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TRANSPORT OF AMINO ACIDS IN EHRLICH ASCITES CELLS: COMPETITIVE STIMULATION

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SUMMARY

1. The term 'competitive stimulation' refers to an experimental finding in which the uptake of one amino acid is increased in the presence of another amino acid when both are initially present extracellularly. The uptake of both amino acids of such pairs was investigated with use of double-label techniques.

2. For all amino acid pairs examined which showed competitive stimulation, the uptake of one amino acid was increased whereas that of the other was decreased. Furthermore, the decrease in uptake of the latter was considerably greater than the increase in uptake of the former.

3. In time studies it was found that the distribution ratio of the amino acid whose uptake was stimulated was increased at all incubation times up to 30 min. The amino acid whose uptake was inhibited showed not only a markedly decreased uptake but also a significantly altered curve of time dependence of uptake.

INTRODUCTION

The term 'competitive stimulation' has been applied to an experimental finding with ascites tumor cells[1,2] in which the uptake of an amino acid is increased in the presence of another amino acid when both are initially present extracellularly. CHRISTENSEN *et al.*[3] reported in 1952 that several L-diamino acids increased the steady-state distribution ratios of glycine, L-alanine and L-histidine. JACQUEZ[4,5] showed that several neutral L-amino acids as well as the amino acid analog azaserine, increased the 2-min distribution ratio of L-tryptophan. He showed that although stimulation of uptake of one amino acid occurred when both were present at approximately equimolar concentrations, inhibition of uptake of the stimulated amino acid occurred when the concentration of the other amino acid was raised sufficiently[5]. In one experiment JACQUEZ[4] measured the 1-min uptakes of L-trypto-

phan and of azaserine, both present at the same initial extracellular concentration, and noted that whereas the initial uptake of L-tryptophan was increased in the presence of azaserine, the initial uptake of azaserine was decreased in the presence of L-tryptophan, and that the decrease in uptake of azaserine was more than twice the increase in uptake of L-tryptophan. As will be shown in this paper this has held true for all pairs of amino acids showing competitive stimulation which we have examined. In order to distinguish between the two amino acids in competitive stimulation and to avoid the use of such long expressions as, 'the amino acid whose uptake was stimulated', and 'the amino acid whose uptake was inhibited', we will use the terms 'stimulated amino acid' and 'inhibited amino acid' in the remainder of this paper.

CHIRIGOS, FANNING AND GUROFF[6] and GUROFF, FANNING AND CHIRIGOS[2] reported that the neutral amino acid analog DL-*p*-fluorophenylalanine increased the 30-min distribution ratios of L-tyrosine, L-*p*-aminophenylalanine, L-tryptophan, L-phenylalanine and L-lysine. GUROFF, FANNING AND CHIRIGOS[2] also measured the DL-*p*-fluorophenylalanine and L-tryptophan 30-min distribution ratios in a competitive-stimulation experiment and found as did JACQUEZ that while the uptake of L-tryptophan increased that of the DL-*p*-fluorophenylalanine decreased. However, they reported that the increase in uptake of the L-tryptophan was approximately equal to the decrease in uptake of the DL-*p*-fluorophenylalanine.

The stoichiometric relation between the changes in uptake of the two amino acids in competitive stimulation is important for the discrimination of possible carrier models[5,7]. However, the information on relative changes in uptake of the two amino acids rests on only two experiments[4,2] and they appear to give contradictory results. The present work was undertaken to provide measurements of initial uptake rates as well as of the time course of uptake on a number of pairs of amino acids which show competitive stimulation.

MATERIALS AND METHODS

In these experiments we used a hypotetraploid line of Ehrlich ascites carried in this laboratory for approx. 300 generations by weekly intraperitoneal inoculation of 0.1 ml of ascites (diluted 1:1 or 2:1 with sterile saline) into female Swiss albino mice. The collection and preparation of the ascites was conducted as described by JACQUEZ AND SHERMAN[1] with the exception that after each centrifugation the top layer of the packed cells was removed by a suction pipette. After the final centrifugation the cells were resuspended to a cytocrit of 0.18–0.32 in KRP solution (pH = 7.0)*.

Design of experiments

All experiments involved two test amino acids each made to 4 mM in a separate KRP solution. A fraction of the amino acid in one solution was labelled with ^{3}H, approx. 3 μC/ml, a fraction in the other solution with ^{14}C, approx. 0.15 μC/ml. In 18 experiments, referred to as 'competition studies' throughout this paper, 14 two-

* The solution referred to as KRP is a modified Krebs–Ringer phosphate buffer medium of the following composition: NaCl, 118.4 mM; KCl, 4.73 mM; $CaCl_2$, 0.84 mM; KH_2PO_4, 1.17 mM; $MgSO_4 \cdot 7H_2O$, 1.17 mM; $NaH_2PO_4 \cdot H_2O$, 5.44 mM; Na_2HPO_4, 14.15 mM. The pH of this buffer was consistently measured at 6.95–7.04.

armed Heinicke tubes were set up as shown in Table I. All tubes were preincubated for 3 min at 37°, then the solutions in the two arms were mixed by tipping all tubes simultaneously and incubated for 2 min at 37°. Three time studies were also carried out in which uptakes were measured after 1, 5, 10, 20 and 30 min of incubation.

To terminate the incubations, the Heinicke tubes were plunged into ice-water agitated to insure fast chilling. (Measurements with a thermocouple showed the temperature of the solution inside the Heinicke tube reached 15° within 10–11 sec.) All subsequent operations were conducted in a cold room at 2–4°. The methods of sample preparation and extraction were the same as those described in a previous paper[1] with the following exception. The packed pellet of cells used for extraction and analysis was obtained by diluting 1 ml of cell suspension with 12 ml of cold KRP solution and centrifuging in the cold. This modification was introduced to reduce the correction for the amino acid retained in the extracellular space of the pellet.

TABLE I

PROTOCOL FOR COMPETITION STUDIES

Tube No.	*Arm 1 of Heinicke tube*	*Arm 2 of Heinicke tube*		
	Ascites suspension (ml)	*KRP solution (ml)*	*Amino acid 1 (ml) (4 mM)*	*Amino acid 2 (ml) (4 mM)*
1	3.0	3.0	—	—
2–4	3.0	1.5	1.5	—
5, 6	3.0	—	3.0	—
7–9	3.0	1.5	—	1.5
10, 11	3.0	—	—	3.0
12–14	3.0	—	1.5	1.5

Scintillation counting procedures

The analysis of sample activity was carried out with use of a Nuclear-Chicago Model No. 725 liquid-scintillation system. For those samples which contained only one isotope, the channels ratio procedure was used; the counting efficiencies were 54–60% for ^{14}C and 10–14% for ^{3}H. For samples which contained both ^{14}C and ^{3}H the modification of the method of OKITA *et al.*[8] given in the Nuclear-Chicago instruction manual was followed. Our recoveries of ^{14}C and ^{3}H from samples containing both always fell in the range 94–104%.

Calculations

The methods of calculation of the intracellular concentrations of the amino acids have been described[4]. The methods used in the present investigation differed only in that the dilution of the extracellular concentrations in the pellets obtained by the modified sampling procedure had to be taken into account in the calculations. All uptake data are given in terms of μmoles of amino acid taken up per g dry weight of cells.

Sources of materials

The unlabelled amino acids which were used were all grade-A amino acids

from California Biochemicals, except the DL-*p*-fluorophenylalanine which was obtained from K and K Laboratories in reagent grade.

The ^{14}C-labelled amino acids used were: [2-^{14}C]glycine, L-[1-^{14}C]alanine, uniformly labelled L-[^{14}C]leucine, and L-[*ME*-^{14}C]methionine, California Biochemicals; L-[3-^{14}C]tryptophan, New England Nuclear; uniformly labelled L-[^{14}C]phenylalanine, Nuclear-Chicago; DL-*p*-fluoro[3-^{14}C]phenylalanine, CEA France.

The tritiated amino acids used were: generally labelled L-[^{3}H]methionine, generally labelled L-[^{3}H]tryptophan, L-[ring-4-^{3}H]phenylalanine, and generally labelled L-[^{3}H]phenylalanine, Nuclear-Chicago.

RESULTS

Competition studies

Table II summarizes the results of 18 competition studies. Within each experiment, three 2-min uptakes were computed for both amino acids: the uptake of the amino acid when alone at an initial extracellular concentration of 1 mM, the uptake of the amino acid from 2 mM extracellular concentration, and the uptake of the given amino acid when at 1 mM with the second amino acid also present at 1 mM in the extracellular fluid (in the table and often in the text this is referred to as the 1 + 1 mM uptake). It should be noted that all of the data reported in these experiments is in terms of averages: the uptakes of amino acids from 1 mM solutions represent the averages of three replicates, while the 2 mM uptakes are the averages of two replicates. As would be expected, the variation between replicates was found to be considerably less than that between experiments. The coefficient of variation (standard deviation of mean divided by the mean) averaged 2.99% for the means of the triplicate determinations and 1.97% for the means of the duplicates. Also computed in Table II is the sum of the uptakes of the two amino acids when both are present at 1 mM, *i.e.* the sum of the 1 + 1 mM uptakes, this measures the 'total amino acid' uptake in competition. This sum was compared to the sum of the uptakes of both amino acids when each was alone extracellularly at 1 mM. In all cases this latter sum was greater than the sum of the competition 1 + 1 mM uptakes, indicating that even with competitive stimulation of one of the amino acids, the total amino acid transported in competition is less than would be taken up if each amino acid could be transported without interaction from the other amino acid. In addition, the ratio of the uptake of each amino acid in the 1 + 1 mM competition situation to the uptake when alone at an extracellular concentration of 1 mM was computed. Any ratio greater than unity indicates that the particular amino acid is accumulated to a greater extent in the presence of the second amino acid than in its absence. In Table II these ratios indicate that competitive stimulation occurred in ten experiments; however, in no experiment was it found that the uptakes of both amino acids of a pair were stimulated. In the remaining eight experiments, both amino acids showed decreased uptake in the presence of the other amino acid.

The results of a single competitive-stimulation experiment, demonstrating the salient facts seen in all such experiments, are illustrated in Fig. 1. In the experiment shown as well as in all the experiments of Table II, the total uptake from the 1 + 1 mM mixture (last bar in Fig. 1) is less than the uptake of the inhibited amino acid from a 2 mM solution and is greater than the uptake of the stimulated amino

TABLE II

SIMULTANEOUS UPTAKE OF TWO DIFFERENT AMINO ACIDS, EACH AT 1 mM EXTRACELLULAR CONCENTRATION, COMPARED WITH THE UPTAKES OF EACH ALONE AT 1 AND 2 mM

Amino acid uptakes are in μmoles/g dry wt. per 2 min incubation. All experiments were competition studies at 37°. Abbreviation: FPA, DL-*p*-fluorophenylalaine.

Expt. No.	*Extracellular amino acid 1*	*Uptake of amino acid 1*			*Extracellular amino acid 2*	*Uptake of amino acid 2*			*Sum of uptakes of amino acids 1 and 2*		*Ratio of uptakes (1 + 1)mM/1 mM*	
		Alone at 1 mM	*Alone at 2 mM*	*1 mM amino acid 1 + 1 mM amino acid 2*		*Alone at 1 mM*	*Alone at 2 mM*	*1 mM amino acid 1 + 1 mM amino acid 2*	*Each alone at 1 mM*	*Both present at 1 mM (1 + 1)*	*Amino acid 1*	*Amino acid 2*
3	L-[^{3}H]Trp	2.35	5.19	2.84	L-[^{14}C]Leu	6.84	11.97	4.63	9.19	7.47	1.21	0.68
4	L-[^{3}H]Trp	2.46	4.19	2.96	L-[^{14}C]Leu	5.96	9.46	3.70	8.42	6.66	1.20	0.62
5	L-[^{3}H]Trp	2.62	4.68	3.27	L-[^{14}C]Leu	8.46	13.22	4.40	11.08	7.67	1.25	0.52
9	L-[^{3}H]Trp	2.78	4.46	3.20	L-[^{14}C]Leu	6.26	9.41	3.70	9.04	6.90	1.15	0.59
10	L-[^{3}H]Trp	2.60	3.56	4.26	L-[^{14}C]Met	8.65	12.16	5.60	11.25	9.86	1.64	0.65
27	L-[^{14}C]Trp	2.81	3.27	4.33	L-[^{3}H]Met	8.22	12.96	3.91	11.04	8.24	1.54	0.48
13	L-[^{3}H]Trp	3.08	3.91	4.59	DL-[^{14}C]FPA	8.89	13.47	4.96	11.97	9.54	1.49	0.56
26	L-[^{3}H]Trp	3.59	4.43	5.14	DL-[^{14}C]FPA	7.80	12.78	4.04	11.38	9.18	1.43	0.52
19	L-[^{3}H]Trp	3.87	4.79	3.01	L-[^{14}C]Phe	7.66	10.87	4.73	11.53	7.74	0.78	0.62
12	L-[^{3}H]Trp	2.68	3.69	2.31	[^{14}C]Gly	10.34	14.21	10.14	13.01	12.44	0.86	0.98
11	L-[^{3}H]Trp	3.58	4.67	2.80	L-[^{14}C]Ala	9.48	15.72	9.49	13.06	12.30	0.78	1.00
15	L-[^{3}H]Phe	5.91	9.50	6.59	DL-[^{14}C]FPA	9.55	14.75	6.80	15.46	13.39	1.12	0.71
23	L-[^{3}H]Phe	4.29	7.26	4.79	L-[^{14}C]Met	7.93	11.17	6.03	12.22	10.82	1.12	0.76
28	L-[^{3}H]Phe	6.01	8.98	5.49	L-[^{14}C]Met	7.64	11.44	5.88	13.65	11.37	0.91	0.77
17	L-[^{3}H]Phe	5.11	7.69	3.49	L-[^{14}C]Ala	9.58	15.96	9.61	14.69	13.10	0.68	1.00
18	L-[^{3}H]Met	8.32	13.07	7.51	L-[^{14}C]Ala	9.55	15.60	9.36	17.88	16.86	0.90	0.98
25	L-[^{3}H]Met	8.55	12.76	6.62	L-[^{14}C]Ala	11.34	17.58	8.82	19.94	15.44	0.77	0.78
14	L-[^{3}H]Met	9.38	13.79	7.20	DL-[^{14}C]FPA	10.84	15.36	8.12	20.22	15.32	0.77	0.75

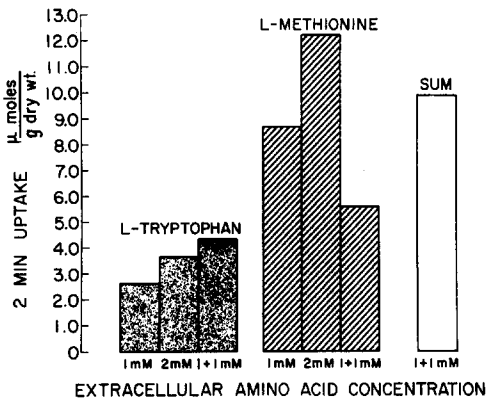

Fig. 1. 2-min uptakes of L-tryptophan and L-methionine measured within the same competition study. The bar labelled 1 mM denotes the uptake of the given amino acid from an extracellular concentration of 1 mM alone, 2 mM denotes the uptake from a 2 mM concentration alone, while 1 + 1 mM indicates uptake of the given amino acid from an extracellular solution containing 1 mM of each of the amino acids. The bar on the far right gives the sum of the 1 + 1 mM uptakes of both amino acids.

acid from a 2 mM solution. The increase in uptake of the stimulated amino acid seen in the 1 + 1 mM situation is significantly less than the decrease in uptake of the inhibited amino acid. This is in contradiction (especially Expts. 13 and 26) to the finding reported by GUROFF, FANNING AND CHIRIGOS[2]. Table III indicates that this holds for all amino acid pairs tested that demonstrate competitive stimulation: *i.e.* that the ratio of the decrease in uptake of the inhibited amino acid to the increase in uptake of the stimulated amino acid is always significantly greater than unity. Another important observation from Table III is that in the experiments in which L-tryptophan uptake was stimulated by DL-*p*-fluorophenylalanine or L-methionine, the increase in uptake of L-tryptophan provided by 1 mM of the inhibited amino acid (Δu_{s2}) was greater than the increase in uptake obtained by increasing the concentration of L-tryptophan by 1 mM (Δu_{s1}). Thus the addition of certain other amino acids can be more effective in increasing L-tryptophan uptake than the addition of more L-tryptophan. Certainly this observation is contrary to what we would expect from most of the simple carrier models which have been used so far.

Time studies

Three experiments were conducted to determine the simultaneous time course of uptake of two competing amino acids. At each of five incubation times the distribution ratio of each amino acid alone at 1 mM, and each amino acid at 1 mM in the presence of the other amino acid also at 1 mM, were determined. In the latter case two replicates were used, in the former only one determination was done because of limitations in the number of Heinicke tubes that could be handled adequately in any one experiment.

Figs. 2 and 3 illustrate the time course of two examples of competitive stimulation. In both cases the stimulated amino acid when alone is taken up more slowly than is the inhibited amino acid, which when alone shows a very rapid initial uptake rate and plateaus within a short time. Examining Figs. 2 and 3, it is seen that in the

TABLE III

COMPARISON OF INCREASED UPTAKE OF THE STIMULATED AMINO ACID TO DECREASED UPTAKE OF THE INHIBITED AMINO ACID

The data presented here is from the same experiments as that reported in Table I. Uptakes are in μmoles/g dry wt. per 2 min incubation. Abbreviation: FPA, DL-*p*-fluorophenylalanine.

Expt. No.	*Stimulated amino acid*	Δu_{s1}*	Δu_{s2}**	*Inhibited amino acid*	Δu_{I1}*	Δu_{I2}**	$\frac{\lvert\Delta u_{I2}\rvert}{\lvert\Delta u_{s2}\rvert}$
3	L-[^{3}H]Trp	2.84	0.49	L-[^{14}C]Leu	5.13	−2.21	4.51
4	L-[^{3}H]Trp	1.73	0.50	L-[^{14}C]Leu	3.50	−2.26	4.52
5	L-[^{3}H]Trp	2.06	0.65	L-[^{14}C]Leu	4.76	−4.06	6.25
9	L-[^{3}H]Trp	1.68	0.42	L-[^{14}C]Leu	3.15	−2.56	6.10
10	L-[^{3}H]Trp	0.97	1.66	L-[^{14}C]Met	3.51	−3.05	1.84
27	L-[^{14}C]Trp	0.46	1.52	L-[^{3}H]Met	4.74	−4.31	2.84
13	L-[^{3}H]Trp	0.83	1.51	DL-[^{14}C]FPA	4.58	−3.93	2.61
26	L-[^{3}H]Trp	0.84	1.55	DL-[^{14}C]FPA	4.98	−3.76	2.42
15	L-[^{3}H]Met	3.59	0.68	DL-[^{14}C]FPA	5.20	−2.75	4.05

* (uptake of amino acid from 2 mM alone) − (uptake of amino acid from 1 mM alone).
** (uptake of amino acid from 1 mM with 1 mM of second amino acid present) − (uptake of amino acid from 1 mM alone).

1 + 1 mM competition situation the initial rate of uptake of the inhibited amino acid is much decreased, and the intracellular concentration plateaus at a decreased level and over a longer time course. In the competition situation the general shape of the uptake curve for the stimulated amino acid does not seem to be greatly altered but rather only shifted upward from the 1 mM uptake curve. The curves also illustrate that at all incubation times the decrease in uptake of the inhibited amino acid is greater than the increased uptake of the stimulated amino acid in the 1 + 1 mM situation. This is especially true in the case shown in Fig. 3. For comparison, Fig. 4 shows a time study in which competitive inhibition is observed. Note that here, in contrast to the competitive-stimulation time studies, the uptake curves of both amino acids are merely depressed uniformly in the presence of the second amino acid. The two curves for L-phenylalanine do not differ significantly at 30 min.

DISCUSSION

Although experimental observation of the phenomenon of competitive stimulation has been limited, several characteristics appear to be quite well established. For several pairs of amino acids showing competitive stimulation the per cent stimulation has been demonstrated to be maximal when the stimulating amino acid is approximately equimolar with respect to the stimulated amino acid[5,2]. Secondly, our results corroborate those of GUROFF, FANNING AND CHIRIGOS[2] which indicate that the inhibited amino acid is taken up much more rapidly and to higher steady-state levels when alone extracellularly than is the stimulated amino acid under the same circumstances.

In these experiments, we have found, as have others[4,2], that with those pairs

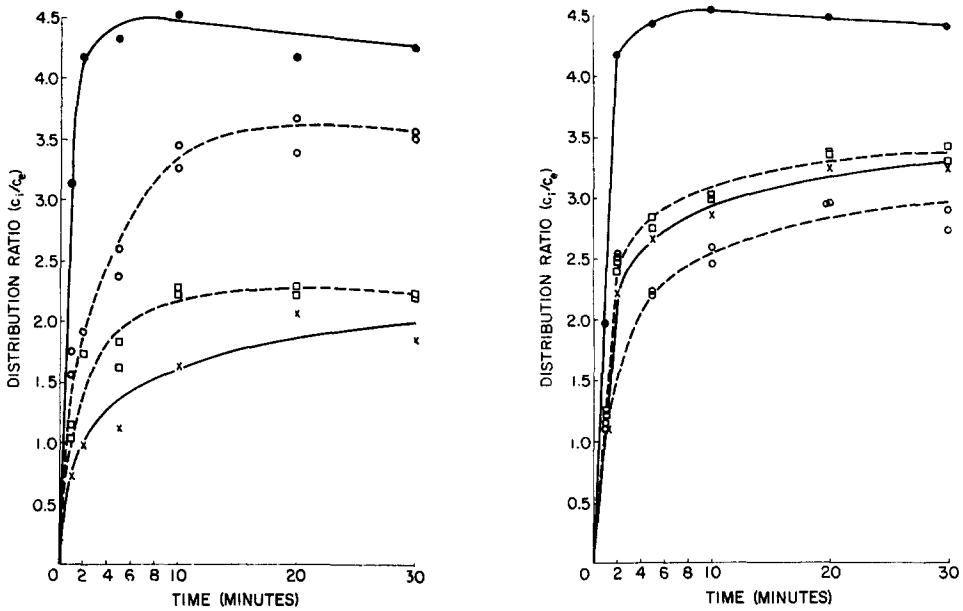

Fig. 2. Time dependence of simultaneously measured distribution ratios for DL-*p*-fluorophenylalanine (FPA) and L-tryptophan for each alone at 1 mM and in the presence of the second amino acid also at 1 mM. ●—●, DL-*p*-fluorophenylalanine when alone at 1 mM; ○— — —○, DL-*p*-fluorophenylalanine when at 1 mM with 1 mM L-tryptophan; ×—×, L-tryptophan alone at 1 mM; □— — —□, L-tryptophan when at 1 mM with DL-*p*-fluorophenylalanine also present at 1 mM. All 2-min distribution ratios are not from the same experiment but are averaged values from competition studies.

Fig. 3. Time dependence of simultaneously measured distribution ratios for DL-*p*-fluorophenylalanine and L-phenylalanine for each alone at 1 mM and in the presence of the second amino acid also at 1 mM. ●—●, DL-*p*-fluorophenylalanine when alone at 1 mM; ○— — —○, DL-*p*-fluorophenylalanine when at 1 mM with L-phenylalanine also present at 1 mM; ×—×, L-phenylalanine when alone at 1 mM; □— — —□, L-phenylalanine when at 1 mM with DL-*p*-fluorophenylalanine also present at 1 mM. All 2-min distribution ratios are not from the same experiment but are averaged values from competition studies.

of amino acids exhibiting competitive stimulation there was a decrease in the uptake of the inhibited amino acid concurrent with the increased uptake of the stimulated amino acid. However, we have found that the decrease in uptake of the inhibited amino acid is always considerably greater than the increase in uptake of the stimulated amino acid whereas GUROFF, FANNING AND CHIRIGOS[2] reported that the two were approximately equal. The finding of GUROFF, FANNING AND CHIRIGOS[2] is based on a 30-min incubation, while the bulk of our data derives from 2-min initial uptakes; nevertheless, our time studies show that the depression of DL-*p*-fluorophenylalanine uptake far exceeds the increase in uptake of L-phenylalanine or L-tryptophan (Fig. 2) out to 30 min of incubation. The time studies also suggest that the percentage stimulation is greater at shorter incubation times.

GUROFF, FANNING AND CHIRIGOS[2] have suggested that the stimulatory effect is occurring extracellularly since they have found that there is a definite stimulation of uptake within 1 min of incubation, supposedly before either amino acid has an appreciable intracellular concentration. Our studies also show significant stimulation

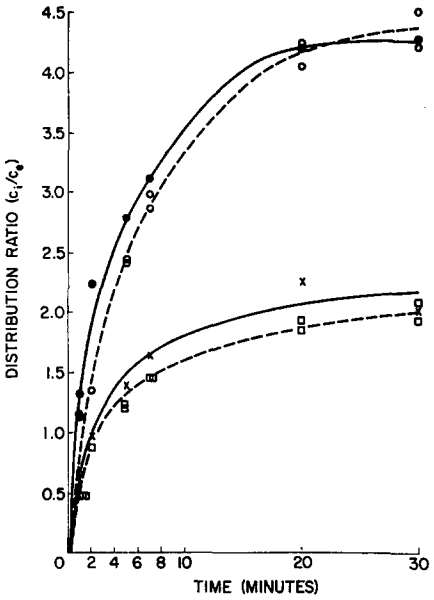

Fig. 4. Time dependence of simultaneously measured distribution ratios for L-phenylalanine and L-tryptophan for each alone at 1 mM and in the presence of the second amino acid also at 1 mM. ●—●, L-phenylalanine when alone at 1 mM; ○— — —○, L-phenylalanine when at 1 mM with 1 mM L-tryptophan also present; ×—×, L-tryptophan when alone at 1 mM; □— — —□, L-tryptophan when at 1 mM with L-phenylalanine also present at 1 mM. All 2-min distribution ratios are not from the same experiment but are averaged values from competition studies.

within 1 min of incubation; however, by 1 min the distribution ratio for the inhibited amino acid is far from negligible (2.0–3.1 in the case of DL-*p*-fluorophenylalanine). GUROFF, FANNING AND CHIRIGOS[2] have also reported that a short preincubation with the inhibited amino acid gives a greater stimulatory effect when the second amino acid is added. This would not only support their hypothesis that some time must be allotted for combination of the inhibited amino acid and its extracellular site, but also the possibility that it has to be accumulated intracellularly to exert its effect. They have suggested an explanation of competitive stimulation based on the postulate that the exchange of one amino acid molecule for another on the carrier is faster than the direct binding of the amino acid to the carrier. Thus one would have to assume that the inhibited amino acid binds to the carrier quite rapidly and that the stimulated amino acid binds slowly but that the exchange reaction between the carrier–amino acid complex and free amino acid is rapid. JACQUEZ[7] has shown that a model of carrier active transport which incorporates these assumptions can indeed predict the phenomenon of competitive stimulation. However, with this explanation one would expect that for each extra molecule of the stimulated amino acid taken up a molecule of the inhibited amino acid would be excluded from the carrier. Thus this model predicts a mole-for-mole replacement. This difficulty might be circumvented if we assume a greater non-exchange efflux rate for the inhibited than for the stimulated amino acid; however, since non-exchange efflux rates are low and are of the same order of magnitude for most amino acids[9] it is unlikely that this is a tenable explanation.

A postulation of an intracellular action of the inhibited amino acid which is consistent with current information is found in the explanation of competitive stimulation offered by OXENDER AND CHRISTENSEN[9]. They suggest that the inhibited amino acid is rapidly accumulated by the non-exchanging 'A-type' carrier for which the stimulated amino acid has lower affinity, and that once inside, the accumulated amino acid drives a counterflow of the stimulated amino acid *via* an exchanging 'L-type' carrier for which both amino acids have considerable affinity. GUROFF, FANNING AND CHIRIGOS[2] have reported obtaining a 50% stimulation of L-tryptophan uptake by L-phenylalanine. Since both phenylalanine and tryptophan are presumably taken up primarily by the L system this finding has remained at variance with the suggestion of OXENDER AND CHRISTENSEN. However, we have found no significant stimulation of the uptake of L-tryptophan in the presence of L-phenylalanine (Fig. 4).

It should be observed that any explanation of competitive stimulation which involves two carriers and exchange diffusion of the intracellular stimulator for extracellular stimulated amino acid would not necessarily imply that the decrease in uptake of the inhibited amino acid must be equal to the increase in stimulated amino acid uptake. Presumably the exchange *via* the 'L-type' carrier would be in a 1:1 manner. However, if it is also assumed that the stimulated amino acid normally has a portion of its uptake mediated by the 'A-type' carrier but that competition by the extracellular inhibited amino acid displaces stimulated amino acid from this carrier, then the increased uptake of stimulated amino acid would be less than the decreased uptake of the inhibited amino acid.

A further test of the OXENDER AND CHRISTENSEN proposal[9] is suggested by the recent investigations of INUI AND CHRISTENSEN[10], who have presented strong evidence for the existence of the two transport systems (A and L) by showing that the flux which they attribute to the A system is dependent on extracellular Na^+ whereas that which they attribute to the L system is independent of extracellular Na^+. If true, this implies that the phenomenon of competitive stimulation should disappear if Na^+ is omitted from the extracellular medium. The results of experiments to test this are reported in the following paper[11].

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