Putrescine Biosynthesis in *Escherichia coli*

REGULATION THROUGH PATHWAY SELECTION*

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SUMMARY

Previous studies have shown that putrescine can be synthesized by two pathways (Fig. 1) and that the rate of urea production by whole cells of *Escherichia coli* reflects the rate of conversion of arginine to putrescine (Pathway II). In this paper, we make use of the latter observation to assess the relative functioning of the two pathways of putrescine synthesis in vivo. In cells growing in unsupplemented minimal medium, the route from ornithine to putrescine (Pathway I) is preferred. On the other hand, when cells are provided exogenous arginine, Pathway II is utilized almost exclusively. Under both conditions of arginine nutrition, the total rate of putrescine synthesis is identical. The degree of utilization of Pathway I in minimal medium is a strain-specific phenomenon, depending on the level of the arginine biosynthetic enzymes. In strains with low levels of ornithine transcarbamylase, greater than 95% of the cellular putrescine is synthesized via Pathway I. In cells with high levels, the preference for Pathway I is less striking or in the extreme case, nonexistent.

The mechanism for this modulation of putrescine pathway selection by the levels of the enzymes of arginine biosynthesis is related to the intracellular pool sizes of ornithine and arginine. Whereas the intracellular arginine level remains relatively invariant, there is a direct correlation between the intracellular ornithine pool and the proportion of putrescine synthesized via Pathway I. Possible mechanisms for this pathway selection are discussed.

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*Escherichia coli* has the potential to synthesize putrescine via four independent routes (Fig. 1) (2, 3). The enzymes of putrescine synthesis fall into two categories: inducible enzymes, which presumably serve a function in amino acid catabolism, and constitutive enzymes, which are considered to be biosynthetic in function. The inducible ornithine and arginine decarboxylases were described by Gale (4). The inducible arginine decarboxylase has been recently purified and characterized (5, 6). These enzymes required specialized culture conditions for formation in *E. coli* (4, 7). Since inducible amino acid decarboxylases were not present in cells grown in minimal medium, while putrescine was formed under all culture conditions, an apparent inconsistency existed. This dilemma was resolved when Morris and Pardee described the presence of ornithine and arginine decarboxylases in *E. coli* which were distinct from the inducible enzymes (2, 3). These biosynthetic decarboxylases differed from the catabolic enzymes in pH optima, thermal stabilities, cofactor requirements, and conditions for synthesis.

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*Fig. 1. Pathways of putrescine synthesis in *E. coli***

In *E. coli* growing in minimal medium, the inducible decarboxylases are not present and putrescine can be synthesized by two pathways which branch off from the arginine biosynthetic pathway (Fig. 1). This unusual situation leads one to question the necessity for the existence of these two routes to putrescine. It was initially proposed that this formed the basis of a unique mechanism for regulation of a branched biosynthetic pathway (3). When *E. coli* is growing in unsupplemented minimal medium, it would be energetically advantageous to use the ornithine to putrescine route (Pathway I). However, in cells growing in arginine-supplemented medium, the pathway from glutamate to ornithine is feedback-inhibited and repressed. The cells would thus be forced to use the now energetically favored route from exogenously supplied arginine to putrescine (Pathway II).

A test of this hypothesis is possible, utilizing the fact that urea synthesis by *E. coli* is solely a result of putrescine biosynthetic pathway II (8). Since this organism does not degrade urea, the rate of urea synthesis can be used as a measure of the rate of functioning of putrescine Pathway II. By comparing the total rate of putrescine synthesis with the rate of urea synthesis, the
relative flux through the two putrescine biosynthetic pathways may be obtained.

The results recorded in this paper confirm our initial hypothesis that *E. coli*, growing in minimal medium, preferentially uses putrescine biosynthetic Pathway I. In arginine-supplemented medium, Pathway II is almost solely used. This pathway selection seems to be regulated, at least in part, by the intracellular pool level of ornithine.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains**—*E. coli* 3000 and 30SO-arg *^4^* are K-12 strains which require thiamine for growth in minimal medium. In addition, 30SO-arg *^4^* requires arginine by virtue of a block between N-acetylglutamic acid and N-acetylglutamic semialdehyde in the pathway of arginine biosynthesis. The strain of *E. coli* B utilized in these studies, AC2514-1, *^3^* is wild type with respect to arginine biosynthesis, but it requires the amino acids isoleucine, leucine, proline, and valine for growth. The homo-arginine-resistant mutants of AC2514-1 were isolated from bacteria plated on homoarginine-containing medium as described by Peyru and Maas (9). These resistant strains are designated AC2514-1 Harg*^a^*1 and AC2514-1 Harg*^a^*8 and have fully and partially derepressed levels, respectively, of ornithine transcarbamylase (Table III).

**Growth of Bacteria**—Growth was routinely carried out with vigorous shaking at 37° *C* in M63 medium of Cohen and Rickenberg (10). Media were routinely supplemented with thiamine (0.5 μg per ml), trace elements (11), and d-glucose (0.2%). Amino acid supplementations (L isomers) were at a level of 20 μg per ml.

**Polyamine Analysis**—For analysis of polyamines, 200-ml cultures were grown from a cell density of approximately 7 × 10^8^ cells per ml to a final concentration of 7 × 10^8^ cells per ml. Cultures were harvested by centrifugation at 0° *C*, and the cells were washed once in 0.1 M potassium phosphate, pH 7.0 *^5^*. Extraction of polyamines with trichloracetic acid was performed as previously described (3). Analysis of polyamines in the trichloracetic acid supernatant solution was performed by an unpublished method utilizing an ion exchange column of Bio-Rex 70 in conjunction with the Technicon amino acid analyzer. *^6^* Polyamine levels were normalized on the basis of cell protein with bovine serum albumin as a standard (Reference 3, 1 mg of cellular protein is equivalent to 3.0 × 10^8^ cells).

**Measurement of Putrescine Turnover**—Cultures were first grown in 200 ml of M63 medium supplemented with 0.1% d-glucose and radioactive precursor from a density of 7 × 10^7^ cells per ml to a concentration of approximately 7 × 10^8^ cells per ml. The specific activity of radioactive precursors in the various experiments were 0.20 μC per μmole of glucose-U-^14^C, *^4^* 0.10 μC of ornithine-5,^14^C, and 0.13 μC of arginine-5,^14^C per μmole. These cultures were centrifuged and resuspended in 1500 ml of prewarmed, unlabeled medium containing 0.2% d-glucose. At zero time and 1 hour, 400-ml samples were taken, and at 2 and 3 hours 200-ml samples were taken. These samples were centrifuged immediately, and polyamines were extracted as described above.

Analysis of putrescine specific activity was accomplished by splitting the effluent stream from the ion exchange column, with one-half going to the amino acid analyzer and the remainder to a fraction collector for subsequent determination of radioactivity. The samples for determination of radioactivity (2 ml) were placed in deionized and distilled water, containing 100 g of naphthalene and 5 g of 2,5-diphenyloxazole per liter. These samples were counted with an efficiency of approximately 75% in a Beckman CPM 100 scintillation counter.

**Measurement of Urea Production**—Samples were prepared and analyzed for urea as previously described (Assay 1, Reference 8).

**Enzyme Assays**—Ornithine decarboxylase, arginine decarboxylase, and agmatine ureohydrolase were assayed as previously described (3).

For determination of ornithine transcarbamylase activity, cells were disrupted by sonic oscillation in 0.05 M potassium phosphate, pH 7.0, 10^-2^ M EDTA, and 10^-4^ M dithiothreitol. Cell extracts were diluted for assay in 0.05 M Tris-HCl, pH 8.0. The assay mixtures (0.5 ml total volume) contained 0.025 M Tris-HCl, pH 8.0, 3 × 10^-4^ M ornithine, and 5 × 10^-4^ M carbamyl phosphate. Reactions were started by the addition of enzyme and incubated for 30 min at 37°. Color development was by the procedure of Gerhart and Pardee (13) for aspartic transcarbamylase. One unit of ornithine transcarbamylase activity is equivalent to 1 μmole of citrulline produced per min.

**Determination of Intracellular Pools of Ornithine and Arginine**—Cells were grown in 1500-ml cultures from a concentration of 1 × 10^8^ cells per ml to a final density of 1 × 10^9^ cells per ml. Cultures were harvested by centrifugation, and the cells were extracted twice at room temperature with 5 ml of 0.3 M trichloracetic acid. The trichloracetic acid supernatant solutions were extracted 3 times with equal volumes of ether and heated at 60° to remove any residual ether. The precipitate from the trichloracetic acid extraction was analyzed for protein as previously described (3), with bovine serum albumin as a standard.

For analysis of ornithine, the trichloracetic acid extract was lyophilized to dryness. The residue was resuspended in the recommended starting buffer* and analyzed with the Technicon amino acid analyzer.

Thin layer chromatographic analysis of the column effluent from the amino acid analyzer indicated that the arginine peak from cell extracts was contaminated with other ninhydrin-positive material. Consequently, it was necessary to use another method for arginine analysis. The trichloracetic acid extract was passed over a water-equilibrated column (1 × 20 cm) of Sephadex G-25, hydrogen form. The column was washed with 75 ml of 0.1 M HCl. The arginine-containing fractions were then eluted with 0.5 M HCl. The combined fractions were dried by rotary evaporation, resuspended in water, and re-evaporated for removal of residual HCl. This sample was then resuspended in an appropriate volume of 0.05 M glycine-NaOH, pH 9.5, containing 5 × 10^-4^ M MnCl_2_. The pH of this solution

* Obtained from Dr. Werner K. Maas.
* Since these manipulations were performed at 0°, a portion of the intracellular spermidine (never more than one-half, as estimated from direct measurements) was converted to acetyl derivatives (12). The values reported in this paper are for spermidine alone. However, as all cultures were treated identically, the comparisons made in this paper are still valid.
* MORRIS, D. R., KOFFRON, K. L., AND OKESTRIN, C. J., Anal. Biochem., 30, 449 (1969). This procedure separates putrescine, 1,3-diaminopropane, cadaverine, spermidine, the acetyl spermidine derivatives, spermine, and acetyl spermine from each other and from the basic amino acids.
* Glucose-U-^14^C refers to the uniformly labeled compound.
Relative growth increment is defined as the time after transfer to
were then incubated with and without arginase (2 Worthington
curves are
with ornithine-5-14C (A — A) or arginine-5-14C (O-O) as
described under
samples
was readjusted with sodium hydroxide if necessary. Samples
were then incubated with and without arginase (2 Worthington
units per ml) for 30 min at 37°. Urea was then determined as
above.

The yield of arginine during sample preparation was routinely
checked by including a tracer amount of 14C-arginine in the
initial trichloracetic acid extract. In all cases, the recovery was
greater than 95%.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Organism</th>
<th>Polyamines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Putrescine</td>
</tr>
<tr>
<td>Minimal</td>
<td>3000a</td>
<td>39</td>
</tr>
<tr>
<td>+ Arginine</td>
<td>3000a</td>
<td>33</td>
</tr>
<tr>
<td>Minimal</td>
<td>+ Ornithine</td>
<td>30SO-arg 4a</td>
</tr>
<tr>
<td>+ Arginine</td>
<td>30SO-arg 4a</td>
<td>33</td>
</tr>
<tr>
<td>+ Citrulline</td>
<td>30SO-arg 4a</td>
<td>31</td>
</tr>
<tr>
<td>+ Arginine, citrulline</td>
<td>30SO-arg 4a</td>
<td>33</td>
</tr>
<tr>
<td>+ Ornithine, arginine</td>
<td>30SO-arg 4a</td>
<td>31</td>
</tr>
<tr>
<td>+ Ornithine, citrulline</td>
<td>30SO-arg 4a</td>
<td>36</td>
</tr>
</tbody>
</table>

* E. coli K-12.

As indicated above, the cells were acid-extracted immediately
after centrifugation from the growth medium. No intermediate
washing step was included in order to minimize pool leakage.
The temperature of centrifugation varied in independent
experiments from 0° to 20° with no significant change in the pool
size.

Materials—d-Glucose-U-14C, DL-arginine-5-14C, and DL-orni-
Thine-5-14C were obtained from New England Nuclear and
diluted to the appropriate specific activity with unlabeled d-
glucose, L-arginine, or L-ornithine. Bovine arginase was pur-
chased from Worthington.

RESULTS

Influence of Arginine and Precursors on Rate of Putrescine Syn-
thesis—Since intermediates of arginine biosynthesis are precursors
of putrescine, it is of interest to know what effect, if any, vari-
ations in arginine supplementation have on the rate of putrescine
synthesis in growing cultures of E. coli. Putrescine and its
metabolic product, spermidine, were measured in cells grown in
minimal medium with various supplements (Table I). With
wild-type E. coli K-12 (strain 3000), there was no significant
difference in polyamine levels in cells grown in minimal and
arginine-supplemented medium. An arginine-requiring mutant of
E. coli K-12 (30SO-arg 4) was grown with various intermediates
of the arginine pathway, and again there was no significant al-
teration in polyamine content (Table I). E. coli strain AC2514-
1 and its homoarginine-resistant derivatives were also found to
have invariant polyamine levels. However, the pool sizes in
these E. coli B strains were somewhat smaller than in K-12 (26
and 1.1 nmoles/10⁸ cells for putrescine and spermidine, re-
spectively).

Since the putrescine pool in E. coli turns over rapidly (14, 15),
the identity of pool levels under these growth conditions cannot
be taken to mean identity of synthetic rates. In order to examine
the rate of turnover of the putrescine pool under the above growth
conditions (Table I), cells were grown from a small inoculum in
media containing radioactive precursors. The cultures were
then shifted to nonradioactive media, and the specific radioac-
tivity of the putrescine pool was monitored as a function of time.
The results are shown in Fig. 2. It is apparent that in both the
K-12 and B strains the decrease in specific activity was greater
than the 50% per generation predicted on the basis of simple
dilution by growth. These results are consistent with a turn-
over of the putrescine pool due either to conversion to other
products or to excretion from the cell. As will be shown below,
the rate of turnover was much greater than can be accounted
for solely on the basis of putrescine conversion to spermidine.
The pattern of dilution of the radioactive putrescine pool is
clearly different in B and K-12. In addition, the dilution rate
seems to be independent of arginine supplementation in the K-12
strains and in the case of the B strains, unaffected by derepression
of the arginine biosynthetic enzymes.

In the case of the B strain of E. coli, the logarithm of putres-
cine specific activity is clearly not a linear function of time.
Applying an F test for linearity (16), one can say with greater
than 99% confidence that this curve is nonlinear. In the case of
E. coli K-12 this nonlinear behavior is not so certain. Applying
the same statistical test, one can only claim nonlinearity with
75% confidence. However, since the same form for this curve
has been observed in three independent experiments, it seems
quite likely that the interpretation of non-linearity is correct

Influence of culture conditions on polyamine levels

![Graph](http://www.jbc.org/)

Fig. 2. Rate of dilution of putrescine pool. E. coli strains 3000 (●●●●), AC2514-1 (□□□□), AC2514-1 Harg81 (■■■■), and AC2514-1 Harg88 (△△△△) were grown in the presence of uniformly labeled 14C-glucose and strain 30SO-arg 4 was grown with ornithine-5-14C (▲▲▲▲) or arginine-5-14C (O——O) as
described under “Experimental Procedures.” The cells were
shifted to unlabeled medium and the specific radioactivity of
putrescine followed with time. The initial putrescine specific
activities (cpm per μ mole) were 222,000 (●●●●), 145,000
(▲▲▲▲), 194,000 (O——O), 131,000 (□□□□), 129,000 (■■■■),
and 149,000 (△△△△). The points are experimental and the
curves are theoretically derived as explained in the text.
The relative growth increment is defined as the time after transfer to
unlabeled medium divided by the doubling time for the particular
culture. Doubling times were 60 min for 3000 and 30SO-arg 4.
54 min for the B strains.
with this strain also. The form of these curves is such that the rate of decline in specific activity at late times approaches 50% per generation, as expected for dilution by growth. However, at early times, this rate is much greater. Since the cultures are growing exponentially throughout the course of the experiment, the data seem to indicate a heterogeneity in the behavior of intracellular putrescine. A model consistent with this behavior is one in which the putrescine precursor \( X \) enters two independent putrescine pools \( P \) and \( P' \). One pool is stable \( (P) \) and, in the experiment represented in Fig. 2, would be diluted 2-fold per generation. The other pool \( (P') \) gives rise to a product (or products) \( (Y) \) and is therefore diluted at a faster rate because of turnover.

\[
\begin{align*}
X & \xrightarrow{k_1} P \quad \xrightarrow{k_2} Y \\
& \quad \xrightarrow{k_1'} P' 
\end{align*}
\]

One can derive a mathematical expression for this model and then produce the curves of Fig. 2, which best fit the experimental points. The parameters which produce this best fit for \( E. coli \) K-12 are consistent with 60% of the intracellular putrescine in pool \( P \) (i.e., turning over). This pool is turning over at a rate \((k_2)\) of 20 nmoles per doubling per \( 10^9 \) cells and is replenished at a rate \((k_1)\) of 40 nmoles per doubling per \( 10^9 \) cells. The over-all rate of putrescine synthesis \((k_{\text{put}} = k_1 + k_2)\) is 53 nmoles per doubling per \( 10^9 \) cells.

Analysis of the data obtained with the \( E. coli \) R strains indicates that again 60% of the intracellular putrescine is in pool \( P \). However, the flux through the unstable pool is somewhat greater than for the K-12 strains, with \( k_1 \) and \( k_2 \) being equal to 47 and 31 nmoles per doubling per \( 10^9 \) cells, respectively. This resulted in a value for \( k_{\text{put}} \) of 57 nmoles per doubling per \( 10^9 \) cells.

There are explanations for the two-component dilution curve other than the two-pool model proposed above. One is that there might be a radioactive contaminant, in the putrescine peak from the ion exchange column, which turns over at a different rate than putrescine. Thin layer chromatography (3) of the isolated putrescine fractions from the ion exchange column, throughout the course of the turnover experiments, showed no radioactivity other than that associated with the putrescine spot. This observation, that the radioactivity fractionated with putrescine during both ion exchange and partition chromatography, together with the fact that identical dilution curves were seen with \(^{14}\)C-glucose, \(^{14}\)C-arginine, or \(^{14}\)C-ornithine as precursors, argues strongly against the possibility of contamination. Another possible interpretation of the data would be a lack of steady state conditions in the bacterial culture during the course of the experiments. Dilution curves identical with those shown in Fig. 2 were obtained when experiments were carried out in larger cultures, so that the cell density was never greater than \( 8 \times 10^9 \) cells per ml. Also, when experiments were designed so that no immediate centrifugation step was necessary, there was again no change in the shape of the dilution curves. Thus it would seem that trivial explanations for the dilution kinetics can be ruled out. However, definitive proof for the two-pool model can only be obtained through other independent experimental approaches.

It is perhaps not surprising that there is some turnover of the putrescine pool, since it is known to be a precursor of spermidine (14). However, the levels of spermidine and its acetyl derivatives in those strains (less than 5 nmoles/10^9 cells for K-12 and less than 2 nmoles/10^9 cells for B) are far too low to account for the observed rates of turnover. The source of this unaccountable turnover is, at present, unknown. It could be due to formation of an unidentified product of putrescine or to excretion of putrescine into the growth medium.

These studies show that the rate of putrescine synthesis in \( E. coli \) K-12 is independent of conditions of arginine nutrition with precursors present in excess. This is also true with changes in state of repression, as shown with the homoarginine-resistant \( E. coli \) B strains. The effect of limiting arginine on the rate of putrescine synthesis has not been studied.

**Influence of Arginine Nutrition on Rate of Putrescine Synthesis via Pathway II**—The level of urea excreted into the medium by a growing culture of \( E. coli \) is a direct measure of the rate of putrescine biosynthesis via Pathway II (8). The experiments summarized in Table II demonstrate the effect of alterations in arginine nutrition on the rate of urea production by various strains of \( E. coli \). In general, cells produced low levels of urea when grown in unsupplemented minimal medium or in minimal medium supplemented with intermediates of arginine biosynthesis earlier than citrulline. On the other hand, when cells were supplemented with intermediates of the arginine pathway later than ornithine, higher levels of urea were produced.

### Table II

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Organism</th>
<th>( k_{\text{urea}} ) (nmoles doubling(^{-1}) (10^9 cells)(^{-1}))</th>
<th>( k_{\text{urea}}/k_{\text{put}} )</th>
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<tbody>
<tr>
<td>Minimal</td>
<td>AC2514**a</td>
<td>1</td>
<td>0.02</td>
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<tr>
<td>+ Arginine</td>
<td>AC2514**a</td>
<td>56</td>
<td>0.98</td>
</tr>
<tr>
<td>Minimal</td>
<td>3000**b</td>
<td>12</td>
<td>0.23</td>
</tr>
<tr>
<td>+ Arginine</td>
<td>3000**b</td>
<td>46</td>
<td>0.87</td>
</tr>
<tr>
<td>Minimal</td>
<td>+ Ornithine</td>
<td>30SO-arg**d</td>
<td>13</td>
</tr>
<tr>
<td>+ Arginine</td>
<td>30SO-arg**d</td>
<td>55</td>
<td>1.04</td>
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<tr>
<td>+ Citrulline</td>
<td>30SO-arg**d</td>
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<td>0.68</td>
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<tr>
<td>+ Arginine, citrulline</td>
<td>30SO-arg**d</td>
<td>46</td>
<td>0.87</td>
</tr>
<tr>
<td>+ Ornithine, arginine</td>
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<td>30SO-arg**d</td>
<td>30</td>
<td>0.57</td>
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</table>

* \( E. coli \) B.  
* \( E. coli \) K-12.
TABLE III

Urea formation by arginine derepressed mutants

<table>
<thead>
<tr>
<th>Organism</th>
<th>Culture conditions</th>
<th>Ornithine transcarbamylase</th>
<th>hurea</th>
<th>hurea/hput</th>
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<tr>
<td>AC2514-1</td>
<td>Minimal</td>
<td>0.47 units/mg protein</td>
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<td>0.02</td>
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<tr>
<td>AC2514-1</td>
<td>Minimal + arginine</td>
<td>*</td>
<td>56</td>
<td>0.98</td>
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<tr>
<td>AC2514-1</td>
<td>Minimal</td>
<td>0.06 units/mg protein</td>
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<td>0.14</td>
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<tr>
<td>Harg&lt;sup&gt;8&lt;/sup&gt;</td>
<td>Misimal + arginine</td>
<td>*</td>
<td>59</td>
<td>1.04</td>
</tr>
<tr>
<td>AC2514-1</td>
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<td>12.3 units/mg protein</td>
<td>47</td>
<td>0.88</td>
</tr>
<tr>
<td>Harg&lt;sup&gt;8&lt;/sup&gt;</td>
<td>Misimal + arginine</td>
<td>*</td>
<td>83</td>
<td>1.46</td>
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<td>3000</td>
<td>Minimal</td>
<td>0.82 units/mg protein</td>
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<td>0.23</td>
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<tr>
<td>3000</td>
<td>Minimal + arginine</td>
<td>*</td>
<td>46</td>
<td>0.87</td>
</tr>
</tbody>
</table>

* Not measured.

TABLE IV

Amino acid pool levels

<table>
<thead>
<tr>
<th>Organism</th>
<th>Amino acid</th>
<th>Ornithine</th>
<th>Arginine</th>
<th>hurea/hput</th>
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<tbody>
<tr>
<td>3000</td>
<td></td>
<td>0.04</td>
<td>0.41</td>
<td>0.23</td>
</tr>
<tr>
<td>3000 + arginine&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>0.00</td>
<td>0.52</td>
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<td>0.10</td>
<td>0.19</td>
<td>0.02</td>
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<tr>
<td>AC2514-1</td>
<td>Harg&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0.04</td>
<td>0.21</td>
<td>0.14</td>
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<tr>
<td>AC2514-1</td>
<td>Harg&lt;sup&gt;8&lt;/sup&gt;</td>
<td>&lt;0.01</td>
<td>0.50</td>
<td>0.82</td>
</tr>
</tbody>
</table>

* Supplemented with arginine during growth.

Combining these data on the rate of urea production with the above estimates of the total rates of putrescine production (hput), one can derive the proportion of putrescine synthesized via Pathway II (hurea/hput). This is included in Table II. E. coli B (strain AC2514-1), grown in minimal medium, produces about 98% of its total putrescine via Pathway I. With E. coli K-12, under these conditions, there was less stringent utilization of Pathway I. Only about 75% of the total putrescine was produced by this route. This strain difference is probably the result of a difference in levels of arginine biosynthetic enzymes, as discussed below.

The value of 60% utilization of Pathway II in strain 3080-arg 4, grown in the presence of both ornithine and arginine (Table II), is in excellent agreement with our previous estimate of 60% derived from isotope dilution studies (3).

Influence of Arginine Supplementation on Levels of Putrescine Biosynthetic Enzymes—The selective utilization of putrescine biosynthetic Pathway I in minimal medium could be due to a modulation of the levels of the enzymes of putrescine biosynthesis. The specific activities of biosynthetic ornithine and arginine decarboxylases and agmatine ureohydrolase were measured in wild-type E. coli K-12 and B and were similar to those found previously (3). No significant variation in the levels of these enzymes was found in cells grown in minimal medium with and without arginine supplementation. Thus, this pathway selection phenomenon cannot be caused by a variation in the rate of synthesis of the putrescine biosynthetic enzymes, and therefore it must involve a modulation of enzyme activity.

Effect of Variations in Arginine Biosynthetic Enzyme Levels on Putrescine Pathway Selection—The data in Table II indicated a striking difference between E. coli B and E. coli K-12 in the relative utilization of Pathway II in minimal medium. An explanation may lie in the fact that these two strains differ in their levels of the enzymes of arginine biosynthesis (17). In order to examine this possibility in a more controlled system, isogenic mutants, which were partially and fully derepressed for the enzymes of arginine biosynthesis, were tested for urea production. These mutants were isolated as homoarginine-resistant mutants of AC2514-1 as described by Peyrn and Maas (9). With ornithine transcarbamylase as an indicator, the level of the arginine biosynthetic enzymes was compared to the relative utilization of putrescine biosynthetic Pathway II (Table III). There is a striking correlation in the B strains between the level of ornithine transcarbamylase in unsupplemented medium and the relative utilization of Pathway II. This correlation extends not only through the derepressed series of E. coli B mutants, but also to wild-type E. coli K-12 (strain 3000). Therefore, preferential utilization of putrescine Pathway I, in cells growing on minimal medium, can be modulated by changes in the levels of the arginine biosynthetic enzymes. Our contention that this is an effect of changes in the arginine enzyme levels is substantiated by the fact that there are no significant differences between these strains in the levels of the enzymes of putrescine biosynthesis.

Intracellular Pool Levels of Ornithine and Arginine—The dependence of the stringency of pathway selection in putrescine biosynthesis on the levels of the enzymes of arginine synthesis indicated that it might be related to pool sizes of arginine intermediates. The intracellular pools of arginine and ornithine were measured in wild-type E. coli K-12 grown in the presence and absence of arginine, and in wild-type and derepressed mutants of E. coli B. These results are summarized in Table IV and compared to the relative utilization of putrescine Pathway II. The most striking feature of these data is the lack of variation in arginine levels. The arginine pool size remains relatively constant within a given strain, in spite of arginine supplementation or derepression of the enzymes of arginine biosynthesis. Ornithine, on the other hand, varies widely in these different instances, and in fact there is a good inverse correlation between ornithine level and percentage of total putrescine synthesized via Pathway II. The change in steady state ornithine concentration with state of derepression is probably due to the non-coordinate control of the levels of the arginine biosynthetic enzymes (17–20).

**DISCUSSION**

The validity of the conclusions reached in this paper depends heavily on the assumption that the rate of urea production by whole cells is a direct measure of the functioning of putrescine biosynthetic Pathway II. Previous work has demonstrated that E. coli lacks both urease activity and alternate pathways of urea production (8). Additional support for the validity of the method comes from independent studies by isotope dilution techniques. We previously reported that cells growing in the presence of equal amounts of arginine and ornithine produced...
approximately 60% of their putrescine by way of Pathway II (3). The relative functioning of Pathway II under this growth condition, obtained by the present method (Table II), is in precise agreement with the previous value. One last piece of evidence in support of this method comes from the rate of urea production in cells growing in medium supplemented only with arginine. Under these conditions, the cells would be expected to use Pathway II exclusively. Therefore, putrescine and urea should be produced at equivalent rates. This expectation was clearly fulfilled (Table II).

The results presented in this paper provide a frame of reference in vivo for the study of the regulation of the two pathways of putrescine biosynthesis in E. coli. When E. coli is growing in minimal medium, the cells are synthesizing arginine de novo, and the route of putrescine synthesis directly from ornithine is preferentially utilized. This preferential utilization of putrescine biosynthetic Pathway I is strain-specific, depending on the level of the arginine biosynthetic enzyme. In strains with high enzyme levels, Pathway I is used less than in more repressed strains. When any of these strains is shifted to medium containing arginine, the utilization of putrescine Pathway I is abolished and Pathway II is used solely. This effect of enzyme levels is probably mediated in part through the altered intracellular ornithine concentration observed above.

In considering possible mechanisms for this pathway selection phenomenon, one must put heavy emphasis on the fact that under both conditions, i.e. in minimal medium or arginine-supplemented medium, the total rate of putrescine synthesis is unchanged. One can reasonably argue that in cells growing in arginine-supplemented medium, putrescine Pathway I is shut off owing to the absence of ornithine in the cell. However, when these cells are shifted to minimal medium, there must be a decreased functioning of Pathway II, concomitant with the increased rate through Pathway I, to account for the constant combined rate. This decreased functioning of Pathway II cannot be due to altered feedback inhibition by end products since the intracellular pools of putrescine and spermidine do not vary under these two conditions. We have also shown in this paper that the modulation of Pathway II activity cannot be due to changes in enzyme levels. Two possible models are that regulation of Pathway II would be through the action of a positive or negative effector, or through the levels of available substrate. If the intracellular level of a negative effector increased or a positive effector decreased during the shift of cells from arginine-containing to un-supplemented medium, a decrease in functioning of Pathway II would be observed. No modulation of arginine decarboxylase activity has been observed with any effector other than the end products of the pathway, putrescine and spermidine, and the cofactors for the reaction, pyridoxal phosphate and magnesium ion. A likely candidate for a negative effector would be ornithine, since its levels are low in arginine-grown cells and high in un-supplemented cells. However, we have observed no inhibition of arginine decarboxylase at physiological ornithine concentrations. The second possible mechanism for regulation of Pathway II, modulation of substrate levels, would, to first approximation, seem to be inconsistent with the experimental evidence presented in this paper. The total intracellular concentration of ornithine varies little under conditions where Pathway II is inhibited up to 98%. However, if there were two intracellular pools of ornithine, one derived from endogenously synthesized material and the other from exogenously supplied amino acid, and in addition if Pathway II were fed primarily from the exogenous pool, the present results would be predicted. Sercarz and Gorini have presented evidence, through the study of chemostat-grown cultures of E. coli, that there may indeed be two pools of arginine, one for protein synthesis and one involved in repression (21). Furthermore, the studies reported here on arginine levels in E. coli K-12, grown in unsupplemented and arginine-supplemented medium, support the concept of an independent pool of arginine involved in repression. Under conditions which give maximal repression of the enzymes of arginine biosynthesis, there is at most a 25% increase in total intracellular arginine.

The pathway selection described in this paper, together with feedback inhibition of both routes to putrescine, forms a complex network for regulation of the two pathways of putrescine biosynthesis. The precise regulation of polyamine synthesis argues strongly for an important physiological role for these compounds.

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