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TWO-CARRIER INFLUX OF NEUTRAL AMINO ACIDS INTO RABBIT ILEAL MUCOSA

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SUMMARY

1. The influx of serine, alanine and methionine across the brush border membrane of the rabbit ileal mucosa has been measured during short periods of incubation.

2. A kinetic analysis of the uptake data, assuming one mediated entry mechanism or one mediated entry mechanism plus a diffusion component to be present, does not provide an adequate explanation for the results obtained. Methionine inhibition of serine uptake provided direct evidence that the diffusive entry of serine into the rabbit ileum was small or non-existent.

3. Data taken from amino acid inhibition and substrate-uptake experiments, fitted simultaneously to a double hyperbolic model of amino acid uptake, give good agreement between predicted and experimental results. There is also good quantitative agreement between computer-derived kinetic constants in the present work and similar constants obtained previously using a different method of analysis.

4. Present work supports the general hypothesis that neutral amino acids use two mediated pathways to enter the rabbit ileal mucosa. The possible physiological significance of these results and their probable effect on currently held concepts of how amino acids cross the brush border membrane of the rabbit intestinal mucosa is discussed.

INTRODUCTION

Previous work has considered neutral amino acid influx across the brush border membrane of the intestinal mucosa to take place by one process only (Preston, Schaeffer & Curran, 1974), despite indirect evidence to the contrary (Newey & Smyth, 1964). More recent work has demonstrated that the mucosal influx of neutral amino acids shows selective inhibition by serine. This has been used to distinguish two separate mechanisms for amino acid entry in the rabbit ileal mucosa (Sepúlveda & Smith, 1978). One of these systems (inhibited by serine and referred to as system 1) was used by all amino acids tested, while the other (unihibited by serine and referred to as system 2) appeared to be used by the more hydrophobic amino acids. Uptake of alanine, determined at one concentration only, was predominantly by system 1, but the possibility that high concentrations of alanine might prefer system 2 could not be excluded. Whether alanine uses one or two pathways of entry in the rabbit ileum is of some importance, since system 1 appears to be more Na-dependent than system 2 (Sepúlveda & Smith, 1978) and the model describing Na-amino acid

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coupling in this tissue assumes only one entry mechanism to be present (Curran, Schultz, Chez & Fuisz, 1967). The main aim of the present work was to test this assumption further.

METHODS

Experimental

The source and weight of New Zealand White rabbits used in these experiments; the regime of feeding and starving rabbits before each experiment; the dissection and presentation of distal ileum for the determination of amino acid influx across the brush border membrane and subsequent counting of samples and calculation of results, were all as described previously (Sepúlveda & Smith, 1978).

Differences from the previous protocol involved the use of ^{3}H-labelled polyethylene glycol of molecular weight 900 (PEG 900) as well as ^{3}H-labelled inulin as a marker of extracellular space. Both markers were used without addition of non-radioactive carrier. PEG 900 was purchased from NEN Chemicals Gmbh, D-6072 Dreieichenhain, West Germany.

Some of the experiments involved measurement of amino acid uptake from solutions containing 1–35 mM-amino acid together with 0–40 mM of a second amino acid used as inhibitor. In these cases mannitol was added to solutions containing less than the maximum concentration of amino acids to maintain constant tonicity. Mannitol itself was without effect on amino acid uptake (see Sepúlveda & Smith, 1978, for control experiments illustrating this point). The maximum increase in tonicity was, however, 75 mM in the present work compared with 50 mM in the earlier work. 35 mM-serine or alanine uptake was unchanged if the total increase in tonicity was raised from 50 mM (as in the uptake *vs.* substrate concentration experiment in Fig. 3) to 75 mM (as in the methionine inhibition experiment).

Computational analysis

The use of curve fitting procedures to reveal heterogeneity in intestinal transport systems is already well established (Atkins & Gardner, 1977; Antonioli, Joseph & Robinson, 1978).

The procedure used in the present work was the maximum likelihood method (the programme MLP of G. J. S. Ross, C 1978, Lawes Agricultural Trust, Rothamsted Experimental Station, Harpenden). This was used to estimate the parameters for a double hyperbolic model describing the uptake of an amino acid of concentration S.

$$J_{mc} = \frac{J_{max_1} S}{S + K_{m_1}} + \frac{J_{max_2} S}{S + K_{m_2}},$$

where J_{mc} is the influx of an amino acid across the brush border membrane of the intestinal mucosa, K_{m_1} and K_{m_2} are the apparent affinities of an amino acid for systems 1 and 2 respectively and J_{max_1} and J_{max_2} are the respective maximal velocities for influx by systems 1 and 2.

In the presence of a second amino acid of concentration I the equation for J_{mc} becomes

$$J_{mc} = \frac{J_{max_1} S}{S + K_{m_1}(1 + I/K_{i_1})} + \frac{J_{max_2} S}{S + K_{m_2}(1 + I/K_{i_2})},$$

where K_{i_1} and K_{i_2} are the inhibitory constants of the second amino acid. These are taken, for the purpose of the analysis, to be equal to the respective K_m values of the second amino acid for the uptake systems 1 and 2. An alternative model was tested by fitting one hyperbola plus a linear component to the experimental data using the same method.

Three amino acids were used in all and six K_m values and six J_{max} values fitted simultaneously from a written programme based on the MINIM subroutine (D. E. Shaw, Division of Mathematical Statistics, CSIRO, Sydney). A user-supplied subroutine accepts a set of parameter values from MINIM, derives the predicted rate of uptake $\hat{J}_{mc}$ and calculates and returns to MINIM the weighted sum of squares of residuals $\Sigma w.\ (J_{mc} - \hat{J}_{mc})^2$. This is minimized by the simplex method of Nelder & Mead (1965). Estimation of standard errors of parameters depends on fitting a quadratic surface around the minimum but this could not be achieved with this twelve parameter model.

The variance of the point estimates of rate of uptake was proportional to uptake rate. A linear regression line through the origin was fitted to $Var\ (J_{mc})$ and J_{mc}, and smoothed estimates

of $Var\,(J_{mc})$ taken from this line. The weight for each J_{mc} is the reciprocal of its variance, $w_i = 1/Var\,(J_{mci})$. These initial weights were scaled by $N/\Sigma w$ so that the sum of adjusted weights ΣW was equal to N, the number of J_{mc}.

In order to ensure that MINIM had converged on a true minimum, the programme was run using different starting values of the parameters and different initial steplengths. In all cases the same minimum was located.

RESULTS

Extracellular space markers and amino acid uptake

One of the problems encountered previously when measuring amino acid influx into rabbit ileal mucosa was whether inulin was an adequate marker of the space immediately adjacent to the mucosal surface of the intestine (Sepúlveda & Smith, 1978). A slight underestimate of this space would lead to an undercorrection of total counts representing amino acid uptake. This possible undercorrection would give an overestimate of amino acid uptake which is directly proportional to the mucosal concentration of amino acid and this would be seen as a non-saturating component of amino acid uptake.

TABLE 1. Serine uptake by rabbit ileal mucosa. Uptake of ^{14}C-labelled serine was measured using tritiated inulin or PEG 900 to correct for extracellular space. Uptakes and extracellular spaces were measured after 45 sec incubations. Values give means ± S.E. of eight determinations

Serine concentration (mM)	Serine uptake (n-mole cm^{-2} min^{-1})		Extracellular space (μl. cm^{-2})	
	Inulin corrected	PEG 900 corrected	Inulin	PEG 900
1	10·5 ± 1·1	11·5 ± 1·2	1·57 ± 0·14	1·52 ± 0·16
6	41·9 ± 5·2	40·3 ± 5·7	1·62 ± 0·20	1·54 ± 0·27
16	69·5 ± 8·3	72·5 ± 10·7	1·64 ± 0·17	1·50 ± 0·22
26	75·6 ± 14·3	85·9 ± 12·8	1·95 ± 0·29	1·62 ± 0·22
36	84·0 ± 9·1	94·5 ± 13·4	1·73 ± 0·21	1·64 ± 0·19
51	100·6 ± 7·7	107·4 ± 19·0	1·65 ± 0·16	1·46 ± 0·16

To test this possibility, serine uptake was determined over a wide range of serine concentrations using either inulin or PEG 900 to correct for extracellular space. The results obtained are shown in Table 1. The uptake of serine ranged from 10·5 to 100·6 n-mole cm^{-2} min^{-1} using inulin and 11·5–107·4 n-mole cm^{-2} min^{-1} using PEG 900 as space marker (uptakes measured from 1 and 51 mM-serine respectively). The mean space volume measured by inulin and PEG 900 in these experiments was 1·69 and 1·55 μl. cm^{-2} respectively. This represented a correction of uptake of about 14% using 1 mM and 43% using 51 mM-serine in the incubation medium. Uptakes for each concentration of serine had been determined using adjacent pieces of tissue, one using inulin and the other PEG 900 to correct for extracellular space. This allowed the significance of differences between the two values for space marker volumes and for serine uptakes, determined for six concentrations of serine, to be assessed using paired t tests. None of the differences were statistically significant. Reducing the molecular weight of the space marker from about 5200 to 900 made no difference to the results obtained. The two markers appear to be inter-

changeable, but it was decided to use PEG 900 in subsequent experiments because of its greater uniformity of size.

Additional evidence that an apparent linear component of amino acid uptake cannot arise as a space marker artifact is described below in relation to results presented in Fig. 1.

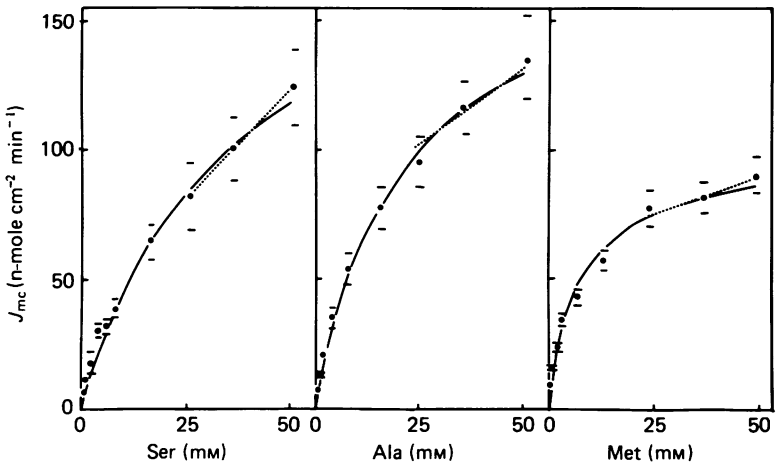

Fig. 1. Concentration dependence of neutral amino acid uptake by rabbit ileal mucosa. Uptake (J_{mc}) of serine, alanine and methionine (Ser, Ala and Met) was measured over a 45 sec period of incubation. Each point gives the mean ± S.E. of eight (Ser), seven (Ala) and fifteen (Met) estimations. The curves give calculated fits to the experimental points assuming one carrier system to be present. The dotted lines show separate estimates of a possible linear component, calculated as described in the text.

Uptake of neutral amino acids described by a single mediated entry system

Previous workers, using a relatively restricted range of amino acid concentrations, have come to the conclusion that neutral amino acids enter the intestinal mucosa of the rabbit ileum through a single mediated process (Preston *et al.* 1974). Experiments presented in Fig. 1 show the concentration dependence of amino acid uptake, determined over a wide concentration range, for serine, alanine and methionine. The curves give the best calculated fit to these points assuming only one process for entry. The fit is satisfactory and the corresponding kinetic constants for these three amino acids are shown in Table 2. Analysed for the whole concentration range the K_m values are 8, 18 and 31 mM for methionine, alanine and serine respectively. Each value for K_m has an acceptable standard error. There is, nevertheless, something much wrong with these results. Fitting a hyperbola to experimental results obtained over restricted ranges of amino acid concentrations (0–8 and 0–25 mM) produces equally acceptable values for K_m which are, however, considerably less than those found when analysing curves over the whole concentration range (Table 2). The assumption that neutral amino acids use only one mediated system to enter the rabbit ileal mucosa is clearly not correct.

Uptake of neutral amino acids described by a single mediated entry system added to a diffusion component

The results presented in Fig. 1 can be fitted equally well using the model, one hyperbola plus a linear component, as described in the Methods section. The calculated linear component for each amino acid is shown as a series of dotted lines in Fig. 1. The calculated values for these linear components are $1{\cdot}55 \pm 0{\cdot}18$, $0{\cdot}83 \pm 0{\cdot}09$

TABLE 2. Kinetic constants for neutral amino acid uptake by rabbit ileal mucosa calculated using the iterative technique of Bliss & James (1966) assuming only one mediated entry system to be present

	Concentration range (mM)					
	0–8		0–25		0–50	
Amino acid	K_m	J_{max}	K_m	J_{max}	K_m	J_{max}
Serine	$5{\cdot}0 \pm 1{\cdot}4$	61 ± 9	$17{\cdot}9 \pm 3{\cdot}6$	136 ± 15	$30{\cdot}7 \pm 5{\cdot}4$	189 ± 17
Alanine	$8{\cdot}2 \pm 1{\cdot}1$	111 ± 9	$11{\cdot}4 \pm 0{\cdot}9$	137 ± 5	$18{\cdot}2 \pm 2{\cdot}6$	176 ± 10
Methionine	$2{\cdot}5 \pm 0{\cdot}2$	57 ± 2	$6{\cdot}6 \pm 1{\cdot}8$	92 ± 10	$8{\cdot}1 \pm 1{\cdot}3$	101 ± 5

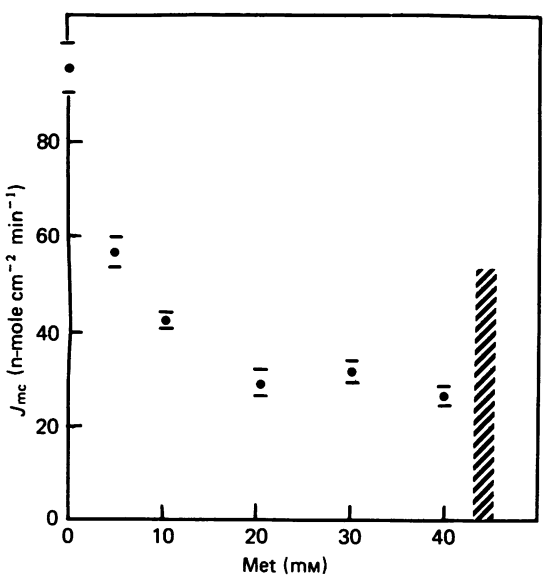

Fig. 2. Methionine inhibition of serine uptake by rabbit ileal mucosa. Serine uptakes were measured from solutions containing 35 mM-serine and different concentrations of methionine. Each point gives the mean ± S.E. of sixteen estimations. The histogram gives the expected serine uptake were methionine to inhibit *all* the mediated uptake of serine leaving a diffusion component equal to that plotted in Fig. 1.

and $0{\cdot}61 \pm 0{\cdot}21$ n-mole cm^{-2} min^{-1} m-mole^{-1} for serine, alanine and methionine respectively. The value for serine, 1·55, corresponds to the value of $1{\cdot}80 \pm 0{\cdot}05$ n-mole cm^{-2} min^{-1} m-mole^{-1}, calculated previously (Sepúlveda & Smith, 1978). The fact that these values vary by as much as 250% shows that they cannot arise as a space marker artifact. Any linear component appearing from this source would be expected to be constant for all amino acids tested.

The question as to whether these linear components are real and if so whether they represent amino acid diffusion or a second system for mediated entry is best answered by carrying out inhibition experiments using selected amino acids. Serine was chosen as the amino acid whose uptake was to be inhibited, since the apparent linear component was greatest for this amino acid. Methionine was used as the inhibiting amino acid, since it appeared from Fig. 1 to have the highest affinity for the entry mechanism(s). The results obtained, using 35 mM-serine and concentrations of methionine varying from 0 to 40 mM are shown in Fig. 2. Serine uptake, measured in the presence of 40 mM-methionine, represented only half the uptake

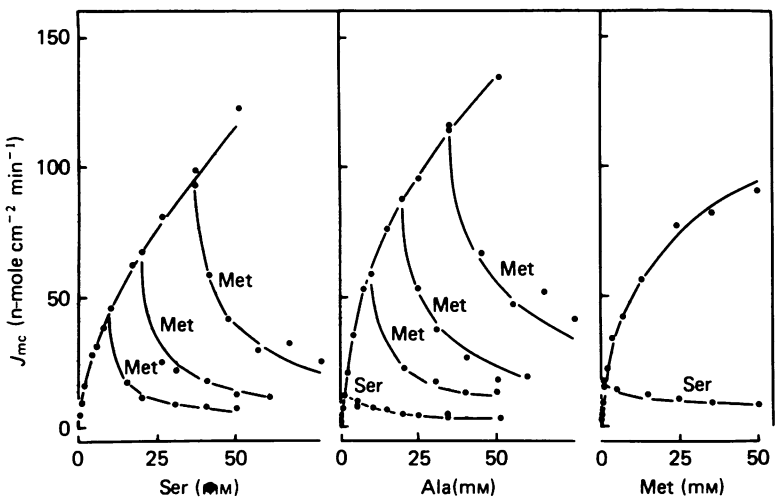

Fig. 3. Summarized results showing serine, alanine and methionine uptake measured alone or in the presence of different concentrations of methionine or serine. The concentration of inhibitor is zero on the substrate-uptake curves for different test amino acids. It then increases to a maximum of 40 mM for methionine and 50 mM for serine. Points on the inhibition plots represent means of eight to fifteen (Met inhibiting Ser), seven to eight (Met inhibiting Ala) or four to eight (Ser inhibiting Ala or Met) estimations. Curves give the calculated lines of best fit assuming two mediated entry systems for each of the three test amino acids to be present.

predicted if the linear portion of serine uptake were to take place by diffusion. This difference could presumably be increased using higher concentrations of methionine in the incubation medium, but it was considered unwise to increase further the over-all tonicity of the incubation medium.

It was concluded from this experiment that the so-called linear component to amino acid uptake could not be due to diffusion. Subsequent analysis, shown in Fig. 3, describes how a model involving two mediated entry systems, with no diffusive element, can fully describe the inhibition curve shown in Fig. 2.

Uptake of neutral amino acids described by two mediated entry systems

Experiments measuring the inhibitory effect of one amino acid on the uptake of another have been used successfully in the past to define different carrier systems for amino acid transport in Ehrlich ascites tumour cells (see the review by Christen-

sen, 1975). The previous section shows that this sort of experiment also provides, in the absence of other criteria, sound evidence allowing a distinction to be made between diffusion and a mediated entry process for an individual amino acid. It was therefore decided to carry out several more inhibition experiments, using methionine as an inhibitor of serine and alanine uptake and serine as an inhibitor of alanine and methionine uptake, before subjecting the complete data to a more comprehensive analysis assuming two mediated entry processes to be present. The results obtained, together with the original uptake versus substrate concentration points, are shown in Fig. 3. The abscissae in this Figure refer to the concentrations of amino acids used to measure amino acid uptakes in the absence of an inhibiting amino acid. When an inhibiting amino acid is present the concentration used can also be read from the abscissae, taking its concentration to be zero at the point where the respective uptake and inhibited uptake curves intersect.

TABLE 3. Kinetic constants for neutral amino acid uptake by rabbit ileal mucosa

	Computer-derived constants				Serine inhibition experiments (Sepúlveda & Smith, 1978)	
	System 1		System 2		System 1	
Amino acid	K_m	J_{max}	K_m	J_{max}	K_m	J_{max}
Serine	6·3	53	(2931)	(4090)	3·2	44
Alanine	4·6	52	91	247	0·7	18
Methionine	0·4	14	22	115	0·3	13

Inspection of the experimental points suggests that methionine is more effective as an inhibitor of serine than of alanine uptake and that serine is more effective as an inhibitor of alanine than of methionine uptake. All the results (eighty-three data points each based on an average of eight influx measurements) have been processed through the computer programme described in Methods assuming two mediated entry processes to be present. The kinetic constants produced from this analysis, shown in Table 3, have been used to plot theoretical curves to the experimental results shown in Fig. 3. The fit of these curves to the experimental points is generally very good.

It is particularly interesting to note that the best fit to all these points involves the generation, by the computer, of a series of low K_m, low J_{max} constants for the uptake of the three amino acids. These correspond quite closely to previously published constants for the system 1 uptake of the three neutral amino acids (Table 3), which were derived by an entirely different method of analysis. The comparison between the computer-derived K_m and J_{max} values and those emerging from the serine inhibition experiment is especially good for methionine and serine, but there is a discrepancy between the two sets of values obtained for alanine. The reason for this is not known at present, but the computer-derived constants are to be preferred, since they are calculated from a greater number of results obtained using a wider variety of experimental conditions.

The system 2 uptake of both methionine and alanine takes place with an apparent

affinity considerably less than that seen for system 1 (Table 3). The apparent affinity of serine for system 2 is too low to be determined accurately. The J_{max} values for methionine and alanine, determined on system 2, are considerably greater than those determined for system 1. The J_{max} for serine by system 2 is too great to be determined with any accuracy.

Curves describing the two-carrier uptake of serine, alanine and methionine in Fig. 3 can be separated into their different components using the constants described in Table 3. The results obtained are shown in Fig. 4. The system 2 uptake of serine (Fig. 4*A*) is directly proportional to the concentration of amino acid. It looks like diffusion but it can be inhibited by methionine. Serine is without effect on the system 2 uptake of alanine and methionine. This agrees with the model proposed previously on the basis of inhibition experiments using serine (Sepúlveda & Smith, 1978).

Serine has a high enough affinity for system 1 to cause significant inhibition of both alanine and methionine uptake. Finally, it can be seen that alanine uptake takes place by both systems 1 and 2, the ratio of system 1/system 2 uptake varying from 10 at an alanine concentration of 1 mM to 1 at an alanine concentration of 20 mM.

DISCUSSION

Fitting theoretical models to real results

The present work illustrates how dangerous it is to base conclusions concerning the number of pathways responsible for amino acid influx on a simple kinetic model. A good fit of experimental points to uptake *vs.* amino acid concentration plots can be obtained assuming a single mediated; a single mediated plus linear component or a double mediated transfer model. It is only by further analysis that two of these models can be shown to be invalid. Extending the concentration range over which kinetic constants are calculated produced results difficult to reconcile with a single mediated entry system in the present series of experiments. A similar discrepancy has been noted previously when measuring phenylalanine uptake into rabbit ileal mucosa (Sepúlveda & Smith, 1978). Results similar to this have been used previously to suggest the presence of more than one mediated entry system for glucose and galactose in hamster small intestine (Honegger & Semenza, 1973).

The reasoning leading to the rejection of the single mediated entry plus linear component model depends mainly on the experiment showing methionine to inhibit serine influx by an amount much greater than could be assumed, were the linear component of serine entry to represent diffusion. This experiment does not, unfortunately, eliminate the possibility that some entry of serine takes place by diffusion and it is not possible to increase the methionine concentration to a point where it would be predicted that all serine entry should be inhibited. A similar difficulty has been encountered when dealing with Ehrlich ascites tumour cells, where the inhibition of a linear component to uptake has been accepted as evidence that no diffusive entry of amino acid takes place (see below).

The main strength of the two-system model for amino acid uptake by rabbit ileal mucosa lies in the close agreement found between the kinetic constants it

produces and constants found previously using serine as a specific inhibitor of system 1 uptake (Sepúlveda & Smith, 1978). It would be quite remarkable if such an agreement were to occur by coincidence. A second powerful reason for accepting the two-system model in preference to the other two is that this was the only model which continued to generate believable constants when presented with results obtained from inhibition experiments.

TABLE 4. Comparison of previously published kinetic constants for neutral amino acid uptake by rabbit ileal mucosa with constants calculated by Bliss-James analysis of present results assuming a single mediated system of entry. The ranges of amino acid concentrations used for these analyses corresponded to those used in previously published work. Numbers 1, 2 and 3 refer to the work of Schultz, Yu-tu & Strecker (1972), Curran *et al.* (1967) and Preston *et al.* (1974) respectively. The methodology used to determine amino acid uptake in past and present work was directly comparable. Values of K_m and J_{max} are given as mM and n-mole cm^{-2} min^{-1} respectively.

	Kinetic constants					
	Present work		Previously published work			
Amino acid	K_m	J_{max}	K_m	J_{max}	Ref.	Concentration range studied (mM)
Serine	16	134	12	200	1	2–20
Alanine	10	135	9	102	2	1·5–17
	—	—	9	200	1	2·5–20
Methionine	2·4	56	1·3	50	3	0·02–0·9

The actual means by which neutral amino acids enter the rabbit ileal mucosa might, in fact, be more complicated than suggested. The assumption of three or more mediated entry pathways would, undoubtedly, give equally good or marginally better fits to the experimental points. The reason for choosing the two-carrier model in preference to these models is that it provides the simplest explanation for the facts as they stand at present.

Comparison of past with present measurements of amino acid uptake into rabbit ileal mucosa

The rabbit ileum has been used extensively in the past to study the kinetics of amino acid uptake (see below for references). The interpretation of these earlier results differs considerably from that presented in this and previous publications (Sepúlveda & Smith, 1978). There were slight differences in the composition and tonicity of the incubation media used in this and earlier work (high K in the earlier work compared with Krebs–Henseleit medium used in the present experiments; mannitol supplemented medium in the present work). It is important to establish whether these differences could affect uptake measurements before commenting further on the earlier results.

Table 4 summarizes kinetic data calculated by previous workers and compares these with present data calculated assuming a single mediated system of entry for *comparable ranges of amino acid concentration*. The agreement between previously published and present results is impressive showing that the minor changes in methodology used did not affect the results obtained.

Certain anomalies in previously published work in the rabbit ileum become explicable, once it is accepted that two pathways exist for the entry of all neutral amino acids. The presence of a second mediated entry system for alanine, for instance, could explain why Schultz & Markscheid-Kaspi (1971) found a discrepancy between measured K_i and K_m values for alanine in this tissue. The second system for leucine uptake, identified previously and thought to represent a high affinity overlapping specificity for a lysine carrier (Munck & Schultz, 1969), can now be probably classified as a system 1 uptake process. The same might also apply to the high affinity system thought previously to be specific for glycine entry (Peterson, Goldner & Curran, 1970). The reason why these interesting results have not been given greater prominence in the past remains obscure.

The fact that alanine entry into rabbit ileal mucosa takes place through two pathways, with system 2 predominating over system 1 at high alanine concentrations, raises specific questions about the nature of Na-amino acid coupling during transport. There *may* be some coupling between the system 2 entry of alanine and Na (see below for results on kidney tubules), but the degree of coupling is likely to be less than that seen for system 1 (Sepúlveda & Smith, 1978). What this might mean to currently held views on Na-amino acid coupling during influx into the rabbit ileum (Curran *et al.* 1967) is at present being investigated.

Amino acid influx in tissues other than the small intestine

The idea that part of the entry of neutral amino acids is mediated by a system having a high K_m and high J_{max} has already been reported for cells other than the enterocyte. Experiments designed to show that this low affinity system cannot be equated with diffusion include studies of the temperature and pH dependence and chemical specificity of amino acid entry into Ehrlich cells (Christensen & Liang, 1966). Further work by Christensen & Handlogten (1977), using conformational isomers of a synthetic amino acid, with the uncharged form of one isomer being 4 times more abundant than the other isomer at a neutral pH, has also shown that the apparent linear component to uptake does not arise from diffusion of the uncharged molecule. Present results add the rabbit ileal enterocyte to the list of cells known to possess a low affinity system for neutral amino acid uptake.

Evidence that this kind of system could also be coupled to Na entry comes from the work of Samaržija (1978), who measured microvillar membrane potentials in kidney proximal tubule cells in the presence and absence of a number of different concentrations of several neutral amino acids. Kinetic analysis of the concentration dependence of the amino acid-induced membrane depolarization allowed her to postulate the presence of two entry systems for glycine, one with a K_m of 0·31 and the other with a K_m of $>$ 1000 mM. These indirect measurements of glycine transport assume that electrical depolarization is caused by the co-transport of glycine with Na ions. The interest here is, first, that these measurements exclude any diffusion component of transport and, second, that they suggest that the low affinity system for glycine entry is electrogenic. Experiments are currently underway to test whether this also applies to the rabbit ileum.

Finally it should be pointed out that, though much is known about amino acid transport in other tissues, these studies suffer from the disadvantage that no natur-

ally occurring amino acid behaves as a specific inhibitor of either system. The rabbit ileal enterocyte has a real advantage over other cells in this respect, serine having such a low affinity for system 2 that it can be used as a specific inhibitor of system 1 uptake (Sepúlveda & Smith, 1978 and Fig. 4 of the present paper). This increases considerably the power of analysis.

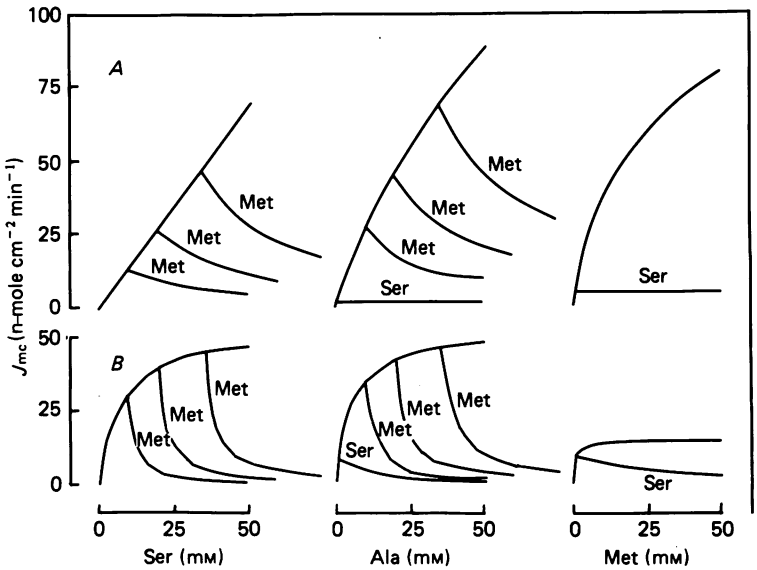

Fig. 4. Calculated values for system 1 (*B*) and system 2 (*A*) uptake of serine, alanine and methionine by rabbit ileal mucosa. The composite curves describing the two-system uptake of these three amino acids, shown in Fig. 3, have been broken down into their separate components using the kinetic constants described in Table 3.

The possible physiological importance of using two distinct systems to transport different neutral amino acids

The affinity of system 1 for neutral amino acid uptake by rabbit ileal mucosa is about two orders of magnitude greater than for system 2 and the maximal rate of transfer is correspondingly less. Within each system, however, the affinities for the small, hydrophilic amino acids are less than for the larger, hydrophobic, amino acids. The smaller amino acids also happen, with the exception of threonine, to be non-essential to the growth of the animal, while the larger amino acids (Val, Met, Ile, Leu, Tyr and Phe) are all essential. It can be seen, from superficial examination of the kinetic constants obtained for the two systems, that the uptake of essential amino acids will be preferred to the non-essential amino acids, when the total concentration of amino acids is low. System 1 has, however, a low capacity for transport and one could imagine a situation where some amino acids would be excreted, were it not for the presence of a second low affinity, high capacity, transport system. The system 2 uptake mechanism will, however, still prefer essential to non-essential amino acids. The possibility that system 1 might be subject to regulation and the effects this might have on over-all amino acid uptake have been discussed elsewhere (Sepúlveda & Smith, 1979).

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REFERENCES

ATKINS, G. L. & GARDNER, M. L. G. (1977). The computation of saturable and linear components of intestinal and other transport kinetics. *Biochim. biophys. Acta* **468**, 127–145.

ANTONIOLI, J.-A., JOSEPH, CL. & ROBINSON, J. W. L. (1978). Kinetics of the absorption of amino acids by the rat intestine in vivo. *Biochim. biophys. Acta* **512**, 172–191.

BLISS, C. I. & JAMES, A. T. (1966). Fitting the rectangular hyperbola. *Biometrics* **22**, 573–602.

CHRISTENSEN, H. N. (1975). Recognition sites for material transport and information transfer. *Curr. Top. mem. Trans.* **6**, 227–258.

CHRISTENSEN, H. N. & HANDLOGTEN, M. E. (1977). Does the non-saturable cell entry apply to the charge-free form of amino acids. *Biochim. biophys. Acta* **469**, 216–220.

CHRISTENSEN, H. N. & LIANG, M. (1966). On the nature of the 'non-saturable' migration of amino acids into Erhlich cells and into rat jejunum. *Biochim. biophys. Acta* **112**, 524–531.

CURRAN, P. F., SCHULTZ, S. G., CHEZ, R. A. & FUISZ, R. E. (1967). Kinetic relations of the Na-amino acid interaction at the mucosal border of intestine. *J. gen. Physiol.* **50**, 1261–1286.

HONEGGER, P. & SEMENZA, G. (1973). Multiplicity of carriers for free glucalogues in hamster small intestine. *Biochim. biophys. Acta* **318**, 390–410.

MUNCK, B. G. & SCHULTZ, S. G. (1969). Interactions between leucine and lysine transport in rabbit ileum. *Biochim. biophys. Acta* **183**, 182–193.

NELDER, J. A. & MEAD, R. (1965). A simplex method for function minimization. *Comp. J.* **7**, 308–318.

NEWEY, H. & SMYTH, D. H. (1964). The transfer system for neutral amino acids in the rat small intestine. *J. Physiol.* **170**, 328–343.

PETERSON, S. C., GOLDNER, A. M. & CURRAN, P. F. (1970). Glycine transport in rabbit ileum. *Am. J. Physiol.* **219**, 1027–1032.

PRESTON, R. L., SCHAEFFER, J. F. & CURRAN, P. F. (1974). Structure-affinity relationships of substrates for the neutral amino acid transport system in rabbit ileum. *J. gen. Physiol.* **64**, 443–467.

SAMARŽIJA, I. (1978). Experimentelle Untersuchungen zum Aminosäuretransport im proximalen Tubulus der Rattenniere. Inaugural Dissertation. Johann Wolfgang Goethe-Universität, Frankfurt am Main.

SCHULTZ, S. G. & MARKSCHEID-KASPI, L. (1971). Competitive interactions between L-alanine and L-phenylalanine in rabbit ileum. *Biochim. biophys. Acta* **241**, 857–860.

SCHULTZ, S. G., YU-TU, L. & STRECKER, C. K. (1972). Influx of neutral amino acids across the brush border of rabbit ileum. Stereospecificity and the roles of the α-amino and α-carboxylate groups. *Biochim. biophys. Acta* **288**, 367–379.

SEPÚLVEDA, F. V. & SMITH, M. W. (1978). Discrimination between different entry mechanisms for neutral amino acids in rabbit ileal mucosa. *J. Physiol.* **282**, 73–90.

SEPÚLVEDA, F. V. & SMITH, M. W. 1979). Different mechanisms for neutral amino acid uptake by new-born pig colon. *J. Physiol.* **286**, 479–490.