THE INVOLVEMENT OF SEVERAL systems in mediated intestinal transport of amino acids was demonstrated by experiments with sacs of everted small intestine, primarily from the golden hamster (37). Evidence was also presented suggesting active transport of L-glutamate and L-aspartate, but the rapid transamination of these amino acids made the evidence inconclusive (40). Sodium-dependent transport of L-glutamate and L-aspartate was first described for rabbit jejunal BBMV, leading to estimates of affinity constant ($K_i$) values of 7 and 5 mM for L-glutamate and L-aspartate, respectively, for influx across the brush-border membrane (BBM) (32). L-Glutamate uptake by segments of the chicken small intestine was described in terms of one high-affinity process and a large, nonsaturable contribution (12), while uptake by isolated chicken enterocytes was described as the result of two transport processes, one with a high affinity ($K_i = 16 \mu M$) and one with a low affinity ($K_i = 4.1 \mu M$) (38, 39). In BBM vesicles (BBMV) from the rat small intestine, the $K_i$ value of uptake of D-aspartate and L-glutamate was found to be between 1 and 2 mM (5); in addition, L-glutamate uptake appeared to be partly chloride dependent (6). Uptake of L-glutamate by BBMV from the human jejunum measured at concentrations between 0.005 and 0.2 mM corresponded to a $K_i$ of 90 µM (9). More recent studies of D-aspartate and L-glutamate uptake by rabbit jejunal BBMV have clearly demonstrated the presence of a high-affinity carrier ($K_i = 60$ and 80 µM for L-glutamate and D-aspartate, respectively), and, at pH 6.0, a low-affinity carrier ($K_i = 7 \mu M$) for L-glutamate (14). The latter is not accessible to D-aspartate, while data on inhibition of L-glutamate uptake by L-aspartate correspond to an inhibitor constant ($K_i$) of $\sim 75 \mu M$ (14). The characteristics described for the high-affinity transporter correspond to those described for system X_{AG} (3). Transport by the low-affinity system was observed at pH 6.0 but was undetectable at pH 8.0. It could be inhibited by most bipolar amino acids, with alanine and serine being as effective as leucine and methionine. These characteristics correspond to those described for system ASC (36). It was, however, proposed that the low-affinity transport of L-glutamate was served by a protonated form of the transport system variously described as “carrier of neutral amino acids,” system NBB, and, most recently, system B^0. Data on pH-sensitive uptake of glutamate by Xenopus laevis oocytes injected with cDNA from human placenta and rabbit small intestine were interpreted similarly (10, 11).

The aim of the present study was to test the proposal of chloride dependence of L-glutamate transport and to examine whether the characteristics of D-aspartate and L-glutamate transport across the luminal membrane of the intact rabbit intestinal epithelium were similar to those described for rabbit jejunal BBMV (13, 14). Of prime interest were the relative capacities of the two transport systems, the unique capacity of bipolar amino acids to cis-stimulate the vesicle uptake of D-aspartate, the effects of pH on a high-capacity system, and the identity of this system. The detailed study was performed using the distal ileum from the rabbit, which with this technique has the highest transport capacity (25, 26). To allow comparison with the results previously reported for jejunal BBMV (13, 14), we also examined the most important points using sheets of the jejunum.
MATERIALS AND METHODS

Animals and Materials

Female albino rabbits with a body weight of 2,500–3,000 g were raised and maintained with free access to water and food and killed by intravenous phenobarbital. For examination of chloride dependence and sodium activation of L-glutamate transport in rabbit ileum, the solutions were made from a buffer (pH 7.2) composed of (in mM) 140 NaCl, 2.6 CaCl₂, 1 MgCl₂, and 140 Cl⁻ or 140 isethionate, 8 phosphate, and 1 SO₄²⁻. For the sodium activation experiments N-methyl-D-glucamine (NMDG)-HCl was substituted for sodium chloride. For all other experiments, 20 mM MES-Tris (pH 5.65 and pH 7.2) or 20 mM HEPES-Tris (pH 8.2) were used instead of phosphate for buffering pH. The total anion concentration was kept constant within each series of experiments by varying the concentrations of isethionate, D-aspartate, and L-glutamate. D-Glucose (5 mM) was present in all solutions, except when measuring the transport of α-methylglucoside. ¹⁴C- and ³H-labeled chemicals were purchased from NEN.

Unidirectional Influx Across BBM

Unidirectional influx across the BBM (Jₘc) of intact rabbit ileum (0–30 cm from the ileocecal junction) or jejunum (100–130 cm from the ileocecal junction) was measured as previously described (27, 32). The excised intestine was mounted between Lucite plates so that the mucosa was exposed in the bottom of wells with an area of 0.62 cm² in which the solution was oxygenated and stirred by high rates of 100% O₂ flow at 37°C. After preincubation for 20 min, the tissues were incubated for 0.50 min. The incubation was stopped by flushing with ice-cold 300 mM d-mannitol. The tissues were incubated for 18 h in 0.1 mM HNO₃. The extract and the retracted incubation fluid were analyzed in a liquid scintillation counter (Tri-Carb 2200CA, Packard). The content of [³H]polyethylene glycol 4000 or [¹⁴C]activity, in the tissue extract was used to calculate the rate of amino acid influx across the BBM.

Sodium Activation of Glutamate Transport

The estimates of transport kinetics were made by nonlinear least squares fitting to this model of the experimentally determined relationship between Jₘc of A and [A]ₘ weighted by the inverse of the standard deviation (SD). The estimates were evaluated by the even distribution of residuals and by calculation of the χ² value with degrees of freedom (df) being the number of experimental points minus the number of parameters estimated. The errors of these estimates are 1 SD. The Kᵢ values were calculated from the ratios between inhibited and uninhibited fluxes, assuming that these were described by the sum of a saturable process conforming to Michaelis-Menten kinetics and diffusion. Errors on fluxes and estimates of Kᵢ are standard errors with the number of observations in parentheses. All influx results are pooled data from at least two rabbits.

RESULTS

Chloride and Sodium Dependence of Glutamate Transport

Chloride dependence of transport of anionic amino acids could complicate measurements of transport at high concentrations unless parallel increases in total osmotic activities were accepted. Consequently, L-glutamate influx was measured at 20 µM in rabbit ileum. Unidirectional influx across the BBM of L-glutamate measured at 140 mM NaCl was 91.0 ± 11.2 nmol·cm⁻²·h⁻¹ (means ± SE of n = 10), 78.5 ± 10.4 nmol·cm⁻²·h⁻¹ at 140 mM sodium isethionate, and 0.9 ± 0.2 nmol·cm⁻²·h⁻¹ when sodium was substituted with NMDG. L-glutamate measured at 1.0 mM L-glutamate at 140 mM NaCl and 140 mM sodium isethionate in rabbit ileum was 1.15 ± 0.08 and 1.14 ± 0.06 µmol·cm⁻²·h⁻¹, respectively (n = 8). The chloride independence of L-glutamate influx allowed the use of isethionate instead of chloride, and, whenever L-glutamate or D-aspartate were used in concentrations above 10 mM, to reduce the concentration of isethionate to keep the sum of the concentrations of isethionate and L-glutamate/D-aspartate constant. The sodium-independent rate of transport corresponds to a permeability of 0.045 cm/h, which is well within the range of our estimates of diffusive contributions to influx of amino acids (19, 21, 22). Thus a separate sodium-independent transport of anionic amino acids is not present in the BBM of rabbit small intestine.

Sodium Activation of Glutamate Transport

The influx of L-glutamate was measured at 20 µM glutamate at pH 7.2 at concentrations of sodium between 0 and 140 mM with preincubations and test incubations made at the same concentrations of sodium (Fig. 1). Analyzed in terms of Eq. 2a, the results are best (χ² = 2.896; df = 5) described by

\[ J_{\text{L-Glu}} = \left( \frac{[\text{Na}^+]^{nH}}{[\text{Na}^+]^{nH} + [\text{Na}^+]^{nH}} \right) + 0.66 \text{nmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1} \]  

(3)

where nH = 1.73 ± 0.25, indicating a sodium: L-glutamate stoichiometry of 2:1. As demonstrated below L-glutamate transport at 20 µM is almost exclu-
Effects of pH on transport of anionic amino acids across rabbit ileal BBM

Otherwise undetectable contamination by basolateral the low-affinity system, it might have represented an maximum rate of transport of only 1% or less of that of capacity of the high-affinity transport (14). With a comparison with function of BBMV agree with those obtained with BBMV (14).

The Concept of Two Transporters

To briefly test the concept of two transporters of anionic amino acids, we performed paired measurements of influx of 50 mM D-aspartate and 50 mM L-glutamate at pH 5.65 and 7.2, assuming that this concentration would provide for high degrees of saturation. At pH 5.65 influx of L-glutamate and D-aspartate was 9.67 ± 0.98 and 2.08 ± 0.29 µmol·cm⁻²·h⁻¹, respectively (n = 4 each). At pH 7.2 influx of L-glutamate and D-aspartate was 9.61 ± 0.52 and 2.40 ± 0.34 µmol·cm⁻²·h⁻¹, respectively (n = 4 each). Influx of L-glutamate was about four times that of D-aspartate at both pH values consistent with the presence of two pathways of transport, of which only one is accessible to both D-aspartate and L-glutamate (14). Consequently, at pH 7.2 paired measurements were made at 1 mM L-glutamate alone and with 50 mM D-aspartate or 50 mM L-glutamate as inhibitors (n = 6). The rate of transport was 1.03 ± 0.08 µmol·cm⁻²·h⁻¹ at 1 mM L-glutamate, which was reduced to 0.14 ± 0.01 µmol·cm⁻²·h⁻¹ when inhibited by 50 mM L-glutamate and to 0.17 ± 0.03 µmol·cm⁻²·h⁻¹ in the presence of 50 mM D-aspartate. These results agree with those on BBMV (14) indicating that the transporter of D-aspartate is shared with L-glutamate with very similar affinities. Furthermore, at 1 mM L-glutamate this common transporter appears to be responsible for >80% of the total transport.

Effects of pH on Transport Across BBM

The possibility of an effect of pH on transport of anionic amino acids was examined by using the ability of amiloride at 1 mM to inhibit the Na⁺/H⁺ exchanger and thereby increase pH of the outside microclimate of the luminal membrane (15). Paired measurements of influx were made at 50 mM L-glutamate or 1 mM D-aspartate at pH 7.2 and 140 mM sodium with or without 1 mM amiloride, with amiloride present in both preincubation and test incubation (Table 1). A second experiment was performed using the same procedure, but reducing the sodium concentration to 70 mM sodium to increase the efficiency of amiloride. Paired measurements were made of D-aspartate at 0.1 mM D-aspartate and of L-glutamate at 0.1 mM L-glutamate plus 70 mM D-aspartate at pH 7.2 or at pH 7.2 plus 1 mM amiloride (Table 1). Confirming the

Comparison With Function of BBMV

Our attention was especially drawn to the very low capacity of the high-affinity transport (14). With a maximum rate of transport of only 1% or less of that of the low-affinity system, it might have represented an otherwise undetectable contamination by basolateral membrane vesicles. The cis-effect of bipolar amino acids on vesicle uptake of D-aspartate was also intriguing, partly because of its low specificity and partly because it was related to the effect of leucine and methionine on intestinal transport of lysine, an effect which at least in the case of transport across the BBM most likely represents a trans-effect made possible by the parallel functions of systems B and b (4, 22). Finally, although lowering the pH is known to stimulate transport of anionic amino acids in several cellular systems (34, 36), reducing the pH had previously been seen to reduce intestinal transport of bipolar amino acids (7). A series of experiments was performed to provide guidance for the further study of these aspects of intestinal function.

![Image](http://ajpgi.physiology.org/)
observations on BBMV (14), the results of these measurements (Table 1) demonstrate that the presumed increase of pH reduced influx of L-glutamate without affecting influx of \( \alpha \)-aspartate. The relative effects at 0.1 and 50 mM L-glutamate suggested that the effect of decreasing proton concentration might be to decrease the affinity of L-glutamate for the transporter not shared with \( \alpha \)-aspartate. This suggestion was further tested by paired measurements of influx of \( \alpha \)-aspartate and L-glutamate both at 50 mM. Influx of L-glutamate at pH 7.2 was 8.13 ± 0.22 \( \mu \)mol·cm\(^{-2}\)·h\(^{-1} \) (n = 8), which was reduced to 4.24 ± 0.35 \( \mu \)mol·cm\(^{-2}\)·h\(^{-1} \) (n = 8) at pH 8.2, whereas \( \alpha \)-aspartate influx at pH 8.2 was 2.85 ± 0.29 \( \mu \)mol·cm\(^{-2}\)·h\(^{-1} \) (n = 8). It is seen that, although greatly reduced at pH 8.2, the influx of L-glutamate remains significantly higher than that of \( \alpha \)-aspartate, indicating persistent function of the glutamate transporter at pH 8.2.

High concentrations of glutamate might by partial dissipation of the electrochemical gradient of sodium have unspecific effects. Also, the effects of amiloride and the effects of changing pH might be unspecific. These possibilities were examined in two series of paired measurements. One in which, at 140 mM sodium and 1 mM serine, influx of serine was measured with and without the presence of 70 mM L-glutamate at pH 5.65, 7.2, 8.2, and 8.2 with 1 mM amiloride (Table 2). The same protocol was used in the second series in which 1 mM \( \alpha \)-methyl-\( \alpha \)-glucoside was used as substrate instead of serine and the influx of this sugar was measured (Table 2). It can be seen (Table 2) that 70 mM L-glutamate significantly inhibits transport of serine at pH 5.65 and 7.2 but not at pH 8.2. At pH 5.65 influx of serine is much reduced compared with pH 7.2, while the glutamate-sensitive contribution is increased by 67%. Also, the influx of \( \alpha \)-methyl-\( \alpha \)-glucoside is reduced at pH 5.65. However, at all the pH values tested, its transport is unaffected by L-glutamate. Amiloride did not significantly affect the transport of serine or \( \alpha \)-methyl-\( \alpha \)-glucoside.

This series of experiments has provided data consistent with the presence of two transporters of anionic amino acids of which only one is accessible to \( \alpha \)-aspartate. They demonstrate pH sensitivity of the exclusive glutamate transporter and suggest an effect of increasing affinity with increasing proton concentration. This suggestion gained considerable support from the relatively high efficiency of glutamate as inhibitor of serine transport at pH 5.65. Unspecific effects of glutamate or amiloride were not seen.

### Specificity of Bipolar Amino Acids as Inhibitors of Glutamate Transport

If the inhibitory effects of bipolar amino acids on transport of L-glutamate were caused by competition for system B, then their \( K_{i} \) values against L-glutamate influx should be identical to their \( K_{i} \) values for system B. Therefore, paired measurements of influx of L-glutamate were made at pH 7.2, 140 mM sodium, and 1 mM L-glutamate alone or in the presence of 10 mM leucine, phenylalanine, or serine. These amino acids were chosen to cover a relatively wide range of \( K_{i} \); 1 mM for leucine (19), 4 mM for phenylalanine (21), and 25 mM for serine (31). Influx of L-glutamate was 1.32 ± 0.09 \( \mu \)mol·cm\(^{-2}\)·h\(^{-1} \) in the absence of inhibitor and 1.09 ± 0.03, 0.92 ± 0.04, and 0.91 ± 0.08 \( \mu \)mol·cm\(^{-2}\)·h\(^{-1} \) in the presence of 10 mM leucine, phenylalanine, and serine, respectively (means ± SE of 6 paired observations). All three inhibitors reduced influx of L-glutamate to a level corresponding to transport by the transporter shared with \( \alpha \)-aspartate. The inhibition of transport by the glutamate transporter must, therefore, be almost complete and the \( K_{i} \) values for all three bipolar amino acids must be well below 10 mM.

### A Quest for Cis-Stimulation of \( \alpha \)-Aspartate Influx by Bipolar Amino Acids

At 0.05 mM \( \alpha \)-aspartate, cis-stimulation of \( \alpha \)-aspartate uptake by jejunal BBMV was demonstrated, using several bipolar amino acids in concentrations between 5 and 50 mM (13, 14). Here, influx of \( \alpha \)-aspartate in the distal ileum and distal jejunum was measured at 140 mM sodium at either 0.05 or 0.10 mM \( \alpha \)-aspartate and at pH 5.65 or 7.2 using various bipolar amino acids as inhibitors (Table 3). In these experiments, neither cis-stimulation nor cis-inhibition was observed. Recent studies (4, 22) have indicated that recycling of bipolar amino acids between the cytoplasm and the outside unstirred water layer by sodium-coupled uptake by system B and exit in exchange with lysine by system \( b^{+} \) is responsible for the apparent sodium dependence of lysine influx and for the apparent cis-stimulation of lysine influx by leucine and methionine (22). Similarly, cis-stimulation by bipolar amino acids present in the unstirred layer might be at hand already in our control situation, masking any effect of additional exogenous amino acid. Precubination at 0 mM sodium will reduce reuptake from the outside unstirred water layer of amino acids leaking from the enterocytes, reducing the cytoplasmic pool as source for the outside concentration of endogenous amino acids during the subsequent period of incubation. Consequently, to examine the possibility that processes of either cis- or trans-stimulation were at hand already in our control situation, tissue (the ileum) were precubicated at 0 mM.
sodium for at least 20 min. After a brief wash at 140 mM sodium, influx was measured at 140 mM sodium, pH 7.2, and 0.05 mM D-aspartate with 0 or 10 mM valine (Table 3). Once again, cis-stimulation was not demonstrated. Instead, a small inhibitory effect was seen.

The results presented above suggested that decreasing pH caused an increase of the affinity of L-glutamate for the transporter it shares with bipolar amino acids. In addition, the relative efficiencies of leucine, phenylalanine, and serine as inhibitors of L-glutamate transport questioned the identity of this common transporter as system B. These questions were subsequently examined.

Kinetics of D-Aspartate and L-Glutamate Transport

Kinetics at pH 7.2. D-Aspartate influx was measured at 140 mM sodium and at concentrations of D-aspartate between 0.05 and 50 mM. The results (Fig. 2) are best ($\chi^2 = 1.284; df = 6$) described as

$$[(2.04 \pm 0.33) \cdot [\text{D-Asp}]/([0.28 \pm 0.07] + [\text{D-Asp}])]$$

$$+ (0.017 \pm 0.006) \cdot [\text{D-Asp}] \, \text{mmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$$

D-Aspartate influx was then measured at 0.05 mM D-aspartate in the presence of 0–10 mM L-glutamate. The results (Fig. 3A) correspond to a $K_i$ of glutamate of 0.34 ± 0.04 mM.

L-Glutamate influx was now measured at 140 mM sodium and concentrations of glutamate between 0.07 and 140 mM. The results of these experiments (Fig. 4) are best ($\chi^2 = 0.267; df = 6$) described as the sum of two saturable processes and diffusion

$$[(0.91 \pm 0.12) \cdot [\text{L-Glu}]/([0.24 \pm 0.12] + [\text{L-Glu}])$$

$$+ [(11.00 \pm 2.35) \cdot [\text{L-Glu}]/(53.8 \pm 16.5) + [\text{L-Glu}])]$$

$$+ (0.033 \pm 0.001) \cdot [\text{L-Glu}] \, \text{mmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$$

Kinetics at pH 5.65. In agreement with the observed identity of $K_i$ and maximum rate of uptake of D-aspartate by BBMV at pH 6 and 8 (14), our data (Table 1) on D-aspartate influx at pH 8.2, 7.2, and 5.65 did not indicate any pH effect on this function of the intact epithelium. The kinetics of D-aspartate transport were, therefore, not examined at pH 5.65. L-Glutamate influx was measured at 140 mM sodium and at 0.1–140 mM L-glutamate. Assuming a passive permeability to L-glutamate of 0.03 cm$^2$/h, the results (Fig. 5) are best ($\chi^2 = 1.862; df = 8$) described as

$$[(1.08 \pm 0.72) \cdot [\text{L-Glu}]/([0.63 \pm 0.78] + [\text{L-Glu}])$$

$$+ [(7.41 \pm 0.57) \cdot [\text{L-Glu}]/(22.02 \pm 7.09)$$

$$+ [\text{L-Glu}])] + 0.033 \cdot [\text{L-Glu}] \, \text{mmol} \cdot \text{cm}^{-2} \cdot \text{h}^{-1}$$

These results confirm that transport of D-aspartate is restricted to one pH-independent, high-affinity transporter, which is shared with L-glutamate (14). They demonstrate that the vanishing of the low-affinity transport of L-glutamate at increasing pH reflects a decrease in affinity alone and that relative to the capacity of the low-affinity transporter that of the high-affinity system is more than ten times as high as determined for BBMV.

Kinetics of Interactions Between L-Glutamate, Leucine, and Serine

To restrict transport of L-glutamate to the low-affinity transporter, we measured influx at 0.1 mM L-glutamate in the presence of 70 mM D-aspartate. Because of the need to use up to 200 mM of glutamate

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Table 3. A quest for cis-stimulation of D-Asp influx by bipolar amino acids in rabbit distal ileum

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>D-Asp, mM</th>
<th>Ileum</th>
<th>Jejunum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intracellular (nmol/cm$^2$/h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 5.65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mM</td>
<td>0.10</td>
<td>77 ± 5 (8)</td>
<td>67 ± 11 (5)</td>
</tr>
<tr>
<td>5 mM</td>
<td>0.10</td>
<td>70 ± 8 (8)</td>
<td>70 ± 11 (5)</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.10</td>
<td>76 ± 5 (8)</td>
<td>70 ± 11 (5)</td>
</tr>
<tr>
<td>Phe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mM</td>
<td>0.10</td>
<td>67 ± 11 (4)</td>
<td>73 ± 11 (4)</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.10</td>
<td>121 ± 6 (6)</td>
<td>118 ± 16 (6)</td>
</tr>
<tr>
<td>20 mM</td>
<td>0.10</td>
<td>107 ± 15 (6)</td>
<td>107 ± 15 (6)</td>
</tr>
<tr>
<td>pH 7.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mM</td>
<td>0.10</td>
<td>161 ± 31 (6)</td>
<td>31 (6)</td>
</tr>
<tr>
<td>1 mM</td>
<td></td>
<td>166 ± 32 (6)</td>
<td>32 (6)</td>
</tr>
<tr>
<td>5 mM</td>
<td></td>
<td>147 ± 31 (6)</td>
<td>31 (6)</td>
</tr>
<tr>
<td>10 mM</td>
<td></td>
<td>132 ± 30 (6)</td>
<td>30 (6)</td>
</tr>
<tr>
<td>Val*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mM</td>
<td>0.05</td>
<td>166 ± 6 (12)</td>
<td>6 (12)</td>
</tr>
<tr>
<td>10 mM</td>
<td></td>
<td>142 ± 7 (12)</td>
<td>7 (12)</td>
</tr>
</tbody>
</table>

Values are means ± SE of n paired observations (given in parentheses). Val, valine. Phe, phenylalanine. Ile, isoleucine. *Preincubation in 0 mM Na+, with wash and incubation in 140 mM Na+. |
as inhibitor, we measured influx of serine at 200 mM sodium.

Kinetics at pH 7.2. Paired measurements of influx of L-glutamate were made at 140 mM sodium and 0.1 mM L-glutamate and 70 mM D-aspartate with 0–20 mM of serine or leucine. Assuming a $K_{i}$ of L-glutamate of 54 mM, the results (Fig. 3B) correspond to a $K_{i}$ of 4.9 ± 0.8 and 2.0 ± 0.8 mM for serine and leucine, respectively.

The cited estimate of 25 mM as $K_{i}$ of serine for system B was based on measurements that probably included contributions by systems $b_{0}^{-}$ and $B_{0}^{-}$ (31). To obtain estimates comparable to those cited for leucine and phenylalanine, we measured influx of leucine at 140 mM sodium, 100 mM lysine, 0.5 mM leucine, 0–200 mM serine, and 200–0 mM mannitol. In these measurements, lysine served to exclude leucine from systems $b_{0}^{+}$ and $B_{0}^{+}$, while mannitol served as an osmotic compensation for serine. The results (Fig. 6) do, therefore, describe the effect of serine on system B for which they indicate a $K_{i}$ of 18 ± 2 mM. To the extent that leucine may be transported by the low-affinity transporter of L-glutamate, this number overestimates the affinity of serine for system B.

The efficiency of glutamate as inhibitor of influx of serine was examined in paired measurements at 1 mM serine and 200 mM sodium with 0–200 mM L-glutamate (Fig. 6). Assuming a $K_{i}$ for L-glutamate of 58 ± 10 mM and a glutamate-sensitive transport of 0.8 µmol·cm$^{-2}$·h$^{-1}$, the results correspond to an inhibitor constant ($K_{i}$) for L-glutamate of 58 ± 10 mM and a glutamate-sensitive transport of 0.8 µmol·cm$^{-2}$·h$^{-1}$.

To examine the assumption that the L-glutamate-resistant transport of serine is accomplished by systems B and $b_{0}^{+}$, influx of serine at 1 mM was measured at 200 mM sodium glutamate and pH 7.2 serine and...
0–100 mM leucine. The results (Fig. 7) demonstrate that at 50 and 100 mM leucine, influx of serine is reduced to the level of the usual diffusive contributions. On the basis of this assumption and using 18 mM as the \( K_{1/2} \) for serine, the results correspond to a \( K_i \) for leucine of 1.71 ± 0.14 mM. This value does not differ from our previous estimates of the affinity of leucine for systems B and bo,

Kinetics at pH 5.65. At pH 5.65 only interactions between serine and anionic amino acids were examined. Influx of L-glutamate was measured at 140 mM sodium, 0.1 mM L-glutamate, and 70 mM D-aspartate in the presence of 0–20 mM serine (Fig. 8). Assuming a \( K_{1/2} \) for glutamate of 22 mM, the results correspond to a \( K_i \) for serine of 4.8 ± 0.8 mM.

Influx of serine was measured at 200 mM sodium and 1 mM serine in the presence of 0–200 mM L-glutamate (Fig. 9). Assuming a \( K_{1/2} \) of 4.8 mM for serine and a glutamate-resistant transport of serine of 0.2 \( \mu \)mol·cm\(^{-2} \)·h\(^{-1} \), the results correspond to a \( K_i \) for glutamate of 9.9 ± 1.5 mM and a glutamate-sensitive serine transport of 0.8 \( \mu \)mol·cm\(^{-2} \)·h\(^{-1} \). Thus both the affinity of serine for the glutamate transporter and the magnitude of its glutamate-sensitive transport are independent of pH. In contrast, at pH 5.65 the glutamate-resistant flux of serine is reduced to a level within
the range observed for transport of bipolar amino acids by system B\(^{0+}\), indicating that transport by system B is eliminated at pH 5.65. This interpretation was examined in paired measurements of serine influx at pH 5.65 and 7.2 at 140 or 0 mM sodium. Reducing sodium from 140 to 0 mM reduced serine influx at pH 5.65 from 1.34 ± 0.06 to 0.15 ± 0.01 μmol·cm\(^{-2}\)·h\(^{-1}\) (n = 6) and at pH 7.2 from 2.27 ± 0.18 to 0.31 ± 0.03 μmol·cm\(^{-2}\)·h\(^{-1}\) (n = 6). Thus at both pH values sodium-independent serine influx is close to the glutamate-resistant influx seen at pH 5.65 (see Table 2). This supports the notion that at pH 5.65 transport of serine is restricted to system B\(^{0+}\), the low-affinity transporter of L-glutamate, and diffusion.

The data obtained with rabbit BBMV indicate that at pH 6.0 K\(_i\) of L-aspartate against the low-affinity transport of L-glutamate is ~75 mM. To allow comparison with these results, we measured influx of serine at 1 mM at pH 5.65 and 200 mM sodium with 0–200 mM L-aspartate present. Assuming an L-aspartate-resistant contribution to the transport of serine of 0.2 μmol·cm\(^{-2}\)·h\(^{-1}\), the results correspond to a K\(_i\) of L-aspartate of 79 ± 5 mM and an L-aspartate-sensitive influx of 0.8 μmol·cm\(^{-2}\)·h\(^{-1}\).

**Jejunal Transport of D-Aspartate, L-Glutamate, and Serine**

In the rabbit small intestine, system B\(^{0+}\) is present only in the ileum (25). Influx of other amino acids is characterized by an aborally increasing capacity with constant affinity (23, 25–27). Conceivably, the differences between the present study and those performed with jejunal BBMV (13, 14) might reflect this topographic variation, while the possibility of cis-stimulation was excluded by the data in Table 3. The questions as to the relative capacities of the two transporters of anionic amino acids and the pH effects on transport of glutamate and serine were dealt with in the following two series of experiments.

The pH effect and the question of relative capacities were examined in paired measurements of influx of D-aspartate and L-glutamate at 140 mM sodium and 50 mM of the amino acids at pH 5.65 and 8.2. Influx of L-glutamate was 5.51 ± 0.32 and 3.07 ± 0.33 μmol·cm\(^{-2}\)·h\(^{-1}\) at pH 5.65 and pH 8.2, respectively (n = 6). Influx of D-aspartate was 1.73 ± 0.24 and 1.52 ± 0.40 μmol·cm\(^{-2}\)·h\(^{-1}\) at pH 5.65 and 8.2, respectively (n = 6). It is seen that increasing pH from 5.65 does not affect the influx of D-aspartate, which is significantly exceeded by the influx of L-glutamate at both pH values. At pH 5.65 the difference between the two fluxes is 3.8 μmol·cm\(^{-2}\)·h\(^{-1}\), which assuming identical diffusive permeabilities of D-aspartate and L-glutamate represents the function of the high-capacity system. Assuming a diffusional permeability of 0.03 cm/h, the mediated transport of D-aspartate is 0.7 μmol·cm\(^{-2}\)·h\(^{-1}\), corresponding to ~20% of transport of L-glutamate by its low-affinity transporter.

The pH effect on transport of serine was examined by paired measurements at 200 mM sodium and 5 mM serine with 0 or 200 mM L-glutamate at pH 7.2 and 5.65 (Table 4). Under these conditions influx of serine was increased also in the distal ileum. As with the distal ileum (Tables 4 and 2), at pH 5.65 glutamate reduces the jejunal influx of serine to a level close to that expected for system B\(^{0+}\) together with diffusion (21), and L-glutamate is a more efficient inhibitor at pH 5.65 than at pH 7.2.

**DISCUSSION**

The present study confirmed the observations of anionic amino acid transport by the rabbit small intestine as being served by a high- and a low-affinity system and of proton stimulation of the latter system (13, 14). In contrast, cis-stimulation by bipolar amino acids of the high-affinity system could not be confirmed, and while the ratio between the capacities of the two systems was ~1:200 with BBMV (14), it was ~1:10 with the intact epithelium. Nor was the specificity of interactions between bipolar amino acids and the low-affinity transport consistent with system B being responsible for this function.

**The High-Affinity System**

Consistent with the observations made with rabbit BBMV (14), a component of the passage of D-aspartate and L-glutamate across the BBM of the intact epithelium is characterized by equally high affinities, insensitivity to changes in pH between 5.65 and 8.2, and to inhibition by bipolar amino acids, by an activation by sodium indicating a sodium:L-glutamate stoichiometry of 2:1, and by independence of the presence of chloride. The difference by a factor of 3–5 between the present estimates of the affinities of the two anionic amino acids and those made with BBMV is similar to the difference between previous kinetic estimates for the high-affinity transport of taurine and β-alanine in rabbit intestine (17, 27), which with respect to affinity and capacity is comparable to the high-affinity transporter of D-aspartate and L-glutamate. Therefore, and because all our kinetic observations on the low-affinity transport agree with those made with BBMV, we would expect a J\(_{\text{max}}\) for D-aspartate between 0.1 and 0.2 μmol·cm\(^{-2}\)·h\(^{-1}\) and that the model proposed to account for cis-stimulation by bipolar amino acids of D-aspartate uptake by BBMV would apply to its passage across the BBM of the intact epithelium. With a J\(_{\text{max}}\) of ~1

<table>
<thead>
<tr>
<th>pH</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.65</td>
<td>(0.170) ± 0.09</td>
<td>(0.740) ± 0.21</td>
</tr>
<tr>
<td>7.2</td>
<td>(0.120) ± 0.12</td>
<td>(0.600) ± 0.05</td>
</tr>
<tr>
<td>5.65</td>
<td>(0.230) ± 0.26</td>
<td>(0.190) ± 0.11</td>
</tr>
<tr>
<td>7.2</td>
<td>(0.430) ± 0.37</td>
<td>(0.280) ± 0.29</td>
</tr>
</tbody>
</table>

Values are means ± SE of 5 paired measurements. Ser was measured at a concentration of 5 mM in the presence of 200 mM mannitol or L-Glu.
It is well established that effects of cotransport of sodium with bipolar amino acids and glucose on the electrical potential difference across the mucosal membrane suffice to cause large mutual inhibition of uptake by BBMV between these nutrients (16). With the intact epithelium such inhibition reaches only barely significant levels (19). In the present study, the passive contribution to transport at 0.05 and 0.1 mM D-aspartate amounts to <3%, while with the BBMV the passive contribution at 0.05 mM can be as high as 30% of the total uptake. However, since the cis-effect seems to be subject to self inhibition by D-aspartate, it cannot be accounted for by a potential difference effect on the passive contribution to uptake. An electrostatic interpretation of the cis-effect would therefore have the unlikely requirement that sodium-coupled D-aspartate uptake represents a net transfer of negative charge. The electrostatic interpretation of the cis-effect would also require a similar effect of D-glucose, a test for which there are no published results. It appears then that the procedures used to prepare BBMV reduce the capacity of the high-affinity transporter in a way that can, at least partly, be remedied by imposing cotransport of sodium and a variety of bipolar amino acids. Recirculation, using systems B and bo,

\[ \text{cis} \]

of the endogenous pool of bipolar amino acids between the cytoplasm and the outside, unstirred microclimate seems to be a significant stimulus for lysine influx and to be responsible for its apparent cis-stimulation by bipolar amino acids (22). Although preincubation at 0 mM sodium reduced this effect on lysine transport, it did not affect D-aspartate influx. Therefore, it seems unlikely that our failure to demonstrate this cis-stimulation can be explained by it being present already under the control conditions of our experiments.

The Low-Affinity System

Stimulation of transport of anionic amino acids by reduction of the ambient pH has been described for Chinese hamster ovary cells (34), Ehrlich ascites tumor cells (36), and HeLa cells and Xenopus laevis oocytes injected with cDNA from several organs and species, such as mouse testis (35), human coriocarcinoma cell line (10), and rabbit small intestine (11). The effect of pH has been ascribed to protonation of the anionic amino acid (33), but mostly to protonation of the transporter (10, 11, 14, 34, 36). The mechanism of the pH influence has, however, not been examined.

The observation that amiloride at pH 7.2 had a relatively much greater effect on L-glutamate influx at 0.1 mM than at 50 mM suggested that lowering pH led to a decrease of \( K_v \) of L-glutamate for the low-affinity transport. The present results proved this to be the case (Figs. 4 and 6), leaving the \( J_{\text{max}} \) unaffected. The effect of pH changes on transport of bipolar amino acids by the pH-sensitive transporter has not been examined in detail. However, measurements at one concentration in Ehrlich ascites tumor cells (36) and in a Chinese hamster ovary cell line (34) indicate that for transport of bipolar amino acids by system ASC the kinetic parameters are unaffected by lowering pH to 6.0, while transport by system A is greatly reduced. In these cells system A is the closest equivalent to the intestinal system B. These results can, therefore, be seen as consistent with the reduction of alanine transport by the rabbit ileum seen with decreasing pH (7). In agreement with this interpretation of the studies on ovary cells and Ehrlich ascites tumor cells, the present results demonstrate that both affinity for and maximum rate of transport of serine by the transporter shared with glutamate are unaffected by the changes in pH used in this study.

The present results provide in addition the important information that at pH 7.2 L-glutamate can eliminate only one-half or a little less of the total transport of serine. This raises the question of the identity of at least two transporters involved in serine transport. At pH 7.2 system bo,

\[ \text{cis} \]

can account for only 25–33% of the L-glutamate-resistant transport of serine. System bo,

\[ \text{cis} \]

does not function in the absence of chloride (24), and the imino acid carrier is inaccessible to serine (20). Thus only system B is left as purveyor of the L-glutamate-resistant, sodium-dependent transporter of serine. This conclusion is supported by the difference between a \( K_i \) for serine of 5 mM against the transport of L-glutamate and of 18 mM against leucine transport by systems bo,

\[ \text{cis} \]

and B as well as by the \( K_i \) of 1.7 mM for leucine against the L-glutamate-resistant transport of serine. These estimates do not differ from previous estimates of the affinity for systems bo,

\[ \text{cis} \]

and B. It follows that it is not system B but system ASC that is the transporter shared by L-glutamate and serine. This conclusion is supported by the difference between a \( K_i \) of 5 mM for serine against L-glutamate transport and its \( K_i \) of 18 mM against the chloride-independent and lysine-resistant transport of leucine. It is also consistent with the observation on BBMV and cDNA-injected HeLa cells and Xenopus laevis oocytes that alanine, serine, and threonine have as high or higher affinities than leucine and methionine as inhibitors of the low-affinity L-glutamate transport by these cellular systems (10, 11, 14). In contrast, in the rabbit small intestine the affinities of leucine and methionine for system B by far exceed those of alanine, serine, and threonine (19, 21, 22, 31, 34). In addition, the relatively very high degree of L-glutamate inhibition of phenylalanine uptake by BBMV observed at pH 6.0 can be explained partly by the higher affinity of L-glutamate and partly by the decrease in transport by system B seen at reduced pH. In particular, this nearly complete inhibition of phenylalanine uptake led us previously (27, 28) to believe that system B was responsible for the low-affinity transport of L-glutamate and therefore to discard system ASC as a BBM transporter, since direct evidence for its presence there had not been published.

The Topographic Problem

The most detailed part of the present study rests on the use of rabbit distal ileum, although its stepping...
stone is a study performed with rabbit jejunal BBMV (14). This was done because the distal ileum is the most clearly defined section, the easiest to prepare, the most thoroughly examined (19, 20, 23), and the section in which the amino acid transporters have the highest capacities (25, 26, 27). The decline in transport capacity observed with increasing distance from the ileocecal junction is larger for system B(o) and for the imino acid transporter than for system B (25–27). However, one study of transport of anionic amino acids by the rabbit small intestine presented both jejunal and ileal observations (33). These data did not indicate a topographic difference sufficient to account for the differences between the present results and those reported for BBMV. Nevertheless, the possibility of such an explanation was examined. Clearly, cis-stimulation of D-aspartate influx was not present in the jejunum. Corrected for diffusive contributions the ratio between influx of D-aspartate and L-glutamate at 50 mM was the same in the jejunum as in the ileum, and the pH effects on influx of D-aspartate, L-glutamate, and serine were the same in the two sections. Thus the differences discussed seem to reflect methodological differences.

We have previously defined system B as a sodium-dependent, chloride-independent, lysine-resistant transporter of bipolar amino acids (28). Now the quality of L-glutamate resistance must be added. It may be of some interest to note that this is the third case of detection of a transporter different from what was first seen as the carrier of neutral amino acids (37). The first being system b(o) (19, 30) and the next system B(o), initially termed b(alanine) carrier (20). That system ASC does transport bipolar amino acids in general has some effect on the estimates of their K1/2 on system B. In the case of leucine, the effect will be a slight overestimate of K1/2, while for serine (Fig. 6) and probably alanine and threonine K1/2 will be underestimated.

In Table 5, the present and previously published (19, 20) estimates of the affinities of the amino acids used in this study for the amino acid transporters of the luminal membrane of the rabbit small intestine are summarized.

### pH Problems

With the intact epithelium the buffering capacity of the molecular constituents of the membrane surface and the function of the sodium-proton exchanger prevent absolute control of the pH of the microclimate at the membrane surface. It is known that with a pH of 7 in the bathing solution the sodium-proton exchanger maintains a slightly lower pH at the membrane and that this difference can be greatly reduced when the exchanger is inhibited by 1 mM amiloride (15). It is likely that the same effect of the exchanger is present at a solution pH of 8.2, while with a solution pH of 5.65 the pH at the membrane will deviate less. At this pH 4% of the L-glutamate and <1% of the D-aspartate will exist in the bipolar form.

### Comparison With Other Studies

The presence of a low-affinity transport of L-glutamate (K1/2 = 7 mM) and L-aspartate (K1/2 = 5 mM) was previously indicated by data on influx of these amino acids in the rabbit ileum (32). The conclusiveness of this study was, however, limited by the use of a too narrow (2.5–25 mM) concentration range. A study of glutamate uptake by BBMV prepared from human jejunum (9) was conducted using concentrations below 1 mM. The results reflected the function of a high-affinity system with characteristics similar to those described for system XAG. Also, transport of L-aspartate and L-glutamate across monolayers of human Caco-2 cells (29) seems to reflect the function of a system XAG alone. In the rat a common transporter has been described for L-glutamate and D-aspartate with K1/2 values between 1 and 2 mM for uptake by BBMV used with concentrations not exceeding 5 mM (6). However, using the rat intestinal cryptlike cell line IEC-17, L-glutamate transport systems have been described with characteristics corresponding to systems XAG and ASC (18). Whether these two transporters are both present in the luminal membrane of the mature enterocyte in its proper location is not known. In the isolated chicken enterocyte a high-affinity system (K1/2 = 16 µM) and a low-affinity system (K1/2 = 4.1 mM) have been described (38, 39). However, except for the K1/2 values, the two systems have identical characteristics; in particular they both accept D-aspartate. Rather than being an equivalent of system ASC, the lower-affinity system might as well be a system XAG located on the basolateral part of the enterocyte membrane.

### Perspectives

The presentation of this study may give the impression of too much confidence in the case of intact epithelia as opposed to the use of BBMV. It clearly remains, however, for further studies to determine

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**Table 5. Affinities for known amino acid transporters in luminal membrane of rabbit small intestine**

<table>
<thead>
<tr>
<th>Carrier</th>
<th>Imino</th>
<th>B</th>
<th>B^o</th>
<th>XAG</th>
<th>ASC</th>
<th>b^o</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu</td>
<td>23</td>
<td>1</td>
<td>0.1</td>
<td>∞</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Phe</td>
<td>3</td>
<td>0.3</td>
<td>∞</td>
<td>&lt;10</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>257</td>
<td>∞</td>
<td>0.3</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Glu</td>
<td>∞</td>
<td>0.3</td>
<td>54</td>
<td>∞</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Asp</td>
<td>∞</td>
<td>0.3</td>
<td>∞</td>
<td>∞</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data shown are from current and previous studies (19–21). The amino acid transporters in the luminal membrane of the rabbit small intestine can be briefly defined as follows (8, 19–21, 23, 27). Imino, Na^+^- and Cl^-^-dependent carrier of cyclic and aliphatic imino acids with low to very low affinities for bipolar amino acids. System B, Na^+^-dependent and Cl^-^-independent, pH-sensitive, Glu^-^- and lysine-resistant carrier of bipolar amino acids. System B^o, Na^+^-dependent and Cl^-^-independent, pH-sensitive, Glu^-^- and lysine-resistant carrier of bipolar amino acids. System ASC, Na^+^-dependent and Cl^-^-independent, pH-insensitive carrier of bipolar amino acids. System ASC, Na^+^-dependent and Cl^-^-independent, pH-insensitive carrier of bipolar amino acids and for the imino acid transporters have the highest capacities (25, 26, 27). The decline in transport capacity observed with increasing distance from the ileocecal junction is larger for system B(o) and for the imino acid transporter than for system B (25–27). However, one study of transport of anionic amino acids by the rabbit small intestine presented both jejunal and ileal observations (33). These data did not indicate a topographic difference sufficient to account for the differences between the present results and those reported for BBMV. Nevertheless, the possibility of such an explanation was examined. Clearly, cis-stimulation of D-aspartate influx was not present in the jejunum. Corrected for diffusive contributions the ratio between influx of D-aspartate and L-glutamate at 50 mM was the same in the jejunum as in the ileum, and the pH effects on influx of D-aspartate, L-glutamate, and serine were the same in the two sections. Thus the differences discussed seem to reflect methodological differences.

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### Perspectives

The presentation of this study may give the impression of too much confidence in the case of intact epithelia as opposed to the use of BBMV. It clearly remains, however, for further studies to determine
whether cis-stimulation reflects a methodological artifact specific for the BBMV preparation and whether the present technique overestimates the capacity of the high-affinity system $X_{AG}$ relative to the low-affinity system ASC. However, it is evident that a proper understanding of the function of the intestinal BBM rests on the use of several independent methods. It has previously been demonstrated that intestinal transport functions expressed in Xenopus laevis oocytes do not all necessarily belong in the BBM of the enterocyte (22). In the present study, it is demonstrated that even when intestinal transport functions do belong there, they must be classified with a glance to older observations on intact epithelia.

The possibility that the present estimate of the relative capacity of system $X_{AG}$ is correct has functional implications. Although a transport of anionic amino acids with a capacity of $-0.1 \mu$mol $\cdot cm^{-2} \cdot h^{-1}$ seems of limited use to an animal, a 10 times greater capacity with a relatively high affinity could be essential. This is especially important since other amino acids do not compete for use of system $X_{AG}$, while all bipolar amino acids efficiently compete for transport by system ASC.

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