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# THE MECHANISM OF TRANSFER FOR L-LEUCINE INTO THE VASCULAR BED OF THE *ANURAN* SMALL INTESTINE

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## SUMMARY

1. The vascularly perfused small intestine of *Rana pipiens* was used to investigate the movement of the amino acid L-leucine from the epithelium into the vascular bed. It was found that only a few amino acids when present in the lumen inhibited the wash-out of leucine into the vascular bed. The series of amino acids which had this effect belonged to the group previously shown to be transported by 'L-type' carrier systems.

2. Nearly all amino acids when present in the lumen accelerated the flux of leucine from the vascular bed to the lumen and there was little correlation between the amino acids which caused this effect and those which inhibited leucine wash-out into the vascular bed. Replacement of luminal sodium also promoted serosal-to-mucosal leucine flux.

3. The effect of the presence of amino acids in the lumen on the uptake of leucine from the vascular bed was measured using a fractional extraction technique; sucrose was the extracellular marker. There was complete correlation between the amino acids which promoted the extraction of leucine from the vascular bed and those which inhibited leucine wash-out into the vascular bed.

4. In contrast, the wash-out of leucine into the vascular bed was not accelerated by the addition of amino acids to the vascular perfusate, and the presence of 10 mM-leucine in the vascular bed had very little effect upon the mucosal-to-serosal flux of leucine.

5. These results are discussed with regard to the specificity of an exit system for leucine, in the intestinal epithelium, which appears to have an energy requirement.

## INTRODUCTION

The transfer of amino acids from the lumen of the small intestine to the vascular bed involves a series of steps. First, the substrate has to cross the unstirred layer of fluid adjacent to the mucosal cell membrane and then it has to be transported into the epithelial cell across the brush border. Both of these steps have been extensively investigated, and it is now well accepted that there are several translocating systems with different specificities within the brush-border membrane. Several of these systems are active, i.e. they can move the substrate uphill against a concentration gradient. In many species of animals, co-transport with sodium (down its concentration

gradient) and the influence of the membrane potential appear to supply the required energy for this active transport.

In contrast, until recently, there has been little information on the subsequent steps involved in the movement of amino acids from the epithelial cells (enterocytes) into the vascular bed. Munck & Schultz (1969) indicated by indirect measurement that the exit of lysine at the serosal pole of rabbit intestine is carrier-mediated, while Hajjar, Khuri & Curran (1972) came to a similar conclusion for the movement of alanine. However, these experiments did not allow for direct access to the basolateral membranes of the enterocytes; the amino acids also had to penetrate thick layers of connective tissue and muscle, even in so-called 'stripped' preparations.

Recently, Cheeseman (1979) showed that the movement of L-leucine from the frog intestinal epithelium into the perfused vascular bed could be followed using a wash-out technique. The present study has made use of this approach to examine the specificity of the exit system used by L-leucine. In addition, the possibility of asymmetry of the system has been examined by measuring the entry of the amino acid into the epithelial cells from the vascular bed.

The findings suggest that L-leucine exits from the enterocyte via a system which exhibits an 'L-type' specificity on the intracellular side of the membrane but has a much lower affinity for leucine on the extracellular side.

## METHODS

### *Animals*

*Rana pipiens*, weight range 15–60 g, were kept at 22 °C in tanks containing frequently changed filtered tap water. They were fed once a week with live locusts.

### *Experimental procedure*

The vascularly perfused *Anuran* small intestine was used as described by Boyd, Cheeseman & Parsons (1975). Single-pass flow systems were employed for both the perfusion of the lumen and the vascular bed. The rate of flow through both systems was the same and fell within the range of 3–10 ml . g dry wt. $gut^{-1}$ $min^{-1}$.

The composition of the frog Ringer solution used in the perfusates was as follows (mM): NaCl, 93; KCl, 5; $MgSO_4$, 1; $MgCl_2$, 0·8; $NaH_2PO_4$, 2·15; $Na_2HPO_4$, 0·85; $NaHCO_3$, 25; $CaCl_2$, 0·5. In sodium-free solutions, the NaCl was replaced with choline chloride, the $NaHCO_3$ with choline bicarbonate and the sodium phosphate salts with potassium phosphates. In addition, the vascular perfusate always contained 2 mM-glucose and 1 % w/v bovine serum albumin, fraction V.

### *Wash-out experiments*

These were performed in the manner described by Cheeseman (1979). The substrate (1 mM-L-leucine) was perfused through the lumen for 30 min to load the tissue and at the end of this loading period the luminal solution was abruptly switched to one which was leucine-free. The wash-out of the leucine into the vascular bed was then followed for 80 min. This procedure could normally be performed three times on one preparation, enabling one control and two 'test' determinations to be made. The effect of other amino acids on the leucine wash-out was assessed by including them in the luminal solution at a concentration of 10 mM during the 30 min loading period and in the subsequent 80 min wash-out. During control estimates, 10 mM-mannitol was used to maintain the osmolarity of the luminal solutions.

### *Wash-in experiments*

The entry of L-leucine into the epithelium from the vascular bed (wash-in) was measured using the technique previously described by Boyd & Parsons (1979). The fractional extraction of the substrate was estimated with respect to an extracellular marker, in this case $^3H$-labelled sucrose,

which is not hydrolysed by the frog small intestine (Parsons & Pritchard, 1968). L-leucine and sucrose were added to the vascular perfusate, which was continuously infused, while various other substances were added to either the luminal or the vascular solutions.

*Measurement of serosal-to-mucosal fluxes*

The flux of leucine from the vascular bed to the lumen was assessed by the addition of radiolabelled amino acid to the vascular perfusate. Fractional collections were made of the effluent from the lumen and different amino acids added to the luminal perfusate to assess their effect, if any, on the serosal-to-mucosal flux.

*Estimation of tissue concentration of leucine during perfusion*

The preparation was perfused with $^{14}$C-labelled 1 mM-leucine in the lumen and initially a vascular solution containing 10 mM mannitol for 45 min to allow a steady state to be achieved. The vascular solution was then switched to one containing 10 mM-leucine (unlabelled) and the perfusion continued for a further 30 min. The lumen was then flushed out with ice-cold choline Ringer solution, opened along its length and mucosal scrapings taken with a microscope slide. The scrapings were weighed, dried, reweighed and then extracted in 0·05 N-nitric acid, aliquots of which were taken for liquid scintillation counting.

The luminal and vascular extracellular spaces were determined by the addition of $^{3}$H-labelled sucrose to either the vascular or luminal solutions and the results pooled for the calculation of a mean intracellular water content of the tissues.

*Materials*

Bovine serum albumin, fraction V, and all the amino acids were supplied by Sigma Ltd. Bio-Solv BBS3 was obtained from Beckman. ACS scintillant (aqueous counting scintillant), L-[1-$^{14}$C]leucine and [6,6′(n)-$^{3}$H] sucrose were from Amersham Corp. (Arlington Heights, Illinois).

*Scintillation counting*

Aliquots of vascular and luminal effluent were added to either ACS scintillant or a toluene-based one made according to the recipe of Carter & Van Dyke (1971) and counted in a Beckman LS 3133T scintillation counter.

*Calculation and expression of results*

The rate constants ($K_1$ and $K_2$) for the wash-out and their respective pool sizes ($S_{01}$ and $S_{02}$) were calculated as previously described (Cheeseman, 1979). The rate of appearance of substrate in either the vascular bed or the lumen is expressed as $\mu$mole g dry wt. tissue$^{-1}$ hr$^{-1}$ unless otherwise indicated. In the case of vascular appearance, it was assumed that 100 % recovery of the arterial infusate from the portal vein was made for all samples.

To test for the significance of an effect upon the wash-out of leucine, the following procedure was adopted. The rate constants were determined for an individual experiment and were then expressed as a ratio of the control values for the preparation. This was done to compensate for the variation found between animals, a common problem with *Anuran* tissue. The normalized values were then pooled and a Student's $t$ test applied to assess if the groups of data were significantly different from the control group.

A similar procedure was adopted for the analysis of $J_{ms}$, $J_{sm}$ and the 'wash-in' experiments for the same reason.

*Fractional extraction of leucine from the vascular bed*

Steady-state leucine disappearance from the vascular bed was estimated in the presence of the extracellular marker sucrose (Boyd & Parsons, 1979). The concentration of leucine in the vascular effluent was corrected using the known *versus* the observed concentration of sucrose in the vascular solution. The amount of leucine which entered a compartment larger than the extracellular space could then be calculated from the observed concentration of leucine in the effluent, the known concentration of leucine infused into the artery and the flow rate.

*Terminology*

$K_1$ and $K_2$ refer respectively to the rate constants for the fast and slow components of the wash-out into the vascular bed. $S_{01}$ and $S_{02}$ represent the sizes of the pools contributing to the wash-out.

$J$ is used to refer to fluxes of substrates with the following subscripts: *m*, mucosal compartment; *c*, cell compartment and *s*, serosal compartment. Hence, for example, $J_{ms}$ represents the flux of substrate from the mucosal solution to the vascular bed.

AIB refers to the amino acid α-aminoisobutyric acid and MeAIB is the abbreviation for α-(methylamino)-isobutyric acid. All enantiomorphic amino acids referred to in this paper are of the L-configuration.

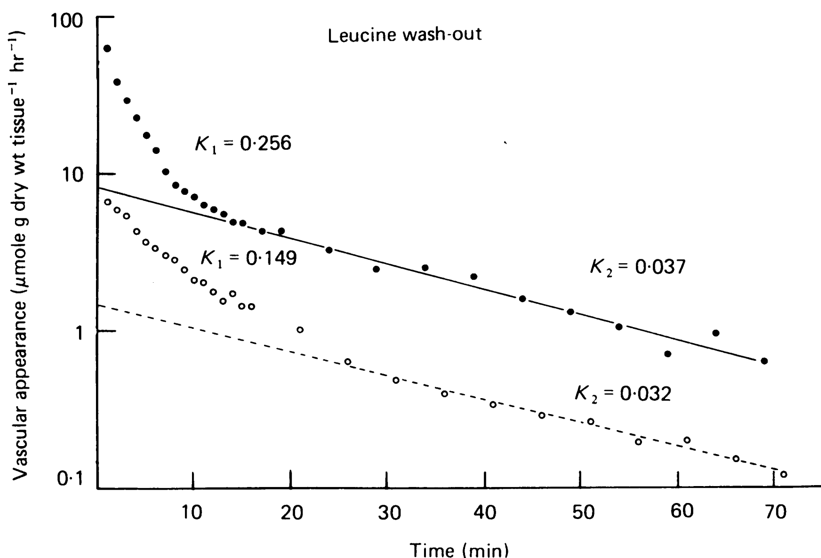


Fig. 1. The wash-out of L-leucine into the vascular bed in the presence and absence of 10 mM-methionine in the lumen. The (●) show the appearance of leucine in the vascular bed when 10 mM-mannitol was perfused through the lumen. The (○) show the appearance of leucine in the vascular bed when 10 mM-methionine was perfused through the lumen. The wash-out is plotted as the rate of appearance as μmole g dry wt. tissue$^{-1}$ hr$^{-1}$ at given time intervals after the luminal solution was changed to one containing no leucine. $K_1$ and $K_2$ refer to the fast and slow rate constants and the continuous lines indicate the slow components for both wash-outs.

## RESULTS

### *Effect of amino acids in the lumen on the vascular wash-out of L-leucine*

When L-methionine was included with L-leucine in the luminal perfusate during the 30 min loading period, the subsequent wash-out of leucine into the vascular bed was found to be slower than the control wash-out (Fig. 1). Analysis of both wash-outs showed that the presence of methionine had reduced the rate constant for the fast component ($K_1$) by nearly 50 % but had no effect upon the slower component ($K_2$). Table 1 gives the rate constants for L-leucine wash-out in the presence of a series of other amino acids, which can be divided into three groups. Methionine, tryptophan, isoleucine, phenylalanine, alanine and histidine all significantly reduced the rate constant for the fast component ($K_1$). β-alanine, threonine, glycine, proline, hydroxyproline and lysine appear to have a small but not significant effect upon leucine

wash-out, while the third group, which includes AIB, MeAIB, serine, sarcosine and arginine, had no observable effect upon the wash-out into the vascular bed.

In contrast, the effects of these amino acids on the pool sizes of the wash-out components ($S_{01}$ and $S_{02}$) and on the steady-state transfer of L-leucine do not fall into the same groupings. Proline, which had little effect upon the wash-out of leucine,

TABLE 1. Data from the analysis of leucine wash-out into the vascular bed. $K_1$ and $K_2$ are the rate constants for the fast and slow components of the wash-out expressed per min. $S_{01}$ and $S_{02}$ are the estimated pool sizes for the two components contributing to the wash-out given as $\mu$mole/g dry wt. $J_{ms}$ is the steady-state flux of leucine into the vascular bed from the lumen achieved during the loading phase and is expressed as $\mu$mole g dry wt. tissue$^{-1}$ hr$^{-1}$. In the experiments labelled 'vascular amino acid', the 10 mM-solution of test amino acid was present in the vascular bed, not the lumen, during the last 15 min of the loading phase and for the entire wash-out period. 'Sodium-free' indicates a choline vascular solution during the loading and wash-out phases. * denotes $P < 0.05$

| Amino acid | ($n$) | $K_1$ | $S_{01}$ | $K_2$ | $S_{02}$ | $J_{ms}$ |
|---|---|---|---|---|---|---|
| Control | (20) | 1·00 (0·270 ± 0·014) | 7·96 ± 0·80 | 1·00 (0·041 ± 0·003) | 2·92 ± 0·41 | 100 (97·3 ± 7·2) |
| Methionine | (2) | 0·54 ± 0·02* | 1·72 ± 1·46* | 0·93 ± 0·24 | 0·66 ± 0·01 | 10 ± 1* |
| Tryptophan | (3) | 0·49 ± 0·03* | 4·55 ± 2·10 | 1·32 ± 0·04 | 2·16 ± 2·39 | 45 ± 13* |
| Isoleucine | (3) | 0·66 ± 0·16* | 3·38 ± 1·38* | 1·12 ± 0·11 | 2·78 ± 2·39 | 23 ± 10* |
| Phenylalanine | (4) | 0·51 ± 0·06* | 3·13 ± 1·18* | 1·10 ± 0·11 | 3·01 ± 0·28 | 27 ± 12* |
| Alanine | (3) | 0·52 ± 0·01* | 3·01 ± 1·81* | 1·05 ± 0·05 | 1·74 ± 0·56 | 31 ± 14* |
| Histidine | (2) | 0·49 ± 0·04* | 4·01 ± 1·07 | 1·12 ± 0·02 | 1·44 ± 0·63 | 29 ± 7* |
| β-alanine | (4) | 0·79 ± 0·17 | 8·59 ± 2·77 | 1·00 ± 0·22 | 3·45 ± 1·10 | 97 ± 17 |
| Threonine | (3) | 0·79 ± 0·10 | 7·01 ± 1·74 | 0·83 ± 0·18 | 1·49 ± 0·47 | 51 ± 13* |
| Glycine | (5) | 0·78 ± 0·20 | 8·70 ± 2·90 | 0·90 ± 0·11 | 2·50 ± 1·50 | 88 ± 20 |
| Proline | (3) | 0·84 ± 0·10 | 3·70 ± 0·85* | 1·07 ± 0·11 | 3·72 ± 1·07 | 51 ± 11* |
| Hydroxyproline | (4) | 0·83 ± 0·15 | 8·80 ± 2·03 | 1·20 ± 0·12 | 2·67 ± 1·54 | 88 ± 17 |
| Lysine | (3) | 0·85 ± 0·06 | 5·76 ± 2·11 | 1·15 ± 0·04 | 2·11 ± 0·13 | 86 ± 13 |
| AIB | (4) | 1·14 ± 0·30 | 6·10 ± 2·10 | 0·80 ± 0·15 | 4·41 ± 1·72 | 108 ± 36 |
| MeAIB | (3) | 1·17 ± 0·16 | 7·32 ± 2·25 | 0·73 ± 0·13 | 1·67 ± 0·76 | 102 ± 6 |
| Serine | (3) | 1·00 ± 0·18 | 7·94 ± 3·44 | 0·78 ± 0·19 | 1·64 ± 0·86 | 74 ± 10 |
| Sarcosine | (4) | 1·07 ± 0·09 | 7·71 ± 1·40 | 0·83 ± 0·12 | 2·65 ± 1·48 | 84 ± 22 |
| Arginine | (2) | 0·95 ± 0·04 | 5·90 ± 1·00 | 0·98 ± 0·13 | 3·20 ± 1·39 | 79 ± 29 |
| Vascular amino acid | | | | | | |
| Methionine | (5) | 0·98 ± 0·10 | 3·6 ± 0·8 | 1·29 ± 0·09 | 4·4 ± 1·9 | 85 ± 11 |
| Leucine | (5) | 0·99 ± 0·10 | 6·8 ± 0·8 | 1·15 ± 0·11 | 3·2 ± 1·5 | 88 ± 6 |
| MeAIB | (4) | 1·04 ± 0·12 | 6·7 ± 0·5 | 1·9 ± 0·35 | 2·4 ± 0·6 | 96 ± 5 |
| Sodium-free | (4) | 1·05 ± 0·17 | 6·07 ± 1·52 | 1·34 ± 0·20 | 1·43 ± 0·77 | 100 ± 17 |

significantly inhibited the steady-state transfer of this amino acid, and this inhibition was also reflected in the reduced size of the fast wash-out component pool size $S_{01}$. If the inhibition of the mucosal-to-serosal steady-state flux ($J_{ms}$) of leucine is a consequence of competition for the transport step, then a reduction in the pool size (e.g. $S_{01}$) contributing to the vascular wash-out of leucine would suggest that the entry of leucine into the epithelium is the locus of the inhibited transport step. Hence, it is probable that a reduction in the pool size $S_{01}$ reflects competition between leucine and the test amino acid for transport across the brush-border membrane.

In contrast, it is likely that changes in the wash-out rate constant for the fast component ($K_1$) reflects alterations in the movement of the leucine from the epithelial cells into the vascular bed. Thus, a reduction of $K_1$ in the presence of a second amino

acid may well represent competition between the two amino acids for an exit system in the basolateral membranes of the enterocyte. Subsequent experiments were designed to test this hypothesis.

*The effect of amino acids in the lumen on the serosal-to-mucosal flux of L-leucine* ($J_{sm}$)

Leucine solution (10 mM) was perfused through the vascular bed and its rate of appearance in the lumen measured while different amino acids were added to the luminal perfusate, also at a concentration of 10 mM. As shown in Table 2, only three of the amino acids tested had no significant effect on $J_{sm}$: sarcosine, proline and AIB.

TABLE 2. The effect of amino acids in the lumen on the serosal-to-mucosal flux ($J_{sm}$) of leucine. Details of the procedure are given in the Methods. Sodium-free indicates a luminal solution in which the sodium was replaced with potassium. Values are expressed as a ratio of the control ± S.E. of the mean. $n$ is the number of observations and * indicates $P < 0.05$

| | $J_{sm}$ | $n$ |
|---|---|---|
| Control (10 mM-mannitol) | 1·00 (28·1 ± 4·2) | 5 |
| Methionine | 7·5 ± 2·5* | 3 |
| Histidine | 3·6 ± 0·8* | 3 |
| Phenylalanine | 7·3 ± 1·4* | 3 |
| Proline | 1·4 ± 0·1 | 4 |
| Lysine | 4·7 ± 1·0* | 4 |
| Valine | 8·6 ± 4·6* | 3 |
| Sarcosine | 1·1 ± 0·2 | 3 |
| AIB | 1·3 ± 0·1 | 4 |
| Serine | 3·2 ± 0·6* | 3 |
| Alanine | 4·3 ± 0·6* | 4 |
| Sodium-free | 1·9 ± 0·4* | 3 |

Replacement of luminal sodium also caused a significant stimulation of the leucine flux from the vascular bed to the lumen ($J_{sm}$).

The role of paracellular movements in this flux was assessed by measuring the appearance of [$^{14}$C]inulin in the lumen when it was perfused through the vascular bed. The replacement of 10 mM-mannitol with 10 mM-methionine in the lumen had no effect upon the $J_{sm}$ of inulin ($J_{sm}$ + methionine = 102% ± 2% of the control).

*Vascular wash-in of leucine* ($J_{sc}$)

If the wash-out of leucine into the vascular bed represents the movement of that amino acid out of the epithelial cells, the wash-in from the vascular bed should give a measure of the movement of the leucine into the epithelial cells across their serosal pole. This was done using sodium-free vascular solutions to eliminate the possible complications of active entry mechanisms in the basolateral membranes of the enterocyte as demonstrated by Mircheff, van Os & Wright (1980).

Fig. 2 shows the accumulated total of intracellular leucine as measured by its fractional extraction from the vascular bed. During the periods indicated by the filled bars, 10 mM-AIB was first introduced into the luminal solution and subsequently methionine was introduced. AIB appeared to have no discernible effect upon the rate of movement of leucine out of the vascular bed, but methionine caused a marked stimulation. Table 3 summarizes the results of a series of such experiments, all of

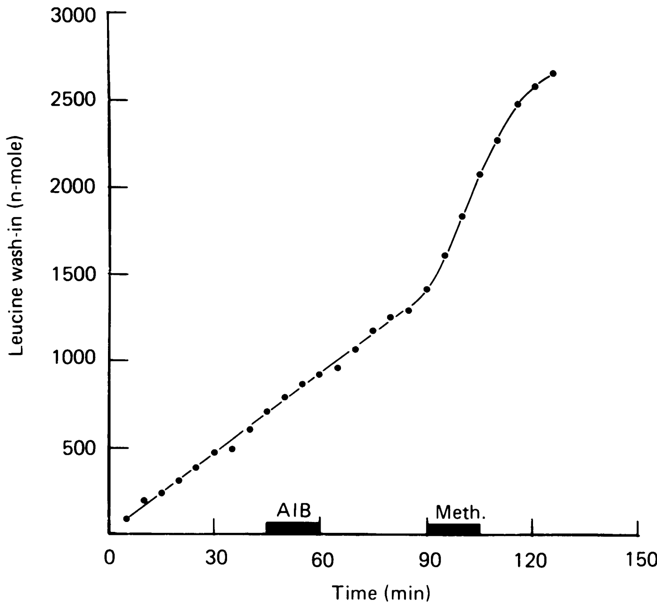


Fig. 2. The disappearance of leucine from the vascular bed and the effect of amino acids introduced into the lumen. The accumulated total leucine having disappeared from the vascular bed is plotted against time. The filled bars indicate the periods when either 10 mM-AIB or 10-methionine was perfused through the lumen in place of 10 mM-mannitol. Leucine disappearance is expressed in n-moles.

TABLE 3. The effects of various amino acids added to the lumen on the fractional extraction of leucine from the vascular bed. All rates of disappearance of leucine from the vascular bed are expressed relative to a control value of unity for that experiment ± the S.E. of the mean and the significance of any change was assessed using the Student's $t$ test. $n$ respresents the number of estimates that were made; no single amino acid was used more than once in any experiment

| Amino acid | $J_{sc}$ | $n$ | $P$ |
|---|---|---|---|
| Control | 1·00 (29·3 ± 2·87) | | |
| Leucine | 1·49 ± 0·12 | 3 | < 0·02 |
| Methionine | 1·47 ± 0·14 | 3 | < 0·05 |
| Tryptophan | 1·56 ± 0·28 | 4 | < 0·05 |
| Isoleucine | 1·50 ± 0·19 | 5 | < 0·05 |
| Phenylalanine | 1·44 ± 0·08 | 3 | < 0·01 |
| Alanine | 1·27 ± 0·01 | 3 | < 0·001 |
| Histidine | 1·88 ± 0·30 | 7 | < 0·05 |
| β-alanine | 1·08 ± 0·11 | 3 | n.s. |
| Threonine | 1·17 ± 0·12 | 3 | n.s. |
| Proline | 0·99 ± 0·03 | 3 | n.s. |
| Hydroxyproline | 0·98 ± 0·11 | 3 | n.s. |
| Lysine | 1·11 ± 0·10 | 3 | n.s. |
| AIB | 1·09 ± 0·11 | 4 | n.s. |
| MeAIB | 1·00 ± 0·03 | 3 | n.s. |
| Serine | 1·28 ± 0·18 | 4 | n.s. |
| Sarcosine | 1·06 ± 0·13 | 3 | n.s. |

which were performed with a choline Ringer vascular solution which contained no sodium. The effects observed directly paralleled those seen on the vascular wash-out fast rate constant $K_1$. Those amino acids which had little or no effect upon the wash-out (like MeAIB, serine, sarcosine, lysine, etc.) similarly had very little or no inhibitory effect upon the wash-in of leucine from the vascular bed, whereas methionine, alanine, phenylalanine, etc. each of which inhibited the wash-out, caused a significant stimulation of the entry of leucine into the tissue.

*Effect of vascular amino acids on the wash-out of L-leucine*

The inhibition of wash-out of L-leucine into the vascular bed and the trans-stimulation of leucine uptake from the vascular bed both indicated interaction between the leucine and the other amino acids at the intracellular side of the basolateral membrane. To test for possible interaction at the other side of the membrane, the effect of amino acids in the vascular bed on the wash-out of leucine was investigated. Table 1 shows the data for the effect of leucine, methionine and MeAIB in the vascular bed at a concentration of 10 mM during the wash-out of leucine from the epithelium into the vascular bed. None of these amino acids had a significant effect on the movement of leucine from the cell to the vascular bed, as measured by steady-state transfer or wash-out into the vascular bed, i.e. counter-transport could not be demonstrated.

*Effect of vascular leucine on intracellular leucine concentration*

The intracellular leucine content of mucosal scrapings taken at the end of steady-state perfusion experiments was estimated to see if indeed leucine could move up a concentration gradient from the tissue to the vascular bed. The estimate of intracellular leucine concentration was made with the assumption that the specific activity of the radiolabelled amino acid within the tissue was essentially unchanged from that in the luminal perfusion fluid. This assumption was made on the basis that the rate of movement from the vascular bed to the tissue had already been shown by the fractional extraction experiments to be very slow and that the affinity of the transport system in the basolateral membranes for leucine on the serosal side of the membrane is very low. However, this possible inaccuracy must be borne in mind.

The concentration of leucine in the mucosal scrapings and perfusion fluids from tissue perfused with radiolabelled leucine (1 mM) in the lumen and 'cold' leucine (10 mM) in the vascular bed was as follows: $1{\cdot}57 \pm 0{\cdot}70$ mM ($n = 5$) within the tissue, $0{\cdot}93 \pm 0{\cdot}01$ mM ($n = 5$) in the lumen and $9{\cdot}99 \pm 0{\cdot}02$ mM in the vascular bed. Hence, there was an apparent concentration gradient from the tissue to the vascular bed of 1:6·4.

## DISCUSSION

It has been suggested previously by Cheeseman (1979) that the fast component of the wash-out of amino acid into the vascular bed of the *Anuran* small intestine represents movement of the amino acid from the epithelial cells across the basolateral membrane, into the intracellular spaces and finally into the vascular bed. The data presented here support that contention. The inhibitory effect of other amino acids in the lumen on the wash-out of leucine shows a specificity which is similar to the

specificity for an 'L-type' transport system, as defined by Christensen (1975). That alone, however, does not prove that the observed effect represents interaction between amino acids and a transport system in the basolateral membrane. The inhibition of leucine wash-out by amino acids like methionine could have been a consequence of an effect at the brush-border membrane. During the leucine wash-out, the test amino acid was continuously perfused through the lumen, and if the two amino acids shared a common carrier system in the brush-border membrane, a counter-transport effect could accelerate the backflux of leucine into the lumen (Robinson, 1974). An accelerated unloading of leucine into the lumen from the epithelium would then leave less intracellular amino acid available for exit into the vascular bed. However, several lines of evidence suggest that although just such an acceleration of leucine flux into the lumen was observed (Table 2), it could not account for the reduced rate constant $K_1$ for leucine.

First, the movement of leucine from the vascular bed to the lumen ($J_{sm}$) was accelerated not only by amino acids which inhibited the $K_1$ for vascular wash-out (e.g. methionine and alanine), but it was also increased by lysine, which had no significant effect upon the $K_1$. This means that this interaction, which may represent counter-transport across the brush-border membrane, cannot be responsible for the reduced rate of appearance of leucine in the vascular bed. The $J_{sm}$ was also accelerated by the replacement of luminal sodium, presumably by reducing the vectorial nature of the carrier systems in the brush-border membrane; Cheeseman (1979) has already shown that luminal sodium replacement has no effect upon the vascular wash-out of leucine. The accelerated movement of leucine from the vascular bed to the lumen did not appear to be a consequence of an increased paracellular flux because the simultaneously measured inulin flux from the vascular bed to the lumen did not change when methionine was perfused through the lumen.

Secondly, the estimated size of the pool contributing to the fast component of the wash-out ($S_{01}$) does not appear to parallel changes in the fast rate constant $K_1$. Methionine, which has the same inhibitory effect on the $K_1$ for leucine wash-out as histidine, reduces the $S_{01}$ to a far greater degree than histidine. Also, proline, which has no significant effect upon the $K_1$, reduces the $S_{01}$ by 50%. The reduction in $S_{01}$ probably represents a reduced entry of leucine into the epithelium as a consequence of competition between leucine and the second amino acid, which was present in the lumen during the loading phase of the experiment.

Consequently, it is difficult to account for the reduced $K_1$ for leucine in the presence of various amino acids in terms of events taking place at the brush-border membrane which either promote $J_{cm}$ or reduce the intracellular pool of substrate. The exclusion of the brush-border membrane as a site of interaction leaves movement across the basolateral membrane as the next most likely locus of the events which influence the $K_1$ for leucine wash-out. If the effect of the amino acids present initially in the lumen on the wash-out fast rate constant $K_1$ does represent competition for an exit mechanism in the basolateral membrane, then it would appear that the specificity of that transport system on the intracellular side of the membrane resembles that specified by Christensen (1975) for an 'L-type' system. In addition, the wash-out of leucine is unaffected by the replacement of sodium by choline in the vascular bed, so the mechanism for exit appears to be sodium-independent.

Comparison of $\Delta J_{ms}$ and $\Delta S_{01}$ shows a correlation coefficient of 0·87, which suggests

that in most cases the reduction in net transfer of leucine was the consequence of inhibition of entry into the tissue, i.e. movement across the brush-border membrane was the rate-limiting step. There is, however, one notable exception which is of interest. Threonine had no effect upon the $S_{01}$ for leucine but it did reduce both the $K_1$ and the $J_{ms}$, suggesting that this amino acid can inhibit leucine transfer but its locus of action is at the basolateral membrane. Hence, *in vivo*, there are at least two possible sites of interaction for amino acids during their movement from the lumen to the vascular bed.

Mircheff *et al.* (1980) have recently shown the existence of a transport system for L-alanine in vesicles of basolateral membranes from rat enterocytes. They were able to demonstrate counter-transport for alanine uptake in the absence of sodium by these vesicles when they were preloaded with amino acids which shared the transport pathway with alanine.

Similar experiments were performed with this preparation to assess the ability of intracellular amino acid to accelerate the entry of leucine into the epithelium from the vascular bed. All of these experiments were performed in the absence of sodium in the vascular perfusate to eliminate possible interaction with sodium-dependent entry processes which Mircheff *et al.* also found. All of the amino acids which had inhibited leucine wash-out also accelerated leucine entry across the serosal side of the epithelium, whereas those which had no effect upon the wash-out similarly had no effect upon the wash-in. Hence, counter-transport accelerating $J_{sc}$ of leucine could be demonstrated, just as Mircheff *et al.* have found.

The general assumption has been made in the past that any exit system in the basolateral membrane for amino acids needs only to be a facilitated mechanism with no energy input. This hypothesis appears to stem from two observations: first, that *in vitro* the sodium-dependent entry systems for amino acids can accumulate the substrate to high concentrations intracellularly, which would imply that exit across the serosal pole is therefore 'downhill'. Secondly, exit is not influenced by the extracellular sodium concentration. Both observations are well documented. Munck & Schultz (1969) showed that lysine accumulated within the rabbit ileal epithelium during steady-state transport and that $J_{cs}$ appeared to be a saturable process, suggesting carrier mediation. They also suggested that the system might have a low sodium dependency. Subsequently, Hajjar *et al.* (1972) investigated alanine serosal efflux in the turtle intestine and showed that it was a process unaffected by the concentration of sodium in the serosal bathing medium, but again appeared to be carrier-mediated. More recently, Danisi, Tai & Curran (1976) again showed that the serosal flux of alanine in the rabbit ileum was independent of sodium but a saturable process.

This lack of sodium dependency, its specificity and the strong trans-stimulation of fluxes via this system have led Mircheff *et al.* (1980) to suggest that this is indeed a facilitated exchange system of the type seen in red cells and *Ascites* tumour cells. However, despite the lack of sodium dependency, Hajjar *et al.* (1972) did comment that there was a possibility that the exit transfer system might be asymmetric because the fluxes of alanine in opposite directions across the serosal pole were not equivalent for a given concentration on one side of the membrane, and high concentrations of 'cold' alanine in the serosal bathing medium did not accelerate the exit of [$^3$H]labelled alanine from the tissue. They also drew attention to the conclusion

of Newey & Smyth (1962) that the exit process for glycine from the rat intestine required metabolic energy.

The counter-transport experiments of Mircheff *et al.* (1980) in no way contradict the findings of Hajjar *et al.* (1972). Counter-transport in this system has only been demonstrated with a high concentration of amino acid intracellularly, while the flux $J_{sc}$ is measured. In the experiments reported here, nearly all of the above observations have been confirmed. The leucine exit system is shared by a number of other amino acids, including alanine, methionine and histidine, but not lysine, proline, AIB or MeAIB. Also, the system is not affected by changes in the concentration of sodium in the vascular bed. However, in this vascularly perfused preparation, it has been possible to investigate counter-transport phenomena in both directions across the basolateral membrane. Counter-transport could only be demonstrated in one direction: the movement of leucine can only be accelerated into the cell, not out of it. The presence of 10 mM-leucine, methionine or MeAIB in the vascular perfusate had little effect upon the steady-state mucosal-to-serosal flux of leucine ($J_{sm}$) and no effect on the wash-out of the leucine into the vascular bed as measured by the fast and slow rate constants ($K_1$ and $K_2$). Indeed, the measurement of intracellular leucine concentraton at the end of steady-state perfusion when 10 mM-leucine is present in the vascular bed indicates that there is a lower concentration of the amino acid inside the cells than in the vascular bed. This strongly suggests that the system can transport against a concentration gradient and that the affinity of the system is different on the inside of the membrane to that on the outside, i.e. it is asymmetric. If this is the case, then the exit system for leucine is an 'active' and not a 'passive' facilitated mechanism, just as was claimed by Newey & Smyth (1962).

It should also be noted that in 1973, Esposito, Faelli & Capraro reported very similar observations for the concentration profile of glucose when transported across the rat small intestine *in vivo*. They showed that the concentration of glucose in the tissue was always lower than that in the blood and concluded that the exit mechanism for sugars must be active.

The possibility of an active exit mechanism for amino acids in the basolateral membranes raises at least two important questions: why should the exit system need to be an active step, and if it is active, what is the source of energy for the exit mechanism? It is possible, at least in part, to answer the first question.

Although *in vitro* preparations show very high levels of accumulation of transported substrate intracellularly, little accumulation, if any, is found in the vascularly perfused small intestine (Cheeseman, 1980). Hence, although entry across the brush border is an active sodium-dependent system, the substrate is cleared sufficiently rapidly into the vascular bed to stop accumulation. This must mean that exit is as fast as entry and possibly a facilitated mechanism would not be sufficiently rapid. It is well known that the serosal-to-mucosal flux of substrates is very low compared to the mucosal-to-serosal flux, i.e. the system is highly rectified, and in these experiments there is almost a fiftyfold difference between these two fluxes. If the exit system as well as the entry system had a vectorial component, then the absorption of amino acids from the lumen to the bloodstream would be in the highly preferred direction of movement, which is observed.

The lack of sodium dependence for a transport system in the small intestine appears to have become synonymous with the interpretation that the system in question is

therefore 'passive'. But co-transport with sodium would hardly be useful in driving the exit of amino acids in view of the fact that the sodium gradient between the cell interior and the vascular bed promotes passive sodium flux into the cell, not out of it. There are, however, many other possible ways in which an energy input could be coupled to an exit system for amino acids. Christensen, Cespedes, Handlogten & Ronquist (1973) have proposed that the accumulation of amino acids by *Ascites* tumour cells is driven by a coupling to proton movements, and Eddy & Nowacki (1971) have suggested that amino acid entry in yeast cells is coupled to the movement of hydrogen and potassium ions.

The data presented in this paper do not conclusively demonstrate that the exit of the amino acid L-leucine across the serosal pole of the enterocyte is an active process, but the ability of the system to move the amino acid against a concentration gradient and its apparent asymmetry certainly raises the possibility that this exit system requires an energy input.

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