0031-3998/90/2705-0519$02.00/0
PEDIATRIC RESEARCH

Vol. 27, No. 5, 1990
Printed in U.S.A.

Maturational Changes in Glutamine Transport by Rat Jejunal Brush Border Membrane Vesicles

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ABSTRACT. The ontogeny of glutamine uptake by jejunal brush border membrane vesicles was studied in suckling and weanling rats and compared with the data obtained from previous studies done on adult rats in our laboratory. Glutamine uptake represented transport into the intravesicular space rather than mere binding into the membrane as evident by osmolality study. The process of glutamine uptake was temperature dependent suggesting a carrier-mediated process with a pH optimum at 7.0. Glutamine uptake was driven by Na^+ and K^+ gradient in both suckling and weanling rats. Both processes exhibited saturation kinetics and were inhibited by other neutral amino acids suggesting the presence of Na^+-dependent neutral brush border system and Na^+-independent (L)-like system. The V_{max} of Na^+-dependent and Na^+-independent processes were significantly greater in suckling rats with V_{max} of 4.9 ± 0.36 nmol · mg protein^{-1} · 7 s^{-1} compared to weanling rats with V_{max} of 2.4 ± 0.2 nmol · mg protein^{-1} · 7 s^{-1} and adult rats with V_{max} of 0.70 nmol · mg protein^{-1} · 7 s^{-1}. The greater V_{max} in suckling rats is also evident when the kinetic parameters are analyzed by subtracting the sodium-dependent uptake values from the sodium-independent values. V_{max} of 1.59 ± 0.3 and 0.76 ± 0.01 nmol · mg protein^{-1} · 7 s^{-1} in suckling and weanling rats, respectively, $p < 0.01$. Km values were not different at 2.5 ± 0.6 and 3.5 ± 0.6 mM, respectively). The data suggest that the activity and/or the number of transporters are greater during the period of active growth and development. We conclude that glutamine transport in brush border membrane vesicles undergoes age-dependent changes with greater maximal capacity to transport glutamine in the suckling period. (*Pediatr Res* 27: 519–524, 1990)

Abbreviations

BBMV, brush border membrane vesicles
NBB, neutral brush border system
HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid
BCH, (β-2 amino bicyclo (2,2,1) heptane-2-carboxylic acid)

The developing intestine is characterized by very rapid growth with a daily protein turnover that exceeds 100% (1). The maturational process in the gastrointestinal tract includes morphologic, biochemical, and functional changes to meet the demand of the organism for growth and development.

One of the important aspects of the metabolic activities in the intestine relates to glutamine metabolism. Although most body tissues use glucose as a source of energy, the intestine derives >70% of its energy supply from glutamine metabolism (arterial and luminal) (2) and the resultant nitrogen atoms are used for the synthesis of citrulline, which is used for the synthesis of arginine in the kidney (3). Arginine is of importance in protein synthesis, especially during active periods of growth.

Because of this increased recognition of the importance of glutamine for the growing intestine, the present studies were designed to investigate the maturational processes in glutamine transport by brush border membranes of the jejunal enterocytes of the rat.

MATERIALS AND METHODS

Materials. L-[G-^{3}H]-Glutamine (sp act 39 Ci/mmol) and scintillation fluid (ACS) were purchased from Amersham/Searle (Des Plaines, IL). Unlabeled glutamine and other amino acids were purchased from Sigma Chemical Company (St. Louis, MO). Cellulose nitrate filters, 0.45 μm pore size were obtained from Sartorius Filters, Inc (Hayward, CA). All other chemicals and reagents were obtained commercially and were of analytical quality.

Preparation of BBMV. Sprague-Dawley littermate rats were purchased from Sasco Laboratories (Omaha, NE). Suckling rats (2 wk old) and weanling rats (3 wk old) were killed by cervical spine dislocation. The entire jejunum from the ligament of Treitz to approximately 13 cm distally was removed and flushed with ice-cold normal saline and then everted on a plastic rod. The mucosal scrapings were obtained from 8–10 rats and the BBMV were isolated by the modified Kessler's divalent cations (Mg^{2+}) precipitation technique (4). Preparations were maintained at approximately 4°C throughout the procedure. The mucosal scrapings were placed in 30 mL of buffer I, which consisted of 300 mM mannitol, 12 mM Tris-HCl (pH 7.4) and 5 mM EGTA, then 120 mL of ice-cold distilled water was added and the prep was homogenized for 3 min at maximum speed in an Omni mixer (Fisher Scientific Co., Pittsburgh, PA). The homogenate was then treated with 1.5 mL of 1 M $MgCl_2$ and allowed to stand for 15 min, followed by centrifugation at 3 000 *g* for 15 min in a centrifuge model J2-21 (Beckman Instruments, Fullerton, CA). The pellet was discarded and the supernatant centrifuged at 27 000 *g* for 30 min. The resulting pellet was resuspended in 30 mL of buffer II, which consisted of 60 mM mannitol, 12 mM Tris-HCl, and 5 mM EGTA, pH 7.4, and homogenized in a Potter-Elvehjem homogenizer for 10 strokes at maximum speed. The homogenate was treated with 0.3 mL of 1 M $MgCl_2$, allowed to stand for 15 min at 0°C, then centrifuged at 3 000 × *g* for 15 min. The pellet was discarded and the supernatant centrifuged at 27 000 × *g* for 30 min. The resulting pellet was resuspended

Received June 30, 1989; accepted December 27, 1989.
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Supported by NIH Grant DK 41274-02.

in 30 mL of buffer III, which consisted of 300 mM mannitol and 30 mM HEPES/Tris at pH 7.4 and homogenized in a Potter homogenizer for 10 strokes at the highest speed. The homogenate was then centrifuged at 50 000 × *g* for 30 min and the resulting pellet was resuspended in the desired volume of transport buffer, using tuberculin syringe with a 25-gauge needle.

Purity of membrane vesicle preparation was assessed by the measurement of disaccharidases as described by Dahlquist (5), leucine aminopeptidase by Boehringer kit no. 124869 (Boehringer-Mannheim Biochemicals, Indianapolis, IN), Na^+-K^+ATPase by the method of Scharschmidt *et al.* (6), and cytochrome-C-oxidase and NADPH cytochrome-C-reductase as described by Beaufay *et al.* (7). Protein assay was done by the method of Lowry *et al.* (8).

Transport measurements. Uptake of ^{3}H-glutamine by BBMV was measured by a rapid filtration technique (5). All experiments were performed at 25°C unless specified otherwise in the figure legends. Transport was initiated by the addition of 20 μL aliquot of membrane vesicle suspension to 80 μL of the desired incubation media containing ^{3}H-glutamine as described in the figure legends. At the desired time intervals, the reaction was stopped by the addition of 1 mL ice-cold stop solution. The suspension containing the vesicle was immediately pipetted onto the middle of a cellulose nitrate filter (0.45 mm pore size Sartorius filters) kept under suction and immediately washed with 5 mL ice-cold stop solution. The stop solution consisted of 100 mM mannitol, 100 mM NaCl, and 20 mM HEPES/Tris, pH 7.4. A liquid scintillation counter (Model LS 3801, Beckman Instruments, Palo Alto, CA) was used to determine the amount of radioactive substrate remaining on the filter with Ready protein$^+$ (Beckman Instruments) used as a liquid scintillant. Radioactivity remaining in the filters after pipetting, an identical solution without vesicles, was used as a background and subtracted from the uptake data. Ten to 12 suckling and six to eight weanling rats were used for each vesicle preparation. The values depicted in the tables and figures represent mean of uptake points from two to three vesicle preparations. Each vesicle preparation was run in triplicates (n = 6–9). The uptake data are expressed in pmol/mg protein per unit time.

Statistical evaluation. All data were statistically analyzed and expressed as mean ± SE. Two-tailed t test was used to evaluate the significance of data differences. A p value < 0.05 was considered statistically significant.

RESULTS

Purity of membrane vesicles. The brush border enzyme markers (leucine aminopeptidase and disaccharidases) showed a 10- to 14-fold enrichment in BBMV compared to mucosal homogenate. There was an impoverishment in Na^+-K^+ ATPase enzyme activity, a marker for basolateral membranes, cytochrome oxidase, a marker of mitochondrial enzymes, and NADH-cytochrome-C-reductase, a marker for endoplasmic reticulum (9).

Glutamine transport versus binding. To determine whether glutamine uptake represents binding or transmembrane movement, two studies were conducted in 3-wk-old rats. First, an osmotic sensitivity was done in which the intravesicular space was decreased by increasing the osmolarity of the incubation media from 200–700 mosmol by the addition of mannitol in increasing concentration. The uptake of glutamine was then determined at 10 min. Figure 1 shows that the uptake of glutamine was inversely related to media 1/osmolarity, as depicted by the equation Y = −8.5 + 13x with r = 0.98. Second, a temperature dependency study was done in which glutamine uptake was determined at 0 and 25°C. Figure 2 shows that glutamine uptake was significantly greater at 25°C compared to 0°C (p < 0.05–0.001 for all values).

To evaluate the degree of glutamine metabolized by brush border membranes, vesicles were incubated with 25 mM glutamine. Glutamine and glutamate were determined at various time points by a well-validated enzymatic method (10). At 10 min, no significant degree of metabolism could be noted. At 20 min, 10% of the glutamine was recovered as glutamate.

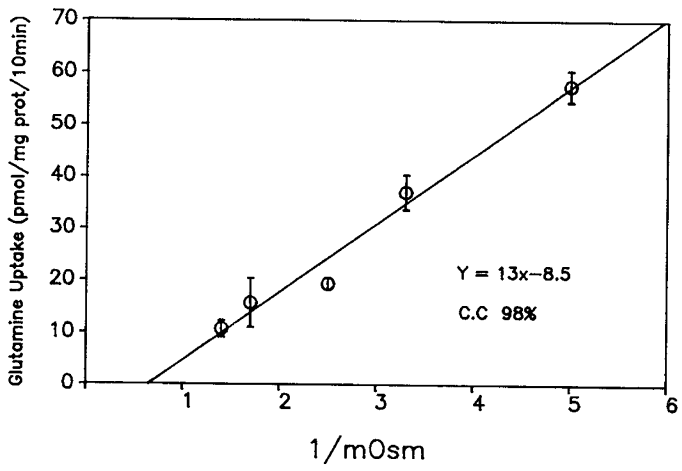

Fig. 1. Effect of media osmolality on glutamine uptake. BBMV from weanling rats were preincubated in 300 mM mannitol and 20 mM HEPES/Tris buffer, pH 7.4. The incubation media consisted of 100 mM NaCl, 20 mM HEPES/Tris buffer, and varying concentrations of mannitol (0–500) to yield osmolarity from 200–700 mosmol in addition to 0.02 mM glutamine and 0.5 μCi ^{3}H glutamine. The reaction was started by the addition of 20 μL vesicles to the variable incubation medium and the reaction stopped at 10 min. The experiments were done in triplicate and the data represents the mean ± SE.

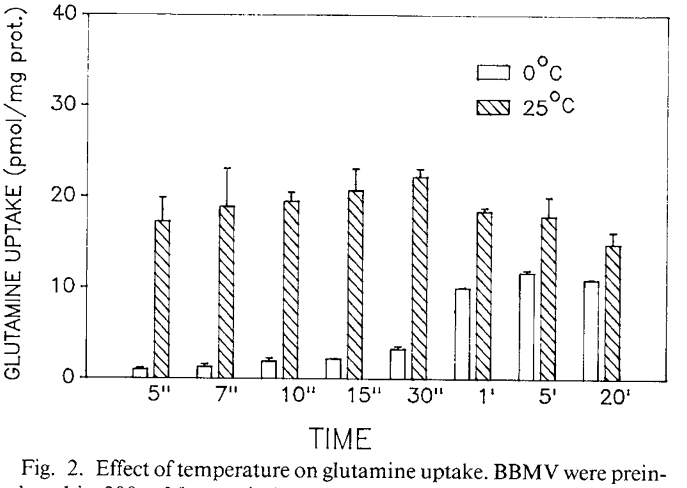

Fig. 2. Effect of temperature on glutamine uptake. BBMV were preincubated in 300 mM mannitol and 20 mM HEPES/Tris buffer pH 7.4. The incubation media consisted of 100 mM mannitol, 100 mM NaCl, and 20 mM HEPES/Tris buffer, pH 7.4, in addition to 0.02 mM glutamine and 0.5 μCi ^{3}H glutamine. Reaction was started by the addition of 20 μL membrane vesicles to the incubation medium and the reaction stopped at the time interval shown in the figure. The experiments were done in triplicate and the data represents the mean ± SE.

Effect of Na^+ and K^+ gradients on glutamine uptake. Glutamine uptake was determined in both suckling and weanling rats under inwardly directed sodium and potassium gradients. The uptake of glutamine was stimulated by both sodium and potassium gradient with "overshoot" phenomena. The "overshoot" with K^+ is a quite unusual phenomenon in transport studies using vesicles. The magnitude of the uptake was greater with Na^+ gradient compared with K^+ gradient (Fig. 3). In addition, in suckling rats the peak "overshoot" was significantly greater than in corresponding values in weanling rats 68.0 ± 8.0 *versus* 21 ± 0.3 pmol·mg protein^{-1} with p < 0.05. Although values at 20 min were different between Na^+ and K^+ gradient, previous studies in

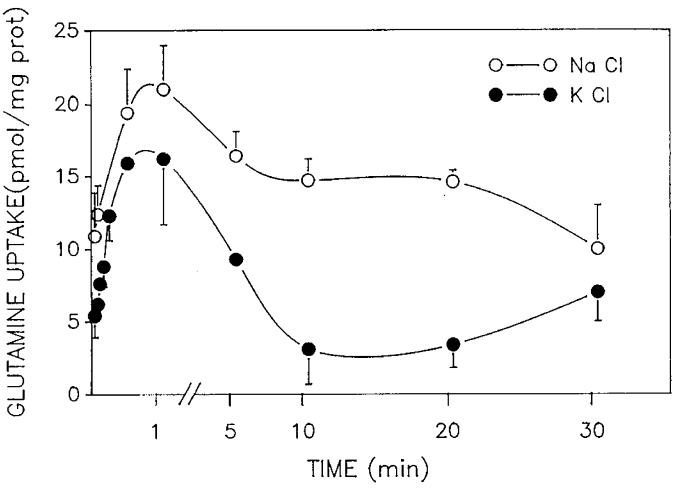

Fig. 3. Effect of Na^+ and K^+ gradient on glutamine uptake. BBMV were prepared from weanling rats and preincubated in 300 mM mannitol and 20 mM HEPES/Tris buffer, pH 7.4. The reaction was started by the addition of 20 μL membrane vesicles to either 100 mM NaCl, 100 mM mannitol, and 20 mM HEPES/Tris buffer, pH 7.4 or 100 mM KCl, 100 mM mannitol, and 20 mM HEPES/Tris buffer, pH 7.4. Glutamine concentration was 0.02 mM. Reaction was stopped at variable points of time as shown. The experiments were done in triplicate and the data represent the mean ± SE.

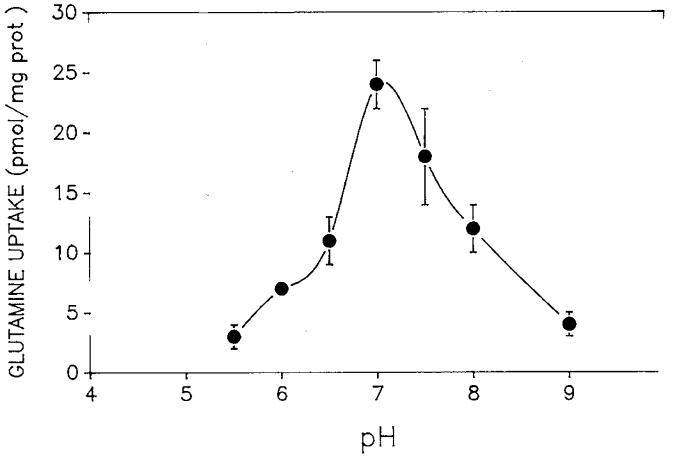

Fig. 4. Effect of pH on glutamine uptake. BBMV were prepared in 300 mM mannitol and 20 mM HEPES/Tris buffer, pH 7.4. The incubation media consisted of 100 mM NaCl, 100 mM mannitol, and 20 mM HEPES/Tris buffer, pH 5.5–9, in addition to 0.02 mM and 0.5 μCi ^3H glutamine. Reaction was started by the addition of 20 μL membrane vesicles to each incubation medium and the reaction was stopped at 15 s. The experiments were done in triplicate and the data represents the mean ± SE.

our laboratory indicated similar vesicle size in membranes of suckling and adult rats using mannitol uptake studies (9). To study the effect of an outwardly directed K^+ gradient on glutamine uptake, vesicles were loaded with KCl solution of various concentrations (100, 50, 0 mM) and glutamine uptake was measured under an inwardly directed Na^+ gradient. Uptake values were 11.6 ± 2, 11.3 ± 2 and 10 ± 2 pmol·mg protein^{-1}·10^{-1} respectively ($p > 0.05$).

pH optimum of glutamine uptake. Figure 4 shows glutamine uptake in weanling rats at different incubation media pH that varied from 5.5 to 9. Optimal uptake was noted at a pH of 7.0 with severe inhibition at 5.5 and 9.0.

Effect of membrane potential on glutamine uptake. To demonstrate the electrical potential dependence of sodium-coupled glutamine transport, two studies were conducted. In the first study, valinomycin, a potassium selective ionophore that mediates the electrogenic movement of K^+ down its concentration gradient, was used to create an electrochemical potential across the membrane (11–16). In Figure 5, with increased interior negativity (K^+ inside > K^+ outside in the presence of valinomycin), there was a significant enhancement of Na^+-coupled glutamine uptake compared to voltage clamp condition (K inside = K outside + valinomycin).

The second study was an anion substitution study. Sodium thiocyanate is a highly permeable anion compared to sodium sulfate with sodium chloride being intermediate (12, 14). A faster influx of thiocyanate into the BBMV creates a negatively charged inside with more uptake of glutamine compared with the sodium sulfate and sodium chloride. This study showed that glutamine uptake was 46 ± 3 pmol·mg protein^{-1}·30 s^{-1} with sodium thiocyanate compared with 24 ± 3 pmol·mg protein^{-1}·30 s^{-1} with sodium sulfate. Both of these studies are strong indicators that Na^+-coupled glutamine transport is rheogenic in nature. A similar study with K^+ gradient showed no difference in glutamine uptake with different anions indicating that the Na^+ independent uptake of glutamine is electroneutral.

Kinetics of glutamine uptake. Kinetic experiments were carried out under initial rate conditions with a substrate concentration that ranged from 0.2–10 mM. Kinetic parameters were analyzed using a computerized model of Michaelis-Menten kinetics (17). In suckling and weanling rats, a saturable process was observed in the presence and absence of sodium gradient. This is in agreement with our previous findings in adult rats where a saturable process was observed under both Na^+ gradient and K^+ gradient conditions. However, as shown in Figure 6 for the Na^+-dependent system, the V_{max} in suckling rats was significantly greater compared to corresponding mean values in weanling rats, 4.9 ± 0.36 *versus* 2.4 ± 0.2 nmol·mg protein^{-1}·7 s^{-1} ($p < 0.01$), whereas the Km being 3.84 ± 0.6 mM in suckling rats *versus* 2.7 ± 0.6 mM in weanling rats. Furthermore, our previous studies with adult rats (18) showed a lower V_{max} of 0.70 nmol·mg protein^{-1}·s^{-1} while the Km was similar at 3.5 mM for the Na^+-dependent system. Figure 7 depicts the Na^+-independent system in suckling and weanling rats. The V_{max} in suckling rats was

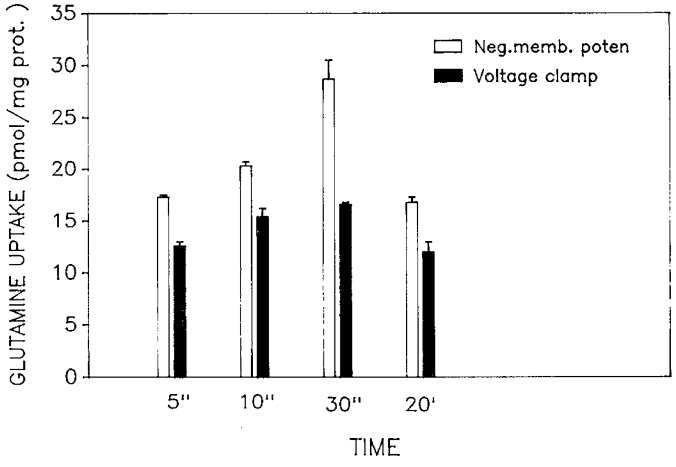

Fig. 5. Effect of membrane potential on glutamine uptake. BBMV were preincubated in 50 mM KCl, 200 mM mannitol, and 20 mM HEPES/Tris buffer, pH 7.4. BBMV were then incubated in a media containing either 100 mM NaCl, 50 mM KCl, 20 mM HEPES/Tris buffer, pH 7, 0.5 μCi ^3H glutamine, 0.02 mM glutamine, and valinomycin 10 μg/mg protein (voltage clamp) or in a media containing 100 mM NaCl, 10 mM KCl, 20 mM HEPES, pH 7.0, 0.5 μCi ^3H glutamine, 0.02 mM glutamine, and valinomycin (negative membrane potential). Reaction was started by the addition of 20 μL membrane vesicles to each incubation medium and the reaction was stopped at the time intervals shown above. The experiments were done in triplicate and the data represent the mean ± SE.

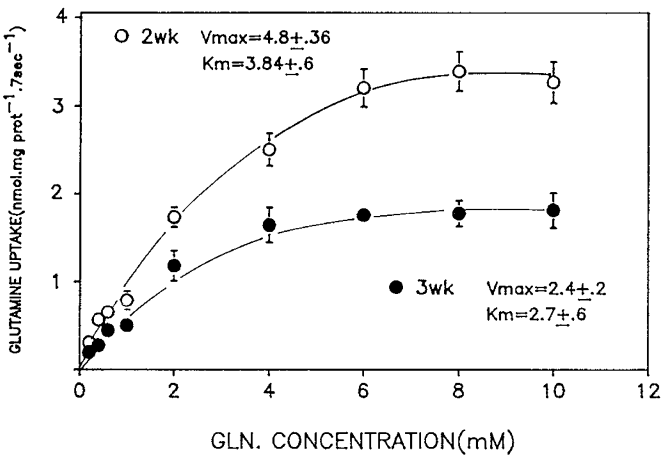

Fig. 6. Kinetics of the Na^+ dependent system in 2- and 3-wk old rats. BBMV were preincubated in 300 mM mannitol and 20 mM HEPES/Tris buffer, pH 7.4. The incubation media consisted of 100 mM mannitol, 100 mM NaCl, and 20 mM HEPES/Tris buffer, pH 7.4. In addition to 0.5 μCi ^3H glutamine, the media contained different glutamine concentrations that ranged from 0.1–10 mM. The reaction was started by the addition of 20 μL membrane vesicles to the different incubation media and the reaction was stopped at 7 s. The experiments were done in triplicate and the data represent the mean ± SE.

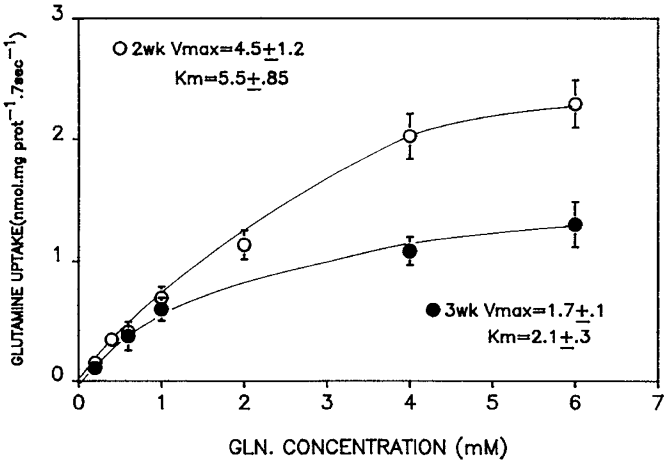

Fig. 7. Kinetics of the Na^+-independent System in 2- and 3-wk old rats. BBMV were preincubated in 300 mM mannitol and 20 mM HEPES/Tris buffer, pH 7.4. The incubation media consisted of 100 mM mannitol, 100 mM KCl, and 20 mM HEPES/Tris buffer, pH 7.4. In addition to 0.5 μCi ^3H glutamine, the media contained different glutamine concentrations that ranged from 0.1–6 mM. The reaction was started by the addition of 20 μL membrane vesicles to the different incubation media and the reaction was stopped at 7 s. The experiments were done in triplicate and the data represent the mean ± SE.

significantly greater compared with corresponding values in weanling rats, 4.5 ± 0.1 *versus* 1.7 ± 0.1 nmoles·mg protein^{-1}·7 s^{-1} ($p < 0.001$) whereas the Km was 5.5 ± 0.85 *versus* 2.1 ± 0.3, respectively. Similarly, adult rat studies showed a lower V_{max} of 0.281 nmol·mg protein^{-1}·7 s^{-1}, whereas the Km was similar at 3.34 mM for the Na^+-independent system (18). When the kinetic parameters were subjected to analysis using the values representing the differences between uptake in the presence of sodium gradient minus the sodium-independent values, the V_{max} in the suckling rat was still significantly greater, 1.59 ± 0.3 compared to corresponding values in weanling rats of 0.76 ± 0.1 nmol·mg protein^{-1}·7 s^{-1} ($p < 0.01$). Km values were not different at 2.5 ± 0.6 and 3.5 ± 0.6 mM, respectively.

Effect of neutral amino acids on glutamine uptake. Table 1 shows that most neutral amino acids, especially asparagine and histidine, inhibited ^3H glutamine uptake. In contrast, 2-(methyl amino)isobuturic acid, β-alanine, BCH, methionine, and phenylalanine showed minimal inhibition of glutamine uptake in adults, suckling, and weanling rats. This pattern is suggestive of system NBB and not system A (19, 20).

Table 2 similarly shows that several neutral amino acids inhibited glutamine uptake. The degree of inhibition was greater with leucine and BCH. This pattern is suggestive of an L-like system (21) and was similar in all age groups.

Effect of lithium substitution on glutamine uptake. In this study, glutamine uptake was determined under inwardly directed Na^+, lithium, and choline gradient. As shown in Figure 8, the uptake of glutamine was significantly lower with lithium and choline in adult, weanling, and suckling rats.

DISCUSSION

The present studies were designed to characterize the maturational changes in glutamine transport by intestinal BBMV in suckling and weanling rats.

The enterocyte of the jejunum receives large amounts of glutamine from two sources: the luminal side and the basolateral side. Both sources utilize the same enzyme within the enterocytes. Windmueller *et al.* (22) described the regulation of glutamine metabolism by the enterocytes by both sources of glutamine. At luminal concentration of 6 mM in the luminal side, glutamine uptake and metabolism from the arterial side was reduced by 40%. Therefore, both sources of glutamine act as a metabolic unit for the jejunum. More recently, Taylor *et al.* (23) presented data to suggest that glutamine transport by the basolateral membranes is not affected by the presence of glutamine in the lumen, arguing against the concept of a common pool of glutamine from luminal and basolateral sources. Intestinal energy production is required to support a number of functions: rapid cellular growth, differentiation, cell migration, and absorptive processes. Active glutamine metabolism has been correlated with rapid cellular growth. For example, activity of glutaminase increases 3-fold during log phase growth in human diploid fibroblasts compared to confluent cultures (24). Moreover, glutaminase activity doubles between d 12 and 15 after birth (25). This observation correlates with that period of intestinal development when there are marked increases in proliferation (1). Therefore, the current studies were designed to investigate and characterize glutamine transport in suckling and weanling rats.

The uptake of a solute by an isolated membrane fraction can be composed of transport into an intravesicular compartment and/or binding to the membrane surface (26). Two possible ways to distinguish between these two components of uptake are osmotic sensitivity and temperature dependency. The osmolarity study based on the fact that the amount of glutamine transported into the intravesicular space should be, under equilibrium conditions, in direct relation to the intravesicular volume. With decreasing the intravesicular volume, the uptake of glutamine should decrease proportionately to the decrease in the vesicular space. The equation depicting the relationship between uptake and 1/osmolarity suggests clearly that the uptake of glutamine by the BBMV represents a transport into the intravesicular space rather than binding into the membrane surface. These findings were substantiated by studies of temperature dependency showing a significant inhibition of uptake at 0°C compared with 25°C, which indicates the presence of a carrier-mediated transport system (27). Similar findings were obtained with adult rats (18).

The imposition of inwardly directed sodium and potassium gradients stimulated glutamine uptake with "overshoot" phenomena under both conditions in suckling and weanling rats alike. However, the magnitude of the enhancement of uptake was greater with sodium gradient compared with potassium gradient in weanling rats, while it was the same in suckling rats. In addition, in suckling rats the peak of "overshoot" under both

Table 1. *Percent of inhibition of ^3H Gln uptake by various amino acids with inwardly directed Na^+ gradient**

		Amino acid													
Rats	Control	Gln	Gly	Met	Phe	MeAIB	β-Ala	Asn	His	Leu	Val	BCH	Ser	Cys	Systems
Adult	100	68 ± 6	16 ± 2	0	8 ± 0.4	0	5 ± 0.5	49 ± 3	79 ± 6	29 ± 1	50 ± 4	3 ± 0.4	34 ± 2	28 ± 2	NBB
3-wk-old	100	61 ± 6	13 ± 5	3.6 ± 1	4 ± 2	0	2.5 ± 0.1	44 ± 2	40 ± 3	0	24 ± 3	10 ± 1	26 ± 2	31 ± 3	NBB
2-wk-old	100	69 ± 4	26 ± 1	0	10 ± 0.4	0	2.6 ± 0.2	40 ± 2	30 ± 1	2.6 ± 0.4	13 ± 1	26 ± 2	32 ± 2	26 ± 3	NBB

* BBMV from suckling, weanling, and adult rats were preincubated in 300 mM mannitol and 20 mM HEPES/Tris, pH 7.4. The incubation media consisted of 100 mM NaCl, 100 mM mannitol, and 20 mM HEPES/Tris, pH 7.4, in addition to 2 mM glutamine, 0.5 μCi, ^3H glutamine, and various amino acids at a concentration of 20 mM. The reaction was started by the addition of 20 μL vesicles to the incubation media and the reaction was stopped at 60 s. The experiments were done in triplicate and the data represents the mean ± SE. MeAIB, 2-(methyl amino)isobuturic acid.

Table 2. *Percent of inhibition of ^3H Gln uptake by various amino acids with inwardly directed K^+ gradient**

		Amino acid													
Rats	Control	Gln	Gly	Met	Phe	MeAIB	β-Ala	Asn	His	Leu	Val	BCH	Ser	Cys	Systems
Adult	100	67 ± 3	5 ± 0.5	0	38 ± 3	0	16 ± 2	10 ± 1	23 ± 2	61 ± 6	0	68 ± 7	23 ± 2	3 ± 0.3	L
3-wk-old	100	73 ± 6	15 ± 2	1.5 ± 0.2	22 ± 0.5	9.5 ± 1	15 ± 1	11 ± 0.2	17 ± 1	76 ± 5	42 ± 2	34 ± 2	30 ± 2	36 ± 3	L
2-wk-old	100	72 ± 5	35 ± 3	0	23 ± 1	18 ± 2	14 ± 0.8	7 ± 0.6	4.6 ± 0.4	81 ± 6	27 ± 2	72 ± 4	27 ± 3	25 ± 2	L

* BBMV from suckling, weanling, and adult rats were preincubated in 300 mM mannitol and 20 mM HEPES/Tris, pH 7.4. The incubation media consisted of 100 mM KCl, 100 mM mannitol, and 20 mM HEPES/Tris, pH 7.4, in addition to 2 mM glutamine, 0.5 μCi, ^3H glutamine, and various amino acids at a concentration of 20 mM. The reaction was started by the addition of 20 μL vesicles to the incubation media and the reaction was stopped at 60 s. The experiments were done in triplicate and the data represents the mean ± SE. MeAIB, 2-(methyl amino)isobuturic acid.

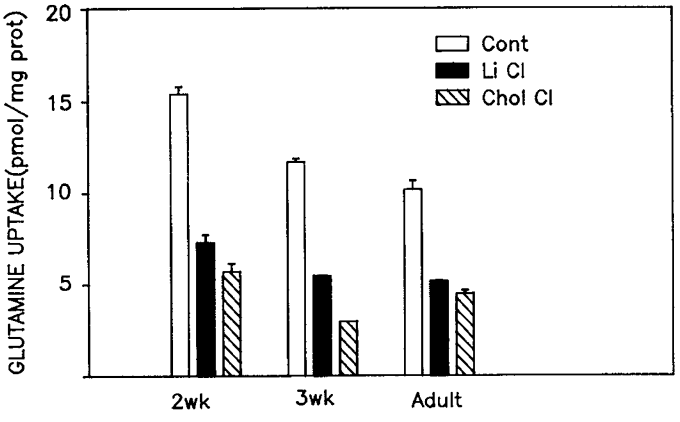

Fig. 8. BBMV from suckling, weanling, and adult rats were preincubated in 300 mM mannitol and 20 mM HEPES/Tris buffer, pH 7.4. The incubation media consisted of 100 mM of either NaCl, LiCl or choline chloride in addition to 0.02 mM glutamine and 0.5 μCi ^3H glutamine. The reaction was started by the addition of 20 μL vesicles to the variable incubation media and the reaction was stopped at 1 min. The experiments were done in triplicate and the data represent the mean ± SE.

Na^+ and K^+ gradient was significantly greater compared with values in weanling rats 68 ± 8 *versus* 21 ± 0.3 pmol·mg protein^{-1}. These results differ from our previous findings in adult rats, which showed a distinct enhancement of uptake only with Na^+ gradient and not with K^+ gradient.

These findings suggest that glutamine uptake by suckling and weanling BBMV is both sodium dependent and sodium independent. Both processes exhibit concentrative overshooting phenomena indicating an active, energy-dependent process. The kinetic properties of both processes exhibit ontogenic changes, with the V_{max} of both sodium-dependent and -independent processes being greater in suckling rats compared with weanling. The sodium-dependent process is electrogenic and pH dependent with maximum uptake at pH 7.0, while the sodium-independent is electroneutral. Moreover, the rate of glutamine uptake in suckling rats is significantly greater compared with membranes in weanling rats. The finding of a greater V_{max} in the suckling rats suggest that the capacity and/or the activity of the transport carrier is greater compared with weanling and adult rats. These ontogenic changes may be related to greater demands during the active period of growth in the suckling period. These observations are supported by the finding of Kimura (28), which showed greater glutamine oxidation by jejunal slices of suckling rats compared to weanling. Although Kimura used jejunal slices that reflect events at both brush border and basolateral membranes, our unpublished observation suggests that the maturational events at the basolateral membranes mirrors that of the brush border membranes.

As shown in Table 1, most neutral amino acids, especially asparagine and histidine, inhibited ^3H glutamine uptake and showed minimal interaction with BCH, a synthetic amino acid transported in a Na^+-independent fashion. This pattern of inhibition is suggestive of the Na^+-dependent NBB system as described in rabbit jejunal BBMV by Stevens *et al.* (21) and in dog jejunal BBMV as described by Bulus *et al.* (29). 2-(Methyl amino)isobuturic acid, the typical substrate for system A, which is well-characterized in other plasma cells, showed no inhibition. Lack or minimal inhibition by phenylalanine and methionine, the typical substrates of system PHE, indicates that glutamine is not transported via this Na^+-dependent system either. The pattern of inhibition is similar in adult, weanling, and suckling rats. As shown in Figure 8, lithium failed to substitute for Na^+ in the three age groups, suggesting absolute specificity for Na^+, unlike system N in other plasma cells, where lithium could substitute for Na^+.

Although system L, in its original description in Ehrlich ascites tumor cells, is believed to be stereospecific for leucine, our study as well as others (21) showed that this system transports several neutral amino acids in intestinal BBMV. However, the degree of inhibition was larger with leucine and BCH. This pattern is suggestive of the presence of an L-like system as described in intestinal BBMV in rabbits (21) and dogs (29).

We conclude that glutamine transport by BBMV undergoes maturational changes consistent with the existence of two different glutamine transporters that have greater capacity in younger groups. The first system is Na^+-dependent NBB, which transports most neutral amino acids, especially asparagine and histidine. This system is pH sensitive and lithium intolerant. The second

system is Na^+-independent L-like system, which transports several other neutral amino acids, especially leucine and BCH.

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