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D-mannose transport and metabolism in isolated enterocytes

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D-mannose transport and metabolism has been studied in enterocytes isolated from chicken small intestine. In the presence of Na^+, the mannose taken up by the cells either remains free, is phosphorylated, is catabolized to H_2O, or becomes part of membrane components. The mannose remaining free in the cytosol is released when the cells are transferred to an ice bath. The Na^+-dependent D-mannose transport is electrogenic and inhibited by ouabain and dinitrophenol; its substrate specificity differs from SGLT-1 transporter. The Glut2 transporter inhibitors phloretin and cytochalasin B added following 30-min mannose uptake reduced the previously accumulated D-mannose, whereas these two agents increased the cell to external medium 3-O-methyl-glucose (3-OMG) concentration ratio. D-mannose efflux rate from preloaded D-[2-^{3}H]-mannose enterocytes is Na^+-independent. Phloretin did not affect D-mannose efflux rate, whereas it inhibited that of 3-OMG. Neither mannose uptake nor efflux rate were affected by fructose. It is concluded that part of the mannose taken up by the enterocytes is rapidly metabolized and that enterocytes have two D-mannose transport systems: one is concentrative and Na^+-dependent and the other is Na^+-independent and passive.

Key words: D-mannose/enterocytes/intestine

Introduction

In mammals, the serum D-mannose concentration ranges from 28–161 μM (Alton *et al*., 1998) and the most likely sources are the diet and production from glucose. Until recently, the general belief was that the D-mannose needed for the biosynthesis of asparagine-linked glycans originated mostly from intracellular D-glucose and that mannose entry the cells via the glucose transporters (Gould and Holman, 1993; Hopfer, 1987). However, human fibroblasts (Panneerselvam and Freeze, 1996) and hepatoma cells (Alton *et al*., 1998) efficiently transport D-mannose by D-mannose-specific transporter(s) and they prefer mannose to glucose for N-glycosylation (Panneerselvam *et al*., 1997).

Early studies using epithelial cells revealed that rodent small intestine absorbed D-mannose at a rate lower than that of glucose (Cori, 1925; Deuel *et al*., 1938; Wilson and Vincent, 1955). Alton *et al*. (1997) observed in rats that following a gavage dose containing ^{3}H-mannose, nearly all of the radioactivity first appearing in the blood was ^{3}H-D-mannose, rather than ^{3}HOH, and that less than 1% of total ^{3}H-mannose was in either feces or intestinal contents. They concluded that D-mannose crosses the enterocytes very efficiently. These studies, however, did not address the mechanisms that mediate transepithelial D-mannose transport.

The transepithelial transport of D-mannose requires transporters located at the apical and basolateral membrane of the epithelial cells. An apical Na^+-dependent D-mannose transport system has been described in flounder (Pritchard *et al*., 1982), dog (Mendelssohn and Silverman, 1989; Silverman and Ho, 1993), and rat (Blasco *et al*., 2000; De la Horra *et al*., 2001) kidney; in LLC-PK1 cells (Saito *et al*., 1996); in Caco-2 cells (Ogier-Denis *et al*., 1994); and in chicken (Cano *et al*., 2001) and rat (De la Horra *et al*., 2001) small intestine. This Na^+/D-mannose transport system is saturable, is electrogenic, and has a substrate specificity and kinetic properties different from those of SGLT-1 transporter. Ogier-Denis *et al*. (1994) also described the presence of a Na^+-independent D-mannose transport system in the basolateral membrane of Caco-2 cells.

The mannose concentration in the mammalian intestinal lumen, ready to use the Na^+/D-mannose cotransporter, is unknown because data on the content and bioavailibity of mannose in the foods and on intestinal glycoproteins digestion are not available. However, the identification of α-mannosidase I and II on the enterocytes brush border (Velasco *et al*., 1993) suggests that they could function in glycoprotein digestion and therefore provide mannose to the apical Na^+/D-mannose transporter.

The current study was designed to further investigate intestinal D-mannose transport. Avian enterocytes were preferred to those from mammals, because, at least in our hands, the rat enterocyte preparations give very low yields and do not remain alive for more than 15 min. In addition previous studies revealed the presence of Na^+/mannose cotransport activity in both, chicken (Cano *et al*., 2001) and rat (De la Horra *et al*., 2001) small intestine.

Results

Time course and ion dependence of D-mannose uptake into isolated enterocytes

D-mannose is taken up by the enterocytes from nominally Na^+-free medium, and at 90 min the total soluble radiolabel

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[^{3}H] cell content (free and phosphorylated sugar plus ^3H_2O) was twice that in the incubation buffer (Figure 1A). Extracellular Na^+ stimulates D-mannose transport, and at 90 min the total soluble label cell content value was 4.6 ± 0.2 pmol/mg protein (1.3 ± 0.08 μmol/L cell water), which corresponds to an inside:outside sugar ratio of 6.5:1.

The transference of cells that have been incubated with D-[2-^{3}H]-mannose for 30 min to ice bath releases D-mannose until its cell concentration equals that measured in Na^+-free conditions (Figure 1A). These observations indicate that part of the mannose taken up by the cells remains in a readily diffusible form, at a concentration greater than that in the incubation buffer at 90 min.

The data shown in Figure 1A represent total soluble cell [^{3}H]-substrate. However, under either Na^+-free or ice-bath conditions, soluble label [^{3}H] cell content was higher than that in the external medium, indicating that part of the [^{3}H]-sugar it is not in a readily diffusible form. In some experiments cell pellets were extracted with barium/zinc to precipitate phosphorylated sugar (Somogy, 1945). Also, the amount of label converted to ^3H_2O during its entry into glycolysis was estimated as described in *Materials and methods* and subtracted from the total soluble label. After these corrections were done, the inside:outside sugar ratio is 1:1 in Na^+-free conditions (Figure 1B), and the transference of those cells, which have been transporting mannose in the presence of Na^+, to ice bath releases the previously taken mannose until the intra- to extracellular mannose concentration ratio was 1 (Figure 1B). These observations indicate that part of the mannose taken up by the enterocytes is phosphorylated and converted to water.

Identification of enterocyte radioactivity

Experiments were carried out to further determine what proportion of D-mannose taken by the cells remains either as free mannose, is converted to H_2O, is phosphorylated, or is bound to membrane components.

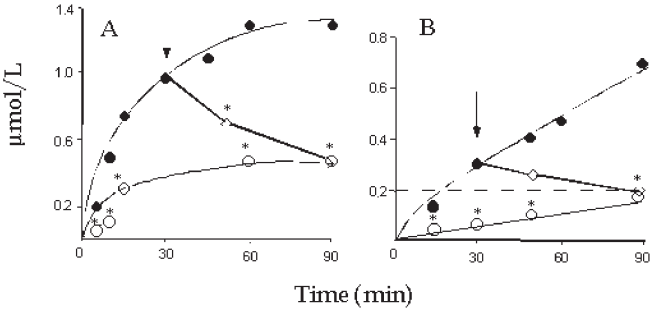

Fig. 1. Time-course of D-mannose uptake into isolated chicken enterocytes. Uptake was measured with (solid circles) or without (open circles) extracellular Na^+ (Na^+ replaced isosmotically by NMG^+). At the time indicated by the arrow cells were transferred to ice bath (diamonds). The dashed line represents the uptake value expected at equilibrium. (**A**) PCA extraction, which gives total soluble ^{3}H-label (free plus phosphorilated sugar plus ^3H_2O); (**B**) barium/zinc precipitation and measureaments of ^3H_2O content (see *Materials and methods*) allows estimation of cell-free mannose concentration. Mean ± SEM of five independent experiments. $^*p < 0.001$ as compared with uptake in the presence of sodium.

To evaluate the D-[2-^{3}H]-mannose associated to membrane components (glycoproteins), cells were precipitated by perchloric acid (PCA) and the radioactivity present in the supernatant (free mannose, phosphorylated sugar, and ^3H_2O) and that in the pellet (bound to membranes) was measured. As mentioned, extraction with barium/zinc was used to evaluate the sugar-phosphate content. Label conversion to ^3H_2O was evaluated as described in *Materials and methods*. To evaluate total mannose uptake (free plus phosphorylated plus bound to cell membranes plus metabolized to water), the enterocytes were separated from the incubation buffer by a rapid filtration technique (De la Horra *et al.*, 2001), and the filters containing the cells were placed in the scintillation fluid.

The results (Figure 2) reveal that after 5 min incubation ~40% of the total mannose taken by the cells is incorporated into membrane components, and this amount increases with time. In all the times tested the phosphorylated sugar represents ~7% of the total label taken by the cells, that is, 12% of the total radioactivity present in the supernatant. At 5 min the free mannose represents 36% of the total taken up by the cells, and it decreased to 25% at 60 min. The amount of label that corresponds to ^3H_2O represents a 16%, 13%, and 11% of the total label present in the cells at 5, 15, and

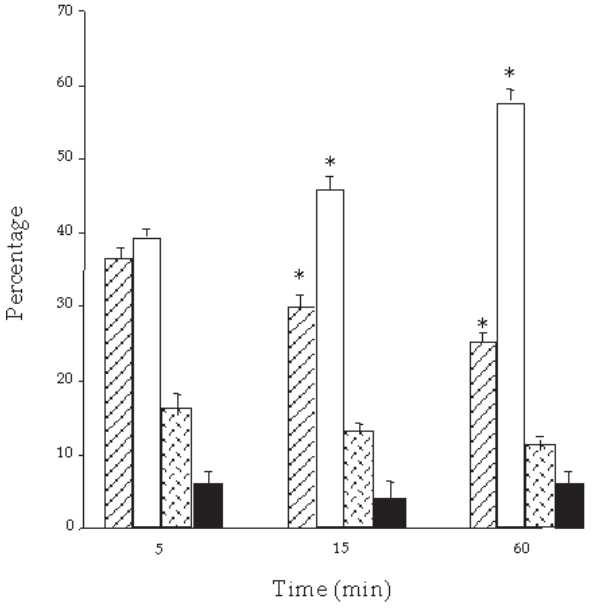

Fig. 2. Identification of enterocyte [^{3}H]-material. Cells were incubated with D-[2-^{3}H]-mannose for 5, 15, and 60 min. Total enterocytes [^{3}H]-material was measured by a rapid filtration technique. Calculations of the amount of label present as free mannose (shaded bars), phosphorylated sugar (solid bars), bound to membranes (open bars), or converted to ^3H_2O (dotted bars) were normalized to total [^{3}H]-material in enterocytes. Cell PCA extraction was used to measure the radioactivity present in the cell pellets (bound to membranes) and in the supernatant (free mannose plus phosphorylated monosaccharides plus ^3H_2O). Cell extraction with barium/zinc was used to measure the cell-free mannose content, and this was subtracted from the radioactivity measured in the PCA cell extracts to estimate the phosphorylated monosaccharides. ^3H_2O was measured as indicated in *Materials and methods*. Mean ± SEM of five independent experiments. $^*p < 0.001$ as compared with values obtained at 5 min.

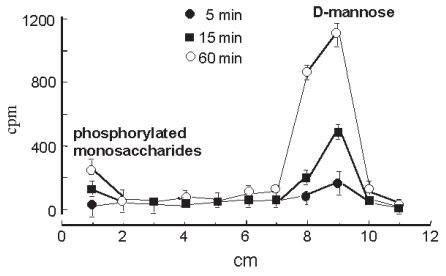

Fig. 3. Thin-layer chromatographic analysis. Chicken enterocytes were incubated with D-[2-^{3}H]-mannose for the times indicated and the cells extracted with ethanol:water as described in *Materials and methods*.

60 min, respectively, or 30% of label present in the cell lysate. Altogether these results indicate that part of the mannose taken up by the cells is rapidly metabolized.

Thin-layer chromatography of the ethanol-soluble phase revealed two peaks, one (Rf: 0.81) corresponding to D-mannose and the other (Rf: 0.1) to phosphorylated monosaccharides (Figure 3). From 15 min onward ~80% of the soluble mannose remains in the free form, corroborating the previously discussed observations.

Energetics of Na^+-dependent mannose uptake

The effects of the Na^+, K^+, ATPase inhibitor ouabain, of the metabolic inhibitor dinitrophenol, and membrane depolarization on mannose uptake were measured. In these experiments cell pellets were extracted with barium/zinc to precipitate phosphorylated sugar. One millimolar ouabain and 1 mM dinitrophenol reduced cell free-mannose concentration from 0.5 ± 0.03 to 0.20 ± 0.01 and to 0.23 ± 0.01 µmol/L cell water/30 min, $n = 5$, respectively. Forty millimolar external K^+ plus 20 µM valinomycin, a condition expected to depolarize the cells, reduces D-mannose uptake from 0.49 ± 0.03 to 0.15 ± 0.01 µmol/L cell water/30 min, $n = 5$.

Specificity of D-mannose transport

D-mannose and 3-O-methyl-glucose (3-OMG) uptakes were measured in the absence and presence of the compounds listed in Table I. The results show that D-mannose uptake was nearly abolished by unlabeled D-mannose (97% inhibition), inhibited by α-methyl-glucopyranoside (44% inhibition) and phlorizin (40% inhibition), and unaffected by D-fructose. The phlorizine-insensitive mannose uptake was nearly abolished by D-mannose but not by α-methyl-glucopyranoside.

On the other hand, 3-OMG uptake was inhibited by α-methyl-glucopyranoside (67% inhibition) and phlorizin (75% inhibition), but not by D-mannose.

Effect of phloretin and cytochalasin B on the steady-state sugar cell concentration

Glut2 mediates glucose efflux from the enterocytes. This transporter is Na^+-independent and phloretin- and cytochalasin B–sensitive. Figure 4 reveals that, as shown by Kimmich and Randles (1975), addition of either phloretin or cytochalasin B at the steady-state 3-OMG cell

Table I. Specificity of the Na^+-D-mannose transport in chicken enterocytes

Modifiers	D-mannose	3-OMG
None	100	100
D-mannose (10 mM)	$3 \pm 0.2^*$	90 ± 5
α-MG (10 mM)	$56 \pm 4^*$	$33 \pm 2^*$
D-fructose (10 mM)	100	—
Phlorizin (0.1 mM)	$60 \pm 3^*$	$25 \pm 2^*$
Phlorizin + D-mannose	$1 \pm 0.01^*$	—
Phlorizin + α-MG	$49 \pm 2^*$	—

Uptake of either 0.2 µM D-mannose or 1 µM 3-OMG was measured in the presence of Na^+ during 30 min in the absence and presence of various unlabeled modifiers. α-MG: α-methyl-glucopyranoside. Labeled sugar uptake obtained in the absence of modifiers was set at 100%.
Means ± SEM, $n = 4$.
$^*p < 0.001$, as compared with the first row.

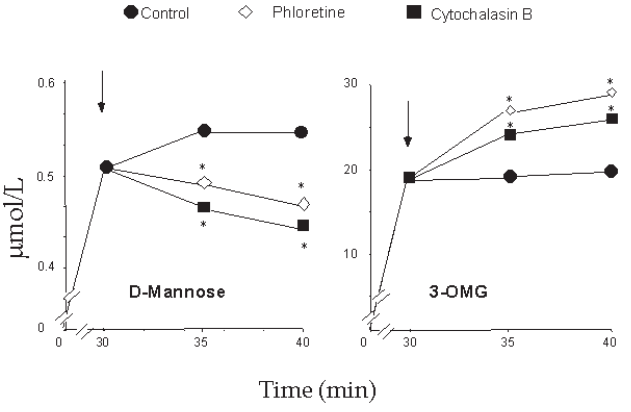

Fig. 4. Effect of phloretin and cytochalasin B on cell sugar content. Cells were allowed to take either D-mannose or 3-OMG for 30 min and then, either phloretin (diamonds) or cytochalasin B (squares) were added. Dots, control. Mean ± SEM of five independent experiments. $^*p < 0.001$ as compared with control conditions.

accumulation induced a transition to a new steady state, in which a higher cellular 3-OMG concentration is maintained because its basolateral efflux is prevented. However, phloretin or cytochalasin B reduced cell mannose concentration (Figure 4).

Sugar efflux rate

Sugar efflux rate was measured in cells loaded with either D-[2-^{3}H]-mannose and [^{14}C]-3-OMG. A semilogarithmic plot of the sugar remaining in the cells as a function of time fits well with a straight line (Figure 5), indicating that either D-mannose or 3-OMG efflux follows first-order kinetics within the time interval studied. However, whereas phloretin inhibited 3-OMG efflux rate, it had no effect on the D-mannose efflux rate (Table II). Efflux rate of either 3-OMG or D-mannose were not affected by the presence of Na^+, fructose, or phlorizin (Table II).

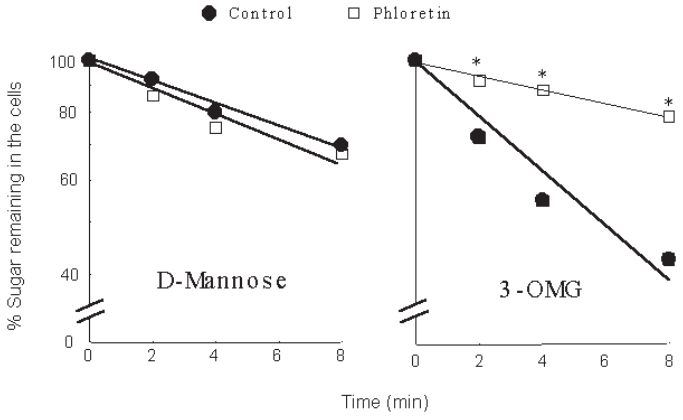

Fig. 5. Sugar efflux from enterocytes loaded with either 0.2 μM D-[2-^{3}H]-mannose or 1 μM 3-[^{14}C]-OMG. 100% is the initial radioactivity present in the samples taken at time 0 min. Control (dots), phloretin (squares). The efflux was measured in Na^+-free conditions (Na^+ substituted by NMG^+). Means ± SEM of four independent experiments. $^*p < 0.001$ as compared with control conditions.

Table II. Effect of sugar and sugar transport inhibitors on either D-mannose or 3-OMG efflux rate from chicken enterocytes

	Efflux rate (min^{-1}) × 10^{-2}	
Modifiers	D-mannose	3-OMG
None	5.03 ± 0.17	11.0 ± 0.5
D-mannose (10 mM)	5.51 ± 0.32	
D-fructose (10 mM)	5.05 ± 0.1	10.0 ± 0.1
Phloretin (0.1 mM)	4.80 ± 0.2	2.5 ± 0.1*
Phlorizin (0.1 mM)	4.90 ± 0.3	10.0 ± 0.5
$Na^+ = 100$ mM	5.03 ± 0.1	11.0 ± 0.1

The values represent the sugar efflux rate constant in min^{-1} in the absence and presence of the indicated modifiers. Unless otherwise stated, efflux was measured in Na^+-free conditions. Means ± SEM, $n = 4$.
$^*p < 0.001$ as compared with control (first row).

Enterocytes phosphomannose isomerase activity

The phosphomannose isomerase (PMI) activity, measured in isolated chicken and rat enterocytes as described in *Materials and methods*, was 10.9 ± 0.06 and 20 ± 0.1 nmol/min/mg protein, respectively. These values are within the range of values (8–34 nmol/mg protein · min) reported for mouse intestine (Davis and Freeze, 2001), human fibroblasts, and leucocytes (Niehues *et al.*, 1998; Westphal *et al.*, 2001) and validate the PMI activity measurements reported here.

Discussion

D-mannose-specific transporters are present in nonpolarized (Alton *et al.*, 1998; Panneerselvam and Freeze, 1996; Panneerselvam *et al.*, 1997) and polarized (Blasco *et al.*, 2000; De la Horra *et al.*, 2001; Mendelssohn and Silverman, 1989; Ogier-Denis *et al.*, 1994; Pritchard *et al.*, 1982; Silverman and Ho, 1993) cells. The current results corroborate those previously obtained in mammalian and avian small intestinal brush-border membrane vesicles (Cano *et al.*, 2001; De la Horra *et al.*, 2001) revealing the presence of a Na^+-dependent, electrogenic, and specific D-mannose transporter. Thus in the presence of an inwardly directed electrochemical Na^+ gradient, enterocytes take up D-mannose against its concentration gradient. This uptake was inhibited by dinitrophenol, ouabain, Na^+-free buffers, and voltage-clamped conditions, and its substrate specificity differs to that of 3-OMG.

However, at a difference from membrane vesicles, intact cells provide information regarding the fate of the mannose taken up by the apical membrane transporter. The current results revealed that under either Na^+-free or ice-bath conditions mannose concentration within the cells was higher than in the external medium, suggesting that once in the cells part of the mannose is converted into a nonreadily diffusible form. Chromatographic studies and radioisotope measurements revealed that part of the mannose taken up by the cells is either bound to cell membranes, phosphorylated, or converted to ^3H_2O, and the rest remains as free mannose. Whereas the amount of phosphorylated sugar remains relatively constant, that bound to cell membranes increased and that remaining as free mannose decreased with time. Additionally, the percentage of label that corresponds to ^3H_2O decreases from 16% at 5 min to 11% at 60 min.

These findings indicate that part of the mannose taken up by the enterocytes remains as free mannose, ready to cross the basolateral membrane toward the blood. Therefore, mannose somehow escapes enterocyte metabolism, a conclusion that can also been reached by comparing enterocyte PMI activity and mannose transport values. Thus the measured PMI activity (10 nmol/min/mg protein) is at least 10,000 times higher than total mannose uptake (around 0.5 pmol/min/mg protein). How can mannose remain free in the cytosol? One could speculate that mannose and PMI are in separate cell compartments. Contrary to the current observations, Panneerselvam *et al.* (1997) found that human fibroblasts convert 85–90% of the transported mannose into water. However, at a difference from fibroblasts, enterocytes are designed to undergo net transepithelial transport of substrates.

Previous reports agree with the aforementioned point of view. Thus, Alton *et al.* (1997, 1998) observed that 8 h after a gavage dose of [2-^{3}H]mannose, only less than 1% of total label was found in rat feces and intestinal contents, indicating that intestinal absorption of mannose was highly efficient. They also concluded that mannose transcytose the enterocytes because nearly all the radioactivity first appearing in the blood was found as [2-^{3}H]mannose rather than as ^3H_2O. Additionally, oral mannose intake leads to an increase in blood mannose levels (Davis and Freeze, 2001; Westphal *et al.*, 2001).

Since the molecular characterization of the first facilitative-diffusion glucose transporter (GLUT1), 12 other hexose transporters have been identified: GLUT2 to GLUT12 and the proton-myoinositol symporter (Joost *et al.*, 2002). Of these 13 transporters, GLUT2 and GLUT5 are expressed in the enterocyte basolateral and

apical membranes, respectively (see Thorens, 1996 for review; Rogers *et al*., 2001). Very low amounts mRNA GLUT8 have also been detected in small intestine (Doege *et al*., 2000; Ibberson *et al*., 2000). The tissue distribution and hexose expecificity of GLUT12 is so far unknown. The following observations suggest that neither GLUT1, GLUT2, GLUT5, nor GLUT8 mediate mannose efflux from enterocytes. Thus the GLUT1 and GLUT2 inhibitor phloretin reduced D-mannose cell concentration and did not affect mannose efflux rate; however, it enhanced the steady-state Na^+-dependent gradient of 3-OMG and inhibited glucose efflux rate. Furthermore, fructose, the GLUT5 substrate (Burant *et al*., 1992) also transported by GLUT8 (Ibberson *et al*., 2000), does not affect mannose efflux rate. The current results do not agree with those previously reported in differentiated Caco-2 cells (Ogier-Denis *et al*., 1994), wherein phloretin and fructose highly inhibited the basolateral membrane Na^+-independent mannose uptake.

In conclusion, enterocytes transport mannose, and 51–67% of the mannose taken up by the cells is metabolized. For mannose transport, enterocytes present at least two D-mannose-specific carrier-mediated transport systems. One is concentrative, Na^+- and voltage-dependent, and located at the apical membrane. In contrast, D-mannose efflux from the enterocytes resembles a facilitated diffusion process that is mediated by a phloretin- and cytochalasin B–insensitive and Na^+-independent transport system. The current results offer no clue on the nature and cellular location of the Na^+-independent D-mannose transport system(s). Locus of the Na^+-independent transport system is tentatively ascribed to the serosal cell surface, where it would serve for mannose transfer between enterocyte and lamina propia of the villus.

Materials and methods

Materials

D-[2-^{3}H]-mannose and 3-[^{14}C]OMG were purchased from Amersham (Little Chalfont, U.K.). The other compounds and salts used were obtained from Sigma (Madrid).

Reagents and solutions

Unless otherwise stated the incubation buffer contained, in mM: 100 NaCl, 1 $CaCl_2$, 70 mannitol, 3 K_2PO_4, 1 $MgCl_2$, 20 HEPES-Tris, pH 7.4, and 1 mg/ml bovine serum albumin. One millimolar L-glutamine was present in all the solutions as passively transported nutrient. The uptake buffer also contained either 0.2 μM D-mannose with tracers of D-[2-^{3}H]-mannose. When used, the concentration [^{14}C]-3-OMG was 1 μM.

Animals, cell isolation, and D-mannose uptake measurements

The experiments were performed in accordance with national/local ethical guidelines. Hubbard chickens, 4–6 weeks old, were killed by decapitation. Enterocytes were isolated by hyaluronidase incubation as described by Calonge *et al*. (1989) and incubated at 37°C, under the desired experimental condition. Uptake was terminated by diluting 200 μl cell suspension in 800 μl ice-cold buffer, and the cells were separated by centrifugation through a layer of an oil mixture (Calonge *et al*., 1989). Cell pellets were extracted with either 3% PCA or with $Ba(OH)_2$ and $ZnSO_4$ (barium/zinc). Aliquots (100 μl) of the supernatants were added to vials for scintillation counting. D-mannose uptake was calculated taking into account the trapped extracellular volume and cell water volume, as previously estimated (Calonge *et al*., 1989). The pellet protein was measured by the method of Bradford (1976).

Chromatographic identification of cell-free D-mannose and phosphorylated monosaccharides

The enterocytes were incubated with D-[2-^{3}H]-mannose and treated as described, except that the cell pellets were extracted with ethanol:water (1:1). Aliquots of the extract were spotted on silica gel thin-layer chromatographic plates along with 2 μg nonradioactive D-mannose and 5 μg mannose-6-P. Ascending chromatography was performed on the plates using a solvent system consisting of (v/v), 20 benthene:20 acetic acid:60 methanol. The lanes, to which radioactivity was applied, were cut into 1-cm sections. Each section was placed in scintillation vial and counted. The remaining portion of the plate, which contained nonradioactive D-mannose and mannose-6-P standards, was visualized with anisaldehyde sulfuric acid. The relative mobilities were calculated from the visualized spots and from radioactivity measurements.

Quantification of glycoprotein-associated [2-^{3}H]-mannose

Radioactivity incorporated into glycoproteins was determined by adding an equal volume of 3% PCA to an aliquot of cells. After vortexing and standing 10 min on ice bath, the precipitated protein was collected by centrifugation. The pellet was washed twice with 3% PCA and resuspended in protosol (Dupont, Boston) for radioactivity counting.

PMI assays

PMI activity was measured as described by Davis and Freeze (2000) in both rat and chicken enterocytes. Briefly, PMI was obtained by homogenization of isolated enterocytes in 50 mM HEPES (pH 7.1) and collecting the supernatant from a 100,000 × *g* centrifugation (1 h). PMI activity assay was carried out in 50 mM HEPES (pH 7.1) containing 50 μg protein, 5 mg $MgCl_2$, 0.25 Nicotinamide adenine dinucleotide phosphate (NADP), and 0.5 U/ml each of phosphoglucoisomerase and glucose 6-phosphate dehydrogenase. The reaction was initiated by addition of 1 μmol/ml D-mannose-6-phosphate. The samples were incubated at room temperature and the optical density at 340 nm was measured.

^3H_2O Determination

At the end of the experiment, aliquots from either the incubation medium or the cell lysate were evaporated to dryness, suspended in water, and counted. The amount of ^3H_2O formed was taken as the difference between the initial amount of radioactivity in either the incubation medium

or cell lysate and that remaining after evaporation. Both methods provide similar results.

Sugar efflux measurements

Enterocytes were loaded by incubation with either 0.2 μM D-[2-^{3}H]-mannose or 1 μM 3-[^{14}C]-OMG in a shaking water bath at 37°C for 30 min. The cells were then washed twice in radioisotope-free ice-cold buffer and resuspended in the desired buffer. The rate of sugar loss from the cells was measured by diluting 0.5 ml of the sugar-loaded cell suspension into 2.5 ml of radioisotope-free buffer kept at 37°C. Aliquots of 200 μl were taken at 0, 2, 4, and 8 min, and the radiolabeled sugar in the cell pellet was measured as indicated. Sugar efflux was evaluated as percent of radiolabeled sugar remaining in the cells and expressed as an apparent efflux rate coefficient that has the units of min^{-1}.

Acknowledgments

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Abbreviations

OMG, O-methyl-glucose; PCA, perchloric acid; PMI, phosphomannose isomerase.

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