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Kinetic evidence for heterogeneity in Na^+-D-glucose cotransport systems in the normal human fetal small intestine

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Zero-*trans* kinetic studies of Na^+-D-glucose cotransport have been performed under voltage-clamped conditions in brush-border membrane vesicles isolated from both jejunum and ileum of 17–20-week-old normal human fetuses. Varying glucose concentrations in the incubation medium led to curvilinear Eadie-Hofstee plots in the jejunum only, thus suggesting the presence of both high-affinity, low-capacity (K_m 0.37 mM; V_{max} 8.3 nmol/min per mg protein) and low-affinity, high-capacity (K_m 4.2 mM; V_{max} 30.9 nmol/min per mg protein) systems in the proximal small intestine, and of a single carrier (K_m 1.2 mM; V_{max} 4.9 nmol/min per mg protein) in the distal small intestine. Sodium activation curves provide further evidence for heterogeneity in glucose transport systems in the fetal small intestine: Hill coefficients of 2 and 1 were found for the jejunal high-affinity and ileal systems, and for the jejunal low-affinity system, respectively. It is concluded that there is early differentiation of a functional heterogeneity in glucose transport capacity along the human fetal small intestine.

Introduction

During the last fifteen years, transport studies using brush-border membrane vesicles have proved valuable in defining the kinetic and molecular mechanisms of Na^+-cotransport systems in both intestinal and renal tissues [1–3]. Most of these studies have been done on animal models [1–3] and only few experiments have been performed on the adult human small intestine [4–7] and kidney [8–9].

The presence of two Na^+-D-glucose carriers has been demonstrated in human [8] and rabbit kidney [10] but the question as to whether the small intestine is endowed with one or two D-glucose transport system(s) remains unanswered. Some kinetic arguments have been given to support the existence of multiple D-glucose transport pathways in hamster everted gut sacs [11], as well as in bovine [12], rabbit [13] and adult human [7] small intestinal brush-border membrane vesicles. Furthermore, Honegger and Gershon [14] have reported some data supporting the existence of two distinct carriers in hamster everted gut sacs. This demonstration was based on partial physical separation of the systems by means of differential specificity for glucose (carriers 1 and 2) and 6-deoxyglucose (carrier 1 only) and their distribution along the length of the small intestine.

Based on this kinetic evidence for two distinct

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

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carriers in the small intestine, Semenza and Corcelli [15] have proposed the following hypothesis to explain the hereditary glucose-galactose malabsorption syndrome: only one Na^+-D-glucose cotransporter would be present in the human gut at birth (the one missing or non-functional in glucose-galactose malabsorption). This system would be characterized by a high affinity for glucose, a 2:1 stoichiometry for Na^+ and inhibition by D-galactose. A second cotransporter, specific for D-glucose would develop later, after a few years. The confirmation of this developmental pattern would allow the characterization of a single Na^+-D-glucose cotransporter in the normal gut. However, this hypothesis has never been verified directly during the ontogeny of the human intestinal tract.

Using brush-border membrane vesicles isolated from both jejunum and ileum of human fetuses, we have recently demonstrated the existence of a fully functional Na^+-D-glucose cotransport system in the human small intestine and the early presence of a proximo-distal gradient of sugar transport activity [16]. Furthermore, we have established that the aboral differences observed for D-glucose uptake between jejunal and ileal brush-border membrane vesicles are best explained by different kinetic properties of the Na^+-dependent transport system(s) present in proximal and distal segments.

In the present study our objective was two-fold: (i) to complete our previous work [16] by determination of the kinetic characteristics of the Na^+-D-glucose cotransport system(s) in brush-border membrane vesicles isolated from both fetal jejunum and ileum, and (ii) to verify directly the hypothesis of Semenza and Corcelli [15] on normal human tissues. Our results suggest the early appearance of multiple sugar transport systems in the normal human small intestine during the gestational period.

Materials and Methods

Chemicals

Labeled compounds, namely D-[U-^{14}C]glucose (315 mCi/mmol) and D-[1(n)-^{3}H]mannitol (19.1 mCi/mmol) were purchased from new England Nuclear Corporation. Valinomycin was obtained from Sigma Chemical Company and FCCP from Aldrich Chemical. All salts and chemicals used for buffer preparation were of the highest purity available.

Preparation of brush-border membrane vesicles

Fresh, 17–20-week-old normal human fetal small intestines from social abortions were kindly provided by Dr. Michel Potier from Ste-Justine Hospital and by Dr. Charlotte Branchaud from the Montreal Children's Hospital. The jejunum and ileum were separated and prepared as previously described [16]. Brush-border membranes were purified by the $CaCl_2$ precipitation method [17] and vesicles were prepared as described previously [16]. Based on sucrase (apical membrane marker) and ($Na^+ + K^+$)-ATPase (basolateral membrane marker) activities, enrichment factors in the range of 12–19-fold over the homogenate were routinely obtained and contamination by basolateral membranes represented less than 5%.

Transport studies

Time course studies of D-[^{14}C]glucose were performed as described previously [16] using the rapid filtration technique of Hopfer et al. [18] as modified by Berteloot [19]. Freshly purified brush-border membrane vesicles were resuspended to a final protein concentration of 6–8 mg/ml in the final resuspension buffer. Aliquots were added to the incubation medium kept at room temperature to start the transport experiments. The exact composition of the resuspension and incubation media are given in the legends of the figures. Uptakes were terminated by the addition of quenched ice-cold stop solution as previously described [16]. For kinetic studies, membrane potential was clamped to zero by K^+-valinomycin (5 μM) and H^+-FCCP (10 μM) as suggested by Kaunitz et al. [20]. Results are expressed as nmol solute uptake/mg protein. Initial rates of uptake as well as Eadie-Hofstee plots have been analyzed by linear regression using an Apple II desk computer and a curve fitter program (P.K. Warme Copyright (C) 1980, Interactive Microware Inc.). Curvilinear Eadie-Hofstee plots have been analyzed by a non-linear least-square curve-fitting iterative procedure based on Feldman's parameter fitting [21,22] using the same computer. Na^+ dependency of D-glucose

uptake has been analyzed by FIT [23], a computer program based on an iterative procedure originally developed by Parker and Waud [24], and by Hill analysis [25].

Assays

Marker enzyme for the brush-border membrane, namely sucrase, was assayed by the method of Dahlqvist [26] as modified by Lloyd and Whelan [27]. $(Na^+ + K^+)$-ATPase activity (basolateral membrane marker) was determined according to Lowry et al. [29] using bovine serum albumin as standard.

Results

Kinetic studies of D-glucose uptake

In previous work [16], we have shown that the time course of D-glucose uptake was linear up to at least 30 s. The initial rate of D-glucose uptake was thus determined at 6 s as a function of increasing concentrations of D-glucose in the incubation medium, ranging from 20 μM up to 12 mM. Fig. 1 illustrates the results from three different experiments, each done in duplicate, on brush-border membrane vesicles isolated from the proximal part of the small intestine. The total uptake of D-glucose (dashed line) did not saturate over the concentration range studied. When outside NaCl was replaced by an equivalent concentration of KCl, a straight line (correlation coefficient 0.94), corresponding to the diffusive component (broken line) was obtained. There was no evidence for carrier-mediated Na^+-independent D-glucose transport under this condition. From this line, a K_d of 1.72 nmol/min per mg protein per mM was determined by linear regression analysis. When this diffusive component was subtracted from the total uptake ($V - K_d \cdot S$), saturation of the D-glucose transport could be observed (solid line). This Na^+-dependent D-glucose transport was then transformed according to the (modified) Eadie-Hofstee graphical representation, as illustrated in Fig. 2. This curve is clearly curvilinear and can be resolved in two distinct components with different characteristics: (i) a high-affinity, low-capacity system with a K_m of 0.27 mM and a V_{max} of 8.3 nmol/min per mg protein, and (ii) a low-affinity, high-capacity system with a K_m of

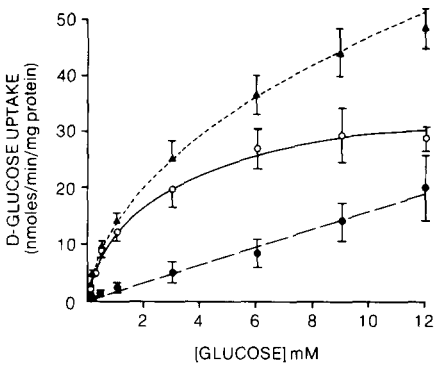

Fig. 1. Initial rates of D-glucose transport as a function of D-glucose concentrations in the incubation medium in the human fetal jejunum. Vesicles were resuspended in 50 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 100 mM KCl, 300 mM choline chloride, 5 μM valinomycin and 10 μM FCCP. Final concentrations in the incubation media were: 50 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 100 mM KCl, 20 or 50 μM D-[^{14}C]glucose and various concentrations of D-glucose (from 50 μM to 12 mM), and either 200 mM NaCl and 100 mM choline chloride (▲- - - - -▲) or 300 mM choline chloride (●— — —●). The solid line (○ ——— ○) represents the difference between D-glucose uptake in the presence or absence of NaCl. Points are Mean ± S.D. from three different experiments each done in duplicate.

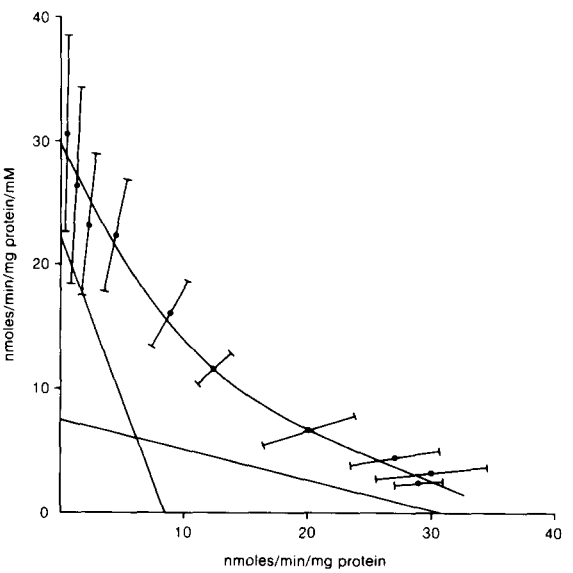

Fig. 2. Eadie-Hofstee (modified) plot of the saturable component of D-glucose transport in the proximal part of the small intestine. Kinetic parameters have been determined by non-linear least-square curve-fitting iterative procedure as described under Materials and Methods. K_{m_1}: 0.37 ± 0.06 mM, V_{max_1}: 8.3 ± 2.3 nmol/min per mg protein; K_{m_2}: 4.2 ± 1.0 mM, V_{max_2}: 30.9 ± 1.9 nmol/min per mg protein.

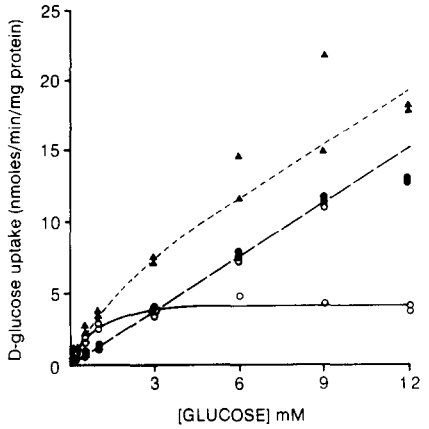

Fig. 3. Initial rate of D-glucose transport in brush border membrane vesicles isolated from human fetal ileum as a function of increasing concentrations of D-glucose in the incubation medium. Experimental conditions and symbols were as described for Fig. 1, except that there was a single point at 20 μM D-glucose.

4.2 mM and a V_{max} of 30.9 nmol/min per mg protein. These results are thus compatible with the existence of two distinct carriers in the proximal part of the small intestine of human fetuses.

Similar kinetic studies have been done for the distal part of the small intestine. Fig. 3 illustrates a typical experiment in which the initial rate of D-glucose transport was determined as a function of D-glucose concentration. The saturable fraction of transport, corrected for the diffusional component (K_d 1.25 nmol/min per mg protein per mM; correlation coefficient 0.99), corresponds to a Michaelian hyperbola which has been transformed into a Eadie-Hofstee plot, as shown in Fig. 4. In this case, the plot appears linear and the kinetic parameters determined were a K_m of 1.2 mM and V_{max} of 4.9 nmol/min per mg protein. In an other set of experiment, the Eadie-Hofstee plot was also linear (correlation coefficient 0.93) and the K_m

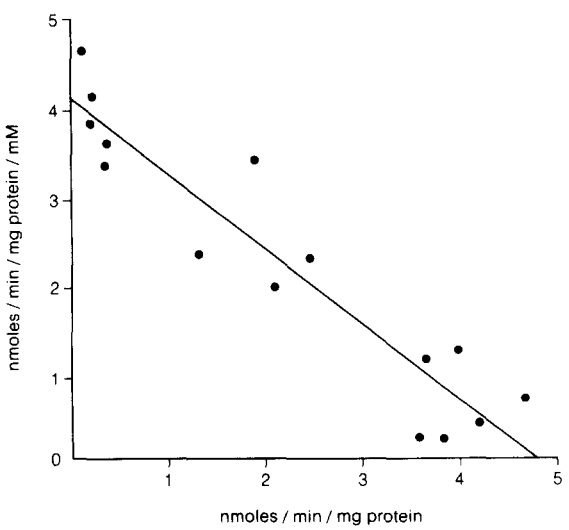

Fig. 4. Eadie-Hofstee plot of the saturable component of initial D-glucose transport in the distal part of the small intestine. Linear regression analysis was performed as described under Materials and Methods. K_m: 1.2±0.2 mM, V_{max}: 4.9±0.3 nmol/min per mg protein, r: 0.939.

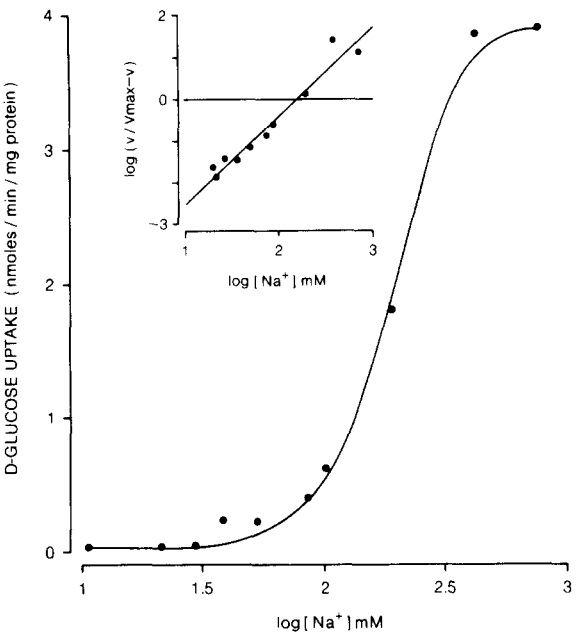

Fig. 5. Na^+ dependency of the high-affinity transport system for D-glucose uptake in the jejunum at 50 μM D-glucose. Vesicles were resuspended in 50 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 100 mM KCl, 1 M choline chloride, 5 μM valinomycin, and 10 μM FCCP. Final concentrations in the incubation media were: 50 mM Tris-Hepes buffer (pH 7.5), 0.1 mM $MgSO_4$, 100 mM KCl, 50 μM D-[^{14}C]glucose, and various concentrations of NaCl (from 20 to 800 mM). Isoosmolarity was maintained with choline chloride. Data were corrected for the diffusive component determined in the complete absence of Na^+. Kinetic parameters were determined by FIT, as described under Materials and Methods. K_m: 198±12 mM; V_{max}: 4.1±0.2 nmol/min per mg protein; r: 0.991. The inset shows the Hill plot of the same data. K_m: 176 mM; Hill coefficient: 2.1±0.1; r: 0.961.

TABLE I

KINETIC PARAMETERS OF Na^+-D-GLUCOSE COTRANSPORT SYSTEMS IN THE HUMAN FETAL SMALL INTESTINE

Results are expressed as mean ± S.E. HAS, high-affinity system; LAS, low-affinity system.

	Jejunum		Ileum
	HAS	LAS	
K_m for D-glucose (mM)	0.37 ± 0.06	4.2 ± 1.0	1.2 ± 0.2
V_{max} (nmol/min per mg protein) [a]	8.3 ± 2.3	30.9 ± 1.9	4.9 ± 0.3 8.7 ± 0.6 [b]
K_m for Na^+ (mM)	198.0 ± 19.0	156.0 ± 57.0	188.0 ± 39.0
V_{max} (nmol/min per mg protein) [c]	4.1 ± 0.2	35.9 ± 5.4	10.4 ± 0.1
Stoichiometry Na^+/glucose	2:1	1:1	2:1

[a] V_{max} for D-glucose determined at fixed concentration of Na^+ (200 mM).
[b] Values from two different experiments.
[c] V_{max} for D-glucose determined at varying concentration of Na^+ (10–800 mM).

determined was identical (1.2 mM). However, the V_{max} was significantly higher (8.7 nmol/min per mg protein). Nevertheless, we have been unable, in both cases, to fit these data with Feldman's iterative analysis for two sites, but the same analytic procedure for one site gave similar results.

Sodium dependency of D-glucose uptake

In order to better characterize the two transport systems present in the proximal part of the small intestine, Na^+ dependency of D-glucose uptake has been evaluated at two different concentrations of glucose. At 50 μM D-glucose, the high-affinity, low-capacity system is responsible for 75% of the total uptake. Under this condition, and after correction of the data for the diffusive component measured in the complete absence of Na^+, the analysis of the Na^+-dependent D-glucose uptake as a function of varying Na^+ concentrations into the incubation medium (from 10 to 800 mM) gave a K_m for Na^+ of 198 mM and a V_{max} of 4.1 nmol/min per mg protein (Fig. 5). By Hill analysis ($n = 2.1 \pm 0.1$), a minimal stoichiometry of 2 Na^+ : 1 glucose can be determined (Fig. 5, inset) for the high-affinity system. The K_m value determined by this method (176 mM) was close to the one found previously (Table I).

At 12 mM D-glucose, the low-affinity, high-capacity system is favoured and contributes to 74% of the total uptake. The analysis of kinetic constants revealed a K_m for sodium of 156 mM

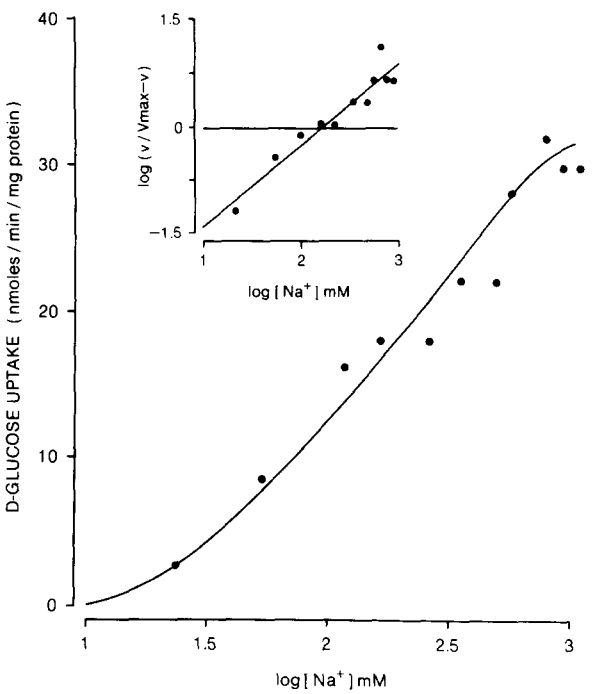

Fig. 6. Na^+ dependency of the low-affinity transport system for D-glucose in the jejunum at 12 mM D-glucose. Experimental conditions were similar as the one depicted in Fig. 5, except for the addition of 12 mM D-glucose to the incubation media. K_m: 156 ± 57 mM; V_{max}: 35.9 ± 5.4 nmol/min per mg protein; r: 0.975. The inset shows the Hill plot of the same data. K_m: 164 mM; Hill coefficient: 1.2 ± 0.1; r: 0.997.

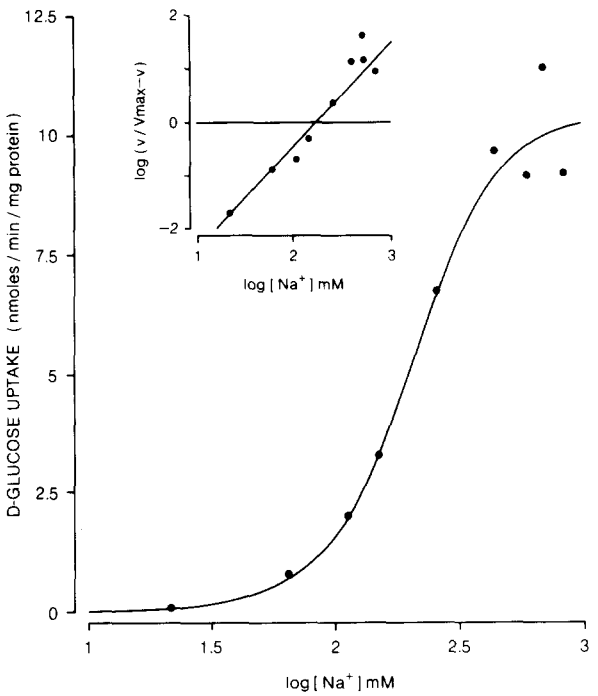

Fig. 7. Na^+ dependency of D-glucose uptake in the ileum. Conditions were as described in Fig. 6. K_m: 188 ± 39 mM; V_{max}: 10.4 ± 0.1 nmol/min per mg protein; r:0.983. Hill plot of the same data (inset): K_m: 189 mM; Hill coefficient: 1.94 ± 0.19; r: 0.999.

and a V_{max} of 35.9 nmol/min per mg protein (Fig. 6 and Table I). The Hill coefficient was 1.2 ± 0.1, suggesting a minimal stoichiometry of 1 Na^+ : 1 glucose (Fig. 6, inset).

In the distal part of the small intestine, the Na^+ dependency of D-glucose uptake has been evaluated at saturating substrate concentration (12 mM). The K_m for Na^+ determined was 188 mM and the V_{max} 10.4 nmol/min per mg protein (Fig. 7 and Table I). The Hill coefficient was 1.9 ± 0.2, suggesting a minimal stoichiometry of 2 Na^+ : 1 glucose (Fig. 7, inset). The kinetic parameters of the different Na^+-D-glucose cotransport systems present along the length of the human fetal small intestine are summarized in Table I.

Discussion

In the present paper we report some kinetic arguments which suggest the existence of multiple Na^+-D-glucose cotransport systems in brush-border membranes of the human small intestine during the early gestation period. Since changes in either glucose, sodium and/or electrical gradients during the course of the experiment can alter transport kinetics [10,12], determinations were made under initial rate and voltage-clamped conditions to avoid these problems. Our results suggest the presence of both high-affinity, low-capacity and low-affinity, high-capacity systems in the jejunum and of only one carrier with intermediate affinity in the ileum.

In adult human kidney, D-glucose transport would be carried out by two systems: a high-affinity, low-capacity one (K_m 0.3 mM; V_{max} 2.5 nmol/min per mg protein), and a low-affinity, high-capacity one (K_m 6 mM; V_{max} 8 nmol/min per mg protein) [8]. Based on physical separation, Turner and Moran [10] have produced conclusive evidence for the existence of two Na^+-D-glucose transporters in rabbit kidney proximal tubule: one prevailing in the outer cortex (a low-affinity system with a K_m of 6 mM and a stoichiometry of 1 Na^+ : 1 glucose) and the other prevailing in the outer medulla (a high-affinity system with a K_m of 0.35 mM and a stoichiometry of 2 : 1). Nevertheless, no such complete physical separation has been reported for the small intestine and the debate about the existence of multiple pathways for sugar transport in the small intestine has never been settled.

Notwithstanding, kinetic studies on sugar transport seem to agree where the presence of multiple pathways for glucose uptake in both kidney and small intestine is concerned, and the comparison of the kinetic properties of these systems revealed close similarities. In adult human small intestine [7] the presence of two distinct carriers in the jejunum and of a single system in the ileum has also been reported. It is quite interesting to compare the K_m value reported for the high-affinity system in our study (0.37 mM), in the adult small intestine (0.35 mM) [7] and in the human kidney (0.3 mM) [8] with the K_m value reported for the high-affinity system present in the outer medulla in rabbit (0.35 mM) [10]. It seems that this high-affinity system is remarkably constant and well preserved in the course of development and in different tissues and species.

The low-affinity system appears more variable.

The K_m values reported were 4.2, 6 and 18 mM for human fetal small intestine, human kidney [8] and human adult small intestine [7], respectively. However, it is clear that the determination of kinetic constants is subject to the mathematical tool used and of the experimental errors. In fact, variations are always more important at high substrate concentrations, which is the region of the Eadie-Hofstee plot which serves to evaluate the low-affinity system. Nevertheless, our results support the existence of two distinct Na^+-D-glucose transport pathways in the proximal part of the small intestine, as reported for hamster [11,14], bovine [12], rabbit [13] and human adult [7] small intestine.

In the distal part of the small intestine, our data indicate the existence of a single system, which confirms the results of Honegger and Semenza in hamster [11] and of Harig et al. [7] in adult human small intestine. The K_m value reported for this system in adult (0.87 mM) [7] is not really different from the one present in the human fetus (1.2 mM), which suggests that this Na^+-D-glucose cotransporter would remain unchanged until the adult period. However, the V_{max} value showed large variation from one experiment to the other, which was not the case for the jejunum. It is well known that the differentiation of the small intestine proceeds toward a proximo-distal gradient [16,30] and that the ileum is less differentiated than the jejunum, as reflected by the activities of the brush-border membrane enzyme markers at this stage of the human development [31]. Thus, variations of the V_{max} value could be best explained by a different density of the membrane carriers as a function of the state of differentiation, as suggested previously [16].

The determination of the exact coupling ratio of Na^+-D-glucose transport system would require different approaches such as the direct method or the 'static head method' as introduced by Turner and Moran [32]. However, the heterogeneity of glucose transport in the proximal part of the human small intestine does not allow the utilization of these methods in which uptake measurements are made under varying concentrations of both substrate and activator. The only way to discriminate between the high- and low-affinity systems in the jejunum is to work under experimental conditions which favor one or the other cotransporter, which means at low and high glucose concentrations. It thus appears difficult, until we find a way to separate these two components, to evaluate more precisely the Na^+ dependency of D-glucose uptake. However, the activation method used in this study and the determination of the Hill coefficients are indicative of different stoichiometries for the systems studied. A Hill coefficient of 2.1 ± 0.1 for the jejunal high-affinity system does not prove by itself a 2 : 1 stoichiometry but is in agreement with previously reported data supporting a coupling ratio of 2 for the high-affinity system in rabbit [13,20] and bovine [12] small intestine. Likewise, the existence of a low-affinity system in the jejunum is supported by the sodium activation curve (mean Hill coefficient $n = 1.2 \pm 0.1$) which suggests a minimal stoichiometry of 1 Na^+: 1 glucose, as proposed for the low-affinity system in rabbit [13] and bovine [12] small intestine as well as in the renal outer cortex [32].

Our data suggesting the existence of multiple Na^+-D-glucose cotransport pathways in the human fetal small intestine argue against the hypothesis of Semenza and Corcelli [15] as to the existence of only one D-glucose carrier in the human gut at birth. This behaviour implies that children lacking the glucose-galactose carrier at birth, such as in glucose-galactose malabsorption syndrome, would remain D-galactose intolerant since the transporter which develops later is thought to be specific for D-glucose. However, this behaviour has never been reported. It is interesting to note that in renal glucosuria (which affects the low-affinity system), there is no aberration of the glucose transport in the small intestine [33]. This discrepancy could be explained either by the absence of a low-affinity system in the small intestine (which seems unlikely), by a mutation which does not affect the intestinal carrier or by the existence of a distinct carrier bearing different properties. Further experiments about substrate specificity, particularly for D-galactose, need to be done to complete the characterization of these systems and to elucidate this point. Moreover, it would be quite interesting, from a genetic and ontogenic point of view, to compare the appearance and the characteristics of the Na^+-D-glu-

cose cotransporter(s) present in the human fetal kidney with those present in the human small intestine at the same gestational age.

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