THE PHOSPHOGLUCONATE PATHWAY OF CARBOHYDRATE METABOLISM IN THE MULTIPLICATION OF BACTERIAL VIRUSES

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Studies in this laboratory (Cohen, 1951a) have revealed the existence of two pathways for the utilization of glucose in Escherichia coli, strain B. No more than two such pathways diverging from glucose-6-phosphate have been demonstrated in this organism. One of these is the well known Embden-Meyerhof scheme, in which glucose is converted anaerobically in the presence of appropriate enzymes and adenosine triphosphate to 2 moles of triose phosphate. The second pathway has been variously termed the hexose monophosphate shunt, the oxidative pathway, or the phosphogluconate pathway. It has been demonstrated to involve oxidation of glucose-6-phosphate to 6-phosphogluconate, oxidation and decarboxylation of the latter to ribulose-5-phosphate, followed by cleavage to one mol of triose phosphate and an unidentified C4 fragment. These relations are summarized in figure 1.

Other ramifications of the pathway require comment. It may be seen that ribulose-5-phosphate may be converted to ribose-5-phosphate, ribose-1-phosphate, and ribose nucleosides, as in ribose nucleic acid. On the other hand, deoxyribose-5-phosphate is derived from the condensation of triose phosphate and acetaldehyde (Racker, 1951). Recent studies on the growth of E. coli on glucose-1-C14 (Lanning and Cohen, 1952a) have revealed that the ribose of ribose nucleic acid is mainly derived from a path in which the C4 of glucose has been lost, such as the phosphogluconate pathway. On the other hand, the deoxyribose of deoxyribonucleic acid of E. coli or of T6r+ bacteriophage produced in E. coli is derived mainly from a path in which C3 had been conserved, such as the Embden-Meyerhof scheme. Indeed these results had been predicted as a result of studies on the isotopic content of CO2 produced by E. coli metabolizing glucose-1-C14. In normal growth when large amounts of ribose nucleic acid were produced, the amount of isotope found in the CO2 suggested an extensive use of the oxidative pathway. It has been found that sufficient enzymes of this pathway are present in E. coli to be consistent with this result (Scott and Cohen, 1952). However, in viral synthesis when ribose nucleic acid synthesis was inhibited, and large amounts of deoxyribose nucleic acid were formed, the use of the oxidative pathway was markedly reduced (Cohen, 1951b).

As presented in figure 1, substrates such as gluconate, ribose, and D-arabinose are utilized by E. coli by insertion into the oxidative pathway as a result of certain conversions and phosphorylations. Gluconate is phosphorylated by adenosine triphosphate in the presence of an adaptive gluconokinase to form 6-phosphogluconate (Cohen, 1951c). D-Arabinose is converted by an adaptive pentose isomerase to form D-ribulose (Cohen, 1952) which then is phosphorylated by a ribulokinase (Lanning and Cohen, 1952b) to form ribulose phosphate. Ribose is phosphorylated by a ribokinase to form ribose phosphate (Cohen, Scott, and Lanning, 1951). It has been demonstrated that when gluconate-1-C14 is utilized by E. coli for growth or multiplication of virus, essentially all of the C3 appears in CO2. Thus, the step of glucose-6-phosphate to 6-phosphogluconate is essentially irreversible in intact cells of E. coli, and the utilization of gluconate, ribose, or D-arabinose must involve the formation of triose phosphate via the oxidative pathway as described in figure 1.

The study of the individual steps of carbohydrate metabolism in E. coli described above was undertaken originally in an attempt to understand the mechanism of inhibition of ribose nucleic acid synthesis under conditions of viral infection with T-even viruses. It is evident that these two pathways do appear to determine the rate of utilization of glucose and the amounts of ribose phosphate and deoxyribose phosphate produced in E. coli under various conditions.

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The total inhibition of ribose nucleic acid synthesis in T6r+ infected *E. coli* may be accounted for in one of several ways. It may be supposed that glucose-6-phosphate may be diverted to the Embden-Meyerhof scheme by the trapping of triose phosphate in the desoxyribonucleic acid of virus. Although this may explain the increased use of this pathway in viral infection as contrasted with the use in normal growth, this does not explain the total inhibition of ribose nucleic acid synthesis. It has been found that ribose nucleic acid synthesis is inhibited in all instances of T-even virus infection of *E. coli*, strain B, in circumstances where desoxyribonucleic acid synthesis may be inhibited markedly or even stopped completely (Cohen and Arbogast 1950c; Fowler, 1952).

Another possible explanation has been tested in this laboratory and is the subject of this report. It has been postulated that since the ribose of ribose nucleic acid appears to be derived from the oxidative pathway, some enzyme in this pathway has been inhibited during viral infection. The present results show that *E. coli* can produce virus and desoxyribonucleic acid from gluconate, ribose, D-arabinose, or nucleosides as sole sources of carbon. It is concluded, therefore, that no enzymatic step of the oxidative pathway is inhibited during the multiplication of virus since these substrates must be converted to triose phosphate and deoxyribose through these reactions.

**MATERIALS AND METHODS**

*Preparation of bacterial strains.* *E. coli*, strain B, was subcultured from stock slants on nutrient agar to 10 ml of a mineral medium containing glucose as sole source of carbon (Cohen and...
Arbogast, 1950; Cohen and Raff, 1951). The growth of strain B in this medium containing glucose and gluconate has been described (Cohen and Raff, 1951). This organism is unable to multiply at an appreciable rate on D-ribose or D-arabinose.

Two mutants of strain B were isolated. The first, B.14, was selected from the parent by subculturing in the mineral medium containing D-arabinose as the sole source of carbon. An isolate from a single colony on agar was passed 15 times in a medium containing arabinose, and maintained on agar. It was subcultured to mineral medium containing glucose prior to use. The ability of B.14 to use D-arabinose is adaptive as revealed by diauxic growth in mixtures of glucose and arabinose. The second mutant, B.15, was isolated by plating 10⁶ cells of strain B on agar containing 1 per cent ribose as the sole source of carbon. Inocula from the colonies which grew out were streaked on agar slants and then transferred to the mineral medium with glucose as sole source of carbon. Only those cultures were maintained which showed diauxic growth in glucose-ribose mixtures. One such culture was transferred 6 times in mineral medium plus ribose, and this selected strain, B.15, was maintained on broth agar prior to subculture to mineral medium containing glucose. Strain B.15 was able to adapt to growth on gluconate, ribose, and D-arabinose. Strain B.14 could adapt to growth on gluconate and D-arabinose, but not to ribose.

To test multiplication of virus on various adaptive substrates, the organisms were subcultured from the medium from which they had exhausted glucose, while still in the exponential phase, to a medium containing glucose plus the desired substrate. The organisms were harvested for use after the primary substrate, glucose, had been exhausted and the culture was in the exponential phase of growth on the second substrate.

The growth of strain B.15 on glucose and mixtures of glucose and gluconate, ribose, D-arabinose, or adenosine is presented in figure 2. Cultures were aerated continually in sterile Klett tubes, and their turbidities were measured in the Klett photometer with a 420 filter. The data show that there is no lag after exhaustion of glucose before growth on gluconate begins. Adaptation to this substrate occurs during growth on glucose. Growth on adenosine similarly does not involve a lag after exhaustion of glucose; these two substrates appear to be used simultaneously. Growth on adenosine alone is slower than on glucose. Normal diauxic growth is evident with the glucose-pentose mixtures.

Preparation of bacteriophages. The viruses, T2r⁺ and T4r, provided a system most suitable for comparison of one step growth curves. The isolation and properties of the viruses, as well as the techniques employed, have been described (Cohen and Arbogast, 1950a,b). In experiments involving T4r, 50 μg of tryptophan per ml was added as an adsorption cofactor to cultures immediately prior to infection.

RESULTS

One step growth curves. The burst sizes and latent periods of T4r multiplication in various adapted strains of E. coli are presented in table 1. The growth of virus in cells grown on glucose and infected in glucose was compared with that in cells grown in the substrate to be tested as described above, and infected in this substrate. As can be seen, production of virus was possible on gluconate although yielding less virus and revealing a longer latent period. Similar results were obtained with one step growth experiments with T2r⁺; however, burst sizes on glucose were quite low (30 to 50) as reported earlier, and it is not felt that comparison of burst size in experiments with our strain of T2r⁺ is interpretable readily.
Of particular interest is the absence of a burst observed with ribose and D-arabinose. Two types of effect were noted. In five experiments the numbers of infectious centers in media containing ribose slowly fell off to 0 in 80 to 90 minutes. In three experiments the numbers of infectious centers in media containing arabinose fell off less slowly in most instances and only once, as in an experiment with strain B₄₁₈ listed in table 1, showed a small burst. In one step growth experiments with T₂r⁺ in D-arabinose, no burst was detected. In two experiments attempts at lysis with cyanide (Doermann, 1952) were unsuccessful in revealing possibly unliberated virus.

**TABLE 1**

<table>
<thead>
<tr>
<th>ESCHERICHIA COLI, STRAIN</th>
<th>SUBSTRATE</th>
<th>LATENT PERIOD</th>
<th>BURST SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Glucose</td>
<td>27–28</td>
<td>82–118</td>
</tr>
<tr>
<td></td>
<td>Gluconate</td>
<td>43</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>Guanosine</td>
<td>39</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Adenosine</td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td>B₄</td>
<td>Glucose</td>
<td>23</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Ribose</td>
<td>no burst</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D-Arabinose</td>
<td>no burst</td>
<td></td>
</tr>
<tr>
<td>B₄₁₈</td>
<td>Glucose</td>
<td>29</td>
<td>190</td>
</tr>
<tr>
<td></td>
<td>D-Arabinose</td>
<td>44</td>
<td>18</td>
</tr>
</tbody>
</table>

**Lysis of multiply-infected bacteria.** In figure 3 is presented a typical experiment describing the lysis of cultures of 2 × 10⁶ bacteria (strain B₄) per ml infected by 10⁶ T₄r viral particles. It is evident that under these conditions the cells lysed readily regardless of substrate although the latent periods of cells infected in D-arabinose and ribose were somewhat prolonged. Furthermore, assay of the lysates at a 2 hour interval revealed more virus than originally had been placed in the system. The titers of the lysates in this instance were: (a) glucose—2.2 × 10⁶ per ml; (b) gluconate—1.7 × 10⁶ per ml; (c) adenosine—2.2 × 10⁶ per ml; (d) ribose—1.0 × 10⁶ per ml; (e) arabinose—7.7 × 10⁵ per ml.

**Desoxyribonucleic acid synthesis by multiply-infected bacteria.** When 2 × 10⁶ bacteria per ml are infected by 10⁶ T₂r⁺ viral particles, a marked stimulation of desoxyribonucleic synthesis occurs after a brief lag of about 7 to 10 minutes. Most of the desoxyribonucleic synthesis occurs at a constant rate. Cells were infected in the presence of the various substrates, and the desoxyribonucleic contents of the cultures were determined (Cohen, 1948). The rates of synthesis of desoxyribonucleic acid were obtained, and a comparison of these rates on different substrates is presented in table 2.

**TABLE 2**

<table>
<thead>
<tr>
<th>BACTERIAL STRAIN</th>
<th>SUBSTRATE</th>
<th>RATE OF DESOXYRIBONUCLEIC ACID SYNTHESIS</th>
<th>MASS DOUBLING TIME</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Glucose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Gluconate</td>
<td>77–85</td>
<td>114</td>
</tr>
<tr>
<td></td>
<td>Adenosine</td>
<td>78</td>
<td>167</td>
</tr>
<tr>
<td>B₄</td>
<td>Glucose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Ribose</td>
<td>35</td>
<td>435</td>
</tr>
<tr>
<td></td>
<td>D-Arabinose</td>
<td>41*</td>
<td>300</td>
</tr>
<tr>
<td>B₄₁₈</td>
<td>Glucose</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>D-Arabinose</td>
<td>63</td>
<td>257</td>
</tr>
</tbody>
</table>

* Determined with T₄r.

2. In the same table are presented comparisons of the mass doubling time determined from data comparable to those given in figure 2.

It appears that in these systems, T₂r⁺ infected cells are able to synthesize desoxyribonucleic
acid. The rates of deoxyribonucleic acid synthesis in a given substrate are roughly inversely related to the mass doubling times of the organism in that carbon source.

**DISCUSSION**

It is not clear why adapted cells infected with a single viral particle in ribose or arabinose are unable to liberate virus. However, under conditions of multiple infection in more concentrated suspensions, deoxyribonucleic acid synthesis, lysis, and liberation of virus may be demonstrated readily although these functions are quantitatively less than in glucose.

Let us assume that *E. coli* metabolizes the carbohydrates used in this study only by the mechanisms presented in figure 1. The conversion of the substrates to deoxyribonucleic acid then involves the following steps: (a) gluconate → 6-phosphogluconate → ribulose-5-phosphate → triose phosphate; (b) d-arabinose → ribulose → ribulose-5-phosphate → triose phosphate; (c) ribose → ribose-5-phosphate → ribulose-5-phosphate → triose phosphate; (d) nucleoside → ribose-1-phosphate → ribose-5-phosphate → ribulose-5-phosphate → triose phosphate.

Furthermore, it has been shown that the phosphogluconate pathway is not eliminated totally in glucose utilization by infected cells since in the metabolism of glucose-1-C¹⁴ by such cells markedly more isotope appears in CO₂ than can be accounted for by mechanisms involving the Embden-Meyerhof scheme (Cohen, 1951b). It is possible that this use of the phosphogluconate pathway leads to the ribose of adenosine triphosphate but not of nucleic acid. In any case, the steps of glucose → glucose-6-phosphate → 6-phosphogluconate → ribulose-5-phosphate can function in infected cells.

Thus, it has been demonstrated that infected cells can use the oxidative pathway if compelled to by the appropriate choice of substrate, even though they do not do so to a great extent when using glucose. All known steps to ribose nucleic acid formation have been shown not to be inhibited by infection. It appears that the total inhibition of ribose nucleic acid synthesis by T-even phage infection is due to redirection of metabolism at steps not directly involving steps of carbohydrate metabolism.

**SUMMARY**

Strains of *Escherichia coli* capable of growth on glucose, gluconate, d-arabinose, ribose, and purine ribosides were tested for their ability to reproduce T₄r bacteriophage, or synthesize deoxyribose nucleic acid when infected in these substrates. Since they were capable of doing so, it was concluded that the inhibition of the phosphogluconate pathway of carbohydrate metabolism was not the immediate cause of the inhibition of synthesis of ribose nucleic acid in some bacteria infected with bacteriophages.

**REFERENCES**


by enzyme systems of *Escherichia coli*. Federation Proc., 10, 173.


Lanning, M., and Cohen, S. S. 1952a The origin of the ribose and deoxyribose of the nucleic acid of *E. coli* and T6r+ virus. Abstracts of the American Chemical Society, Division of Biological Chemistry 2c.

