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Pathways for Alanine Transport in Intestinal Basal Lateral Membrane Vesicles

Austin K. Mircheff*, Carel H. van Os**, and Ernest M. Wright
Department of Physiology, University of California Medical Center, Los Angeles, California 90024

Summary. Membrane vesicles obtained from the basal lateral membranes of the rat intestinal epithelium were used to study the pathways for neutral amino acid transport.

In the absence of sodium there was a stereospecific uptake of L-alanine which exhibited saturation kinetics (K_m 0.73 mM and V_{max} 5.3 nmol/mg min at 22 °C). The activation energy for this process was 8.1 kcal/mole between 5 and 25 °C. Preloading the vesicles with alanine increased the unidirectional influx of alanine into the vesicle. Competition experiments indicated that the affinity of the sodium-independent transport system was glutamine > threonine > alanine > phenylalanine > valine > methionine > glycine > histidine > proline, N-MeAIB. These are the characteristics of the classical *"L"* transport system.

External sodium increased the rate of the stereospecific L-alanine uptake. The Na-dependent flux had a K_m of 0.04 mM and a V_{max} of 0.26 nmol/mg min at 22°, and an activation energy of 9.1 kcal/mole between 5 and 25 °C. Competition experiments suggest the existence of three separate pathways for alanine transport in the presence of sodium. A major pathway is shared by all other amino acids tested (i.e., threonine, glutamine, methionine, phenylalanine, valine, proline and N-MeAIB). This resembles the classical *"A"* system. A second pathway is unavailable to either phenylalanine or N-MeAIB; this is reminiscent of the classical *"ASC"* system; and the third is a novel pathway which is shared by N-MeAIB but not phenylalanine.

The sodium-independent and the sodium-dependent transport of L-alanine was blocked by PCMBS and significantly inhibited by DTP and NEM. It is concluded that the sodium-independent system (the *"L"*-like system) accounts for the efflux of neutral amino acids from the epithelium to the blood during the absorption of amino acids from the gut, and that the sodium-dependent transport processes may play an important role in the supply of amino acids to the epithelium in the absence of amino acids from the gut lumen.

Neutral amino acids are actively transported across the intestinal epithelium from the gut lumen into the blood by a two-stage process: (i) entry into the epithelium across the brush border membrane and (ii) transport from cell to blood across the basal lateral membrane. Studies of uptake into intact epithelium (Schultz & Curran, 1970) and isolated brush-border membrane vesicles (Sigrist-Nelson, Murer & Hopfer, 1975; Evers, Murer & Kinne, 1976; Hopfer *et al.*; 1976, Fass, Hammerman & Sacktor, 1977; Hammerman & Sacktor, 1977, 1978; Slack *et al.*, 1977) have shown the first step to be "active" accumulation by sodium-coupled mechanisms. In principle, facilitated diffusion driven by the amino acid concentration gradients would be sufficient for the second, basal lateral membrane exit step.

It has been impossible to obtain direct information about the exit step from studies with the intact epithelium, since the serosal surface is obscured by muscle and connective tissue layers. However, studies with isolated plasma membrane vesicles have provided a preliminary view of amino acid transport by renal and intestinal basal lateral membranes. Hopfer *et al.* (1976) observed that uptake of valine by intestinal basal lateral membranes was saturable, stimulated by preloading, and independent of sodium. Similarly, Evers *et al.* (1976) and Slack *et al.* (1977) obtained evidence for mediated uptake of phenylalanine and

* *Present address:* Department of Physiology & Biophysics, School of Medicine, University of Southern California, Los Angeles, California 90033.
** *Present address:* Department of Physiology, University of Nijmegen, Nijmegen, The Netherlands.

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Table 1. Occurrence and properties of neutral amino acid transport systems (after Christensen, 1975)

	A	*ASC*	*L*
a) *Occurrence*			
e.g., Ehrlich tumor cell	+	+	+
Avian erythrocytes	−	+	+
Reticulocytes	+	+	+
b) *Specificity*			
Alanine, glutamine threonine	+	+	+
Methionine	+	+[a]	+
Proline	+	+	−
Phenylalanine, valine	+	−	+
MeAIB	+	−	−
c) *Sodium-dependence*	+	+	−

[a] Methionine interacts with, but is not transported by, system *ASC*.

proline in preparations of basal lateral membranes from proximal tubule; sodium accelerated amino acid uptake by the renal preparations, but this was attributed to contamination by brush border membranes.

Since a number of epithelial transport processes, e.g., facilitated diffusion of glucose (Wright, van Os & Mircheff, 1980) and active exchange of sodium and potassium (Harms & Wright, 1980), depend on transport mechanisms which are shared with other cell types, an important question about the amino acid transport systems of basal lateral membranes is the extent to which they resemble the more thoroughly characterized amino acid transport systems of other cells. The work of Christensen (1969, 1975 and 1979) has provided the paradigm within which we have framed this question. Alanine is one of several amino acids which, in a variety of cells, have available three distinct transport systems (Table 1). These systems, designated *L*, *A* and *ASC*, are distinguished by their dependence on sodium and by the nature of the amino acids which they transport. *L* is a sodium-independent system; it has a broad specificity, interacting with most L, α-amino acids; notably, it is unable to interact with proline and with N-methylated derivatives, including the model substrate MeAIB.

Systems *A* and *ASC* are both sodium-dependent, and by coupling amino acid and sodium fluxes, they lead to accumulation of amino acids within cells. System *ASC* prefers three and four carbon amino acids; notably, it excludes glycine, valine, phenylalanine, and MeAIB. System *A* has a broad specificity, interacting with all of the neutral L, α-amino acids and their N-methyl derivatives.

We have recently devised procedures for obtaining preparations of intestinal basal lateral plasma membrane vesicles which are relatively free of contamination by brush border membranes (Mircheff *et al.*, 1979*a*; Wright *et al.*, 1980). We now report that, as had been anticipated, the major mode of alanine transport by these vesicles is a sodium-independent equilibrating system. Following Christensen, we have measured the ability of selected amino acids to inhibit transport of alanine, and we have found that this sodium-independent system closely resembles system *L*. We have also obtained evidence for the presence of sodium-coupled systems resembling systems *A* and *ASC*, and for a novel system which interacts with short chain and N-methylated amino acids but not with long chain or aromatic amino acids. These latter mechanisms may be important in providing amino acids to the epithelium in the absence of dietary protein. A preliminary report of this work has been published elsewhere (Mircheff, van Os & Wright, 1979).

Materials and Methods

Membrane Isolation Procedure

Male Sprague-Dawley rats (140–160 g) were killed by cervical dislocation, and the small intestines were removed following perfusion of the lumen with ice-cold 154 mM NaCl. Fifteen cm of proximal duodenum and distal ileum were discarded, and upper villus cells were isolated from the remaining jejunum according to the procedure of Stern (1966).

Basal lateral membrane preparations were obtained with modifications of previously described methods (Mircheff *et al.*, 1979*a*; Wright *et al.*, 1980). Briefly, cells from four rats were suspended in 200 ml of bomb buffer (25 mM NaCl, and 1 mM Tris-HCl, pH 8.0) and disrupted by nitrogen cavitation after equilibration at 300 lb/in^2. After equilibration under vacuum for 60′, the homogenate was centrifuged at $450 \times g \times 10$ min; the $450 \times g$ pellet was washed once with 100 ml bomb buffer. The pooled $450 \times g$ supernatants were centrifuged at $95{,}000 \times g \times 20$ min, and the resulting pellet was resuspended in 40 ml isolation buffer (250 mM sorbitol, 12.5 mM NaCl, 0.5 mM EDTA, and 5 mM histidine-imidazole buffer, pH 7.5). After its sorbitol concentration had been increased to 40% by addition of 1.4 volumes of 65% sorbitol, the resuspended pellet was distributed to centrifuge tubes, overlayered with 25% sorbitol, and centrifuged at $95{,}000 \times g \times 60$ min. We have reported previously (Mircheff *et al.*, 1979) that the resulting supernatant contained 64% of the initial Na,K-ATPase, 13% of the sucrase, 5% of the succinic dehydrogenase, and 6% of the protein and that equilibrium density gradient centrifugation of this fraction in a high capacity zonal rotor would have yielded a preparation containing 48% of the initial Na,K-ATPase, 1.8% of the sucrase, 1.2% of the succinic dehydrogenase, and 2.6% of the protein. For rapid isolation of basal lateral membranes for the present study, the zonal rotor step was eliminated and material collecting in a band at the interface between 25% and 40% sorbitol was collected. In a typical preparation, this band contained 13% of the initial Na,K-ATPase, 1.1% of the sucrase, 0.3% of the succinic dehydrogenase, and 1.3% of the protein; overall marker recoveries were, respectively, 62%, 78%, 68%, and 87%. Since factors as high as 40 have been obtained for enrichment of sucrase specific activity

in brush border membrane preparations (Mircheff & Wright, 1976), brush border membranes represent, at most, 2% of the protein in the basal lateral membrane preparations. As with previously described basal lateral membrane preparations (Mircheff *et al.*, 1979*b*) use of density perturbation with digitonin as a physical criterion for purity indicates that the only major contaminant in the preparation is a population of membranes of unknown, but presumed intracellular, origin; this population represents approximately 30% of the protein (Mircheff *et al.*, 1979*b*).

The basic buffer system for all subsequent steps was 5 mM Tris-HEPES, pH 7.5, containing 0.5 mM NaN_3.[1]

The 25%–40% interfacial band was diluted sixfold with 200 mM sorbitol-THA, and the membranes were harvested by 30 min centrifugation at 95,000 × *g*. The pellet was resuspended to a protein concentration of 4.5 mg/ml in 30 mM KCl, 340 mM sorbitol-THA; stereospecific L-alanine uptake persisted for three days.

Uptake Measurements

All uptake reactions were performed with double label techniques, permitting simultaneous measurements of a test solute, (e.g., ^{14}C-L-alanine) and a passively permeating solute (e.g., ^{3}H-L-glucose). The final uptake reaction contained 1 mg/ml protein, 40 μCi/ml ^{3}H, and 4 μCi/ml ^{14}C in 30 mM KCl, 340 mM sorbitol-THA; unless otherwise indicated, radioactive solutes were carrier free (^{3}H-L-alanine = 1 μM and ^{14}C-L-alanine = 22 μM.) Reactions were started by addition of protein to otherwise complete media. At appropriate times, 50 μl aliquots were quenched into 1.0 ml of an ice-cold stop solution; stop solution contained 1.0 mM $HgCl_2$, 400 mM sorbitol-THA. 0.9 ml of the quenched reaction was deposited on 0.45 μm nitrocellulose filters (Sartorius, SM 11306) which had been rinsed with two 4-ml aliquots of ice-cold stop solution. The sample was rinsed with a 4-ml aliquot of ice-cold stop solution. Additional filters, rinsed with stop solution, were used to prepare standard samples of the quenched reactions and of ^{14}C. Filters were allowed to dissolve overnight in a commercial cocktail (PCS, Amersham), and radioactivity, determined by liquid scintillation counting, was used to calculate apparent spaces for ^{3}H and ^{14}C. The equilibrium spaces for D- and L-glucose were 3.5 μl/mg protein.

For quantitation of the inhibitory effects of sulfhydryl reagents and competing amino acids, the L-alanine or MeAIB space on each filter was expressed relative to the simultaneously determined space of a passively permeating marker, i.e. L-glucose or D-alanine; this strategy eliminated the effects of variability in membrane retention among filters.

At early time points, the difference between relative space and the relative space measured in the presence of 50 mM, i.e. saturating, L-alanine is a measure of stereospecific alanine uptake, and this difference will be referred to as the relative specific space. Sodium-stimulated uptake was expressed as the increment in relative space obtained when NaCl replaced KCl in the uptake medium.

Competition experiments employed appropriate dilutions of primary stock solutions which consisted of 100 mM amino acid in 350 mM sorbitol-THA, when necessary adjusted to pH 7.5 with Tris or HEPES.

L-alanine (2,3-^{3}H and U-^{14}C) and D-alanine (U-^{14}C), were obtained from Amersham, Arlington Heights, Ill. N-methyl-aminoisobutyric acid (1-^{14}C), L-glucose [1-^{3}H(N)], and D-glucose (U-^{14}C) were obtained from New England Nuclear, Boston, Mass. N-methyl-aminoisobutyric acid was from Sigma, St. Louis, Mo; all other amino acids were from Calbiochem, La Jolla, Calif.

[1] *Abbreviations:* The basic buffer system will be referred to as THA; 2-(methylamino)-isobutyric acid will be abbreviated MeAIB.

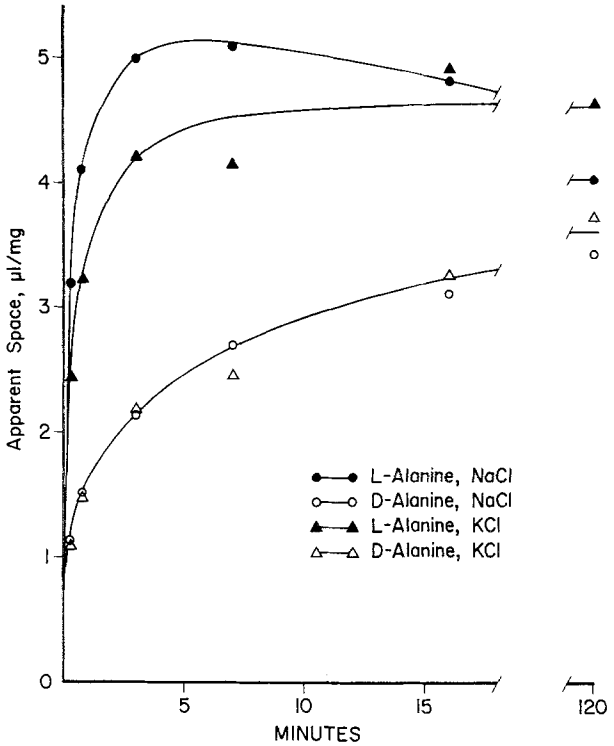


Fig. 1. Time course of D-^{14}C- and L-^{3}H-alanine uptake. Uptake, expressed as apparent space, was determined as described in Materials and Methods. Basal lateral membrane had been equilibrated overnight with 30 mM KCl, 340 mM sorbitol-THA; in NaCl media, 30 mM NaCl replaced 30 mM KCl. Each point is the mean of duplicate determinations. Comparison of the time courses of uptake of D-alanine and L-glucose (Fig. 2) indicates no specific uptake of D-alanine; this was confirmed by direct measurement of time courses of ^{14}C-D-alanine and ^{3}H-L-glucose uptake and by a demonstration that D-alanine uptake is not saturable between 0.1 and 8 mM (Fig. 3). In subsequent experiments, D-alanine and L-glucose were used interchangeably as markers of nonspecific permeation

Results

Time Course of Alanine Uptake

The preparation of basal lateral plasma membrane vesicles exhibits a stereospecific uptake of L-alanine (Fig. 1). In the absence of sodium, the half time for equilibration of L-alanine was less than 0.25 min, while the half time for D-alanine uptake was more than 1.5 min. The apparent equilibrium space for L-alanine was 20% greater than the D-alanine space.[2] The presence of 30 mM NaCl in the uptake medium resulted in a 25% increase in the stereospecific uptake of L-alanine after 0.25 min. A slight overshoot was typically present in the time course of L-alanine uptake in the presence, but not the absence, of NaCl.

[2] The reason for the discrepancy between the equilibrium space for L-alanine and the D-glucose, L-glucose, and D-alanine spaces is not clear. It is not specific binding as the L-alanine space is independent of concentration (1 μM–5 mM) and of the isotope used.

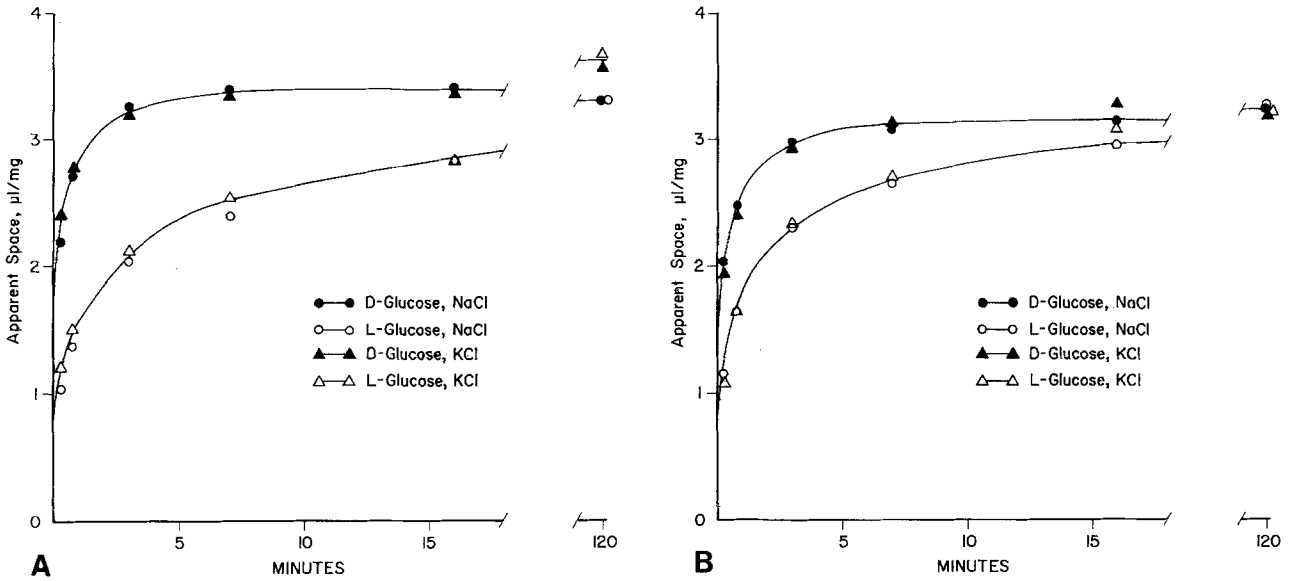


Fig. 2. Time course of ^{14}C-D- and ^{3}H-L-glucose uptake. Basal lateral membranes loaded with 30 mM KCl, 340 mM sorbitol-THA were from the preparation used in Fig. 1. (*A*): The external media contained either 30 mM NaCl or 30 mM KCl in 340 mM sorbitol-THA. (*B*): The external media contained 100 mM NaCl or 100 mM KCl in 200 mM sorbitol-THA. Each point is the mean of duplicate determinations. The failure of sodium to stimulate D-glucose uptake confirms previous results (Wright *et al.*, 1980) and indicates that brush border contamination is not the reason for observed effects of sodium (Fig. 1) on uptake of L-alanine

Table 2. Effects of sulfhydryl reagents on sodium-independent and sodium-stimulated L-alanine uptake

	Na-independent		Na-stimulated	
	Uptake	Inhibition	Uptake	Inhibition
Control	1.79±0.03		0.91±0.06	
p-Chloromercuriphenylsulfonate	0.06±0.01	97%	0.05±0.03	95%
Dithiodipyridine	0.77±0.01	57%	0.44±0.02	52%
N-ethylmaleimide	1.43±0.01	20%	0.79±0.06	13%

4.5 mg/ml basal lateral membranes were incubated with inhibitors for 60 min at 0 °C. *p*-Chloromercuriphenylsulfonate and dithiodipyridine concentrations were 1.0 mM, and the N-ethylmaleimide concentration was 2.2 mM. Uptake (^{14}C-L-alanine and ^{3}H-L-glucose) was measured after incubation of 0.5 min under conditions described in Materials and Methods; results presented are relative stereospecific L-alanine uptake with the mean of three determinations ±SEM.

Effect of Sodium of D-*Glucose Uptake*

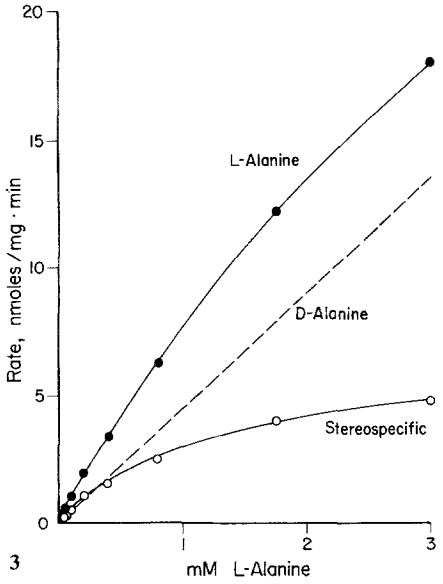
We have previously reported that external sodium had no effect on the stereospecific uptake of D-glucose by intestinal basal lateral membrane vesicles (Wright *et al.*, 1980). The experiments summarized in Fig. 2 confirm that neither 30 mM NaCl, a concentration which gives a marked stimulation of the initial rate of alanine uptake, nor 100 mM NaCl, a concentration used in demonstrating sodium-dependent transport in brush border membrane preparations, stimulated D-glucose uptake. Comparison of the time courses of D- and L-glucose uptake in the presence of either 30 or 100 mM KCl suggests that elevated ionic strength may decrease the size and increase the passive permeability of the basal lateral membrane vesicles.

Effects of Sulfhydryl Reagents on L-*Alanine Uptake*

The sensitivity of stereospecific L-alanine uptake to dithiodipyridine and to *p*-chloromercuriphenylsulfonate ranged from 52 to 97%, and the sodium-independent and the sodium-stimulated components of L-alanine uptake were similarly sensitive to the two reagents (Table 2). N-ethylmaleimide inhibited 13% of the sodium-stimulated uptake and 20% of the sodium-independent uptake.

Concentration Dependence of L-*Alanine Uptake Rate*

Although the time resolution of the membrane ultrafiltration technique is not entirely satisfactory for measurements of initial rates, measurements of L- and D-alanine uptake after 0.25-min incubation suggest that L-alanine uptake is the result of superposition of a saturable and a linear process (Fig. 3). The concentration dependence of sodium-independent, stereospecific L-alanine uptake at 0.25 min suggests the hyperbola characteristic of saturation kinetics, and an Eadie-Hofstee transformation of the data in Fig. 3 extrapolates to a maximal velocity of 5.3 nmol/mg min and a half-saturation constant of 0.73 mM



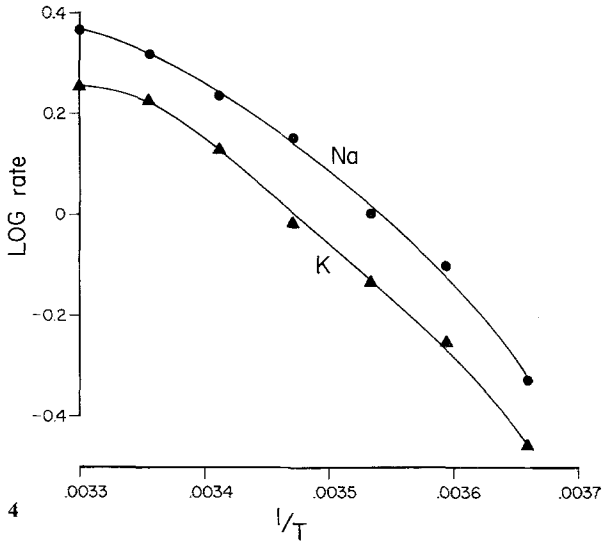


Fig. 3. Concentration dependence of sodium-independent alanine uptake. ^{3}H-L- and ^{14}C-D-alanine concentrations were varied by addition of nonradioactive isomers to give a constant total (D+L) alanine concentration. As described in the text, uptake was measured after 0.25 min incubation and results were first expressed as apparent spaces; each point is the mean of quadruplicate determinations. Rates were calculated by multiplying the apparent space by the medium alanine concentration and dividing by 0.25 min. After 0.25 min the apparent spaces for D-alanine were 1.15 ± 0.03 µl/mg at 8 mM D-alanine and 1.11 ± 0.02 µl/mg at 0.1 mM D-alanine i.e., the rate of D-alanine uptake is linear through this concentration range. Stereospecific L-alanine uptake was calculated by subtracting the simultaneously measured D-alanine space from the total L-alanine space

Fig. 4. Arrhenius plots of stereospecific L-alanine uptake. Uptake of ^{14}C-L-alanine and ^{3}H-0-glucose after 0.25 min reaction was measured as described under Materials and Methods. Temperature was controlled between 0 and 30 °C with a Lauda K-2/R refrigerated water bath (Brinkmann). Each point is the mean of duplicate determinations

($R=0.961$). Experiments discussed below demonstate that the sodium-stimulated component of alanine uptake is also saturable.

Temperature Dependence of L-*Alanine Uptake Rate*

Stereospecific L-alanine uptake at 0.25 min was measured over a temperature range of 0 to 30 °C. The resulting Arrhenius plots are presented in Fig. 4. Between 5 and 25 °C, the apparent activation energies are 8.1 kcal/mole ($R=0.995$) in 30 mM NaCl and 9.2 kcal/mole ($R=0.999$) in 30 mM KCl. Activation energies derived from the simultaneously measured L-glucose spaces were 2.9 and 3.1 kcal/mole, respectively, values similar to that measured in a study of glucose transport by basal lateral membrane vesicles (Wright *et al.*, 1980).

Trans-Stimulation of Alanine Uptake

When basal lateral membranes had been equilibrated with 4.0 mM L-alanine (Fig. 5), the rate of ^{14}C-L-alanine uptake was double the control rate, and there was a twofold overshoot in the ^{14}C-L-alanine content of the vesicles.

Specificity of Sodium-Independent Alanine Uptake

The ability of histidine and of selected neutral amino acids to inhibit the sodium-independent component of stereospecific L-alanine uptake is presented in Fig. 6. MeAIB did not inhibit sodium-independent uptake. Proline inhibited a small component of L-alanine uptake. In preliminary experiments lysine had no consistent effect on alanine uptake; aspartic acid and glutamic acid inhibited L-alanine uptake, but it was not possible to demonstrate any uptake of these amino acids beyond that attributable to passive diffusion. (C.H. Van Os, A.K. Mircheff, and E.M. Wright, *unpublished*). At concentrations of 50 mM glutamine, methionine, threonine, glycine, phenylalanine, and valine were as effective as alanine in inhibiting all stereospecific ^{14}C-L-alanine uptake, and, in preliminary experiments, glycine, phenylalanine, and valine were taken up more rapidly than L-glucose. Histidine inhibited weakly, but the pattern of inhibition between 1 and 25 mM (Fig. 6) gives no indication

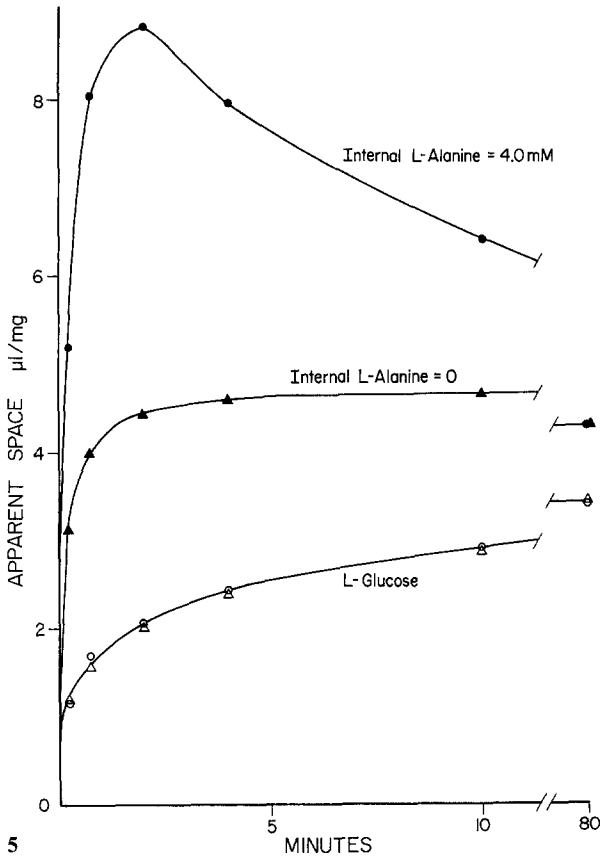


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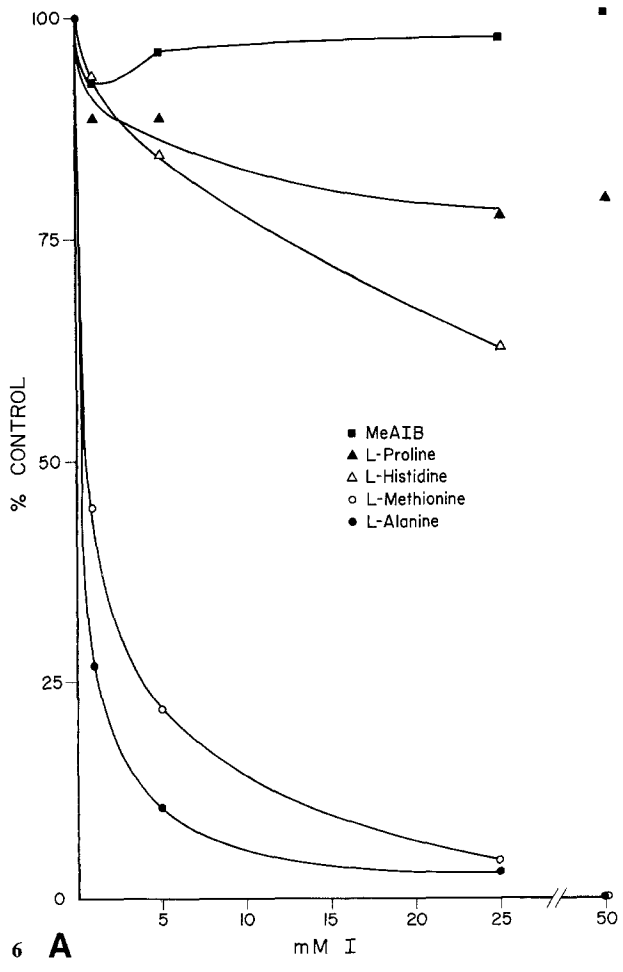
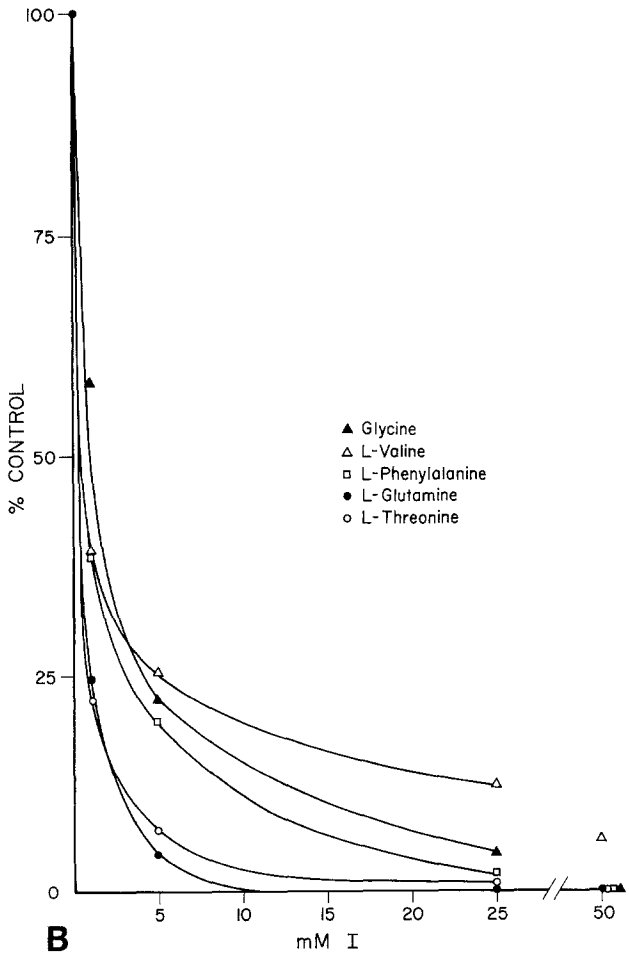
Fig. 5. Trans-stimulation phenomenon in L-alanine uptake. Basal lateral membranes at a concentration of 9.0 mg protein/ml were incubated for 3 hr at 0 ° in 30 mM KCl, 340 mM sorbitol-THA containing either 0 L-alanine (control) or 4.0 mM L-alanine (preloaded). Uptake reactions were started by diluting 7.5 µl of the control or preloaded basal lateral membranes into a final volume of 120 µl uptake medium containing, respectively, either 0.25 mM or 0 nonradioactive L-alanine and the usual concentrations of ^{3}H-L-glucose and ^{14}C-L-alanine. 105-µl aliquots of the uptake reactions were quenched into 1.0 ml ice-cold stop solution, and 950-µl aliquots of the quenched reaction were filtered, rinsed, and counted as described under Materials and Methods. Each point is the mean of duplicate determinations

Fig. 6. Inhibition of sodium-independent ^{14}C-L-alanine uptake by neutral amino acids. Relative stereospecific L-alanine uptake was measured after 0.5 min incubation in the presence of increasing concentrations of N-methyl-aminoisobutyric acid and L-amino acids as described under Materials and Methods. Each point is the mean of triplicate determinations. The broad specificity to the mechanism responsible for L-alanine uptake and its inability to interact with proline and MeAIB is characteristic of system *L* in Ehrlich ascites cells



6 **A**



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of a component of Na-independent, stereospecific L-alanine uptake which is not accessible to L-histidine. Linear regressions of Hanes transformations (e.g., Muflih & Widdas, 1976; Sepulveda & Smith, 1978) of the data from Fig. 6 had r values in the range of 0.98 to 0.99. The inhibitor constants and order of decreasing affinity calculated from these regressions are glutamine, 0.26 mM > threonine, 0.44 mM > alanine, 0.59 mM > phenylalanine, 0.97 mM > valine, 1.18 mM > methionine, 1.25 mM > glycine, 1.57 mM > histidine, 7.97 mM. The inhibitor constant calculated for alanine agrees well with the half-saturation constant estimated from an Eadie-Hofstee transformation of the data from Fig. 3 (0.73 mM).

Specificity of Sodium-Stimulated Alanine Transport Systems

Increasing concentrations of L-alanine also inhibit the sodium-stimulated component of ^{14}C-L-alanine uptake (Fig. 7). Extrapolation of a Hanes plot of the data from Fig. 7 leads to an estimated half saturation constant of 0.04 mM, and insertion of this value into the Michaelis-Menten equation, along with an estimated initial rate of 0.09 nmol/mg min at 0.022 mM L-alanine (Fig. 1), yields a $V_{max}=0.26$ nmol/mg min. Since, as will be discussed below, the sodium-stimulated component of L-alanine uptake is the result of several parallel transport systems, these kinetic parameters give only a qualitative estimate of the characteristics of sodium-stimulated alanine transport in the basal lateral membrane preparation.

At concentrations of 50 mM, threonine, glutamine, and methionine inhibit 91–98% of the sodium-stimulated L-alanine uptake (Table 3). The concentration dependence of inhibition of L-alanine transport by phenylalanine (Fig. 7) reveals the existence of a component of sodium-stimulated alanine transport which is insensitive to phenylalanine, i.e., of a sodium-stimulated transport system which interacts with alanine but not phenylalanine. Valine and proline also appear to inhibit only a portion (73 to 81%) of the sodium-stimulated alanine transport (Table 3), and addition of 25 mM phenylalanine to 25 mM valine or 25 mM proline produced no inhibition beyond that produced by 50 mM concentrations of valine and proline (Table 3).

In a separate experiment (Table 4), MeAIB at 25 mM inhibited a larger component of Na-dependent alanine uptake than did phenylalanine at concentrations of 25 or 50 mM; consistent with this result, addition of 5 mM MeAIB to an uptake medium containing 25 mM phenylalanine resulted in an increased inhibition of sodium-dependent alanine uptake. MeAIB

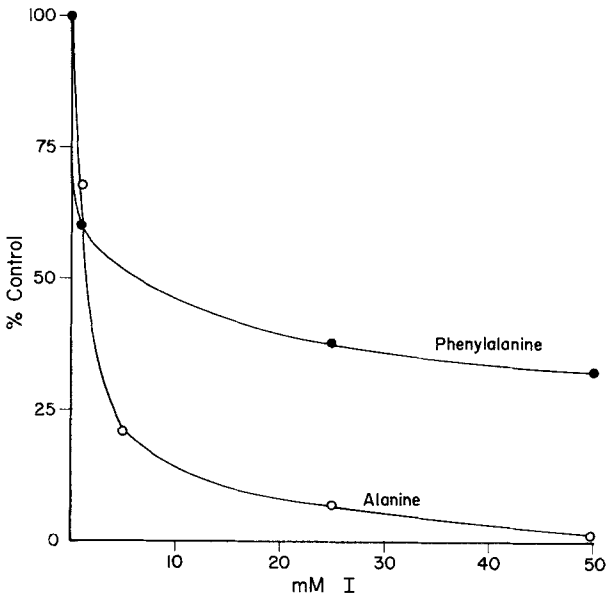

Fig. 7. Inhibition of sodium-dependent ^{14}C-L-alanine uptake by L-alanine and L-phenylalanine. Sodium-dependent uptake was expressed as the difference between the relative specific L-alanine spaces in 30 mM NaCl, 340 mM sorbitol-THS *vs.* 30 mM KCl, 340 mM sorbitol-THS after 0.5 min

Table 3. Inhibition of sodium-stimulated alanine uptake by neutral amino acids

	50 mM amino acid		25 mM amino acid +25 mM phenylalanine	
	Uptake	Inhibition	Uptake	Inhibition
Control	0.88±0.04			
L-Alanine	0.05±0.02	95%		
L-Glutamine	0.02±0.02	98%	0.01±0.02	99%
L-Methionine	0.02±0.02	98%	0.00±0.01	100%
L-Threonine	0.08±0.01	91%	0.10±0.02	88%
L-Phenylalanine	0.24±0.02	73%		
L-Valine	0.18±0.02	79%	0.16±0.02	82%
L-Proline	0.17±0.07	81%	0.13±0.01	85%

Uptake (^{14}C-L-alanine and ^{3}H-L-glucose) was measured after 0.5 min incubation. Results are sodium-stimulated relative L-alanine uptake, mean of three determinations, ±SEM.

Table 4. Inhibition of sodium-stimulated alanine uptake by L-phenylalanine and N-methyl-aminoisobutyric acid (MeAIB)

L-Phenylalanine	MeAIB	Uptake	Inhibition
0	0	1.13±0.11	
25 mM	0	0.54±0.02	52%
50 mM	0	0.42±0.03	63%
0	25 mM	0.30±0.04	74%
25 mM	5 mM	0.19±0.03	83%
25 mM	25 mM	0.11±0.03	90%

L-Alanine uptake was measured after 0.5 min incubation. Results presented are in Tables 1 and 2.

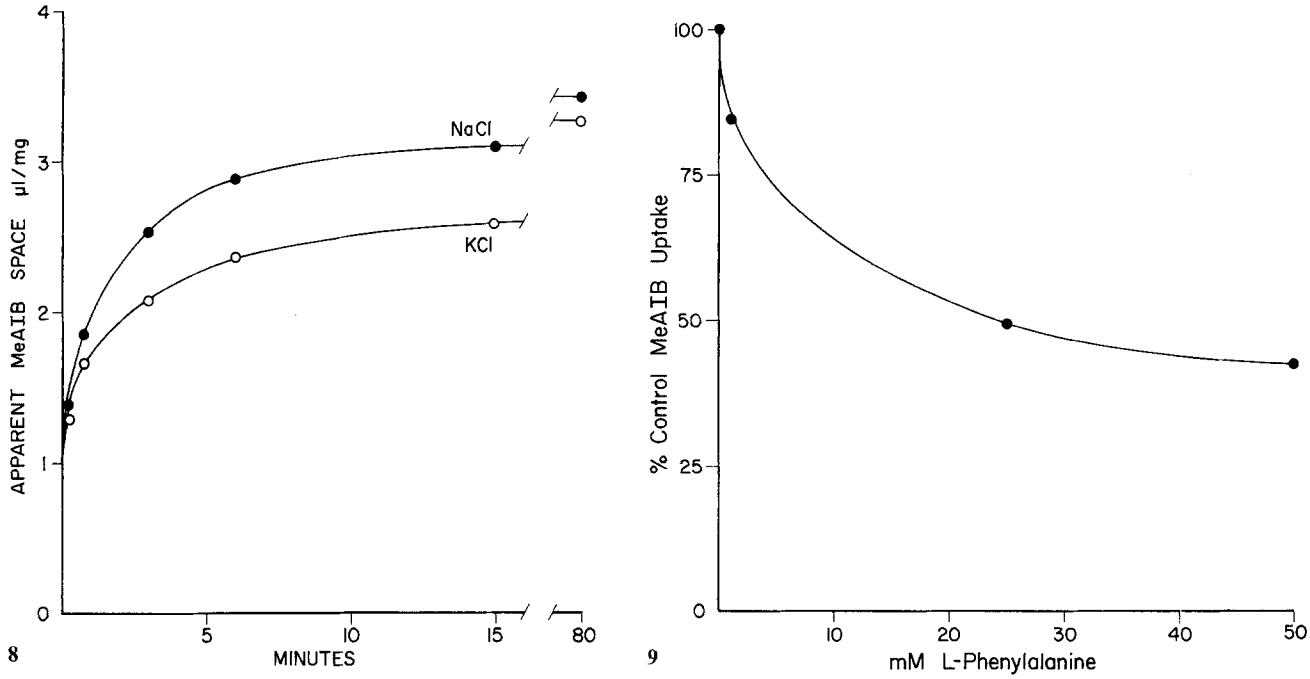

Fig. 8. Time course of MeAIB uptake Standard procedure, described under Materials and Methods, was used to measure N-methyl-aminoisobutyric acid uptake in the presence of 30 mM NaCl, 340 mM sorbitol-THA or 30 mM KCl, 340 mM sorbitol-THA. Each point is mean of two determinations. Time course in the presence of KCl is similar to the time courses of uptake of D-alanine and L-glucose

Fig. 9. Inhibition of sodium-dependent MeAIB uptake by L-phenylalanine. Procedures described unter Materials and Methods were used to determine relative specific methyl-aminoisobutyric acid uptake after 3.0 min incubation. The nonzero asymptote indicates that Na-dependent uptake occurs by at least two transport systems, one which is also able to interact with phenylalanine and one which is not; the first is similar to system *A* described in Ehrlich ascites cells, and the second appears to be novel

(Fig. 8) is taken up by the basal lateral membranes. In the absence of sodium, MeAIB uptake resembles L-glucose and D-alanine uptake. This process is slow compared to L-alanine uptake, and Na-dependent increments in MeAIB uptake at each time point are an order of magnitude smaller than the Na-dependent increments in L-alanine uptake; however, at 3 min the Na-dependent increment was large enough to permit a survey of the competitive effects of other amino acids. Phenylalanine at 50 mM (Fig. 9) inhibited 57% of the Na-dependent MeAIB uptake. In a separate preparation of basal lateral membranes (Table 5) 50 mM concentrations of threonine, methionine, and alanine inhibited virtually all of the MeAIB uptake, while phenylalanine inhibited 59% of the sodium-dependent MeAIB uptake.

Discussion

Uptake of L-alanine by preparations of isolated intestinal basal lateral membrane vesicles has a number of characteristics conventionally taken as diagnostic of carrier-mediated transport. Both the sodium-independent and the sodium-stimulated components of alanine uptake are saturable, stereospecific, and inhibited by sulfhydryl reactive agents, and they have higher activation energies than simple diffusional processes. The equilibrium space for alanine is similar to the simultaneously measured equilibrium spaces of passively permeating, nonmetabolized solutes. The sodium-independent component of stereospecific L-alanine uptake is subject to a trans-stimulation phenomenon large enough to transiently increase the intravesicular ^{14}C-L-alanine content to double its equilibrium value. These results extend previous reports that valine is taken up by preparations of intestinal basal lateral membranes (Hopfer *et al.*, 1976) and that phenylalanine (Evers *et al.*, 1976) and proline (Slack *et al.*, 1977) are taken up by proximal tubular basal lateral membranes.

Two lines of evidence exclude the possibility that the sodium-stimulated component of L-alanine uptake might be attributed to contamination by brush border membrane vesicles. First, brush border membranes contain a sodium-coupled mechanism for D-glucose uptake, yet there is no indication of a sodium-stimulated component in the time course of D-glucose uptake by basal lateral membrane preparations (Fig. 2). Secondly, it is possible to estimate the magnitude

Table 5. Inhibition of sodium-stimulated N-methyl-aminoisobutyric acid (MeAIB) uptake by neutral amino acids

	Uptake	Inhibition
Control	0.09 ± 0.02	
MeAIB	-0.04 ± 0.003	145%
L-Alanine	0.00 ± 0.02	100%
L-Methionine	-0.02 ± 0.02	100%
L-Threonine	0.01 ± 0.03	91%
L-Phenylalanine	0.05 ± 0.02	59%

Uptake of ^{14}C-MeAIB and ^{3}H-L-glucose was measured after 3.0 min reaction in the presence of 50 mM concentrations of the competing amino acids. Results presented are relative specific MeAIB uptake, mean of three determinations ± SEM.

Table 6. Summary of mediated permeation pathways for L-alanine in intestinal basal lateral membranes

	Sodium-dependent systems			Sodium-independent systems
	A-like	*ASC*-Like	Novel	*L*-Like
Alanine	+	+	+	+
Glutamine	+	+	+	+
Methionine	+	+	+	+
Threonine	+	+	+	+
MeAIB	+	−	+	−
Phenylalanine	+	−	−	+
Valine	+	−	−	+
Proline	+	−	−	−

of the sodium-stimulated flux of alanine into the brush border membrane vesicles present as contaminants in the basal lateral membrane preparation. From the data of Sigrist-Nelson *et al.* (1975), the peak of sodium-stimulated uptake of alanine by brush border vesicles occurs at 0.25 min. When the alanine concentration was 1 mM, and sodium-stimulated component of alanine uptake was 3 nmol/mg, i.e., the apparent space was 3 µl/mg. Since brush borders account for 2% of the protein in the basal lateral membrane preparations, at 0.25 min the brush border contribution to the sodium-stimulated alanine space would be 0.06 µl/mg, i.e., negligible compared to the observed total sodium-dependent increment of 1.0 µl/mg. It is not yet certain, however, that the sodium-stimulated transport apparent in the membrane preparation is, in fact, associated with the basal lateral membranes, since it is known that roughly 30% of the protein in the preparation is associated with a population of membranes derived from an as yet unidentified intracellular structure. Attempts to measure transport in highly purified basal lateral membranes obtained by density perturbation with digitonin (Mircheff *et al.*, 1979*b*) were unsuccessful *(unpublished results)*, apparently because digitonin destroys the membrane barrier to nonspecific permeation.

The sodium-independent and the sodium-stimulated components of L-alanine transport represent the parallel actions of several distinct transport systems. Although both components are similarly sensitive to each of three sulfhydryl reagents (*p*-chloromercuriphenylsulfonic acid, dithiodipyridine and N-ethylmaleimide), they can be distinguished by their abilities to interact with selected neutral amino acids.

The characteristics of the sodium-independent alanine transporting system – its broad sensitivity, its exclusion of proline and MeAIB, and its strong trans-stimulation phenomenon – are all similar to the characteristics of system *L* defined in Ehrlich ascites cells (Christensen, 1969, 1975, 1979).

Sodium-stimulated alanine uptake can be resolved into three components. A major portion of the sodium-stimulated alanine influx is inhibited by all of the neutral amino acids tested, including phenylalanine, proline, and MeAIB; this broad specificity is characteristic of the classical system *A* of Ehrlich ascites cells (Christensen, 1969, 1975, 1979). The flux which is insensitive to phenylalanine can be seen as partitioned between two components, one which can be inhibited by MeAIB, and one which cannot. A transport system which is insensitive to both phenylalanine and MeAIB is reminiscent of system *ASC* (Christensen, 1969, 1975, 1979). This system which is inhibited by MeAIB but not phenylalanine resembles neither *A* nor *ASC;* corroborating evidence for such a novel system derives from the demonstration that a component of sodium-stimulated MeAIB uptake is insensitive to phenylalanine. Table 6 contains a summary of the alanine transporting systems which the present work indicates to be present in preparations of basal lateral plasma membranes of rat intestinal epithelial cells.

The *L*-like system, because it is sodium-independent, has properties expected of the basal lateral membrane exit step in the overall process of amino acid active absorption. That this system provides the major pathway for transport of alanine across the basal lateral membranes may be seen, qualitatively, from Fig. 1, and, quantitatively, from a comparison of the kinetic parameters of the sodium-independent (K_m 0.7 mM and V_{max} 5.3 nmol/mg min) and sodium-stimulated (K_m 0.04 mM and V_{max} 0.26 nmol/mg min) transport systems. The presence of sodium-stimulated amino acid transport systems in the basal lateral plasma membranes would not be necessary for active absorption; in fact, such systems would tend to op-

pose net cell-to-blood flux by providing pathways for sodium gradient-driven fluxes back into the cell. However, it is plausible that sodium-stimulated transport across the basal lateral membranes might become significant in providing the cells of the intestinal epithelium with amino acids in the absence of dietary protein. This interpretation is supported by a recent observation *(in preparation)* that there is a gradient of sodium-stimulated alanine transport activity from proximal duodenum to terminal ileum. Christensen (1979) has also discussed evidence that System *L* participates in concentrative amino acid uptake involving a direct input of metabolic energy.

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