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CHARACTERISTICS OF RAT JEJUNAL TRANSPORT OF TRYPTOPHAN

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SUMMARY

The parameters of rat jejunal transport of tryptophan have been examined. The interactions between tryptophan and lysine or methionine have been reexamined, and some aspects of the trans effects of cellularly accumulated amino acids have been studied.

It has been demonstrated that:

(1) The influx of tryptophan across the jejunal brush border (J_{mc}^{Trp}) can be accounted for by the carrier of α-aminomonocarboxylic acids alone.

(2) Tryptophan competes with lysine for the carrier of basic amino acids across the brush border membrane without itself being transported by this carrier.

(3) Lysine has neither cis nor trans effects on J_{mc}^{Trp}, whereas intracellular tryptophan is highly inhibitory to J_{mc}^{Lys}.

(4) The intracellular concentration of lysine and of tryptophan, $[Lys]_c$ and $[Trp]_c$, are unaffected by tryptophan and lysine, respectively, although the transmural fluxes, from the mucosal side to the serosal side, J_{ms}, of lysine, J_{ms}^{Lys}, and of tryptophan, J_{ms}^{Trp}, are inhibited by tryptophan and lysine, respectively. The latter effects thus represent inhibitory interactions at the basolateral membrane.

(5) Methionine is a potent cis and trans inhibitor of J_{mc}^{Trp}, but stimulates J_{ms}^{Trp} and reduces $[Trp]_c$.

(6) Methionine causes trans acceleration of the influx of lysine across the brush border membrane, J_{mc}^{Lys}, but has no effect on the influx of galactose, J_{mc}^{Gal}.

(7) Leucine causes trans inhibition of J_{mc}^{Leu}.

(8) Tryptophan does not cause cis inhibition of J_{mc}^{Gal}, but is a strong trans inhibitor of J_{mc}^{Gal}.

(9) Cellularly accumulated tryptophan appears to accelerate the eventual decline in transepithelial potential difference and short-circuit current.

These results are consistent with the conclusions that:

(1) Tryptophan is transported across the brush border membrane by the carrier of neutral amino acids alone, but leaves the cell across the basolateral membrane by a mechanism used by lysine also.

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(2) Leucine, methionine and probably tryptophan have a transeffect on the transport of neutral amino acids across the brush border membrane which may represent a phenomenon which can appropriately be termed decelerating exchange diffusion.

(3) Cellularly accumulated tryptophan has a strong and indiscriminate depressive effect on all transport functions of rat jejunal epithelium.

INTRODUCTION

The mechanism of tryptophan transport across small intestinal epithelium and the cellular localization of the inhibitory interactions between tryptophan and lysine transport have not been elucidated adequately by the data available so far [1–4]. The present investigation is an attempt to elucidate the various steps involved in the transepithelial transport of tryptophan and its relation to the transport of lysine. To judge the limits of its success this attempt must be evaluated in the light of three effects of intracellularly located amino acids, two of which have not previously been described: (I) the previously described [5, 6] stimulation of the influx of lysine across the brush border membrane, (II) an inhibitor effect of neutral amino acids on influx across the brush border membrane which probably is specific for the transport mechanism for neutral amino acids and (III) a non-specific tryptophan inhibition of the transport functions of the epithelial cells. These effects, which appear as trans effects with respect to the luminal side of the brush border membrane, are also described in the present report.

MATERIALS AND METHODS

Inorganic chemicals used were of analytical grade. Sugars and amino acids were of the highest purity commercially available. ^{14}C-labelled amino acids and galactose and ^{3}H-labelled methoxyinulin were obtained from New England Nuclear Co. Male albino rats of body weight 125–150 g were used. Prior to use, the rats were kept in the laboratory for at least 18 h with free access to food and water. The total small intestine was removed under nembutal sodium anaesthesia, whereupon the rats were killed.

Unidirectional transmural fluxes

Unidirectional transmural fluxes (J_{ms}, J_{sm}) were measured by the Ussing-Zerahn technique modified as described by Schultz and Zalusky [7] for the rabbit ileum. The chamber into which a piece of gut wall was mounted consisted of two half-chambers, each with the shape of a cylindrical cone with an apex to base distance of 25 mm and a base area of 0.62 cm^2. Each half-chamber was connected to water-jacketed reservoirs thermostated to give a temperature in the half-chambers of 37 °C. The potentiometer was connected via calomel electrodes and agar bridges to the half-chambers, and the source of the short-circuiting current via Ag/AgCl electrodes and agar bridges. The agar bridges contained in each case the medium used for the experiment, gelatinized with 2 % agar, except that sugars and amino acids were omitted.

During some measurements of leucine flux it was found (Munck, B.G., unpublished) that leucine was taken up very effectively by rubber and the silicone rubber tubing used to connect the half-chambers with the water-jacketed reservoirs. This problem was eliminated when thin-walled polyethylene tubing was used instead.

Transmural fluxes were calculated from the rate of increase in radioactivity on the initially unlabelled side, in which the final activity never exceeded 1 % of that on the initially labelled side. The change in radioactivity on the initially unlabelled side was followed by taking 1-ml samples every fifth or tenth min for 60–80 min. The volume on the side from which samples were drawn was kept constant by appropriate replacement. The rate of increase in activity reached a steady state in 30 min or less. Each flux measurement, therefore, is based on at least three separate samplings. In all experiments four preparations were made from each rat, using the mid 10 cm (approximately) of the total small intestine. These four preparations were used simultaneously, and, in most of the experimental series, were used to provide two pairs of unidirectional transmural fluxes. J_{ms} and J_{sm} are used as symbols for mucosa-to-serosa, and serosa-to-mucosa fluxes, respectively.

The preparations were in all cases kept short circuited by means of an automatic voltage-clamp apparatus with four channels. The apparatus was constructed to correct for the resistance of the medium and to adjust for changes in tissue resistance. The short-circuiting current (I_{sc}) was recorded together with the potential difference (PD), which was recorded for 24-s periods every fifth min during automatic unclamping of the preparation.

Influx across the brush border membrane

Influx across the brush border membrane (J_{mc}) was measured by the method [8] described for rabbit ileum, reducing the area of exposed mucosa from 1.13 to 0.62 cm^2. From each rat the mid 15–20 cm of the total small intestine were used to provide 2×4 experimental areas. The intestine was cut lengthwise along the line of mesentery attachment. With the mucosal side facing upwards, the preparation was mounted on a lucite plate. On top of the plate a lucite block was clamped, in which four wells were drilled, exposing four mucosal areas of 0.62 cm^2. On one side of the block, with the centre lines 0.2 cm above the lower surface, two drillings were made into each well. One of these was used to inject and withdraw solutions for measurements of influx. Immediately upon withdrawal of the test solution, ice-cold mannitol solution was injected through the other drilling to stop fluxes and to wash away surplus test solution. The exposed area was punched out, removed with a pair of forceps, briefly washed in ice-cold mannitol solution, blotted on a piece of hard filter paper and placed in 2 ml of 0.1 M HNO_3. After shaking overnight, a sample was taken for analysis of radioactivity. The content of [^{3}H]methoxyinulin was used to correct for surface contamination and, thus corrected, the content of ^{14}C-labelled sugar or amino acid was used to calculate the rate of flux across the brush border membrane. Incubation periods of 0.5 to 0.7 min were used.

On the other side of the lucite block a drilling which fits a hypodermic needle is made into each well. Through the hypodermic needle vigorous stirring and oxygenation of the test solution is maintained by means of a large flow of O_2.

In order to prevent spilling from one well to another 0.7-cm high transverse septa have been inserted between the openings of the wells.

Steady-state epithelial uptake ($[A]_c$) is measured using the isolated mucosal tissue as described by Schultz et al. [9]. The same part of the intestine is used as for measurements of influx and transmural fluxes. The isolated intestine is cut lengthwise along the line of attachment of the mesentery. After being washed briefly, it is placed on a plate of glass with the mucosa facing upwards. The mucosa is separated from the rest of the tissues by means of two glass slides. The isolated mucosa is cut into pieces of approx. 100 mg wet weight. These pieces, 6–12 from each rat, are incubated for 40–80 min in 25-ml flasks containing 4 ml preoxygenated solution. After the incubation, each piece is divided into three equally large pieces. This is done on pieces of filter paper. Each piece of tissue is weighed on preweighed pieces of alumina foil, two used for extraction and the third to estimate the dry weight, i.e. the weight after 24 h at 105 °C. The extraction is performed in 2 ml of 0.1 M HNO_3 during constant shaking for 16–24 h. For each piece of tissue, 1 ml of the extract is analyzed for ^3H and ^{14}C in a Tri-carb scintillation spectrometer. From this analysis the uptake of labelled amino acid is calculated, assuming that the tritiated methoxyinulin has access to the extracellular space only, and that the specific activity and absolute concentration of amino acid is the same in the extracellular space and in the incubation medium. It is further assumed that the ratio between wet and dry weight is the same for all three pieces of each of the incubated tissue fragments.

Fluxes of sugar and amino acids are calculated on the basis of measured fluxes of radioactive tracers. The radioactivities of appropriate samples are analyzed in a Tri-Carb liquid scintillation spectrometer, using the scintillation fluid described by Bray [10].

Sacs of everted small intestine [11] were prepared from the mid 15 cm of the total small intestine. Samples of tissue extract and of serosal fluids from these sacs were examined by ascending paper chromatography at room temperature in a *sec*-butanol/formic acid/water (75 : 15 : 10) v/v system [12]. The chromatograms were either coloured by ninhydrin or cut into 0.5-cm long segments which were eluted in the scintillation fluid and analyzed for radioactivity.

In all experiments a Krebs phosphate buffer was used with 8 mM phosphate at pH 7.4. The temperature was 37 °C.

The results are stated as mean values ±S.E., with the number of observations in parentheses. P values less than 0.05 according to Student's t-test are taken as indication of statistical significance.

The following equations [8] are used to calculate the unidirectional fluxes, J_{cs} and J_{sc}, across the basolateral membrane of the epithelial cell:

$$J_{net} = J_{ms} - J_{sm} = J_{mc} - J_{cm} = J_{cs} - J_{sc} \tag{1}$$

$$J_{ms} = \frac{J_{mc} \cdot J_{cs}}{J_{cm} + J_{cs}} \tag{2}$$

$$J_{sm} = \frac{J_{sc} \cdot J_{cm}}{J_{cs} + J_{cm}} \tag{3}$$

These equations are derived for a three-compartment system in steady state. Eqn 1 is valid for the preparations. But the use of Eqns 2 and 3 is based on the assumptions that the non-epithelial tissues constitute a well stirred extension of the

fluid bathing the serosal side of the preparation, that transport takes place across an epithelium made up by a homogeneous cell population, and that passage through paracellular shunts can be neglected.

EXPERIMENTS AND RESULTS

Influx of lysine and tryptophan across the brush border membrane

Lysine influx was measured at different concentrations between 0.1 and 50 mM. In a plot (Fig. 1) of J_{mc}^{Lys} vs J_{mc}^{Lys}/Lys, the data are well described by

$$J_{mc}^{Lys} = 2.5 \cdot \frac{[Lys]_m}{3+[Lys]_m}$$

Accordingly, J_{mc}^{Lys} is assigned a K_t of 3.0 mM and a J_{max} of 2.5 μmol/cm^2 per h.

Tryptophan influx was measured at eight different concentrations between 0.1 and 40 mM. In these experiments, eight different concentrations were used for the eight areas provided by each rat. The results are shown in Fig. 2 which also includes the mean value of several series of influx measurements at 5 mM tryptophan, a concentration not included in the series of eight. In addition, the mean value of a separate series at 40 mM is included in the figure. Plots of $1/J_{mc}^{Trp}$ vs $1/[Trp]_m$ did not provide evidence of inhomogeneity for J_{mc}^{Trp}, and as shown by the figure the experimental data are well described by

$$J_{mc}^{Trp} = 11.5 \cdot \frac{[Trp]_m}{8+[Trp]_m}$$

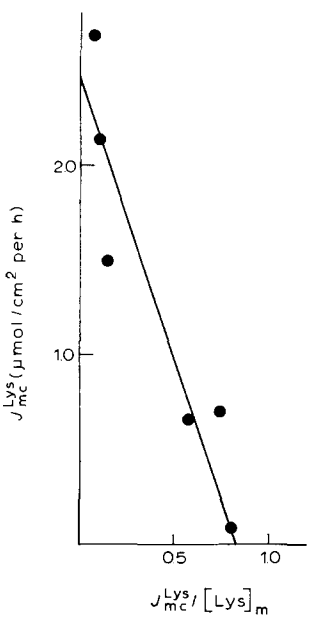

Fig. 1. Plot of J_{mc}^{Lys} vs $J_{mc}^{Lys}/[Lys]_m$. The straight line represents the linear transformation of $J_{ms}^{Lys} = 2.5\ [Lys]_m/3+[Lys]_m$.

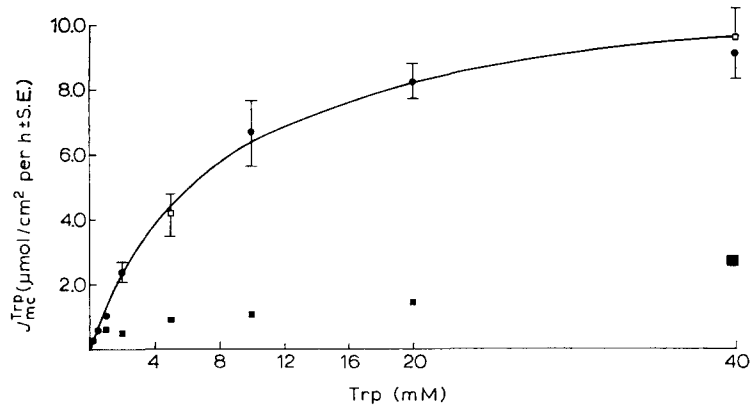

Fig. 2. Plot of J_{mc}^{Trp} as a function of $[Trp]_m$. Each point represents the mean of at least seven observations. The vertical bars indicate ±1 S.E. The curve represents the equation $J_{mc}^{Trp} = 11.5\ [Try]_m/8 + [Try]_m$. The filled circles describe data from a single experimental series. The open quadrangles represent means of separate series. Both symbols describe measurements made after preincubation in sugar and amino acid-free Krebs-phosphate buffer. The filled quadrangles represent means of at least six measurements of influx made subsequently to 30 min of preincubation at the same concentration of unlabelled tryptophan, both preincubations and test incubation were at 17 mM (−)-glucose. The symbols are larger than or equal to ± 1 S.E.

The inhibitory effect of tryptophan on J_{mc}^{Lys} was examined in a separate experimental series. Here J_{mc}^{Lys} was measured at 1 m M lysine plus 0, 2, 20 or 30 mM tryptophan in each experiment, changing the sequence of the solutions in each of the two sets of four areas provided by each rat. Based on the transport constants for lysine given above, K_i for tryptophan against lysine was calculated to be 26 mM. Accordingly, the data shown in Fig. 3 are reasonably well described by

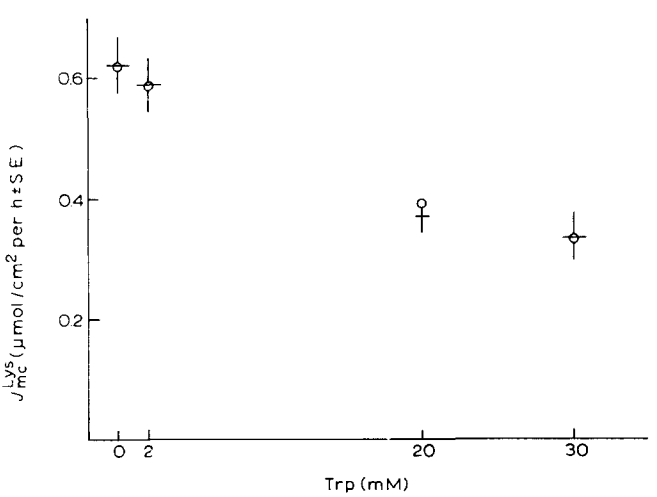

Fig. 3. Plot of J_{mc}^{Lys} at 1 mM lysine as a function of $[Trp]_m$. Horizontal bars represent means of eight measurements. Vertical bars ±1 S.E. The open circles indicate data calculated from the equation $J_{mc}^{Lys} = 2.5\ [Lys]/3 + [Lys] + 3[Trp]/26$.

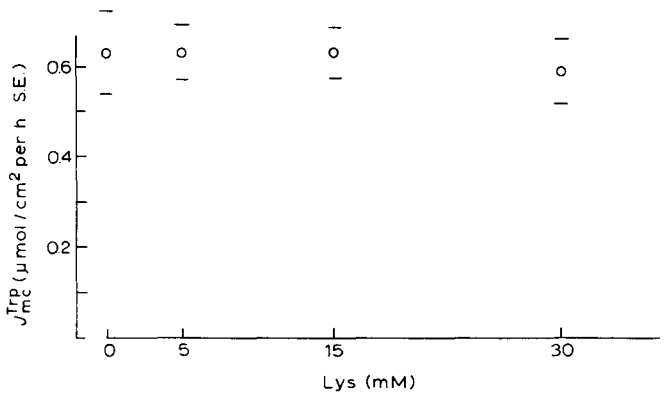

Fig. 4. Plot of J_{mc}^{Trp} at $[Trp]_m = 0.5$ mM as a function of $[Lys]_m$. Open circles indicate means of six experiments. Horizontal bars indicate ± 1 S.E.

$$J_{mc}^{Lys} = 2.5 \frac{[Lys]_m}{3+[Lys]_m+3[Trp]_m/26}$$

The inhibitory effect of lysine on J_{mc}^{Trp} was tested in experiments of the same design as above, using 0.5 mM tryptophan plus 0, 2, 15 or 30 mM lysine. It was found that lysine did not inhibit J_{mc}^{Trp} (Fig. 4). In a second series of experiments alternate areas were exposed to either 20 mM tryptophan or 20 mM tryptophan+20 mM lysine.

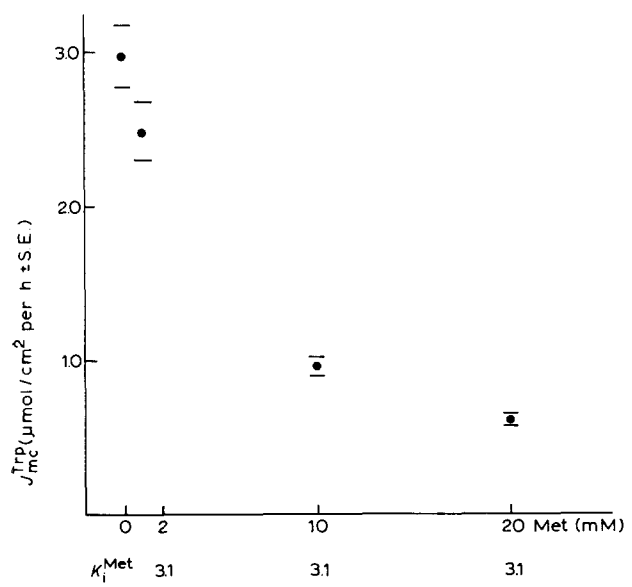

Fig. 5. Plot of J_{mc}^{Trp}, at $[Trp]_m = 5$ mM as a function of $[Met]_m$. The symbols indicate means of eight measurements ± 1 S.E. K_i^{Met} is calculated from the mean values using the equation: $J_o^{Trp}/J_i^{Trp} = ([Trp]_m+K_t^{Trp}(1+[Met]_m/K_i^{Met}))([Trp]_m+K_t^{Trp})^{-1}$ assuming $K_t^{Trp} = 8$ mM.

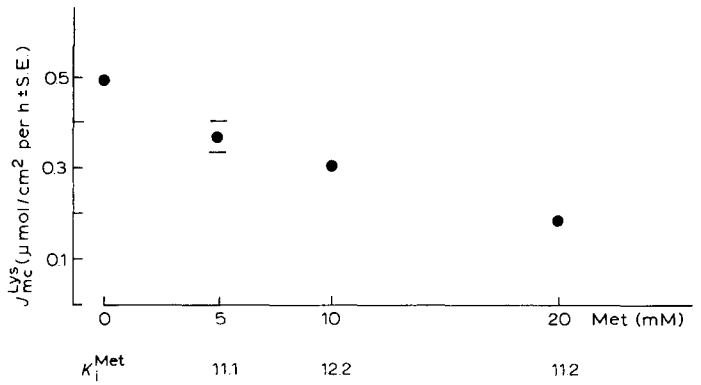

Fig. 6. Plot of J_{mc}^{Lys} at $[Lys]_m = 1$ mM as a function of $[Met]_m$. The symbols indicate means of four measurements ±1 S.E. K_i^{Met} is calculated as described for Fig. 5 assuming that $K_t^{Lys} = 3$ mM.

The fluxes were 3.56±0.12 ($n = 8$) and 3.67±0.18 ($n = 7$) μmol/cm² per h, respectively. Thus J_{mc}^{Trp} cannot be inhibited by lysine.

In order to substantiate the description of the kinetics of J_{mc}^{Trp} and to facilitate the interpretation of the preloading effects described below for J_{mc}, several series of experiments were made to estimate the kinetics of mutual inhibition between a number of amino acids.

In two series, the inhibitory effects of methionine on J_{mc}^{Trp} and J_{mc}^{Lys} were examined. For tryptophan, each experiment of 2×4 areas was made with 5 mM tryptophan plus 0, 1, 10 or 20 mM methionine. For lysine, the experiments were made with 1 mM lysine plus 0, 5, 10 or 20 mM methionine. From the results (Figs 5 and 6), K_i values for methionine were calculated using the K_t values for lysine and tryptophan stated above. The curves of the figures are based on the means of the calculated values for K_i. These were 3 and 11 mM against tryptophan and lysine, respectively. The value of 3 mM agrees well with results from a parallel study which indicated a K_t between 2 and 4 mM for the influx of methionine across the brush border membrane.

The inhibitory effect of tryptophan on J_{mc}^{Met} was examined by measuring J_{mc}^{Met} at 1 mM methionine plus 0, 5, 20 or 40 mM tryptophan. Based on the means of four observations at each concentration and using the procedure described in the legend to Fig. 5, K_t^{Met} was calculated to be 2.0±0.1 mM ($n = 3$). In the calculation, it is assumed that K_i for tryptophan is 8 mM. The fact that methionine may be transported by the carrier of basic amino acids is neglected. This approximation suffices to account for the difference between the estimates of 2 mM for K_t^{Met} and 3 mM for K_i^{Met}.

The inhibitory effect of leucine on J_{mc}^{Lys} was examined by measuring J_{mc}^{Lys} at 1 mM lysine plus 0, 5, 10 or 20 mM leucine. Based on the mean values of seven observations at each concentration and assuming a K_t for lysine of 3 mM, K_i^{Leu} was calculated to be 5.1±0.5 mM ($n = 3$). Finally, in a parallel study, J_{mc}^{Leu} was measured at concentrations between 0.1 and 40 mM. Assuming that leucine was transported by the lysine carrier with a J_{max} of 2.5 μmol/cm² per h, and a K_t of 5 mM, and ascribing the carrier of neutral amino acids a J_{max} for leucine of 6 μmol/cm² per h, the data on J_{mc}^{Leu} were well described by a K_t^{Leu} of 4 mM for the neutral carrier; the difference between observed and calculated values for the eight concentrations was 10±3 %.

Transconcentration effects on the influx across the brush border of tryptophan, lysine, leucine and galactose

In these experiments, alternate areas were preincubated for 30 min with control solutions, mostly plain Krebs buffer, and experimental solutions as stated in Table I. After a brief wash with plain buffer, influxes were measured under conditions as stated in the table.

The effects of preloading at 1 and 2 mM methionine on J_{mc}^{Trp} were measured, because the results together with the estimated K_i^{Met} against J_{mc}^{Trp} (Fig. 5) could be used to distinguish between an effect from the cytoplasmic side of the brush border membrane, a true trans effect, and an effect of methionine leaking from inside this membrane to its outside unstirred layer, a simple cis inhibition. It was found that preincubation at 1 or 2 mM methionine resulted in 30 and 50 % inhibition, respectively, of J_{mc}^{Trp} measured at 5 mM tryptophan. From the kinetics described above, the degrees of cis inhibition should not exceed 20 and 30 %, respectively. In a further attempt to distinguish between cis and trans effects of the preloading procedure the additive effect of cis inhibition was examined. After preincubation at 2 mM methionine, J_{mc}^{Trp} was measured at 1 mM tryptophan or at 1 mM tryptophan plus 2 mM methionine. Supporting the concept of a true trans effect, the degree of cis inhibition is only 6 % less than predicted by the kinetic constants.

The effects of methionine preloading on J_{mc}^{Gal} and L_{mc}^{Lys}, the effects of tryptophan preloading on J_{mc}^{Gal} and J_{mc}^{Lys} and the cis effect of tryptophan on J_{mc}^{Gal} were also examined. It was the purpose of these experiments to examine the specificity of the trans effects, particularly in the case of tryptophan.

Indicating that the trans inhibition by methionine is specific for the carrier of neutral amino acids, it was seen that preloading with methionine enhanced J_{mc}^{Lys} by 50 %, but had no effect on J_{mc}^{Gal}.

Preloading with tryptophan (2 mM) strongly inhibited both J_{mc}^{Lys} and J_{mc}^{Gal}. The high K_i^{Trp} against J_{mc}^{Lys} (Fig. 3) and the inability of tryptophan to cis inhibit J_{mc}^{Gal} clearly demonstrate that the inhibition of J_{mc}^{Gal} and J_{mc}^{Lys} seen after preincubation at 2 mM tryptophan represents a kind of trans effect.

It has previously been demonstrated [5, 13] that preloading with leucine enhances J_{mc}^{Lys} without affecting J_{mc}^{Leu}. The demonstration that leucine has very similar K_t values for the carriers of neutral and basic amino acids suggested that the apparent lack of leucine-induced trans inhibition of J_{mc}^{Leu} might result from an approximately equal inhibition of influx by the carrier of neutral amino acids and stimulation of influx by the carrier of basic amino acids. The validity of this explanation was tested by examining the effect of preloading at 2 mM leucine on J_{mc}^{Leu} measured at 2 mM leucine plus 15 mM lysine, 15 mM lysine being used to competitively exclude leucine from transport by the carrier of basic amino acids.

In agreement with the explanation offered above, this procedure made it possible to inhibit J_{mc}^{Leu} by means of preincubation with leucine (2 mM). This inhibition slightly exceeded the cis inhibition expected for 2 mM of an inhibitor with a K_i equal to K_t^{Leu}. It can, therefore, safely be considered an example of trans inhibition.

Unless trans effects can be ruled out, steady-state values for J_{mc} must be used when Eqns 1–3 are used to estimate J_{cs} and J_{sc}. Accordingly, therefore, with the aim of evaluating the effects of methionine and lysine on J_{cs}^{Trp} and of tryptophan on J_{cs}^{Lys}, the fifth series from above of Table I, was supplemented with the last three series of

TABLE I

TRANSCONCENTRATION EFFECTS ON PROCESSES OF INFLUX ACROSS THE BRUSH BORDER MEMBRANE

Numbers in parentheses indicate number of measurements. Errors are ±S.E. KP, Krebs phosphate buffer (pH 7.4).

Preincubation		Incubation		J_{mc} (μmol/cm^2 per h±S.E.)	
Control	Test	Control	Test	Control	Test
KP	1 mM Met	5 mM Trp	5 mM Trp	4.76±0.61 (8)	3.29±0.35 (8)
KP	2 mM Met	5 mM Trp	5 mM Trp	5.15±0.18 (4)	2.57±0.22 (4)
2 mM Met	2 mM Met	1 mM Trp	1 mM Trp+2 mM Met	0.37±0.03 (8)	0.25±0.01 (8)
KP	2 mM Met	10 mM Lys	10 mM Lys	2.11±0.13 (8)	3.05±0.10 (8)
KP	2 mM Try	1 mM Lys	1 mM Lys	0.34±0.02 (8)	0.15±0.01 (8)
KP	5 mM Try	5 mM Trp	5 mM Trp	2.84±0.19 (8)	0.78±0.04 (8)
KP	KP	1 mM Gal	1 mM Gal+5 mM Trp	0.52±0.05 (4)	0.59±0.03 (4)
KP	2 mM Try	1 mM Gal	1 mM Gal	0.51±0.02 (4)	0.34±0.04 (4)
1 mM Gal	1 mM Gal+5 mM Trp	1 mM Gal	1 mM Gal+5 mM Trp	0.45±0.04 (7)	0.19±0.01 (8)
KP	2 mM Met	1 mM Gal	1 mM Gal	0.57±0.06 (4)	0.64±0.03 (4)
KP	2 mM Leu	2 mM Leu+15 mM Lys	2 mM Leu+15 mM Lys	1.11±0.06 (10)	0.80±0.03 (10)
5 mM Trp	5 mM Trp + 5 mM Met	5 mM Trp	5 mM Trp + 5 mM Met	1.63±0.19 (8)	0.76±0.06 (8)
2 mM Trp + 17 mM Glc	2 mM Trp + 17 mM Glc + 20 mM Lys	2 mM Trp + 17 mM Glc	2 mM Trp + 17 mM Glc + 20 mM Lys	1.13±0.10 (8)	0.92±0.22 (8)
KP	20 mM Lys	20 mM Lys	20 mM Lys	1.93±0.13 (8)	1.74±0.09 (8)

TABLE II

EFFECTS OF TRYPTOPHAN ON STEADY-STATE TRANSPORT PARAMETERS OF LYSINE

It is assumed that measurements of J_{mc}, made after 30 min preincubation, represent the steady-state rate of unidirectional influx. J_{cs} and J_{sc} are calculated from Eqns 1 and 2 on the basis of this assumption. Numbers of experiments are given in parentheses. Errors are ±S.E.

	1 mM lysine	1 mM lysine + 2 mM tryptophan	20 mM lysine	20 mM lysine + 2 mM tryptophan
$[Lys]_c$ mM ± S.E.	5.1 ± 0.7 (12)	6.0 ± 0.7 (12)	50 ± 3 (10)	48 ± 1 (8)
J_{ms}^{Lys} μmol/cm² per h ± S.E.	0.12 ± 0.02 (4)	0.06 ± 0.01 (4)	0.56 ± 0.09 (5)	0.45 ± 0.07 (5)
J_{sm}^{Lys} μmol/cm² per h ± S.E.	0.01 ± 0.001 (4)	0.02 ± 0.001 (4)	0.84 ± 0.10 (5)	0.70 ± 0.08 (5)
J_{mc}^{Lys} μmol/cm² per h ± S.E.	0.34 ± 0.02 (8)	0.15 ± 0.01 (8)		
J_{cs}^{Lys} μmol/cm² per h ± S.E.	0.13	0.07		
J_{sc}^{Lys} μmol/cm² per h ± S.E.	0.02	0.03		

the table. The results of these experiments are incorporated in Tables II–IV. Here it suffices to note that neither J_{mc}^{Trp} nor J_{mc}^{Lys} was significantly affected by the procedure of preincubating at 20 mM lysine.

TABLE III

EFFECTS OF LYSINE ON STEADY-STATE TRANSPORT PARAMETERS OF TRYPTOPHAN

Numbers of experiments are given in parenthesis. Errors are ±S.E.

	2 mM tryptophan	2 mM tryptophan±20 mM lysine
$[Trp]_c$ mM±S.E.	9.6±0.8 (12)	8.3±0.6 (12)
J_{ms}^{Trp} μmol/cm² per h±S.E.	0.17±0.01 (8)	0.12±0.01 (8)
J_{sm}^{Trp} μmol/cm² per h±S.E.	0.032±0.004 (6)	0.038±0.005 (6)
J_{mc}^{Trp} μmol/cm² per h±S.E.	1.13±0.10 (8)	0.92±0.22 (8)
J_{cs}^{Trp} μmol/cm² per h±S.E.	0.17	0.12
J_{sc}^{Trp} μmol/cm² per h±S.E.	0.04	0.04

TABLE IV

EFFECTS OF METHIONINE ON STEADY-STATE TRANSPORT PARAMETERS OF TRYPTOPHAN

Assumption and calculation as explained for Table II.

	5 mM tryptophan	5 mM tryptophan+5 mM methionine
$[Trp]_c$ mM±S.E.	16.3±1.9 (12)	8.2±0.6 (12)
J_{ms}^{Trp} μmol/cm² per h±S.E.	0.21±0.01 (8)	0.28±0.02 (8)
J_{sm}^{Trp} μmol/cm² per h±S.E.	0.12±0.01 (7)	0.13±0.03 (9)
J_{mc}^{Trp} μmol/cm² per h±S.E.	1.63±0.12 (8)	0.76±0.06 (8)
J_{cs}^{Trp} μmol/cm² per h±S.E.	0.23	0.36
J_{sc}^{Trp} μmol/cm² per h±S.E.	0.14	0.21

Transmural unidirectional fluxes of tryptophan and lysine

As an essential step in describing the transport of tryptophan by a small intestinal epithelium, J_{ms} and J_{sm} were measured at concentrations from 1 to 40 mM. J_{ms} and J_{sm} were measured on preparations from the same rat. The use of four preparations thus provided two pairs of fluxes for each rat and made it possible to obtain for each experimental animal pairs of J_{ms} and J_{sm} for two concentrations. The results are shown in Fig. 7A. The data for J_{sm} are suggestive of a saturable process. The data for J_{ms} suggest the parallel operation of an easily saturated process and of a process which cannot, with certainty, be distinguished from diffusion.

Fig. 7A also demonstrated a relative minimum for J_{ms}^{Trp} at about 5 mM tryptophan. In a separate series of experiments this phenomenon was examined by

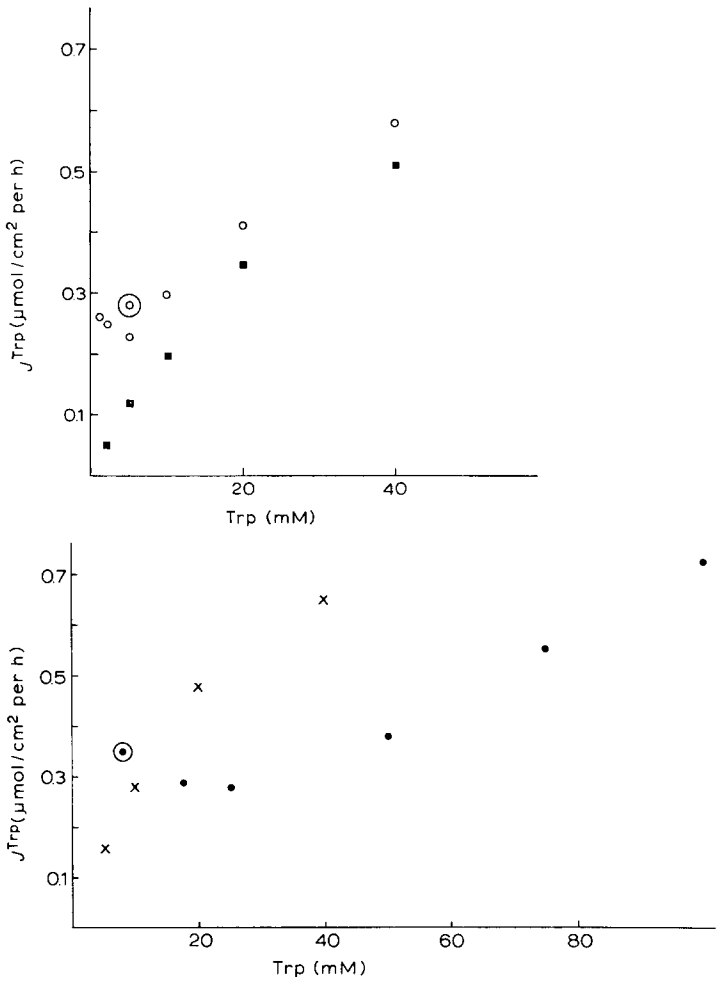

Fig. 7. Plots of tryptophan fluxes. (A) J_{ms}^{Trp} vs $[Trp]_m$ is symbolized by open circles. J_{sm}^{Trp} vs $[Trp]_s$ is symbolized by closed quadrangles. J_{ms}^{Trp} measured at 5 mM tryptophan plus 5 mM methionine is indicated by an encircled open circle. Means of 4–13 measurements are shown. The S.E. values were 5–10%. (B) J_{cs}^{Trp} vs estimates of $[Trp]_c$ are indicated by closed circles. J_{sc}^{Trp} vs $[Trp]_s$ is indicated by crosses. J_{cs}^{Trp} at 5 mM tryptophan plus 5 mM methionine in the media is indicated by the encircled closed circle. The values for J_{cs} and J_{sc} were calculated as described in the text.

measuring, in paired experiments, J_{ms}^{Trp} at 1, 2, 5 and 10 mM tryptophan. The flux values were (μmol/cm^2 per h) 0.21 ± 0.02 ($n = 6$), 0.21 ± 0.04 ($n = 6$), 0.27 ± 0.06 ($n = 5$), and 0.25 ± 0.04 ($n = 6$), respectively. These values were not significantly different from each other, and the relative minimum was not confirmed.

With the purpose of analyzing the interactions between tryptophan and lysine four experimental series were made. J_{ms}^{Lys} and J_{sm}^{Lys} were measured in paired experiments at 1 mM lysine with or without 2 mM tryptophan, and at 20 mM lysine with or without 2 mM tryptophan; J_{ms}^{Trp} and J_{sm}^{Trp} were measured in paired experiments at 2 mM tryptophan with or without 20 mM lysine, and likewise in paired expe-

riments J_{ms}^{Trp} and J_{sm}^{Trp} were measured at 5 mM tryptophan with or without 5 mM methionine. It was found that 2 mM tryptophan significantly inhibited J_{ms}^{Lys} at 1 mM lysine, but not at 20 mM (Table II), that at 2 mM tryptophan J_{ms}^{Trp} was significantly inhibited by 20 mM lysine (Table II), and that J_{ms}^{Trp} was significantly stimulated by the presence of 5 mM methionine (Table IV).

All transmural unidirectional fluxes were measured with 17 mM glucose in the medium.

Steady-state accumulation of tryptophan and lysine

The steady-state epithelial accumulation of tryptophan or lysine was measured with the purpose of completing the analysis of the epithelial transport of tryptophan and of its relationships to lysine transport. Steady-state accumulation of tryptophan was measured at 1, 2, 5, 10, 20 and 40 mM tryptophan. The results of these measurements are used in Fig. 7B for the plot of J_{cs} vs $[Trp]_c$. Estimates of the effect of tryptophan (2 mM) on lysine accumulation at 1 mM lysine are shown in Table II. The effect of 20 mM lysine on the accumulation of tryptophan at 2 mM tryptophan is shown in Table III, and the effect of 5 mM methionine on the accumulation of tryptophan at 5 mM tryptophan is shown in Table IV. It was found that tryptophan and lysine did not affect the steady-state accumulation of each other. The accumulation of tryptophan, however, was markedly inhibited by methionine.

Using the data on J_{ms}^{Trp}, J_{sm}^{Trp} and J_{mc}^{Trp} after preincubation, J_{cs} and J_{sc} were calculated by means of Eqns 1 and 2. In Fig. 7B the results are plotted against the estimated steady-state tissue concentrations and the serosal concentrations, respectively. These curves indicate that the basolateral membrane of the epithelial cell is capable of active transport of tryptophan into the cells.

A search for a non-specific effect of intracellular tryptophan

All measurements of transmural fluxes were made under short-circuit conditions, and the short-circuit currents were recorded. These recordings demonstrated that at concentrations of tryptophan of 2 mM or more the decline in short-circuit current was unusually rapid. As demonstrated by Fig. 8 the decline in I_{sc} at 20 mM tryptophan plus 17 mM glucose is so rapid that ionic fluxes and I_{sc} can hardly be correlated. Nevertheless, unidirectional transmural fluxes of sodium and chloride were measured in paired experiments at 20 mM tryptophan plus 17 mM glucose. On 12 out of 24 preparations the flux rates were measured for the first 10 min of incubation, and in contrast to the situation usual for this period the J_{ms}^{Na} and J_{ms}^{Cl} were higher than during any subsequent period. Furthermore, for all preparations, during any 10-min period measured the ionic fluxes were higher than during any subsequent period. Disregarding the absence of a steady state for the fluxes of chloride and sodium, mean rates were calculated for the first 60 min of incubation. $J_{net} = J_{ms} - J_{sm}$ were 1.7 ± 0.5 ($n = 6$) μequiv/cm^2 per h for sodium and -1.4 ± 0.3 ($n = 6$) μequiv/cm^2 per h for chloride, equivalent to an I_{sc} of 3.1 μequiv/cm^2 per h or 84 μA/cm^2. The mean maximum value for I_{sc}, usually reached within the first 2–4 min of incubation, was 240 ± 18 μA/cm^2 ($n = 20$); after 30 min of incubation the mean for these 20 preparations was 80 ± 18 μA/cm^2, and after 60 min of incubation the mean was 26 ± 4 μA/cm^2.

Of these results only the early maximum is within the range seen in com-

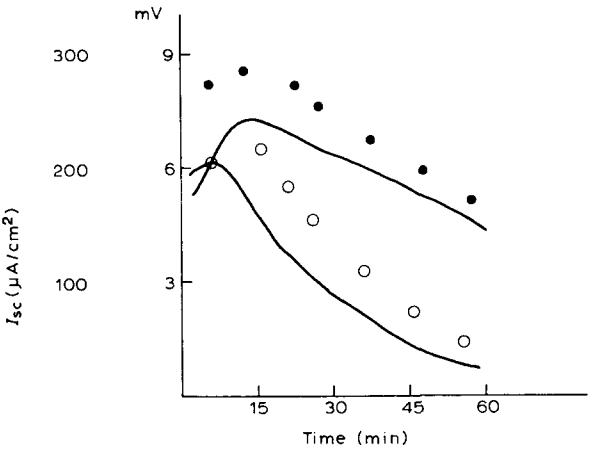

Fig. 8. Tracings of recordings of transmural potential difference (PD) and short-circuit current (I_{sc}) of rat jejunum. Upper curve and closed circles recorded at 2 mM tryptophan plus 17 mM (+)-glucose. Lower curve and open circles recorded at 20 mM tryptophan plus 17 mM (+)-glucose.

parable experiments [14]. After 30 min of incubation I_{sc} is only 50 % of the value which would have been found during incubation at 17 mM glucose alone. After 60 min of incubation, the I_{sc} is reduced to about 50 % of the value expected for this time of incubation in the absence of both sugars and amino acids. The net flux of sodium is a little higher than previously observed under comparable conditions [14], whereas the net flux of chloride is much smaller than that usually observed for rat jejunum, in the absence of sugars and amino acids as well as during transport of these substances. It is thus seen that within the first 10 min of incubation in spite of a normal

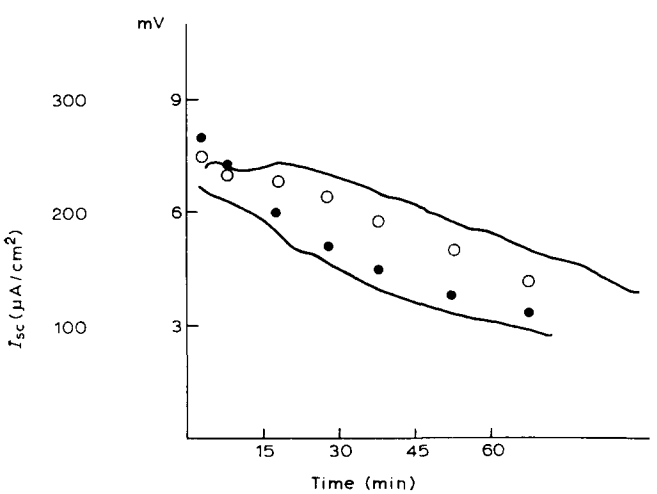

Fig. 9. Tracings of recordings of rat jejunal PD and I_{sc}. Upper curve and open circles recorded at 5 mM tryptophan plus 5 mM methionine plus 17 mM (+)-glucose. Lower curve and closed circles recorded at 5 mM tryptophan plus 17 mM (+)-glucose.

initial stimulation of I_{sc} the presence of 20 mM tryptophan leads to a dramatic decline in I_{sc}, and that this decline is primarily reflected in a reduction of J_{net}^{Cl}.

Fig. 9 serves to demonstrate that the presence of 5 mM methionine reduced the rate of decline of I_{sc} induced by 5 mM tryptophan and delayed its onset.

Metabolism of tryptophan

Ascending paper chromatograms of extracts of everted sacs incubated for 2 h with labelled tryptophan, and of samples of the serosal fluids of these sacs, revealed the presence of a metabolite with an R_F value of 0.86, against 0.54 for tryptophan. The metabolite accounted for 2–3 % of the total radioactivity.

DISCUSSION

In the course of the present investigation we have confirmed the previous observation [3] that tryptophan effectively inhibits transepithelial transport of lysine, and that transepithelial transport of tryptophan can be stimulated by methionine and inhibited by lysine (Tables II–IV). We shall attempt to interpret these data, and to describe the characteristics of tryptophan transport. Before this, however, two other aspects of the study must be dealt with.

Active transport across the basolateral membrane

Firstly, the plots in Fig. 7B of the calculated values for J_{cs} and J_{sc} indicated the existence of a mechanism for the active transport of tryptophan into the epithelial cell across the basolateral membrane. Obviously, a number of factors could contribute to this conclusion.

One factor could be that the estimates of intracellular concentrations of tryptophan used in Fig. 7B are not representative of the concentrations in the transporting cells. Another and more important factor could be that the diffusion barrier and unstirred layer of the villous cores, submucosal, muscular, and serosal strata constitute a fourth compartment. The effect of this compartment will be that influx across the basolateral membrane can be significant and that consequently the values given for J_{ms} underestimate the true unidirectional efflux across the basolateral membrane of the epithelium. Such underestimates, of course, will be carried through to the calculated J_{cs} values. In measuring J_{ms}^{Trp} and J_{sm}^{Trp}, 17 mM glucose was used in order to reduce the effect of the non-epithelial tissues as a diffusion barrier. It is possible, as will be explained elsewhere in a forthcoming publication that glucose creates a fluid circuit [15] between the tissues and the mucosal fluid. By enhancing a paracellular fraction of J_{sm} such a fluid circuit could contribute to the direction of the J_{net}^{Trp} deduced for the basolateral membrane. However, Naftalin and Curran [16] have very recently proposed an active transport of galactose across the basolateral membrane into the rabbit ileal epithelium. And further, on the basis of autoradiographic studies with sacs of everted hamster small intestine, Kintner and Wilson [17] made the same proposal for this epithelium.

Transconcentration effects on influx across the brush border membrane

Secondly, it was observed that preincubating the tissues with a number of amino acids profoundly affected the subsequent influx of amino acid across the brush border membrane.

That preincubation with methionine enhances J_{mc}^{Lys} (Table I) confirms the similar observation made by Reiser and Christiansen [18] on isolated cells from rat small intestine. And the clear cis inhibition of J_{mc}^{Lys} by methionine shows that the stimulation of J_{mc}^{Lys} induced by preincubating with methionine is a true trans effect. It has previously been shown that methionine stimulates transepithelial transport of lysine [19]. The present observation makes it very likely then that the effects of methionine on transport of basic amino acids [19, 20] are similar in details to those of leucine [5].

The effects of tryptophan preloading are almost as unambiguously defined as trans effects as that of methionine preloading on J_{mc}^{Lys}. It appears to be a phenomenon of general inhibition of all epithelial transport functions. This conclusion is based on observation of the preloading-induced inhibition of J_{mc}^{Lys}, J_{mc}^{Trp} and J_{mc}^{Gal} which all by far exceed the possible degrees of cis inhibition. In addition, the time course of the tryptophan effect on I_{sc} demonstrates that the inhibition of ion transport is also secondary to the epithelial accumulation of tryptophan.

It was demonstrated that a metabolite of tryptophan occurred during incubation with epithelial tissues. It, therefore, remains an open question whether tryptophan or a metabolite of this amino acid is responsible for the trans effects observed.

The nature of the type of preloading effect demonstrated for methionine against J_{mc}^{Trp} is more difficult to interpret. The reason is that, because methionine is also a very effective cis inhibitor of J_{mc}^{Trp}, effects of amino acids, e.g. methionine, contained in the unstirred layer at the epithelial surface can probably not be ruled out completely. However, the procedure of vigorous stirring reduces the thickness of the unstirred layer [21], and the procedure of washing between pre- and test incubations can be expected to remove remnants of the preincubation medium; but hereafter, the unstirred layer is replenished by means of a backflux from the epithelium. However, between the termination of the preincubation and the beginning of the test incubation, the epithelium must to some extent discharge the accumulated amino acid to its serosal side. The maximum concentration attainable on the luminal side of the brush border membrane must, therefore, be considerably lower than that used for preincubation. With methionine, therefore, the concept of trans inhibition is significantly supported by the observation that the inhibitory effects of preloading exceeded the degree of cis inhibition one would expect from the concentrations used for preincubation. Furthermore, if the effects of preloading were just an expression of cis inhibition, then, as it was seen at 1 mM tryptophan with or without 2 mM methionine after preincubation at 2 mM methionine, the inhibition, which is undoubtedly of the cis type, should not be completely additive to the inhibitory effect of preloading. In the case of methionine it thus seems reasonable to conclude that a true trans effect has been observed.

In the case of leucine, the preloading procedure reduced the influx of ^{14}C-labelled leucine slightly more than could be accounted for as cis inhibition by 2 mM leucine, strongly indicating a true trans effect. That this trans effect is not seen in the absence of lysine [5, 13] strongly indicates that, as for the transport of lysine, leucine transport by the lysine carrier is enhanced by leucine preloading.

The data presented thus indicate that in general the carrier of neutral amino acids is susceptible to trans inhibition and that of basic amino acids to trans stimulation whether transporting basic or neutral amino acids. The nature of these effects is

unsettled. But the absence of an effect of lysine preloading on J_{mc}^{Lys}, rules out that in the case of the lysine carrier a process of simple competitive exchange diffusion [22] is involved.

Tryptophan transport

The data on J_{mc}^{Trp} (Fig. 2) were well described by an equation for a single, saturable process. Therefore, they do not support the idea that tryptophan should cross the brush border membrane by more than one carrier [3].

Plots of J_{ms} and J_{sm} vs the concentrations of the incubation media are suggestive of saturability. The plot of J_{sc} vs the serosal concentration confirms this impression, indicating that influx across the basolateral membrane is a carrier-mediated process. For J_{cs} plotted against the estimated intraepithelial concentrations the picture is less clear. But this plot together with the plot of J_{ms} vs the mucosal concentration indicates that two processes may be involved in the transfer of tryptophan across the basolateral membrane, one with a K_t well below 20 mM and one with a considerably higher K_t. It is, however, not possible separately to estimate the J_{max} or K_t values for these two processes.

Interactions between tryptophan and lysine

Fig. 3 indicates that tryptophan is a competitive inhibitor of the binding of lysine to its carrier with a K_i of 26 mM. Consistent with the homogeneity of any plot of J_{mc}^{Trp} vs $[Trp]_m$ it was, however, demonstrated that tryptophan is not transported by the lysine carrier. This controversy may indicate either that tryptophan, on binding, does not induce changes necessary for translocation of the substrate carrier complex across the membrane, or that it induces changes which are prohibitive to this translocation. Similar interactions have been proposed to explain the characteristics of sugar transport by hamster [23] and rabbit [24] small intestine.

The K_i^{Trp} of 26 mM shows that the tryptophan inhibition of J_{ms}^{Lys} (Table II) cannot be secondary to the inhibition of J_{mc}^{Lys}. That $[Lys]_c$ is unaffected by tryptophan leads to the same conclusion. It, furthermore, demonstrates that interactions at the basolateral membrane lead to the observed inhibition. That at 20 mM lysine, J_{ms}^{Lys} is inhibited relatively less by 2 mM tryptophan than at 1 mM lysine, and that at 2 mM tryptophan, J_{ms}^{Trp} is inhibited by 20 mM lysine, indicate that these two amino acids compete for a mutual mechanism of transport across the basolateral membrane. Most likely methionine stimulates transepithelial transport of lysine by direct interaction with the process of J_{cs}^{Lys} as described for the leucine-lysine interaction [5]. It is, therefore, consistent with the concept of a mutual carrier for lysine and tryptophan that the presence of methionine evidently (Table IV) leads to a stimulation of J_{cs}^{Trp}. However, considering the general inhibitory effect of intraepithelial tryptophan, the methionine stimulation of J_{cs}^{Trp} could be a consequence of a lower degree of autoinhibition of this flux by tryptophan, made possible by the methionine inhibition of the steady-state epithelial uptake of tryptophan (Table IV). The possibility of such a mechanism is supported by the unusual concentration dependence of J_{cs}^{Trp} (Fig. 7B). Thus, in spite of good support for a competitive relationship between tryptophan and lysine at the basolateral membrane, it remains a possibility that tryptophan inhibition of J_{ms}^{Lys} is a reflection, at the basolateral membrane, of the general inhibitory action of intraepithelially located tryptophan.

In conclusion, we have seen that J_{mc}^{Trp} can be accounted for by the function of the carrier of neutral amino acids alone, and further, that tryptophan appears to bind competitively to the carrier of basic amino acids and, in doing so, to make this carrier inoperative. For methionine, leucine and probably tryptophan, a type of trans inhibitory effect has been demonstrated. It may represent a phenomenon, which in analogy with the terminology introduced by Stein [22], could be called decelerating exchange diffusion. The specificity of this effect is best illustrated by the case of methionine, which intracellularly has the effect of stimulating J_{mc}^{Lys}, and of inhibiting J_{mc}^{Trp} without affecting J_{mc}^{Gal}. In contrast to this type of trans effect, cellular tryptophan is shown to have a marked, but apparently totally indiscriminate inhibitory effect on all epithelial transport functions.

The combined results of the examination of interactions between lysine, tryptophan, and methionine leads to the conclusion that interactions at the basolateral membrane are decisive for the inhibition of J_{ms}^{Trp} and J_{ms}^{Lys} by lysine and tryptophan, respectively, and for the methionine stimulation of J_{ms}^{Trp}. The data furthermore suggest that tryptophan uses the carrier of basic amino acids for passage across the basolateral membrane.

The data for the transport of tryptophan across the basolateral membrane indicate the participation of a saturable process, but a detailed interpretation is too hazardous at this stage. Firstly, because of the general inhibitory effect of cellularly accumulated tryptophan, and, secondly, because of the possibility of a significant diffusional component in both J_{ms} and J_{sm}.

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REFERENCES

1 Spencer, R. P. and Samiy, A. H. (1960) Am. J. Physiol. 199, 1033–1036
2 Cohen, L. L. and Huang, K. C. (1964) Am. J. Physiol. 206, 647–652
3 Munck, B. G. (1966) Biochim. Biophys. Acta 126, 299–307
4 Reiser, S. and Christiansen, P. A. (1971) Biochim. Biophys. Acta 241, 102–113
5 Munck, B. G. and Schultz, S. G. (1969) Biochim. Biophys. Acta 183, 182–193
6 Munck, B. G. (1972) in Transport Across The Intestine (Burland, W. L. and Samuel, P. D., eds), pp. 187–194, Churchill Livingstone, London
7 Schultz, S. G. and Zalusky, R. (1964) J. Gen. Physiol. 47, 567–584
8 Schultz, S. G., Curran, P. F., Chez, R. A. and Fuisz, R. E. (1967) J. Gen. Physiol. 50, 1241–1260
9 Schultz, S. G., Fuisz, R. E. and Curran, P. F. (1966) J. Gen. Physiol. 49, 849–866
10 Bray, G. A. (1960) Ann. Biochem. Exp. Med. 1, 279–285
11 Wilson, T. H. and Wiseman, G. (1954) J. Physiol. London 123, 116–125
12 Stein, W. H. (1953) J. Biol. Chem. 201, 45–58
13 Munck, B. G. (1972) Biochim. Biophys. Acta 266, 639–648
14 Munck, B. G. (1972) J. Physiol. London 223, 699–717
15 Ussing, H. H. (1969) Q. Rev. Biophys. 1, 365–376
16 Naftalin, R. and Curran, P. F. (1974) J. Membrane Biol. 16, 257–278
17 Kinter, W. B. and Wilson, T. H. (1965) J. Cell Biol. 25, 2, 19–39
18 Reiser, S. and Christiansen, P. A. (1972) Biochim. Biophys. Acta 266, 217–229

19 Munck, B. G. (1972) in Transport Across The Intestine (Burland, W. L. and Samuel, P. D., eds), pp. 169–185, Churchill Livingstone, London
20 Robinson, J. W. L. (1968) Eur. J. Biochem. 7, 78–89
21 Preston, R. L., Schaeffer, J. F. and Curran, P. F. (1974) J. Gen. Physiol. 64, 443–467
22 Stein, W. D. (1967) in Movement of Molecules Across Cell Membranes, Vol. 6, Theoretical and Experimental Biology (Danielli, J. F., ed.), p. 156, Academic Press, New York
23 Caspary, W. F., Stevenson, N. R. and Crane, R. K. (1969) Biochim. Biophys. Acta 193, 168–178
24 Goldner, A. M., Schultz, S. G. and Curran, P. F. (1969) J. Gen. Physiol. 53, 362–383