. Relative sugar transport by the two systems depends largely on luminal hexose concentration. At high sugar concentrations diffusional D-glucose transfer occurs by the passive GLUT2 process (Kellet and Helliwell, 2000), but as concentrations drop, increased sugar uptake takes place by co-transport with sodium by the SGLT1 system (Wright and Turk, 2004). D-Fructose transfer across the intestinal luminal membrane takes place by the combination of two sodium-independent facilitated diffusional processes, involving GLUT2 and GLUT5. In mammal intestine, both D-glucose and D-fructose exit the absorptive epithelial cell by a shared sodium-independent GLUT2 transporter to the blood. Considerably less is currently known about which sugar transporters are responsible for net transcellular transport of D-glucose and D-fructose across invertebrate digestive tract or renal epithelia or how these transporters are arranged in brush border and basolateral membranes of these epithelia.

Recently, a detailed investigation of transepithelial D-glucose and D-fructose transport across invertebrate epithelial cells, and how transporter members of the SLC5 and SLC2 gene families might be involved in these processes, was reported for the larval midgut of the parasitic wasp *A. ervi* (Caccia et al., 2007). This study used the combination of radiolabeled hexoses and immunocytochemical localization methods to define the sugar transport systems present on both brush border and basolateral membranes of this gastrointestinal epithelium. Basolateral D-glucose transport by this tissue was suppressed by cytochalasin B (an inhibitor of GLUT2), but was unaffected by phloridizin, and this cell pole transported both sugars with a higher specificity for D-glucose. Brush border D-glucose transport was sodium dependent and inhibited by both phloridizin and D-galactose, while D-fructose transport by the luminal membrane was only partially inhibited by cytochalasin B. Immunohistochemical methods were used to disclose both SGLT1-like and GLUT5-like transporters on the brush border membrane. As a group, these experiments suggested that in this invertebrate a similar sugar transporter ensemble for gastrointestinal absorption of D-glucose and D-fructose is present, as previously described for mammalian epithelia, supporting the notion of an evolutionarily conserved mechanism across phyla for obtaining these substrates for biological metabolism.

Additional support for evolutionary transporter conservation of epithelial D-glucose and D-fructose transport by digestive and renal organ systems is provided by the results of the present investigation of sugar transport by lobster intestine. The presence of an SGLT1-like glucose transporter on the mucosal membrane of the lobster intestine, as shown by the inhibition of glucose transport in the presence of phloridizin (Fig. 3A), has previously been reported in mammals (Drozodowski and Thomson, 2006; Wright et al., 2004; Helliwell and Kellett, 2002; Turk et al., 1994; Turk and Wright, 1997), parasitic wasp larvae (Caccia et al., 2007), blue crabs (Chu, 1986), freshwater prawns (Ahearn and Maginniss, 1977) and a variety of other vertebrates and invertebrates. Although mammalian and crustacean intestines appear to have similar mucosal glucose and fructose transporters, SGLT1 and GLUT5 respectively, the kinetic constants for these transporters in these organisms differ. In mammals, the apparent binding affinity, K_m, of both intestinal glucose and fructose transport was reported to be in the millimolar range (Kellett, 2001; Au et al., 2002), while in lobster, prawn and crab intestines the K_m values of these sugars are in the micromolar range (Fig. 1A and Fig. 2A) (Ahearn and Maginniss, 1977; Chu, 1986). In contrast, sugar transport K_m values in crustacean hepatopancreatic epithelial cells are much higher than those in the intestine of the same species, and even approximate those reported

for the mammalian digestive tract (Ahearn et al., 1985; Verri et al., 2001). These findings suggest that sugar absorption in the digestive tract may occur sequentially as food travels from stomach to rectum, its transport characteristics being determined by substrate concentration in each location. In the more anterior hepatopancreas, where luminal substrate concentrations are high, brush border sugar transport occurs by low affinity transporters, as shown in mammalian intestine. However, by the time the luminal constituents are delivered to the intestine, sugar concentrations have been significantly reduced and additional transfer across intestinal epithelial cells occurs by high affinity systems. Intestinal K_m values for glucose and fructose lower than those displayed in the hepatopancreas would facilitate near-complete withdrawal of hexoses from dietary constituents during their transit through the digestive tract.

A similar linear arrangement of sodium-dependent sugar transporter apparent binding affinities has been reported along teleost fish digestive tract. In rockfish (*Sebastes caurinus*) digestive tract brush boarder membrane vesicles, D-glucose transport K_m was $0.58\pm0.12\,mmol\,l^{-1}$ in the more anterior pyloric caeca (analogous to the crustacean hepatopancreas), and $0.14\pm0.02\,mmol\,l^{-1}$ in the upper intestine (Ahearn et al., 1992b). Furthermore, the sodium coupling coefficient with transported D-glucose varied from 1:1 in the pyloric caeca to 2:1 for the upper intestine, providing for an efficient and complete sugar withdrawal process as the sugar moved along the substrate gradient. A similar linear heterogeneous arrangement of sodium-dependent D-glucose transporters has been reported for mammalian proximal tubule where a lower affinity SGLT2, displaying a 1:1 sodium co-transport stoichiometry, occurs immediately after the glomerulus, and is followed by a high affinity SGLT1 transporter with a 2:1 co-transport stoichiometry, together effectively removing all of the filtered D-glucose from the proximal tubule prior to the loop of Henle (Turner and Moran, 1982a; Turner and Moran, 1982b).

In previous studies, mucosal GLUT2 has been shown to be a transient transporter that is only inserted on the intestinal brush border membrane in the event of excess amounts of luminal glucose (Caccia et al., 2007; Au et al., 2002; Kellett, 2001). In the present study, the sensitivity of fructose transport to luminal phloretin (Fig. 3D), a GLUT2 inhibitor, suggests the presence of a mucosal GLUT2-like transporter in lobster intestine. The same response was not observed for glucose transport in the presence of phloretin (Fig. 3C). The lack of sensitivity of glucose to phloretin as shown in Fig. 3C is possibly due to affinity differences in the binding of the two sugars to the transporter, with the transporter displaying a higher affinity for fructose than for glucose. A similar affinity difference of GLUT2 for the two sugars has been reported in previous studies in vertebrates (Wood and Trayhurn, 2003). These results suggest that brush border glucose transport in lobster intestine is predominantly mediated by a sodium-dependent SGLT1-like transporter.

Mucosal GLUT5 is a known fructose transporter previously identified in the mammalian intestinal epithelial brush-border membrane (Manolescu et al., 2007; Corpe et al., 2002; Miyamoto et al., 1994). As shown in the immunohistochemical analysis in Fig. 5B, the anti-GLUT5 antibody localization to the lobster epithelial brush border membrane suggests that an ortholog fructose transport system occurs in lobster intestine and facilitates the uptake of fructose from dietary constituents in this location. Therefore, the lobster intestine displays a suite of brush border sugar transporters (SGLT1-like, GLUT2-like and GLUT5-like) with similar substrate and pharmacological properties reported for mammalian intestine.

The seasonal effects on transepithelial intestinal sugar transport observed in our study have been reported in a previous study that examined intestinal transport of the organic cation TEA^+ (tetraethylammonium) across lobster intestinal epithelium (Piersol et al., 2007). In this earlier study, summer animals had significantly greater MS fluxes of TEA^+ than winter animals, which is similar to the findings presented in Fig. 3D and Fig. 4. Not only was transepithelial MS fructose transport lower in animals in the autumn/winter but also there was no apparent trafficking of phloretin-sensitive GLUT2-like transport proteins to the mucosal membrane during cold months (Fig. 4). This observation is not surprising because lobsters are known to reduce their metabolism and eat less in the winter season (Factor, 1995), and under these conditions the housekeeping GLUT5 fructose transporter may be sufficient for absorbing any limited sugar available. Therefore, during environmentally cold conditions, and a reduced overall metabolic rate, there would be little apparent need to express the transient GLUT2 protein on the mucosal intestinal membrane. Additional future studies are needed to further strengthen the notion of seasonal variation in transporter expression in animals such as the lobster that are exposed to marked environmental temperature changes throughout the year.

On the serosal membrane of mammalian intestinal epithelial cells, the presence of GLUT2 allows for transmural transport of both glucose and fructose from the cell to the blood during their transit from luminal dietary constituents (Au et al., 2002; Drozdowski and Thomson, 2006; Caccia et al., 2007). In the present study, the exponential decay graphs shown in Fig. 6 revealed that about 70–73% of serosal to mucosal glucose transport was *via* a serosal GLUT2-like transporter, while only about 51–52% of fructose transport was mediated by the same apparent process. At the present time the mechanism for the remaining fraction of serosal to mucosal transport of each sugar is unclear, but it may be due to diffusional transfer through paracellular spaces between intestinal epithelial cells. As a group, the results obtained in this study of transmural D-glucose and D-fructose transport across lobster intestine support the concept of sugar transport conservation across phyla as originally outlined by the mammalian transport paradigm (Wright, 1998).

Two studies involving marine invertebrates, of considerably different phylogenetic histories, were recently carried out to investigate epithelial sugar transport processes of their respective digestive tracts (Sterling et al., 2009; Sterling and Ahearn, 2011). These studies used a combination of isolated hepatopancreatic cell suspensions and purified membrane vesicles of epithelial brush border and basolateral membranes of the 'living fossil' *Limulus polyphemus* (horseshoe crab) and *H. americanus* to define the nature of sugar transport in similar gastrointestinal organs from arthropod species evolutionarily separated from each other by at least 250 million years. These studies showed that D-glucose absorption across hepatopancreatic epithelia of both species appeared to follow the mammalian paradigm involving luminal SGLT1-like and GLUT2-like carrier systems, and GLUT2-like transport at the basolateral cell pole. In contrast, epithelial transport of D-fructose by both invertebrate epithelial cells significantly departed from the mammalian paradigm by displaying sodium-dependent transport at both cell poles that was unaffected by a 10-fold excess concentration of D-glucose. As discussed in Sterling and Ahearn's paper (Sterling and Ahearn, 2011), BLAST sequence alignments of horseshoe crab cDNA with mouse SGLT4 protein sequence and lobster sequence alignments with human SGLT4 protein sequence (Sterling et al., 2009) suggested the high probability of orthologs of this sodium-

dependent mammalian D-fructose transporter (Tazawa et al., 2005) being present in both invertebrates. Sodium-dependent D-glucose and D-fructose transport in the same digestive tract epithelium is a significant departure from the sugar transport properties characterized in many vertebrate and invertebrate species, suggesting a potentially more diverse arrangement of sugar transport proteins at sites of nutrient absorption than strictly defined by the mammalian paradigm.

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REFERENCES

Ahearn, G. A. and Maginniss, L. A. (1977). Kinetics of glucose transport by the perfused mid-gut of the freshwater prawn *Macrobrachium rosenbergii*. *J. Physiol.* **271**, 319-336.

Ahearn, G. A., Grover, M. L. and Dunn, R. E. (1985). Glucose transport by lobster hepatopancreatic brush-border membrane vesicles. *Am. J. Physiol.* **248**, R133-R141.

Ahearn, G. A., Gerencser, G. A., Thamotharan, M., Behnke, R. D. and Lemme, T. H. (1992a). Invertebrate gut diverticula are nutrient absorptive organs. *Am. J. Physiol.* **263**, R472-R481.

Ahearn, G. A., Behnke, R. D., Zonno, V. and Storelli, C. (1992b). Kinetic heterogeneity of Na-D-glucose cotransport in teleost gastrointestinal tract. *Am. J. Physiol.* **263**, R1018-R1023.

Au, A., Gupta, A., Schembri, P. and Cheeseman, C. I. (2002). Rapid insertion of GLUT 2 into the rat jejunal brush-border membrane promoted by glucagon-like peptide 2. *Biochem. J.* **367**, 247-254.

Brown, G. K. (2000). Glucose transporters: structure, function and consequences of deficiency. *J. Inherit. Metab. Dis.* **23**, 237-246.

Caccia, S., Casartelli, A., Losa, E., de Eguileor, M., Pennacchio, F. and Giordana, B. (2007). The unexpected similarity of intestinal sugar absorption by SGLT 1 and apical GLUT 2 in an insect (*Aphidius ervi*, Hymenoptra) and mammals. *Am. J. Physiol.* **292**, R2284-R2291.

Chu, K. H. (1986). Glucose transport by the *in vitro* perfused midgut of the blue crab, *Callinectes sapidus*. *J. Exp. Biol.* **123**, 325-344.

Conklin, D. E. (1995). Digestive physiology and nutrition. In *Biology of the Lobster, Homarus americanus* (ed. J. R. Factor), pp. 441-463. New York: Academic Press.

Conrad, E. M. and Ahearn, G. A. (2005). ^{3}H-histidine and $^{65}Zn^{2+}$ are cotransported by a dipeptide transport system in the intestine of lobster *Homarus americanus*. *J. Exp. Biol.* **208**, 287-296.

Corpe, C. P., Bovelander, F. J., Munoz, C. M., Hoekstra, J. H., Simpson, I. A., Kwon, O., Levine, M. and Burant, C. F. (2002). Cloning and functional characterization of the mouse fructose transporter, GLUT 5. *Biochim. Biophys. Acta* **1576**, 191-197.

Drozdowski, L. A. and Thomson, A. B. (2006). Intestinal sugar transport. *World J. Gastroenterol.* **12**, 1657-1670.

Factor, J. R. (1995). *The Biology of the Lobster, Homarus americanus*. New York: Academic Press.

Helliwell, P. A. and Kellet, G. L. (2002). The active and passive components of glucose absorption in rat jejunum under low and high prefusion stress. *J. Physiol.* **544**, 579-589.

Henderson, P. J. F. (1990). Proton-linked sugar transport systems in bacteria. *Bioenerget. Biomembr.* **22**, 525-569.

Kellet, G. L. (2001). The facilitated component ofintestinal glucose absorption. *J. Physiol.* **531**, 585-595.

Kellet, G. L. and Helliwell, P. A. (2000). The diffusive component of intestinal glucose absorption is mediated by the glucose-induced recruitment of GLUT2 to the brush border membrane. *Biochem. J.* **350**, 155-162.

Maiden, M. C. J., Davis, E. O., Baldwin, S. A., Moore, D. C. M. and Henderson, P. J. F. (1987). Mammalian and bacteria sugar transport proteins are homologous. *Nature* **325**, 641-643.

Manolescu, A. R., Witkowska, K., Kinnaird, A., Cessford, T. and Cheeseman, C. (2007). Facilitated hexose transporters: new perspective on form and function. *Physiology* **22**, 234-240.

Miyamoto, K. I., Tatsumi, S., Morimoto, A., Minami, H., Yamamoto, H., Sone, K., Taketani, Y., Nakabou, Y., Oka, T. and Takeda, E. (1994). Characterization of the rabbit intestinal fructose transporter (GLUT 5). *Biochem. J.* **303**, 877-883.

Piersol, M. C., Sterling, K. M. and Ahearn, G. A. (2007). Absorption of tetraethylammonium (TEA^{+}) by perfused lobster intestine. *J. Exp. Zool.* **307A**, 176-186.

Sterling, E. M. and Ahearn, G. A. (2011). Glucose and fructose uptake by *Limulus polyphemus* hepatopancreatic brush border and baso-lateral membrane vesicles: evidence for Na^{+}-dependent sugar transport activity. *J. Comp. Physiol. B* **181**, 467-475.

Sterling, K. M., Cheeseman, C. I. and Ahearn, G. A. (2009). Identification of a novel sodium-dependent fructose tansport activity in the hepatopancreas of the Atlantic lobster *Homarus americanus*. *J. Exp. Biol.* **212**, 1912-1920.

Tazawa, S., Yamato, T., Fujikura, H., Hiratochi, M., Itoh, F., Tomae, M., Takemura, Y., Maruyama, H., Sugiyama, T., Wakamatsu, A. et al. (2005). SLC5A9/SGLT4, a new Na^{+}-dependent glucose transporter, is an essential transporter for mannose, 1,5-anhydro-D-glucitol, and fructose. *Life Sci.* **76**, 1039-1050.

Turk, E. and Wright, E. M. (1997). Membrane topology motifs in the SGLT cotransporter family. *J. Membr. Biol.* **159**, 1-20.

Turk, E., Martín, M. G. and Wright, E. M. (1994). Structure of the human Na+/glucose cotransporter gene SGLT1. *J. Biol. Chem.* **269**, 15204-15209.

Turner, R. J. and Moran, A. (1982a). Heterogeneity of sodium-dependent D-glucose transport sites along the proximal tubule: evidence from vesicle studies. *Am. J. Physiol.* **242**, F406-F414.

Turner, R. J. and Moran, A. (1982b). Further studies of proximal tubule brush border membrane D-glucose transport heterogeneity. *J. Membr. Biol.* **70**, 37-45.

Verri, T., Mandal, A., Zilli, L., Bossa, D., Mandal, P. K., Ingrosso, L., Zonno, V., Vilelli, S., Ahearn, G. A. and Storelli, C. (2001). D-Glucose transport in decapod crustacean hepatopancreas. *Comp. Biochem. Physiol.* **130A**, 585-606.

Wood, S. and Trayhurn, P. (2003). Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. *Br. J. Nutr.* **89**, 3-9.

Wright, E. M. (1998). Glucose-galactose malabsorption. *Am. J. Physiol.* **275**, G879-G881.

Wright. E. M. and Turk, E. (2004). The sodium/glucose cotransport family SLC5. *Pflugers Arch.* **447**, 510-518.

Wright, E. M., Loo, D. D. F., Hirayama, B. A. and Turk, E. (2004). Surprising versatility of Na^{+}-glucose cotransporters: SLC5. *Int. Union Physiol. Sci.* **19**, 370-376.

Wright, E. M., Hirayama, B. A. and Loo, D. F. (2007). Active sugar transport in health and disease. *J. Intern. Med.* **261**, 32-43.

Wright, S. H. and Ahearn, G. A. (1997). Nutrient absorption in invertebrates. In *Handbook of Physiology*: Section 13, *Comparative Physiology*, Vol. 2 (ed. W. H. Dantzler), pp. 1137-1206. New York: Oxford University Press.