Biosynthesis of Ribose and Deoxyribose in Escherichia coli*

Fillmore K. Bagatell,† Elmer M. Wright,‡ and Henry Z. Sable§

From the Department of Biochemistry, Western Reserve University, School of Medicine, Cleveland, Ohio

(Received for publication, January 23, 1959)

The studies reported here are concerned with the biosynthesis of ribose and deoxyribose in Escherichia coli R-2. When these studies were undertaken the evidence available allowed no general conclusions to be drawn as to a common biosynthetic pathway for the two pentoses. Since that time numerous studies have contributed to a better understanding of the problem, Tracer studies on the origin of ribose in glucose-grown E. coli by Lanning and Cohen (1) and Bernstein (2) indicate that the sugar arises to a large extent from the oxidative decarboxylation of 6-phosphogluconate to ribulose 5-phosphate (hexose monophosphate oxidation).1 Sowden et al. (3) and David and Renault (8) on the basis of tracer studies with glycine-C14, considered the formation of pentose by this oxidative decarboxylation as sole carbon source. All cultures required vigorous aeration which was provided by continuous swirling maintained by a reciprocal shaker. The stock culture was maintained on nutrient agar slants. In order to adapt the cells to grow well on acetate, the culture was transferred from the slant to the acetate-salts medium containing 0.05 per cent glucose and was grown for 18 hours. A 10 per cent inoculum was then added to fresh acetate-salts medium without glucose. The second culture was used to inoculate additional acetate-salts medium, and the third culture was used to inoculate the medium used in the tracer experiments. The optical density of the cultures was measured in a Klett-Summerson photometer with a No. 66 filter. Cells were harvested by centrifugation at 2°C and were washed twice with 1 per cent KCl and twice with distilled water. The cells were extracted twice with ethanol-ether (3:1) at 37°C, and then twice with cold 5 per cent trichloroacetic acid. The trichloroacetic acid extracts were combined and neutralized with NaOH to pH 8.5. These extracts contain acid soluble hexose phosphates and some polysaccharide.

Separation of Cellular Constituents

Hexose Phosphates—Preliminary experiments indicated that the amount of hexose phosphates present in 10 gm. of cells was very small, and therefore 0.5 mmole each of glucose-6-P, fructose-6-P, and HDP3 were added to the neutralized trichloroacetic acid extract and the hexose phosphates were separated on a column of Dowex 1-chloride as described by Bartlett.4

1 The term hexose monophosphate oxidation is used here to describe the formation of pentose by this oxidative decarboxylation and excludes any resynthesis of hexose.
2 The designation TK-TA is used here to refer only to the conversion of hexose to pentose and not the reverse, according to the equation 2H6O + 3 pentose = hexose + 3 pentose.
3 The abbreviation used is: HDP, fructose 1,6-diphosphate.
4 G. R. Bartlett, unpublished procedure.
RNA—The residue of the trichloroacetic acid extracts was suspended in 1 N KOH (1 ml. per 100 mg. of original wet weight of cells), and incubated at 37° for 18 hours. The solution was chilled and adjusted to pH 6.5 by addition of 1 N HCl. The precipitate which formed contained DNA, protein, and polysaccharide and was collected by centrifugation in the cold and washed once with water. The supernatant solution and wash water, which contained ribonucleotides were combined. The ribonucleotides were absorbed on charcoal (Darco G-60) and eluted with ammoniacal ethanol as a means of removing salts before separation on Dowex 1-chloride according to the procedure of Cohn (17).

DNA—The precipitate obtained in the HCl step described above was dissolved in water by adjusting the pH to 7.0. Three mmoles of sodium acetate and 400 mmoles of mixed ribonucleotides were added, and the material was then reprecipitated with acid. The precipitate was redissolved and the whole process was then repeated. Finally, the precipitate was redissolved, carrier, "cold" acetate and ribonucleotides were again added and then the solution was dialyzed overnight against cold distilled water. The solution was made 5 mM in MgC12, adjusted to pH 7.2, deoxyribonuclease was added, and the solution was incubated at 37° until all the deoxyribose in an aliquot was soluble in 5 per cent HClO4. Trichloroacetic acid was added to a final concentration of 5 per cent and the precipitate containing protein and polysaccharide was removed by centrifugation. The supernatant solution was extracted with ether, concentrated, adjusted to pH 9.0, and treated with phosphodiesterase (18). The nucleotides were absorbed on charcoal, eluted with ammoniacal ethanol, and separated on columns of Dowex 1-acetate (19) by a gradient elution procedure.

Isolation and Degradation of Cellular Sugars—Purine ribonucleotides were combined, the ribose determined, and the nucleotides diluted with known amounts of nonradioactive nucleotides, and then hydrolyzed with 1 N H2SO4 at 100° for 2 hours. The solution was deionized with Dowex 50-H+ and Duolite A-4 OH- resins and the residual purine bases were removed by centrifugation. The supernatant solution was then treated with carrier, "cold" acetate and ribonucleotides were added, and the sugar purified further by chromatography on a cellulose column. The distribution of C14 was determined as described by Bernstein et al. (20) or Topper and Hastings (21).

Hexose monophosphates were converted to glucose as follows. To the mixed hexose monophosphates obtained from the column were added barium acetate, phosphohexose isomerase, and a seed crystal of Ba glucose-6-P. The combination of the enzymatic action with constant removal of Ba glucose-6-P by crystallization converted most of the hexose monophosphate to glucose-6-P. After removal of Ba++ with Dowex 50-H+, the glucose-6-P was hydrolyzed with phosphatase and the glucose purified and degraded as described above. Hexose diphasate was converted to fructose by treatment with the phosphatase. This sugar was also purified by chromatography on a cellulose column and degraded with Leuconostoc mesenteroides.

Determination of Radioactivity—Radioactivity was determined on BaO3 plates with a windowless gas flow counter to a standard counting error of 3 per cent or less. Self-absorption corrections to infinite thinness were made. In the case of low activity samples (hexose phosphates) the counting was performed in a gas phase counter (22).

Quantitative Analysis—Ribose was determined by the method of Mejaub (29) with ribose as standard, and deoxyribose by the method of Stumpf (30) with thymidine as a standard. Glucose was determined by the method of Somogyi (31) or by an anthranic method (32). Purines, pyrimidines, and their derivatives were assayed quantitatively in a Beckman DU spectrophotometer. The molar extinction coefficients used were those reported by Beaven et al. (33).

RESULTS

Distribution of C14 in Sugars Derived from E. coli Grown on Acetate-1-C14—A typical experiment was carried out as follows. To 1500 ml. of salts-acetate medium in a 6-l. Erlenmeyer flask, 200 μC. of acetate-1-C14 were added. The medium was inoculated with 150 ml. of a 20-hour culture grown in nonisotopic medium, and the cells were permitted to grow for 22 hours with shaking. Ribose and deoxyribose were isolated from the culture. The results of two such experiments are summarized in Table I. It is usually considered that the administration of acetate-1-C14 to animals gives rise to glucose-3,4-C14 and on the basis of Bernstein's results (2) we had expected that the ribose would be formed by hexose monophosphate oxidation and be labeled only in positions 2 and 3, but the ribose was labeled in positions 1, 2, and 3. The possibilities existed that under our experimental conditions the E. coli had employed another mechanism of pentose synthesis, or that the glucose formed from acetate was not labeled exclusively in positions 2 and 3. Therefore, in the remaining experiments, the polyglucosan as well as the RNA and DNA were isolated from the culture. One of these experiments is summarized in Table II. Glucose-3,4-C14 was produced and the ribose and deoxyribose were labeled similarly to those from the 22-hour culture.

In the experiments described above, isotopic acetate was present in the medium at the time of inoculation. It was conceivable that during the lag phase one set of biosynthetic re-
300 ml. of a 20-hour culture of acetate-grown E. coli. After 8
lated from this culture. Unfortunately, the level of radioactivity
distribution in the pentoses, glycogen, and hexose phosphate
that at the time of addition of the tracer not more than 30 per
cent of the initial acetate had been utilized. Ribose, deoxy-
ribose was almost identical, which suggested that both pentoses
had a common origin or that ribose was reduced to
deoxyribose. Racker (7) suggested that deoxyribose might be
synthesized by the condensation of triose phosphate and acetale-
dehyde. An abbreviated scheme is presented in Fig. 1 to show
that hexose-6-C\textsuperscript{14} would give rise to acetaldehyde-2-C\textsuperscript{14} and triose
phosphate-3-C\textsuperscript{14}, and that by the condensation of these com-
pounds deoxyribose-2,5-C\textsuperscript{14} would be produced. It therefore
appeared likely that the use of glucose-6-C\textsuperscript{14} would permit a
better assessment of the contribution of the condensation path-
way in the biosynthesis of deoxyribose than was possible from
the data obtained with acetate-1-C\textsuperscript{14}. An experiment was car-
ried out in which the cells were grown in nonradioactive acetate,
and when the culture was in the logarithm stage of growth
tracer glucose-6-C\textsuperscript{14} was added in two portions. Approximately
90 per cent of the tracer was added at once and the culture al-
lowed to grow for an additional 2.5 hours during which time the
optical density increased about 40 per cent. It was expected that
this first portion of tracer would introduce significant
amounts of C\textsuperscript{14} into the RNA, DNA, and polyglucosan. The
remaining 10 per cent of the tracer was then added to the medium
and 5 minutes later growth was stopped by acidification to pH
5.0. The last portion of tracer presumably served to intro-
duce measurable amounts of C\textsuperscript{14} into the hexose phosphate pools.
The ribose, deoxyribose, polyglucosan glucose, and hexose phos-
phates were isolated and degraded. The results are presented
in Table III. All the sugars were labeled principally in the last
carbon atom. The ribose and deoxyribose were labeled almost
identically with C-1 containing about 10 per cent and C-5 about
90 per cent of the radioactivity. Thus the results again indicate
that the deoxyribose is formed by reduction of ribose or from
some common precursor. If the pathway suggested by Racker
was important, there should have been a large amount of radio-
activity in C-2, as illustrated in Fig. 1. Since the radioactivity
of C-2 of deoxyribose was very low in comparison with C-5, this
experiment does not indicate a significant participation of the
actions is favored, which leads to 3,4-labeled hexose, while in
the logarithmic phase, when large quantities of nucleic acids are
synthesized different reactions prevail and therefore the poly-
glucon does not represent the distribution of isotope in the
hexoses utilized for pentose synthesis. This hypothesis was
tested by adding acetate-1-C\textsuperscript{14} to the medium while the culture
was in the logarithmic phase of growth and examining the C\textsuperscript{14}
distribution in the pentoses, glycogen, and hexose phosphate
pools. Subsequent analysis of an aliquot of the culture showed
that at the time of addition of the tracer not more than 30 per
cent of the initial acetate had been utilized. Ribose, deoxy-
ribose, polyglucosan glucose, and hexose phosphates were iso-
lated from this culture. Unfortunately, the level of radioactivity
found in the hexose phosphates was not sufficiently high for
accurate counting. The C\textsuperscript{14} distribution in the glucose, ribose,
and deoxyribose was almost identical with that found in the 8-
hour culture reported in Table II. The results of this experi-
ment indicate that the biosynthetic pathways are similar in the
lag and logarithmic phases of growth.

Distribution of C\textsuperscript{14} in sugars Derived from E. coli Grown on
Acetate in Presence of Tracer Amount of Glucose-6-C\textsuperscript{14}—In all the
experiments with acetate-1-C\textsuperscript{14}, the C\textsuperscript{14} distribution in ribose
and deoxyribose was almost identical, which suggested that both
pentoses had a common origin or that ribose was reduced to
deoxyribose. Racker (7) suggested that deoxyribose might be
synthesized by the condensation of triose phosphate and acetale-
dehyde. An abbreviated scheme is presented in Fig. 1 to show
that hexose-6-C\textsuperscript{14} would give rise to acetaldehyde-2-C\textsuperscript{14} and triose
phosphate-3-C\textsuperscript{14}, and that by the condensation of these com-
pounds deoxyribose-2,5-C\textsuperscript{14} would be produced. It therefore
appeared likely that the use of glucose-6-C\textsuperscript{14} would permit a
better assessment of the contribution of the condensation path-
way in the biosynthesis of deoxyribose than was possible from
the data obtained with acetate-1-C\textsuperscript{14}. An experiment was car-
ried out in which the cells were grown in nonradioactive acetate,
and when the culture was in the logarithm stage of growth
tracer glucose-6-C\textsuperscript{14} was added in two portions. Approximately
90 per cent of the tracer was added at once and the culture al-
lowed to grow for an additional 2.5 hours during which time the
optical density increased about 40 per cent. It was expected that
this first portion of tracer would introduce significant
amounts of C\textsuperscript{14} into the RNA, DNA, and polyglucosan. The
remaining 10 per cent of the tracer was then added to the medium
and 5 minutes later growth was stopped by acidification to pH
5.0. The last portion of tracer presumably served to intro-
duce measurable amounts of C\textsuperscript{14} into the hexose phosphate pools.
The ribose, deoxyribose, polyglucosan glucose, and hexose phos-
phates were isolated and degraded. The results are presented
in Table III. All the sugars were labeled principally in the last
carbon atom. The ribose and deoxyribose were labeled almost
identically with C-1 containing about 10 per cent and C-5 about
90 per cent of the radioactivity. Thus the results again indicate
that the deoxyribose is formed by reduction of ribose or from
some common precursor. If the pathway suggested by Racker
was important, there should have been a large amount of radio-
activity in C-2, as illustrated in Fig. 1. Since the radioactivity
of C-2 of deoxyribose was very low in comparison with C-5, this
experiment does not indicate a significant participation of the

\begin{table}
\centering
\caption{Distribution of C\textsuperscript{14} in pentoses from 22-hour cultures of
E. coli grown on acetate-1-C\textsuperscript{14}}
\begin{tabular}{|c|c|c|c|}
\hline
Carbon No. & Relative specific activity* & & \\
\hline & Purine-bound & Pyrimidine-bound & Deoxyribose & \\
& ribose & ribose & & \\
\hline 1 & 23 & 11 & 24 & \\
2 & 45 & 46 & 47 & \\
3 & 100 & 100 & 100 & \\
4 & 1.3 & 2.5 & 1.5 & \\
5 & 1.5 & 3.3 & 4.0 & \\
\hline
\end{tabular}
\end{table}

* For each sugar the carbon atom with the highest specific activity is arbitrarily assigned a value of 100.
\dag Average value from two separate experiments.

\begin{table}
\centering
\caption{Distribution of C\textsuperscript{14} in sugars synthesized by E. coli from
acetate-1-C\textsuperscript{14} in 8-hour growth period}
\begin{tabular}{|c|c|c|c|}
\hline
Carbon No. & Relative specific activity & & \\
& Glycogen & Ribose & Deoxyribose & \\
\hline 1 & 1.6 & 23.7 & 21.9 & \\
2 & 3.1 & 49 & 47 & \\
3 & 100 & 100 & 100 & \\
4 & 97.5 & 8.7 & 1.7 & \\
5 & 0 & 3.4 & 3.2 & \\
6 & 0 & & & \\
\hline
\end{tabular}
\end{table}

* For each sugar the carbon atom with the highest specific activity is arbitrarily assigned a value of 100.
Cells were adapted to grow on acetate by repeated transfer in liquid culture as described in the text. 180 ml. of a 22-hour culture were inoculated into 1600 ml. of nonradioactive acetate-salts medium. The optical density immediately after inoculation was 10 Klett units. After 5.8 hours, when the optical density had reached 71 Klett units, 30 mg. of glucose containing 105 PC. of glucose-6-C14 were added. Five minutes after this glucose-6-C14 were added (with the counting equipment used 1 PC. = 4.5 x 10^6 c.p.m.). Growth was permitted to continue for another 2.5 hours, at which time the optical density was 90 Klett units and an additional amount of 3 mg. of glucose containing 10.5 PC. of glucose-6-C14 were added. Five minutes after this addition, growth was stopped by acidification of the medium. The sugars were isolated and degraded as described in the text.

The hexose monophosphate and hexose diphosphate pools were isolated by a carrier technique, therefore the specific activities of these pools could not be determined.

The total radioactivity in the glucose-6-C14 added in the experiment was 5.4 x 10^6 c.p.m. The acetate remaining in the medium at the end of the experiment was isolated and was found to contain a total of 7.8 x 10^6 c.p.m., indicating that at least 14 per cent (and probably much more) of the glucose added was metabolized by the cells to a 2-carbon unit in metabolic equilibrium with acetate. Therefore, it appears quite certain that the 2-carbon unit would be labeled and that the deoxyribose was not formed by CB and CZ condensation. In this laboratory, the biosynthesis of deoxyribose by E. coli infected with T2H bacteriophage has been studied. E. coli was grown on nonisotopic acetate, transferred to glucose medium, and was found to contain a total of 7.8 x 10^6 c.p.m. The acetate re-oxidized from 3,4-labeled hexose. It is seen in Table IV that a second mole of hexose phosphate, under the influence of transketolase-catalyzed reaction of a triose phosphate and hexose phosphate-1,2-C14. In Step II the tetrose phosphate reacts with a second mole of hexose phosphate, under the influence of transaldolase, to yield (H) heptulose phosphate-3-C14 and triose phosphate. In Step III, again catalyzed by transketolase the products of Step II react to yield (2) pentose phosphate-1,2,3-C14 and (3) pentose phosphate-3-C14.

**TABLE III**

<table>
<thead>
<tr>
<th>C atom</th>
<th>Relative specific activity</th>
<th>C atom</th>
<th>Relative specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>Hexose monophosphate</td>
<td>Hexose diphosphate</td>
<td>Ribose</td>
</tr>
<tr>
<td>1</td>
<td>15.4</td>
<td>21</td>
<td>14.4</td>
</tr>
<tr>
<td>2</td>
<td>7.2</td>
<td>8.7</td>
<td>4.5</td>
</tr>
<tr>
<td>3</td>
<td>5.2</td>
<td>6.0</td>
<td>4.6</td>
</tr>
<tr>
<td>4</td>
<td>0.8</td>
<td>3.8</td>
<td>3.5</td>
</tr>
<tr>
<td>5</td>
<td>6.5</td>
<td>4.4</td>
<td>3.5</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

- a C-5 of ribose = 6.44 x 10^6 c.p.m. per mmole of carbon.
- b C-5 of deoxyribose = 3.9 x 10^6 c.p.m. per mmole of carbon.
- c C-6 = 13 x 10^6 c.p.m. per mmole of carbon.
- d The hexose monophosphate and hexose diphosphate pools were isolated by a carrier technique, therefore the specific activities of these pools could not be determined.

**Hypothetical C14 distribution in pentose formed from hexose-3,4-C14 by various combinations of oxidative and nonoxidative pathways.**

The TK-TA sequence is considered to yield a C14 distribution of 33.3:33.3:100 in C-1, C-2, and C-3, respectively, and the hexose monophosphate oxidation is considered to yield a distribution of 0:100:100, from glucose-3,4-C14. For example, in the case proposed where there is a 3:1 preponderance of the TK-TA sequence, the calculation is made as follows: C-1 = 33.3 x 3 + 0 x 1 = 33.3; C-2 = 33.3 x 3 + 100 x 1 = 100; C-3 = 100 x 3 + 100 x 1 = 100.

**TABLE IV**

<table>
<thead>
<tr>
<th>Contribution made by</th>
<th>Relative specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TK-TA pathway</td>
<td>HMP oxidative pathway</td>
</tr>
<tr>
<td>1</td>
<td>10.7</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>26.6</td>
</tr>
<tr>
<td>5</td>
<td>29.3</td>
</tr>
</tbody>
</table>

**Discussion**

Bernstein (2) found that in E. coli, glucose-3,4-C14 gives rise exclusively to ribose-3-C14. Our finding that the pentoses were labeled in the ratio of approximately 25:50:100 in carbons 1, 2, and 3 was therefore unexpected although Bernstein (34) had reported a very similar isotope distribution in the pentose of rats to which NaHCO3 had been administered. It is apparent that neither HMP oxidation nor TK-TA pathways (Fig. 2) alone can account for the isotope distribution observed, but a combination of the two pathways could explain the labeling in pentose produced from 3,4-labeled hexose. It is seen in Table IV that a
The preponderance of TK-TA over HMP oxidation of about 3:1 is compatible with the data obtained. A similar argument has been used by Marks and Frndlson (35) to explain their data. The absence of significant amounts of label in C-1 and C-2 of glycerogen suggests that very little resynthesis of hexose from pentose has occurred in these cells.

The experiment in which a tracer amount of glucose-6-C\textsuperscript{14} was added to a culture of E. coli growing in salts-acetate medium permits several other conclusions to be drawn: (a) the distribution of C\textsuperscript{14} in the hexose phosphate pools indicates that the polyglucosan accurately reflects the C\textsuperscript{14} distribution of the hexose phosphates, and (b) on the basis of the C\textsuperscript{14} distribution in the glucose derived from the polyglucosan and from the hexose phosphates, carbon 1 of hexose contributes to carbon 1 of pentose; again indicating a significant contribution of the TK-TA sequence in E. coli growing on acetate.

The similarity of C\textsuperscript{14} distribution in the purine- and pyrimidine-bound ribose was to be expected. The compound 5-phosphoribosyl-1-pyrophosphate has been implicated in the synthesis of both pyrimidine nucleotides (37) and purine nucleotides (38), and the existence of a common precursor suggests that ribose when formed is utilized for synthesis of all ribose-containing compounds in the cells. This concept has been borne out in these studies. All our experiments indicate that ribose and deoxyribose arise from a common precursor or that the ribose gives rise to deoxyribose. In experiments in which it was possible to calculate the actual radioactivity of the sugars synthesized in the cells, the specific activity of the ribose was always greater than that of the deoxyribose, so that one may postulate that ribose is a precursor of deoxyribose and not the reverse. This supports the finding of Grossman and Hawkins (14) that ribose derivatives are reduced enzymatically to deoxyribose derivatives. Bernstein (39) has drawn the same conclusion on the basis of studies with E. coli utilizing variously labeled glucose and lactate. The possibility remains that deoxyribose is synthesized by a different mechanism in other organisms. Shreve and Grossman (8) and Bernstein (39) have reported that the pattern of isotope distribution in deoxyribose differs from that of ribose in rats fed C\textsuperscript{14}-labeled glycine and NaHCO\textsubscript{3}. Horecker et al. (40) have recently reported that the ribose and deoxyribose of regenerating liver and mammalian tumor cells show moderate differences in their labeling patterns. They present arguments in favor of the conversion of ribose to deoxyribose, and believe that the deoxyribose aldolase mechanism is probably not very important in the biosynthesis of deoxyribose in these tissues. It should be noted, however, that recent enzymatic studies by Doxer and Snouk (41) suggest that deoxyribose aldolase may be involved in normal liver and malignant hepatomas. The latter authors propose an important role for threonine aldolase in supplying acctaldehyde. These considerations may also have a bearing on the present work, because of the prevailing uncertainty as to whether a pyruvic carboxylate is present in E. coli.

**SUMMARY**

1. The synthesis of ribose and deoxyribose in E. coli grown in a synthetic medium for varying lengths of time has been studied. In all the studies sodium acetate was the sole source of carbon. When either acetate-1-C\textsuperscript{14} or glucose-6-C\textsuperscript{14} was the tracer, the distribution of C\textsuperscript{14} in the deoxyribose was very similar to that of the ribose, supporting the concept of reduction of ribose to deoxyribose. A combination of the transketolase-transaldolase and hexose monophosphate oxidative pathways was found to best fit the data obtained.

2. The polyglucosan-glucose and hexose monophosphate pools were found to have a similar distribution of C\textsuperscript{14}, indicating the reliability of the use of polyglucose compounds as indicators of the isotope distribution within the hexose phosphate pools.

3. The data suggest that the condensation of 3-carbon and 2 carbon fragments did not occur to an appreciable extent in the synthesis of deoxyribose in these cells.

**Acknowledgment** —The expert technical assistance of Miss Joy L. Bailey in many of these experiments is acknowledged.

**REFERENCES**


\* The authors are grateful to the reviewing editor for calling this matter to their attention.
Biosynthesis of Ribose and Deoxyribose in *Escherichia coli*

Fillmore K. Bagatell, Elmer M. Wright and Henry Z. Sable