Biochimica et Biophysica Acta, 512 (1978) 172—191

BBA 78115

KINETICS OF THE ABSORPTION OF AMINO ACIDS BY THE RAT INTESTINE IN VIVO

J.-A. ANTONIOLI *, Cl. JOSEPH and J.W.L. ROBINSON

Départements de Pharmacologie Clinique et de Chirurgie Expérimentale, Centre Hospitalier Universitaire Vaudois, and Institut de Physique Nucléaire, Faculté des Sciences de l'Université, Lausanne (Switzerland)

(Received November 7th, 1977)
(Revised manuscript received April 21st, 1978)

Summary

The kinetics of L-phenylalanine and L-lysine absorption by the rat small intestine in vivo have been studied by perfusing intestinal segments and monitoring simultaneously the uptake of the substrate into the intestinal tissue and its disappearance from the perfusate.

The rate of phenylalanine disappearance is a linear function of the substrate concentration. Its uptake into the tissue is rapid and obeys saturation kinetics, but is not concentrative. Both tissue uptake and disappearance rate can be inhibited by leucine or methionine, but are not influenced by hydrophilic neutral or dibasic amino acids.

Lysine disappearance from the perfusate and its uptake into the tissue both display saturation kinetics. Lysine transport is quantitatively smaller than that of phenylalanine. Both uptake and disappearance are inhibited by arginine and leucine, but are unaffected by other neutral amino acids or sugars.

To analyse the kinetic results, integrated equations were developed to express the final concentration in the perfusate in terms of the original concentration. The disappearance rate was considered as a mixed process (saturable and non-saturable in parallel) in a one-compartment system, and the uptake by the tissue was treated as a two-compartment system in which the amino acid entered the cells by a mixed process but left them by a pure non-saturable mechanism.

The results concerning disappearance from the lumen are compatible with the one-compartment model. Phenylalanine absorption can be described by a major non-saturable component and a minor saturable one, while lysine absorption occurs almost entirely by a saturable process. The two-compartment model does not adequately describe the tissue uptake results.

* Present address: Laboratoire Biologique, Société d'Assistance technique pour Produits Nestlé S.A., CH-1350 Orbe, Suisse.

Introduction

The uptake of amino acids by excised intestine in vitro involves accumulation in the mucosa against a concentration gradient, whilst their velocity of uptake is a saturable function of the substrate concentration, obeying Michaelis-Menten kinetics, and subject to competitive inhibition by homologous amino acids [1]. However, the relevance of the mechanisms elucidated from studies in vitro to the overall process of absorption in vivo remains a challenging problem, as testified for instance by studies with diethylglycine [2] which is well absorbed in vivo but not in vitro. In man, perfusion techniques are commonly used for clinical investigations [3,4], but such methods do not always provide kinetic parameters in agreement with those obtained in vitro [5].

Few studies on the kinetics of amino acid transport from perfused loops of rat intestine have been published, despite available methodology [6]. Isolated results have indicated that the absorption of certain amino acids by the perfused intestine is either non-saturable or described by a very high K_m [7—10]. However, the evaluation of most of these data is questionable since the direction of the fluxes of substrate during perfusion has only seldom been controlled, and allowance has usually not been made for the drop in substrate concentration of the perfusate. Certain authors have recently pointed out that amino acid uptake from a perfusate into the intestinal tissue does not follow the same pattern as its disappearance rate from the lumen [11—13]; furthermore, the level of the amino acid in question in the plasma could play a role in the regulation of this absorption [14].

These various results appear to question the widespread interpretation of absorption rates in vivo in terms of a carrier-mediated transcellular transport mechanism equivalent to that observed in vitro. Accordingly, we have examined the kinetics of L-phenylalanine and L-lysine absorption from the perfused rat intestine, comparing the characteristics of absorption from the perfusate with those of uptake into the tissue in vivo. We also present integrated equations to describe our results for luminal disappearance or tissue uptake. An iterative method is used to fit these equations to the experimental data in order that the kinetic constants of the models can be evaluated. This novel approach has enabled us to define the kinetic nature of amino acid absorption in the living animal.

Theory

Symbols and units

Q	amount of substrate transferred	mmol
s	substrate concentration	mmol · l^{-1}
V	volume of a compartment	l
C'	first-order constant	l · min^{-1}
C	diffusion constant	min^{-1} ($=C'/V$)
D	diffusion constant	min^{-1}
J'	maximum rate for amount	mmol · min^{-1}
J	maximum rate for concentration (so-called maximal velocity)	mmol · l^{-1} · min^{-1} ($=J'/V$)
K_m	concentration of substrate for half maximum rate (so-called Michaelis constant)	mmol · l^{-1}

The first subscript refers to a specific compartment (i, j), and in the case of a variable concentration, a second subscript refers to time (0, t). By definition, a "compartment" is of finite volume in which concentration is maintained homogeneous. It is assumed that non-saturable or saturable transport can contribute alone or in combination to the transfer of a solute (= substrate) from the intestinal lumen to the blood.

One-compartment model

The substrate is assumed to be transferred from the intestinal lumen (compartment 1 with a finite volume) into the blood (a sink with an assumed infinite volume) by either a non-saturable, a saturable or a mixed process; the concentration of the substrate in the blood is zero at zero time. The differential equations and the integrated relations to permit the computation of $s_{1,t}$, the concentration in the lumen at time, t, are presented below:

Non-saturable process:

$$\text{equation: } \frac{ds_1}{dt} = -C_1 s_1 \tag{1}$$

$$\text{integrated relation: } C_1 t = \ln(s_{1,0}/s_{1,t}) \tag{2}$$

Saturable process:

$$\text{equation: } \frac{ds_1}{dt} = -\frac{J_1 s_1}{K_m + s_1} \tag{3}$$

$$\text{integrated relation: } s_{1,0} - s_{1,t} = J_1 t - K_m \cdot \ln(s_{1,0}/s_{1,t}) \tag{4}$$

Mixed process:

$$\text{equation: } \frac{ds_1}{dt} = -\frac{J_1 s_1}{K_m + s_1} - C_1 s_1 \tag{5}$$

$$\text{integrated relation: } \left(\frac{s_{1,t}}{s_{1,0}}\right)^{K_m C_1} \cdot \left(\frac{C_1 \cdot s_{1,t} + K_m C_1 + J_1}{C_1 \cdot s_{1,0} + K_m C_1 + J_1}\right)^{J_1} = e^{-C_1(K_m C_1 + J_1)t} \tag{6}$$

Two-compartment model

The transfer occurs from the intestinal lumen (compartment 1 of finite volume) through the intestinal wall (compartment 2 of finite volume) into the blood (a sink of infinite volume). At zero time, the concentration of substrate is zero in the intestinal wall and in the blood. The transfer from the intestinal lumen into the intestinal wall may occur by either non-saturable, saturable or mixed transport; the transfer from enterocyte into the blood is assumed to occur only by a non-saturable process. The substrate concentration in the lumen is assumed to remain constant throughout the experiment; the corresponding situation in which the luminal substrate concentration was variable gave rise to equations that could not in all three cases be solved analytically. The relations, involving the amount transferred from the lumen (Q), are:

Non-saturable process:
equations:

$$\frac{dQ}{dt} = C'(s_1 - s_2) \tag{7a}$$

$$\frac{ds_2}{dt} = C_2(s_1 - s_2) - D_2 s_2 \tag{7b}$$

integrated relation: $s_{2,t} = \frac{C_2 s_1}{C_2 + D_2} \cdot \{1 - e^{-(C_2 + D_2)t}\}$ (where $C_2 = C'/V_2$) (8)

Saturable process:
equations:

$$\frac{dQ}{dt} = \frac{J' s_1}{K_m + s_1} \tag{9a}$$

$$\frac{ds_2}{dt} = \frac{J_2 s_1}{K_m + s_1} - D_2 s_2 \tag{9b}$$

integrated relation: $s_{2,t} = \frac{J_2 s_1}{D_2(K_m + s_1)} \cdot \{1 - e^{-D_2 t}\}$ (10)

Mixed process:
equations:

$$\frac{dQ}{dt} = \frac{J' s_1}{K_m + s_1} + C'(s_1 - s_2) \tag{11a}$$

$$\frac{ds_2}{dt} = \frac{J_2 s_1}{K_m + s_1} + C_2(s_1 - s_2) - D_2 s_2 \tag{11b}$$

integrated relation: $s_{2,t} = \{1 - e^{-(C_2 + D_2)t}\} \left\{ \frac{J_2 \cdot s_1}{(K_m + s_1)(C_2 + D_2)} + \frac{C_2 \cdot s_1}{C_2 + D_2} \right\}$ (12)

Note that these equations contain a term, $J_2 = J'/V_2$, which is dependent on the volume of the cellular compartment; it therefore differs from the J_1 term of Eqn. 6. For this reason, comparisons are focused particularly on the values of K_m, which always refer to compartment 1.

The one-compartment model is applicable to the disappearance of substrate from the perfusate, whilst the two-compartment model with determination of $s_{2,t}$, the concentration in compartment 2, may be used for study of uptake into the tissue. The results concerning the disappearance of substrate from the lumen have been analysed by means of Eqn. 6, whereas those involving tissue uptake have been examined by applying Eqn. 12. Figs. 1 and 2 present simulations of these functions at different values of the two variables, t and s_0.

Methods

Male Sprague-Dawley rats (150—300 g) were fasted for 24 h, anaesthetised with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and subjected to tracheal cannulation. Two or three intestinal segments (10—15 cm in

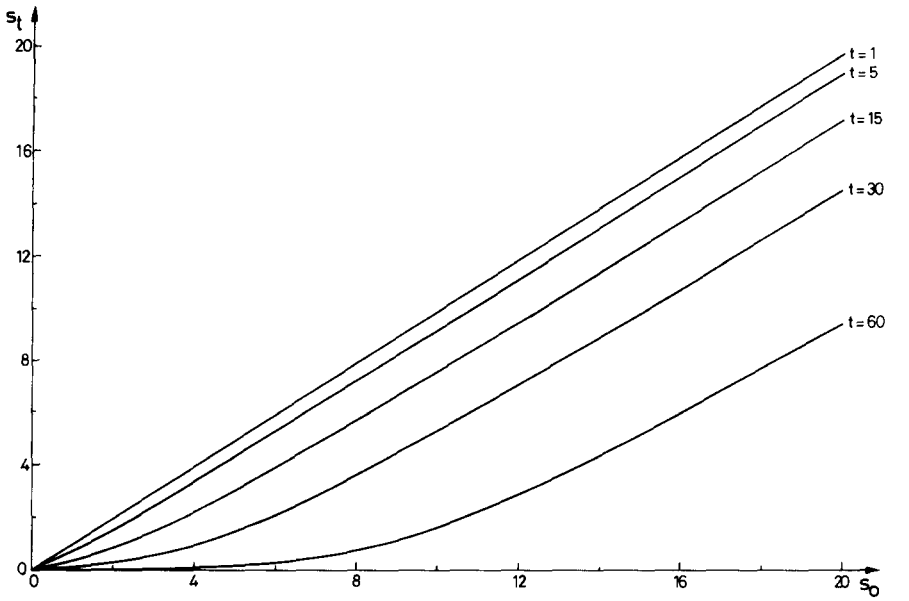

Fig. 1. Simulation of Eqn. 6 for mixed absorption of amino acid from a perfusate when the non-saturable component tends to zero. Values assigned are: $J = 0.20$; $K_m = 2.0$; $C = 1 \cdot 10^{-6}$. The final concentration in the perfusate at different times, t, is plotted against the initial substrate concentration. Note the principal properties of this function are an approximately linear relationship at early time and concavity at low substrate concentrations. An important portion of the function is almost linear.

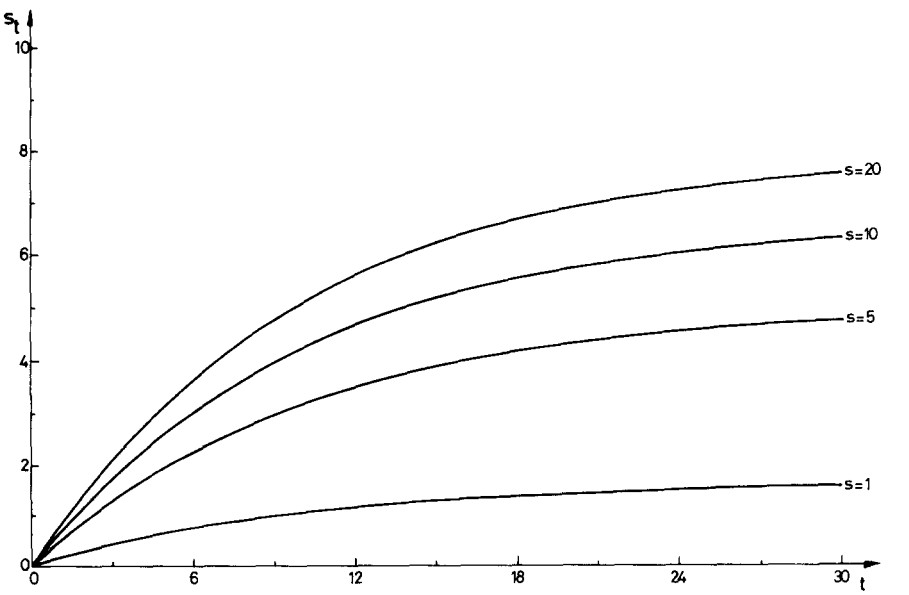

Fig. 2. Simulation of Eqn. 12 for the uptake of an amino acid into the tissue. Values assigned are: $J = 1.0$; $K_m = 50$; $D = 0.1$; $C = 1 \cdot 10^{-6}$. The substrate concentration in the lumen is plotted against time at various values of s_0. This function approaches an asymptotic value with increasing time and with increasing substrate concentration.

length) were isolated and cannulated, care being taken not to damage their vascularisation. The segments were chosen in the upper jejunum, in the lower ileum, and in a midway position. After cannulation, the loops were replaced and the abdomen was closed. They were perfused inside the abdominal cavity, generally at a flow rate of 1 or 2 ml/min, with pre-warmed perfusate.

After the segments had been rinsed for 10 min with isotonic saline, the perfusion was performed either by single-pass or multiple-pass techniques (Table I). Separate aliquots of effluent were colllected every 2 min in the single-pass experiments, or the perfusate was returned to a magnetically stirred reservoir in the multiple-pass series; the reservoir was sampled at 10-min intervals. In this latter case, the circulating volume of the perfusate was 10 ml. At the end of the perfusion, the length of the segments was measured, then samples of the intestinal wall were cut out, rinsed in ice-cold saline, gently blotted, weighed and assayed for radioactivity. In addition, some specific techniques were employed for special problems (Table I), as will be discussed more fully below.

The perfusate consisted of a mixture of 135 mM NaCl and 5 mM KCl whose pH was maintained at 7.4 with a phosphate buffer (15 mM), and which usually contained 1 mM L-phenylalanine or 0.5 mM L-lysine, labelled with a tracer dose of the corresponding uniformly ^{14}C-labelled amino acid. The osmolarity of the medium after addition of more than 20 mM amino acid was maintained by corresponding decreases in the NaCl concentration; however, the sodium was appropriately balanced at other substrate concentrations by replacement with choline chloride, so that the sodium concentration was constant within each experiment. Only amino acids in the L-configuration and sugars in the D-form were employed, and are therefore referred to henceforth without mentioning their stereoisomerism.

To determine net water movements, phenol red was added to the perfusate at a concentration of 8 mg/100 ml and determined spectrophotometrically

TABLE I

PERFUSION METHODS

The table indicates the experimental sequences in the various types of perfusion technique employed. MP, multiple pass; SP, single pass. Three segments were perfused in each rat (two in the MPa series), at a rate of 1 ml/min (or 2 ml/min in MPa series), unless otherwise stated in the text. The perfusion sequence was only repeated in the same animal in the MPa series.

Type	Sequence	Fluid	Duration (min)	Recirculation
SP	Perfusion	Medium	14—16	no
MPa	Equilibration	Medium	5	no
	Perfusion	Medium	60	yes
	Rinse	Saline	10	no
MPb	Equilibration	Medium	5	no
	Perfusion	Medium	30	yes
MPc	Equilibration	Loading	5	no
	Perfusion	Loading	15	yes
	Equilibration	Exchange	1.5	no
	Perfusion	Exchange	30	yes

[15]. In preliminary experiments, phenol red was compared with polyethyleneglycol (PEG-4000) and found to be suitable for routine work, as reported by Miller and Schedl [16].

Radioactivity was measured by conventional liquid scintillation techniques, both in the perfusate and in samples of intestinal tissue hydrolysed in boiling 30% KOH [17]. To calculate the quantity of substrate remaining in the perfusate, corrections for net water movements were applied. The rate of disappearance is expressed in μmol/m intestine per min of collection period (2 min in single-pass and 10 min in multiple-pass). The concentration of the substrate in the tissue water is calculated on the basis of 80.5% tissue water content. For the preliminary analyses, a value for the velocity of uptake was obtained by dividing this uptake by the duration of the perfusion and expressed in μmol/ml tissue water per min. When comparing results concerning intestinal weight, the average wet weight of the intestine was taken as 4.5 g/m.

Experimental design, statistical evaluation and kinetic analysis. All experiments were designed as random blocks with replicates and submitted to a two-way analysis of variance; the different treatments and the different segments constitute the two entries, and the different animals the replicates. The standard error of the means (reported as S.E. in the tables) is derived from the mean square for error in the analysis of variance; a multiple t-test was performed in order to compare different means whenever the F-test showed a statistically significant difference [18].

Kinetic analysis of the experiments in which transport was studied as a function of the initial substrate concentration was performed in two steps. First, the data were plotted on two graphs: direct plot of rate of uptake against substrate concentration, and the Woolf linearisation of the Michaelis-Menten equation [19]. Linearity was checked by regression analysis, and the respective constants were derived from the least squares fit.

Secondly, the appropriate integrated equations (for perfusate, Eqn. 6 and for tissue, Eqn. 12) were used in a search for the best values of the constants by "iteration" (using an HP 9830 desk computer). This was performed by varying the constants systematically around the values found from the aforementioned plots, in order to minimise the χ^2 values, where χ^2 is defined as the sum of the squares of the difference between the observed and calculated values divided by the standard error of the observed value [20].

Results

Preliminary considerations

Two signs of intestinal damage are easily spotted: irregular outflow of the perfusate, or its acidification, as indicated by a colour change in the medium. They are only occasionally observed, such as in the case of respiratory failure. The experiment was then discarded. In addition, routine histological controls of perfused samples and of tissues blotted for uptake measurements generally revealed only slight oedema of the lamina propria following successful perfusion.

The fate of the radioactive amino acid introduced into the intestinal lumen was investigated in three different ways: Thin-layer chromatography of effluent

perfusates and of mucosal homogenates showed that neither phenylalanine nor lysine was significantly metabolised, in confirmation of reports of other workers [21]. Secondly, the incorporation of the amino acid into mucosal protein during the perfusion was monitored by homogenising mucosal scrapings in trichloroacetic acid following a perfusion with 10 mM [^{14}C]phenylalanine for 20 min. The following values (means of three experiments) were obtained: Concentration of phenylalanine incorporated into the protein precipitate, 0.03 ± 0 mM; free phenylalanine in mucosal supernatant, 7.2 ± 3.49 mM; phenylalanine in underlying muscular layer, 3.8 ± 1.11 mM. Finally, the production of radioactive CO_2 by the rat following introduction of a single dose of radioactive phenylalanine or lysine into the duodenum was assessed by cannulating the trachea and capturing all the exhaled gas. When phenylalanine was used, the total radioactive CO_2 produced by the whole body was less than 4% of the quantity introduced in the first 30 min, and less than 10% in 2 h, whereas when lysine was employed, the values were even lower.

During single-pass perfusions with 1 mM [^{14}C]mannitol, the radioactivity in the perfusate does not change appreciably (1.0 ± 0.012 mM, $n = 6$), and the mannitol found in the tissue represents only a small fraction (2%) of the tissue water space.

Net water movements were observed during the perfusion: during the 16 min of a single-pass experiment, the concentration of phenol red increased by about 4%, and during the 60 min of a multiple-pass perfusion, the increase was about 20%.

In all experiments, the anatomical location of the test segments was randomly rotated. The two-way analysis of variance seldom revealed significant differences between the amino acid absorptions due to the anatomy of the various segments.

Directional fluxes of amino acids

In order to ascertain the degree of reversibility of the absorptive process, we have attempted to induce efflux into the lumen in various ways. In the first experiment shown in Table II, the rat intestines were preloaded (design MPc in Table I) by perfusion with 20 mM labelled phenylalanine or lysine. Then the efflux of labelled amino acid into a second perfusate containing unlabelled amino acid, which might be expected to elicit counterflow, was measured. It was found that the rate of efflux across the mucosa was less than 10% of the rate of influx, in good agreement with findings in vitro [22]. Secondly, measurements of the radioactivity in the tissue suggested that the amino acid must move preferentially towards the blood rather than towards the lumen, despite the presence of an unlabelled amino acid in the perfusate.

In addition, the movement of phenylalanine or lysine from the blood into the lumen was studied in six rats where 100 mM ^{14}C-labelled amino acid was infused into the jugular vein (experiment 2 of Table II). Despite high concentrations in the intestinal tissue, only traces of radioactivity appeared in the perfusate, which nevertheless contained 10 mM unlabelled amino acid in an attempt to elicit efflux.

Inhibition of phenylalanine absorption

The disappearance of phenylalanine in multiple-pass experiments was

TABLE II

EFFLUX OF AMINO ACID FROM TISSUE OR BLOOD INTO THE LUMINAL PERFUSATE

In the first experiment, the tissue was preloaded by perfusing the lumen for 15 min with 20 mM ^{14}C-labelled amino acid. The lumen was rinsed for 1.5 min with saline, and then perfused again with a solution containing 20 mM unlabelled amino acid (MPc). The concentrations of labelled amino acid in the blood and tissue water after the preloading perfusion and after the exchange perfusion, and the change in luminal concentration occurring during the perfusion, are given in the table. In the second experiment, 100 mM labelled amino acid was infused into the blood at a rate of 0.1 ml/min for 60 min, whilst the lumen was perfused with 10 ml of a 20 mM solution of the unlabelled amino acid. At the end of the experiment, the concentrations of labelled amino acid were determined in each compartment. Results are means ± S.E. of at least six rats in each case.

	Concentration in blood (mM)		Concentration in tissue water (mM)		Concentration change in lumen (mM)	
	Phenyl-alanine	Lysine	Phenyl-alanine	Lysine	Phenyl-alanine	Lysine
Experiment 1						
Following preloading	1.2	0.2	9.0 ± 0.7	4.9 ± 0.6	−1.8 ± 0.1	−1.4 ± 0.2
Following exchange	0.3	0.1	1.8 ± 0.2	1.7 ± 0.3	+0.1 ± 0.03	+0.3 ± 0.06
Experiment 2						
Following intravenous infusion	2.9 ± 0.4	4.4 ± 0.3	4.1 ± 0.4	4.9 ± 0.3	+0.03 ± 0.02	+0.07 ± 0.01

inhibited by leucine and methionine (Table III), but not by lysine and galactose. This observation was confirmed in single-pass experiments (Table IV), where it was also demonstrated that hydrophilic neutral amino acids, such as alanine, serine and histidine, had no effect on phenylalanine absorption. Galactose,

TABLE III

INHIBITION OF PHENYLALANINE OR LYSINE DISAPPEARANCE DURING MULTIPLE-PASS PERFUSION

MPa technique, substrate concentrations determined at sampling times stated. Inhibitors added at a concentration of 20 mM. Results are the means ± S.E. of eight determinations.

Substrate	Sampling time (min)	Concentration (mM)	Perfusate concentration as percentage of controls in the presence of:			
			Galactose	Leucine	Methionine	Lysine
Phenylalanine 1 mM	10	0.81 ± 0.145	126	70	49	102
	20	0.70 ± 0.075	109	61	68	90
	30	0.57 ± 0.055	105	71	64	93
	40	0.49 ± 0.046	100	64	55	102
	50	0.42 ± 0.034	112	70	69	88
	60	0.36 ± 0.033	84	73	67	102
			Galactose	Leucine	Methionine	Arginine
Lysine 0.5 mM	10	0.42 ± 0.136	121	51	100	92
	20	0.36 ± 0.058	128	35	64	52
	30	0.32 ± 0.032	104	43	132	47
	40	0.28 ± 0.023	118	50	96	35
	50	0.25 ± 0.024	114	56	91	62
	60	0.21 ± 0.018	104	50	91	60

TABLE IV

INHIBITION OF PHENYLALANINE ABSORPTION IN VIVO

Single-pass perfusions with 1 mM L-[^{14}C]phenylalanine. In the first experiment, galactose and the amino acids were added at a concentration of 20 mM, and the table represents the means of experiments on 27 rats. In the second experiment, nine rats were employed, and two different concentrations of leucine were tested. The results are means presented +S.E. computed from the analysis of variance; the asterisk indicates values significantly different from the controls.

Addition	Concentration in tissue water (mM)	Rate of disappearance (μmol/m per min)
None (control)	0.64	1.42
Phenylalanine	0.41 *	1.39
Leucine	0.41 *	0.78 *
Methionine	0.41 *	0.61 *
Alanine	0.55	1.39
Serine	0.59	1.27
Lysine	0.51	1.21
Histidine	0.51	0.94
Galactose	0.54	2.33 *
	±0.047	±0.132
None (control)	0.53	1.58
20 mM leucine	0.36 *	0.83
50 mM leucine	0.21 *	0.32 *
	±0.014	±0.164

which slightly stimulated phenylalanine absorption at the beginning of the multiple-pass experiments, enhanced its absorption in the single-pass series, but this was accompanied by a marked change in water movements and can tentatively be ascribed simply to solvent drag. The inhibition by leucine was dose dependent, as demonstrated by the results in the lower part of Table IV. Note that phenylalanine does not inhibit its own transport, suggesting that the absorption of this amino acid is not saturable within the 1—20 mM range.

The situation in the tissue is similar to that of the perfusate, with two exceptions. Galactose has no influence on tissue uptake, which is not surprising if solvent drag is responsible for the effect on disappearance rate. More importantly, phenylalanine inhibits its own uptake into the tissue, testifying to the saturable nature of this process.

Kinetics of phenylalanine absorption

Disappearance from the perfusate. No evidence of saturation was revealed in multiple-pass experiments over three different ranges of concentration (0.05—100 mM phenylalanine): disappearance rate increased linearly with substrate concentration ($r = 0.886$ at $s = 0.05$—1.0; $r = 0.921$ at $s = 1.0$—20; $r = 0.698$ at $s = 10$—100 mM, where r is the correlation coefficient on the direct plot).

The single-pass technique allows early and close measurements with a minimal drop in substrate concentration. Actually a 2-min interval between samples was selected; constant results were obtained from the 4th min of perfusion onwards during the next 10 min, since 4 min perfusion is needed to replace the rinsing fluid by the perfusion medium. Single-pass perfusions were performed using six different phenylalanine concentrations from 0.1 to 50 mM.

TABLE V

KINETICS OF PHENYLALANINE DISAPPEARANCE RATE AND TISSUE UPTAKE

Rat intestine was perfused by the single-pass technique with the concentrations of phenylalanine stated. Measurements were made on 2-min samples. Tissue concentration was determined after a perfusion lasting 15 min. The "calculated" values were obtained from Eqns. 6 and 12 by use of the constants given in Table VI. Results are the means ± S.E. of nine determinations on different animals.

Original luminal concentration (mM)	Final luminal concentration (mM)		Tissue concentration (mM)	
	Observed	Calculated	Observed	Calculated
0.1	0.09 ± 0.006	0.09	0.06 ± 0.004	0.07
1.0	0.87 ± 0.089	0.89	0.60 ± 0.051	0.64
5.0	4.5 ± 1.12	4.50	3.5 ± 0.35	3.01
10	8.9 ± 1.16	9.05	6.3 ± 0.51	5.43
25	23.3 ± 3.49	22.8	10.9 ± 1.48	10.9
50	45.7 ± 7.64	45.7	16.9 ± 1.02	16.4

The experimental observations are presented in Table V and the kinetic analysis of the results is given in Table VI. A high correlation on the direct plot is found, whereas only a moderate correlation occurs on the Woolf plot. Using the integrated equation for mixed transport at variable substrate concentration (Eqn. 6), the best fit of the data by iteration was obtained with a relatively

TABLE VI

KINETIC ANALYSIS OF RESULTS CONCERNING LUMINAL DISAPPEARANCE AND TISSUE UPTAKE DURING PHENYLALANINE OR LYSINE PERFUSION

All linear regressions and iteration were performed by using experimental points obtained at six different substrate concentrations, except for lysine luminal disappearance where the results at the highest lysine concentration could not validly be used for linear regression analysis. For the fitting of the tissue uptake, the arithmetic mean concentration of the substrate at the beginning and end of the perfusion was used. The number of degrees of freedom (DF) for the χ^2 is taken as the number of points minus the number of parameters. The units of the various constants are given in Theory; note that $J = J_1$ for disappearance rate and $J = J_2$ for tissue uptake.

	Phenylalanine		Lysine		
	Luminal disappearance (2 min)	Tissue uptake (15 min)	Luminal disappearance (30 min)	Tissue uptake (5 min)	Tissue uptake (35 min)
Linear regression					
Woolf plot					
r	0.595	0.960	0.978	0.948	0.948
K_m	122	48	2.7	23	24
J	6.6	2.3	0.03	2.9	0.73
Direct plot					
r	0.990	0.980	0.975	0.974	0.995
C	0.041	0.023	0.002	0.07	0.02
Iteration					
Equation	16	22	16	22	22
χ^2 (DF)	0.149 (3)	8.90 (2) *	0.497 (3)	4.155 (2)	3.694 (2)
Goodness of fit	$P > 0.975$	$P < 0.05$	$P > 0.900$	$P > 0.10$	$P > 0.10$
K_m	6	36	1.66	23	23.8
J	0.1	3.3	0.023	2.9	1.07
C	0.044	0.01	$1 \cdot 10^{-6}$	$1 \cdot 10^{-6}$	$1 \cdot 10^{-6}$
D	—	0.11	—	0.02	0.02

high diffusion constant and a minor saturable component with a K_m of 6 mM (Fig. 3).

Uptake by the intestine. Contrary to the disappearance rate which increases almost linearly with substrate concentration, the uptake by the tissue is a saturable phenomenon (Table V). However, the concentration within the tissue always remains below that of the perfusate. Good correlations are observed on both direct and Woolf plots. The best fit using Eqn. 12 is obtained by a combination of a saturable transport with a rather high K_m and a diffusive component with a smaller constant than that obtained for the disappearance rate (Fig. 3); the diffusion constant for exit is markedly higher, giving a half life of about 6 min (Table VI). The amount of radioactivity recovered from the tissue represents about 20% of that disappearing from the perfusate.

The constants derived above were used to simulate the results of an independent experiment in which the time course of tissue uptake of phenylalanine was measured at three times (5, 15 and 30 min) and at three different substrate concentrations (0.1, 1 and 20 mM). The values found after 15 and 30 min are in good agreement with those expected, but the real 5-min values are significantly higher than those predicted (Table VII). The data with the exception of the earliest measurement could therefore be compatible with the model. The diffusion constant for exit could also be derived from an independent observation, namely experiment 1 of Table II where movement into the blood is determined directly, and a value for this constant of the same order of magnitude was evaluated (0.062 as compared with 0.110).

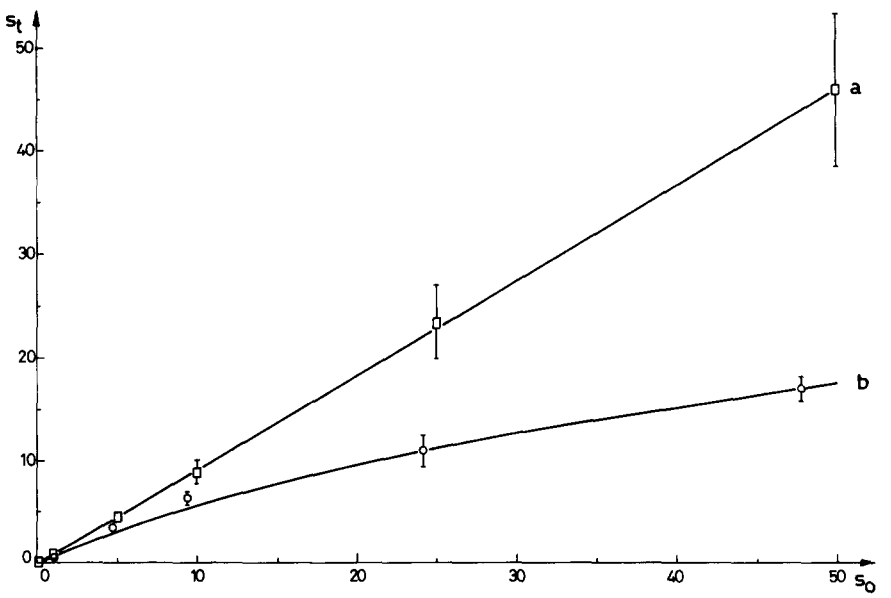

Fig. 3. Disappearance of phenylalanine from the perfusate (curve a) and uptake into the tissue (curve b) during single-pass perfusions. The experimental points are means ± S.E. of nine determinations. The lines are calculated from the best fits to Eqns. 6 and 12, as given in Table VI. For curve a, the ordinate represents the final concentration and the abscissa the original concentration in the perfusate. For curve b, the ordinate is the final concentration in the tissue, and the abscissa the mean concentration in the perfusate.

TABLE VII

PHENYLALANINE UPTAKE INTO THE TISSUE AS A FUNCTION OF PERFUSION TIME

Intestines perfused with phenylalanine at different concentrations for different periods. Concentration in the tissue water determined at the end of the perfusion. Results in mM, presented as the range ± 2 standard errors, given in right-hand column. Simulated concentrations computed from Eqn. 12, using the parameters reported in Table VI.

External concentration (mM)	Perfusion time (min)	Simulated concentration (mM)	Experimental values (± 2 S.E.)
0.1	5	0.04	0.04—0.08
0.1	15	0.06	0.05—0.09
0.1	30	0.08	0.07—0.11
1.0	5	0.36 *	0.63—1.13
1.0	15	0.56	0.29—0.79
1.0	30	0.80	0.47—0.97
20	5	5.40 *	9.7 —15.7
20	15	9.58	7.6 —13.6
20	30	11.26	5.5 —12.0

Finally, one other experiment underlined the difference between rate of disappearance from the perfusate and uptake into the tissue. When the rate of perfusion was altered, the disappearance rate changed, but the uptake into the tissue remained constant (Table VIII).

Inhibition of lysine absorption

In experiments with the MPa design, the absorption of lysine is inhibited by arginine or leucine, but is unaffected by methionine or galactose (Table III). When experiments were performed with the MPb technique (Table I) the disappearance rate of lysine is unaffected by serine or phenylalanine, but is inhibited by arginine and leucine (Table IX). In contrast to phenylalanine, lysine also inhibits its own disappearance, suggesting the presence of a saturable processs. Parallel results were obtained when the uptake into the tissue was examined: no inhibition by serine or phenylalanine, inhibition by leucine and arginine, and saturation of its own uptake.

Kinetics of lysine absorption

Disappearance from the perfusate. The rate of disappearance of lysine is

TABLE VIII

DEPENDENCE OF PHENYLALANINE ABSORPTION ON THE RATE OF PERFUSION

Single-pass perfusions with 1 mM phenylalanine. Results are the means of 12 rats, presented with the standard error derived from analysis of variance.

Rate of perfusion (ml/min)	Concentration in tissue water (mM)	Rate of disappearance (μmol/m per min)
0.5	0.61	0.91
1.0	0.78	1.37
2.0	0.69	1.67
3.0	0.76	2.18
	±0.060	±0.140

TABLE IX

INHIBITION OF LYSINE ABSORPTION

Multiple-pass perfusions (MPb) with 0.5 mM L-[^{14}C]lysine, with addition of 20 mM other amino acids. Two lots of 18 rats were employed. Results are means presented with the standard error derived from the analysis of variance; an asterisk indicates a significant inhibition.

Addition	Equilibration	Perfusion	
	Tissue concentration (mM)	Tissue concentration (mM)	Rate of disappearance (μmol/m per min)
None (control)	0.25	0.46	0.30
Serine	0.27	0.41	0.25
Phenylalanine	0.22	0.43	0.23
Leucine	0.17 *	0.35 *	0.18 *
Lysine	0.13 *	0.21 *	0.11 *
Arginine	0.10 *	0.22 *	0.09 *
	±0.017	±0.021	±0.028

much lower than that of phenylalanine, and so a multiple-pass system (MPb) had to be used for the kinetic study. The absorption of lysine appears to be saturated at a concentration of 20 mM, and it was therefore studied over a range of 0.1—10 mM. The experimental observations are presented in Table X and the kinetic analyses are included in Table VI. Good correlations are found with both graphical analyses. The best fit with Eqn. 6 (Fig. 4) is obtained assuming an almost pure saturable transport with a rather low K_m of 1.7 mM and a negligible value of C. (Indeed, the same χ^2 residue is obtained by introducing the same values of K_m and J into Eqn. 4).

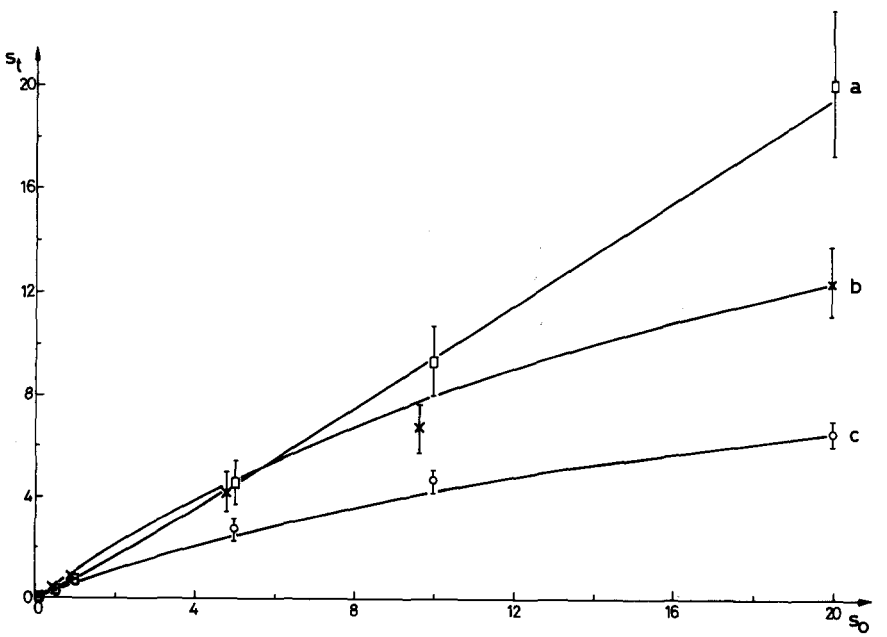

Fig. 4. Disappearance of lysine from the perfusate (curve a) and uptake into the tissue at 35 min (curve b) and 5 min (curve c) during multiple-pass perfusions. The lines are calculated from the best fits to Eqns. 6 and 12, as given in Table VI. The axes have the same significance as in Fig. 3.

TABLE X

KINETICS OF LYSINE DISAPPEARANCE RATE AND TISSUE UPTAKE

Rat intestine was perfused with the lysine concentration stated for 35 min, results being the means ± S.E. of nine animals. Tissue concentrations were determined after 5 min or 35 min in two different series of animals. The "calculated" values were obtained from Eqns. 6 and 12 using the constants given in Table VI.

Original luminal concentration (mM)	Final luminal concentration (mM)		Tissue concentration (mM)			
	Observed	Calculated	After 5 min		After 35 min	
			Observed	Calculated	Observed	Calculated
0.1	0.07 ± 0.006	0.07	0.06 ± 0.006	0.06	0.11 ± 0.010	0.10
0.5	0.34 ± 0.039	0.36	0.26 ± 0.026	0.29	0.44 ± 0.057	0.47
1.0	0.77 ± 0.086	0.76	0.68 ± 0.090	0.58	0.89 ± 0.201	0.97
5.0	4.6 ± 0.82	4.5	2.7 ± 0.46	2.5	4.2 ± 0.72	4.5
10	9.3 ± 1.35	9.4	4.6 ± 0.47	4.2	6.7 ± 1.01	7.8
20	19.9 ± 2.80	19.4	6.5 ± 0.48	6.5	12.3 ± 1.25	12.3

Uptake by the tissue. Tissue uptake was measured in two series of rats, the first being sampled after 5 min perfusion and the second after 35 min (Table X). Both results show a high correlation on the direct plot (Table VI); on the Woolf plot the correlation is better after 35 min than after 5 min. Using Eqn. 12, the best fit for both time periods is obtained by assuming an almost pure saturable transport (Fig. 4); the same rather high value of K_m (23 mM) is obtained after 5 min and after 35 min, but J is higher after 5 min. The diffusion constant for exit is also lower than the one found for phenylalanine, yielding a half life of about 35 min. A very similar value (28 min) is derived from the independent experiment (Table II, experiment 1).

Using the constants calculated from the data obtained after 35 min perfusion, it is possible to investigate whether the model predicts reasonably the tissue content of lysine after 5 min perfusion. As with phenylalanine, the real values after 5 min are higher than those predicted by the model, though the difference is greater in the case of lysine.

As for phenylalanine, the amount of radioactivity recovered from the tissue corresponds to about 20% of that disappearing from the perfusate. In contrast to phenylalanine, lysine is able to establish distribution ratios between tissue and luminal perfusate slightly greater than unity, when the external concentration is between 0.1 and 1.0 mM.

Discussion

Kinetic analysis by way of iteration with integrated equations

Kinetic analysis is generally performed by representing the velocity of a reaction in terms of the substrate concentration. If a pure saturable process is assumed, evaluation can be carried out by one of several linear transformations of the Michaelis-Menten equation. This type of analysis has been widely employed for the study of transport processes in vitro. Alternatively, when a combination of a saturable and a non-saturable process is postulated, the non-saturable component can be derived from observations made at very high substrate concentrations [23]. In a complex system, such manoeuvres suffer from serious drawbacks. For instance, the transformation of data using different graphical methods can provide widely different results for the values of the kinetic constants [24,25]; the velocity of absorption is dependent on a constantly changing concentration; the "peeling" of a mixed system by assessing the diffusional component at high substrate concentrations reduces the number of available points for kinetic analysis, and is at best a subjective matter; and finally discrimination between different models is often performed by use of the correlation coefficient, which is insensitive and cannot be used to spot curvature in some part of a function. On a direct plot, the general trend is always towards a rise in v with an increase in s. Experimental discrimination is not easy, as solubility problems and physiological or pharmacological effects restrict the utilisable concentration range.

The main advantage of using the appropriate integrated equation lies in the possibility of discriminating between saturable and non-saturable processes, when both are present simultaneously. It should be noted (Table VI) that the value of the constants describing the predominant process is given fairly

accurately by the non-integrated equations under our experimental conditions, where the drop in substrate concentration is relatively unimportant (<20%); with the integrated equation, however, minor components are revealed which might otherwise pass undetected.

The simulation of a process described by a single integrated equation and solved for s_t permits the computation of the different constants involved in a theoretical model by minimishing the χ^2 value. During this process a proper weight is given to each experimental value by introducing its variance. Since the minimisation is rapidly convergent, there is no subjective interference. Apart from this fundamental property, the method provides a means of comparing different models and testing their adequacy. It supplies the best numerical values of the constants to fit the experimental data and can be used to predict the results of independent experiments. Theoretically, the fiducial limits of the constants can be obtained by inverting a matrix composed of the second partial derivatives of the equation used in the minimisation procedure.

Kinetic analysis of amino acid absorption from the intestine in vivo

Kinetic analysis of intestinal absorption processes in vivo is fraught with difficulties [26,27], particularly since most investigators have considered absorption as a single saturable system, analogous to that examined in vitro, according to which all substrate is absorbed by a transcellular route. The present results show that the disappearance of phenylalanine from a perfusate, for instance, does not follow a single saturable function, but can be satisfactorily fitted to an integrated equation involving both a minor saturable and a major non-saturable component.

In applying a single integrated equation for a mixed transport system to each set of measurements (Eqn. 6 for disappearance from the lumen and Eqn. 12 for tissue uptake), taking into account two variables, substrate concentration and perfusion time, it is important to be sure of the validity of the experimental design. It is assumed, first, that the blood compartment is of infinite volume; this seems justified by the relatively short perfusion times and the small size of the perfused segment. The use of labelled phenylalanine or lysine as substrate is vindicated by the lack of metabolism within the mucosa. The assumption of an almost irreversible process of absorption is justified by the results in Table II. The very good fit of Eqn. 6 to the experimental observations (Table VI) underlines the adequacy of the mathematical model for this experimental situation. Clearly, the fact that the experimental observations are consistent with a simple model does not exclude the possibility of their compatibility with a more complicated one, including such factors as laminar flow and the effect of unstirred layers, which might be revealed by the use of different experimental conditions.

Paradoxically, although most workers have assumed that absorption in vivo occurs by a rate-limiting transcellular passage through the enterocyte, no mathematical model seems to have been developed to explore the characteristics of tissue uptake in vivo. Indeed only few workers have examined this process at all [11,13]. In contrast to the model for disappearance from the lumen, the one developed for the analysis of tissue uptake makes at least one assumption that is not justified, namely that the concentration in the perfusate

remains unchanged throughout the experiment. Unfortunately, a convenient equation could not be developed for a two-compartment model with variable concentration in the first compartment and mixed transport from compartment 1 to compartment 2. As a compromise, we were obliged to use an experimental situation in which the drop in substrate concentration was maintained as small as possible (see Tables V and X). Furthermore, it is obvious that considering the intestinal tissue as a single compartment represents a gross oversimplification. Thus it is not surprising that the deviation between the theoretical curves and the experimental observations is considerably greater in the case of tissue measurements (Eqn. 12) than in the case of substrate disappearance (Eqn. 6). Indeed, the χ^2 residues for phenylalanine give a significant value. Nevertheless, several observations indicate that this model should not be rejected out of hand: First, it predicts that the tissue concentration tends towards a plateau with increasing time; this was observed experimentally (Table VII) in confirmation of the data of Winne [13] who also studied phenylalanine absorption in the rat intestine in vivo. Secondly, the efflux constants determined from the iterative process agree quite closely with those measured experimentally in separate experiments.

Characteristics of the absorptive processes in vivo

The kinetic analysis employed has revealed certain features of amino acid absorption in vivo that remained undetected in earlier investigations, where graphical methods were used for the evaluation. In particular, there is a very important difference in the absorptive properties of the various amino acids.

The rate of disappearance of lysine from a perfusate can be well described by a pure saturable process; the fit to Eqn. 6 improves as the value of C tends to zero. This mechanism is characterised by a K_m of 1.7 mM, which agrees with values for lysine obtained with rings of excised rat intestine in vitro [21,28]. It would thus appear appropriate to conclude that the majority of lysine absorbed in vivo follows a trancellular route, utilising the same pathway as that studied in vitro.

In contrast to that of lysine, phenylalanine absorption from a perfusate cannot be described by a single saturable mechanism. Preliminary analyses indicated that a very high K_m might characterise this process, as indeed has been reported by other workers in the field [5,8]. It is possible, as suggested elsewhere [29], that such a high value could be attributed to an unstirred layer at the surface of the mucosa, the effect of which is to raise the K_m considerably [30,31], but this explanation appears untenable on two grounds: First, it is difficult to imagine the presence of an unstirred layer in vivo which selectively affects the movement of neutral but not dibasic amino acids; and secondly, an unstirred layer would also decrease the rates of absorption [30], which are, however, in the present work, two or three times greater than those reported for the rat intestine in vitro [21]. Nevertheless, it must be admitted that our finding of greater rates of absorption when the perfusion rate is increased is compatible with a role of the unstirred layer in preventing maximal absorption [29].

Although the linearity of disappearance rate with substrate concentration and the lack of self-inhibition suggested that phenylalanine absorption occurred

principally by a non-saturable process, analysis of the results in accordance with Eqn. 6 revealed two components, a minor saturable one with a K_m of 6 mM and a major non-saturable one. It is probable that the saturable component corresponds to that studied in vitro, and indeed the K_m values obtained under the two experimental conditions are similar [28]. However it is surprising that this pathway only accounts for 25% of the total absorption when the concentration of phenylalanine is 0.1 mM, and only 10% of the absorption when it is raised to 10 mM.

The possibility that amino acids might be absorbed in vivo by a combination of saturable and non-saturable processes in parallel was first proposed by Booth and Kanaghinis [32], but the nature of the non-saturable component is still unclear. It is tempting to surmise that it involves movement between the cells, across the tight junctions, as has been suggested by certain investigators studying sugar transport [33,34], but the existence of leucine inhibition of phenylalanine absorption in vivo and the absence of mannitol absorption show that this interpretation must be treated with caution. If it is true, then the conclusion to be drawn from the present findings is that most of the cationic amino acid, lysine, passes through the epithelial cells, perhaps in response to the electronegativity of the cytoplasm; indeed, Munck and Schultz [35] have recently observed little or no movement of lysine through paracellular shunts in the rat jejunum in vitro. In contrast, most of the phenylalanine would pass through the intercellular spaces; the existence of a paracellular component of phenylalanine flux, which would be rapidly drained and not retained within the mucosa, could explain the difference between phenylalanine disappearance rate and its uptake into the tissue.

The characteristics of the tissue uptake of lysine and phenylalanine in vivo are very similar. Uptake is saturable, inhibited by analogues that are transported by the same transport system, and unaffected by the rate of perfusion. Distribution ratios greater than unity are not established, but this can probably be attributed to two factors: First, the enterocytes are efficiently drained in vivo and thus efflux of the amino acid from the epithelial cells is favoured, and secondly the tissue, as analysed, contains many inert layers (lamina propria, musculature) in which no amino acid would be expected in vivo, but which may be accessible in vitro. In addition, the K_m values calculated by fitting the results to Eqn. 12 are uniformly higher than those obtained in vitro, and those obtained from fitting Eqn. 6 to the disappearance rate. This result probably reflects the inadequacies of the two-compartment model employed and the equation applied.

Acknowledgements

We are grateful to Nestlé S.A. for financial support for this project, to Professor D. Winne (Tübingen) for stimulating discussions, to Mme. S. Salvi and Mlle. M. Genier for skilful technical assistance, and to Mlle. W. Happee for secretarial aid.

References

1 Matthews, D.M. (1969) Klin. Wschr. 47, 397—414
2 Antonioli, J.A. and Christensen, H.N. (1968) Am. J. Physiol. 215, 951—958
3 Modigliani, R., Rambaud, J.C. and Bernier, J.J. (1973) Digestion 9, 176—192
4 Modigliani, R., Rambaud, J.C. and Bernier, J.J. (1973) Digestion 9, 264—290
5 Adibi, S.A. (1969) Gastroenterology 56, 903—913
6 Jacobs, F.A. (1968) Adv. Tracer Methodol. 4, 255—272
7 Jervis, E.L. and Smyth, D.H. (1969) J. Physiol. Lond. 149, 433—441
8 Kakemi, K., Sezaki, H., Nakano, M. and Suzuki, E. (1970) Chem. Pharm. Bull. 18, 2176—2182
9 Pénzes, L. (1969) Exp. Gerontol. 4, 223—230
10 Pénzes, L. (1972) Rev. Roum. Méd. Int. 9, 187—190
11 Schedl, H.P., Miller, D.L., Wilson, M.D. and Flores, P. (1969) Am. J. Physiol. 216, 1131—1138
12 Winne, D. (1972) FEBS Lett. 27, 94—96
13 Winne, D. (1973) Naunyn-Schmiedeberg's Arch. Pharmacol. 277, 113—138
14 Wapnir, R.A., Hawkins, R.L. and Lifshitz, F. (1972) Am. J. Physiol. 223, 788—793
15 Kunze, H. and Vogt, W. (1967) Naunyn-Schmiedeberg's Arch. Pharmakol. Exp. Pathol. 256, 139—148
16 Miller, D.L. and Schedl, H.P. (1970) Gastroenterology 58, 40—46
17 Robinson, J.W.L. and Felber, J.-P. (1965) Gastroenterologia (Basel) 104, 335—342
18 Snedecor, G.W. and Cochran, W.G. (1967) Statistical Methods, 6th edn., pp. 258—275, Iowa State University Press, Ames
19 Dixon, M. and Webb, E.C. (1964) Enzymes, 2nd edn., pp. 63—70, Longmans Green and Co., London
20 Cramér, H. (1955) The Elements of Probability Theory, Chapter 13, p. 181, J. Wiley, New York
21 Reed Larsen, P., Ross, J.E. and Tapley, D.F. (1964) Biochim. Biophys. Acta 88, 570—577
22 Robinson, J.W.L. (1974) Biochim. Biophys. Acta 367, 88—101
23 Akedo, H. and Christensen, H.N. (1962) J. Biol. Chem. 237, 118—122
24 Wilkinson, G.N. (1961) Biochem. J. 80, 324—332
25 Dowd, J.E. and Riggs, D.S. (1965) J. Biol. Chem. 240, 863—869
26 Fisher, R.B. and Parsons, D.S. (1953) J. Physiol. Lond. 119, 210—223
27 Curran, P.F. (1972) Arch. Int. Med. 129, 258—269
28 Finch, L.R. and Hird, F.J.R. (1960) Biochim. Biophys. Acta 43, 278—287
29 Rey, F., Drillet, F., Schmitz, J. and Rey, J. (1974) Gastroenterology 66, 79—85
30 Winne, D. (1973) Biochim. Biophys. Acta 298, 27—31
31 Wilson, F.A. and Dietschy, J.M. (1974) Biochim. Biophys. Acta 363, 112—126
32 Booth, C.C. and Kanaghinis, T. (1963) J. Physiol. Lond. 167, 18P
33 Axon, A.T.R. and Creamer, B. (1975) Gut 16, 99—104
34 Rinaldo, J.E., Jennings, B.L., Frizzell, R.A. and Schultz, S.G. (1975) Am. J. Physiol. 228, 854—860
35 Munck, B.G. and Schultz, S.G. (1974) J. Membrane Biol. 16, 163—174