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Ontogeny of D-Mannose Transport and Metabolism in Rat Small Intestine

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Abstract Oral mannose therapy is used to treat congenital disorders of glycosylation caused by a deficiency in phosphomannose isomerase. The segmental distribution and ontogenic regulation of D-mannose transport, phosphomannose isomerase, and phosphomannose mutase is investigated in the small intestine of fetuses, newborn, suckling, 1-month-old, and adult rats. The small intestine transports D-mannose by both Na^+-dependent and Na^+-independent transport mechanisms. The activities of both systems normalized to intestinal weight peak at birth and thereafter they decreased. In all the ages tested, the activity of the Na^+-independent mechanism was higher than that of the Na^+/mannose transport system. At birth, the Na^+-independent D-mannose transport in the ileum was significantly higher than that in jejunum. Phosphomannose isomerase activity and mRNA levels increased at 1 month, and the values in the ileum were lower than in jejunum. Phosphomannose mutase activity in jejunum increased during the early stages of life, and it decreased at 1 month old, as does the amount of mannose incorporated into glycoproteins, whereas in the ileum, they were not affected by age. The phosphomannose isomerase/phosphomannose mutase activity ratio decreased at birth and during the suckling period, and increased at 1 month old. In conclusion, intestinal D-mannose transport activity and metabolism were affected by ontogeny and intestinal segment.

A preliminary report of some of these results was published in abstract form (Cano et al. 2005).

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The D-mannose needed for the biosynthesis of asparagine-linked glycans originates from serum D-mannose (Alton et al. 1998), from glucose metabolism (Davis and Freeze 2001) and from degradation of glycoconjugates (Fujita et al. 2008).

Within the cells, the phosphomannose mutase (PMM) catalyzes the conversion of mannose-6-phosphate to mannose-1-phosphate (Fig. 1). There are two isozymes of PMM, the PMM1 and the PMM2, which differ in their kinetic properties and tissue distribution. PMM1 has phosphatase, phosphoglucomutase, phosphomannomutase, and glucose-1,6-biphosphatase activities (Pirard et al. 1999; Veiga-da-Cunha et al. 2008). High levels of PMM1 mRNA were found in human liver, heart, brain, and pancreas (Matthijs et al. 1997a) and in rat and mouse brain (Pirard et al. 1999; Heykants et al. 2001). PMM2 is essentially a phosphomannomutase, and its mRNA has been found in all tissues tested, being strongly expressed in human pancreas and liver (Matthijs et al. 1997b) and in rat intestine and mouse testis and liver (Pirard et al. 1999; Heykants et al. 2001). The physiological relevance of the two isozymes is quite different because PMM2 inactivation causes embryonic lethality (Thiel et al. 2006), whereas PMM1-deficient mice develop normally (Cromphout et al. 2006).

The biosynthesis of asparagine-linked glycans from glucose requires the phosphomannose isomerase (PMI) that interconverts fructose-6-P and mannose-6-P (Fig. 1). This reaction allows mannose and glucose to fuel either glycolysis or glycoconjugates synthesis. PMI mRNA is

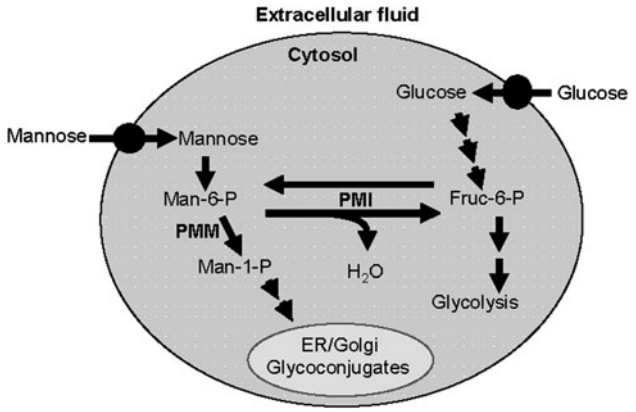

Fig. 1 Mannose metabolism in mammalian cells. Mannose in glycoconjugates can be derived from mannose imported via transport of both mannose and glucose and from internal glycogen stores. Mannose is phosphorylated to Man-6-P. Phosphomannose isomerase (PMI) interconverts Fru-6-P and Man-6-P in a reaction that causes irreversible loss of ^3H_2O from Man-6-P. This reaction allows either mannose or glucose to contribute to either glycolysis or to glycoconjugate synthesis. Man-6-P is converted into Man-1-P by phosphomannomutase (PMM) for its incorporation into glycoconjugates (from Davis and Freeze 2001)

present in all human and mouse tissues examined, being more abundant in testis, brain, and heart (Davis et al. 2002).

The serum D-mannose concentration in mammals ranges 28–161 μM (Alton et al. 1998), and the most likely source is the diet. Mannose is effectively absorbed from the gastrointestinal tract (Alton et al. 1997; Niehues et al. 1998; Davis and Freeze 2001) via the Na^+/D-mannose cotransporter located at the apical membrane of the intestinal epithelia (Cano et al. 2001; De la Horra et al. 2001; Durán et al. 2004). The importance of intestinal transport of mannose becomes evident in patients with the congenital disorder of glycosylation type Ib, which is caused by a deficiency in PMI (Niehues et al. 1998; Jaeken et al. 1998; de Koning et al. 1998). These patients can only synthesize mannose-6-P from mannose, and they had been successfully treated with oral mannose therapy (Alton et al. 1997; Niehues et al. 1998; Freeze 2001; Westphal et al. 2001).

In spite of the role played by the intestine on mannose body homeostasis, to our knowledge, there are no data available on the ontogeny of intestinal mannose transport and metabolism.

The current work addresses the segmental distribution and ontogeny of D-mannose transport, PMI activity and mRNA levels, and PMM activity and mRNA levels in rat small intestine. The rat was used as experimental animal model because of the similarity of its intestinal transport physiology to that of humans. The age of the rats studied spanned five phases of intestinal development: gestation, neonatal, suckling, 1 month old, and adulthood.

Materials and Methods

Materials

D-^{3}H-mannose was purchased from Amersham (Buckinghamshire, UK). The other compounds and salts were obtained from Sigma (Madrid, Spain).

Animals and Tissues

The experiments were performed in accordance with national/local ethical guidelines. Jejunum and ileum of fetuses of 19 days' gestation; 1 day postpartum (newborn); 15-day postpartum (suckling); and 1- and 8-month postpartum (adult) Wistar rats were used. Rats were weaned on a rat chow diet (Panlab 04) ad libitum with free access to water.

D-Mannose Uptake Measurements

Rats were anaesthetized by ether inhalation during 5–10 min. Except for the duodenum, the whole small intestine was removed, opened longitudinally, rinsed clean with ice-cold saline solution (0.9% NaCl), everted, and divided into three similar parts. The first two thirds of the small intestine (following the duodenum) were considered as jejunum, and the last one third ileum. Pieces (1–2 cm) of jejunum and ileum were incubated during 3 min at 37°C in a thermostatic bath with continuous shaking in Ringer solution containing, in mM: 140 NaCl, 1.2 $CaCl_2$, 2.4 K_2HPO_4, 0.4 KH_2PO_4, 10 $KHCO_3$, 1.2 $MgCl_2$, pH 7.4, 0.2 μM D-mannose, and tracers of D-[$^{2-3}$H]-mannose, and it was continuously bubbled with 95% O_2/5% CO_2. When required, NaCl was isosmotically substituted by mannitol. Thereafter, the tissues were washed in ice-cold Na^+-free Ringer solution and blotted carefully on both sides to remove excess moisture. The tissue was weighed wet and extracted by shaking for 15 h in 1 ml of 0.1 M HNO_3. The HNO_3 extract was treated with $Ba(OH)_2$ and $ZnSO_4$ (barium/zinc), as previously described (Somogy 1945), to precipitate phosphorylated sugar. Samples were taken from the bathing solution and from the extracts of the tissues for radioactivity counting.

The glycoprotein-associated [^{3}H]-mannose was quantified by washing the previously extracted tissue with 0.1 M HNO_3 and resuspension in protosol (Dupont) for radioactivity counting.

Total mannose transport was calculated as the sum of the mannose content in the tissue extracts plus that measured in the tissue.

^3H_2O Determination

After extraction with barium/zinc, the ^3H present in the extracts corresponds to D-^3H-mannose and ^3H_2O. To estimate the amount of ^3H_2O, aliquots from tissue extracts were evaporated to dryness, suspended in water, and counted. The amount of ^3H_2O was the difference between the amounts of radioactivity measured in the tissue extract before and after evaporation.

The amount of label remaining in the tissue extracts after evaporation corresponds to D-^3H-mannose.

PMI and PMM Assays

PMI and PMM activities were measured in jejunum and ileum as described (Davis and Freeze 2001). Briefly, the intestinal mucosa was scrapped off, homogenized in 50 mM HEPES (pH 7.1) containing protease inhibitors and centrifuged at 100,000×*g* for an hour. The supernatant was collected and the protein content measured. PMI activity was determined at room temperature in 50 mM HEPES (pH 7.1) containing 50 µg protein, 5 mM $MgCl_2$, 0.25 mM NADP, 0.5 U/ml phosphoglucose isomerase, and 0.5 U/ml glucose 6-phosphate dehydrogenase. The reaction was initiated by addition of 1 µmol/ml D-mannose-6-phosphate.

PMM activity was determined at room temperature in 50 mM HEPES (pH 7.1) containing 50 µg protein, 5 mM $MgCl_2$, 0.25 mM NADP, 0.5 U/ml PMI, 0.5 U/ml phosphoglucose isomerase, 0.5 U/ml glucose 6-phosphate dehydrogenase, and 10 µM glucose 1,6-diphosphate as cofactor. The reaction was initiated by addition of 1 µmol/ml D-mannose-1-phosphate.

In both assays, the optical density of the NADPH produced was measured at A_{340nm}.

Relative Quantification of Real-Time Polymerase Chain Reaction

Total RNA was extracted from both jejunum and ileum of rats of different ages using RNeasy kit (Qiagen, Barcelona, Spain). cDNA was synthesized from 1 µg of total RNA using QuantiTest reverse transcription kit (Qiagen, Barcelona, Spain) as described by the manufacturer. Real-time polymerase chain reaction (PCR) was performed with iQ-SYBR Green Supermix (BioRad, Madrid, Spain), 0.4 µM primers, and 1 µl cDNA, as described (García-Delgado et al. 2007). Controls were carried out without cDNA. Amplification was run in a MiniOpticon System (BioRad) thermal cycler (94°C/3 min; 35 cycles of 94°C/40 s; 58°C/40 s, and 72°C/40 s). After amplification, a melting curve analysis was performed by heating the reactions from 65 to 95°C in 1°C intervals while monitoring fluorescence. The primers for PMI (antisense -cagctttccgttaaaggtgtc- and sense -agtgttcccactttcctgtg-), PMM1 (antisense -aattgatcaagtcctgtaggag- and sense -attgatcctgaggtatcagcc-) and PMM2 (antisense -ccagatgaccttgaatattctg- and sense -ctctgtctctttgacatgga-) were chosen according to the rat cDNA sequences entered in GenBank and designed by the PerlPrimer program. β-Actin (antisense -acccacactgtgcccatcta- and sense -cggaaccgctcattgcc-) and GAPDH (antisense -tgcaccaccaactgcttagc- and sense -ggcatggactgtggtcatgag-) served as reference genes for sample normalization, both reference genes are invariably expressed during development of the intestine. The cycle at which each sample crossed a fluorescence threshold, Ct, was determined, and the triplicate values for each cDNA were averaged. Analyses of real-time PCR were performed using the comparative Ct method with the Gene Expression Macro software supplied by BioRad.

Statistics

Data are presented as means ± standard error of the mean (SEM) for *n* separated animals. Comparison between different experimental groups was evaluated by the two-tailed Student's *t*-test and two-way analysis of variance (ANOVA) followed by the Newman-Keuls test (GraphPad Prism program). Differences were set to be significant for $P < 0.05$.

Results

Glucose Effect on D-Mannose Intestinal Transport

D-^3H-mannose was used to measure intestinal D-mannose transport activity. The amount of tissue ^3H that corresponds to free mannose is a function of both transport and intracellular trapping. As described in "Materials and methods", the tissue radioactivity was extracted with barium/zinc to precipitate phosphorylated sugar, and therefore the ^3H present in the tissue extracts corresponds to free D-^3H-mannose and ^3H_2O.

Figure 2 shows the amount of ^3H taken up by the jejunum of newborn rats after a 3-min incubation period. In the absence of glucose in the incubation buffer, a large percentage of the tissue ^3H corresponds to ^3H_2O (about 75% under Na^+-free conditions and about 90% in the presence of Na^+). The addition of 5 mM glucose to the incubation buffer reduced tissue ^3H_2O content and increased the amount of ^3H that remains as D-[^3H]-mannose. Similar results were obtained with 10 mM glucose in the incubation buffer.

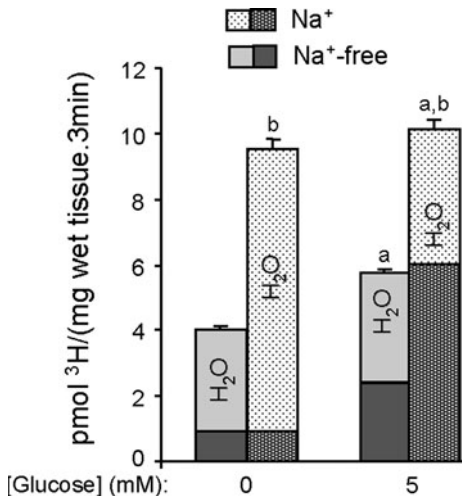

Fig. 2 Effect of glucose on intestinal D-mannose transport. D-^{3}H-mannose transport was measured in the jejunum of newborn rats in the presence and absence of Na^+ and with or without 5 mM glucose in the incubation buffer. The tissue radioactivity was extracted with barium/zinc to precipitate the phosphorylated sugar. Free D-^{3}H-mannose and ^3H_2O content were calculated as indicated in Methods. Data are means ± SEM of 4 separated animals. (*a*) $P < 0.001$ versus glucose-free conditions; (*b*) $P < 0.01$ versus nominally Na^+-free conditions

These observations indicate that glucose reduces the conversion of mannose to fructose-6-P and as a consequence increases the amount of free D-mannose within the tissue. The rest of the experiments described in the current work were carried out in the presence of 5 mM glucose in the incubation buffer to minimize the rapidly conversion of mannose to fructose-6-P.

To find out whether the Na^+-independent component of D-mannose transport was mediated by a carrier system, the effect of 5 mM of mannose, fructose, or 3-O-methyl-glucose (3-OMG) on D-mannose transport was measured under Na^+- and glucose-free conditions. Na^+-independent D-mannose transport activity was inhibited by mannose by 44% and by 3-OMG by 25% (data not shown), but it was unaffected by fructose. These observations indicate that about 45% of the Na^+-independent D-mannose transport is carrier mediated.

Intestinal D-Mannose Transport Versus Age

Intestinal D-mannose transport was measured in jejunum and ileum of fetuses, newborn, suckling, 1-month-old, and adult rats. The experiments were carried out with and without Na^+ in the incubation buffer and in the presence of 5 mM glucose. HNO_3 treatment extracts the total soluble ^{3}H (D-^{3}H-mannose, ^{3}H-phosphorilated sugar, and ^3H_2O) and after barium/zinc treatment the extracts contain D-^{3}H-mannose and ^3H_2O. The amount radioactivity obtained after barium/zinc treatment did not significantly differ from that extracted with HNO_3 (data not shown), suggesting that the amount of phosphorylated sugar is too low to be measurable. The ^{3}H associated with protein was also evaluated, as described in Methods, and added to that measured in the HNO_3 extracts. The results are given in Fig. 3 and represent the total tissue radioactivity (D-^{3}H-manose, ^{3}H-phosphorilated sugar, ^{3}H-protein, and ^3H_2O). The Na^+-dependent D-mannose transport was calculated as the difference between the transport measured in the presence of Na^+ and that measured in Na^+-free conditions.

Figure 3a shows that both, the Na^+-dependent and the Na^+-independent D-mannose transport activities, normalized to intestinal weight, increased during the late gestation period, reaching a peak at birth. Thereafter, they decreased and remain fairly constant from 1- to 8-month-old rats.

At birth, the Na^+-independent mannose transport activity in the ileum was significantly higher than that measured in the jejunum. No significant differences between jejunal and ileal Na^+-dependent mannose transport activities were observed.

By means of previous measurements of intestinal weight versus age (Peral et al. 2005), the data shown in Fig. 3a were expressed as mannose transported by the whole intestinal segment (Fig. 3b). The results reveal that intestinal mannose transport increased with age.

The amount of D-^{3}H-mannose incorporated into protein is shown in Fig. 3c. It was not affected by the presence of Na^+ in the incubation buffer. In newborn rats, it was higher in the jejunum than in the ileum. In the jejunum, the ^{3}H incorporated into protein significantly decreased in 1-month-old rat. In the ileum, it was not significantly affected by age.

Ontogeny of Intestinal PMI Activity and mRNA Levels

PMI activity and mRNA levels were measured in jejunum and ileum of fetuses, newborn, suckling, and 1-month-old rats. Figure 4a shows that PMI activity does not significantly change during the early stages of life, but it significantly increased at 1 month old. Jejunum and ileum have similar PMI activity values.

The effect of ontogeny on PMI mRNA levels was examined by real-time PCR assays. Figure 4b reveals that PMI mRNA levels vs. age show the same pattern as activity: there is no change at birth and suckling period, and they increased at 1 month old. At difference from activity, the abundance of PMI mRNA in jejunum was significantly higher than in ileum.

Ontogeny of Intestinal Phosphomannomutase (PMM) Activity and PMM1 and PMM2 mRNA Levels

The PMM activity and mRNA levels were measured in jejunum and ileum of rats of different ages. In jejunum,

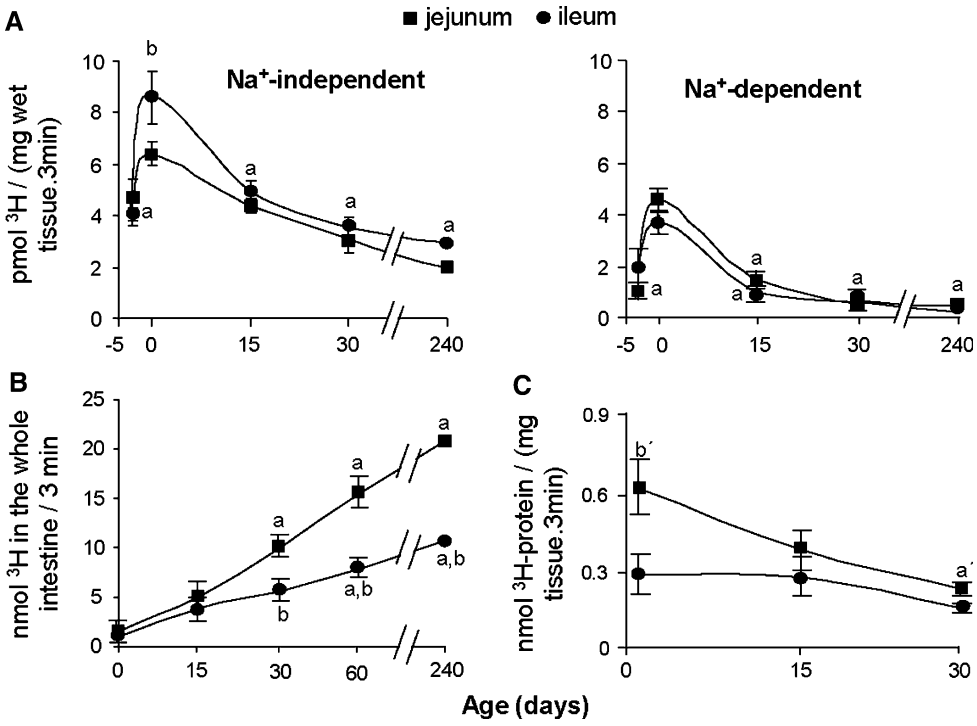

Fig. 3 Intestinal D-mannose transport versus age. D-^{3}H-mannose transport was measured in jejunum and ileum of fetuses, newborn, suckling, and 1- and 8-month-old rats. Experiments were carried out with and without Na^{+} in the incubation buffer and in the presence of 5 mM glucose. **a** Transport expressed per mg of weight tissue. **b** Total ^{3}H taken by the whole small intestine. **c** ^{3}H incorporated into proteins. Means ± SEM. The number of animals used per age was eight and four 8-month-old rats. Two-way ANOVA showed an effect of maturation on small intestinal D-mannose transport ($P < 0.0001$). Newman-Keuls test showed significant differences: (*a*) $P < 0.001$, (*a′*), $P < 0.05$ versus newborn; (*b*) $P < 0.001$, (*b′*) $P < 0.05$ versus jejunum

PMM activity increased at birth and it decreased at 1 month (Fig. 5). In the ileum, PMM activity was not significantly affected by maturation. In all the ages tested, the intestinal PMM activity was lower than that of PMI, and the PMI/PMM activities ratio decreased at birth, remained low during the suckling period, and increased at 1 month (Table 1).

The effect of ontogeny on PMM mRNA levels was examined by real-time PCR assays. PMM1 mRNA levels were not significantly modified by maturation in the jejunum, whereas in the ileum, their abundance significantly decreased after birth.

In the jejunum, the PMM2 mRNA levels were increased by age, whereas those in the ileum were not modified.

Discussion

The adaptation of a great variety of morphological and functional parameters of the digestive system to development is known for long, including the transport of monosaccharides. However, to our knowledge, no data are currently available on the ontogeny of intestinal mannose transport and metabolism. This information is of interest because oral mannose supplementation is used to treat congenital disorders of glycosylation type Ib (Alton et al. 1997; Niehues et al. 1998; Freeze 2001; Westphal et al. 2001). The present study reveals that in the rat small intestine D-mannose metabolism and transport varies with age.

The uptake of mannose by the cells is a complex process that couples the membrane transport of mannose to its intracellular utilization (Fig. 1). This is also the case in intestinal cells because part of the mannose that has entered the cells has been incorporated into glycoprotein, and because the presence of glucose in the incubation buffer significantly reduced the production of water due to mannose metabolism.

The current experiments have used everted small intestine to reduce the entry of mannose to the enterocytes across the serosal side. Therefore, the majority of the D-^{3}H-mannose measured in the intestine has entered the epithelial cells across their apical membrane. As previously reported (Durán et al. 2004), mannose used the Na^{+}-dependent transport system to enter the epithelial cells. Mannose is also taken up by the cells via a Na^{+}-independent mechanism or mechanisms, which was inhibited by mannose and 3-OMG but unaffected by fructose.

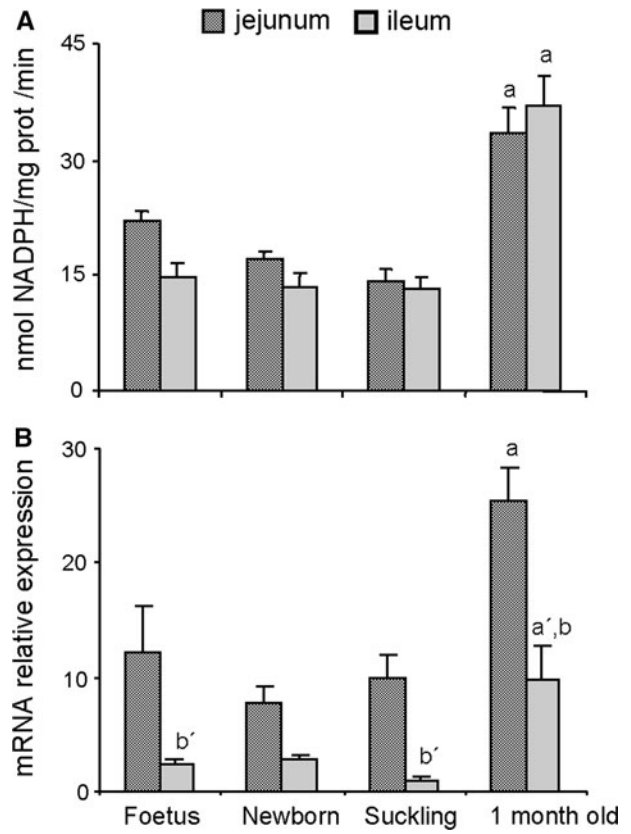

Fig. 4 Intestinal phosphomannose isomerase (PMI) activity and mRNA levels versus age. **a** Data are means ± SEM of PMI activity in jejunum and ileum versus age. **b** Histograms represent the means ± SEM of arbitrary units of PMI mRNA levels versus age. The mRNA levels measured in the ileum of suckling rats were set at 1. The number of animals per age was at least 4. Two-way ANOVA showed an effect of maturation on small intestinal PMI activity and mRNA levels ($P < 0.001$). Newman-Keuls test showed significant differences: (a) $P < 0.001$, (a') $P < 0.05$ as compared with newborn values; (b) $P < 0.001$; (b') $P < 0.05$ as compared with jejunum

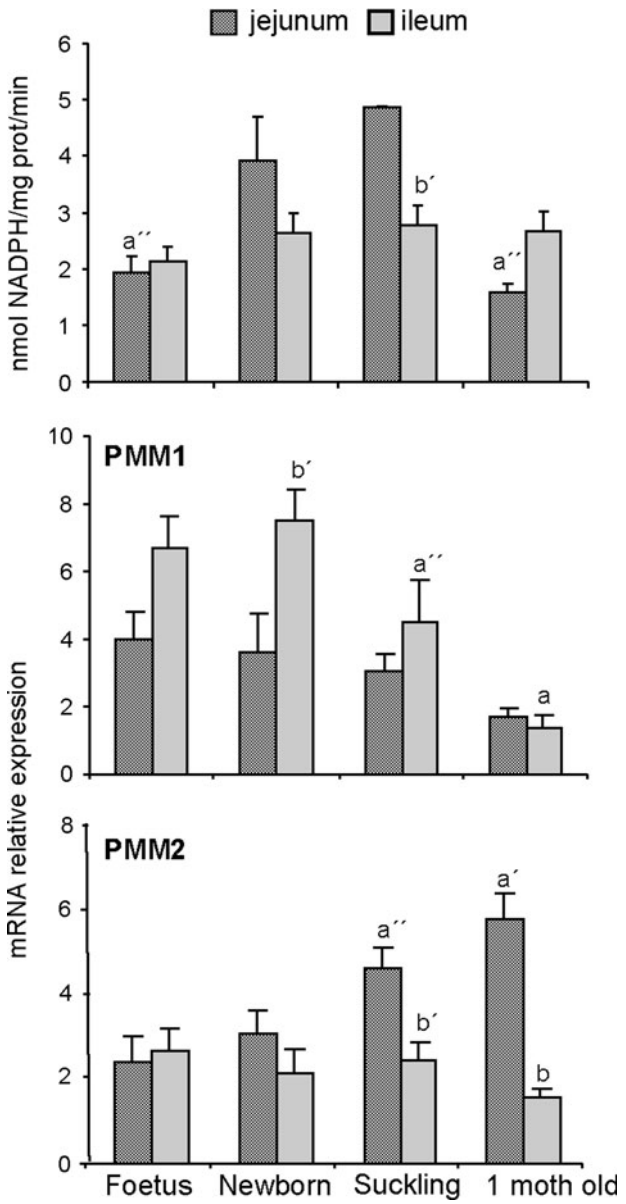

Fig. 5 Intestinal phosphomannomutase (PMM) activity and mRNA levels of PMM1 and PMM2 versus age. Other details as in Fig. 4. The mRNA levels measured in the ileum of 1-month-old rats were set at 1. Two-way ANOVA showed an effect of maturation on small intestinal PMM activity and mRNA levels ($P < 0.001$). Newman-Keuls test showed significant differences: (a) $P < 0.001$, (a'), $P < 0.01$, (a'') $P < 0.05$ as compared with newborn; (b) $P < 0.001$; (b') $P < 0.05$ as compared with jejunum

At least two Na^+-independent sugar transporters are present at the apical membrane of the intestinal epithelia: Glut2 and Glut5. Glut2 transports glucose and glucose analogues, including mannose (Uldry et al. 2002). Glut5 transports fructose (Burant et al. 1992). In Caco-2 cells (Ogier-Denis et al. 1994) and in the brush-border membrane of dog kidney epithelia (Mendelssohn and Silverman 1989), the Na^+-independent mannose transport activity was inhibited by fructose, indicating that Glut-5 is the underlying transporter. In the intestine, however, D-mannose does not appear to use Glut-5 because the Na^+-independent mannose transport activity was not inhibited by fructose. Therefore, Glut2 may be the transporter that mediates the Na^+-independent mannose transport activity.

When using whole tissue, the mannose filling the submucosal space can reenter the enterocytes via basolateral transporters and be reprocessed by the cells. If this were the case, our data would overestimate the mannose transport. The mechanism underlying mannose transport across the basolateral membrane remains unknown. Rodríguez et al. (2005) reported that Glut transporters mediate the Na^+-independent mannose transport activity in several cell lines. Our previous studies, however, suggested that neither Glut1, Glut2, Glut5, nor Glut8 mediate mannose efflux from chicken enterocytes (Durán et al. 2004).

Table 1 Ratio between specific activities of PMI and PMM[a]

Site	PMI/PMM values			
	Fetus	Newborn	Suckling	1 Month old
Jejunum	11.4** ± 0.82	4.37 ± 0.88	2.61 ± 0.96	21.2* ± 1.68
Ileum	7.0 ± 0.97	5.1 ± 1.15	4.3 ± 1.02	13.9* ± 2.14

PMI phosphomannose isomerase, *PMM* phosphomannose mutase

* $P < 0.001$, ** $P < 0.01$ versus newborn

[a] Data (mean ± SEM) are the ratio of the PMI and PMM activities values shown in Figs. 4 and 5

Both the Na^+-dependent and Na^+-independent mannose transport activities normalized to milligram of wet tissue were affected by maturation. The main maturation-related changes in intestinal mannose transport activity were as follows: (1) an increase during late gestation period that leads to maximal values at birth (similar changes have been reported for intestinal Na^+-glucose and amino acids transport activities; Buddington et al. 2001); (2) a decrease during the suckling and 1-month periods (the latter coincides with the shift from milk to an adult diet); and (3) very low mannose transport activity after 1 month.

Signals involved in telling the intestine to turn on the correct transporters at the correct time might be external (e.g., changes in dietary solute inputs associated with weaning) and internal. The latter are genetically programmed to occur at a certain time, independent of external circumstances. Direct effects of nutrients on the postnatal gene expression shift and gene products processing, have been implicated on the expression of SGLT1 and Glut5 (Ferraris 2001). Declines in rates of transport could also arise from nonspecific adaptive changes, such as different distribution of transporters along the crypt–villus axis in the immature and adult intestine. Changes in phospholipids composition and fluidity of the plasma membrane could also contribute to the observed changes (Schwarz et al. 1985; Dudeja et al. 1991). The current results cannot differentiate between all these possibilities.

The low intestinal mannose transport activity per milligram of wet tissue observed in the adult rat contrasts with the use of oral mannose to treat metabolic disorders and with previous studies, revealing that mannose is effectively absorbed by the intestine of mammals (Alton et al. 1997; Niehues et al. 1998; Davis and Freeze 2001). These authors measured the amount of mannose appearing in blood; therefore, they evaluated the mannose absorbed by the whole intestine. The normalization of the data to milligram of wet tissue, however, does not represent total intestinal absorption. When the data were expressed as mannose transported by the whole intestine, the total mannose transport remains fairly constant during the first month of life, and it steeply increased with age. Therefore, the increase in mucosal surface area with maturation compensates the observed decrease in mannose transport per milligram of weight tissue.

As reported by Alton et al. (1998) for several tissues of adult rat, including the intestine, the intestinal PMM activity was lower than that of PMI. The current data also reveal that the activity and the mRNA levels of PMI and PMM varied with age. The PMI activity slightly decreased at birth and it increased at 1 month, whereas that of PMM increased at birth and decreased at 1 month. As a result, the PMI/PMM activities ratio decreased at birth, remained low during the suckling period, and increased at 1 month. These observations suggest that the amount of mannose used by the intestinal mucosa for glycosylation and glycolysis varies with age. The amount of intestinal mannose entry to glycosylation seems to be higher in newborn and suckling rats than in adults because they show higher PMM activity and higher amount of ^3H incorporated into proteins, and higher in the jejunum than in ileum. Suckling is a period characterized by a rapid growth of the intestine (Ferraris and Diamond 1997; Yu et al. 1992) and by changes in the composition and dynamics of the enterocyte population (Smith 1988), which probably demands high levels of glycoprotein.

The molecular assays suggest that ontogeny regulates PMI at the transcriptional level because the age-induced changes on activity and those on mRNA levels show a similar pattern.

In the case of PMM, the molecular assays and the activity measurements are not easy to relate. It is generally accepted that PMM2 accounts for essentially all of the PMM activity (Pirard et al. 1999). However, the observed changes in the mRNA levels of either PMM1 or PMM2 vs. age do not keep any relationship with those found in PMM activity. As a result, neither the level of regulation of PMM by ontogeny nor the PMM isozyme (PMM1 and/or PMM2) responsible for the observed intestinal PMM activity can be inferred from the current results.

In conclusion, the observations described herein revealed that ontogeny affects intestinal D-mannose transport activity and metabolism.

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