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KINETICS OF THE UPTAKE OF SOME NEUTRAL AMINO ACIDS BY SPIRODELA POLYRHIZA

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SUMMARY

Uptake of glycine, L-alanine, L-leucine, L-isoleucine, L-valine, and D-valine by intact fronds of the duckweed *Spirodela polyrhiza* was investigated.

1. The time-course of amino acid uptake showed that the uptake rate (= net influx) was apparently not affected by accumulation of amino acid in the fronds, suggesting that there was no trans-inhibition of the influx, and that the efflux was negligible.
2. Efflux was measured from fronds preloaded with L-(^{14}C)leucine. When the external and internal concentration were both about 2.5 mM the efflux was $\leqslant 1\,\%$ of the influx.
3. Uptake rates were measured over a wide concentration range, 1 µM to 10 mM. For each amino acid the relation between uptake rate and external concentration could be described as the sum of two Michaelis-Menten processes. It is suggested that the duckweed possesses two transport systems common to the neutral amino acids tested: a low Km, low capacity system (system 1), and a high Km, high capacity system (system 2).

1. INTRODUCTION

The existence of transport systems for the uptake of amino acids into the cell has been well established both in microorganisms and in animal cells (for reviews see OXENDER 1972 and HEINZ 1972). For higher plant cells the need for such transport systems might seem less obvious, since the external solution from which the plant absorbs its nutrients does not contain amino acids in appreciable amounts, while transport from cell to cell may occur symplasmatically.

Long distance transport of amino acids and amides, however, proceeds through the vascular bundles. If it occurs via the xylem, but possibly also when it occurs via the phloem (GEIGER et al. 1974), the translocated solutes first move into the apoplasmatic spaces, from where they are subsequently taken up by contiguous cells. It is likely, therefore, that plant cells in general are equipped with systems which transfer amino acids across the plasmalemma.

The results of studies on amino acid uptake by various plant tissues are consistent with this view. Thus, it has been found that plant tissues can take up amino acids against a concentration gradient, and that the uptake process shows specificity, saturation kinetics, and sensitivity to metabolic inhibitors. However, the number of transport systems involved in the uptake of amino acids by higher plant cells has not yet been established. The present paper describes two transport systems involved in the uptake of neutral amino acids by fronds of *Spirodela polyrhiza.*

2. MATERIAL AND METHODS

2.1. Chemicals

^{14}C-labelled amino acids of various specific activities were obtained from the Radiochemical Centre, Amersham, U.K. D-(1-^{14}C) valine was prepared from DL-(1-^{14}C)valine (37.5 mCi/mmol) with L-amino acid oxidase from *Bothrops atrox* venom (crude, type II; Sigma, St. Louis, U.S.A.). At least 98% of the L-isomer was oxidized. The D-valine obtained was isolated from the reaction mixture using a 5 × 0.6 cm cation-exchange column (Dowex 50W-X8, 200–400 mesh, H^+-form).

2.2. Uptake experiments

The growing of axenic cultures of *S. polyrhiza* and the design of the uptake experiments have been described (BORSTLAP 1970, 1974). The growth medium (mineral salts, 1% sucrose and 167 mg Na-EDTA/l; pH 5.3) was also used in the uptake experiments. Illumination and temperature during uptake were the same as those at which the duckweed was grown. Uptake experiments which lasted more than 10 hr were performed under axenic conditions.

2.3. Kinetic data

Kinetic data, which relate uptake rate and external concentration, were obtained as follows. The uptake rate of an amino acid was calculated from the amount of ^{14}C-labelled amino acid which disappeared from the medium during the experiment. When the amino acid concentration in the medium decreases from S_o at zero time to S_t at time t, the average uptake rate $\bar{v}$ over that period is given by

$$\bar{v} = V\,(S_o - S_t)/(w \cdot t) \tag{1}$$

where V is the volume of the medium, and w is the total dry weight of the fronds incubated on the medium. As the concentration changes considerably during the experiment, the concentration S_x at which the uptake rate is equal to the average uptake rate $\bar{v}$, has to be calculated. Provided that the uptake process shows Michaelis-Menten kinetics, S_x can be calculated from S_o and S_t. From the integrated form of the Michaelis-Menten equation (LEE & WILSON 1971) it follows

$$S_x = (S_o - S_t)/\ln\,(S_o/S_t) \tag{2}$$

During the present investigation, however, it became apparent that the relation between uptake rate, v, and concentration, S, could be described by

$$v = \frac{Vm_1 \cdot S}{Km_1 + S} + \frac{Vm_2 \cdot S}{Km_2 + S} \tag{3}$$

rather than by the simple Michaelis-Menten equation. It could be shown that in case of two Michaelis-Menten processes, operating simultaneously, S_x in addition depends on the parameters by which these processes are defined, *i.e.* Km_1, Vm_1, Km_2, and Vm_2. Nevertheless, the right-hand side of Eqn (2) appeared to be a good estimate for S_x at various realistic combinations of parameter values (A. C. Borstlap & P. G. Doucet, unpublished). Hence, kinetic data were obtained by measuring average uptake rates, and calculating the corresponding concentrations according to Eqn (2).

2.4. Curve fitting and simulation

Experimental values of the uptake rate, v, and the external concentration, S, were fitted to Eqn (3) by means of an iterative computer program for non-linear least squares regression, essentially as described by CLELAND (1967). This fitting procedure may be applied if the error in S is small with respect to that in v, and if the values for v are appropriately weighted. These conditions were fulfilled. The variability of the plant material, considered to be the main source of error in the measurement of the uptake rates, did not affect the precision by which the concentrations were known. Since uptake rates were calculated from the relative change in concentration it was expected that the error in v will be proportional to v itself, so that values for v had to be weighted by factors equal to $1/v^2$.

The time-courses of amino acid uptake by exponentially growing cultures were simulated on a digital computer by means of a program written in Leans-G.

2.5. Amino acid determinations

Extraction of free amino acids and their quantitative analysis by means of gas-liquid chromatography has been described before (BORSTLAP 1972).

3. RESULTS

3.1. Time-course of amino acid uptake

Uptake of amino acids was measured from the decrease of the amino acid concentration in the medium, so that the uptake rates calculated represent net influxes. This method has obvious advantages: (1) samples to be analysed can be obtained by simple operations, (2) the contribution of uptake into a possible free space may be neglected if the ratio of tissue volume to medium volume is sufficiently low. However, this method may have the disadvantage that, owing to accumulation of amino acid in the cells, the net influx decreases during the experiment by either an increasing efflux, or by trans-inhibition of the influx. It will be shown from the time-course of uptake that these processes do not play a significant part in the uptake of neutral amino acids by the duckweed.

At low concentrations ($\leqslant 10 \mu M$) the uptake showed first order kinetics *(fig. 1a)*. When the logarithm of the external concentration was plotted against time a straight line was obtained (*fig. 1a*, inset). This means that the uptake rate at these low concentrations was proportional to the amino acid

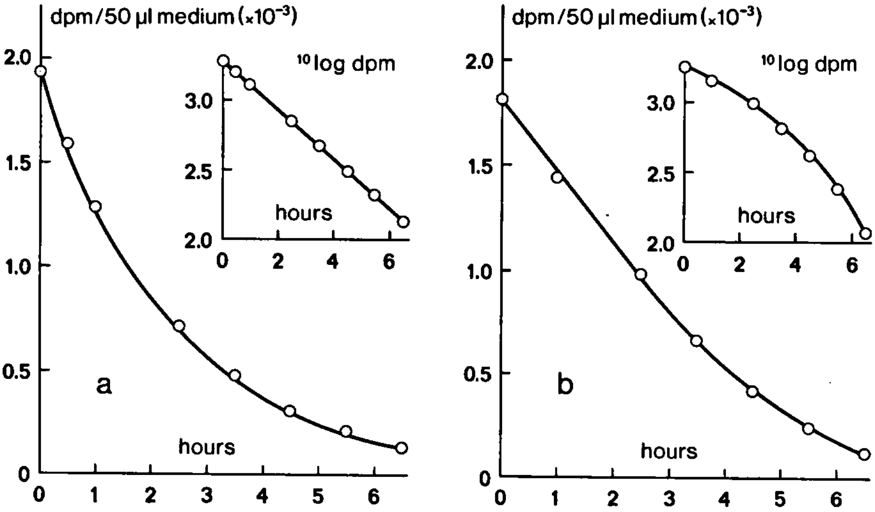

Fig. 1. Time-course of L-leucine uptake. The graphs show the decrease of L-leucine concentration in the medium. (a) Fronds (49 mg dry wt) were incubated on 25 ml medium with 0.01 mM L-(U-^{14}C) leucine. (b) Fronds (78 mg dry wt) were incubated on 25 ml medium with 0.1 mM L-(U-^{14}C)leucine. The insets show semilogarithmic plots of the L-leucine concentration in the medium against time.

concentration in the medium. The decrease in the uptake rate during this experiment seems, therefore, only due to the decrease in the external concentration.

At high concentrations (10 mM) the uptake showed zero order kinetics *(fig. 2b)*. The uptake rate was constant for at least 6 hours, although the external concentration decreased by 25 % during that period. In a parallel experiment fronds were incubated on medium with 10 mM L-leucine and the time-course of the leucine content of the fronds was determined *(fig. 2b)*. The rate at which the leucine content increased during the first hour was 30 nmoles.hr^{-1}. mg^{-1}dry wt. This value is close to the uptake rate at 10 mM, calculated according to Eqn (3) using the data given in *table 2*, *viz.* 32 nmoles.hr^{-1}.mg^{-1}dry wt. After 1 hr the rate at which the leucine content increased declined gradually, suggesting that the rate of leucine breakdown increased during the experiment. After 6 hrs of incubation the leucine content had reached a value of 110 nmoles/mg dry wt. corresponding to an average leucine concentration in the plants of 14 mM (1 mg dry wt is equivalent to about 8 µl of tissue water). At that time the leucine concentration in the medium had decreased to 7.5 mM. It is clear that leucine was taken up against a concentration gradient, and that the uptake rate was not affected by the strong increase of the leucine concentration in the plants.

Similar conclusions can be drawn from the results shown in *figs. 1b* and *2a*. The course of leucine uptake at an initial concentration of 0.1 mM is shown in

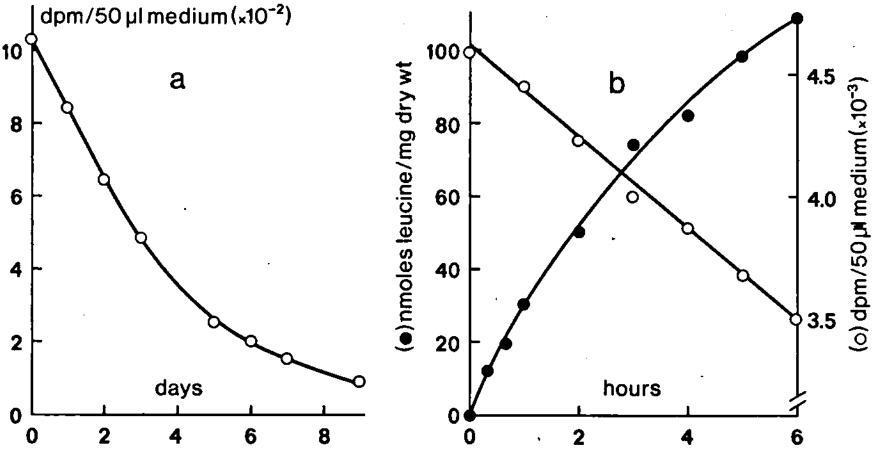

Fig. 2. Time-course of L-leucine uptake. (a) To a *Spirodela* culture (about 30 fronds on 100 ml medium) L-(U-^{14}C)leucine was added to a final concentration of 1 mM. At this leucine concentration frond multiplication was completely inhibited. The number of fronds was, therefore, essentially constant during the experiment. The graph shows the decrease of the L-leucine concentration in the medium. (b) Time-course of L-leucine uptake (○) and of the leucine content of the fronds (●) at 10 mM. The course of leucine uptake is shown as the decrease in the external leucine concentration when fronds (103 mg dry wt) were incubated on 10 ml medium with 10 mM L-(U-^{14}C)leucine. The time-course of the leucine content was determined as follows. About 250 fronds (180 mg dry wt) were incubated on 100 ml medium with 10 mM L-leucine. Samples of the fronds, taken at various times, were assayed for their leucine content; the points represent average values of three independent experiments.

fig. 1b. The uptake rate was essentially constant during the first two hours. An equilibrium between external and internal concentration (where influx and efflux cancel each other) was not established. The time-course of leucine uptake at an initial concentration of 1 mM is even more suggestive *(fig. 2a)*. The uptake rate was constant during the first two days. External concentrations at day 1 and day 2 were about 0.8 and 0.6 mM respectively. The leucine concentration in the plants at these times was 18 mM, as could be calculated from determinations of the leucine content of the fronds in similar experiments. Thus, leucine was taken up against a steep concentration gradient, and the uptake rate was not influenced by the relatively high leucine concentration in the plants. It is evident that the decline in the uptake rate observed in these experiments *(figs. 1b* and *2a)* was due to the decline in the external concentration.

The time-course experiments demonstrate that the uptake rate was merely determined by the amino acid concentration in the medium. In other words, the relation between uptake rate (= net influx) and external concentration did not change during the experiments. This also means that neither efflux of amino acid nor trans-inhibition of the influx were involved in the uptake process.

Table 1. Exit of L-(^{14}C)leucine from preloaded fronds.

To each of two *Spirodela* cultures (about 80 fronds on 100 ml medium) 1 ml 0.01 M L-leucine *plus* 0.5 µCi L-(U-^{14}C)leucine was added. After 40 hr the cultures were rinsed with tap water for 15 sec. Each culture was divided into three parts which were placed on 25 ml of freshly prepared medium, whether or not supplied with 10 mM L-leucine or 10 mM L-alanine. – The figures are therefore mean values of two determinations. – At various times 0.5 ml samples were taken from the medium and assayed for ^{14}C. At the end of the experiment the fronds were lyophilized and free amino acid fractions were prepared in which the amount of ^{14}C was determined. Paperchromatograms of these fractions showed that radioactivity was exclusively found in leucine. The leucine content of the fronds, 19–20 nmoles/mg dry wt, was calculated from the amount of ^{14}C in the amino acid fractions, assuming that the specific activity was the same as that of the leucine supplied. The exit is expressed both as percentage of the amount of leucine in the fronds, and as nmoles leucine/mg dry wt.

	efflux medium supplied with					
time	no amino acid		10 mM L-alanine		10 mM L-leucine	
(hr)	%	nmoles per mg dry wt	%	nmoles per mg dry wt	%	nmoles per mg dry wt
1	0.2	0.04	1.2	0.24	1.5	0.30
2	0.4	0.08	1.4	0.28	1.7	0.34
4	0.5	0.09	2.5	0.50	3.0	0.60

3.2. Efflux of amino acid

The exit of leucine was determined with fronds which had previously accumulated L-(^{14}C)leucine *(table 1)*. The leucine content of the fronds was 19–20 nmoles/mg dry wt, which is equivalent to an average internal leucine concentration of about 2.5 mM. When the preloaded fronds were incubated on medium without amino acid the initial efflux was 0.04 nmoles.hr^{-1}.mg^{-1}dry wt. The net influx of L-leucine at an external concentration of 2.5 mM, calculated from the data in *table 2*, amounts to 26 nmoles.hr^{-1}.mg^{-1}dry wt. Therefore, when the external and internal concentration are both 2.5 mM the influx will be about 600 times larger than the efflux.

Exit of leucine was stimulated by the presence of high concentrations (10 mM) of L-alanine or L-leucine in the medium, possibly because re-uptake of L-(^{14}C)leucine was inhibited. If so, the efflux observed in these cases will be closer to the real efflux of leucine. But even then the efflux amounts to only 1% of the influx.

3.3. Free space

Uptake into a possible free space did not contribute appreciably to the uptake rates measured in this investigation. This can be reasoned as follows. In most experiments not more than 70 mg dry wt of fronds were incubated on 25 ml of medium. This amount of fronds is equivalent to about 0.6 ml of tissue water and, assuming that as much as 25% of the tissue volume is free space (LÜTTGE 1973), a free space volume of 0.15 ml. Equilibration of amino acid between

Table 2. Kinetic parameters for the uptake of some neutral amino acids by *S. polyrhiza*. The kinetic data were fitted to Eqn (3). The parameters for D-valine which were obtained by fitting the kinetic data to the simple Michaelis-Menten equation are in italics. The ranges given are computer-derived standard errors.

	system 1		system 2	
amino acid	Km$_1$ (µM)	Vm$_1$ (nmoles.hr^{-1}.mg^{-1})	Km$_2$ (mM)	Vm$_2$ (nmoles.hr^{-1}.mg^{-1})
Glycine	77.0 ± 12.0	12.8 ± 1.9	4.2 ± 1.5	75.9 ± 11.8
L-alanine	35.3 ± 4.5	7.8 ± 0.9	1.5 ± 0.4	30.5 ± 3.2
L-leucine	27.5 ± 3.2	5.7 ± 0.6	1.0 ± 0.2	29.5 ± 1.5
L-isoleucine	73.5 ± 6.6	13.6 ± 1.1	3.5 ± 1.2	36.0 ± 4.9
L-valine	63.0 ± 5.6	13.2 ± 1.1	3.9 ± 1.5	39.5 ± 7.5
D-valine	517.0 ± 179.0	13.9 ± 6.6	8.2 ± 9.4	37.8 ± 12.5
			1.1 ± 0.1	*30.5 ± 1.8*

medium and free space would then result in a 0.6% decrease of the amino acid concentration in the medium. Uptake rates were determined when at least 30% of the amino acid was taken up by the plants. Thus, not more than 2% of the uptake rates measured might have been due to uptake into the free space.

Apart from these considerations, uptake into a free space seems to play no part at all in our experiments. This can be concluded from the experiment in which the time-course of L-(^{14}C)leucine at 10 mM was followed (*fig. 2b*). In this experiment the presumed free space volume was 2% of the medium volume. It is clear that there was no initial burst of uptake due to equilibration of amino acid with the free space. It is likely, therefore, that the free space of the duckweed fronds represents much less than 25% of the tissue volume. In this respect may be pointed to the work of YOUNG & SIMS (1972), who studied the uptake of K$^+$ by *Lemna minor* and concluded that the fronds did not contain any detectable free space.

3.4. Concentration dependence of the uptake rate

From the time-course of amino acid uptake it was concluded that the relation between uptake rate and external concentration did not change during the uptake experiments. Hence, the procedure outlined in Material and Methods, measurement of the average uptake rates and calculation of the corresponding concentrations, can be used.

Data relating uptake rate and external concentration were obtained for glycine, L-alanine, L-leucine, L-isoleucine, L-valine, and D-valine. In *fig. 3* the uptake rate of L-leucine is plotted against the L-leucine concentration in the medium. Apparently, the uptake rate levels off in the low concentration range at about 0.03 mM, and in the high concentration range at about 1 mM. This biphasic saturation is also illustrated by the two-limbed curve obtained in the double reciprocal plot (*fig. 3*, inset), but is demonstrated more clearly by Hofstee plots (HOFSTEE 1952), as in such plots the experimental points are better

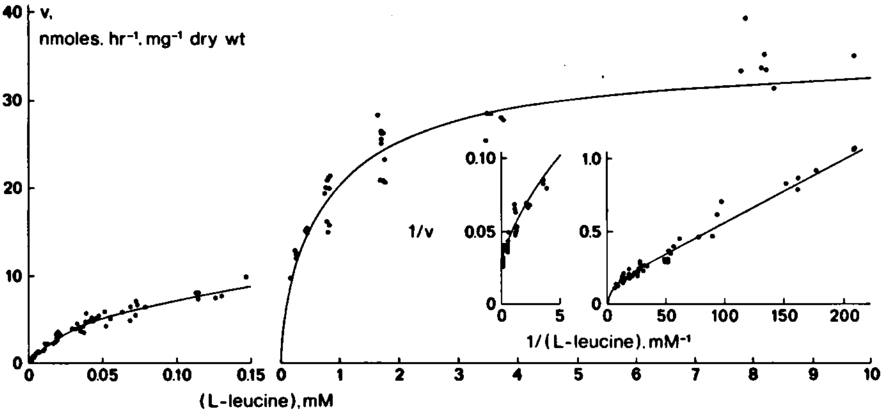

Fig. 3. The uptake rate of L-leucine, v, as a function of the concentration in the medium. The concentration on the abscissa is given from 0–0.15 mM, and then, on a different scale from 0–10 mM. The points shown in the left-hand figure were omitted in the right-hand figure. Inset: Double reciprocal plots of the same data. Experimental values at low concentrations up to 0.2 mM (= 5 mM^{-1}) are shown in the right-hand figure. The remaining values are shown in the left-hand figure, in which the scales for both abscissa and ordinate are expanded 10 times in comparison with those in the right-hand figure. The lines drawn were computed by means of the kinetic parameters for L-leucine uptake given in table 2.

distributed. Hofstee plots for the uptake of the neutral amino acids studied are shown in *fig. 4*. It can be seen that the uptake of all amino acids shows biphasic saturation, but less pronounced for D-valine.

The kinetics observed can be explained by the simultaneous operation of two transport systems, each system showing Michaelis-Menten kinetics. The parameters which characterize these transport systems, obtained by fitting the kinetic data to Eqn (3), are given in *table 2*. The respective parameters for the various amino acids are of the same order of magnitude, except for D-valine, where the Km-values for both systems were much higher. The kinetic data for D-valine were also analysed assuming that this amino acid is taken up by only one system. It is true that the resulting parameter values were more reliable but, as will be shown below, inhibition studies indicate that the uptake of D-valine by two transport systems is more likely.

As pointed out in the preceding section, the component in the uptake rate which becomes predominant at high concentrations cannot be due to diffusion into the free space. On the strength of its saturation kinetics it seems justifiable to consider this uptake component to be carrier mediated. Accordingly, it will be shown below that uptake of L-leucine at high concentration (10 mM) is inhibitable by other amino acids.

Fronds were incubated for 1 hr on medium with 10 mM L-leucine in the absence or presence of 20 mM of another amino acid. The uptake rate of leucine was determined from the increase of the leucine content of the fronds.

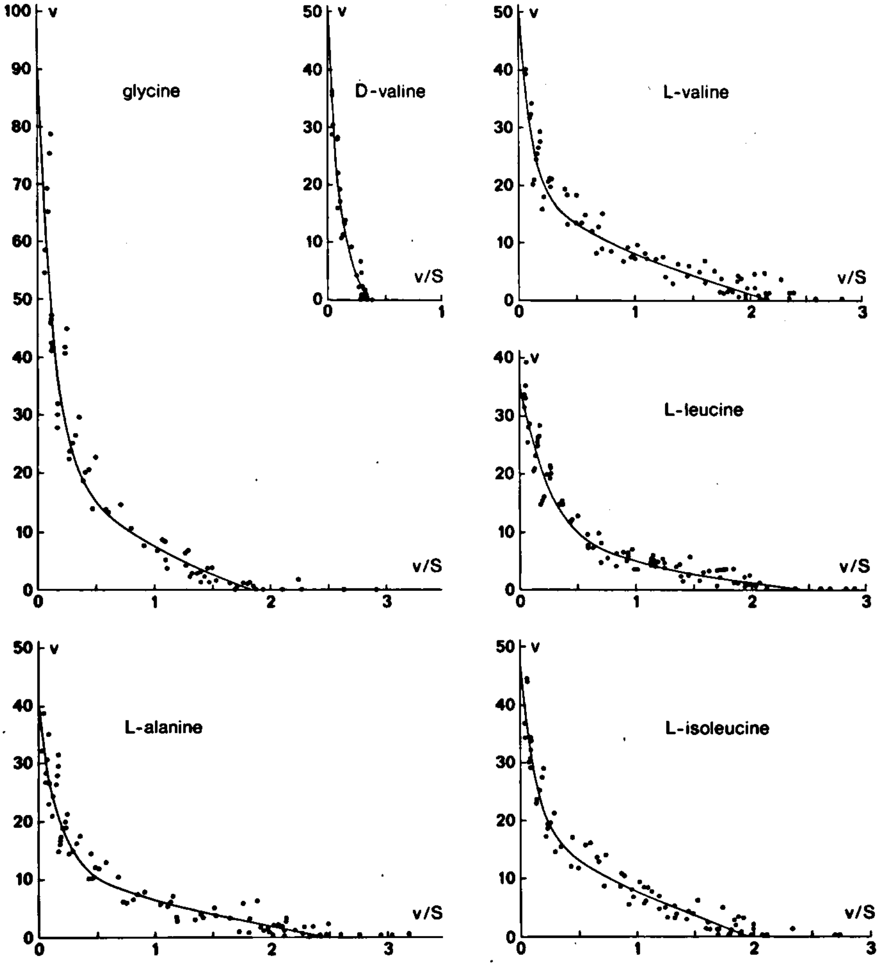

Fig. 4. Hofstee plots for the uptake of neutral amino acids. The uptake rate v (nmoles.hr^{-1}. mg^{-1}dry wt) is plotted against v/S, where S is the amino acid concentration in the medium in units of 10^{-5} M. The lines drawn were calculated by means of the parameters given in table 2. The line for D-valine was calculated from the parameters for two transport systems.

This method could be used as the amount of leucine metabolized was apparently low with respect to the amount of leucine taken up (compare *fig. 2b*). This is also true of the other branched-chain amino acids, so that their uptake rates could be measured similarly. Using the parameter values from *table 2*, the uptake rates expected in this experiment were calculated from the formula

$$v = \frac{Vm_1 \cdot S}{Km_1\left(1 + \frac{I}{Ki_1}\right) + S} + \frac{Vm_2 \cdot S}{Km_2\left(1 + \frac{I}{Ki_2}\right) + S} \qquad (4)$$

Table 3. Inhibition of L-leucine uptake at 10 mM by some neutral amino acids.
Fronds were incubated for 1 hr on medium supplied with 10 mM L-leucine *plus* 20 mM of inhibitor as indicated. Observed uptake rates, v_{obs}, were determined from the increase of the amino acid content of the fronds. Predicted uptake rates, v_{pred}, were calculated assuming two transport systems common to all neutral amino acids tested, and using the kinetic parameters given in table 2. Predicted uptake rates of L-leucine by system 1 and system 2 are given separately. For the experiment with D-valine uptake rates were also predicted assuming that this amino acid was only taken up by system 2; the resulting values are in italics.

	uptake rate of L-leucine (nmoles.hr^{-1}.mg^{-1}dry wt)				uptake rate of inhibitor (nmoles.hr^{-1}.mg^{-1}dry wt)	
inhibitor (20 mM)	v_{obs}	v_{pred}	system 1	system 2	v_{obs}	v_{ped}
None	30.9	32.4	5.7	26.7		
Glycine	22.7	21.9	3.3	18.6		
L-alanine	11.4	14.3	2.2	12.1		
L-leucine	11.6*	11.4	1.9	9.5		
L-isoleucine	20.5	20.7	3.2	17.5	19.1	18.3
L-valine	19.0	21.2	3.1	18.1	21.0	18.9
D-valine	24.8	27.0	5.2	21.8	6.5	8.2
		15.5	*5.7*	*9.8*		*19.4*

* 1/3 × uptake rate of L-leucine at 30 mM

where S is the concentration, Km_1, Km_2, Vm_1, and Vm_2 are the kinetic parameters of the amino acid whose uptake rate, v, is calculated, and I is the concentration, and Ki_1 and Ki_2 are the respective Km-values of the inhibiting amino acid. The results *(table 3)* show that the observed uptake rates are in reasonable agreement with the uptake rates predicted. L-leucine and L-alanine, which have a relatively high affinity (low Km's) with respect to system 2, inhibited L-leucine uptake more strongly than the other amino acids. It also appears that the mutual uptake inhibition of D-valine and L-leucine is only predictable when it is assumed that D-valine is taken up by two transport systems.

3.5. Simulation of amino acid uptake by exponentially growing cultures

The time-course of the uptake of L-valine and L-isoleucine, added simultaneously, by exponentially growing *Spirodela* cultures was determined experimentally, and was simulated by using the kinetic parameters for the uptake of these amino acids.

Two fronds were inoculated on each of four growth media (100 ml) supplied with 0.4 mM L-valine *plus* 0.1 mM L-isoleucine, or with 0.8 mM L-valine *plus* 0.2 mM L-isoleucine. Either of the amino acids was supplied as the ^{14}C-labelled compound. The number of fronds in each culture was counted daily

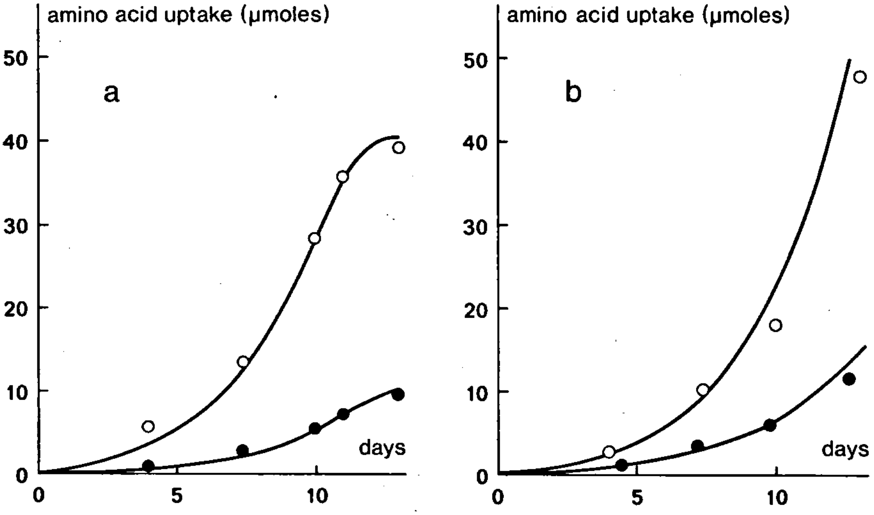

Fig. 5. Uptake of L-valine (○) and L-isoleucine (●), added simultaneously, by exponentially growing *Spirodela* cultures. The initial amounts of amino acids in the growth medium were (a) 40 µmoles L-valine *plus* 10 µmoles L-isoleucine, and (b) 80 µmoles L-valine *plus* 20 µmoles L-isoleucine. The points represent experimental values. The lines drawn show the course of uptake determined by simulation.

and at various times samples were taken from the medium so as to determine the amount of ^{14}C-labelled amino acid taken up. In each culture the number of fronds increased exponentially, and the fronds produced were of normal size and shape. At the end of the experiment the dry weights of the cultures were determined.

The time-course of the uptake of both L-valine and L-isoleucine was simulated for each experiment individually. The growth of the culture in the simulation was determined by the number of fronds at zero time and the multiplication rate of the fronds. For each culture individually, these data were obtained from semilogarithmic plots of the number of fronds against time. Uptake rates of L-valine and L-isoleucine were calculated in the simulation according to Eqn (4) by substituting the appropriate kinetic parameters given in *table 2*. The results show *(fig. 5)* that the time-course of amino acid uptake could be reasonably well predicted by the simulations.

4. DISCUSSION

The time-course experiments with L-leucine showed that the uptake was not affected by high leucine concentrations in the plants. Similar results have been obtained for amino acid uptake by several plant tissues: carrot slices (Birt & Hird 1956), mustard roots (Wright 1962), leaf fragments of barley (Shtarkshall et al. 1970) and of pea (Cheung & Nobel 1973), tomato stem segments (van Bel 1974), and isolated leaves of *Egeria densa* (Petzold & Jacob 1975).

These results indicate that amino acid uptake by cells from higher plants is characterized by the absence of both efflux and trans-inhibition of influx. The absence of efflux has been established for amino acid uptake by yeasts and other fungi (WILEY & MATCHETT 1966; CRABEEL & GRENSON 1970; KOTYK & ŘÍHOVÁ 1972), and seems, therefore, a characteristic of amino acid uptake by plant cells in general.

In fungi, however, high concentrations of amino acid in the cell trans-inhibit amino acid influx (RING & HEINZ 1966; CRABEEL & GRENSON 1970; PALL 1971; PALL & KELLY 1971; KOTYK & ŘÍHOVÁ 1972). The effect of trans-inhibition becomes apparent when the time-course of amino acid uptake is followed. For example, KOTYK & ŘÍHOVÁ (1972), who incubated yeast with 50 mM α-aminoisobutyric acid, found that after 4 hr the uptake rate had decreased by 90%. This result is in sharp contrast to the present results with *Spirodela*.

Biphasic saturation kinetics has been reported for amino acid uptake in various microorganisms (OXENDER 1972) and in animal cells or tissues (HEINZ 1972). It has also been found for the uptake of α-aminoisobutyric acid by barley leaf strips (REINHOLD et al. 1970) and by isolated leaves of *E. densa* (PETZOLD & JACOB 1975), and for L-alanine uptake by soybean root cells (KING 1976). Biphasic kinetics were also observed in the uptake of leucine in *Lemna* root tips by NEWTON (1974) who, unfortunately, used DL-leucine. In other studies with plant tissues the biphasic kinetics may have escaped notice either because uptake was investigated over a too small concentration range (KING & OLENIUK 1973), or because the concentration intervals were taken too large (MARETZKI & THOM 1970; STEWART 1971).

It is suggested that *S. polyrhiza* possesses two transport systems common to the neutral amino acids. The results of inhibition experiments are in agreement with this view. Thus, it has been shown (BORSTLAP 1974) that the uptake rate of L-leucine, L-valine, and L-isoleucine at 30 μM, 80–90% of which is due to system 1, was appreciably reduced by glycine and L-alanine, L-alanine being the more potent inhibitor, but was only slightly reduced by D-valine. The affinity of glycine, L-alanine, and D-valine to system 1 is therefore reflected by their ability to inhibit the uptake of a competing amino acid by this system. The mutual uptake inhibition of the neutral amino acids at high concentrations (*table 3*) could also be explained by the two-carrier model.

Although the kinetics of a process cannot give conclusive evidence about the underlying mechanism (CRAM 1973), this does not imply that the kinetics has no indicative value in this respect. Since the pioneering work of Epstein and co-workers (EPSTEIN et al. 1963) the model of two independent transport systems is still the most simple one to explain biphasic saturation kinetics. Genetic analyses (SLAYMAN 1973) and kinetic analyses (HONEGGER & SEMENZA 1973; QUAY & CHRISTENSEN 1974; HOLDEN et al. 1975; MATTHEWS et al. 1975; WOOD 1975) of the uptake of sugars and amino acids favour the interpretation of biphasic kinetics in terms of multiple carriers.

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