# THE UPTAKE OF AMINO ACIDS BY ISOLATED SEGMENTS OF RAT INTESTINE

## II. A SURVEY OF AFFINITY FOR UPTAKE FROM RATES OF UPTAKE AND COMPETITION FOR UPTAKE

L. R. FINCH AND F. J. R. HIRD

*Department of Biochemistry and School of Agriculture, University of Melbourne, Victoria (Australia)*

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SUMMARY

Average rates have been determined for the uptake by segments of rat small intestine of 17 amino acids at the concentrations of 1 m*M* and 10 m*M*. At 10 m*M* the order is such that the more lipophilic amino acids show the smaller rates, but at 1 m*M* the order is distinctly reversed, with the more lipophilic amino acids having the greater rates. On the assumption that MICHAELIS-MENTEN kinetics apply to the rate of uptake, MICHAELIS constants have been calculated from the average rates at 1 m*M* and 10 m*M*. From these constants, assuming that the amino acids compete for uptake by a MICHAELIS-MENTEN mechanism, predictions have been made of the effect of one amino acid on the other and have been compared with observed values. In general, the predictions agree well with the observed values, suggesting that the assumptions may be correct; but exceptions to the agreement suggest that L-lysine, and probably L-ornithine and L-arginine, may not compete for a common mechanism with the other L-amino acids. If the agreement is taken to implicate a rate-limiting, reversible combination with a common site in the uptake of most L-amino acids, then such a site shows a higher affinity for the more lipophilic amino acids. Limited studies with D-amino acids suggest that they may also combine with the site for uptake of L-amino acids, but have a lower affinity for it.

## INTRODUCTION

The specificity of the active process for transport of amino acids by isolated intestine has not been clearly defined by previous work. WISEMAN[1,2] has given evidence of different rates for different amino acids in the transfer by isolated sacs of hamster small intestine. Since the study was carried out at a single concentration, information of the changes of the rate with concentration, from which an order of affinities might be inferred, is not available. With the techniques of both transfer[1] and uptake[3], and probably of absorption[4], the more lipophilic amino acids have the greater effectiveness as inhibitors of the transport, suggestive of a greater affinity. Agreement between the affinities intrinsic to rates of transport and inhibition of transport would suggest

that the amino acids are combined only with common sites in the process of transport. The present paper represents an attempt to compare affinities for uptake and inhibition of uptake. The comparison has been made on the basis of MICHAELIS-MENTEN kinetics.

## EXPERIMENTAL METHODS

The techniques used and the allowances for water exchange were the same as in the previous paper[5].

## RESULTS

### *Comparisons of rates at different concentrations*

From preliminary studies of the uptake at 4 min of several amino acids over a range of concentrations (Figs. 1, 2 and 3), it was decided to make a survey of rates of uptake at concentrations of 1 m$M$ and 10 m$M$, using the uptake at 4 min as a measure of the rate of uptake. Individual determinations were made for each amino acid on several preparations of tissue, comparing as many amino acids as possible on the one tissue. The average values for the uptakes at 1 m$M$ and at 10 m$M$ are presented in Table I. It is apparent that at both concentrations L-amino acids possessing similar charges and oil–water partition characteristics show similar rates of

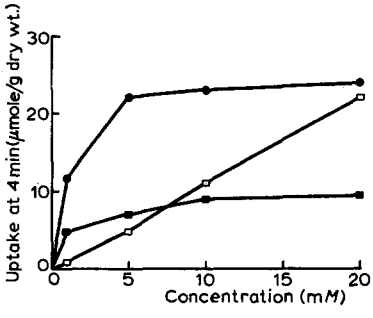


Fig. 1. The effect of concentration on the rate of uptake of L-lysine, L-glutamic acid and L-isoleucine. Segments of small intestine incubated with amino acid at the given concentrations. ■—■, L-lysine; □—□, L-glutamic acid; ●—●, L-isoleucine.

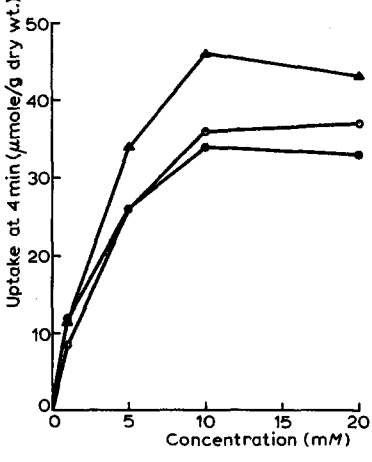


Fig. 2. The effect of concentration on the rate of uptake of L-histidine, L-alanine and L-isoleucine. Segments of small intestine incubated with amino acid at the given concentrations. O—O, L-histidine; ▲—▲, L-alanine; ●—●, L-isoleucine.

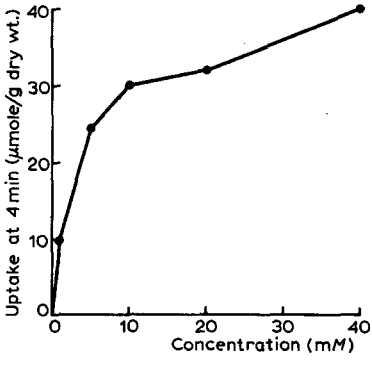


Fig. 3. The effect of concentration on the rate of uptake of L-phenylalanine. Segments of small intestine incubated with [$^{14}$C]amino acid at the given concentrations. Change in concentration of amino acid in the external solution measured by direct plating and counting in a gas flow counter.

uptake, except in so far as metabolism of the amino acids may have affected the determination[5]. For the amino acids without a third charged group at pH 7.4 the general trend at 10 m$M$ is that the more lipophilic amino acids have the lower rates of uptake; the order being closely similar to that for transfer at 20 m$M$ (see ref. 1, 2). The trend at 1 m$M$, however, is reversed.

TABLE I

RATES OF UPTAKE OF L-AMINO ACIDS

Segments of small intestine incubated with amino acid at given concentration, uptake at 4 min taken as a measure of the rate of uptake. Results presented as the mean ± S.E.M. for the bracketed number of experiments.

| *Amino acid* | *Uptake at 10 mM (μmole/g dry wt.)* | *Uptake at 1 mM (μmole/g dry wt.)* |
|---|---|---|
| Lysine | 10.0 ± 1.96 (5) | 6.8 ± 1.37 (3) |
| Ornithine | 23.2 ± 2.72 (4) | 5.3 ± 2.40 (2) |
| Arginine | 16.7 ± 1.80 (4) | 7.8 ± 1.41 (3) |
| Aspartic acid | 14.4 ± 2.05 (3) | 1.6 ± 1.02 (3) |
| Glutamic acid | 13.6 ± 2.17 (5) | 1.8 ± 0.53 (3) |
| Asparagine | 57.3 ± 8.49 (3) | 8.6 ± 0.97 (3) |
| Glutamine | 51.0 ± 6.32 (4) | 11.3 ± 2.20 (2) |
| Glycine | 35.7 ± 4.08 (9) | 2.7 ± 0.77 (3) |
| Serine | 54.7 ± 9.13 (4) | 8.1 ± 1.11 (3) |
| Proline | 43.6 ± 1.87 (5) | 7.8 ± 0.65 (2) |
| Histidine | 41.1 ± 2.58 (15) | 7.35 ± 0.85 (8) |
| Alanine | 45.3 ± 1.57 (16) | 11.3 ± 0.49 (9) |
| Phenylalanine | 35.5 ± 1.50 (2) | 10.9 ± 0.70 (2) |
| Valine | 34.7 ± 3.88 (4) | 13.6 ± 1.92 (3) |
| Isoleucine | 25.2 ± 2.06 (9) | 12.7 ± 0.54 (8) |
| Methionine | 24.0 ± 1.88 (5) | 13.7 ± 0.50 (2) |
| Leucine | 21.5 ± 3.50 (2) | 13.9 ± 1.70 (2) |

*Comparison between data for rates of uptake and for inhibition of uptake*

Predictions of the competition between amino acids have been made by assuming that the rate of uptake measured follows MICHAELIS-MENTEN kinetics with respect to the external concentration of amino acid. That is, the rate of uptake,

$$v = \frac{V_{\max} S}{K_m + S} \tag{1}$$

where $S$ is the external concentration of the amino acid, $V_{\max}$ is a constant and $K_m$ is the MICHAELIS constant. If two amino acids, $A'$ and $A''$, at concentrations $S'$ and $S''$ with MICHAELIS constants $K'_m$ and $K''_m$, respectively, are competing for such a mechanism, the relationship,

$$\frac{v'_{A''}}{v'} = \frac{K'_m + S'}{K'_m + S' + \dfrac{K'_m S''}{K''_m}} \tag{2}$$

derived from THORNE[6], will apply. Here $v'_{A''}$ is the rate of uptake of $A'$ per unit of tissue in the presence of $A''$ and $v'$ is the rate of uptake of $A'$ per unit of tissue when alone.

The results presented in Figs. 1–3 suggest that it may be reasonable to assume MICHAELIS-MENTEN kinetics in considering the inhibition of uptake. As a partial test of the assumptions of MICHAELIS-MENTEN kinetics and competition for a common site in the uptake of L-histidine and L-isoleucine, the uptakes of L-histidine in the presence of varying concentrations of L-isoleucine were determined, and compared (Fig. 4) with values predicted from the average control uptake and the MICHAELIS constants derived from the rates of uptake at 1 m$M$ and 10 m$M$ (see Table II). The agreement gives support for the assumptions.

Table II presents values for the MICHAELIS constants calculated from the average rates in Table I, together with the predicted uptake of L-histidine at 10 m$M$ in the presence of other amino acids at 10 m$M$. For comparison the experimental values of

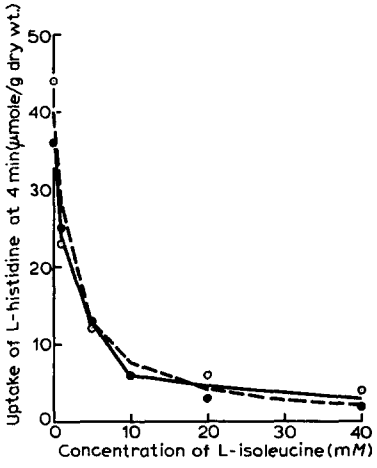


Fig. 4. The effect of various concentrations of L-isoleucine on the rate of uptake of L-histidine. Segments of small intestine incubated with L-histidine at 10 m$M$ and the given concentrations of L-isoleucine. Results presented for experiments on two different preparations of tissue. ●—●, O—O, experimental points; ———, average of experimental points; – – – –, values predicted from the MICHAELIS constants and the average uptake of L-histidine in the absence of L-isoleucine.

AGAR *et al.*[3] for uptake of L-histidine in the presence of other amino acids are included. Calculation of a MICHAELIS constant from the rates of uptake for glycine yields a negative value, since the rate at 1 m$M$ is less than 0.1 the rate at 10 m$M$. If a constant is calculated from the data for its inhibitory effect on the uptake of L-histidine, the value 34 m$M$ is obtained. This value coupled with the average rate at 10 m$M$ predicts a rate of 4.5 $\mu$moles/g dry weight at 1 m$M$ as compared with the average value of 2.7 found for three determinations. It is possible that the errors involved in the different values determined could account for this discrepancy. The value of the MICHAELIS constant derived from the effect of glycine on L-histidine uptake, that is, 34 m$M$, has been used in later calculations for glycine.

To examine whether the effect of amino acids on the uptake of other amino acids would be predicted from the data for rate of uptake, some further series of experiments were performed. The results for combinations of the amino acids, L-lysine, L-glutamic acid, L-histidine, L-alanine and L-isoleucine, are given in Table III. Table IV gives values for the uptake of L-amino acids in the presence of 10 m$M$ L-isoleucine. The

TABLE II

MICHAELIS CONSTANTS FOR THE UPTAKE OF L-AMINO ACIDS WITH PREDICTED AND OBSERVED UPTAKES OF L-HISTIDINE IN THE PRESENCE OF THE AMINO ACIDS

MICHAELIS constants calculated from the rates of uptake at 1 m*M* and 10 m*M* (Table I). Values for the uptake of L-histidine at 10 m*M* in the presence of L-amino acid at 10 m*M* expressed as a percentage of the uptake in control (L-histidine alone at 10 m*M*). Predicted values calculated from the MICHAELIS constants as described in text, experimental values from AGAR *et al.*[3]. No corrections for water exchange.

| *Amino acid* | *Michaelis constant (mM)* | *Uptake of L-histidine in presence of other amino acid (% control uptake)* | | |
|---|---|---|---|---|
| | | *Predicted* | *Experimental* | |
| | | | *at 15 min* | *at 45 min* |
| Lysine | 0.55 | 10 | 83 | 76 |
| Ornithine | 6.0 | 54 | — | — |
| Arginine | 1.5 | 23 | 79 | 77 |
| Aspartic acid | 80 | 94 | 81 | 87 |
| Glutamic acid | 26 | 84 | 110 | 105 |
| Asparagine | 17 | 77 | 74 | 73 |
| Glutamine | 6.4 | 57 | 76 | 81 |
| Glycine | — | — | 78 | 87 |
| Serine | 18 | 77 | 84 | 78 |
| Proline | 10 | 66 | 75 | 74 |
| Histidine | 10.4 | — | — | — |
| Alanine | 5.0 | 49 | 66 | 57 |
| Phenylalanine | 3.3 | 39 | 45 | 49 |
| Valine | 2.1 | 28 | 38 | 43 |
| Isoleucine | 1.2 | 19 | 30 | 22 |
| Methionine | 0.91 | 15 | 14 | 23 |
| Leucine | 0.65 | 11 | 9 | 21 |

results of further studies of inhibitions, including some on the uptake of L-isoleucine, are given in Table V. In each of these Tables, the inhibited uptake is expressed as a percentage of the control uptake. The average inhibited uptake is also given in $\mu$mole/g dry weight to allow an estimate of the absolute magnitude of the percentage values. Thus in some cases apparently large differences between the predicted and experimental percentage values would require only small differences in absolute uptake.

Predictions involving L-lysine, L-ornithine and L-arginine show some marked disagreement with experimental values (Tables II, III and IV). Adsorption and, for L-ornithine and L-arginine, metabolism[5] of the amino acid may play a part in the observed uptake of these three amino acids, but, from the small inhibition of uptake by L-isoleucine, uptake by a process involving combination with a site common with L-isoleucine is unimportant.

L-aspartic and L-glutamic acids have low rates of uptake (Table I) and low affinities in uptake (Fig. 1, Tables II and III). The non-recovery of these two amino acids from the tissue[5] means that the uptake may substantially represent metabolism rather than accumulation within the tissue, so that the comparisons of data may not be valid. However, qualitatively, the strong inhibition by L-isoleucine and L-histidine (Table IV) suggests that an uptake, involving the same site as does the uptake of L-isoleucine, is a rate limiting step in the removal from the medium.

## TABLE III

THE UPTAKE OF SOME L-AMINO ACIDS IN THE PRESENCE OF ONE ANOTHER

Segments of small intestine incubated 4 min with the given concentrations of the amino acids under study. Controls contained the amino acids singly. Predicted values calculated from the MICHAELIS constants (Table II) as described in text.

| *Amino acid studied, concentration (mM)* | *Additional amino acid, concentration (mM)* | *Uptake in the presence of additional amino acid* | | |
|---|---|---|---|---|
| | | *Experimental* | | *Predicted* |
| | | *(μmole/g dry wt.)* | *(% control uptake)* | *(% control uptake)* |
| Glutamic acid 20 | Isoleucine 2 | 5.0 | 26 | 52 |
| Glutamic acid 5 | Isoleucine 5 | 1.5 | 31 | 22 |
| Glutamic acid 5 | Lysine 5 | 5.1 | 104 | 12 |
| Glutamic acid 20 | Lysine 20 | 21 | 110 | 5 |
| Glutamic acid 5 | Glutamic acid 5 | 5.5* | 112* | 86* |
| Lysine 5 | Glutamic acid 5 | 11 | 153 | 99 |
| Lysine 20 | Glutamic acid 20 | 21 | 162 | 98 |
| Lysine 20 | Isoleucine 5 | 9.5 | 73 | 91 |
| Lysine 5 | Isoleucine 5 | 6.5 | 93 | 73 |
| Lysine 5 | Lysine 5 | 4.5* | 64* | 57* |
| Isoleucine 5 | Lysine 5 | 20 | 87 | 36 |
| Isoleucine 5 | Glutamic acid 5 | 22 | 96 | 97 |
| Isoleucine 5 | Alanine 5 | 24 | 92 | 84 |
| Isoleucine 5 | Histidine 5 | 25 | 96 | 91 |
| Isoleucine 5 | Isoleucine 5 | 11.5* | 52* | 55* |
| Isoleucine 5 | Isoleucine 5 | 17* | 65* | 55* |
| Alanine 5 | Isoleucine 5 | 6.4 | 19 | 32 |
| Alanine 5 | Histidine 5 | 29 | 85 | 80 |
| Alanine 5 | Alanine 5 | 23* | 67* | 67* |
| Histidine 5 | Alanine 5 | 20 | 77 | 60 |
| Histidine 5 | Isoleucine 5 | 2.6 | 10 | 26 |
| Histidine 5 | Histidine 5 | 18* | 69* | 75* |

* These values based on half the uptake at 10 m*M*, using the uptake at 5 m*M* as control.

## TABLE IV

THE UPTAKE OF L-AMINO ACIDS IN THE PRESENCE OF L-ISOLEUCINE

Segments of small intestine incubated 4 min with amino acid (10 m*M*) and L-isoleucine (10 m*M*). L-isoleucine omitted in the control. Predicted values calculated from the MICHAELIS constants (Table II) as described in text.

| *Amino acid* | *Uptake in the presence of 10 mM L-isoleucine* | | |
|---|---|---|---|
| | *Average uptake (μmole/g dry wt.)* | *Individual experiments (% control uptake)* | *Predicted values (% control uptake)* |
| Lysine | 8.5 | 69, 75 | 69 |
| Ornithine | 15 | 65, 74 | 24 |
| Arginine | 19 | 108, 132 | 48 |
| Aspartic acid | 6.5 | 44, 33 | 12 |
| Glutamic acid | —2.5 | —18, —25 | 12 |
| Asparagine | 12 | 20, 30 | 16 |
| Glutamine | 9 | 29, 16 | 24 |
| Glycine | 7 | 14, 23, 31, 26 | 14 |
| Serine | 5 | 13, 15 | 16 |
| Proline | 5 | 16, 9 | 19 |
| Histidine | 6 | 17, 13 | 19 |
| Alanine | 13 | 43, 31 | 26 |
| Valine | 7 | 20, 29 | 41 |
| Isoleucine | 14* | 50*, 52* | 53* |
| Methionine | 16 | 60, 77 | 59 |

* These values are based on half the uptake at 20 m*M*, using the uptake at 10 m*M* as the control.

TABLE V

THE UPTAKE OF SOME L-AMINO ACIDS IN THE PRESENCE OF ANOTHER AMINO ACID

Segments of small intestine incubated 4 min with the amino acid studied (10 m*M*) and the given concentration of an additional amino acid. No additional amino acid in control. Predicted values calculated from the MICHAELIS constants (Table II) as described in text.

| *Amino acid studied* | *Additional amino acid present, Concentration (mM)* | | *Average uptake (μmole/g dry wt.)* | *Individual experiments (% control uptake)* | *Predicted values (% control uptake)* |
|---|---|---|---|---|---|
| Isoleucine | L-proline | 10 | 24 | 83 | 90 |
| Isoleucine | L-alanine | 10 | 17 | 95 | 82 |
| Isoleucine | L-valine | 10 | 8 | 43, 33 | 66 |
| Isoleucine | L-methionine | 10 | 12 | 50, 45 | 42 |
| Phenylalanine | L-phenylalanine | 10 | 16* | 53* | 57* |
| Aspartic acid | L-histidine | 10 | 6 | 27 | 53 |
| Glycine | L-histidine | 10 | 6 | 21 | 58 |
| Valine | L-histidine | 10 | 32 | 75 | 85 |
| Histidine | DL-α-amino-octanoic acid | 2 | 15 | 44, 34 | — |

* These values based on half the uptake at 20 m*M*, using the uptake at 10 m*M* as control.

The two amides L-asparagine and L-glutamine show higher affinities and much greater rates of uptake than the corresponding acids (Tables I and II). For L-asparagine there is good agreement between the predicted and observed effects (Tables II and III). For L-glutamine, agreement with prediction might not be expected, since this amide is metabolised by the tissue[5]; but since the uptake of L-glutamine is strongly inhibited by L-isoleucine it seems likely that combination with a site common to other amino acids is a rate limiting step in the uptake process.

For the remaining L-amino acids studied the experimental results obtained show a reasonable agreement with the predictions. The large number of experiments with L-histidine and L-isoleucine, separately and together, and with other amino acids almost all give data in good agreement with the predictions. The general trend of agreement between the predicted and experimental values, except for the amino acids, lysine, arginine and ornithine, provides further support for the two assumptions of MICHAELIS-MENTEN kinetics and a common site in the uptake of most L-amino acids.

Considered as inverse measures of the affinity, the MICHAELIS constants for the process of uptake (Table II) show the affinity of this site to be greater for the more lipophilic L-amino acids. An interesting indication that this high affinity for lipophilic L-amino acids is not restricted to the naturally occurring amino acids comes from the effectiveness of DL-α-amino-octanoic acid as an inhibitor of the uptake of L-histidine (Table V).

The importance of the external concentration of amino acid (amino acid in the medium) rather than the internal concentration (amino acid taken up) in inhibiting the uptake of a second amino acid is shown by the experiment presented in Fig. 5. The results show that L-isoleucine taken up during a prior incubation is not an effective inhibitor of the uptake of L-histidine, whereas L-isoleucine present in the medium is an effective inhibitor. The results would appear to exclude the importance of retention sites within the tissue in the mechanism of uptake of L-histidine.

*The uptake of and inhibition by* D-*amino acids*

Some investigations of the uptake of certain D-amino acids are presented in Table VI. Experiments were carried out on the effect of various amino acids at 10 m*M*, including some D-amino acids, on the uptake of L-histidine at 0.5 m*M*. The experiments were unsatisfactory in that the correction for release of histidine from the tissue makes up a large proportion (about 60 %) of the uptake at this concentration, so that relatively small variations in this release may cause considerable errors in the uptake determined. The results are presented in Table VII as being of qualitative value in making comparisons of the effectiveness of these amino acids as inhibitors of the uptake of L-histidine.

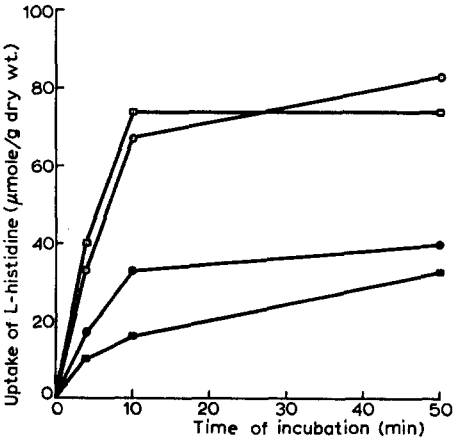


Fig. 5. The effect of preincubation with L-isoleucine on the uptake of L-histidine. Segments of small intestine preincubated and incubated with amino acids as specified below. 10 min preincubation: ■—■, □—□, with L-isoleucine (10 m*M*); ●—●, ○—○, without L-isoleucine. Incubation with L-histidine (10 m*M*): ■—■, ●—●, plus L-isoleucine (10 m*M*); □—□, ○—○, without L-isoleucine.

TABLE VI

THE UPTAKE AND INHIBITION OF UPTAKE OF SOME D-AMINO ACIDS

Segments of small intestine incubated 4 min with D-amino acid at the given concentrations. The effects of 10 m*M* L-isoleucine and 2,4-dinitrophenol[5] determined as for L-amino acids.

| *Amino acid* | *Uptake at 10 mM (μmole/g dry wt.)* | *Uptake at 1 mM (μmole/g dry wt.)* | *Uptake at 10 mM in presence of DNP (% control uptake)* | *Uptake at 10 mM in presence of 10 mM L-isoleucine (% control uptake)* |
|---|---|---|---|---|
| D-serine | 8, 13 | — | — | 50, 38 |
| D-threonine | 3, 7 | — | — | 0, —29 |
| D-histidine | 6, 7 | — | — | —16, —14 |
| D-alanine | 8, 13, 16 | 5.5 | 100 | 62, 44 |
| D-phenylalanine | 6, 2 | 0.6 | — | — |
| D-valine | 10, 11 | — | — | — |
| D-isoleucine | 7, 12 | 2.0, 2.7 | 171, 42 | — |
| D-methionine | 25, 24, 21 | 2.0, 3.3 | 19 | 16, 29 |
| D-leucine | 23, 22 | — | — | — |

TABLE VII

THE EFFECTS OF D-AMINO ACIDS AND SOME OTHER AMINO ACIDS ON THE UPTAKE OF L-HISTIDINE

Segments of small intestine incubated 4 min with 0.5 m*M* L-histidine plus an additional amino acid at 10 m*M*. Additional amino acid omitted in the control. Values corrected for the blank released from tissue incubated in medium without amino acid and estimated as 2.5 and 2.4 μmoles of histidine/g dry wt. in Expt. 1, and 2.2 and 2.1 μmoles of histidine/g dry wt. in Expt. 2.

| *Additional amino acid* | *Uptake of L-histidine (μmole/g dry wt.)* | |
|---|---|---|
| | *Expt. 1* | *Expt. 2* |
| Control (L-histidine alone) | 3.9, 4.4 | 3.2, 3.7 |
| α-amino-isobutyric acid | 2.0 | 2.2 |
| L-2,4-diamino-butyric acid | 3.9 | 3.0 |
| Glycine | 2.4 | 1.5 |
| D-serine | 4.1 | 3.4 |
| D-threonine | 4.3 | 3.5 |
| D-alanine | 3.7 | 2.6 |
| D-phenylalanine | 3.4 | 2.1 |
| D-valine | 4.3 | 1.9 |
| D-isoleucine | 3.7 | 3.2 |
| D-methionine | 1.5 | 1.0 |
| D-leucine | 2.6 | 1.8 |
| D-tryptophane | 1.3 | 1.5 |

The results, together with those of previous workers[3,7,8], indicate that D-amino acids may be taken up through a process involving combination with the site concerned in the uptake of L-amino acids, but that the affinity for the site is relatively low. The rapid uptake of D-leucine and D-methionine at 10 m*M* suggests a particular importance of the process for these amino acids. This suggestion for D-methionine is supported by the considerable inhibition of uptake both by L-isoleucine and 2,4-dinitrophenol. The effects on the uptake of L-histidine suggest that D-methionine, D-leucine and D-tryptophane have about the same affinity for the site as have glycine and L-amino-isobutyric acid, whereas the other D-amino acids and L-2,4-diamino-butyric acid have a lower affinity or none at all.

## DISCUSSION

The conclusions drawn for the uptake of the individual amino acids may be summarised to state that the process involves combination with a site which appears to be available to all of the L-amino acids tested, with the possible exceptions of lysine, ornithine and arginine. There is evidence that some D-amino acids also combine with the site. The higher affinity for the more lipophilic L-amino acids is in agreement with previous results on the uptake, transfer and absorption of L-histidine[3,4], and on competition in the transfer of amino acids[1].

An important aspect of the results obtained is the reversal of the order of rates with change in concentration. The effect that the rate of uptake at high concentrations is in the inverse order to the affinity, and at low concentrations is in the same order as the affinity has been predicted by WILBRANDT[9] for uptake by carrier transport. In such a system the rate would be determined by the degree of saturation of the carrier and the net rate of dissociation from it. The first of these would be increased,

and the second decreased, with greater affinity for the carrier, so lowering the maximum rate on complete saturation of the carrier system, but raising the rate at concentrations well below saturation. The similar time-progresses of uptake of amino acids[5], or the concentration of amino acids against a gradient by intestinal tissue[1-3], are not predicted by the mechanism proposed by WILBRANDT[9], but are explicable by relatively simple modifications of it.

On the data derived in the present work, suggestions may be made as to the nature of the site involved in the uptake of amino acids by small intestine. It is proposed that the site has specific points of attachment for the L-amino and L-carboxyl groups, with an adjacent lipophilic region situated so as to allow concurrent attachment of the lipophilic side chains of the L-amino acids. Since glycine attaches to the site, a side chain is not an essential for attachment. Moreover, if the inhibitory effect of L-amino-isobutyric acid on the uptake of L-histidine can be taken to indicate combination with the site, then the α-hydrogen is also non-essential for the attachment. For L-amino acids, the greater the length of the carbon chain the more strongly is it held to the lipophilic area, so resulting in a higher affinity for the site and a slower dissociation from it to effect transport. Hydrophilic groups on the side chain will have the reverse effect. The flexibility of the side chain may also be of importance in determining the readiness of attachment to the site.

It is reasonable to suggest that the site functions to determine the rate of transport of the amino acid by binding it to a mobile carrier. It is possible that the transport of amino acids may be the unique function of the site, or that the site may also be the binding centre of an enzyme involving amino acids, *e.g.* a peptidase or transaminase. It is conceivable that the location of an enzyme on a mobile carrier could "adsorb" an amino acid and, without the presence of an acceptor molecule (*e.g.* keto acid in the case of transaminase), move it from point A and release it at point B, without structural change. Previous information[1-5] and that in the present paper is consistent with the existence of a mobile carrier for the movement of amino acids into the tissue and of some further process for the movement from the tissue (possibly as a solute in a water stream) at a rate proportional to the amount in the tissue. The concentration gradient obtained would be a resultant of these two processes. A plausible suggestion for the mechanism of carrier movement is that it involves the flow and vesiculation of cell membranes, which might support the specific carrier sites[10,11].

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