Multiple Pathways of Putrescine Biosynthesis in Escherichia coli

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SUMMARY

Two routes of putrescine biosynthesis exist in Escherichia coli both emanating from the latter portion of the arginine-biosynthetic pathway. Isotope competition experiments indicate that both arginine and ornithine can give rise to putrescine. The formation of putrescine from ornithine is independent of the conversion of the latter to arginine.

The decarboxylation of ornithine to yield putrescine is catalyzed by cell-free preparations. This enzyme has been termed "biosynthetic" ornithine decarboxylase to distinguish it from the inducible degradative ornithine decarboxylase, from which it differs in many properties.

Cell-free extracts also carry out the conversion of arginine to putrescine. In this pathway, the arginine carboxyl group is first released as CO₂, and the amidine group is then removed as urea. The arginine decarboxylase has optimal activity at pH 8 and an absolute magnesium ion requirement. Both of these characteristics distinguish it from the catabolic arginine decarboxylase. Agmatine ureohydrolase, the enzyme catalyzing the second step of this pathway, is also present in cell-free extracts.

Arginine biosynthesis in E. coli involves a branched pathway, the branch point to putrescine and arginine occurring at ornithine. The existence of two pathways of putrescine biosynthesis is interpreted in relation to control mechanisms of the arginine biosynthetic pathway.

The polyamines are found in almost all living cells. In many, they are a major low molecular weight component. Spermidine, \( \text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2 \), is found in bacteria, fungi, and animals (2, 3). Spermine, \( \text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2 \), has been reported in only one bacterial species (4), but is commonly found in animals and fungi (2, 3). Putrescine, \( \text{NH}_2(\text{CH}_2)_4\text{NH}_2 \), a metabolic precursor of spermidine and spermine (5, 6), has been extracted from gram-negative bacteria and fungi (3). These compounds are presumed to play an important metabolic role because of their broad distribution in high concentration and because certain microorganisms require them for growth (7-9). Little is known of their physiological function, however.

To study the function of these compounds, one should be able to control their intracellular concentration. With this in mind, we have initiated a study of the biosynthesis of putrescine, the basic unit of the polyamines. In this paper, we will show that in Escherichia coli there are two major pathways of putrescine biosynthesis under nonspecialized culture conditions. The first route of putrescine biosynthesis is the decarboxylation of ornithine (1). The second pathway is from arginine to putrescine, independent of ornithine. This conversion appears to involve agmatine (decarboxylated arginine) as an intermediate.

EXPERIMENTAL PROCEDURE

Growth of Bacteria—These studies were performed with strain 3080-arg 4 of E. coli K-12 (obtained from Dr. W. Maas). This is an Hfr strain which requires arginine because of a block between N-acetylglutamic acid and N-acetylglutamic semialdehyde. This organism was routinely grown at 37° with vigorous shaking in minimal medium (Medium E, described by Vogel and Bonner (10)). The medium was supplemented with trace elements (11), thiamine (0.5 µg per ml), and arginine (20 µg per ml) or ornithine (20 µg per ml).

Extraction and Separation of Polyamines—The cells from log phase cultures (approximately 5 \( \times 10^9 \) cells per ml) were harvested by centrifugation and washed once with 0.9% NaCl at 0°. The cells were then extracted with 0.3 N trichloracetic acid as described by Dubin and Rosenthal (12). The protein precipitate was removed by centrifugation and was saved for analysis. After the trichloracetic acid supernatant solution was extracted three times with 2 volumes of ether, it was applied to a column (1.4 \( \times 20 \) cm) of Bio-Rad AG 50W-X8 (100 to 200 mesh, hydrogen form). The first elution was by a linear gradient formed with 400 ml of water in the mixing chamber and 400 ml of 2.5 N HCl in the reservoir. A typical elution pattern of a mixture of putrescine and basic amino acids is shown in Fig. 1. Lysine was the only amino acid which cochromatographed with putrescine under these conditions. Agmatine was eluted after arginine. The neutral and acidic amino acids were eluted in the earlier fractions. After completion of the gradient elution, 4 N HCl was passed through the column to elute spermidine. The putrescine- and spermidine-containing fractions were located.
Fig. 1. Ion exchange chromatography of putrescine and basic amino acids. A mixture of 1 mg each of \(^{14}C\)-putrescine-2HCl (200,000 cpm), \(^{14}C\)-ornithine-HCl (480,000 cpm), \(^{14}C\)-arginine-HCl (500,000 cpm), \(^{14}C\)-lysine-HCl (100,000 cpm), and \(^{14}C\)-diaminopimelic acid (220,000 cpm) was applied to a column of Bio-Rad AG 50W and eluted as described in the text. Samples of approximately 5 ml each were collected, and 0.1-ml aliquots were counted. The radioactive peaks were identified by thin layer chromatography. Peak 1 contained ornithine and diaminopimelic acid, Peak 2 contained putrescine and lysine, and Peak 3 was composed solely of arginine.

Either by radioactivity measurements or by amine analysis. The pooled putrescine and spermidine fractions were concentrated on a rotary evaporator and were decolorized with Darco G-60. These pooled fractions were then analyzed for radioactivity and amine content.

Estimation of Amines—Aliquots of the putrescine- and spermidine-containing column fractions were evaporated under reduced pressure over NaOH. The amine content of these fractions was then determined by formation of their dinitrophenyl derivatives (13).

Protein Analysis—The precipitates from the trichloracetic acid extractions were dissolved in 0.5 N NaOH to give a protein concentration of approximately 3 to 5 mg per ml. An aliquot of this solution was then diluted in 0.5 N NaOH and was analyzed for protein content by the method of Lowry et al. (14). The remainder of the NaOH solution was saved for radioactivity analysis.

Thin Layer Chromatography—Thin layer chromatography of putrescine and spermidine was carried out on cellulose layers 0.25 mm thick. A solvent consisting of 1-butanol, acetic acid, pyridine, and water (4:1:1:2) was used for development. After development, the plates were dried at room temperature, sprayed with 0.5% ninhydrin in 70% ethanol, and heated at 100°C for 5 min. With the use of this solvent system, a separation of arginine, ornithine, agmatine, putrescine, and spermidine was effected.

\(^{14}C\) Determinations—For the determination of radioactivity present in the HCl effluents from the ion exchange columns, aliquots were placed in scintillation vials and evaporated under reduced pressure over NaOH. The residues were redissolved in 0.1 ml of water, and 15 ml of Bray's scintillation fluid (15) were added to each vial. The samples were counted in a Packard scintillation counter.

The amount of radioactivity incorporated into protein was measured by counting 5 ml of the NaOH solution of the trichloracetic acid precipitate. This quantity of 0.5 N NaOH in 15 ml of scintillation fluid did not quench.

For the determination of radioactivity on thin layer plates, the radioactive spots were excised and placed in scintillation vials with 15 ml of Bray's solution (15). The observed counts were corrected for 30% quenching.

Radioactive CO\(_2\) was trapped and counted as previously described (1).

Cell-free Extracts and Enzymatic Assays—The preparation, dialysis, and assay of ornithine decarboxylase have been previously described (1). A unit of ornithine decarboxylase activity is defined as that quantity of enzyme which catalyzes the formation of 1 \(\mu\) mole of CO\(_2\) per min under the standard assay conditions.

For the preparation of extracts capable of catalyzing the conversion of arginine to putrescine, the cells were ground for seven minutes in a mortar with alumina (approximately 2.5 times their bacterial weight). The mixture was suspended in 0.05 M \(\text{Tris-HCl}, \ pH\ 7.6\) containing \(10^{-2} \text{ M EDTA and } 10^{-3} \text{ M dithiothreitol}\) it was centrifuged twice for 15 min at 18,000 \(\times\) g. The supernatant solution was used as a source of enzymes. When it was necessary to remove low molecular weight cofactors, 2 ml of the supernatant solution were passed through a column (1.4 x 13 cm) of coarse Sephadex G-25 which had been equilibrated with the extraction buffer.

The reaction mixture for the enzymatic conversion of arginine to putrescine contained 30 \(\mu\) moles of Tris-HCl buffer (pH 7.5) 2.22 \(\mu\) moles of arginine-U-\(^{14}C\) (7.75 \(\times\) 100 \(\mu\) mole per \(\mu\) mole 0.012 \(\mu\) mole of pyridoxal phosphate, and 0.60 \(\mu\) mole of magnesium sulfate, in a total volume of 0.30 ml. The reaction was stopped, after incubation at 37°C, by the addition of 0.02 ml to 100\% (w/v) trichloroacetic acid.

For the determination of agmatine ureohydrolase activity cell-free extracts were prepared as described above for \(\text{arginine to putrescine conversion. The assay mixture contained 30 }\mu\text{ moles of Tris-HCl, }\ p\text{H 7.5, and 2.2 }\mu\text{ moles of agmatine sulfate, in a total volume of 0.30 ml. The reaction was started by the addition of enzyme. After incubation at 37°C for 10 min 0.10 ml of 7% perchloric acid was added. The protein precipitate was removed by centrifugation. To the supernatant solution were then added 0.10 ml of 3 N sodium acetate, }\ p\text{H 5, and 0.25 ml of 3,3/4-dinitrobenzidine reagent (Hyced blood urea nitrogen reagent). After they were heated in a boiling water bath for 2 min, the tubes were placed on ice for 3 min. To each tube was added 0.5 ml of water, and the absorbance at 470 my was determined. The amount of urea produced was determined by comparison with a standard curve. The range of this assay was between 0.2 and 0.8 \(\mu\) mole of urea.\textsuperscript{2} A unit of agmatine ureohydrolase activity is defined as that amount of enzyme which catalyzes the formation of 1 \(\mu\) mole of urea in 1 min under the standard assay conditions.

Materials—Ornithine-5-\(^{14}C\), arginine-5-\(^{14}C\), and lysine-U-\(^{14}C\) were obtained from Calbiochem. Arginine-U-\(^{14}C\) was purchased from Volk. Ornithine-U-\(^{14}C\) was synthesized as previously described (1) by the method of Rivard and Carter (16). Putrescine-1,4-\(^{14}C\) and diaminopimelic acid-2,6-\(^{14}C\) were purchased from New England Nuclear. Merck supplied the glucose-U-\(^{14}C\) and glucose-U-\(^{14}C\) refer to the uniformly labeled compounds.

1 Arginine-U-\(^{14}C\), lysine-U-\(^{14}C\), ornithine-U-\(^{14}C\), and glucose-U-\(^{14}C\) refer to the uniformly labeled compounds.

2 This assay is a modification of the arginine assay recommended by Worthington.
RESULTS

Conversion of Labeled Precursors to Putrescine—Studies by Tabor, Rosenthal, and Tabor demonstrated that 14C-ornithine is incorporated into the putrescine residue of spermidine in intact E. coli (6). These results indicate that putrescine arises from a late intermediate in the arginine-biosynthetic pathway. Table I confirms and extends the experiments of these workers. The first two experiments show that both ornithine and arginine are converted to putrescine with equal efficiency, the specific activities of putrescine and spermidine in both cases being equal to that of the intracellular arginine pool, measured by the radioactivity of the arginine in cell protein.

Since arginine is converted to putrescine in Experiment 2, two possibilities exist for the conversion of ornithine to putrescine (Experiment 1): (a) conversion to arginine and then to putrescine or (b) direct decarboxylation to yield putrescine. The last two experiments in Table I were designed to distinguish between these two possibilities. If ornithine gave rise to putrescine solely by its conversion to arginine, the specific activity of putrescine and, therefore, spermidine would be expected to be equal to that of the arginine pool under all growth conditions. This was not the case. When the cells were grown with 14C-ornithine and unlabeled arginine (Experiment 3), the specific activities of the polyamines were much greater than that of the arginine pool. The low radioactivity content of the arginine pool in this experiment is due to the repression of the arginine biosynthetic enzymes by the arginine present in the culture medium (18–20). In the inverse experiment (No. 4), with cells grown on 14C-arginine and unlabeled ornithine, the specific activities of putrescine and spermidine were lower than that of the arginine pool. These experiments clearly show that E. coli has a mechanism for the conversion of ornithine to putrescine which does not involve arginine as an intermediate.

Although the above experiments show that putrescine can arise from intermediates of the arginine-biosynthetic pathway, it was necessary to exclude the possibility of an additional route to putrescine which is independent of arginine biosynthesis. To investigate this possibility, the incorporation of 14C-glucose into putrescine was studied with the use of the ornithine-requiring mutant (Table II). In Experiments 1 and 2, the incorporation of 14C-glucose into polyamines was examined in cells grown in the presence of ornithine and arginine, respectively. In Experiment 3, cells were incubated with 14C-glucose during arginine starvation. The incorporation into putrescine was quite low in Experiments 1 and 2, being 3 and 1.5%, respectively, of the theoretical value. The small amount of radioactivity which did appear in the putrescine fraction was probably due to lysine, which cochromatographs with putrescine on ion exchange chromatography (Fig. 1). The radioactivity of the spermidine in these experiments was at the level expected for labeling of the 3-carbon unit which arises from methionine (6). It appears, therefore, that the sole source of putrescine in E. coli is from the pathway of arginine biosynthesis.

Cell-free Conversion of Ornithine to Putrescine—Since the labeling data in vivo indicated a direct conversion of ornithine to putrescine, ornithine decarboxylase activity was sought in cell-free extracts of E. coli (1). The liberation of CO2 from ornithine, catalyzed by a dialyzed crude extract, is shown in Fig. 2. The rate of CO2 evolution was proportional to the concentration of enzyme, and the reaction had an absolute requirement for pyridoxal phosphate. The Km for ornithine in this reaction is approximately 1 × 10–6 M.

To show that putrescine, in addition to CO2, was a product of
Crude extracts of strain 3080-arg 4 were examined for the ability to release CO$_2$ from arginine, a reaction which may take place regardless of the route of arginine conversion to putrescine. Fig. 3 shows that crude extracts do indeed catalyze the formation of $^{14}$CO$_2$ from arginine-$^{14}$C. The reaction, when carried out with Sephadex-treated extracts, showed an absolute requirement for magnesium and a partial pyridoxal phosphate requirement. As can also be seen from this figure, the kinetics of $^{14}$CO$_2$ evolution was not altered when unlabeled ornithine was included in the reaction mixture, suggesting that the latter compound is not an intermediate in this reaction. The reaction had optimal activity at pH 8 and a $K_m$ for arginine of about $1 \times 10^{-3}$ M.

In order to identify the products of this reaction, arginine-$^{14}$C was incubated with a cell-free extract, and the products were separated by ion exchange chromatography. The elution profile of radioactivity from the column is shown in Fig. 4. Putrescine and unreacted arginine were identified by thin layer chromatography and by their elution volumes from the column. Urea was identified by its stoichiometric degradation to CO$_2$ and NH$_3$ during incubation with urease (Table IV). The first peak of radioactivity from the column was an impurity present in the $^{14}$C-arginine. The stoichiometry of the conversion of arginine-$^{14}$C to putrescine, urea, and CO$_2$ is shown in Table V. It was found that 1 mole each of putrescine, urea, and CO$_2$ was formed per mole of arginine which disappeared. The specific activities of the putrescine, urea, and recovered arginine were approximately equal to the theoretical values.

These results show that cell-free extracts of $E. coli$, strain 3080-arg 4, are capable of converting arginine to putrescine. In this reaction, the carboxyl group of arginine is released as CO$_2$ and the amine group is converted to urea. Cell-free extracts of wild-type $E. coli$ B and K-12 were also examined for the ability to evolve $^{14}$CO$_2$ from arginine-$^{14}$C. This activity was present in cells grown in minimal medium at approximately the same level as in 3080-arg 4. (1.2 $\times 10^{-3}$ m mole of CO$_2$ per min per mg protein for B and 9.0 $\times 10^{-3}$ m mole per min per mg for K-12, respectively.)

<table>
<thead>
<tr>
<th>Compound determined</th>
<th>Change (+Enzyme)</th>
<th>Change (-Enzyme)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Putrescine</td>
<td>1.3</td>
<td>0.0</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>1.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Ornithine</td>
<td>-1.5</td>
<td>0.0</td>
</tr>
</tbody>
</table>

The reaction, ornithine-$^{14}$C was incubated with a crude extract, and thin layer chromatography was performed on the reaction mixture. Putrescine was the sole nonvolatile ninhydrin-positive product of the reaction. It was found that 1 m mole of CO$_2$ and putrescine formed per m mole of ornithine which disappeared (Table III).

These results show that cell-free extracts of an ornithine-requiring mutant of $E. coli$ K-12 are capable of carrying out the conversion of ornithine to putrescine. Approximately the same levels of ornithine decarboxylase activity have been found in cells of wild type $E. coli$ K-12 and $E. coli$ B which have been grown in minimal medium ($3.5 \times 10^{-2}$ and $3.2 \times 10^{-2}$ unit per mg of protein, respectively, as compared with $3.5 \times 10^{-2}$ unit per mg for 3080-arg 4). These results show that this mode of putrescine biosynthesis is not peculiar to the $E. coli$ mutant used in these studies.

**Cell-free Conversion of Arginine to Putrescine**—The labeling studies with intact cells reported in this paper showed that $E. coli$ possesses a mechanism for the conversion of arginine to putrescine. Crude extracts of strain 3080-arg 4 were examined for the ability to release CO$_2$ from arginine, a reaction which may take place regardless of the route of arginine conversion to putrescine. Fig. 3 shows that crude extracts do indeed catalyze the formation of $^{14}$CO$_2$ from arginine-$^{14}$C. The reaction, when carried out with Sephadex-treated extracts, showed an absolute requirement for magnesium and a partial pyridoxal phosphate requirement. As can also be seen from this figure, the kinetics of $^{14}$CO$_2$ evolution was not altered when unlabeled ornithine was included in the reaction mixture, suggesting that the latter compound is not an intermediate in this reaction. The reaction had optimal activity at pH 8 and a $K_m$ for arginine of about $1 \times 10^{-3}$ M.
Degradation of enzymatically produced urea by urease

The reaction mixtures containing 30 μmoles of Tris-HCl buffer (pH 7.5), 2.2 μmoles of agmatine, and 1.1 mg of a crude extract of 30S0-arg 4 in a total volume of 0.3 ml were incubated at 37. The reactions were stopped at the indicated times by the addition of 0.02 ml of 100% (w/v) trichloroacetic acid. The 14CO2 was trapped and counted. The reaction mixtures were assayed directly for CO2, NH3 and urea (A — A) and agmatine (O — O). For the guanidino group of agmatine (Curve 2, O — O) by the procedure of Conn and Davis (24). Reaction mixtures incubated in the absence of enzyme were also analyzed for urea (Δ + Δ) and agmatine (O — O).

Effect of agmatine on conversion of 14C-arginine to putrescine

A crude extract of 30S0-arg 4 (9.7 mg of protein) was incubated for 30 min as described in Fig. 4, with and without the addition of 30 μmoles of unlabeled agmatine sulfate. Analysis of 14CO2 and ion exchange chromatography were carried out as in Fig. 4. The results are expressed as micrograms of the indicated product arising from arginine-U-14C. This was calculated from the specific activity of the arginine-U-14C (7.75 × 10^4 cpm per μmole).

TABLE V

<table>
<thead>
<tr>
<th>Compound determined</th>
<th>From 14C-arginine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No addition</td>
</tr>
<tr>
<td>μmoles</td>
<td></td>
</tr>
<tr>
<td>CO2</td>
<td>2.90</td>
</tr>
<tr>
<td>Urea</td>
<td>2.04</td>
</tr>
<tr>
<td>Putrescine</td>
<td>1.96</td>
</tr>
<tr>
<td>Agmatine</td>
<td>1.52</td>
</tr>
</tbody>
</table>

Fig. 5. Agmatine ureohydrolase activity in E. coli extracts. Reaction mixtures containing 30 μmoles of Tris-HCl buffer (pH 7.5), 2.2 μmoles of agmatine, and 1.1 mg of a crude extract of 30S0-arg 4 in a total volume of 0.3 ml were incubated at 37°C. The reactions were stopped at the indicated times by the addition of 0.02 ml of 100% (w/v) trichloroacetic acid. The 14CO2 was trapped and counted. The reaction mixtures were assayed directly for CO2, NH3 and urea (A — A) and agmatine (O — O).

TABLE IV

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>CO2 μmoles</th>
<th>NH3 μmoles</th>
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</thead>
<tbody>
<tr>
<td>10</td>
<td>0.44</td>
<td>0.84</td>
</tr>
<tr>
<td>20</td>
<td>0.59</td>
<td>1.17</td>
</tr>
</tbody>
</table>
Putrescine Biosynthesis

Putrescine and spermidine are present in E. coli at a combined concentration of approximately 0.1 μmole per mg of protein under the culture conditions described here. This means that in one generation time (approximately 60 min in minimal medium), 1 mg of cell protein must synthesize at least 0.1 μmole of putrescine. The rates in vivo of putrescine synthesis observed in cell-free extracts are compatible with this requirement. A examination of the specific activities of the three enzymes reported in this paper shows that the optimal assay conditions for the three pathways are capable of making an amount of putrescine far in excess of the cellular requirement. Ornithine dehydrogenase has a specific activity of approximately 2 μmoles/min per mg; arginine decarboxylase and agmatine decarboxylases specific activities of 0.8 and 5 μmoles/60 min per mg, respectively.

The specific activities of the enzymes of the two putrescine biosynthetic pathways, measured under conditions of optimal activity, cannot be extrapolated to their activities in vivo. However, it is possible to obtain the flux through the two pathways from the isotope competition experiments reported in this paper. From the data presented in Table I, approximately 40% of cellular putrescine was formed via the ornithine route, and 60% came from arginine when surplus precursors were present.

Radioactive anagmatine in an unlabeled pool of agmatine (Table VI). Therefore, the transformation of arginine to putrescine appears to involve decarboxylation of arginine (Equation 2) followed by hydrolysis of agmatine (Equation 3).

\[
\begin{align*}
\text{NH}_2\text{CNH}_2\text{(CH}_2\text{)}_3\text{CHCOOH} \rightarrow \text{NH}_2\text{(CH}_2\text{)}_3\text{NH}_2 + \text{CO}_2 \\
\text{NH}_2\text{(CH}_2\text{)}_3\text{NH}_2 + \text{H}_2\text{O} \rightarrow \text{NH}_2\text{(CH}_2\text{)}_3\text{NH}_2
\end{align*}
\]

In 1940, Gale reported the presence of arginine and ornithine decarboxylases in cultures of E. coli grown under special conditions (26). The biosynthetic decarboxylases described in the present paper appear to be distinct from the induced enzymes found by Gale. A previous report from this laboratory showed that the biosynthetic and induced ornithine decarboxylases differ in their pH optima, heat stabilities, and conditions for formation (1). The biosynthetic ornithine decarboxylase, postulated to be involved in the arginine to putrescine transformation, appears to differ from the arginine decarboxylases described by Gale in its pH optimum (8 as opposed to 5) and its high degree of stimulation by magnesium ion (Fig. 3) (28). Both catabolic decarboxylases are inducible and require special conditions for formation (26, 29), whereas both biosynthetic enzymes are present in cells grown in neutral minimal medium. It seems, therefore, that E. coli possesses the genetic information for two arginine and two ornithine decarboxylases.

The formation of putrescine from arginine in E. coli appears to take place by a mechanism identical with the pathway of arginine degradation by mycobacteria (25). Plants also seem to follow a similar route (30, 31). However, they hydrolyse arginine to N-carbamylputrescine with subsequent conversion to putrescine (32), rather than directly to putrescine and spermidine as in the bacterial cases. Only in E. coli has a second pathway from ornithine to putrescine been reported.

Putrescine and spermidine are present in E. coli at a concentration of approximately 0.1 μmole per mg of protein under the culture conditions described here. This means that in one generation time (approximately 60 min in minimal medium), 1 mg of cell protein must synthesize at least 0.1 μmole of putrescine. The rates in vivo of putrescine synthesis observed in cell-free extracts are compatible with this requirement. Examination of the specific activities of the three enzymes reported in this paper shows that the optimal assay conditions for the three pathways are capable of making an amount of putrescine far in excess of the cellular requirement. Ornithine dehydrogenase has a specific activity of approximately 2 μmoles/min per mg; arginine decarboxylase and agmatine decarboxylase specific activities of 0.8 and 5 μmoles/60 min per mg, respectively.

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is not surprising that the relative flux in vivo through the two pathways deviates from that predicted from the specific activities of the enzymes in crude extracts. One would expect that the activities of the enzymes in vivo would be modulated by the levels of substrates, cofactors, and inhibitors, while, under the optimal conditions of the assay in vitro, these would not be factors.

The existence of the two pathways of putrescine biosynthesis appears to provide a unique answer to the regulatory problems encountered in branched biosynthetic pathways. As can be seen from Fig. 6, the pathway of arginine biosynthesis in E. coli K-12 is indeed branched, the branch point occurring at ornithine. The most economical route of putrescine synthesis, from the standpoint of energy conservation, is the direct decarboxylation of ornithine. However, when repressible strains of E. coli are grown in the presence of arginine, the synthesis of the arginine-biosynthetic enzymes is inhibited (18-20). Since the formation of ornithine is blocked during growth on arginine, the organism can no longer synthesize putrescine from this source, but uses the now energetically favorable route from preformed arginine. Consequently, under these conditions, the conversion of arginine to putrescine is the only route of polyamine biosynthesis. This provides a reasonable explanation for the existence of the two pathways of putrescine biosynthesis.

REFERENCES

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