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*A TRANSPORT SYSTEM SERVING FOR MONO- AND DIAMINO ACIDS**

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A recent investigation showed that the mediated entry of neutral amino acids into the Ehrlich cell divides itself between two distinct transport systems of heavily overlapping affinities.[1, 2] A third system, to be referred to as the *lysine-accepting* system, has now been differentiated from the *leucine-preferring* system by similar procedures. It serves especially for the entry of diamino acids, but because it differs from the *leucine-* and the *alanine-preferring* systems in tolerating a positively charged sidechain, it retains a role in the transport of neutral amino acids.

Methods.—Details of propagation and study of the Ehrlich ascites tumor cell have been described.[2, 3] Migrations were studied during 1-5-min intervals at 37° and pH 7.4, in 1–4% cell suspensions in Krebs-Ringer bicarbonate solutions of uniform osmolarity. For experiments below pH 7, a phosphate buffer was substituted. The intervals were terminated by diluting with ice-cold saline, and immediate brief centrifugation.[2] Measurement and calculation of amino acid levels by scintillation counting have been described.[2] L-α,γ-diaminobutyric acid (DAB[4]) and L-α,β-diaminopropionic acid (DAP[4]) were obtained by applying the Hofmann degradation[5] to uniformly labeled L-glutamine and L-asparagine. The α-aminoisobutyric acid-1-C^{14} was also our own preparation. Carboxyl-labeled glycine, L-leucine and L-phenylalanine, and DL-ornithine-2-C^{14} were obtained from Calbiochem, and uniformly labeled L-lysine and L-arginine from Volk Radiochemical Company. The preparations of β-methyl-α,γ-diaminobutyric and α,γ-diaminoheptanoic acids (each presumably containing 4 isomers) were the gift of Dr. Herbert C. Carter, the ϵ-hydroxynorleucine, of Dr. Roger Gaudry.

Results.—Figure 1 shows the time course of the uptake of three diamino acids, and Figure 2 the inhibitory actions of 6 diamino acids on the uptake of 5 basic amino acids. The latter results show a progressive increase in inhibitory action with increase in chain-length in the homologous series, DAP[4], DAB[4], ornithine, and lysine, DAP having no significant action on the uptake of ornithine, arginine, and lysine, and being itself insensitive to the presence of most of the other diamino acids; but it is notably sensitive to the increase of its own concentration. Figure 1 shows, however, that the initial rates of uptake and the total extents of accumulation fall almost in the reverse order, a situation very similar to the one that led to discovery of the dichotomy in the transport of the neutral amino acids.[2] If such a dichotomy proves to be characteristic of other amino acid transport systems, an important indication as to mechanism may be gained.

Figures 3–6 put forward the basis for recognizing that the transport of lysine occurs by a system distinct from the *A* and *L* systems.[2] Figure 3 illustrates the observation that the neutral amino acids act to inhibit the entry of lysine into the Ehrlich cell. This suppression is very extensive, given a sufficient concentration of the inhibitor

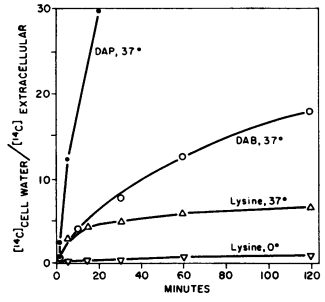

FIG. 1.—Time course of the uptake of 3 diamino acids by Ehrlich cells. Each amino acid was at 1 mM in the suspending medium.

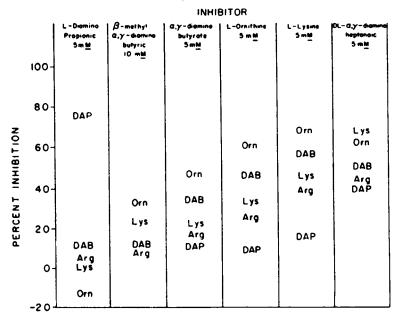

FIG. 2.—Relative effectiveness of 6 diamino acids as inhibitors of the uptake of 5 basic amino acids by the Ehrlich cell. Uptake measured during 1 min and expressed as per cent of that occurring in the absence of any added inhibitor.

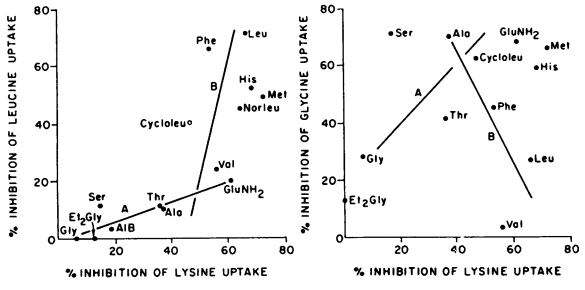

FIG. 3.—Attempts to correlate inhibitory actions of a group of neutral amino acids on the uptake of lysine and leucine, respectively (*left*), and on the uptake of lysine and glycine, respectively (*right*). The uptake of the 3 test amino acids was measured during 1 min from 1 mM solutions. The naturally occurring and optically inactive amino acids were tested as inhibitors at 5 mM levels. DL-Norleucine was tested at a 10 mM level. "Cycloleu" represents 1-aminocyclopentanecarboxylic acid. The points stand for averages for duplicate experiments. The lines *A* and *B* represent efforts to correlate separately the inhibitory actions of amino acids having predominately either *A*- or *L*-system affinity.[2]

(Fig. 4), if a small nonsaturable fixation of C^{14}, complete in 1 min even at 0° (Fig. 1), is deducted. The extent of the inhibitory action on lysine uptake of a group of amino acids does not correlate with their action on the uptake of either leucine or glycine (Fig. 3). The results show that the competition certainly does not occur by the *A* system, nor to any major extent by the *L* system. If we limit consideration to amino acids showing distinctly more *A* system affinity than *L* system affinity in our earlier classification,[2] a better correlation between inhibitory action on lysine and leucine uptake is obtained (line *A*, Fig. 3, *left*), but probably because a substantial portion of the inhibitory action of these amino acids on leucine uptake takes place within the new system, i. e., by their partial saturation of a previously unrecognized portion of leucine uptake that occurs by the distinct mediating system accessible to lysine.

In contrast to the major suppression of lysine transport by a sufficient level of almost any neutral amino acid (as in Fig. 4), lysine is able to eliminate only a clearly defined portion of the uptake of neutral amino acids (Fig. 5). The major portion of the entry of these amino acids remains fully sensitive to phenylalanine on one hand, or to AIB on the other, in the presence of even 50 mM lysine. Hence,

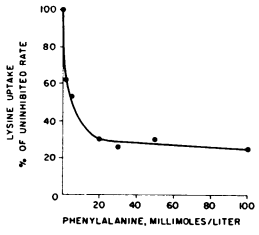

FIG. 4.—Suppression of L-lysine-U-C^{14} uptake by phenylalanine. Lysine concentration, 1 mM. The points represent average values from duplicate experiments. In the presence of 50 mM phenylalanine, the residual uptake of lysine was not significantly affected by the addition of 10 mM DAB or AIB.

entry by the *A* or *L* systems remains essentially intact. This result is the basis for our conclusion that these two transport systems do not tolerate the charged sidechains of cationic amino acids, and that the circumscribed portion of the uptake of neutral amino acids susceptible to inhibition by lysine occurs by a distinct lysine-accepting system. Because AIB and glycine have very little affinity for the *L* system, the results of Figure 5 show the partition of the mediated entry of phenylalanine at a 1-millimolar level between the *L*, *A*, and lysine-accepting systems, the first of these predominating strongly for phenylalanine but only moderately for leucine. Standard kinetic parameters need to be evaluated to provide these proportions to various concentrations. It is important to note that in no case have we observed one amino acid to inhibit the uptake of another, without finding reciprocal effects of an excess of the second on the entry of the first.

The results of Figure 3 tempt one to think of the system accepting lysine at 1 mM as a variant of the *L* system which is able, perhaps because of the absence of a positive-charge barrier, to tolerate a positive charge on the hydrocarbon sidechain. This modification may also account for a greater ability of the system to tolerate uncharged but polar groups on the hydrocarbon sidechain. Whereas the inhibitory action of 10 mM DL-norleucine on the uptake of 1 mM phenylalanine is sharply reduced (by 40 and 56%, respectively) when an ϵ-hydroxy or an ϵ-form amido group is introduced on the norleucine structure, the same structural changes affect only slightly (by 7 and 17%, respectively) the inhibitory action on lysine uptake. Besides their similar responsiveness to increasing hydrocarbon content of the sidechain (Fig. 3) the *L* and the lysine-accepting systems share several attributes, including a low pH sensitivity (Fig. 6), and lively participation in countertransport (Table 1). The sensitivity of lysine uptake to cyanide and 2,4-dinitrophenol (Table 2) is also smaller than that characteristic of uptake by the *A* system.[2]

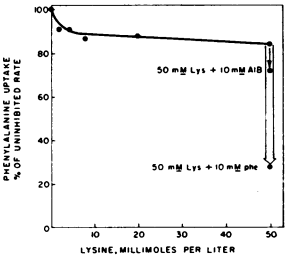

FIG. 5.—Lysine inhibition of the uptake of phenylalanine. The curve shows the extent to which the uptake of phenylalanine from a 1 mM solution is diminished. The arrows show the additional inhibition produced by 10 mM AIB or phenylalanine, as indicated, added to the 50 mM lysine solution. All points represent averages from duplicate experiments. Similar experiments with leucine, glycine, and AIB have shown circumscribed inhibition by lysine, without loss of sensitivity to inhibition by neutral amino acids.

If lysine is brought into the cell by a positive-charge-tolerant variant of the *L* system, we may ask whether this system is accompanied by a counterpart, namely, a similarly tolerant variant of the *A* system. The intense contrast presented between the quickly completed but limited uptake of lysine (Fig. 1) and the much larger, gradual accumulation of DAB at high loads,[6, 7] despite the inferior competitive action of the latter

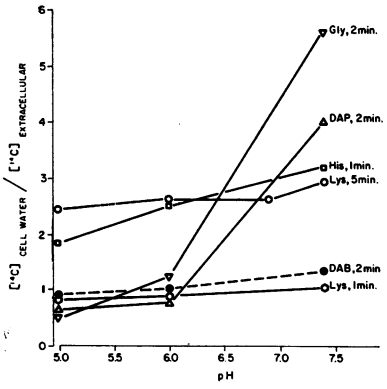

FIG. 6.—pH sensitivity of the entry of several amino acids into Ehrlich cells. Amino acids were at 1 mM in the external medium.

(Fig. 2), is highly reminiscent of the relation between selected neutral amino acids, e.g., leucine and glycine, which led to the discrimination of the *A* and the *L* transport systems.[1, 8]

The unique position of DAP in Figure 2 establishes that its entry occurs largely by a route distinct from that serving for the other diamino acids. Investigation shows, however, that this behavior does not represent the suspected dichotomy among cationic amino acids; the entry of DAP occurs instead mainly by the *A* system, presumably working on the species, $NH_2CH_2CH(N^+H_3)COO^-$, a structural analogue to serine. This conclusion is based on a pH sensitivity of DAP entry much like that of glycine (Fig. 6); by the almost total suppression of its entry by the presence of AIB (Fig. 7), alanine, or glycine; and by the close correlation between inhibitory effects of several amino acids on DAP and alanine (Fig. 8) or glycine uptake. A sharply distorted pH sensitivity would arise if much mediated entry occurred for the species, $N^+H_3CH_2CH(N^+H_3)COO^-$, which predominates only below pH 6.7.[9] Furthermore, very little DAB inhibition of DAP entry is observed, even after the AIB-sensitive transport of DAP has been suppressed (Fig. 7). Accordingly the cationic species *per se* must have a low affinity for transport. The behavior recalls the demonstration that histidine behaves as a neutral amino acid in its uptake[6] (see Fig. 6). A small part of DAP entry is especially β-alanine-sensitive.

Because the lowest member of the series of diamino acids showed so little affinity for transport in its cationic form, our attention turned to the next higher homologue, DAB. A plot of the reciprocal velocity of its mediated uptake shows the intervention at high levels of a second mode of mediated entry (Fig. 9). This behavior contrasts with the close correspondence to a unitarian Michaelis-Menten model,

TABLE 1

LOSS OF LABELED LYSINE AND DAB FROM EHRLICH CELLS INTO AMINO ACID SOLUTIONS

	Initial cellular level, millimoles per kilo water	% Decrease into: 20 mM lysine	20 mM DAB	20 mM other amino acid
Lysine	9.0	23	14	25 (leucine)
DAB	17.1	23	40	19 (phenylalanine)

The time interval was 1 min for the first experiment, 5 min for the second. The depletion of cellular radioactivity into amino acid-free KRB was less than 2%.

TABLE 2

COMPARATIVE INHIBITORY ACTION OF 2,4-DINITROPHENOL AND NaCN ON THE UPTAKE OF DAP AND LYSINE

% inhibition of uptake of	2,4-dinitrophenol 1 mM	NaCN 5 mM
DAP during 2 min	70; 55	68; 58
Lysine during 1 min	40; 39	34; 16

Uptake was measured during 15 min from 1 mM amino acid solutions. A stable vapor pressure of HCN was maintained by passing the 5% CO_2-95% O_2 first through a relatively large volume of 5 mM NaCN in Krebs-Ringer bicarbonate medium at 37°. The results are from 2 successive experiments.

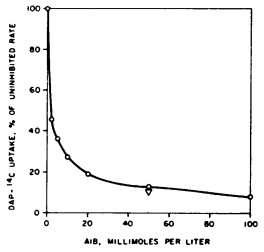

FIG. 7.—Suppression of DAP uptake by α-aminoisobutyric acid. Uptake during 2 min from 1 mM DAP-U-C^{14} is expressed as per cent of that occurring in the absence of any amino acid inhibitor. The triangle shows the effect of adding 10 mM unlabeled DAP plus 50 mM AIB. The points represent averages from duplicate experiments.

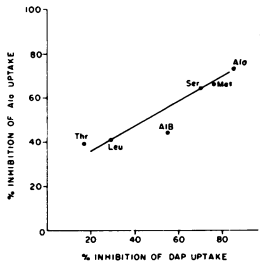

FIG. 8.—Correlation between the inhibitory action of a group of amino acids on the uptake of diaminopropionic acid and of alanine. The uptake of DAP-U-C^{14} was observed during 2 min, that of alanine-1-C^{14} during 1 min, from 1 mM solutions. The inhibitory amino acids were added at 5 mM, all except AIB being the L forms. The points represent averages for duplicate experiments.

of the cellular uptake of many amino acids, including glycine, serine, and AIB, and of the exodus of several others (leucine, valine, and methionine) under the accelerating influence of an extracellular amino acid.[2] Lysine entry also yields a Lineweaver-Burk plot (as in Fig. 9), supporting the action of at least one additional mode of its entry at elevated levels. Whereas the K_m values take the order 0.3 and 0.8 M for the entry of lysine and DAB, respectively, at low levels, the estimated K_m values (crudely, 10–30 mM) describing their entry at high levels take the reverse order. This relationship is extended by Figure 10, which shows that the inhibitory action of a 5-fold excess of lysine on DAB uptake falls sharply, while that of a 5-fold excess of AIB rises, as the external DAB level is raised from 0.3–10 mM.

The appreciable magnitude of this inhibition by AIB is not unexpected, if this second diamino acid transport system tolerates either a charged or an uncharged sidechain (cf. Fig. 3). The converse inhibitory action, rising gradually with DAB concentration to 62 per cent for 100 mM DAB acting on the uptake of 1 mM AIB, might suggest that the second mode of uptake for diamino acids is by the A system *per se*, rather than a positive-charge-tolerating variant. This action, however, and that of the completely cationic γ-guanidino-α-aminobutyric acid, rise so gradually from 10 to 100 mM levels, as to indicate K_m values for the A system probably above 100 mM. Furthermore, if inhibition of the entry of DAB by the presence of AIB occurred mainly in the A system, this inhibitory action should already approach the maximal value at low AIB levels (as in Fig. 7), because AIB shows a K_m of 1–2 mM for the A system.[2] Accordingly the second system for diamino acids indicated by Figures 9 and 10 cannot be the A system.

If two systems for diamino acid transport were to bear a relation to each other exactly like that shown between the two neutral amino acid systems, they might be distinguished by sharp differences in their sensitivity to a lowering of the pH, in their reversibility, and in their participation in countertransport. Although the entry of DAB is appreciably slowed by lowering the pH to 6 or to 5 (Fig. 6), this effect proved not significantly greater at a 20 mM than at a 0.3 mM level. Whether DAB was at higher (Table 1) or lower levels, its exodus was accelerated by the external presence of 20 mM DAB, or of lysine or phenylalanine. Cells brought to

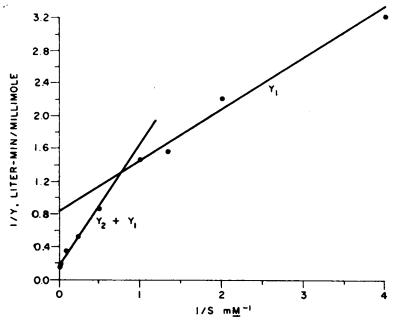

FIG. 9.—Plot of the reciprocal of the velocity of the mediated entry of DAB into Ehrlich cells against the reciprocal of concentration. Y denotes the velocity of mediated entry, after deducting nonsaturable components of uptake.[10] Typical result among 4 similar experiments.

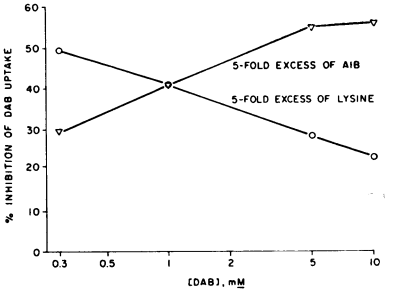

FIG. 10.—Shift in the over-all character of the reactive sites serving for entry of DAB as its concentration is raised from 0.3 to 10 millimolar, as shown by a decreasing inhibitory action of lysine, and an increasing inhibitory action of AIB. The uptake of DAB-U-C^{14} at 37° from Krebs-Ringer bicarbonate medium pH 7.4 was measured during 2 min in the presence and absence of a 5-fold excess of either lysine or AIB. The action of AIB rises from 0.6 of that of lysine to 2.3 times that of lysine.

calculated contents of labeled lysine and DAB, respectively, of 9.5 and 18.5 mM per kilo of water lost only 5 per cent of their C^{14} content in 5 min, whereas at calculated contents of 0.37 and 0.33 mM, the losses were 40 and 28 per cent, respectively.

Discussion.—The foregoing results show that diamino acids are brought into the Ehrlich cell by distinct mediating systems which serve only as an additional, often minor, route for the neutral amino acids. The mutual inhibition between cationic and neutral amino acids thus appears to be limited almost entirely to the new systems, which accept amino acids whether their sidechains carry a positive charge or not. The small extent to which many neutral amino acids ordinarily use these systems, along with the apparent failure of cationic amino acids to react with the transport sites serving characteristically for the neutral amino acids, accounts for the long-standing impression that these two groups enter by entirely different pathways. A prior observation that asparagine and glutamine inhibit lysine uptake by 35–42 per cent (Table 4 in a 1952 article[6]) might well have brought our attention earlier to the asymmetric overlap between the mediations serving principally for cationic and neutral amino acid transport, respectively.

The results suggest that each of the two systems designated L and A exists in a variant form that tolerates positively charged groups. The term, *lysine-accepting system*, has been used here to designate the mediation by which lysine, at external concentrations up to 1 mM, is transported. Presumably transport at that level involves only a minor component of the second diamino acid system, which appears to serve principally at high levels and more extensively for DAB. The tentative terms, L^+ and A^+, for these cation-tolerant transports may assist in explaining the suspected relationships.

The intolerance of the A and L systems to positively charged sidechains apparently applies also to sidechains bearing both a positive and a negative charge,

as shown by the insensitivity of the entry of neutral amino acids to the presence of such amino acids. Their transport may therefore be restricted to the mediation applying to diamino acids. This relationship probably accounts for the wastage into the urine of cystine (and the mixed disulfide of cysteine and homocysteine[11]) when mediated diamino acid transport is defective, as in cystinuria. More detailed resemblances in structure[12] probably need not be invoked. Webber[13] has observed that the infusion of neutral amino acids in some cases increases the urinary excretion of basic amino acids. Comparisons suggest the general extension of the *L*, *A*, and lysine-accepting classification to renal tubular absorption as well as to intestinal absorption and uptake by other cells. Correction for overlapping lysine-accepting affinity promises to bring the description of the *L* system of the Ehrlich cell into closer correspondence with the amino acid transport system seen almost in isolation in the adult human erythrocyte.[14]

The evidence for the existence of a duality in the transport of diamino acids, in several significant aspects similar to that found for the neutral amino acids,[1, 2, 8] strengthens a possibility considered elsewhere[15, 16] that the reactive sites designated *L* and *A* (and the pair here tentatively designated L^+ and A^+) are functionally related as the interconvertible parts of single, complete transport systems. That is, the conversion of the *A* site to the *L* site could serve to unload extensively such amino acids as glycine and alanine from the carrier to produce their uphill transport, although the modified site might still serve for the exchange and a small net migration of many amino acids. If the two are related in this way, however, the situation must be additionally complicated; otherwise one finds it hard to understand why leucine and phenylalanine are not pumped out of the cell while glycine, alanine, and the like, are pumped in. In the case of the diamino acids, the duality has additional interest because no biological advantage to the animal organism is recognized for a traffic in short-chain diamino acids.

Summary.—A *lysine-accepting* transport system in the Ehrlich cell has been differentiated from the previously described *leucine-preferring* system, by the intolerance of the latter to cationic sidechains. The properties of the lysine-accepting system appear to give it importance also for the transport of some of the neutral amino acids. A second mediation operates for the uptake of cationic amino acids, especially short-chain ones, at high levels. The appearance of a dichotomy in diamino acid transport, not unlike that seen for neutral amino acid transport, appears to be significant.

The assistance of Miss Marie Liang and Mrs. B. L. Whitmore is gratefully acknowledged.

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CORRELATION BETWEEN THE SIGNIFICANT LIQUID STRUCTURE THEORY AND THE CELL THEORY

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In 1937 Lennard-Jones and Devonshire[1] (hereafter denoted as L-J & D) proposed the so-called cell theory on a quantitative basis by introducing certain assumptions. The main idea of the assumptions is that an atom in a dense gas is to be regarded as confined for most of its time to a cell whose volume is V/N, where V is the molar volume of the gas and N is the number of atoms, and that its average environment is something like that of an atom in a liquid or a crystal. This picture can only be accepted as a rough first approximation, for it neglects the possibility of the migration or diffusion of atoms from one cell to another. This restriction of ignoring interchange of molecules between cells introduces an error in the entropy of the system, which is usually corrected by arbitrarily adding what is known as the communal entropy factor, e^N. The cell theory has been investigated by several authors[2–10] by introducing holes in the lattice. This procedure eliminated the necessity of introducing the factor e^N. However, none of these procedures shows much improvement over the original cell theory in calculating the compressibility factors, vapor pressures, and various thermodynamic properties, even though theoretically they are an improvement.

In this paper, we introduce Eyring's idea of the "degeneracy factor"[11, 12] into the free volume term in Ono's hole theory based on the earlier L-J & D procedures. In this way, we obtain a new partition function using the Bragg-William approximation and apply it to the equation of state and the thermodynamic properties of rigid sphere molecules. The results obtained are astonishingly good compared to the previous hole theories and to the L-J & D theory. Furthermore, it is shown that the significant liquid structure theory can be related to the L-J & D theory.

Theory.—The cell theory of L-J & D is derived from the classical partition function, Z_N, for an assembly of N monatomic molecules:[13]

$$Z_N = \lambda^{-3N} Q_N. \tag{1}$$

Here, $\lambda^2 = h^2/2\pi mkT$ and Q_N is the associated configurational integral defined as: