THE PURINE METABOLISM OF ESCHERICHIA COLI*

BY ARTHUR L. KOCH, FRANK W. PUTNAM, AND E. A. EVANS, JR.
(From the Department of Biochemistry, University of Chicago, Chicago, Illinois)

(Received for publication, January 24, 1952)

Although many tracer studies on the purine metabolism of higher organisms have been reported, there are only preliminary data (1, 2) on the purine metabolism of Escherichia coli, strain B, the host of the "T series" of bacteriophages. The work presented in this paper was undertaken concomitantly with an investigation of the purine metabolism of the virus-infected cell (3). The study of the purine metabolism of the uninfected cell was undertaken both to investigate the purine metabolism in the host and to prepare purines and bacteria for the study of the infected cell.

Bacteria were grown in synthetic medium supplemented with C\(^4\)\textsuperscript{14}-labeled substrates (CO\(_2\) or purines). The bacteria were harvested while the culture was still in the logarithmic phase of growth, and the purines from the various fractions of the cell were isolated, and the radioactivity determined. It was found that CO\(_2\) was used for the synthesis of both adenine and guanine within the cell and that extracellular adenine and guanine were absorbed, utilized, and interconverted.

EXPERIMENTAL

Eight experiments were performed. In Experiment 1, the supplement was C\(^4\)O\(_2\). In Experiments 2 to 7 and 10 the bacteria were grown in the presence of purines, as indicated in Table I.

Growth Conditions—In Experiments 2 and 3 (Table II), E. coli strain B/3,4,7 was used. However, this strain was found to be genetically unstable with respect to phage resistance, and, consequently, in the other experiments the closely related strain B/1,5 was employed.\(^1\) In Experiment 2 the medium was prepared as described by Putnam, Kozloff, and Neil (6). In the other experiments the amount of NH\(_4\)Cl was increased from 0.01 to 0.1 per cent to insure an adequate nitrogen source. Bacteria were grown with vigorous aeration at 37° to the desired titer, as deter-

* Aided by grants from The National Foundation for Infantile Paralysis, Inc., and from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. This report is from a dissertation submitted by Arthur L. Koch for the degree of Doctor of Philosophy in the Division of Biological Sciences, University of Chicago.

\(^1\) Strains B/3,4,7 and B/1,5 are both one-step mutants of strain B. Apparently the mutation only affects the adsorption of phage (4), but see Anderson (5).
mined by turbidity measurements (7), and then sedimented and washed twice with saline in the cold.

**Chemical Analysis**—The bacteria were partitioned by the Schmidt and Thannhauser method (8) as modified by Schneider. In Experiments 6 and 7 the total nucleic acids were isolated by the method of Schneider (9). The purine-containing fractions in Experiments 1 and 2 were hydrolyzed with 5 per cent H$_2$SO$_4$, the purines precipitated as the silver salts, and the free bases regenerated with HCl. In the other experiments the purine fractions were evaporated almost to dryness and hydrolyzed with perchloric acid as described by Marshak and Vogel (10, 11). The individual bases were isolated by ion exchange chromatography, under the conditions described by Cohn (12). The Dowex 50 cation exchange resin was employed in all experiments except in Experiments 7 and 10, in which Dowex 2 anion exchange resin was used to isolate the pyrimidines in addition to the purines. In Experiment 10 all the bases were further purified by chromatography on Dowex 50 columns. Adequate resolution of the ultraviolet light-absorbing materials was obtained in all cases. The amount of material was estimated with the Beckman spectrophotometer. Extinction coefficients were estimated from chromatographically pure bases.

In Experiment 10, carrier was added to samples of the pure bases for N$^{15}$ analysis. Other samples were deaminated with nitrous acid. Hypoxanthine and xanthine were then isolated from Dowex 50 columns.

**C$^{14}$ Determination**—Unlabeled purines were added as carrier to the samples and the purines were precipitated with silver nitrate in the presence of an excess of NH$_4$OH. The purines were dissolved in HCl and precipitated as above and then dried. The sample was burned in a micro combustion apparatus. Barium carbonate samples were transferred to aluminum cups of 3.5 sq. cm. area with the aid of methyl alcohol; the samples were counted six times with a flow gas counter for approximately 1000 counts. Samples were diluted so that the counting rate was between 90 and 2000 c.p.m. and were corrected to infinite thickness (13). Duplicate determinations agreed within 5 per cent. In Experiment 10, the radioactivity was determined as an infinitely thin sample on aluminum planchets. 10,000 counts were taken on all samples. Duplicate determinations agreed within 3 per cent.

**Radioactive Materials**—Radioactive purines were prepared in two ways. Bacteria were grown in the presence of C$^{14}$O$_2$ and the purines isolated (Experiment 1). The total yield of labeled purines was 0.4 per cent, based on 3 mc. of C$^{14}$O$_2$. Adenine was also synthesized from 0.82 mc. of C$^{14}$-labeled formate and 4,5,6-triaminopyrimidine by the procedure of Clark.

Kindly donated by Dr. George Bosworth Brown of the Sloan-Kettering Institute for Cancer Research. The C$^{14}$-formate and C$^{14}$O$_2$ were obtained on allocation from the Oak Ridge National Laboratory.
A. L. KOCH, F. W. PUTNAM, AND E. A. EVANS, JR.

and Kalckar (14), with a yield of 50 per cent of \( \text{C}^{14}\text{-adenine} \). The adenine was isolated chromatographically on Dowex 50 and its purity established by filter paper chromatography (15). The biosynthetically prepared purines were used in Experiments 2 and 3. C-8-labeled purines were used in the other experiments. In Experiment 7, C-8-labeled guanine, isolated from residues of Experiments 4 and 5, was used.

**Results**

**Incorporation of \( \text{CO}_2 \) into Growing *E. coli*—**Bacteria were grown in a sealed system with \( \text{C}^{14}\text{O}_2 \) counting \( 45 \times 10^6 \text{ c.p.m.} \). At the end of the growth period the specific activity of the \( \text{CO}_2 \) had been reduced to \( 1.5 \times 10^6 \text{ c.p.m.} \). Adenine and guanine from desoxyribonucleic acid (DNA) and ribonucleic acid (RNA) were isolated from the bacteria and all had a specific activity of \( 1 \times 10^6 \text{ c.p.m.} \). A sample of DNA guanine was degraded by the method of Heinrich and Wilson (16) and found to contain 94 per cent of the total activity of the purine in position 6 of the purine nucleus. Therefore, this ring carbon had a specific activity of \( 5 \times 10^6 \text{ c.p.m.} \).

**Incorporation of Purines into Growing *E. coli*—**The results of studies on the incorporation of radioactive purines are given in Table I. As the bacteria were grown from a small inoculum and the culture was harvested while still in the exponential phase of growth, the data are a measure of processes occurring in rapidly growing bacteria. The extent to which the purines present in the growth medium replace the \textit{de novo} synthesis of purines from ammonium lactate and \( \text{CO}_2 \) is a function of the concentration of the purines in the growth medium. In these experiments the final bacterial titer was between 3 and \( 5 \times 10^8 \text{ bacteria per ml.} \), which corresponds to a final total purine content of 30 to 50 \( \mu \text{M} \) per liter. In Experiments 10 and 7, medium purine was present in high concentration and completely or almost completely repressed \textit{de novo} synthesis. In the other experiments the amount of purine in the medium was lower than the final bacterial purine content, and, consequently, purines were synthesized from smaller molecules.

---

3 This experiment was conducted in collaboration with Mrs. Miriam Gollub-Banks.

4 Of the initial activity of the molecule, 4 per cent was found in C-8, 1 per cent in C-2, and 0 per cent in C-4 and C-5. 94 per cent was found in carbons 4, 5, 6. Assuming 5 per cent accuracy for the radioactive determinations, the 6 position contains 94 \( \pm 1 \) per cent of the activity of the molecule.

5 It may be calculated that 80 per cent of the C-6 position of the purine was derived from \( \text{CO}_2 \). The assumptions made in this calculation are that (1) \( \text{CO}_2 \) production per cell is constant, (2) there is no turnover of purines, and (3) \( \text{CO}_2 \) outside the cell is in rapid equilibrium with \( \text{CO}_2 \) inside the cell. In view of these assumptions 80 per cent is a minimum value.
In the third column of Table I the utilization of the radioactive substrate is computed. It is seen that in these experiments the medium was not completely depleted of the labeled purine except in Experiment 6, in which the initial adenine concentration was very small. In a qualitative experiment it was demonstrated that adenine is removed (80 to 100 per cent) from a medium initially only $1.24 \times 10^{-9}$ M in adenine. These results show that in the other experiments the purines of the medium were present throughout the course of the experiment in high enough concentration to be utilized by the bacteria.

The conversion of one purine to the other is also dependent on the purine concentration in the medium. Adenine at concentrations above 2.6 $\mu$M per liter is utilized about equally for the synthesis of cellular guanine and adenine, and below this it is used preferentially for adenine components of the cell. In these experiments non-radioactive guanine was added to the growth medium: in Experiment 4, 72.8 $\mu$M per liter, and in Experiment 5, 183.0 $\mu$M per liter.

### Table I

**Incorporation of Purines by E. coli**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Radioactive substrate $\mu$M per l.</th>
<th>Per cent derived from radioactive substrate $\times 10^9$</th>
<th>Guanine RNA</th>
<th>Guanine DNA</th>
<th>Adenine RNA</th>
<th>Adenine DNA</th>
<th>Ratio of guanine to adenine derived from medium $\times 10^9$</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>249.0</td>
<td>10</td>
<td>100</td>
<td>100</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2.6</td>
<td>53</td>
<td>4.0</td>
<td>5.5</td>
<td>4.5</td>
<td>6.1</td>
<td>0.90 $\pm$ 0.90</td>
</tr>
<tr>
<td>4§</td>
<td>1.63</td>
<td>70</td>
<td>1.72</td>
<td>1.13</td>
<td>4.13</td>
<td>2.74</td>
<td>0.41 $\pm$ 0.41</td>
</tr>
<tr>
<td>5§</td>
<td>1.63</td>
<td>56</td>
<td>1.81</td>
<td>2.11</td>
<td>4.45</td>
<td>4.93</td>
<td>0.41 $\pm$ 0.43</td>
</tr>
<tr>
<td>6</td>
<td>0.124</td>
<td>100</td>
<td>0.233</td>
<td>1.0</td>
<td>0.233</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>13.9</td>
<td>47</td>
<td>29.9</td>
<td>28.9</td>
<td>11.0</td>
<td>10.3</td>
<td>2.73 $\pm$ 2.81</td>
</tr>
<tr>
<td>7</td>
<td>304.0</td>
<td>15</td>
<td>104</td>
<td>83</td>
<td></td>
<td></td>
<td>1.25</td>
</tr>
</tbody>
</table>

* Computed from the radioactivity and the purine content of the bacteria.
† Computed as the ratio of the specific activity of the isolated purine to the administered radioactive purine times 100.
‡ Ratio of data from previous columns.
§ In these experiments non-radioactive guanine was added to the growth medium: in Experiment 4, 72.8 $\mu$M per liter, and in Experiment 5, 183.0 $\mu$M per liter.
the conversion of adenine to guanine, especially at low extracellular concentrations of guanine. When bacteria are grown on a medium containing labeled adenine and a large amount of unlabeled guanine (Experiments 4 and 5), the radioactive adenine continues to be used and converted at a high rate. The conversion of adenine to guanine is not markedly affected by the presence of guanine (compare with Experiments 2 and 6) even though de novo synthesis of both purines is probably largely repressed (compare Experiments 3 and 7).7

It will be seen from Table I that in all the experiments the ratio of the activity of guanine to that of adenine was the same for RNA as for DNA, even though the value of this ratio varies from experiment to experiment, depending on the kind and amount of the radioactive substrate. This is the more remarkable, since the ratio of the specific activity of RNA to DNA varies from 0.73 in Experiment 2 to 1.51 in Experiment 4.

In Experiment 3 the acid-soluble fraction was investigated and the radioactivity of nucleotide adenine determined to be approximately 80 per cent of that of the DNA adenine. Other fractions of the bacterial cell, protein, ether-alcohol-soluble, and pyrimidines, as well as the respiratory carbon dioxide, contained no significant radioactivity. The specific activity of these fractions was always much less than 1 per cent of the specific activity of the purines.

N15 Experiment—In Experiment 10, the bacteria were grown in a medium containing N15HCl and a large amount of radioactive adenine containing no excess N15. Under these conditions essentially all of the purines of the bacteria are derived from the adenine of the medium (see Table I). Hypoxanthine and xanthine obtained from the DNA of the bacteria contained little N15. The nitrogen atoms of the rings of adenine and guanine had less than 1 and 2 per cent, respectively, of their origin in the N15HCl of the medium. This indicates that the nitrogen atoms of the purine ring do not exchange with other nitrogenous components of the bacterial cell.

The N15 in the amino groups of the two purines was calculated by comparing the N15 content of the original and deaminated purine. Approximately 60 per cent of the amino group of guanine and 6 per cent of the amino group of adenine had been derived from the N15 of the medium. Between 85 and 90 per cent of the nitrogen of other constituents of the bacteria had been derived from the N15 of the medium. Details of this experiment are given in the accompanying paper (3) Table IV.

acid in Table I. This indicates that the low value of the specific activity ratio of guanine to adenine is not the result of the adsorption of adenine onto cells at this low concentration.

7 An experiment has subsequently been performed with the same initial concentration of adenine in the absence of guanine. The same ratio of specific activities was obtained as in Experiments 4 and 5.
Nucleic Acid Composition of E. coli—The purine composition of the RNA and DNA and acid-soluble fraction of E. coli is listed in Table II. It is seen that the composition of the DNA is more constant than that of the RNA, and the composition is not noticeably affected by the presence of purines in the medium or by strain differences. No significant amount of a guanine-containing compound is present in the acid-soluble fraction. As the number of bacteria was estimated turbidimetrically and since neither viable nor total bacterial counts were made, the purine content per bacterium may be slightly in error. This, however, does not affect the relative composition.

**Table II**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Bacterial titer, 10^6 per ml.</th>
<th>Purine concentration of medium</th>
<th>DNA</th>
<th>RNA</th>
<th>Acid-soluble</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Guanine (μM per l.)</td>
<td>adenine (μM per l.)</td>
<td>guanine/adenine</td>
<td>guanine (μM per 10^6 B)</td>
</tr>
<tr>
<td>2†</td>
<td>2.8</td>
<td>16.3</td>
<td>11.4</td>
<td>1.43</td>
<td>50.5</td>
</tr>
<tr>
<td>3†</td>
<td>3.3</td>
<td>19.1</td>
<td>13.1</td>
<td>1.46</td>
<td>35.0</td>
</tr>
<tr>
<td>4†</td>
<td>4.0</td>
<td>19.4</td>
<td>13.5</td>
<td>1.29</td>
<td>46.9</td>
</tr>
<tr>
<td>5†</td>
<td>3.0</td>
<td>15.8</td>
<td>12.4</td>
<td>1.27</td>
<td>41.2</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>17.0</td>
<td>12.6</td>
<td>1.35</td>
<td>43.4</td>
</tr>
<tr>
<td>% standard error§</td>
<td></td>
<td>10.0</td>
<td>8.0</td>
<td>1.35</td>
<td>16</td>
</tr>
</tbody>
</table>

* B signifies bacteria.
† Strain B/3,4,7.
‡ Strain B/1,5.
§ Per cent standard error = \( \sqrt{\frac{\sum \Delta^2}{(n - 1)}} \times 100/\text{average} \)

**DISCUSSION**

The conclusions that can be drawn from these results are shown schematically in Fig. 1.

In rapidly growing E. coli, carbon dioxide is used for the synthesis of the 6 position of the purines, as has previously been shown for the rat (16) and the pigeon (18, 19). It appears probable that under these conditions of growth (synthetic ammonium lactate medium and vigorous aeration) CO₂ is the major source of the C-6 position of the purine ring and is only slightly utilized for the synthesis of other positions in the purine nucleus.

The values obtained for the molar ratio of guanine to adenine differ significantly from those obtained by Smith and Wyatt (17) for the DNA of E. coli strain B/r. This probably reflects strain differences, as complete recoveries of bases were obtained in this study as shown by the absence of radioactivities in the non-purine fractions of the chromatograms.
Carbon dioxide enters to an equal extent into both purines of each type of nucleic acid. Therefore it appears likely that CO$_2$ is incorporated into a common precursor of the adenine and guanine of both DNA and RNA.

Both adenine and guanine which are present in the growth medium are incorporated into the nucleic acids of the bacteria. The facility with which purines are utilized and interconverted varies greatly from species to species (20–22). *E. coli* very effectively incorporates purines, although it does not require them. It also converts adenine to guanine with such efficiency that at moderately low levels both adenine and guanine are derived from the medium to an equal extent. The conversion of adenine to guanine is unaffected by the simultaneous incorporation of guanine. The conversion of guanine to adenine is less extensive than the reverse process. Thus the purine metabolism of *E. coli* differs from that of yeast (20), *Lactobacillus casei* (21), and *Tetrahymena geleii* (22).

The finding that neither adenine nor guanine present in the medium at low concentration is equally used for the synthesis of both purines in the nucleic acids of the cell indicates that neither purine is the sole precursor of the other. This suggests that preformed purines enter the purine metabolism of the cell at a later step than that of the "common purine precursor." Possibly isotope initially present in adenine appears in guanine through a reversal to this "common purine precursor," which is subsequently converted to guanine.

In Fig. 1 the stages in purine synthesis in which purines of the medium enter the de novo pathways of synthesis have been designated "intermediates." Nothing is implied of their chemical constitution. The fact that adenine is utilized by the bacteria when present in extremely low concentrations suggests that the free bases themselves are not the "intermediates." From the N$^{15}$ experiment, the conclusion is drawn that the amino group of preformed adenine is maintained in the formation of the DNA adenine of
the cell. Therefore, the “adenine intermediate” must contain the amino group. The small amount of N⁶ present in the amino group of DNA adenine may be attributed to a small degree of deamination and reamination.

From the observation that the ratios of the specific activity of guanine to adenine in the two types of nucleic acid are equal in any experiment, in spite of the large variation of their numerical value, it may be inferred that purines from the “intermediate” pools are common to the synthesis of both types of nucleic acids.

SUMMARY

In rapidly growing Escherichia coli CO₂ is incorporated almost exclusively into the C-6 position of all purines.

Adenine and guanine in the growth medium are utilized for the synthesis of purine compounds in the cell.

Adenine and guanine are interconverted by the cell to an extent which varies with the type and concentration of the purine in the medium.

The purines are drawn from a pool of common intermediates for the synthesis of both types of nucleic acids.

Radioactive purines and bacteria labeled specifically in the purines were prepared for the study of purine metabolism during phage growth.

BIBLIOGRAPHY

THE PURINE METABOLISM OF ESCHERICHIA COLI
Arthur L. Koch, Frank W. Putnam and E. A. Evans, Jr.


Access the most updated version of this article at http://www.jbc.org/content/197/1/105.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/197/1/105.citation.full.html#ref-list-1