The Origin of Purine and Pyrimidine Deoxyribose in T6r+ Bacteriophage*

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(Received for publication, August 28, 1958)

Lanning and Cohen (1) reported that when Escherichia coli strain B is infected with T6r+ bacteriophage in a medium containing glucose-1-C14, the deoxyribose from the deoxyribonucleic acid of the T6r+ particles liberated on lysis of the cells contains 40 to 60 per cent of the specific activity of the exogenous glucose. In parallel experiments, it was shown that the deoxyribose from the deoxyribonucleic acid of growing, uninfected cells contains only 20 to 30 per cent of the specific activity of the exogenous glucose-1-C14. Thus, the infection process, which calls for an increased rate of deoxyribose synthesis, also results in a shift of pathways used for deoxyribose formation from glucose. However, it remained to be shown whether this shift is true of all T6r+ deoxyribose or of only some of the T6r+ deoxyribose. It is of interest to know, for example, whether pyrimidine deoxyribose is made by a different pathway from purine deoxyribose. Experiments performed to decide this point are described in this paper.

Cells grown in C14-glucose were infected with T6r+ particles in media containing glucose-1-C14 as sole carbon source. The T6r+ DNA produced under these conditions was isolated and hydrolyzed to the deoxyribonucleoside level. The specific activity of the deoxyribose moieties of each of the four deoxyribonucleosides was then determined.

Another aspect of deoxyribose synthesis concerns the nature of the deoxyribose precursor. There is much evidence to suggest that this is a ribose compound, i.e. that adenosine 5' phosphorylase is the precursor of deoxyadenosine 5'-phosphate. This hypothesis was tested in a series of experiments reported in this paper. Lastly, this paper also describes the characterization of a new compound isolated uniquely from enzymatic hydrolysates of T6r+ deoxyribonucleic acid, namely, 5-hydroxymethyl-(0-diglucosyl)-cytosine deoxyribonucleoside.

Before these experiments were performed, some aspects of the chemistry and metabolism of T6r+ deoxyribonucleic acid had to be considered. First, T6r+ deoxyribonucleic acid contains the base, hydroxymethylcytosine, instead of cytosine (2). Moreover, most of this hydroxymethylcytosine is glucosylated, i.e. it has attached to its hydroxymethyl group glucosyl moieties (3). When T6r+ deoxyribonucleic acid is hydrolyzed to the nucleotide level, small amounts of hydroxymethylcytosine nucleotides are released. About one-half of this hydroxymethylcytosine fraction contains diglucosylated nucleotide, another half contains unsubstituted nucleotide, and a very small portion consists of monoglucosylated nucleotide.1 The glucosylation of the hydroxymethylcytosine results in a partial resistance of T6r+ deoxyribonucleic acid to hydrolysis by deoxyribonuclease and phosphatases. Moreover, most of the hydroxymethylcytosine residues of the deoxyribonucleic acid are found in the enzymatically resistant core, which also contains some guanine, adenine, and thymine residues. Thus, how yields of hydroxymethylcytosine deoxyribonucleoside are obtained after enzymatic digestion, and further chemical attack on the core is necessary to increase this yield. Secondly, under the experimental conditions reported in this paper, about 23 per cent of the T6r+ deoxyribose is derived from the host, and the remainder from the exogenous glucose (1). Therefore, the data must be corrected for host contributions to T6r+ deoxyribonucleic acid. Thirdly, the infected cell synthesizes excess T6r+ deoxyribonucleic acid (4), i.e. phage deoxyribonucleic acid that is found in the medium, but not within phage particles. This excess deoxyribonucleic acid can be isolated and used as a further source of hydroxymethylcytosine deoxyribonucleoside (5) which is otherwise difficult to obtain.

EXPERIMENTAL

Infection and isolation of C14-labeled T6r+ were carried out in the following manner. E. coli strain B was vigorously aerated in 1 l. of mineral medium (6) containing 1 mg. per ml. of glucose and allowed to grow from 5 x 10⁸ cells per ml. to 2 x 10⁹ cells per ml. These cells, collected by centrifugation and washed with mineral medium, were then resuspended in 1 l. of mineral medium containing 3 mg. per ml. of glucose-1-C14 with a specific activity of 800 c.p.m. per mmole. 50 mg. of L-tryptophan in 10 ml. of 0.02 N KOH were added followed by a 5-fold multiplicity of T6r+ particles. The culture was vigorously aerated at 37° for 6 to 8 hours, by which time lysis had occurred. T6r+ particles were collected by 2 cycles of differential centrifugation, and the DNA was obtained by the urea method (7), modified as follows. T6r+ particles were suspended in a volume of 0.05 M tris(hydroxymethyl)aminomethane at pH 7.5 containing 1 M NaCl.

In unpublished observations by S. S. Cohen and J. Lichtenstein, comparable ratios of non-, mono-, and diglucosylated derivatives of the hydroxymethylcytosine nucleotide have been observed in both r+ and r preparations of T6 bacteria. A similar result concerning the presence of the three nucleotides was reported orally by M. Jesaitis at the Philadelphia meeting of the American Society of Biological Chemists in May, 1958.

* This research was supported by the Commonwealth Fund and the Upjohn Company. The data presented in this paper are taken from a dissertation presented by Marilyn R. Loeb to the Faculty of the Graduate School of Arts and Sciences, the University of Pennsylvania, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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0.85 per cent NaCl such that the final concentration of DNA, as determined by the diphenylamine reaction (8), was less than 0.2 mg. per ml. To each 10-ml. of suspension were added 5 gm. of deionized urea. This caused a 1.4-fold increase in volume so that the final urea concentration was 6 M. The suspension was warmed to 37° and then incubated at this temperature for 2 hours. During this time lysis of more than 99 per cent of the phage occurred with a consequent drop in light scattering by the suspension. The viscosity of the solution increased greatly. 1.4 gm. of NaCl for each 10 ml. of original suspension, i.e. the volume before the addition of the urea, were added. This further reduced the slight turbidity by dissociating nucleoprotein particles. The mixture was then deproteinized by the procedure of Sevag et al. (9) by shaking with an equal volume of an 8:1 mixture of chloroform and caprylic alcohol, centrifuging, and removing the top layer of solution. This solution contained the nucleic acid; it was treated one to two more times in a similar manner to remove protein. Addition of 4 volumes of ethanol precipitated the highly polymerized DNA which was collected around a stirring rod, washed in 70 per cent, 95 per cent, and absolute ethanol successively, and then dried in a vacuum. All preparations from T-even phage made by this method have been highly viscous and have had high molecular weights, estimated from sedimentation and viscosity data.3

The DNA was then hydrolyzed with DNase and calf intestinal phosphatase as described in the previous paper (10). The hydrolysate was reduced to a small volume (approximately 0.5 ml) and extracted three times with absolute ethanol at 50°. This extract contained deoxyribonucleosides, and the non-extractable residue contained the HMC-rich, enzyme-resistant core. In order to obtain HMC and HDR, this DNA core was hydrolyzed in 1.5 N HClO₄ for 1 hour at 100° as described by Cohen (11). The solution was brought to neutrality by addition of 6 x KOH, and after removal of the KClO₄ precipitate, the hydrolysate was treated with calf intestinal phosphatase in order to convert hydroxymethyldeoxyxycytidylic acid to HDR. The hydrolysate was then chromatographed on paper in butanol-ammonia (12). Three bands were obtained, the first of which consisted of HMC, HDR, and guanine and had an Rf of 0.05 to 0.15. The second band was adenine (Rf, 0.30 to 0.40), and the third band was thymine and thymidine (Rf of 0.45 to 0.55). The material in the HMC-HDR-guanine band was eluted and chromatographed on paper in ethanol-acetic acid (13) to yield HDR (Rf, 0.55), HMC (Rf, 0.45), and guanine (Rf, 0.35).

The excess DNA mentioned above was obtained from the lysate after removal of cell debris and T6r+ particles by the method of Green et al. (5).

A summary of the various fractionation procedures employed appears in Fig. 1. The enzymatically released deoxyribonucleosides were applied to a Dowex 1-formate column and eluted as described in the previous paper (10), the only difference being that HDR was obtained in place of CDR. Each of these deoxyribonucleosides was then chromatographed on paper with the following solvent systems: thymidine in butanol-ammonia (12), GDR and HXDR each in water-isoamyl alcohol, and HDR in ethanol-acetic acid (13). The water-isoamyl alcohol solvent is prepared in the same way as the 5 per cent Na₂HP₂O₇-isoamyl alcohol solvent of Carter (14) and is used instead in order to avoid contamination of the nucleosides with phosphate. All of the deoxyribonucleosides gave one spot except HDR which yielded two. This apparent anomaly will be discussed at length below.

The specific activities of the deoxyribose moieties of GDR, HXDR, and thymidine were determined by enzymatically transferring the deoxyribose moieties to a free base to form a

\[ \text{5-hydroxymethyl-(O-diglucosyl)-cytosine deoxyribonucleoside; } \\
\text{CDR, cytosine deoxyribose; } \\
\text{GDR, guanine deoxyribose; } \\
\text{HXDR, hypoxanthine deoxyribonucleoside; and UDR, uracil deoxyribonucleoside.} \]
"new nucleoside," and then measuring the specific activity of this new nucleoside. These reactions were mediated by the nucleoside phosphorylase system in E. coli extracts, and the full details are presented in the previous paper (10). Thus, when thymidine, which contained C14 in both its base and deoxyribose moieties, was incubated with uracil and enzyme, UDR, labeled only its deoxyribose moiety, was formed.

However, this enzymatic method cannot be used with HDR, since HDR is not acted upon by the nucleoside phosphorylase of E. coli. Moreover, although 5-hydroxymethyluracil deoxyribonucleoside, the deamination product of HDR, can be cleaved by nucleoside phosphorylase, attempts to transfer its deoxyribose moiety to a free base (thymine) have been unsuccessful. Therefore, the specific activity of the deoxyribose moiety of HDR was determined as the difference in specific activity between HDR and HMC. This in itself presented difficulties since the exact molar extinction coefficient of HDR is not known. A value of 11.7 × 103 at pH 1 and 2800 A was used, based on the determination of Flaks and Cohen (15) for hydroxymethyl-deoxytacetic acid.

Reducing sugar was determined by the anthrone procedure (16, 17). Pyrimidine-bound deoxyribose was determined by the tryptophan-perchloric acid method (18).

RESULTS

Specific Activity of Deoxyribose Moieties—When the four deoxyribose moieties of the T6r+ DNA obtained from 2 l. of

<table>
<thead>
<tr>
<th>Original deoxyribonucleoside</th>
<th>New deoxyribose moiety</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Corrected specific activity</td>
<td>Per cent* specific activity</td>
</tr>
<tr>
<td>TDR . . . . UDR . . . . . . .</td>
<td>393 49</td>
<td>430 54</td>
<td></td>
</tr>
<tr>
<td>HXDR . . . . TDR . . . . . .</td>
<td>392 48</td>
<td>452 56</td>
<td></td>
</tr>
<tr>
<td>GDR . . . . TDR . . . . . .</td>
<td>386 48</td>
<td>402 50</td>
<td></td>
</tr>
</tbody>
</table>

* Per cent specific activity = (specific activity of compound/specific activity of exogenous glucose) × 100.

TABLE II

Specific activity of deoxyribose moiety of hydroxymethylcytosine deoxyribonucleotide from T6r+ DNA

<table>
<thead>
<tr>
<th>Source of HDR and HMC</th>
<th>Corrected specific activity of HDR</th>
<th>Corrected specific activity of HMC</th>
<th>Specific activity of deoxyribose moiety</th>
<th>Per cent specific activity of deoxyribose moiety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excess DNA</td>
<td>1480</td>
<td>1040</td>
<td>440</td>
<td>55</td>
</tr>
<tr>
<td>Enzyme-resistant DNA</td>
<td>1400</td>
<td>1040</td>
<td>300</td>
<td>45</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzymatically released DNA</td>
<td>1300</td>
<td>975</td>
<td>325</td>
<td>11</td>
</tr>
<tr>
<td>Enzyme-resistant DNA</td>
<td>1330</td>
<td>975</td>
<td>325</td>
<td>44</td>
</tr>
</tbody>
</table>

E. coli infected in a glucose-1-C14 medium were analyzed, it was found that each had approximately the same specific activity. In Table I, it can be seen that the deoxyribose moieties of the three nucleosides capable of undergoing enzymatic exchange reactions with a free base contain about 50 per cent of the specific activity of the exogenous glucose. Table II shows the results obtained for the deoxyribose moiety of HDR, the HDR and HMC having been isolated by the various procedures described above. The values for the deoxyribose moiety of HDR vary somewhat from the values for the deoxyribose moieties of the other three nucleosides, but it is believed that this is largely attributable to certain inherent difficulties in this determination. First, an extinction coefficient of HDR was assumed since the true value has never been measured. Secondly, the determination of the specific activity of the deoxyribose moiety is based on the subtraction of one large number from another large number so that, as can be seen in Table II, a 6 per cent variation in specific activity of HDR can result in a 20 per cent variation in specific activity of its deoxyribose moiety. Nevertheless, it is believed that, within experimental error, the data support the hypothesis that all deoxyribose is formed by the same pathway. It should be noted that all values in Table I and II have been multiplied by a factor of 1.3 to correct for host donations to deoxyribose.

Is Ribose of Acid-soluble Adenosine Precursor of Deoxyribose?—Since all deoxyribose was found to have a similar origin in infected E. coli, it became feasible to test the hypothesis that deoxyribose of DNA is derived from a ribose compound. Although the enzymatic evidence of a conversion of ribonucleosides and ribonucleotides to deoxyribonucleosides and deoxyribonucleotides, respectively, has only recently been found (19), such a pathway had been postulated by several other workers (1, 20-28) on the basis of labeling experiments.

If a compound such as AMP is the precursor of deoxy-AMP, then the ribose moiety of the acid-soluble AMP in the infected cell should approach the same specific activity as the deoxyribose moiety of adenine-bound AMP from the infected cell. It should also be stated as a point of interest that a study of the specific activity of RNA-ribose in the infected cell would not be of value, although such a study was quite feasible in the growing E. coli cell. The reason for this is that the infected cell has a very unusual RNA metabolism with only a small portion of its RNA exhibiting any metabolic activity (29).

Therefore, in order to test the possibility of a ribose compound serving as the deoxyribose precursor, the acid-soluble fraction of 2 l. of T6r-infected E. coli was collected 90 minutes after infection, and the specific activity of the ribose moieties of the adenine nucleotides was determined. It was thought that by waiting for 90 minutes the acid-soluble fraction would have turned over enough times so that all of its carbons would be derived from the C14-glucose in the medium, with no donation from host material. If the ribose of AMP serves as precursor of the deoxyribose of adenine nucleotides in DNA, then its ribose portion should have a per cent specific activity of about 45 to 55.
The experimental details were as follows. 2 l. of cells were grown and then infected in radioactive medium as described in "Experimental" above. 90 minutes after infection the cells were quickly chilled to 5° and collected by centrifugation in the cold. The cells were then suspended in cold 2 per cent HClO₄, and this mixture was centrifuged 20 minutes later. The precipitate was washed once with cold 2 per cent HClO₄, and this washing was added to the acid-soluble fraction. KOH was added until the solution had a pH of 7, and the HClO₄ was then removed by centrifugation. After the pH was adjusted to 4 with HCl, the material was passed through a column (1 x 1 cm.) of activated charcoal (Merck). More than 99 per cent of the ultraviolet-absorbing material of the acid-soluble fraction was adsorbed onto the charcoal. The column was washed with 15 ml. of water without elution of ultraviolet-absorbing material. The column was then washed with ammoniacal ethanol (water: ethanol:concentrated ammonia, 48:50:2, by volume). Approximately 50 per cent of the ultraviolet-absorbing material present in the original acid-soluble fraction was released in this step. This eluate was taken to dryness to remove ammonia and ethanol and then treated with snake venom⁵ to convert adenine nucleotides to adenosine. An ethanol extract of the dried hydrolysate was then chromatographed in butanol-water (12). Four ultraviolet-absorbing bands were obtained, the one with an Rₚ of 0.19 having spectral properties of adenosine. This material was chromatographed in isopropanol-ammonia, and one band with an Rₚ of 0.27 was obtained. This consisted of 2 μmoles of adenosine. After chromatography in a third solvent, water-isooamyl alcohol (see above), the specific activity of the adenosine was determined. After hydrolysis of the adenosine in N HCl for 1 hour at 100°, adenine was isolated and purified by paper chromatography and its specific activity also determined. Ribose specific activity was then calculated as the difference in specific activity between adenosine and adenine. In one experiment adenosine had 890 c.p.m. per μmole and adenine, 590 c.p.m. per μmole, thereby giving the ribose moiety a specific activity of 300 c.p.m. per μmole. In a second experiment these three values were 900, 600, and 300 c.p.m. per μmole, respectively. Thus, the per cent specific activity in the ribose moiety was 33.3 or 38 per cent. A value of about 45 to 55 per cent should be obtained if a ribose compound is to be considered a deoxyribose precursor.

One reason for the slightly low value obtained could be the donation of host material to the acid-soluble nucleotide pool. This possibility was tested by performing an experiment similar to the above but different in that the cells were grown in glucose-1-C¹⁴, harvested, washed, and then infected in nonradioactive medium. The ribose moiety of the 90-minute acid-soluble adenine nucleotides in this experiment had a specific activity of 16 c.p.m. per μmole. Since the ribose of the uninfected cell contains only 25 per cent of the total C-1 of glucose, and since the specific activity of the exogenous glucose was 800 c.p.m. per μmole, then the maximal specific activity expected in the ribose of the adenine nucleotides was 200 c.p.m. per μmole. This is the value that would have been obtained if all of the acid-soluble adenine ribose 90 minutes after infection had been derived from the host. However, only 20 to 8 per cent came from the host; the remaining ribose was derived from glucose of the medium. If this amount of host donation is used to correct the per cent specific activity values obtained in the first two acid-soluble experiments, then the corrected value of the specific activity of ribose is 41 per cent. This comes close to the expected 45 to 55 per cent.

Characterization of 5-Hydroxymethyl-(O-diglucosyl)-cytosine Deoxyribonucleoside (HDRD)—It was mentioned earlier that when the small amount of HDR released enzymatically from phage DNA was chromatographed in ethanol-acetic acid, two bands were obtained. One of these had an Rₚ of 0.55 which is the usual Rₚ of HDR in this solvent, and the other had an Rₚ of 0.21. This latter compound is designated HDRD for reasons to be discussed below.

Both of these compounds had identical spectra, as shown in Fig. 2. However, as noted in Table III, they differed considerably in specific activity, HDRD having a specific activity greater than that of HDR by about 1600 c.p.m. per μmole. Since the HDRD was formed in medium containing glucose-1-C¹⁴ having a specific activity of 800 c.p.m. per μmole, and since the presence of a diglucosylated HMC in TGR+ DNA had been reported by Jesaitis (3), it was suspected that the product was 5-hydroxymethyl-(O-diglucosyl)-cytosine deoxyribonucleoside. Therefore, this compound was isolated in larger amounts and characterized.

90 mg. of TGR+ DNA were hydrolyzed by DNase and intestinal phosphatase, and the alcohol-soluble fraction containing the deoxyribonucleosides was chromatographed on Dowex 1-formate as described under "Experimental." The HDR fraction was then chromatographed on paper in ethanol-acetic acid to yield about 2 μmoles each of HDR and HDRD. After elution from paper the HDRD, in 0.1 M HCl, was applied to a small Dowex 50-H⁺ column. Elution with 0.01 M HCl removed a small amount of ultraviolet-absorbing material as well as contaminants from the paper chromatograms that would inter-

⁵ *Crotalus adamanteus* venom was obtained from Ross Allen’s Reptile Institute, Silver Springs, Florida.
from the column with 2.5 per cent ammonia. This material was then eluted in 6 N KCl in a sealed tube at 100° for 3 hours (4), HMC and fructose. Spraying the chromatogram with the meta-phenylenediamine reagent (31) revealed one spot which had the same RF as a glucose control. When HDRD was hydrolyzed for 1 hour at 100° in N HCl and then chromatographed on paper in water-sugar by hydrolyzing HDRD for 1 hour at 100° in N HCl and then chromatographing the hydrolysate on paper in water.

The properties of HDRD on Dowex 50 and Dowex 1 suggested that its amino group is a slightly weaker base than that of HDR. In order to verify this, the pK of the amino group was determined by its ultraviolet absorption, a molar extinction coefficient of 11.7 X 10³ at pH 1 and 2800 A being used. Glucose was identified as the reducing sugar by hydrolyzing HDRD for 1 hour at 100° in N HCl and then chromatographing the hydrolysate on paper in water-pyridine-butanol (30) which separates glucose from galactose and fructose. Spraying the chromatogram with the meta-phenylenediamine reagent (31) revealed one spot which had the same Rf as a glucose control. When HDRD was hydrolyzed in 6 N HCl in a sealed tube at 100° for 3 hours (4), HMC was found after chromatography in butanol-ammonia.

The properties of HDRD on Dowex 50 and Dowex 1 suggested that its amino group is a slightly weaker base than that of HDR. In order to verify this, the pK of the amino group was determined by the spectral method of Cohn as described by Beaven et al. (32). Fig. 3 shows a plot of the 290:260, 280:260, and 250:260 extinction ratios of HDR and HDRD at various pHs. The values of these ratios change with pH because of the ionization of the amino group. The inflection point of each of the curves represents the pK value of the amino group. For HDR this pK is about 3.5 to 3.6; for HDRD it is about 3.5 to 3.4.

Thus, the analytical data indicate that HDRD is a diglucosylated HDR. The postulated structure of this compound appears in Fig. 4.

DISCUSSION

The essential point of this paper is that all deoxyribose in T6r+ DNA is derived from glucose by a common pathway. Thus, the increased conservation of the C-1 of glucose in deoxyribose that occurs when the cell is infected is not just for pyrimidine deoxyribose or for purine deoxyribose but for all deoxyribose. This is evident from the data which showed that the deoxyribose moieties of each of the four nucleosides of T6r+ DNA had essentially the same specific activity.

Other experiments in this paper suggest that the precursors of the deoxyribose of T6r+ DNA are the ribonucleotides of the acid-soluble fraction. For, within experimental error, the ribose moiety of the adenine of the acid-soluble fraction of the infected cell had nearly the same specific activity as the deoxyribose moieties of the T6r+ DNA. These data support the findings of several other investigators. Furthermore, the interesting finding of a contribution of the host to the acid-soluble nucleotides late in the infection process has also been noted by Hershey (33).

Finally, the isolation of HDRD upon enzymatic hydrolysis of T6r+ illustrates once again the uniqueness of this DNA. While the other three nucleosides are released enzymatically from T6r+ DNA to the extent of 70 to 80 per cent, only 10 per cent, at most, of the HDR fraction is released and only half of this is HDRD. The fact that T6r+ DNA contains small amounts of both free HMC and monoglucosylated HMC in addition to larger portions of diglucosylated HMC means that this DNA consists of six different nucleotides. Thus, this DNA, because of its glucosylation, is more resistant to enzymatic hydrolysis and possesses a more varied structure than do other known DNA’s. The mechanism by which phage DNA, when injected into the cell during the infection process, causes the cell to synthesize HMC instead of cytosine has just recently been elucidated. It has been shown in this laboratory (15) that upon infection a new enzyme is synthesized which has the property of converting deoxycytidylic acid to hydroxymethyldeoxycytidylic acid. However, the question of how and when the glucose residues become attached still remains to be investigated.

One further interesting facet of infected cell metabolism that the data make apparent is that the glucose moieties of HDRD are derived solely from the medium, i.e. there is no host contribution to this glucose. Thus, when cells were infected in media containing glucose-1-C¹⁴ with a specific activity of 800 c.p.m. per pmole, the HDRD from the T6r+ DNA was found to have a specific activity higher than that of HDR by 1600 c.p.m. per pmole. This glucose attached to HDRD, then, represents the only known component of phage that is not derived in any measure from the host. Some unpublished experimental data of Cohen and Lichtenstein also support this finding, for they showed that when cells completely labeled with C¹⁴ were infected in nonradioactive medium, the glucose portions of the HDRD obtained from the phage DNA had no radioactivity.

SUMMARY

1. When Escherichia coli is infected with T6r+ bacteriophage in media containing glucose-1-C¹⁴, it is found that the deoxyribose moieties of each of the four deoxyribonucleosides of the T6r+ deoxyribonucleic acid formed upon infection have the same
specific activity. It is concluded that all deoxyribose is formed from glucose via the same pathway.

2. Evidence is presented that the acid-soluble adenine nucleotides in the infected cell serve as precursors of the deoxyribose of T6r+ deoxyribonucleic acid.

3. The isolation and characterization of a nucleoside, 5-hydroxymethyl-(O-diglucosyl)-cytosine deoxyribonucleoside from enzymatic hydrolysates of T6r+ deoxyribonucleic acid are described.

4. The glucose portions of 5-hydroxymethyl-(O-diglucosyl)-cytosine deoxyribonucleoside receive no contribution from the host, but are derived solely from glucose in the medium.

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