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Tryptophan transport through transport system T in the human erythrocyte, the Ehrlich cell and the rat intestine

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We studied the transport of tryptophan through transport system T in the human red cell, the Ehrlich ascites-tumour cell and in everted sacs of rat intestine. In red cells we confirmed earlier results on Na^+-independence and aromatic amino acid specificity (Rosenberg, R., Young, J.D. and Ellory, J.C. (1980) Biochim. Biophys. Acta 598, 375–384). In addition we observed that N-methylation or N-acetylation did not reduce the affinity of the substrates for system T, hydroxylation could increase or decrease substrate affinity, and system T was insensitive to pH changes in the medium. These results characterize reactive differences between system T and other known amino acid transport systems. We also found that D-isomers were about 1/3 as effective as L-isomers to inhibit L-tryptophan uptake. D-Tryptophan competitively inhibited L-tryptophan uptake, but was not taken up by system T. L-Tryptophan produced trans-stimulation of the uptake (influx) and trans-inhibition of the release (efflux) of L-[^{3}H]tryptophan; D-tryptophan produced trans-inhibition of the efflux but did not affect significantly the uptake. These results show that in red cells the transport properties of transport system T are asymmetric. Transport system T seems to be absent in the other two preparations studied, the Ehrlich ascites-tumour cell and the rat intestine.

Introduction

At least three different transport systems have been described for the transfer of neutral amino acids through the erythrocyte membrane [1,2]. Na^+-dependent systems include a pathway specific for glycine and sarcosine [2,3] first described in the pigeon erythrocyte [4,5] and later in other cell types [6], and another one for alanine, serine, threonine and cysteine [2,7] with higher activity in reticulocytes [8] and similar to the ASC system found in other cells [5,9]. The Na^+-independent pathway [2,10,11] is similar to system L found in many other cell types [12,13].

The transport of tryptophan by cells other than the erythrocytes seems to be divided between system L and the Na^+-dependent systems [12,14–17]. In human red cells, however, the affinity of system L for tryptophan is very poor [2,10,18–20] and the uptake observed was not inhibited by glycine, alanine or serine and did not exhibit Na^+-dependent components [18–20].

Recently, a new pathway for the uptake of tryptophan has been described in the human erythrocyte. This pathway, called system T, is Na^+-independent but distinct from system L. Its substrate specificity is apparently limited to L-tryptophan, L-tyrosine and L-phenylalanine among the natural amino acids. D-Tryptophan was also

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Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; BCH, 2-aminonorbornane 2-carboxylic acid or 2-aminobicyclo(2.2.1)heptane 2-carboxylic acid.

proposed to be a substrate for system T on the basis of its ability to inhibit L-tryptophan uptake [18–20]. Although the possible relevance of system T to explain the differences of intestinal transport found between Hartnup's disease and blue dipper syndrome has been pointed out [20], the presence of system T in cells other than the erythrocytes has not been investigated yet.

In the present paper system T of the human erythrocyte has been further characterized with regard to the structural basis for affinity, stereospecificity, pH-dependence and exchange properties. In addition, the presence of system T in two other cell types, the Ehrlich ascites-tumour cell and the rat intestinal cell was investigated.

Materials and Methods

Experiments with erythrocytes

Fresly drawn human blood was collected using heparin and centrifuged at 3000 rev./min for 5 min. After removal of the plasma and buffy coat the cells were washed three times with 0.15 M KCl and one with incubation medium of the following composition (mM): KCl, 120; $CaCl_2$ 0.5; $MgSO_4$, 1.2; mannitol, 50; K-Hepes buffer, 10 (pH 7.4); the cells were then incubated during 60 min at 37°C, sedimented by centrifugation and resuspended at 50% haematocrit. This cell suspension was used for the transport experiments.

Uptake experiments were started by mixing 1 volume of the 50% cell suspension with 4 volumes of prewarmed incubation medium containing the amino acids and further additions as specified for each experiment. The osmolarity of the medium was kept constant by modifying the mannitol concentrations. The incubation period was terminated either by ice-cold dilution and centrifugation followed by two washes with ice-cold 0.15 M KCl or by centrifugation through di-*n*-butyl phthalate oil [18,21]. Both methods gave similar results. The cells were lysed with distilled water. An aliquot was taken for haemoglobin determination, and the remainder deproteinized with trichloroacetic acid (6%, final concentration). An aliquot of the original cell suspension was also deproteinized with trichloroacetic acid and used to calculate the specific activity.

Loading of the cells with L- or D-tryptophan was performed by suspending the cells at 10% haematocrit and incubating this suspension at 37°C for 1–3 h in the presence of the amino acid. After the incubation the cells were washed three times with ice-cold medium, resuspended at 50% haematocrit in ice-cold medium and used inmediately as described before.

For the exit experiments the cells were first loaded with L-[^{3}H]tryptophan or L-[^{3}H]tyrosine by incubating a 50% cell suspension for 1–3 h at 37°C. After 3 washes with ice-cold medium, the cells were resuspended again at 50% haematocrit. An aliquot was taken for determination of the initial radioactivity and of haemoglobin and the experiment was started inmediately by mixing an aliquot of the 50% cell suspension with excess prewarmed medium (final haematocrit 1–5%) containing different amino acids as specified for each experiment. The incubation period was terminated by either ice-cold dilution and centrifugation or sedimentation through di-*n*-butyl phthalate [21] and the cell pellets processed as described before.

Radioactivity in the cell extracts was determined by liquid scintillation counting [22]. In some experiments tryptophan was determined fluorimetrically [23], the method giving the same fluorescence for both the L- and D-isomers and the *N*-methyl derivative. Haemoglobin was estimated from the absorbance of the lysates at 540 nm. All the data were normalized by reference to the haemoglobin content and are expressed as μmol/l intracellular water assuming that 1 g of haemoglobin is equivalent to 1.32 ml of intracellular water.

Experiments with Ehrlich cells

A tetraploid strain of Ehrlich ascites-tumour cells, kindly provided by Dr. Y. Valladares, Instituto Nacional de Oncología, Madrid, Spain, was used. Methods for preparation, collection and handling the cells were as described by Inui and Christensen [24]. The incubation medium had the following composition (mM): choline chloride, 121; KCl, 5; $CaCl_2$, 0.5; $MgSO_4$, 1.2; mannitol, 20; choline-Hepes buffer (prepared by mixing choline bicarbonate and Hepes, free acid), 10 (pH, 7.4). The incubation period was started by mixing 1 volume of 50% cell suspension with 10 volumes of prewarmed medium containing L-[^{3}H]-

tryptophan and/or [^{14}C]BCH and other amino acids as specified in the results section. After 1 min at 37°C the incubation period was terminated by ice-cold dilution and centrifugation. The supernatant was then removed and the cell pellet weighted and extracted with 6% trichloroacetic acid. Other technical details were as described previously [25]. Radioactivity in the extracts was determined by liquid scintillation counting. All the data were normalized by reference to the fresh cell weight and expressed as μmol/l intracellular water by assuming that 1 g of pelleted cells is equivalent to 0.53 ml of intracellular water and allowing a correction for the extracellular space in the cell pellet of 32% of the fresh weight [26].

Experiments with rat intestine

Intestinal transport was studied in vitro using everted sacs [27] from male wistar rats (about 175 g body weight). Four 4-cm segments were obtained from the most distal portion of the small intestine from each animal and pooled for the different conditions. The incubation medium contained (mM): NaCl, 140; KCl, 5; $CaCl_2$, 1; glucose, 10; sodium phosphate buffer, 10 (pH 7.4). Each sac was filled with 0.4 ml of medium (serosal fluid) and incubated under O_2 atmosphere in 5 ml of medium containing 0.1 mM of each L-[^{3}H]tryptophan and L-[^{14}C]-leucine and other additions as specified with the results (mucosal fluid). After 40 min at 37°C the incubation was terminated by dipping briefly the sacs into a beaker containing ice-cold medium. Each sac was weighed, the content (serosal fluid) collected and weighed again. Then the tissue was extracted with 70% ethanol during 48 h at 4°C [28]. The ^{3}H and ^{14}C contents in the serosal and mucosal fluids and in the ethanol extracts were determined by differential liquid scintillation counting. The data were then expressed as transepithelial transport and tissue accumulation, referred in both cases to the fresh tissue weight.

Chemicals

L-[G-^{3}H]Tryptophan (spec. act. 3.9 Ci/mol), L-[3,5-^{3}H]tyrosine (spec. act., 51 Ci/mol) and L-[U-^{14}C]leucine (spec. act., 334 Ci/mol) were obtained from Amersham International p.l.c.; aminobicyclo(2.2.1)heptane 2-[^{14}C]carboxylic acid (DL-b-2-[*carboxyl*-^{14}C]) (spec. act. 4.8 Ci/mol) was obtained from New England Nuclear. All the unlabelled amino acids and derivatives used were obtained from Sigma London except b-(±)-BCH, which was a generous gift from Dr. H.N. Christensen, The University of Michigan, Ann Arbor, MI, U.S.A. Di-*n*-butyl phthalate was obtained from BDH Chemicals. All other products used were obtained either from Sigma London or from E. Merck, Darmstadt.

Results

In preliminary experiments the uptake of L-tryptophan by the human erythrocyte was found to be linear with time during at least the first 10 min of incubation at 37°C at external concentrations ranging from 0.1 to 30 mM. An incubation period of 3 min was used to estimate the initial rate of uptake in routine experiments. When the influx was studied at different external concentrations of L-tryptophan (0.1 to 30 mM) it could be resolved into two components, a saturable one ($V = 81 \pm 8$ μmol/l cell water per min; $K_m = 1.7 \pm 0.3$ mM) and a nonsaturable one ($k = 0.012 \pm 0.002$ min^{-1}, mean ±S.E. of seven experiments). The values were the same in Na^+-containing medium (KCl replaced by NaCl in the standard incubation medium described in Methods) as in the Na^+-free medium. The addition of excess L-leucine (30–50 mM) to the incubation medium inhibited 75–80% of the uptake through the nonsaturable component, the saturable uptake being little affected. The norbornane amino acid (BCH), a specific substrate for transport system L in the Ehrlich cell [17], had the same effect. These results are consistent with previous reports [18–20]. The influx of L-tryptophan was not significantly modified by changes of the pH of the incubation medium between 6.1 and 7.8 (data not shown).

The effects of other amino acids and amino acid derivatives on the influx of L-tryptophan are shown in Table I. The substrate concentration used (80 μM) secured that 80–90% of the uptake took place through the saturable component (system T) in the control condition. Among the natural amino acids tested only the aromatic ones produced significant inhibition. The apparent affinities were: tryptophan > tyrosine > phenyl-

TABLE I

EFFECTS OF SEVERAL AMINO ACIDS AND ANALOGS ON THE 3-min UPTAKE OF L-[^{3}H]TRYPTOPHAN (80 μM) BY THE HUMAN ERYTHROCYTE

Each value is the mean ±S.E. of three or four individual values. K_i values, determined in independent experiments, were about (mM): (a), 2; (b), 6.5; (c), 7. Leucine, histidine and valine, tested at 10 mM did not have significant effect. The abbreviations L-, D- and (DL)-define the stereoisomeric series used.

Inhibitor	mM	Per cent inhibition		
		L-	D-	(DL)-
Tryptophan	2	61±3	49±3	–
5-Hydroxytryptophan	2	20±4	–	–
Phenylalanine	2	28±1	16±6	–
Tyrosine [a]	2	42±2	16±6	–
DOPA	2	20±5	7±7	–
N-Methyltryptophan	2	58±2	–	–
N-Acetyltryptophan	2	–	–	28±2
N-Acetyltyrosine	2	25±6	–	–
Tryptophan	10	77±3	71±2	–
Phenylalanine [b]	10	55±3	29±2	–
N-Acetylphenylalanine [c]	10	70±3	–	–
α-Methyltryptophan	20	–	–	83±1
α-Methylphenylalanine	20	–	–	62±3

alanine > DOPA ≥ 5-hydroxytryptophan. Alpha-methylation, N-methylation or N-acetylation did not modify substantially the affinity for system T.

The concentration-dependence of the influx of *N*-methyl-L-tryptophan (L-abrine) is shown in Fig. 1. As with L-tryptophan, the influx of L-abrine was fully Na^+-independent and could be resolved into a saturable component ($V = 22$ μmol/l cell water per min; $K_m = 0.55$ mM) and a non-saturable one ($k = 0.003$ min^{-1}). These results are consistent with the effects of L-abrine as an inhibitor of the uptake of L-tryptophan (Table I). The rate of uptake of L-abrine at 1 mM external concentration was about half that of L-tryptophan when measured in the same batch of cells. The leucine-sensitive component was still significant although smaller (about 1/3) than that of L-tryptophan (quadruplicates in two different experiments; results not shown). The competitive nature of the inhibition of the uptake of L-tryptophan by N-substituted derivatives is shown in Fig. 2 for *N*-acetylphenylalanine.

The D-isomers showed significant affinities for system T although smaller than that of the L-forms, as judged from the competition experiments (Table I). The relative affinities of L- and D-tryptophan were studied in some detail in the experiment shown in Fig. 3. Both isomers inhibited the same component of the uptake (about 80% of the total) the L-form being 2–3 times more potent than the D-form. The inhibition by D-tryptophan followed a competitive pattern (Fig. 2).

When the influx of D-tryptophan was compared with that of the L-isomer it was found to be much slower, even under conditions in which both iso-

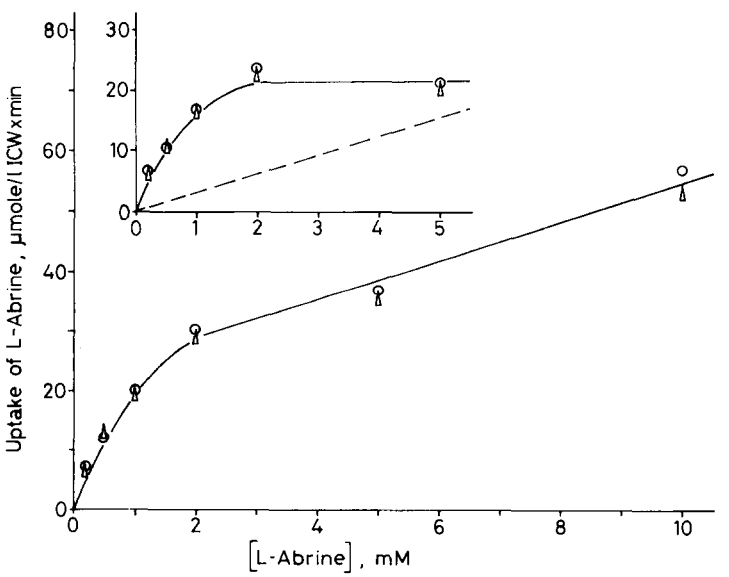

Fig. 1. Concentration-dependence of the uptake of L-abrine by human red cells. The uptake was studied in Na^+-containing (circles) or Na^+-free (triangles) medium for 3 min at 37°C. Abrine was determined fluorimetrically. The inset shows the same data resolved into a saturable component (continous line, $V = 22$ μmol/l intracellular water per min; $K_m = 0.55$ mM) and a non-saturable component (dashed line, $k = 0.003$ min^{-1}).

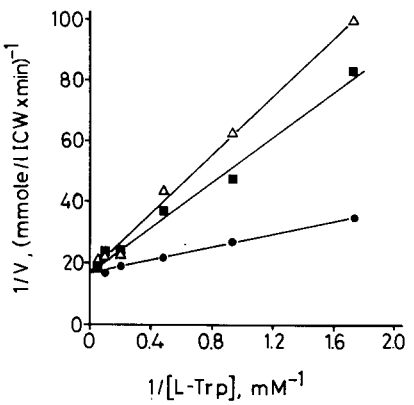

Fig. 2. Double-reciprocal plot of the uptake of L-tryptophan alone (●) or in the presence of 6 mM D-tryptophan (△) or 10 mM *N*-acetyl-L-phenylalanine (■). The uptake was studied during 3 min at 37°C. The non-saturable component has been subtracted. Lines were adjusted by the minimum-squares method. The values of r were 0.992 to 0.997. The values of K_i estimated from this data were (mM): D-tryptophan, 1.7; *N*-acetylphenylalanine, 4.0.

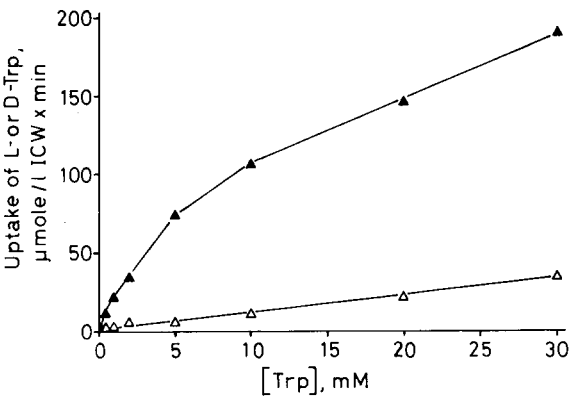

Fig. 4. Concentration-dependence of the uptake of L- (▲) or D-tryptophan (△) by human erythrocytes. The uptake was studied during 3 min at 37°C. Tryptophan was determined fluorimetrically. The values estimated for kinetic parameters from the data are: D-tryptophan, $k = 1.1 \cdot 10^{-3}$ min^{-1}; L-tryptophan, $k = 4.2 \cdot 10^{-3}$ min^{-1}, $V = 60$ µmol/l intracellular water per min, $K_m = 2.0$ mM.

mers produced about the same inhibition of the uptake of tracer L-[^{3}H]tryptophan. For instance, whereas 5 mM D-tryptophan was 90% as effective as the L-isomer to inhibit the uptake of the tracer, its initial rate of uptake was 13-times slower. Fig. 4 shows the concentration-dependence of the influx of both isomers measured in the same batch of cells. The uptake of the D-isomer was much slower and did not show saturation kinetics. These results show that even though D-tryptophan competes

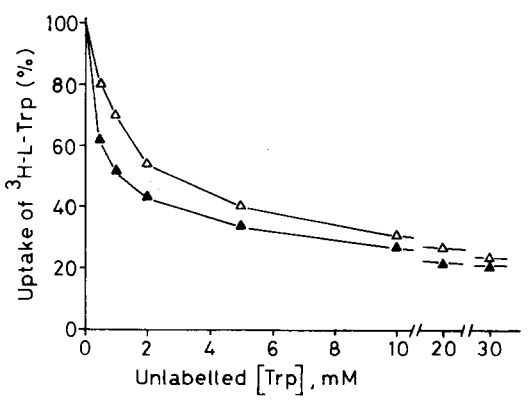

Fig. 3. Inhibition of the uptake of L-[^{3}H]tryptophan (80 µM) by unlabelled L- (▲) or D-tryptophan (△). The uptake was studied during 3 min at 37°C. Half-maximal inhibition was obtained at 0.7 and 1.7 mM, respectively.

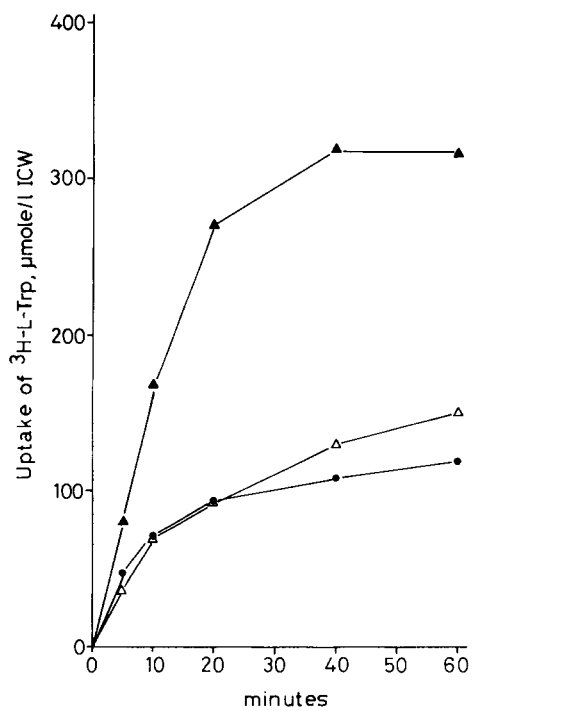

Fig. 5. Uptake of L-[^{3}H]tryptophan (100 µM) by control, unloaded cells (●), and cells loaded with either L-tryptophan (▲) or D-tryptophan (△). The intracellular levels of unlabelled tryptophan (determined fluorimetrically) were 25.2 (▲) and 27.3 mM (△). The initial relative rates for the uptake were (min^{-1}): 0.07 (△), 0.09 (●) and 0.16 (▲).

with the L-isomer for system T it cannot be translocated by this transport system.

The exchange properties of system T were investigated by studying the trans-effects of L- or D-tryptophan on the fluxes of L-[^{3}H]tryptophan. Fig. 5 shows the effects of preloading the cells with either L- or D-tryptophan on the uptake of L-[^{3}H]tryptophan. In this particular experiment the internal concentration of tryptophan was about 25 mM in both cases, a concentration that should saturate the efflux through system T (Ref. 21, see also Fig. 6 below). Note that while D-tryptophan had little effect, intracellular L-tryptophan accelerated the influx of L-[^{3}H]tryptophan 2.3-times. This acceleration could not be explained by the inhibition of the efflux of L-[^{3}H]tryptophan by the internal unlabelled L-tryptophan since the loss of radioactivity during the first 5 min should be very small in any case (see for example Fig. 6). On the other hand, if that were the mechanism, the same stimulation of the uptake should have been observed with D-tryptophan which should be equally able to inhibit the efflux of L-[^{3}H]tryptophan. We must conclude that the stimulation of the uptake of L-[^{3}H]tryptophan by internal L-tryptophan is a true trans-effect (accelerative exchange diffusion in the Stein's nomenclature [29]). In four other similar experiments with different batches of cells loaded with L-tryptophan (14–18.7 mmol/l intracellular water) a trans-stimulation of the uptake of L-[^{3}H]tryptophan was always observed (range: 1.7- to 4.6-times the control value), while loading with D-tryptophan never had significant effect. The presence of excess (50 mM) leucine in the extracellular medium did not modify the above results (three experiments, results not shown).

The effects of extracellular L- or D-tryptophan on the exit of L-[^{3}H]-tryptophan from cells loaded previously at three different levels are shown in Fig. 6. The addition of 10 mM L- or D-tryptophan to the incubation medium decreased the rate of loss of the preloaded L-[^{3}H]tryptophan. The effect was stronger for the D-isomer, which increased the time for half-loss ($T_{1/2}$) 5–8-times (depending on the level of preloading), while external L-tryptophan increased $T_{1/2}$ only 2–3-times. This inhibition of the exit cannot be explained by competition between unlabelled tryptophan and L-[^{3}H]tryptophan at the internal side of the membrane. In the cells loaded with the higher L-[^{3}H]tryptophan concentration, the uptake of unlabelled L-tryptophan could not account for more than 10–15% of the total tryptophan content of the cells by the end of the first 5 min of incubation. On the other hand, D-tryptophan, which is taken up much more slowly, produced stronger trans-inhibition of the efflux. We must conclude that both isomers are able to produce a true trans-inhibition of the efflux of L-tryptophan with a decrease of the apparent V_m for the efflux. The efflux of L-[^{3}H]tryptophan was also inhibited by external 20 mM L-phenylalanine (33%) or 2 mM L-tyrosine (50%).

Rosenberg [21], using a different methodological procedure, showed that external L-tryptophan does not modify the efflux of L-tryptophan measured at 25°C. In order to clear up this apparent

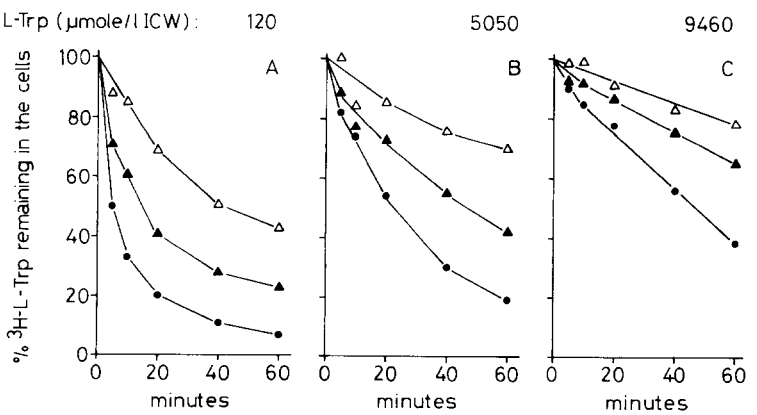

Fig. 6. Efflux of L-[^{3}H]tryptophan from human erythrocytes at 37°C. The cells were first loaded to the levels indicated on top of each panel. The external medium contained no amino acid (●) or 10 mM of either L- (▲) or D-tryptophan (△). The incubation period was terminated by ice-cold dilution. The times for half-loss (min) estimated from the figure for control, L-tryptophan and D-tryptophan are, respectively: (A) 5, 15 and 42; (B) 24, 47 and 122; (C) 47, 90 and approx. 180.

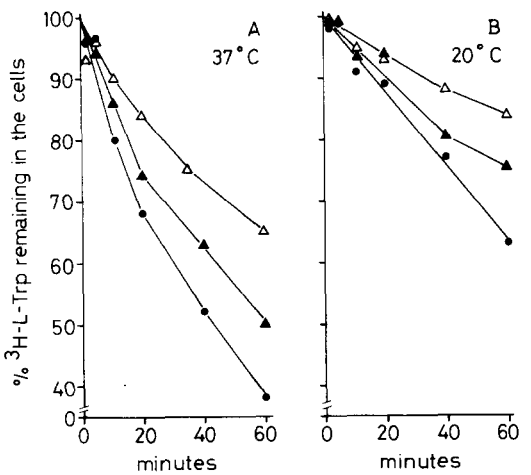

Fig. 7. Efflux of L-[^{3}H]tryptophan from human erythrocytes at 37° and 20°C. Cells were first loaded by incubation with 15 mM L-[^{3}H]tryptophan during 4 h at 37°C. Then the cells were washed and suspended in medium containing no amino acids (●) or 10 mM of either L- (▲) or D-tryptophan (△). The incubation was terminated by centrifugation through di-*n*-butyl phthalate. The initial rates of loss (μmol/l intracellular water per min) for control, L- and D-tryptophan are, respectively: (A) 180, 130 and 90; (B) 60, 48 and 30.

contradiction with our results, experiments were performed under the conditions described by Rosenberg [21]. The results are shown in Fig. 7. A trans-inhibition of the exit of L-[^{3}H]-tryptophan by external L- or D-tryptophan was again obtained although it was somewhat smaller than that shown in Fig. 6. Even from the data reported by Rosenberg [21] in Table I it can be calculated that 13–17% trans-inhibition of the efflux of L-tryptophan should be expected for an internal concentration of the amino acid of 10–20 mM by adding a similar concentration of L-tryptophan to the extracellular medium. This value is not far from the approx. 19% trans-inhibition observed in the right pannel of Fig. 7 in the experiment performed reproducing his conditions.

The trans-effects of L- and D-tryptophan on the transport of L-tyrosine, another substrate of system T (Ref. 20, Table I), were similar to those described for L-tryptophan transport above. Loading the cells with L-tryptophan (11.5 mmol/l cell water) accelerated the influx of L-tyrosine (20 μM) 3.5-times. On the other hand, the exit of L-tyrosine (about 50 μmol/l cell water) was affected by the presence of other amino acids (2 mM) in the incubation medium. D-Tryptophan inhibited the exit ($T_{1/2}$ = 24 min vs. 10 min in the control with no amino acids in the incubation medium), and so did L-tryptophan although somewhat more weakly ($T_{1/2}$ = 17 min). L- and D-tyrosine had a much smaller inhibitory effect ($T_{1/2}$ = 13.5 and 12.5 min, respectively).

The possible existence of transport system T in the Ehrlich cell was investigated by studying the effects of different amino acids on the uptake of L-tryptophan. Experiments were performed in Na^+-free medium to eliminate the operation of system A, a pathway absent in the adult erythrocyte but responsible for some of the uptake of tryptophan in the Ehrlich cell [15]. Using 1 mM L-[^{3}H]tryptophan as the substrate, 20 mM of either L-tryptophan, L-leucine or the norbornane amino acid (BCH) produced about the same inhibition of the 1-min uptake. The effect of leucine was examined in more detail using different concentrations of this amino acid (1 to 20 mM) and showed a pattern of homogeneous and fully-competitive inhibition (data not shown). Table II compares the inhibitory effects of several amino acids on the 1-min uptake of L-[^{3}H]tryptophan and [^{14}C]BCH. The correlation of the inhibitory actions of the various amino acids on the uptake of both substrates was good (r = 0.89) giving no indication of the existence of two different transport pathways. In contrast to the findings in erythrocytes, L-tryp-

TABLE II

INHIBITION OF THE UPTAKE OF L-[^{3}H]TRYPTOPHAN AND [^{14}C]BCH (50 μM EACH) BY SEVERAL AMINO ACIDS (200 μM) IN THE EHRLICH CELL

The uptake of L-[^{3}H]tryptophan and [^{14}C]BCH were measured simultaneously in the same cells during 1 min at 37°C. Two different batches of cells were used.

Inhibitor amino acid	Per cent inhibition of the uptake of	
	Tryptophan	BCH
L-Tryptophan	56–61	79–85
BCH	59	80
L-Histidine	52	79
L-Methionine	50	80
L-Valine	38	75
L-Tyrosine	62	73
L-Phenylalanine	69	81
L-Alanine	5	22
L-Leucine	64	79

TABLE III

EFFECTS OF SEVERAL AMINO ACIDS (10 mM) ON THE INTESTINAL TRANSPORT OF L-[^{3}H]TRYPTOPHAN AND L-[^{14}C]LEUCINE (0.1 mM EACH)

Each value is the mean ± S.E. of four data.

Inhibitor amino acid	% Inhibition of transepithelial transport of		% Inhibition of tissue accumulation of	
	Tryptophan	Leucine	Tryptophan	Leucine
L-Leucine	73 ± 2	61 ± 3	67 ± 5	48 ± 7
L-Tryptophan	85 ± 2	73 ± 4	70 ± 2	57 ± 3
N-Acetylphenylalanine	72 ± 4	61 ± 4	35 ± 9	20 ± 10
Transport without inhibitor (μmol/kg fresh tissue per 40 min)	184 ± 21	227 ± 23	153 ± 26	139 ± 10

tophan showed a very high affinity for system L, evidenced in Table II by its ability to inhibit BCH uptake.

Table III shows the results of the experiments designed to search for the presence of transport system T in the rat intestine. The effects of three selected amino acids, L-leucine, L-tryptophan and *N*-acetyl-L-phenylalanine, on the intestinal transport of L-[^{14}C]leucine and L-[^{3}H]tryptophan were studied. The two substrates and the inhibitor were added to the mucosal medium at concentrations of 0.1 and 10 mM, respectively. The effects of all the tested inhibitors on either transepithelial transport or tissue accumulation were similar for both substrates. These results, of course, do not rule out the existence of system T on the basolateral membrane. In order to check the possibility of a basolateral T-transport system, experiments were performed in which L-leucine or L-tryptophan (10 mM) were added to the serosal medium and transepithelial transport and tissue accumulation of mucosal L-[^{3}H]tryptophan and L-[^{14}C]leucine (0.1 mM each) were studied. Again the effects on the transport of both substrates were similar and both inhibitors blocked only slightly (10–20%) the transepithelial transport. Tissue accumulation was not significantly modified (data not shown).

Discussion

Our results support the view that transport system T is a distinct transport system whose properties do not correspond to any of the transport systems for neutral amino acids described previously. 5-Hydroxylation of tryptophan decreases the affinity, *p*-hydroxylation of phenylalanine increases the affinity and *o*-hydroxylation decreases the affinity again. This lack of a systematic trend is in contrast with the effects previously found for other transport systems where hydroxylation either increases or decreases the affinity [9,12]. α-Methylation, N-methylation or N-acetylation had no pronounced effects on the affinity for transport system T. N-substitution is known to abolish reactivity for transport systems L and ASC while preserving it for system A [2,9,30]. Since transport system A is not present in the human erythrocyte [1,2] we initially thought that *N*-methyl-L-tryptophan (L-abrine) would be a good model substrate for system T in this cell. However, L-abrine retained an unexpectely large fraction of leucine-sensitive transport (about 1/3 as much as L-tryptophan).

The sensitivity of the amino acid uptake to changes in the pH of the medium has also been used to discriminate among different transport systems. Transport system A is very sensitive to medium acidification, whereas systems L and ASC are barely sensitive to pH variations [9,12]. Transport system T behaved as the last two since the uptake of L-tryptophan by the human erythrocyte was not significantly modified by changes of the pH of the incubation medium.

Competition experiments showed that D-isomers were only about a third as effective as L-isomers in causing inhibition of L-tryptophan trans-

port through transport system T. This moderate stereospecificity had been found previously for other transport systems [13]. In the case of D-tryptophan, the only one examined in detail, we were surprised to find that, although it behaved as a competitive inhibitor of L-tryptophan transport, it was not taken up by transport system T. In terms of the mobile carrier hypothesis [29] it should be concluded that D-tryptophan is able to form the carrier-substrate complex, but this complex is unable to translocate through the membrane. A similar situation has been found for methionine and other amino acids acting on transport system ASC [9] and for phlorizin and L-fucose acting on sugar transport [31].

The study of the exchange properties of system T did reveal an unexpected asymmetric behaviour. Extracellular L- or D-tryptophan produced a transinhibition of the exit of L-tryptophan. In the case of D-tryptophan, this could be convincingly explained by the stabilization of the D-tryptophan-carrier complex at the external side of the membrane, as expected from its 'non-substrate but inhibitor' behaviour. In contrast, intracellular L-tryptophan produced trans-stimulation of the uptake, while D-tryptophan was without significant effect. The same behaviour was observed using tyrosine as the substrate. In terms of the mobile carrier hypothesis [29] these results suggest that the rate constant for the translocation from the internal to the external side of the membrane of the L-tryptophan-carrier complex is larger than that of the free carrier, while the reverse situation applies for the translocation in the opposite direction.

The uptake of tryptophan by the human erythrocyte through transport system L presented low-affinity characteristics, as reported previously [20]. This is in contrast with the excellent reactivity for system L found in the Ehrlich cell [15]. Although that could reflect the existence of variants of the same transport protein in different tissues, the unexplored possibility that the lipid composition of the membrane can modulate the affinity of the different substrates for a given carrier should also be considered.

The results obtained in the Ehrlich ascites-tumour cell and the rat intestine did not support the existence of transport system T in these preparations. This leaves open the question of the physiological significance of system T.

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