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ANTAGONISMS BETWEEN AMINO ACIDS IN THE GROWTH OF SPIRODELA POLYRHIZA DUE TO COMPETITIVE AMINO ACID UPTAKE

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SUMMARY

The branched-chain amino acids L-leucine, L-valine and L-isoleucine can, when supplied to the medium, inhibit the growth of the duckweed *Spirodela polyrhiza*. The responses to L-leucine and to L-valine could be described as all-or-none responses, that to L-isoleucine as a graded response. This information permitted unambiguous measurement of the growth responses. The relation between amino acid dose (initial concentration in the medium) and growth response was evaluated by means of probit analysis. Dose response curves were determined for each branched-chain amino acid when supplied singly to the medium. They were also determined when a second amino acid was added to the medium at a fixed initial concentration. It appeared that L-glutamic acid, glycine and L-alanine, in increasing order (1) antagonized, each to a distinct degree, the growth inhibitory action of irrespective which branched-chain amino acid and (2) inhibited the uptake rate of the branched-chain amino acids. D-valine, L-ornithine and L-lysine did not antagonize, nor did they inhibit uptake. Evidence is provided that the observed antagonisms result from inhibition of the uptake of the growth inhibitory amino acids.

1. INTRODUCTION

The growth rate of an organism may depend on exogenous supply of an amino acid in two ways. First, the organism may require the amino acid as an essential nutrient. Secondly, the amino acid may have a deleterious effect on metabolism such that growth is inhibited; this holds especially for the so-called amino acid analogues (RICHMOND 1962; FOWDEN, LEWIS & TRISTRAM 1967). Before an amino acid can exert either its beneficial or its detrimental effect it should first of all pass the cell membrane. It is conceivable that the effect of an amino acid on the growth rate depends on the rate by which the amino acid is taken up by the organism, and that inhibition of the uptake will lead to a diminution of that effect.

Competition between amino acids in the uptake by plant tissues or plant organs has been reported (BIRT & HIRD 1958; WRIGHT 1962; BÖSZÖRMÉNYI & CSEH 1962; STEWART 1971; ÖZER & BASTIN 1972). A complete analysis of amino acid transport systems in plant cells, however, has not yet been made. But the situation may be similar to that in bacterial, fungal and animal cells where amino acids are taken up via a number of transport systems, each system transporting a distinct class of amino acids which are more or less structurally related (CHRISTENSEN 1969; PALL 1969, 1970a, b; GRAY & COOPER 1971; SHORT, WHITE & KABACK 1972; LOMBARDI & KABACK 1972). Amino acids

which compete for the same transport system will depress each other's uptake rates. It can be reasoned therefore that the effect an amino acid has on growth should be antagonized by amino acids belonging to the same transport class.

The notion that antagonism between amino acids can be due to competition at the uptake process has originated from work with microorganisms (for references see discussion). In reports on antagonism between amino acids in the growth of higher plants it has not been considered whether competitive uptake was the cause of antagonism. To investigate whether this might be so seems important because from the observed antagonisms one has been inclined to draw conclusions regarding metabolic processes inside the cell (DUNHAM & BRYAN 1969; MIFLIN 1969; BORSTLAP 1970; WONG & DENNIS 1973; HENKE et al. 1974).

The present paper provides evidence that in *Spirodela polyrhiza* some amino acids antagonize the growth inhibitory action of the branched-chain amino acids by inhibiting their uptake.

2. MATERIAL AND METHODS

2.1. Chemicals

Glycine, L-alanine, L-isoleucine, L-glutamic acid (monosodium salt), L-ornithine. HCl and L-lysine. HCl were obtained from the British Drug Houses Ltd., Poole, Dorset, U.K.; L-valine from E. Merck A. G., Darmstadt, G. F. R. and L-leucine and D-valine from Fluka A.G., Buchs, Switzerland. L-(1-^{14}C)leucine, 59 mCi/mmole, L-(U-^{14}C)valine and L-(U-^{14}C)isoleucine both 10 mCi/mmole were purchased from The Radiochemical Centre, Amersham, U.K.

2.2. Growth experiments

The duckweed was grown as described previously (BORSTLAP 1970). The growth medium used has been described by LACOR (1968) but in addition it contained 36 mg/l $Na_2HPO_4.2H_2O$ and 1 % sucrose. Growth experiments were started by inoculating 100 ml of medium with two fronds, a motherfrond with one daughterfrond. The growth was followed by counting the fronds over a period of ten days; the first count was done one day after inoculation. Fronds were scored as soon as they had attained half their ultimate size. In cultures grown in the presence of L-isoleucine the fronds were smaller than normal; they were scored when they had released from their motherfronds.

2.3. Uptake experiments

Fronds for uptake experiments were, like the inocula for the growth experiments taken from axenic cultures with 80 to 100 fronds. In such cultures the number of fronds still increased exponentially. 30 to 60 fronds were placed in an 100 ml erlenmeyer flask on freshly prepared medium at 27 °C. The appropriate amount (s) of amino acid solution(s) in medium and about 0.5 μCi of ^{14}C-labelled amino acid were added to the medium, the final volume of which was 25 ml. Illumination and temperature during the uptake experiments were the same as for the growth experiments. The number of fronds and the incubation time

were chosen so that the percentage of labelled amino acid expected to be taken up was between 30% and 70%. After incubation three samples of 50 µl were taken from the medium with Drummond microcaps. Detached stolons and root caps were avoided from the samples. Each sample was counted in 15 ml of scintillation liquid (toluene-methanol, 3:1, by volume, containing 4.9 g PPO and 0.1 g POPOP per liter), with a Packard liquid scintillation spectrometer, model 3375. The fronds were lyophilized after which their dry weight was determined.

Extraction of free amino acids and their quantitative determination by gas-liquid chromatography following the method of ROACH & GEHRKE (1969) have been described (BORSTLAP 1972).

3. RESULTS

3.1. Growth responses

Each of the branced-chain amino acids L-leucine, L-valine and L-isoleucine can, when supplied to the medium, inhibit the growth of *S. polyrhiza*. The degree of inhibition depends on the initial amino acid concentration in the medium (BORSTLAP 1970). To find a method for unambiguous measurement of growth responses further inquiries were made about the way in which the course of growth was affected by the exogenously supplied amino acid.

When *S. polyrhiza* was grown on the basal medium (control cultures) the number of fronds increased exponentially. Thus plotting the logarithm of the number of fronds against time yields a straight line *(fig. 1a)*. The slope of this line represents the multiplication rate (MR) of the culture. For 147 control cultures the multiplication rates were determined. For convenience the MR's were calculated from the number of fronds in the inoculum and at the end of the culture period respectively. The mean MR was 0.1405 (S.E. = 0.0017). Apparently the MR's of the control cultures are normally distributed *(fig. 2a)*.

With L-leucine or L-valine added to the medium the growth was either exponential and the fronds produced were of equal size and shape as in the control cultures, or increase in the number of fronds was completely arrested at an early stage of the culture period. So with L-leucine or L-valine in the medium there was at the beginning of the culture period a chance, dependent on the initial amino acid concentration in the medium, that growth would stop. The growth responses to these amino acids can be best expressed as "occurring" or "not occurring", that is as an all-or-none response. Accordingly cultures were said to be "growing" or to be "not growing". In case of growth inhibition an MR-value, calculated from the number of fronds in the inoculum and at the end of the culture period respectively, was yet assigned to the culture. The all-or-none response is well illustrated by the split up of the MR's into two groups *(fig. 2b, c, d)*.

When cultures were grown on medium with relatively high concentrations of L-valine plus glycine or L-alanine another type of growth curve was sometimes found *(fig. 1d)*. The culture started to grow at a normal multiplication rate

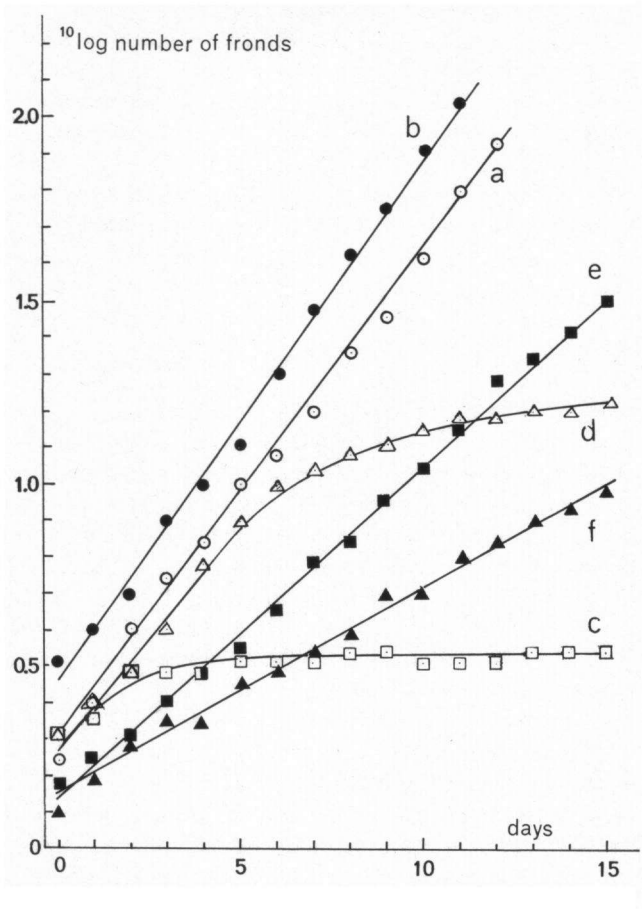

fig. 1

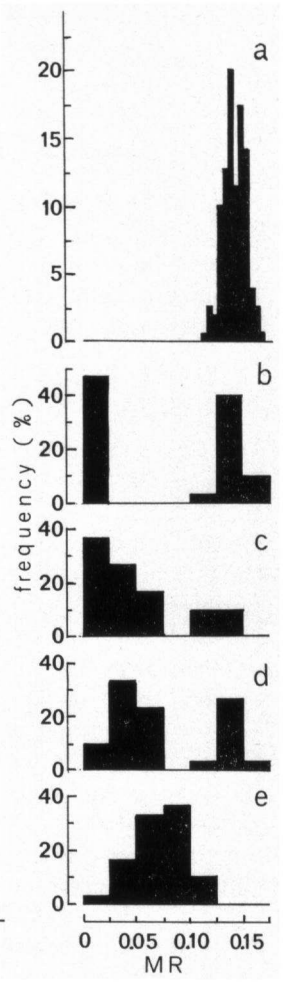

fig. 2

Fig. 1. Growth curves of a control culture, no amino acid added to the medium (a), and of cultures grown on medium supplied with 0.015 mM L-leucine (b, c), 0.225 mM L-valine plus 0.3 mM L-alanine (d), 0.05 mM L-isoleucine (e), and 0.1 mM L-isoleucine (f).

Fig. 2. Distribution of multiplication rates of control cultures (a), and of cultures grown in the presence of 0.015 mM L-leucine (b), 0.15 mM L-leucine plus 0.3 mM L-alanine (c), 0.325 mM L-valine (d), and 0.05 mM L-isoleucine (e). In (a) data were taken from 147 cultures, in (b), (c), (d) and (e) from 30 cultures.

producing fronds of normal size and shape. The growth was maintained for several days but then growth became strongly inhibited. As at the initial amino acid concentrations these cultures grew like the control cultures they have been

scored as "growing". The difference between growth curves of type c and d *(fig. 1)* lies in the time at which growth stopped. Growth curves might be obtained for which it is difficult to decide whether they are of type c or d, but this problem was not encountered in the present study.

In cultures grown on medium supplied with L-isoleucine the number of fronds increased exponentially but slower than in the control cultures *(fig. 1e, f)*. The fronds were smaller according as they developed later on in the culture period, but the multiplication rate remained constant. The response to L-isoleucine can therefore be described as a graded one; MR's of cultures grown on media with a fixed initial concentration of L-isoleucine belong to one group *(fig. 2e)*.

3.2. Dose response curves

As stated by PARKER & WAUD (1971) it is common experience that responses lie on a sigmoid curve when plotted against the logarithm of the concentration. Various functions of a generally sigmoid shape can be used to fit dose response curves, but there is no theoretical curve that can be chosen a priori. Among these functions both the logistic function (PARKER & WAUD 1971) and the normal error curve (GADDUM 1926) have been used. BLISS (1934) has proposed "probit transformation" of experimental data to be fitted to the normal error curve, and in this form the method has been described by FINNEY (1964). The reason to apply the latter method in the present study was that statistical treatment of the experimental results was available from Finney's book. PARKER & WAUD (1971) have mentioned that the normal error curve is more difficult to handle than the logistic function because of the necessary use of tables. In the present work the fitting process was performed with a digital computer so that the cumbrous use of tables could almost completely be omitted.

To obtain the control dose response curves for L-leucine, L-valine and L-isoleucine a number of growth experiments have been done. Fronds were inoculated on media with various concentrations of singly supplied amino acids. Growth responses to L-leucine and L-valine were expressed as the percentage of "growing" cultures, those to L-isoleucine as the percentual MR relative to the MR of control cultures. The procedure for probit analysis, referring to the pages in FINNEY (1964) where the various topics are discussed, was briefly as follows.

The data were plotted on log-probit paper and fitted by eye to a straight line, the provisional probit regression line. For the doses used the expected percentages effect were read from this line, and these values were transformed into expected probits with the aid of table 1 in FINNEY (1964). The data concerning doses, number of cultures per dose, percentages effect, expected percentages effect and expected probits were processed with a computer program[1]. In this way the predicted probit regression line of maximal likelihood (p. 52) was calculated. Data were tested for heterogeneity (p. 55). and from the predicted

[1] This computer program is available on request.

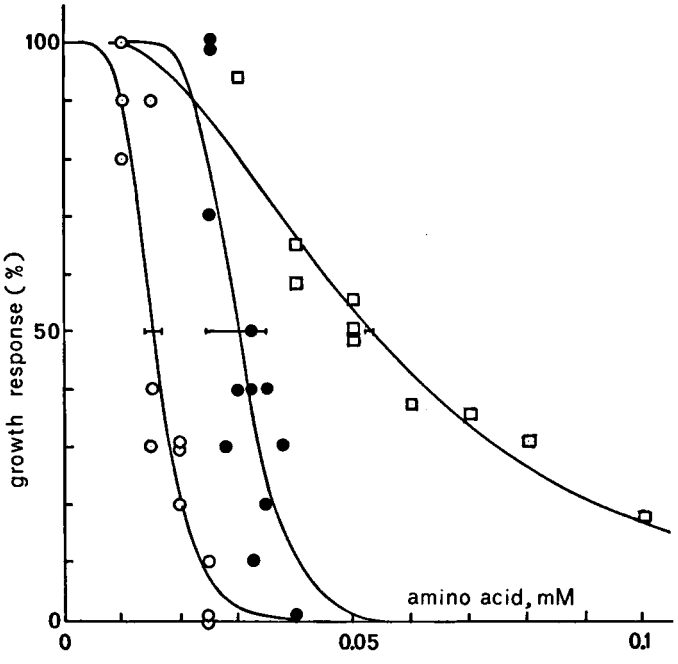

Fig. 3. Dose response curves for L-leucine (○), L-valine (●) and L-isoleucine (□). Each point represents 10 cultures. The 95% fiducial limits of the ED_{50}'s are indicated by bars. The lines drawn are the calculated response curves of maximal likelihood. The curves are asymmetric because of the normal concentration scale.

probit regression line the ED_{50}, i.e. the dose which gives 50% effect, and its exact fiducial limits (p. 63) were calculated. The predicted probit regression lines were finally transformed into the sigmoid dose response curves, in which form they are presented in *fig. 3*. The ED_{50}'s found were for L-leucine 15 μM, for L-valine 30 μM, and for L-isoleucine 52.5 μM.

Although probit analysis has mainly been applied to all-or-none responses it can be equally well used for graded responses. There is, however, a difference between the statistical procedures for the two types of data (p. 185).

Fig. 4. a. Dose response relations for the branched-chain amino acids in the presence of D-valine (■), L-ornithine (△), L-lysine (▲), L-glutamic acid (□), glycine (○) and L-alanine (●), each at an initial concentration of 0.3 mM. The lines drawn represent the calculated dose response curves; from left to right: the control response curves, and the response curves in the presence of L-glutamic acid, glycine and L-alanine respectively. The calculated response curves in the presence of D-valine, L-ornithine and L-lysine are not shown. Each point represents at least 6 cultures. Note the different concentration scales. b. The influence of the same amino acids on the uptake rates (nmoles. hr^{-1}. mg dry wt^{-1}) of the branched-chain amino acids. Same symbols as in a. Uptake rates in the absence of a competing amino acid are means of 6 values.

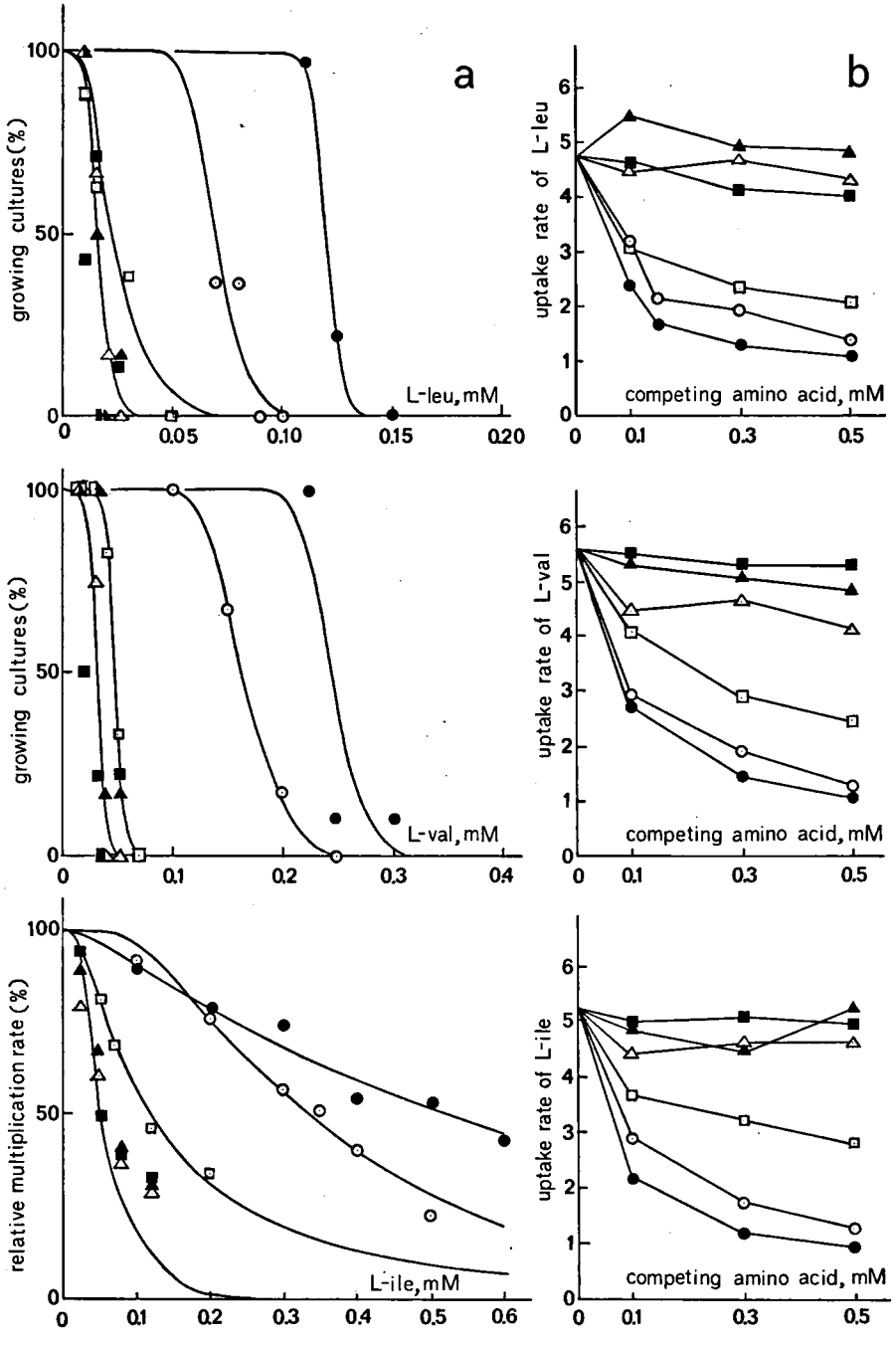
a
b
growing cultures (%)
L-leu, mM
uptake rate of L-leu
competing amino acid, mM
L-val, mM
uptake rate of L-val
relative multiplication rate (%)
L-ile, mM
uptake rate of L-ile

Fig. 4.

Table 1. Multiplication rates, MR, and branched-chain amino acid pools of *Spirodela* cultures grown on media with various amino acids supplied at a concentration of 0.3 mM. Amino acid pools were determined in cultures grown to about 40 fronds; single determinations.

amino acid added	MR [$\bar{x} \pm$ S.E. (n)]	branched-chain amino acid pools (nmoles/mg dry wt)		
		valine	isoleucine	leucine
None	0.141 ± 0.002 (147)	3.0**	0.7**	0.7**
D-valine	0.139 ± 0.004 (6)	381.5***	0.4	0.7
L-ornithine	0.132* ± 0.004 (6)	1.4	0.6	0.5
L-lysine	0.131* ± 0.002 (6)	1.0	0.5	0.4
L-glutamic acid	0.134 ± 0.001 (6)	3.7	0.8	0.6
Glycine	0.138 ± 0.001 (6)	1.4	0.2	0.3
L-alanine	0.129* ± 0.004 (6)	2.8	0.7	0.8

* in Student's t-test with $P<0.05$ significantly different from the mean MR of the control cultures

** data from BORSTLAP (1972)

*** D- and L-valine were not separated in gas chromatographic analysis

A number of amino acids have been tested for their effect on the growth inhibitory action of the branched-chain amino acids. Upon these amino acids two demands were made previously. First, they should not cause severe growth inhibition at the concentrations used, and secondly they should not affect the sizes of the branched-chain amino acid pools, because this might be an indica-

Table 2. ED_{50}-values and dose ratios, DR, for the growth inhibitory action of the branched-chain amino acids. Figures in brackets are the 95% fiducial limits. In some cases these limits could not be calculated.

additional amino acid (0.3 mM)	ED_{50} (μM)			DR		
	L-leucine	L-valine	L-isoleucine	L-leucine	L-valine	L-isoleucine
None	**15.3** (13.9–16.7)	**30.1** (24.3–34.8)	**52.5** (52.1–53.0)			
D-valine	**11.5**	**18.1** (11.7–25.4)	**62.0**	**0.75** (0.57–0.93)	**0.60** (0.21–0.85)	**1.18** (1.16–1.21)
L-ornithine	**16.4** (12.6–19.4)	**31.1**	**61.2** (60.7–61.6)	**1.07** (0.82–1.40)	**1.03** (0.83–1.32)	**1.17** (1.14–1.19)
L-lysine	**15.5** (11.7–19.0)	**38.3** (31.2–45.3)	**69.9** (69.3–70.4)	**1.01** (0.81–1.27)	**1.27** (0.89–1.83)	**1.33** (1.31–1.36)
L-glutamic acid	**22.0** (14.8–30.5)	**46.6** (39.2–60.2)	**118** (118–120)	**1.43** (1.12–1.82)	**1.55** (1.29–1.87)	**2.26** (2.21–2.30)
Glycine	**69.4** (37.6–76.3)	**164** (126–194)	**333** (331–335)	**4.53** (3.66–5.54)	**5.45** (4.53–6.53)	**6.35** (6.25–6.45)
L-alanine	**120** (117–123)	**245**	**516**	**7.85** (6.73–9.22)	**8.15** (5.2–14.3)	**9.84** (9.65–10.0)

tion of interference with the biosynthesis of the branched-chain amino acids.

Glycine, L-alanine, D-valine, L-glutamic acid, L-ornithine and L-lysine satisfied these conditions. When these compounds were supplied singly to the medium at 0.3 mM the growth rate of the duckweed was not very different from that of the control cultures, although L-alanine, L-ornithine and L-lysine yielded growth rates which were significantly lower *(table 1)*. Likewise the branched-chain amino acid pools in these cultures were not very different from those in the control cultures, at least they were not higher *(table 1)*.

Dose response curves for the branched-chain amino acids were again determined but now in the presence of one of the above amino acids supplied to the medium at a concentration of 0.3 mM. The resulting curves, shown in *fig. 4*, were calculated in the same way as the control curves. The slightly lower multiplication rates due to L-alanine, L-ornithine and L-lysine were neglected in the experiments with L-leucine and L-valine, where all-or-none responses were found, but they were accounted for in the experiments with L-isoleucine where the response was graded. In the computer program mentioned above each dose response curve and its appropriate control response curve were tested for parallelism (p. 71). Moreover, for each pair of response curves the dose ratio, DR, was calculated with its exact fiducial limits. The dose ratio is identical with the relative potency as defined by Finney (p. 66). Thus DR is equal to the ratio of branched-chain amino acid concentrations which gave 50% effect in the presence and in the absence of a second amino acid respectively. The value of DR can therefore be taken as a measure of antagonism.

The tests for parallelism showed that the slopes of the various probit regression lines for a particular branched-chain amino acid were not significantly different (data not shown). Moreover the degree to which an amino acid antagonized the growth inhibitory action of each of the branched-chain amino acids, expressed as the DR, was rather constant *(table 2)*. Thus D-valine, L-ornithine and L-lysine did not antagonize, yielding DR's generally not significantly larger than 1.0. L-glutamic acid, glycine and L-alanine, in increasing order and each to a distinct degree, antagonized the growth inhibitory action of irrespective which branched-chain amino acid.

3.3. Uptake experiments

It was considered that the above results might be explained by assuming that the branched-chain amino acids are taken up by the duckweed via a common transport system, to which the antagonists L-glutamic acid, glycine and L-alanine, in this order have increasing affinities, wheras the non-antagonists D-valine, L-ornithine and L-lysine have no affinity.

First it was examined how the branched-chain amino acids affect each other's uptake rates. Fronds were placed on medium supplied with 0.03 mM ^{14}C-labelled L-leucine. The uptake rate of the amino acid was calculated from the disappearance of ^{14}C from the medium. Uptake rates of L-leucine were also determined when various concentrations of L-valine or L-isoleucine were added

to the incubation medium. Analogous experiments were done with L-(^{14}C)valine and L-(^{14}C)isoleucine.

Measurement of uptake rates was based on the results from preliminary experiments which showed that up to 30 μM the uptake rate of a branched-chain amino acid was approximately proportional to the amino acid concentration in the medium. The initial uptake rate, v, may therefore be calculated from the formula

$$v = \frac{N \ln (Do/Dt)}{wt}$$

where D_o and D_t are dpm's, due to ^{14}C-labelled amino acid, in a sample of medium at the start and at the end of the uptake experiment respectively, N is the total amount of amino acid supplied to the medium, w is the dry weight of the fronds and t is the incubation time.

The results *(fig. 5)* clearly demonstrate that the branched-chain amino acids mutually inhibit their uptake, from which it can be deduced that they are taken up via a common transport system.

Uptake rates of the branched-chain amino acids, at 0.03 mM, were also measured in the presence of various concentrations of D-valine, L-ornithine, L-lysine, L-glutamic acid, glycine or L-alanine, the same amino acids which were tested for their effect on the growth inhibitory action of the branched-chain amino acids. As shown in *fig. 4b* the uptake rate of a branched-chain amino acid, whichsoever, was inhibited in increasing order by L-glutamic acid, glycine and L-alanine, the antagonists, whereas the non-antagonists D-valine, L-ornithine and L-lysine did not decrease the uptake rate or, if so, only slightly.

Finally it was questioned whether a decrease in the uptake rate of a growth inhibiting amino acid would ultimately lead to a restricted accumulation of

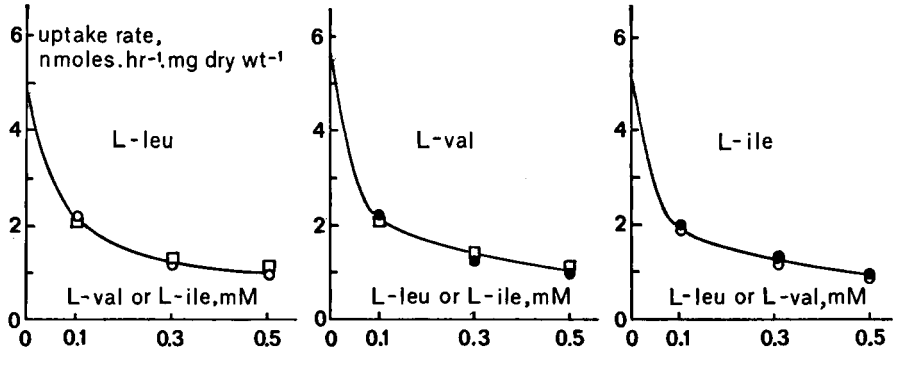

Fig. 5. Influence of the branched-chain amino acids on each other's uptake rates. Uptake rates in the absence of a competing amino acid are means of 6 values. Other points refer to single measurement. The competing amino acids were L-leucine (●), L-valine (○) and L-isoleucine (□).

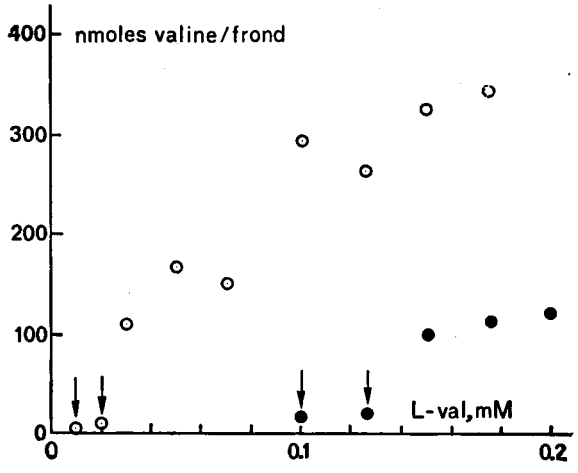

Fig. 6. Accumulation of valine in fronds incubated for three days on media with various concentrations of L-valine (○), and on media with various concentrations of L-valine plus 0.2 mM glycine (●). Arrows refer to inocula which had normally grown: the number of fronds had doubled. In the other inocula growth was inhibited: the number of fronds increased by only 30% or less and, after three days, the proximal parts of the not fully grown fronds were yellowish.

that amino acid after longer periods. This was examined for one amino acid pair: L-valine and glycine. Two series of media were prepared, each series with increasing concentrations of L-valine. One series in addition contained 0.2 mM glycine. All media were inoculated with two fronds. The fronds were cultured as in the growth experiments, harvested three days after inoculation after which the valine pool in the fronds was determined. The results, depicted in *fig. 6*, show that glycine restricted the accumulation of L-valine.

4. DISCUSSION

Various types of growth curves were found for *Spirodela* cultures grown on medium supplied with a branched-chain amino acid. A detailed quantitative knowledge of amino acid uptake, incorporation of amino acid into protein, catabolic breakdown of amino acid, the characteristics of the amino acid pool and its relation to endogenous formation of amino acid and to the growth inhibition might eventually lead to an understanding of the various types of growth curves. However, the observations that responses to L-leucine and L-valine were all-or-none responses and that the response to L-isoleucine was graded were sufficient to measure the growth responses unambiguously, and to apply the appropriate statistical treatment to the experimental results.

The correlation between the ability of an amino acid to inhibit the uptake rate of a branched-chain amino acid and its ability to antagonize the growth

inhibitory effect of that branched-chain amino acid may indicate a causal relation. As uptake rates were measured over relatively short periods one might argue that lower initial uptake rates would not necessarily lead to lower accumulation levels after longer periods. Whether this is true or not was examined for the amino acid pair L-valine and glycine. As indicated in *fig. 5* growth was not inhibited until 0.02 mM of singly supplied L-valine. In the presence of 0.2 mM glycine, L-valine was not inhibitory until a concentration of 0.125 mM. In both cases the fronds which were not growth-inhibited had low valine pools, less than 20 nmoles/fronds, whereas the growth-inhibited fronds had high valine pools of at least 100 nmoles/frond. But this mere observation in not sufficient to conclude that glycine antagonizes the growth inhibitory action of L-valine by inhibiting its uptake. This is so because the consumption of valine for protein synthesis will be less when growth is inhibited. So high valine pools might be the result rather than the cause of growth inhibition. There is, however, additional evidence. First, in the fronds incubated at concentrations of L-valine which just inhibited growth the valine pools were about 100 nmoles/frond. This indicates that the level of the valine pool which just inhibits growth does not depend on the presence of glycine in the medium and, moreover, that the lower valine consumption in growth-inhibited fronds caused an increase in the valine pool of at highest 100 nmoles/frond. Secondly, the valine pools of fronds incubated on medium with 0.1 and 0.125 mM L-valine were about 275 nmoles/frond in the absence of glycine and about 20 nmoles/frond in the presence of glycine, a difference of 255 nmoles/frond. If the lower valine consumption in the growth-inhibited fronds contributed 100 nmoles/frond, the remaining 155 nmoles/frond has to be ascribed to inhibition of valine uptake by glycine. Thirdly, fronds incubated on media with 0.15 and 0.175 mM L-valine were growth inhibited, both in the absence and in the presence of glycine. Glycine reduced the accumulation of valine by about 225 nmoles/frond, which can be only ascribed to inhibition of valine uptake.

The antagonisms found in the present work can be interpreted quantitatively in terms of a model used in pharmacology to describe the action of drugs (WAUD & PARKER 1971). The uptake of an amino acid A, which causes growth inhibition, is thought to be preceded by the reversible binding of the amino acid to a carrier. This results in a fractional carrier occupancy

$$y_A = [A]/([A] + K_A) \quad (1)$$

where K_A is the dissociation constant of the amino acid-carrier complex. The uptake rate of the amino acid, v_A, can be written as

$$v_A = V_m\, y_A = V_m\, [A]/([A] + K_A) \quad (2)$$

where V_m is the maximal uptake rate of the amino acid. Growth inhibition by the amino acid A is considered to be a function (of unknown nature) of the uptake rate of the amino acid, rather than of the amino acid concentration in the medium. Thus

$$E_A = f(v_A) \quad (3)$$

where E_A denotes the degree of growth inhibition. In the presence of an amino acid B which competes with A for occupancy of the carrier

$$v_A' = V_m [A]'/([A]' + K_A (1 + [B]/K_B)) \quad (4)$$

where K_B is the dissociation constant of the complex of B with the carrier, and

$$E_A' = f(v_A') \quad (5)$$

f is unchanged: the competitive amino acid does not alter the relation between carrier occupancy and effect. If effects in the absence and in the presence of B are matched

$$E_A = E_A' \text{ or } f(v_A) = f(v_A')$$

Equating the right-hand sides of Eqns (2) and (4) leads to the expression

$$[A]'/[A] = [B]/K_B + 1 \quad (6)$$

where [A]'/[A] is designated as the dose ratio, DR. It should be noticed that DR, at a given value of [B] only depends on K_B. Thus according to this model a particular amino acid would have the same antagonizing effect on the growth inhibitory action irrespective of which branched-chain amino acid, provided the branched-chain acids are taken up by the same transport system.

The results shown in *table 2* are approximately consistent with this model. The deviations between the experimental results and what is to be expected from the above model are of two kinds. First, the DR-values for D-valine with respect to L-leucine and L-valine are both significantly smaller than 1.0. This might be due to contamination of D-valine with L-valine and/or the formation in the plants of small amounts of L-valine from the accumulated D-valine. The growth inhibitory effects of L-leucine and L-valine were found to be additive (unpublished results). The second deviation is that DR-values with respect to L-leucine tend to be smaller than DR-values with respect to L-valine which in turn tend to be smaller than those with respect to L-isoleucine. It is probable that this deviation is connected with the differences between the branched-chain amino acids regarding their ability to inhibit growth, and with the fact that amino acid uptake by *S. polyrhiza* does not obey simple Michaelis-Menten kinetics, as is assumed in the above model, but can be described by a double Michaelis-Menten equation (unpublished results).

It is nevertheless concluded that uptake inhibition is by far the most important, if not the only cause of antagonism. There are a number of reasons which suggest that other factors did not contribute considerably to the antagonisms. First, one can wonder whether the antagonisms could be partly due to interactions between the amino acids inside the cell. As regards this question the work of FILNER (1966) comes to mind. Filner has found that in tobacco cells, grown in suspension cultures, nitrate reductase was repressed by a number of amino acids, some other amino acids reversing this effect. It is not known whether nitrate reductase in *S. polyrhiza* behaves in the same way, but even if this were so it cannot be the reason for the antagonisms reported here. Not only that the growth inhibition by the branched-chain amino acids can be ascribed to causes

different from an effect on nitrate reductase (BORSTLAP 1972), but also because the medium employed besides nitrate contained an amount of ammonium large enough to maintain growth of the duckweed at multiplication rates of about 70% of those of control cultures (Van Mazijk, personal communication). Secondly, the cause of growth inhibition for each branched-chain amino acid seems to be a different one. An excess of one of these amino acids leads to shortage of one or both of the other branched-chain amino acids (BORSTLAP 1972). It is unlikely that each of these different causes is antagonized by a direct interaction to the same extent by L-glutamic acid or glycine or L-alanine. Finally it might be imagined that the latter amino acids, in increasing order should stimulate the biosynthesis of the three branched-chain amino acids. This would render the plants more refractory to an excess of one of them. But cultures grown on medium with L-glutamic acid, glycine or L-alanine did not have larger pools of the branched-chain amino acids *(table 1)* while the multiplication rates, and by inference the consumption of amino acids for protein synthesis, never exceeded those of the control cultures. The idea of a stimulation of biosynthesis can therefore be rejected.

The hypothesis that antagonism could be due to competitive uptake has first been inferred from the observation that the growth of amino acid auxotrophic mutants of bacteria was competitively inhibited by structurally related amino acids, whereas the growth of wild types was not inhibited (COHEN & MONOD 1957). In later studies observations corroborating this hypothesis have been made. Thus antagonism has not been observed if the required amino acid was supplied as a peptide (PRESCOTT, PETERS & SNELL 1953; HIRSCH & COHEN 1953). Moreover antagonism has not been observed when the cells were first exposed to the one, then to the other amino acid (MATHIESON & CATCHESIDE 1955; BROCK & BROCK 1961). More directly it has been shown by MANDELSTAM (1956) that the inhibiting effect of diamines on the growth of arg^- and lys^- mutants from *Escherichia coli* was correlated with their ability to restrict the accumulation of arginine and lysine. Similarly CYBIS & WEGLENSKI (1969) found that the uptake inhibition of arginine by lysine is the cause of the growth inhibition of *Aspergillus nidulans* arg^- mutants by lysine, and POURQUIE (1970) concluded that growth inhibition of gua^- mutants of *Schizosaccharomyces pombe* by the purines adenine and hypoxanthine was due to inhibition of guanine uptake. Recently MEINS & ABRAMS (1972) have obtained evidence that in *Chlorella vulgaris* L-glutamine and L-methionine act as antagonists of the growth inhibitor methionine sulfoximine by reducing its uptake.

From the present investigation it is concluded that the antagonistic effects of L-glutamic acid, glycine and L-alanine on the growth inhibitory action of the branched-chain amino acids can predominantly, if not completely, be attributed to inhibition of branched-chain amino acid uptake. In growth experiments these amino acids apparently behave as antagonists of growth inhibition, but in fact they prevent growth inhibition. In interpreting antagonisms between amino acids, observed with plants or plant cells, competitive uptake as a possible cause should be seriously considered.

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