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Transport of Neutral and Cationic Amino Acids across the Brush-Border Membrane of the Rabbit Ileum

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Summary. The transport of sugars and amino acids across the brush-border membrane of the distal rabbit ileum has been studied. The kinetics of the transport of glucose demonstrated that the data obtained with the present technique are less distorted by unstirred layers than those obtained with the same technique adapted to the use of magnetic stirring. The role of depolarization of the electrical potential difference across the brush-border membrane in mutual inhibition between different classes of amino acids was estimated by measurements of the effects of high concentrations of alanine and lysine on the transport of galactose. It was found that this role would be insignificant in the present study. By measurements of the transport of alanine, leucine and lysine and the inhibitory interactions between these amino acids the function of three transport systems has been delineated. The transport of lysine is resolved in a high- and a low-affinity contribution. At 140 mM sodium these transport systems may also function as respectively high- and low-affinity contributors to the transport of neutral amino acids. At 0 mM sodium the high-affinity system remains a high-affinity system for cationic and neutral amino acids with reduced capacity especially for the neutral amino acids. At 0 mM sodium the low-affinity system's affinity for lysine is reduced and it is inaccessible to neutral amino acids. In addition to the two systems for lysine transport the existence of a lysine-resistant, sodium-dependent, high-affinity system for the transport of neutral amino acids has been confirmed. It seems unlikely that the distal ileum is equipped with a low-affinity, sodium-independent system for the transport of neutral amino acids.

Key Words rabbit · small intestine · amino acid transport · sugar transport · passive permeability

Introduction

The first unequivocal evidence of mutual inhibition between α-amino-mono-carboxylic acids (neutral a.a.) and cationic a.a. and sharing of one or more transport systems in the brush-border membrane was reported for the rabbit small intestine by Munck and Schultz (1969*a*,*b*), who resolved the transport of both neutral and cationic a.a. into two saturable processes. In these studies it was established that lysine was transported by a high-affinity, low-capacity carrier which appeared sodium-independent and by a sodium-dependent low-affinity, high-capacity carrier; but it was not determined which of these were shared with neutral a.a. Preston, Schaeffer and Curran (1974) observed a 30% inhibition of J_{mc}^{Met} by a series of cationic a.a., but did not incorporate this observation into their evaluation of the characteristics of the transport of neutral a.a. across the rabbit small intestine. The study by Paterson, Sepúlveda and Smith (1981) was the first follow-up on the results of Munck and Schultz (1969*a*,*b*). In this report it was shown that in the absence of sodium, alanine is a competitive inhibitor of a fraction of J_{mc}^{Lys}, and lysine a competitive inhibitor of a fraction of J_{mc}^{Ala}. The situation at normal concentrations of sodium was not examined.

For the rabbit small intestine Sepúlveda and Smith (1978) and Paterson, Sepúlveda and Smith (1979, 1980) proposed that neutral a.a. cross the brush-border membrane by a low-affinity, high-capacity carrier, which is totally sodium-independent and a high-affinity, low-capacity carrier, which is completely sodium-dependent. However, the observations of Curran et al. (1967), Munck and Schultz (1969*a*), Paterson et al. (1981) and those of Christensen, Handlogten and Thomas (1969) suggest for the rabbit ileum an alternative model with three carriers. One is the principal carrier of neutral a.a. At 140 mM sodium it is a high-affinity, high-capacity carrier and at 0 mM sodium a low-affinity, high-capacity carrier of only neutral a.a. The second is both at 140 and 0 mM sodium a high-affinity, low-capacity carrier of both neutral and cationic a.a. The third is the low-affinity, high-capacity carrier of lysine, which at 140 mM sodium is a low-affinity, high-capacity carrier also of neutral a.a., but at 0 mM sodium a low-affinity, high-capacity carrier of lysine, which is inaccessible to neutral a.a.

In principle this model is confirmed by the present study. However, a following study (Munck, accompanying paper) of the transport of β-alanine and 2-(methylamino)isobutyric acid indicates that in addition the rabbit ileum possesses an imino acid carrier, which is also a low-affinity carrier of neutral a.a., and a β-alanine-accepting carrier, which is also a high-affinity carrier of both neutral and cationic a.a. Some of the results have been presented in a review of the comparative aspects of intestinal transport of amino acids (Munck, 1983).

Materials and Methods

Materials

Female albino rabbits with a body weight of 2500 to 3000 g were used. The animals were maintained with free access to food and water; they were killed by intravenous injection of pentobarbital sodium. For a part of the study of sugar transport a section between 60 and 90 cm from the ileo-coecal junction was used; otherwise the most distal 20 to 30 cm of ileum were used.

All solutions were made from a phosphate buffer with a pH of 7.4, and the composition (in mM) of: Na, 140; K, 8; Ca, 2.6; Mg, 1; Cl, 140; phosphate, 8; and SO_4, 1. Except when otherwise stated the solutions contained 5 mM D-glucose. Mannitol was used to create isotonicity between the solutions of each experimental series. Sodium-free solutions were obtained by substituting choline for sodium. L-amino acids, D-glucose, and D-galactose were used.

^{14}C-labeled alanine, leucine, lysine, D-galactose, D-glucose, tetraethylammonium (TEA), and polyethyleneglycol (mol. wt. 4000), and ^{3}H-labeled mannitol and polyethyleneglycol (mol. wt. 4000) were purchased from New England Nuclear Co.

Methods

Using the technique described for the rabbit ileum (Schultz et al., 1967) influx of a substance "*A*" across the brush-border membrane, J^A_{mc}, was measured at different concentrations, $[A]_m$, and at different inhibitor concentrations, $[I]_m$, in the mucosa-bathing solutions. The chambers for this technique were slightly modified by reducing the area of the exposed mucosal surface to 0.62 cm^2, by separating the individual wells by 0.7 cm high septa, and by placing the Lucite® stoppers loosely over the wells. Thus it became possible to use very high rates of O_2 flows to stir and oxygenate the solution in the wells. The tissues were incubated for 30 min with amino acid-free solutions and the incubation fluid was then withdrawn; before injection of the test solution the mucosal surface was gently wiped with soft paper to remove adherent incubation fluid. The test incubation lasted for 0.5 min, except that in one series of measurements of glucose transport incubation periods of 0.25 to 8 min were used. When sodium-free solutions were used the incubation fluid was changed three times, and in several series the withdrawn test solutions were analyzed for sodium by flame photometry.

^{14}C-labeled polyethyleneglycol was used as extracellular marker when ^{3}H-labeled mannitol was used to measure the influx of the latter substance. Otherwise ^{3}H-labeled polyethyleneglycol was used as extracellular marker.

It was assumed that transport across the brush-border membrane could be described as the sum of one or two saturable processes and free diffusion:

$$J^A_{mc} = MM_1(+MM_2) + P[A]_m, \quad (1)$$

where

$$MM = \frac{J_{max}[A]_m}{K_t + [A]_m + \frac{K_t[I]}{K_i}},$$

P = diffusive permeability of A in μmol cm^{-2} (serosal area) · hr^{-1} · $[A]_m^{-1}$, J_{mc} = μmol cm^{-2} (serosal area) hr^{-1}; $[A]_m$, $[I]_m$, K_t and K_i are in mM. The estimates of the transport kinetics presented in Eqs. (2)–(5) were obtained by nonlinear least-square fitting (Wolberg, 1967; Gardner & Atkins, 1982) of this model to the experimental relationships between J^A_{mc} and A_m. The errors of these estimates are SD, and they are evaluated by the chi-square test with the degrees of freedom (f) being the number of concentrations less the number of estimated parameters. The K_i values were calculated from ratios between inhibited and uninhibited fluxes assuming that these are described by Eq. (1). Errors on fluxes and estimates of K_i are SE. For these data P values below 0.05 by Student's t-test are taken as evidence of statistical significance.

Results

Diffusion, Unstirred Layers, PD-Effects

The question of homogeneity of transport of an amino acid or sugar can be judged by graphic plots of J^A_{mc} *vs.* $J^A_{mc}/[A]_m$ which will be upward concave if carriers with different K_t values are involved. However, diffusive contributions to the rates of transport will have the same effect, whereas unstirred layers will have the opposite effect (Preston, 1982). It is therefore of interest to gain information on the diffusive permeability of the amino acids and sugars in the rabbit ileum. For the judgment on the estimates of the transport kinetics presented here it would also be valuable to be able to compare the present technique and those used in other studies with respect to the effects of unstirred layers. If two substances are transported by several carriers but only one of the involved carriers is shared by the two substances, they will be partial competitive inhibitors. However the picture of partial competitive inhibition may also be seen if both substances are subject to rheogenic transport, even if they have no common carrier (Schultz, 1980). This phenomenon has important implications for the evaluation of the role of the lysine carriers in the transport of neutral amino acids.

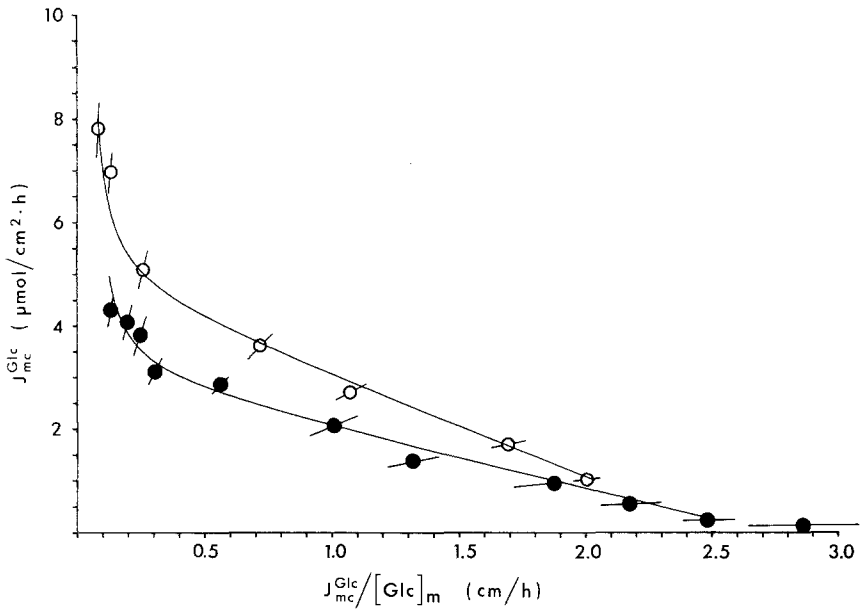

Fig. 1. Influx of glucose across the brush-border membrane of: The section of the rabbit's small intestine 60 to 90 cm from the ileo-coecal junction. The data (●) are means ± SE of 8 to 13 measurements. The curve is described by the Eadie-Hofstee transformation of Eq. (1). The distal 20 to 30 cm rabbit ileum. The data (○) are means ± SE of eight to nine measurements. The curve is described by the Eadie-Hofstee transformation of Eq. (2)

Diffusion

The question of diffusive permeability was examined by measurements of influx of tetraethylammonium which is a monovalent cation with a molecular weight close to that of lysine, and by measuring the influx of mannitol, J_{mc}^{Man}, and that of D-galactose in the presence of 200 mM D-glucose. Both TEA and mannitol as well as D-galactose were used at 1 mM. J_{mc}^{TEA} was 0.015 ± 0.005 μmol/cm² · hr (n = 16), J_{mc}^{man} was 0.034 ± 0.002 μmol/cm² · hr (n = 13), and J_{mc}^{Gal} was 0.025 ± 0.003 μmol/cm² · hr (n = 16). Similarly in the jenunum J_{mc}^{Gal} was measured at 1 mM D-galactose in the presence of 200 mM D-glucose; here J_{mc}^{Gal} was 0.039 ± 0.005 μmol/cm² · hr (n = 8). J_{mc}^{TEA} will be used as an estimate of the diffusive permeability of lysine. The data on the transport of mannitol and galactose indicate that J_{mc}^{Man} may overestimate the diffusive permeability of hexoses such as galactose and glucose.

Unstirred Layers

In the rabbit jejunum J_{mc}^{Glc} has been studied using the present technique modified to allow graded rates of magnetic stirring (Thomson & Dietschy, 1980) and in experiments with microvesicles of brush-border membranes (Wright et al., 1983). With the purpose of comparing with the unstirred layers of these techniques the jejunal J_{mc}^{Glc} was measured in paired experiments using glucose concentrations between 0.1 and 40 mM. Glucose was not added to the preincubation fluid. In these experiments the dry weight of the exposed tissues was determined after the extraction of the labeled substances. Similar experiments were made with ileal preparations using glucose concentrations between 0.5 and 100 mM glucose.

The relatively high values for J_{max}^{Glc} led to a series of experiments, in which, at 0.5 mM glucose, J_{mc}^{Glc} was measured in paired experiments, where exposure times ranging from 0.25 to 8 min were used.

The data of Fig. 1 describe the transport of glucose across the brush-border membrane of the jejunum. The curve of this figure is described by the Eadie-Hofstee transformation of Eq. (2).

$$J_{mc}^{Glc} = \frac{(3.05 \pm 0.20)[Glc]_m}{(1.14 \pm 0.09) + [Glc]_m} + (0.05 \pm 0.01)[Glc]_m \ \mu mol/cm^2 \cdot hr. \quad (2)$$

By the chi-square test the fit (f = 8) between this equation and the experimental data is characterized by a P-value of 0.9.

The dry weight of these preparations was 3.21 ± 0.05 mg/0.62 cm². The J_{max}^{Glc} of 3.05 μmol/cm² · hr then corresponds to 0.98 μmol/100 mg d.w. · min.

The data of Fig. 1 describe the transport of glucose in the rabbit ileum. The curve of this figure is described by the Eadie-Hofstee transformation of Eq. (3).

$$J_{mc}^{Glc} = \frac{(4.8 \pm 0.2)[Glc]_m}{(1.9 \pm 0.1) + [Glc]_m} + (0.033 \pm 0.003)[Glc]_m \mu mol/cm^2 \cdot hr. \quad (3)$$

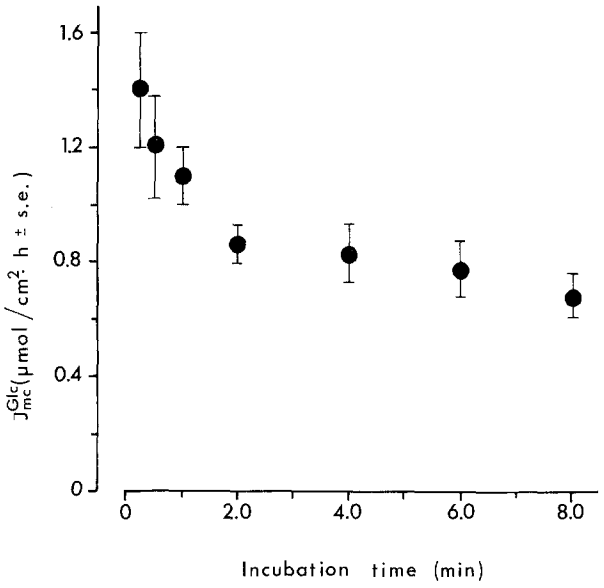

Fig. 2. Time course of the influx of glucose across the brush-border membrane of the distal 20 to 30 cm rabbit ileum measured at 0.5 mM glucose. The data are means ± SE of six measurements

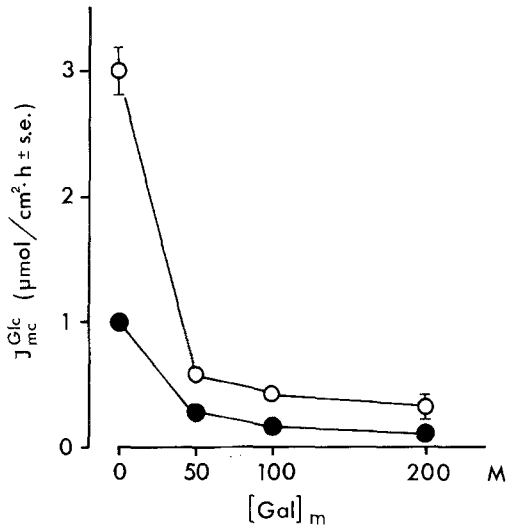

Fig. 3. Efficiency of galactose as inhibitor of the influx of glucose across the brush-border membrane of the distal 20 to 30 cm rabbit ileum (○) for which the data are means ± SE of four measurements, and the section of the small intestine 60 to 90 cm from the ileo-coecal junction (●), for which the data are means ± SE of 10 measurements

By the chi-square test the fit ($f = 4$) between this equation and the experimental data is characterized by a P-value of 0.9.

In the experiments on the rabbit ileum the dry weights were determined on separate pieces of tissues and found to be 4.5 ± 0.2 mg/0.62 cm²; the J_{max}^{Glc} then corresponds to 1.18 μmol/100 mg d.w. · min.

The data of Fig. 2 describe the effect of the duration of the incubation on J_{mc}^{Glc} expressed in μmol/cm² · hr. It is evident that already exposure times of 2 min are too long for initial rates to be measured. If the uptakes are calculated without correction for the extracellular contamination and the ensuing data normalized to the 0.25 min uptake of Fig. 2, the same time course is obtained. Thus the decline of rate of uptake with increasing duration of incubation is not a result of increasing PEG-4000 space (Thomson & Dietschy, 1980*a*). The conflict between these results and those obtained with magnetic stirring (Thomson & Dietschy, 1980*b*) is readily explained by the use in the latter study of 120 mM glucose for measuring the time course of uptake: at this high concentration approximately 75% of the total uptake will be by a process of diffusion.

In both the jejunum and the ileum J_{mc}^{Glc} was measured in paired experiments at 1 mM D-glucose using 0, 50, 100 and 200 mM D-galactose as inhibitor. For both sections the K_i of galactose was the same at all concentrations (Fig. 3), being 8.4 ± 0.2 mM ($n = 3$) and 11 ± 2 mM ($n = 3$), respectively, for the jejunum and the ileum. Thus the use of galactose as inhibitor did not reveal any inhomogeneity in the transport of glucose.

PD-Effects

At 140 mM sodium partial competitive inhibition was observed only for lysine as inhibitor of J_{mc}^{Ala} and J_{mc}^{Leu}. Therefore, inhibitory effects related to inhibitor-induced changes in PD or intracellular concentrations of sodium were of interest primarily for the interpretations of these results. To estimate the magnitude of these effects in the ileum J_{mc}^{Gal} was measured in paired experiments at 1) 1 mM galactose, 2) 1 mM galactose +90 mM alanine, 3) 1 mM galactose +90 mM alanine +200 mM lysine. In these experiments the tissues were incubated at 5 mM glucose, but washed briefly with glucose-free solution before injecting the test solutions. J_{mc}^{Gal} (μmol/cm² · hr ± SE) was in 1) 0.38 ± 0.02 ($n = 16$), in 2) 0.33 ± 0.03 ($n = 14$), and in 3) 0.29 ± 0.02 ($n = 16$). Together 200 mM lysine and 90 mM alanine caused a statistically significant 23% inhibition of J_{mc}^{Gal}, but added in addition to the 90 mM alanine the 200 mM lysine only imposed a statistically insignificant 13% inhibition. This result indicates that neither an effect of lysine on PD nor its possible effect on the intracellular activity of sodium suffice to significantly inhibit the transport of alanine.

Kinetics of Lysine Transport

In paired experiments J_{mc}^{Lys} was measured at 140 mM Na^+ using concentrations of lysine between 0.5 and 160 mM lysine, and similarly at 0 mM sodium using

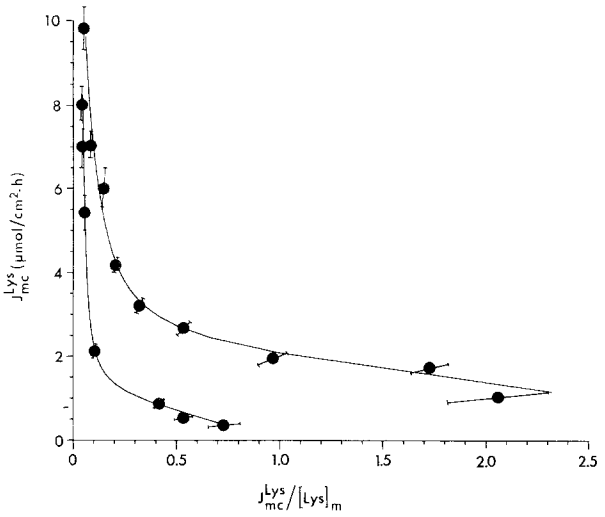

Fig. 4. Influx of lysine across the brush-border membrane of the distal 20 to 30 cm rabbit ileum. Upper curve: measurements at 140 mM sodium; the data are means ± SE of five to eight measurements. The curve is described by the Eadie-Hofstee transformation of Eq. (4). Lower curve: measurements at 0 mM sodium. The data are means ± SE of five to 11 measurements. The curve is described by the Eadie-Hofstee transformation of Eq. (5)

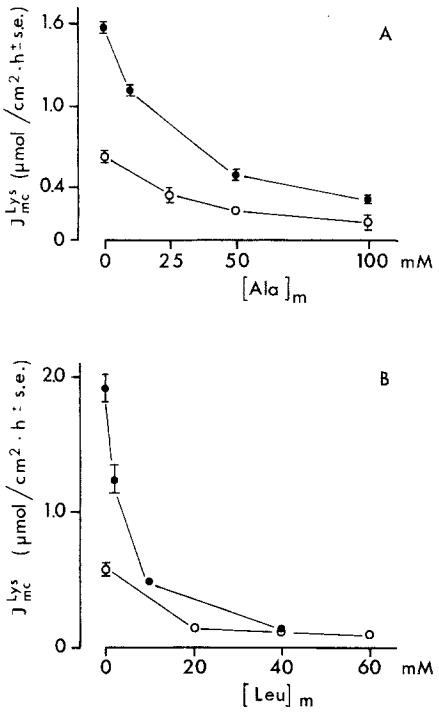

Fig. 5. Efficiencies of alanine (section *A*) and leucine (section *B*) as inhibitors of lysine (1 mM) influx across the distal 20 to 30 cm rabbit ileum measured at 140 mM sodium (●) or 0 mM sodium (○). The data are means ± SE of four measurements

concentrations of lysine between 0.5 and 200 mM. Analyzed by nonlinear regression the results (Fig. 4) could be described only by assuming that at least two saturable transport systems were involved. When, assuming that the diffusive permeability to lysine could be described by J^{TEA}_{mc}, the data of Fig. 4 were corrected by subtracting $(0.015 \pm 0.005)[Ly]_m$, then Eqs. (4) and (5) were obtained as descriptions of lysine transport at 140 mM Na and 0 mM Na, respectively.

$$J^{Lys}_{mc} = \frac{(2.33 \pm 0.34)[Lys]_m}{(0.56 \pm 0.20) + [Lys]_m} + \frac{(6.9 \pm 1.6)[Lys]_m}{(64.4 \pm 34.5) + [Lys]_m} + 0.015 \pm 0.005[Lys]_m \quad (4)$$

$$J^{Lys}_{mc} = \frac{(1.19 \pm 0.15)[Lys]_m}{(1.23 \pm 0.38) + [Lys]_m} + \frac{(8.8 \pm 3.5)[Lys]_m}{(231 \pm 149) + [Lys]_m} + 0.015 \pm 0.005[Lys]_m. \quad (5)$$

By the chi-square test the fit ($f = 5$) between Eq. (4) and the experimental data is characterized by a *P*-value of 0.5 and that of Eq. (5) ($f = 3$) by a *P*-value of 0.85. The curves of Fig. 4 are described by Eadie-Hofstee transformations of Eqs. (4) and (5).

After correction by subtracting $(0.015 \pm 0.005)[Lys]_m$ mol/cm² · hr it was also attempted to fit the data of Fig. 4 obtained at 140 mM sodium to a model of one saturable contribution alone and to one saturable contribution plus diffusion. For these models the best fit had *P*-values of 0.0005 and 0.05, respectively.

Alanine and Leucine as Inhibitors of J^{Lys}_{mc}

Both alanine and leucine were examined as inhibitors of J^{Lys}_{mc} at 1 mM lysine where according to Eqs. (4) and (5) 90% of the total transport should be on the high-affinity carrier, and at 20 mM lysine where the high- and the low-affinity carriers should contribute equally to the transport of lysine.

At 1 mM lysine and 140 mM Na, alanine was used at concentrations of 10, 50 and 100 mM; and leucine at 2, 10 and 40 mM. At 1 mM lysine and 0 mM Na, alanine was used at 25, 50 and 100 mM; and leucine at 20, 40 and 60 mM. The results are shown in Fig. 5. Estimates of the K_i values for alanine and leucine against J^{Lys}_{mc} were made from the mean values obtained at the different concentrations of the inhibitors assuming a K_t of lysine of 0.5 mM both at 140 and at 0 mM sodium. For alanine K_i appeared to be 7.7 ± 0.3 mM ($n = 3$) at 140 mM Na and 8.9 ± 0.7 mM ($n = 3$) at 0 mM Na; for leucine K_i was 1.1 ± 0.1 mM ($n = 6$) at 140 mM Na. These estimates were essentially the same at all inhibitor concentrations. At 0 mM Na the K_i of leucine rose with increasing concentration of leucine. However, if the mean values for this series of experiments were arranged in a plot of $1/(1 - J_i/J_o)$ *vs.* $(1/[I]_m$, where J_i is the inhibited J^{Lys}_{mc} and J_o the control value (Preston et al.,

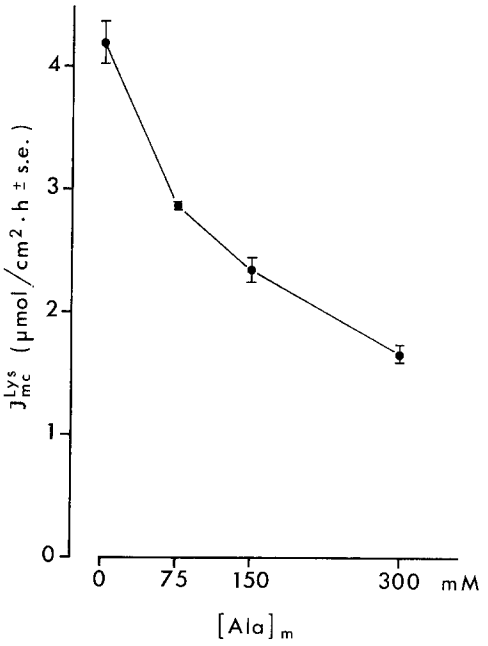

Fig. 6. Alanine inhibition of the influx of lysine (20 mM) across the distal 20 to 30 cm rabbit ileum, measured at 140 mM sodium. The data are means ± SE of six to eight measurements

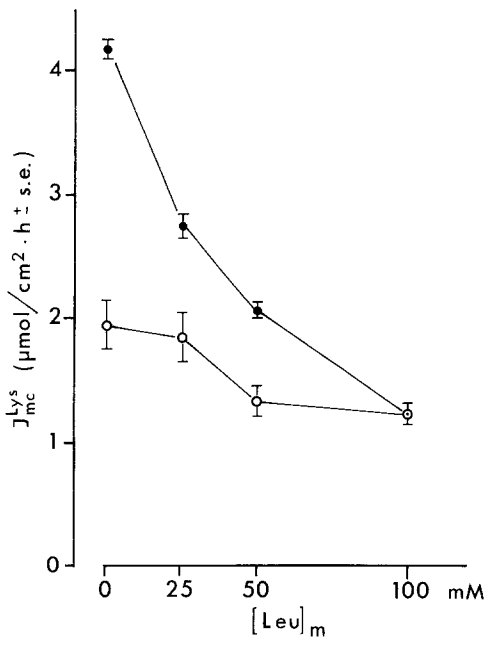

Fig. 7. Leucine inhibition of influx of lysine (20 mM) across the brush-border membrane of the distal 20 to 30 cm rabbit ileum at 140 mM sodium (●) or 0 mM sodium (○). The data are means ± SE of four measurements

1974), a visual extrapolation indicated that in these experiments a fraction of 0.07 μmol/cm² · hr was resistant to inhibition by leucine. Corrected by the 0.07 μmol/cm² · hr, which is close to the contribution to J_{mc}^{Lys} by the low-affinity lysine carrier [Eq. (5)], the data on leucine inhibition of J_{mc}^{Lys} correspond to a K_i of 1.2 ± 0.1 mM (n = 3), which is the same at the three concentrations of leucine. These results indicate that both at 140 and at 0 mM Na the high-affinity carrier of lysine may be a high-affinity carrier also of alanine and leucine.

At 20 mM lysine alanine was used as inhibitor only at 140 mM Na and at concentrations of 75, 150 and 300 mM. Both at 140 and at 0 mM Na, leucine was used at concentrations of 25, 50 and 100 mM. In addition, at 0 mM Na^+, methionine was tested as inhibitor of lysine using inhibitor concentrations of 25, 50, 100, 150 and 200 mM.

The data on alanine as inhibitor are shown in Fig. 6. In order to estimate the affinity of alanine for the low-affinity carrier of lysine the data of this figure were corrected using 2.25 μmol/cm² · hr as J_{mc}^{Lys} by the high-affinity carrier, 0.5 mM as its K_t, 7.7 mM as K_i of alanine for this carrier, 0.26 μmol/cm² · hr as the diffusive contribution to J_{mc}, and 70 mM as K_t of lysine for its low-affinity carrier. With these assumptions the K_i of alanine appeared to be essentially the same at all three inhibitor concentrations, 82 ± 11 mM (n = 3). Hence, also at 20 mM lysine, alanine is a fully competitive inhibitor of J_{mc}^{Lys}. With the same assumptions, except that K_i of leucine on the high-affinity carrier of lysine is 1.1 mM, the data of Fig. 7 demonstrate that at 140 mM Na, leucine acts as a fully competitive inhibitor with a K_i of 36 ± 4 mM (n = 3). The data (Fig. 7) on the inhibitory effect of leucine at 0 mM Na show that under sodium-free conditions only a fraction of J_{mc}^{Lys} with the magnitude of the contribution expected from the high-affinity carrier (Eq. 5) is inhibitable by leucine. Thus the data of Figs. 5 and 7 together demonstrate that it is the low-affinity carrier of lysine which becomes inaccessible to leucine under sodium-free conditions. The data of Fig. 8 demonstrate that this is also the situation in the case of methionine. Thus only in the presence of sodium may the low-affinity carrier of lysine be a low-affinity carrier also of neutral a.a.

Lysine Inhibition of J_{mc}^{Ala} and J_{mc}^{Leu}

Lysine was examined as inhibitor of the influx of alanine (1 mM) and leucine (2 mM) both at 140 mM Na and at 0 mM Na. In paired measurements of J_{mc}^{Ala} lysine was used as inhibitor at concentrations of 0, 2, 20 and 40 mM at 140 mM Na, and at concentrations of 0, 2, 40 and 80 mM at 0 mM Na. J_{mc}^{Leu} was measured under the influence of 0, 20, 40 and 80 mM lysine at 140 mM Na and at 0, 10, 30 and 90 mM

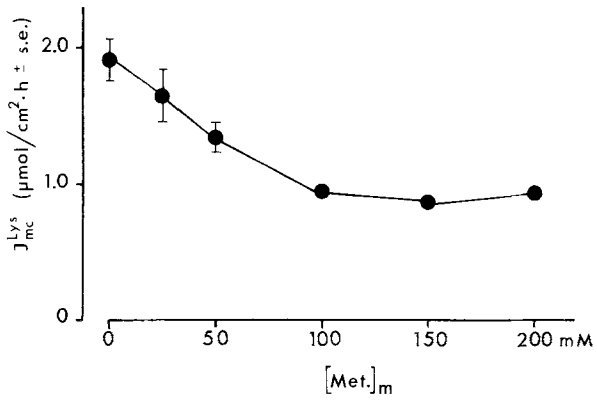

Fig. 8. Methionine inhibition of the influx of lysine (20 mM) across the distal 20 to 30 cm rabbit ileum measured at 0 mM sodium. The data are means ± SE of five to nine measurements

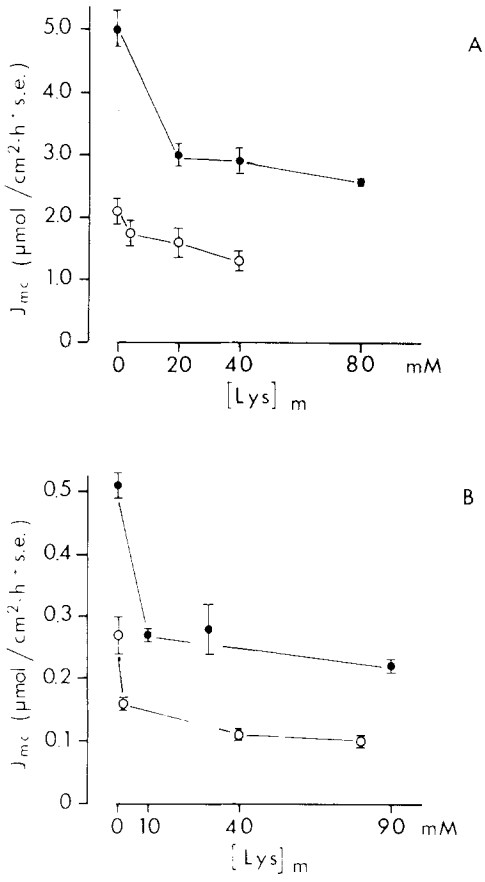

Fig. 9. Efficiency of lysine as inhibitor of the transport of alanine (1 mM) (○), and leucine (2 mM) (●), across the brush-border membrane of the distal 20 to 30 cm rabbit ileum, measured at 140 mM sodium (section *A*) or 0 mM sodium (section *B*). The data are means ± SE of four to eight measurements

lysine at 0 mM Na. The results from these four series of experiments are shown in Fig. 9*A&B*. The data obtained at 140 mM Na (Fig. 9*A*) and at 0 mM Na (Fig. 9*B*) demonstrate that under both conditions lysine is only a partially competitive inhibitor of J^{Ala}_{mc} and J^{Leu}_{mc}. The data of the figures allow reasonably good estimates of the magnitudes of the lysine-resistant transport of both alanine and leucine. At 140 mM Na 2.6 μmol/cm² · hr of a total of 5.0 μmol/cm² · hr of J^{Leu}_{mc} are resistant to inhibition by lysine and 1.25 out of 2.1 μmol/cm² of J^{Ala}_{mc} are resistant to lysine inhibition. At 0 mM Na these values are dramatically reduced, the lysine-resistant fractions of J^{Ala}_{mc} and J^{Leu}_{mc} being, respectively, 0.10 and 0.23 μmol/cm² · hr. Correspondingly, the contributions by the high-affinity system for lysine transport are reduced to, respectively, 0.2 and 0.3 μmol/cm² · hr.

When these estimates of the lysine-resistant contributions are subtracted from the mean values shown in Fig. 9*A&B*, the resulting values can be used for estimates of the K_i of lysine against the transport of alanine and leucine by the high-affinity system for lysine transport, if it is assumed that the previous estimates of K_i of alanine and leucine against the transport of lysine at 1 mM lysine describe the K_t of the two neutral a.a. for the same transport system. With these assumptions the effect of 20 mM lysine on the transport of leucine at 140 mM Na corresponds to a K_i of 1.8 ± 0.8 mM (n = 6); at 0 mM Na the effect of 10 mM lysine corresponds to a K_i of 0.6 ± 0.1 mM (n = 4). At 0 mM Na the effect of 2 mM lysine on J^{Ala}_{mc} corresponds to a K_i of 0.9 ± 0.1 mM (n = 4).

The evaluations of the data of Figs. 5 and 9*A* and *B* demonstrate that the affinities of alanine and leucine for the high-affinity transport system for lysine are sodium-independent. The data of Fig. 9*A* and *B* indicate that neutral a.a. are transported by the high-affinity carrier of lysine both at 140 and at 0 mM sodium. Assuming that both at 140 and at 0 mM sodium the K_t values for alanine and leucine are equal to their K_i values against the high-affinity transport of lysine, the much reduced rate of lysine-inhibitable transport of alanine and leucine seen at 0 mM sodium indicates that the J_{max} values for alanine and leucine by the high-affinity carrier of lysine are sodium-dependent.

Lysine was also examined as inhibitor of J^{Ala}_{mc} at 90 mM alanine and of J^{Leu}_{mc} at 20 mM leucine. The aim was to obtain supplementary evidence to that of Figs. 6 and 7 that at 140 mM Na neutral a.a. can be transported by the low-affinity system for lysine transport. In both cases lysine was used as inhibitor in paired experiments at concentrations of 0 to 200 mM. The results (Fig. 10) show that 200 mM lysine reduces the J_{mc} of both alanine and leucine by considerably more than amounts corresponding to the estimate of the J_{max} of the high-affinity transport of lysine. Thus, the data of Figs. 6, 7 and 10 together

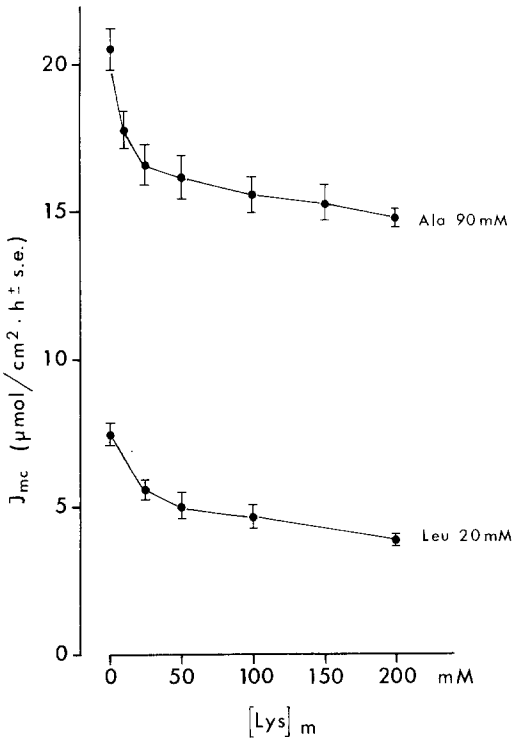

Fig. 10. Lysine inhibition of alanine (90 mM) and leucine (20 mM) influx across the brush-border membrane of the distal 20 to 30 cm rabbit ileum. For alanine the data are means ± SE of eight measurements, for leucine of six to seven measurements

leave little doubt that at 140 mM Na also the low-affinity transport system for lysine can contribute to the transport of neutral amino acids.

Lysine-Resistant Transport of Alanine and Leucine

The data of Figs. 5, 9 and 10 demonstrated that also under sodium-free conditions a system with high affinity for lysine serves as a high-affinity transport system for alanine and leucine. In order to examine whether a sodium-independent, lysine-resistant transport of neutral a.a. takes place, alanine and leucine transport was measured at 0 mM Na and, respectively, 40 and 100 mM lysine under the conditions of self-inhibition. For further clarification similar experiments were carried out at 140 mM Na. The results are shown in Fig. 11*A* (alanine) and Fig. 11*B* (leucine).

At 140 mM Na the average values for J_{mc}^{Ala} expressed as the rate of transport at 1 mM alanine under the inhibitory influence of 0, 5, 15 and 40 mM alanine are shown in Fig. 11*A*. Estimated from these values the K_i of alanine was essentially the same at all three inhibitor concentrations being 12.6 ± 0.1 mM (n = 3). This concentration-independence of the K_i indicates that at 1 mM alanine only one lysine-resistant, medium affinity transport system contributes significantly to J_{mc}^{Ala}.

In contrast to the situation for alanine the data of Fig. 11*B* demonstrate that at 140 mM Na, even in the presence of 100 mM lysine, the K_i of leucine against J_{mc}^{Leu} increases with increasing inhibitor concentration, being 1.6 ± 0.3 (n = 6) at 2 and 5 mM, 2.4 ± 0.5 mM (n = 6) at 10 mM and 3.3 ± 0.4 mM (n = 6) at 25 mM. These estimates indicate that one or more low-affinity transport systems contributes significantly to J_{mc}^{Leu} even in the presence of 100 mM lysine. If it is assumed that J_{max}^{Leu} by the low-affinity transport system for lysine is 4 μmol/cm² · hr with a K_t of 34 mM and 4.8 by the carrier of MeAIB with a K_t of 25 mM (Munck, 1983), the total J_{mc}^{Leu} calculated for 1 mM leucine will decrease from approximately 0.3 μmol/cm² · hr at 1 mM leucine to 0.18 μmol/cm² · hr under inhibition by 25 mM leucine. If the values of Fig. 11*B* are corrected accordingly, the K_i for 5, 10 and 25 mM leucine is 0.6 ± 0.006 mM (n = 3).

For both alanine and leucine the data of Figs. 11*A* & *B* demonstrate self-inhibition at 0 mM Na when lysine is present at a concentration which according to the data of Figs. 10*A* & *B* essentially completely suppresses their transport by the high-affinity lysine carrier. In the case of alanine the estimates of K_i for self-inhibition is the same at all three inhibitor concentrations, being 76 ± 4 mM (n = 3) if it is assumed that the diffusive contribution to J_{mc}^{Ala} is 0.03 μmol/cm² · hr. In the case of leucine only at 20 and 80 mM additional leucine a statistically significant inhibition was seen but neither at 10 nor at 40 mM. In addition 20 and 80 mM leucine had equal effects on J_{mc}^{Leu}.

The estimates of K_i for alanine, leucine and lysine are summarized in the Table.

Discussion

The Role of the Diffusive Contribution to J_{mc}

The magnitude of the diffusive contributions to the measured fluxes is of particular significance in three parts of the present study: 1) The evaluation of the kinetics of J_{mc}^{Glc}, 2) the evaluation of the kinetics of J_{mc}^{Lys}, and 3) the answer to the question whether neutral a.a. are subject to sodium-independent, lysine-resistant transport.

Both for the jejunum and the ileum the influx of galactose at 1 mM galactose +200 mM glucose appears as a good estimate of the diffusive permeability to glucose. For the ileum this conclusion rests on

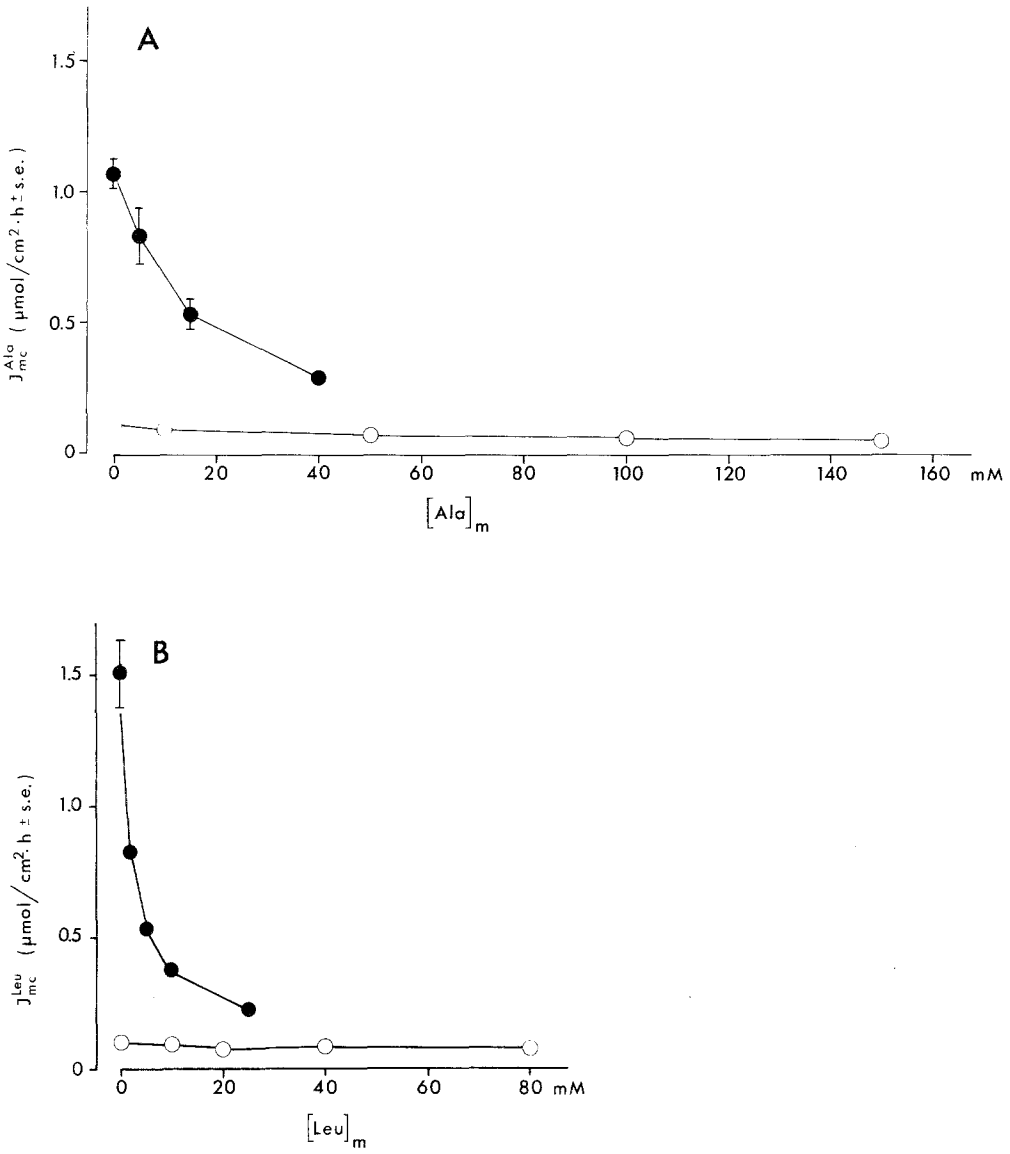

Fig. 11. *A*) Alanine inhibition of the influx of alanine (1 mM) across the brush-border membrane of the distal 20 to 30 cm rabbit ileum measured at 140 mM sodium (●) or 0 mM sodium (○) in the presence of 40 mM lysine. The data are means ± SE of four measurements. *B*) Leucine inhibition of the influx of leucine (1 mM) across the brush-border membrane of the distal 20 to 30 cm rabbit ileum measured at 140 mM sodium (●) or 0 mM sodium (○) in the presence of 100 mM lysine. The data are means ± SE of six to eight measurements

the observation of a slightly higher permeability to mannitol; and for both sections of the intestine the estimates of K_t for glucose and K_i for galactose indicate that under these conditions the mediated fraction of J_{mc}^{Gal} is an insignificant part of the total flux. These independent estimates of the diffusive permeability for glucose lend credibility to the estimates of this parameter in Eqs. (2) and (3).

TEA is a monovalent cation with almost the same molecular weight as lysine. In addition for the rat small intestine it has been demonstrated that J_{mc}^{TEA} is a valid measure of the diffusive permeability of lysine (Munck & Schultz, 1974; Munck & Rasmussen, 1979). It has therefore been assumed that also in the rabbit ileum J_{mc}^{TEA} could be used as a measure of the diffusive contribution to J_{mc}^{Lys}. This assumption was supported by the data on alanine and leucine inhibition of J_{mc}^{Lys} at 140 mM Na^+ both at 1 and at 20 mM lysine (Figs. 5–7). With higher diffusive contributions the estimates of K_i for alanine and leucine would have been increasing with increasing inhibitor concentration.

Contributions by transport systems with very low affinities, apparently nonsaturable transports, cannot be distinguished from diffusive contributions. However, in small intestinal epithelia the

Table. Kinetics of mutual inhibition between lysine, leucine, and alanine (K_i in mM) for transport across the brush-border membrane of rabbit small intestine[a]

Inhibitors	Substrates					
	1 mM lys	20 mM lys	1 mM leu	1 mM leu (+100 mM lys)	1 mM ala	1 mM ala (+40 mM lys)
Leu at 140 mM Na^+	1.1 ± 0.1 (6)	36 ± 4 (3)		0.6 ± 0.006 (3)		
Leu at 0 mM Na^+	1.2 ± 0.1 (3)	∞				
Lys at 140 mM Na^+			1.8 ± 0.8 (6)[b]		5 ± 3 (3)	
Lys at 0 mM Na^+			0.6 ± 0.1 (4)[c]		0.9 ± 0.1 (4)[d]	
Ala at 140 mM Na^+	7.7 ± 0.3 (3)	82 ± 11 (3)				12.6 ± 1.1 (3)
Ala at 0 mM Na^+	8.9 ± 0.7 (3)	∞				76 ± 4 (3)

[a] The assumptions and corrections on which these estimates are based are fully described in the text. The numbers in parentheses indicate number of inhibitor concentrations. For each concentration the K_i was calculated from means of at least four paired measurements. Errors are SE.

[b–d] These estimates are based on the use of only one inhibitor concentration; i.e. b: 20 mM lysine, c: 10 mM lysine, d: 2 mM lysine. The numbers in parentheses indicate number of measurements.

paracellular route is a significant pathway for sugars and amino acids (Munck & Rasmussen, 1979); therefore, the term diffusion is preferred for the less commital term nonsaturable transport.

Unstirred Layers

Neither in the present study nor in that of the accompanying paper were downward bending of the right-hand end of plots of J^A_{mc} *vs.* $J^A_{mc}/[A]_m$ observed. According to Preston et al. (1974) this absence suggests unstirred layers not thicker than 50 μm.

In the study of glucose transport in the rabbit jejunum with the use of magnetic stirring a J^{Glc}_{max} of 0.23 μmol/100 mg d.w. min and a K_t of 1.8 mM were observed at the maximum rate of stirring, and the real K_t was estimated to be 0.8 mM. In spite of a four times higher J^{Glc}_{max} the present technique yielded a K_t of 1.14 mM indicating that with respect to unstirred layer thickness the adaptation to magnetic stirring is inferior to the present use of the influx technique.

In the study using brush-border membrane microvesicles the transport of glucose was resolved into two saturable processes, one with a K_t of 1 mM and one with a K_t of 0.03 mM. Such inhomogeneity of J^{Glc}_{mc} could explain the absence of evidence of unstirred layer effects in Fig. 1. The present failure to accomplish a separation into the two saturable processes would then suggest a considerable unstirred layer. However, in the same microvesicle study the K_i of proline against the transport of MeAIB was determined to be 0.6 mM which is not statistically different from that of 0.8 ± 0.1 reported in the accompanying paper. It is therefore possible that the apparent inhomogeneity of glucose uptake by microvesicles reflects an inhomogeneity in the size of the microvesicles.

Depolarizations of the PD across the Brush-Border Membrane as a Contributor to Mutual Inhibitors between a.a.

In the amphibian small intestine the application of alanine or galactose dramatically depolarizes the PD across the brush-border membrane and can even reverse the control PD of about −50 mV (Gunter-Smith, Grasset & Schultz, 1982); but in 10- to 20-min prolonged exposure an almost complete repolarization takes place. A parallel transient increase in intracellular activity of sodium has also been observed (S. G. Schultz, *personal communication*). In contrast, indicating a faster basolateral response to the increased flux of sodium across the brush-border membrane (Schultz, 1981), only very moderate degrees of depolarizations have been observed in mammalian small intestines (Rose & Schultz, 1971). In agreement with these observations, in the rabbit ileum J^{Ala}_{mc} was not significantly inhibited by glucose or galactose (Chez, Schultz & Curran, 1966) and in the rat and guinea pig the mutual inhibition between sugars and a.a. amounted to only 10 to 15% (Munck, 1972, 1980; Robinson & Alvarado, 1977). The 14% inhibition which lysine caused to the transport of 1 mM galactose in the presence of 90 mM alanine is consistent with these results. However, the higher the membrane con-

ductance the lower the depolarizing effect of any rheogenic process will be (Schultz, 1980). While the lysine inhibition of J_{mc}^{Gal} was studied at 1 mM galactose, the experiments on lysine inhibition of J_{mc}^{Ala} and J_{mc}^{Leu} were made at 5 mM glucose, which corresponds to a higher degree of saturation of the sugar transport and consequently a higher electrical conductance of the brush-border membrane. Therefore, the 13% inhibition that lysine caused to J_{mc}^{Gal} overestimates the fraction of the lysine inhibition of J_{mc}^{Ala} and J_{mc}^{Leu}, which may be related to a depolarization of this membrane induced by lysine. J_{mc}^{A} may be affected by the intracellular activity of sodium; but in this case the data on lysine inhibition of J_{mc}^{Gal} demonstrate that neither is this intracellular activity significantly changed by 200 mM lysine.

Transport of Alanine, Leucine, and Lysine

Here and in the accompanying paper, as in most studies of intestinal transport of amino acids (Preston et al., 1974; Sepúlveda & Smith, 1978) competitive inhibition of the transport of *A* by *B* is seen as evidence that *A* and *B* share one or more transport systems. In the present studies this criterion has in some cases been supplemented by demonstration of inhibition of the transport of *B* by *A*. It remains to be shown that contributions to the transport of *A* and *B* exist which have K_t values which match the observed K_i values, and that for each transport system a substrate *C* has the same K_i value against *A* and *B*. However, when several transport systems are involved, meeting this *A/B/C* set of criteria (Scriver & Wilson, 1964) may be difficult (Heinz, 1972), as it requires that the several contributions to the transport of each amino acid are characterized by their K_t and J_{max} values.

This and the accompanying paper demonstrate that at least three transport systems are involved in the transport of lysine across the distal rabbit ileum, and that at least five are transporting alanine and leucine.

Lysine Transport

The analyses of the data of Fig. 4 clearly demonstrate that in addition to diffusion both high- and low-affinity processes contribute to J_{mc}^{Lys}, both at 140 and at 0 mM sodium. This interpretation confirms previous studies. Equations (4) and (5) indicate that for the low-affinity system only K_t is sodium-dependent, whereas the J_{max} of the high-affinity contribution appears sodium-dependent. In contrast either K_t (Munck & Schultz, 1969*a*) or J_{max} (Paterson et al., 1981) have previously been described as sodium-independent. The accompanying paper indicates that a β-alanine-accepting carrier, which at 140 mM sodium is a high-affinity carrier of lysine, is inoperative at 0 mM sodium. This most likely accounts for the lower J_{max} of the high-affinity transport at 0 mM sodium. It is questionable whether its K_t is also sodium-dependent. Therefore the affinities of alanine and leucine have been evaluated using a K_t for lysine of 0.5 mM both at 140 and at 0 mM sodium.

Lysine-Alanine-Leucine Interactions

When evaluated as described in the results section the data of Fig. 5 clearly demonstrate that at 140 mM sodium, alanine and leucine are high-affinity inhibitors of the high-affinity transport of lysine. However, the estimates of K_i^{Ala} and K_i^{Leu} against J_{mc}^{Lys} of, respectively, 8.7 and 1.1 mM describe the very complex processes of inhibition of the transport of lysine by the low-affinity system as well as by the two high-affinity systems. For one of the latter, the β-alanine-accepting system, the K_i of alanine and leucine against $J_{mc}^{\beta\text{-ala}}$ of 0.1 mM most likely describes the inhibitory efficiency of alanine and leucine against its contribution to lysine transport. It is also clear that alanine and leucine are low-affinity inhibitors of the low-affinity transport of lysine. These inhibitory effects strongly indicate that at 140 mM sodium the low-affinity and the high-affinity carriers of lysine are also, respectively, low- and high-affinity carriers of neutral a.a. Similarly, the almost complete inhibition of J_{mc}^{Lys} by alanine and leucine at 0 mM sodium and 1 mM lysine (Fig. 5) and the clearly only partial competitive inhibition by leucine and methionine of J_{mc}^{Lys} at 0 mM sodium and 20 mM lysine (Figs. 7 and 8) indicate that in the absence of sodium only the high-affinity, β-alanine-inaccessible carrier of lysine is accessible to neutral a.a.

In the case of the high-affinity carriers of lysine the data of Fig. 9 and the data of Fig. 5 of the accompanying paper where inhibition of J_{mc}^{Lys} and J_{mc}^{Ala} by β-alanine is demonstrated confirm these interpretations of the inhibitory effects on J_{mc}^{Lys}. With respect to the transport of neutral a.a. by the low-affinity carrier of lysine the data of Fig. 10 demonstrate that at 20 mM leucine and at 90 mM alanine 200 mM lysine reduces both J_{mc}^{Leu} and J_{mc}^{Ala} much more than corresponding to the estimate of J_{max} for the high-affinity contribution to J_{mc}^{Lys}. These results support the interpretation that the low-affinity carrier of lysine is a sodium-dependent carrier of neutral a.a.

Lysine-Resistant Transport of Neutral a.a.

100 mM lysine will reduce the transport of alanine and leucine, both at 1 mM, by the β-alanine-accessible carrier and by the other high-affinity carrier of lysine to values which are very small when compared with the rates of transport remaining at 100 mM lysine. Therefore the data of Figs. 11*A* & 11*B* allow the conclusion that in addition to these high-affinity carriers the rabbit ileum is equipped with one more high-affinity carrier of neutral a.a. As described in the accompanying paper the rabbit ileum also possesses a carrier of imino acids which may act as a low-affinity carrier of neutral a.a. but is inaccessible to cationic a.a. In addition, even at 100 mM lysine the low-affinity carrier of lysine may contribute measurably to the transport of leucine. These two contributions to J_{mc}^{Leu} suffice to account for the increase in K_i for leucine (Fig. 11*B*) with increasing concentration of leucine. The lack of a similar change in the alanine experiments is well accounted for by the much lower affinity of alanine for these two carriers.

From the data presented here it is evident that at least a part of the previously described sodium-independent transport of neutral a.a. has been accomplished by the sodium-independent contributor to the high-affinity transport of lysine. The question whether a sodium-independent, lysine-resistant, low-affinity transport of neutral a.a. takes place (Paterson et al. 1979, 1980) was addressed by the experiments of Figs. 11*A* & 11*B*. The immediate impression from the experiments on J_{mc}^{Ala} (Fig. 11*A*) was that, indeed, such a transport system exists. However, several of the results from these experiments create severe doubt about the tenability of this impression. First even 90 or 100 mM lysine will not 100% inhibit the transport of neutral a.a. by the high-affinity carriers of lysine; second, the withdrawn test solution contains 0.2 mM sodium; third, at 1 mM alanine or leucine the high lysine concentration reduces the J_{mc}^{Ala} and J_{mc}^{Leu} to values which are not much above the upper range of J_{mc}^{Man} as measured at 1 mM mannitol. Finally the data on leucine self-inhibition at 0 mM sodium (Fig. 11*B*) correspond to a K_i much higher than that estimated for alanine (Fig. 11*A*) (Preston et al., 1974; Paterson et al., 1980). It is thus possible that the sodium-independent transport of neutral a.a. is entirely accomplished by the sodium-independent high-affinity carrier of lysine, and that the high estimates of K_t and J_{max} for this transport are results of too low estimates of the passive permeabilities.

The present demonstration of reciprocal inhibition between neutral and cationic a.a. differ markedly from results obtained with a preparation of isolated brush-border membrane vesicles in which lysine inhibition of the transport of neutral a.a. could not be demonstrated (Stevens, Ross & Wright, 1982). This brush-border preparation represented the entire rabbit small intestine except the uppermost 30 cm and the last 30 cm of the ileum. Therefore the most likely explanation for these differences is that the most distal 30 cm of the rabbit small intestine are differently equipped for the transport of a.a. This interpretation is supported by the observation of the accompanying paper that, also in contrast to the microvesicle preparation, the distal rabbit ileum possesses a transport system for β-alanine, whereas in the segment of the rabbit small intestine between 60 and 90 cm from the ileocoecal junction, this capacity for transport of β-alanine is much reduced.

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