Amino Acid Transport Systems in Escherichia coli K12*

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SUMMARY.

The kinetic analysis of amino acid transport in Escherichia coli K12 cells revealed the presence of several distinct transport systems with very little overlap between them. Separate transport systems were found for the following groups of amino acids: leucine, isoleucine, and valine; alanine, glycine, and serine; phenylalanine, tyrosine, and tryptophan; and methionine. The division of the amino acids into the various systems was supported by differences in competitive interactions among the amino acids for transport and by the ability of various amino acids to accelerate the loss of accumulated labeled amino acids from the cell by countertransport. The $K_m$ values for most of the amino acids were in the range of 1 to 10 $\mu$M. All transport systems with the exception of that serving for alanine, glycine, and serine were highly stereospecific. The maximum velocity of transport occurred in a temperature range of 45-50°C and in a broad pH range of 5.5 to 8.0. About two-thirds of the amino acids accumulated at room temperature or above could be lost in the first few seconds when cells were washed at 0°C instead of room temperature. The presence of sodium azide caused the rapid loss of the previously accumulated labeled amino acids.

In 1965, Cohen and Bienenberg (1, 2) described the competitive interactions among leucine, isoleucine, and valine during their accumulation into Escherichia coli. A review of the interactions among the neutral amino acids has been written by Britten and McClure (3). The isolation of mutants of E. coli which are defective in the transport of D-serine, glycine, and L-alanine has indicated that these three amino acids probably migrate by a common transport system (4, 5). More recently, Ames (6), with Salmonella typhimurium, has shown the presence of specific transport systems for histidine and some of the aromatic amino acids in addition to a less specific system capable of transporting all of the aromatic amino acids.

The existence of several transport systems with overlapping affinities for the neutral amino acids has been shown in detail for Ehrlich cells (7-10), erythrocytes (11, 12), and reticulocytes (13), and indicated also for the intestine (14-16) and the renal tubule (17). The present report is an attempt to establish more completely the number and the specificity of the systems for the transport of the neutral amino acids into E. coli cells.

METHODS

Wild type E. coli K12 bacteria were grown on minimal Medium C as described by Davis and Mingioli (18). The medium contained per liter: 7.0 g of K$_2$HPO$_4$, 3.0 g of KH$_2$PO$_4$, 0.5 g of Na$_2$ citrate-3H$_2$O, 1.0 g of (NH$_4$)$_2$SO$_4$, 0.1 g of MgSO$_4$-7H$_2$O, and 2.0 g of glucose (the latter autoclaved separately). The final pH was 7.0.

Cells were harvested in their late exponential growth phase, washed with Medium C at 4°C, and resuspended in this buffer at 4°C to give a final concentration of 10 mg of cells per ml. Chloramphenicol was added to a level of 300 $\mu$g per ml of cell suspension. The cells retained maximal transport capacity for as long as 75 min when kept cold.

Transport was measured by pipetting 0.2 ml of the warmed cell suspension into a test tube containing 1.3 ml of a $^{14}$C-amino acid solution at 37°C in Medium C containing 0.02% glucose. Incubations were stopped after suitable time intervals by pipetting 1.0 ml of the incubation mixture onto the center of a 1-inch diameter Millipore filter, type HA. The entire filtration was completed in 2 to 3 sec. For most of the kinetic studies incubation times of 30 sec were used to approximate initial rate measurements. Unless otherwise mentioned the cells were washed on the filter with 5 ml of warm (25°C) Medium C immediately after filtering. After drying, the filters were glued onto aluminum...
planchets and counted in a gas flow counter. An aliquot of the radioactive incubation medium was plated and counted to determine specific activity. The wet weight of the cells, calculated from the initial bacterial suspension, was 0.58 to 0.89 mg, which corresponds to approximately 10^9 cells. The transport velocities were usually expressed as millimoles of amino acid transported per 30 sec per kg of cells.

The question whether accumulated amino acids were modified by the bacterial cell was investigated by incubating for various time intervals. Cell suspensions were exposed to 14C-amino acid for 2 min and then centrifuged and extracted with 5 volumes of 75% ethanol at 0° for 30 min. An aliquot of the extract was subjected to ascending paper chromatography in n-butyl alcohol-acetic acid-water (4:1:1). The position of the radioactive substances was determined with a paper strip counter (Vanguard model 880). When the L isomers of the amino acids leucine, isoleucine, phenylalanine, and valine were examined, one radioactive spot corresponding to the test amino acid could account for 96, 96, 98, and 89%, respectively, of the total radioactivity. After 2-min contact of glycine, alanine, and methionine with the cells less than 50% of the radioactivity was recovered in the spot representing the original amino acid, indicating rather extensive metabolic activity of E. coli K12 toward these amino acids. When the incubation time was reduced to 15 sec, the recovery of each of the latter group of labeled amino acids in the unaltered form fell in the range of 75 to 80%.

RESULTS

Time Course of Amino Acid Transport—The time course of the uptake of five different labeled amino acids is shown in Fig. 1. The external concentration of each amino acid is listed in the legend of the figure. The incubation fluid included chloramphenicol to minimize incorporation of the labeled amino acids into protein. The experiments were conducted as described under “Methods” except that the incubation time was varied. The steady state for most of these amino acids is reached between 1 and 2 min. With the techniques used in this study it is difficult to use incubation times of less than 10 to 15 sec, but the results obtained indicated that a 30-sec incubation time approximates the initial rate of entry.

As presented under “Methods” the amino acids leucine, isoleucine, phenylalanine, and valine were not appreciably altered during the incubation, but over 50% of the labeled glycine and methionine appeared to be either altered or incorporated into protein. The continued uptake of label shown for glycine and methionine in Fig. 1 was probably due to rapid metabolism of these amino acids in the cell.

Kinetics of Neutral Amino Acid Entry—The dependence of the initial rates of entry on the external concentration for several amino acids is shown in Fig. 2. The curves obtained for isoleucine, glycine, and phenylalanine did not show an appreciable slope from 20 to 200 μM; however, the results for methionine suggest that some heterogeneity in the mode of uptake may exist. We show that each amino acid is a substrate for a distinct transport system, described below. The reciprocal plots of the initial rate data were made as shown for phenylalanine in Fig. 3, and the kinetic constants describing the entry were obtained. A list of these kinetic constants is presented in Table I. The entry of valine could not be described by a single set of constants since the reciprocal plot produced a biphasic curve. This curve was analyzed provisionally to give the two K_m and V_max values shown.
Kinetic constants for neutral amino acids
Labeled amino acids were tested under the experimental conditions described under "Methods." L-Valine gave a biphasic reciprocal plot for which the two $K_m$ values shown were obtained. The numbers in the parentheses indicate the number of experiments used to establish the range of values listed.

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (μmol/s/30sec/kg, wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Alanine</td>
<td>9.2 ± 1.7</td>
<td>1.17</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.8 ± 1.0</td>
<td>1.08</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>1.22 ± 0.13</td>
<td>0.96</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>1.07 ± 0.18</td>
<td>1.58</td>
</tr>
<tr>
<td>L-Valine</td>
<td>8.0 ± 5.7</td>
<td>1.41</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>0.70 ± 0.10</td>
<td>0.90</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>2.27 ± 0.39</td>
<td>0.39</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>0.72 ± 0.17</td>
<td>0.75</td>
</tr>
<tr>
<td>D-Alanine</td>
<td>0.9 (1)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Fig. 4. Inhibition of leucine uptake. Uptake of L-leucine (5 to 10 μM) was measured as described under "Methods" in the presence of varying concentrations of the inhibitors indicated above.

For valine in the table. No such discrepancies were noted for leucine and isoleucine which appear to share the same transport system.

Kinetics of Amino Acid Inhibition—If two amino acids share a common transport system, the concentration of one of the amino acids necessary to give one-half the maximal inhibition of the entry of the other should be equal to the $K_m$ for the entry of the inhibiting amino acid. Furthermore, the inhibiting amino acid would be expected to give, as a limit, complete inhibition of the test amino acid if all routes of entry are shared. This method of examining the specificity of transport can be used to establish the minimal number of transport systems operative in various cells or tissues. Fig. 4 shows the inhibitory action of five different amino acids on leucine uptake. The cells were incubated for 30 sec with labeled leucine at levels of 5 to 10 μM to which varying concentrations of unlabeled inhibitor amino acids were added. Valine and isoleucine gave essentially complete inhibition of leucine uptake, the data for only isoleucine inhibition of leucine uptake being shown in Fig. 4. Reciprocal plots of the initial velocity of amino acid uptake in the presence of increasing levels of an unlabeled inhibiting amino acid were used to evaluate inhibition constants ($K_i$).

The $K_i$ values for several of the D and L isomers of the amino acids are presented in Table II. Most transport systems that have been described for biological tissues show a preference for the L isomers of the amino acids. Extensive stereospecificity was shown for the uptake of the isomers of leucine, phenylalanine, and methionine into the E. coli cells, as can be seen from the $K_i$ values for these isomers listed in Table II. In contrast to these observations the $K_m$ value for the transport of D-alanine, shown in Table I, was indistinguishable from that obtained for the L isomer, although the maximum velocity of entry for the D isomer was somewhat lower. This same apparent lack of stereospecificity was found for the isomers of serine when their inhibitory action was measured against the uptake of L-alanine shown in Table II.

Effect of Wash Temperature on Accumulated Amino Acids and Partial Protection by Sodium and Calcium—Before the Millipore filter came into common usage the accepted procedure for terminating cell incubations carried out at room temperature or higher was to pour the suspension into a large volume of cold buffer. This procedure was later shown by Britten and McClure (3) to cause the loss of some of the accumulated amino acids from E. coli. In the present study we found that within a few seconds nearly 90% of the leucine accumulated at 37° was released when the cells were washed with 10 ml of buffer at 4°. Table III shows the loss of previously accumulated 14C-L-leucine caused by washing the cells on a Millipore filter with 5-ml portions of buffer at decreasing temperatures. The critical temperature associated with the rapid loss of amino acids appeared to lie somewhere between 8.5° and 3°. Cells that had been treated with cold buffer

Table II
Inhibition constants for amino acids
The bacteria were incubated for 30 sec at 37° with labeled amino acid at a concentration equal to that of the $K_m$ (see Table I). Varying concentrations of unlabeled inhibitor amino acids were present during the uptake. From a plot of the reciprocal velocity against the inhibitor concentrations, the inhibition constants were calculated.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Inhibitor</th>
<th>$K_i$ of inhibitor (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Leucine</td>
<td>L-Valine</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>L-Isoleucine</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Cycloleucine*</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>D-Leucine</td>
<td>27</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>L-Leucine</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>L-Valine</td>
<td>4</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>Glycine</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>L-Serine</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>d-Serine</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>D-Alanine</td>
<td>14</td>
</tr>
<tr>
<td>Glycine</td>
<td>L-Alanine</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>AIB*</td>
<td>4200</td>
</tr>
<tr>
<td>L-Phenylnalanine</td>
<td>d-Phenylalanine</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>L-Tyrosine</td>
<td>3</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>L-Tryptophan</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>D-Methionine</td>
<td>600</td>
</tr>
<tr>
<td></td>
<td>L-Ethionine</td>
<td>23</td>
</tr>
</tbody>
</table>

* Cycloleucine, L-aminocyclopentane carboxylic acid.
* AIB, α-aminoisobutyric acid.
TABLE III

Effect of temperature and composition of washing medium on loss of accumulated amino acids

The cells were incubated in Medium C (see "Methods") containing 7 μM 14C-L-leucine for 30 sec at 37°C. After filtration the cells were washed with 5 ml of Medium C at various temperatures in Experiment 1. In Experiment 2 the Na+ buffer was prepared by substituting sodium salts for the potassium salts normally used for Medium C.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Temperature of wash</th>
<th>Uptake (mmoles/30 sec/kg, wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37°C</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>23.4</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>1.58</td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.44</td>
</tr>
<tr>
<td>2 (Na+, buffer)</td>
<td>4</td>
<td>0.85</td>
</tr>
</tbody>
</table>

TABLE IV

Effect of various treatments on loss of amino acids accumulated by Escherichia coli

Cells were suspended in 10 μM 14C-L-leucine-containing medium for 2 min at 37°C and were then filtered on a Millipore filter and washed two times with 5-ml portions of buffer at 37°C. These cells served as the control for comparison with other cells that were washed a third time with 10 ml of buffer containing the additions shown in the table.

<table>
<thead>
<tr>
<th>Additions to final 10-ml wash</th>
<th>Temperature</th>
<th>Radioactivity retained by filtered cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer medium</td>
<td>37°C</td>
<td>100</td>
</tr>
<tr>
<td>Buffer medium</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>0.6 mM glucose</td>
<td>37</td>
<td>100</td>
</tr>
<tr>
<td>0.5 mM leucine</td>
<td>37</td>
<td>53</td>
</tr>
<tr>
<td>30 mM azide</td>
<td>37</td>
<td>70</td>
</tr>
</tbody>
</table>

recovered their full transport capacity after incubation of the cells at 37°C. Table III also shows that increasing the concentration of the sodium ion in the washing buffer produced a sparing or protective effect on the loss of labeled leucine. This protection was maximal at 0.15 M, further additions being without further effect. Calcium ion (10 mM) added to the potassium phosphate buffer produced similar effects. The addition of both sodium and calcium ions did not produce additional protection, showing that the effects of the 2 ions were not additive.

Effect of Unlabeled Amino Acids and Sodium Azide on Loss of Accumulated Amino Acids—Adding either unlabeled leucine or sodium azide to the washing fluid greatly increased the loss of accumulated 14C-leucine (Table IV). Essentially all of the radioactivity could be removed from the cells by washing them four or five times with 10 ml portions of buffer containing 0.5 mM unlabeled leucine. The leucine presumably participates in "accelerative exchange" as described under "Countertransport of Amino Acids."

pH Dependence of Amino Acid Transport—The effect of hydrogen ion concentration on the uptake of several neutral amino acids was examined. The buffer used for incubation and that used for washing were at the same pH. Fig. 5 shows the pH profile for the entry of isoleucine. The entry rates of all of the amino acids tested showed rather broad pH optima between 6.0 and 8.0. The entry of glycine and alanine seemed to be decreased slightly more than that of isoleucine by lowering the pH to 6 or less.

Effect of Temperature on Amino Acid Entry—The initial rate of entry of leucine as a function of temperature is shown in Fig. 6. The temperature optimum appeared to be at 50°C to 55°C. The temperature optima for the other amino acids were very similar to that shown for leucine. The Q10 between 15°C and 25°C for the amino acids ranged from 1.6 to 2.1. Amino acid entry was minimal below 4°C and above 65°C.

Countertransport of Amino Acids—The finding that a solute added to the medium can accelerate the loss of a second previously accumulated solute by counterflow presumably indicates that these two share a common transport system (19). The ability of analogues to participate in countertransport can thus assist in establishing the number and kinds of transport systems present. For these experiments cells were incubated in 0.01 to 0.1 mM labeled amino acids for 1 min and then filtered and washed immediately with 5 ml of buffer at 37°C containing 0.5 mM unlabeled amino acid. The results are expressed as the percentage of the accumulated radioactivity remaining after the first wash (Table V). The control, which is set at 100% had been washed with 5 ml of Medium C at 37°C. The loss of labeled alanine, serine, and glycine was not increased by other...
TABLE V

Countertransport of amino acids in E. coli

The bacteria were held in 0.01 to 0.1 mM labeled amino acid for 1 min at 37° and then filtered. The filtered cells were then immediately washed at 37° with 5 ml of Medium C containing 0.5 mM unlabeled amino acid. The data are expressed as the percentage of the values for cells which had been washed with 5 ml of Medium C at 37° without the addition of unlabeled amino acids.

<table>
<thead>
<tr>
<th>Preloaded amino acid</th>
<th>Amino acid in wash</th>
<th>Accumulated amino acid remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Leucine</td>
<td>L-Leucine</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>L-Valine</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>L-Valine</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>L-Phenylalanine</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>L-Alanine</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>L-Phenylalanine</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>L-Methionine</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>L-Methionine</td>
<td>94</td>
</tr>
<tr>
<td>L-Valine</td>
<td>L-Valine</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>L-Leucine</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>L-Alanine</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>104</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>L-Methionine</td>
<td>70</td>
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<tr>
<td></td>
<td>L-Leucine</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>L-Alanine</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>L-Phenylalanine</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td>L-Norleucine</td>
<td>96</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>L-Phenylalanine</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>L-Tryptophan</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>L-Tyrosine</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>L-Proline</td>
<td>104</td>
</tr>
<tr>
<td></td>
<td>L-Methionine</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>L-Leucine</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>L-Alanine</td>
<td>102</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>L-Alanine</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>L-Alanine</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>L-Serine</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>L-Leucine</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>L-Methionine</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>L-Phenylalanine</td>
<td>102</td>
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<tr>
<td></td>
<td>L-Valine</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>L-Alanine</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>L-Leucine</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>L-Valine</td>
<td>100</td>
</tr>
</tbody>
</table>

a d-Leucine concentration in the wash media was 50 mM.

DISCUSSION

E. coli K12 cells have the ability to synthesize all of the necessary amino acids from inorganic salts and glucose, yet they maintain the capacity to accumulate external amino acids
actively. The results described here show that the transport-
mediating systems for the neutral amino acids in \textit{E. coli} separate
not into a set of systems specific to one amino acid but into
at least four different systems serving for groups of amino acids,
with little overlap in scope between them. The separation into
distinct systems can be observed not only by an analysis of the
inhibitory actions of one amino acid on another, as shown in
Table II, but also by the differences in the extent to which they
participate in countertransport as shown in Table V.

Our results suggest the presence of the following transport
systems: System I serving for alanine, serine, and glycine;
System II for leucine, isoleucine, and valine; System III for
tryptophan, tyrosine, and phenylalanine and; System IV for
methionine. For the amino acids used in these studies no evi-
dence for a specific transport system for any of the individual
amino acids was observed, except perhaps for methionine.
These results are in contrast to the observation by Ames (6)
with \textit{Salmonella typhimurium} in which specific and general
transport systems were found present together.

The \textit{E. coli} K12 cells are able to distinguish between the
apolar side chains of the branched chain amino acids and the
aromatic amino acids, thereby causing the transport of these
amino acids to fall into two different transport systems. This
result contrasts with the finding for the Ehrlich cell and for
other mammalian tissues in which leucine and phenylalanine
have been found to compete for a common transport system (7).
Despite the test of scores of analogues in this laboratory, no
discrimination has been detected in the handling of leucine and
phenylalanine by the Ehrlich cell. In the \textit{E. coli} cell methio-
nine appears to have relatively strong affinity for each of the two
major neutral amino acid transport systems, whereas in \textit{E. coli}
methionine appears to be transported by a system separate
from that for the two wider scope systems.

In general, we found that amino acids which use one of the
transport systems show very little inhibitory action on the entry
of amino acids by another system. A typical example of the
overlap among the transport systems is presented in Fig. 4, which
shows that glycine and phenylalanine produced relatively little
inhibition of leucine entry even at levels 100 times the concen-
tration of leucine.

The initial rates of entry of most of the amino acids into the
bacteria cells are very high and the steady state level of accumu-
alation is reached between 1 and 2 min. For the kinetic studies
it was necessary to use intervals of 15 to 30 sec to approximate
initial rate conditions. The kinetic experiments presented in
Fig. 2 show a concentration dependence for uptake which can be
described in terms of Michaelis-Menten kinetics. At amino acid
concentrations sufficient to saturate their primary transport
systems only negligible contributions were observed from other
modes of entry such as the previously described nonsaturable
transport system observed for mammalian cells (20, 21). When
the external level of an amino acid was increased 500 to 1000
times the \(K_m\) value for its uptake, an additional entry process
with a rate which appeared to be linear with concentration
could be observed. We were not able to estimate this minor
component of entry accurately. The \(K_m\) values represent the
concentrations of external amino acid necessary to produce one-
half saturation of the maximal rate of entry. A comparison of
the observed \(K_m\) values (Table I) with the \(K_m\) values for amino
acid entry into the Ehrlich cells (7) shows that the transport
systems in bacteria can be saturated at levels of amino acids
300 to 1000 times lower than is usually found for the Ehrlich

cell.

In measuring amino acid transport in either bacterial or
mammalian cells, incubation has commonly been terminated by
diluting the suspension or by washing the cells with cold buffer.
In contrast to the behavior of Ehrlich cells (7) we found that
60\% of the amino acid accumulated by the \textit{E. coli} K12 cells
could be lost in the first few seconds when the cells were washed
on Millipore filters with ice-cold buffer instead of a medium at
room temperature. A study of the effect of temperature during
washing showed that loss of accumulated amino acid sharply
increased at temperatures below 8\(^\circ\)C, suggesting an apparent
transition point at this temperature which may be associated
with a morphological change in the plasma membrane. Some
metalllic ions such as sodium and calcium seemed to protect the
cells partially from the loss of amino acids during washing with
cold buffer. Accumulated radioactive leucine was also rapidly
lost by washing the bacterial cells on the filter with either un-
labeled leucine or 30 mM sodium azide solutions even at room
temperature (Table IV). The effect of adding unlabeled leucine
to the wash fluid can possibly be explained by an accelerated
reorientation of the mediator or “carrier” arising whenever it
is complexed with leucine. An increased rate of carrier reorienta-
tion could explain the increased loss of \(^{14}\text{C}\)-leucine observed
when unlabeled leucine is added to the wash fluid. This phe-
nomenon has been described previously (7, 22) and more recently
referred to as “accelerative exchange” by Stein (23).

The ability of one amino acid to produce “accelerative exchange”
or countertransport with a second previously loaded amino acid
(Table V) served as evidence for the number and kinds of trans-
port systems as noted above. Attempts to show increased
entry of the amino acids by incubating the cells with relatively
high levels of unlabeled amino acids prior to the initial rate of
entry measurements were unsuccessful, probably because the
endogenous levels (Table VI) of most of the neutral amino acids
were already high enough to produce maximal rates of entry by
countertransport.

From earlier studies on amino acid transport in \textit{E. coli} Britten
and McClure (3) concluded that the formation of the large
internal concentrations of the amino acids required metabolic
energy, but that this internal “pool” could be maintained for
relatively long periods without an energy source and at 0\(^\circ\)C.
However, these authors used the technique of chilling the cell
susensions to 0\(^\circ\)C, and therefore excluded from consideration
the large fraction of accumulated amino acids lost upon cooling from
25\(^\circ\)C to 0\(^\circ\)C, as described above. If the cells are washed with a
buffer at room temperature so as to retain the accumulated
amino acids, a subsequent chilling of the cells or exposure to 30
mM sodium azide causes a rapid loss of the “internal” pool.
These results suggest that metabolic energy is required to retain
the accumulated amino acids. The mechanism by which
metabolic energy is used to maintain intracellular levels of the
amino acids is not clear, but two possible explanations have been
considered. The energy-dependent pool may be maintained
either by an energy-coupled re-entry of amino acids through an
undescribed transport system with a high affinity such as that
discovered for sugar transport in \textit{E. coli} by Rotman (24), or the
transport carrier may be converted to a low affinity form at the
inner face of the membrane by coupling to cellular energy.
The technique of rapid washing of the cells that have been
deposited on a Millipore filter minimizes possible re-entry of
amino acids that have already escaped from the cell since the extracellular fluid is constantly being changed. Nevertheless, an experimental demonstration that re-entry is negligible seems desirable in each context. Washing with buffer medium at room temperature causes only minimal losses compared to the more rapid loss when chilled buffer is used or sodium azide or unlabeled amino acid is added to the washing fluid. These results seem to support the hypothesis that the membrane carrier may be kept in an unreactive form by expenditure of metabolic energy.

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Amino Acid Transport Systems in *Escherichia coli* K12
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