THE PURINE AND PYRIMIDINE METABOLISM OF NORMAL AND PHAGE-INFECTED ESCHERICHIA COLI*

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In the Escherichia coli B-coliphage system, duplication of the "T-even" phages involves the active synthesis of desoxypentose nucleic acid (DNA) (1). This virus DNA differs in purine to pyrimidine ratio from that of the host cell (2-4) and cytosine has been reported absent in purified preparations of T2 phage (2). Although the synthesis of virus DNA proceeds mainly from nitrogen (5) and phosphorus (6, 7) assimilated from the external environment of the cell, unequal purine and pyrimidine contributions by nucleotides of degraded host DNA are made early in the infection cycle (8-10). In view of the metabolic alteration by such infected cells a comparative study was made of the purine and pyrimidine metabolism of E. coli B under conditions of growth and virus infection with T4r+ bacteriophage.

Materials and Methods

Materials—Compounds were obtained commercially except that synthetic preparations of 4-amino-5-imidazolecarboxamide hydrochloride and isoguanine sulfate were used. Several were recrystallized as salts. Each preparation gave a single spot when 100 μ were chromatographed on paper in n-butanol, diethylene glycol, and 0.1 N HCl (11) and the eluates gave characteristic spectra.

Analytical Methods—Concentrations are derived from spectra of preparations at least 95 per cent pure, as judged by the ratio of extinction to the maximum when measured at 5 μ intervals between 235 and 290 μ by means of a Beckman spectrophotometer (model DU). The principle of differential spectrophotometry was used to measure deamination of adenine or cytosine and their derivatives and isoguanine; Δε changes at 265 and 240 μ as described by Kalekar were used for adenine (12), those at 280 and 260 μ for cytosine, and at 285 μ for isoguanine. To detect cleavage of

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pyrimidine derivatives, advantage was taken of spectral shifts of the bases at alkaline pH (13). Ascending paper chromatography was run in the above solvent (11) or in 5 per cent KH$_2$PO$_4$ saturated and layered with isoamyl alcohol (14). Diazotizable amine was measured by a modification of the Bratton-Marshall procedure (15) after acetylation with acetic anhydride. To measure desoxypentose, the precipitated bacterial pellet was hydrolyzed in 5 per cent perchloric acid at 100° for 30 minutes, and increments were determined with diphenylamine (16) by using a commercial sperm nucleic acid of 70 per cent purity as standardized by its phosphorus content. The perchloric acid-tryptophan method (17) liberated desoxypentose quantitatively from thymidine and hypoxanthine desoxyriboside. Ammonia was detected by nesslerization.

Organism and Bacteriophage—E. coli, strain B, was maintained on tryptone-yeast extract agar slants and grown and transferred several times through the experimental medium prior to use as an inoculum. Studies were made on cells from vigorously aerated cultures in an early phase of exponential growth. The bacteriophage was prepared by the agar layer method (18) and concentrated by several cycles of low and high speed centrifugation. Suspended in m/15 phosphate buffer, pH 7.2, the preparation had an activity of 1.9 to 3.2 × 10$^{12}$ plaque-forming units per ml. during the course of these studies. Infection statistics, i.e. input, free phage, per cent adsorption, multiplicity, and infected bacteria, were determined for each experiment by standard methods (19).

Experimental Procedures—The reaction system and methods used in the studies with dense cell suspensions have been described (20), except that deaminations were verified by measuring ammonia increments quantitatively, and 4-amino-5-imidazolecarboxamide was determined as a non-acetylatable diazotizable amine.

To determine the comparative metabolism of the two systems, automatic inoculation (21) was used to provide 3.5 to 4.5 hour-old salts-glucose medium$^1$ cultures in the morning. Washed cells from these cultures were resuspended into four 125 ml. volumes of new medium containing 0.3 to 0.5 per cent glucose and 0.5 mg. of tryptophan to give an initial concentration of 1 × 10$^9$ bacteria per ml. Following a 15 minute period of growth, virus was added to half the cultures, and, 10 minutes later, substrate was added to a normal and an infected culture. The two other cultures served as a normal and an infected control, respectively. The cultures were aerated vigorously. At zero time, and intervals thereafter, 12 ml. aliquots were removed and added to cold perchloric acid (3 per cent final concentration) in an ice bath and centrifuged cold within 20 minutes. The supernatant solutions were examined for the various activities, and the drained

$^1$ This medium contained Na$_2$HPO$_4$ 6 gm., KH$_2$PO$_4$ 3 gm., MgSO$_4$.7H$_2$O 0.1 gm., NaCl 1.0 gm., NH$_4$Cl 1.0 gm., glucose 2 gm., and water to make 1 liter.
protein pellets used to determine desoxypentose increments. The virus multiplicity for each experiment was between 6 and 10; hence 99 per cent of the infected cells had adsorbed one or more particles and lysis inhibition existed. During the course of an average experiment, the turbidity of an uninfected system almost doubled, whereas that of an infected system was approximately three-fourths the turbidity of the growing system.

Results

Enzymatic Activities of Host Cell

In view of divergent results reported for different strains of E. coli, the metabolic interconversions which this strain was capable of performing were determined. The results are shown in Table I. The enzymatic activities shown here were also demonstrated with centrifuged cell-free extracts obtained by sonic vibration. No activity in the cell-free systems was found for orotic acid, hypoxanthine, guanine, or 4-aminoptimidazolecarboxamide (with and without formate).

As is seen in Table I, with dense cell suspensions, the greatest incorporation of the original substrate or reaction product following deamination is from the base. Best deaminase activity in all cases occurred with the riboside. The base was isolated following deamination of nucleoside or nucleotide derivatives. Whether nucleoside phosphorylase activity or phosphatase activity preceded deamination could not be determined, although such activity could be demonstrated on riboside or ribotide derivatives of respective reaction products. In a series of experiments with hypoxanthine, xanthine, xanthosine, or guanine, a substance having an absorption maximum of 265 mμ and an Rf value of 0.38 in n-butanol, diethylene glycol, and 0.1 N HCl was regularly found. When xanthine oxidase was incubated with an eluate of this substance from a chromatogram in which guanine had been the original substrate, no rise in absorption at 290 mμ was detected, indicating that it was not xanthine.

Parallel experiments were done with washed suspensions of cells that had just previously been infected with T4r+ bacteriophage (22). In all instances the enzymatic activities found in normal cells were also found in infected ones. The differences, when followed by kinetic studies, were quantitative, and these studies were then extended to growing systems which could actively synthesize desoxypentose and virus.

Comparative Metabolism of Normal and T4r+-Infected E. coli B

Under growth conditions an infected culture duplicating virus shows a desoxypentose increment in 80 minutes that is 4 times greater than the

* The reaction system contained extract equivalent to 0.5 mg. of cells wet weight and 0.5 mg. of substrate in a total volume of 1.3 ml. of M/15 phosphate buffer, pH 7.2.
### Table I

**Enzymatic Activities of Dense Cell Suspensions of E. coli B**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Cells (mg.)</th>
<th>$t_0$ (μM)</th>
<th>Product ($t_{90}$)</th>
<th>Per cent recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Amino-5-imidazolecarboxamide HCl</td>
<td>6.7</td>
<td>1.57</td>
<td>1.55</td>
<td>99.0</td>
</tr>
<tr>
<td>Orotic acid</td>
<td>5.0</td>
<td>4.90</td>
<td>4.60</td>
<td>94.0</td>
</tr>
<tr>
<td>5-Methylcytosine</td>
<td>8.3</td>
<td>3.84</td>
<td>3.76</td>
<td>98.0</td>
</tr>
<tr>
<td>Thymine</td>
<td>5.3</td>
<td>2.83</td>
<td>2.26</td>
<td>80.0</td>
</tr>
<tr>
<td>Adenine HCl</td>
<td>6.2</td>
<td>2.62</td>
<td>2.43</td>
<td>93.0</td>
</tr>
<tr>
<td>Adenosine</td>
<td>3.3</td>
<td>0.74</td>
<td>Hypoxanthine 0.53</td>
<td>72.0</td>
</tr>
<tr>
<td>Adenosine-3'-PO₄</td>
<td>5.0</td>
<td>2.79</td>
<td>&quot;</td>
<td>96.4</td>
</tr>
<tr>
<td>Cytosine</td>
<td>4.5</td>
<td>6.20</td>
<td>Uracil 4.35</td>
<td>71.8</td>
</tr>
<tr>
<td>Cytidine</td>
<td>2.4</td>
<td>2.92</td>
<td>&quot;</td>
<td>86.2</td>
</tr>
<tr>
<td>Cytidylic acid</td>
<td>5.0</td>
<td>1.60</td>
<td>&quot;</td>
<td>98.0</td>
</tr>
<tr>
<td>Isoguanine</td>
<td>5.5</td>
<td>1.90</td>
<td>Xanthine 1.40</td>
<td>73.7</td>
</tr>
<tr>
<td>2,6 Diaminopurine</td>
<td>10.3</td>
<td>3.31</td>
<td>( &quot; )</td>
<td></td>
</tr>
<tr>
<td>Guanosine</td>
<td>5.0</td>
<td>3.52</td>
<td>Guanine 2.94</td>
<td>83.5</td>
</tr>
<tr>
<td>Inosine</td>
<td>6.0</td>
<td>1.04</td>
<td>Hypoxanthine 1.04</td>
<td>100.0</td>
</tr>
<tr>
<td>Uracil</td>
<td>4.5</td>
<td>1.28</td>
<td>Uracil 0.91</td>
<td>71.0</td>
</tr>
<tr>
<td>Thymidine</td>
<td>4.5</td>
<td>1.42</td>
<td>Thymine 1.28</td>
<td>90.0</td>
</tr>
<tr>
<td>Guanylic acid</td>
<td>5.5</td>
<td>1.83</td>
<td>Guanine 1.17</td>
<td>64.0</td>
</tr>
<tr>
<td>Uridylic &quot;</td>
<td>4.5</td>
<td>0.92</td>
<td>Uracil 0.78</td>
<td>85.0</td>
</tr>
<tr>
<td>Inosinic &quot;</td>
<td>5.3</td>
<td>0.58</td>
<td>Hypoxanthine 0.54</td>
<td>93.0</td>
</tr>
</tbody>
</table>

The system contains 1 ml. of cells, 0.5 ml. of substrate, 1.5 ml. of M/15 phosphate buffer, pH 7.2, with 0.1 per cent glucose. The absence of a reaction product indicates no detectable conversion, and the values given are for remaining substrate. The parentheses around a reaction product indicate incomplete conversion. Zero time and 90 minutes from zero time are denoted by $t_0$ and $t_{90}$.

**Fig. 1.** DNA increments in a normal and infected culture
normal (Fig. 1). The addition of a purine or pyrimidine substrate did not influence the rate or extent of synthesis in either system.

A comparison of deaminase activity by the two systems on representative substrates is shown in Fig. 2. In contrast to normal activity, the infected systems show differences in rate or extent of substrate disappearance with adenine, isoguanine, and adenosine. Differences can also be seen in the time of appearance, quantitative amount, or reutilization of the reaction products. Following an initial lag, more of these substrates disappear in the infected systems, the rate being accelerated, with adenine and isoguanine. Similarly, with adenine and adenosine, less product is found and a lag occurs in the appearance and reutilization of the reaction product, hypoxanthine. In these systems, deamination of adenosine-3'-phosphate and deoxyadenylic acid could not be demonstrated, possibly because of the insufficient concentration of cells employed. The deamination of isoguanine was incomplete; the infected system showed relatively less xanthine in the final mixture for substrate utilized. Experimental values for this substrate with the normal and infected systems, respectively, were 0.215 and 0.292 for -Δε at 285 mμ, and 0.049 and 0.063 for +Δε at 260 mμ.

Uracil was detected in both systems after deamination of cytosine, cytidine, and cytidylic acid. The order of deaminase activity was cytidine > cytidylic acid > cytosine, although activity was not complete. In the infected system with 0.29 μM of cytosine per ml., more substrate disappeared following the initial lag, and the shift away from the absorption maximum of cytosine towards uracil was first apparent at 60 minutes, as contrasted with the normal shift at 45 minutes. Similar lags in deamination were found with 0.1 μM per ml. of cytidine and cytidylic acid. Incorporation of these substrates was rapid, and release of reaction product variable and slow.

Results of experiments comparing purine and pyrimidine nucleoside phosphorylase activity in the two systems on various substrates are shown in Fig. 3. With guanosine and uridine, cleavage begins with the first 10 minutes in both systems. This cleavage is rapid with guanosine and 95 per cent complete with uridine at the end of 60 minutes. Although cleavage rates are similar in both systems, in an infected system less uridine and its reaction product disappear. Similarly, less guanine is used. A specific uridine nucleoside phosphorylase has been described (23). Data with 0.14 μM per ml. of uridylic acid indicated that uracil first appeared at 10 minutes in a normal system but was not apparent until 45 minutes in an infected system. The reaction was incomplete at 60 minutes in both systems.

Nucleoside phosphorylase activity on deoxyribosides was then tested.
Fig. 2. Deaminase activity in a normal (●) and infected (×) culture. The solid lines indicate $-\Delta \varepsilon$ changes and the dotted lines $+\Delta \varepsilon$ changes (hypoxanthine).
Fig. 3. Nucleoside phosphorylase activity in a normal (●) and infected (X) culture. With thymidine, the dotted lines denote desoxypentose corresponding to the coordinate on the right. The arrows indicate the times when cleavage is first measurable.
The data with thymidine are shown. Following initial disappearance of substrate, the normal system shows cleavage beginning at 10 minutes and is complete at 20 minutes. There is a concomitant rapid disappearance of substrate and liberated desoxypentose, confirming recently described results (24). In the infected system, an initial lag is followed by rapid disappearance of thymidine during the 10 to 20 minute period. Cleavage begins at 20 minutes and remains incomplete at 50 minutes, and the disappearance of desoxypentose shows a marked lag. The lag in disappearance of desoxypentose was also demonstrated with hypoxanthine deoxyriboside. In the uninfected system, 90 per cent of the freed desoxypentose disappears in 10 minutes from 0.1 µM of substrate per ml., whereas in an infected system all of the desoxypentose was present at the end of 20 minutes and then slowly disappeared, 20 per cent remaining at 40 minutes. The cleavage of 0.2 µM of thymidylic acid per ml. was incomplete in 60 minutes in a normal system, with no demonstrable incorporation of either substrate or reaction product. However, in an infected system, no cleavage occurred and 39 per cent of the substrate disappeared in 60 minutes. It may be noted that incorporation of the cleaved base following nucleoside phosphorylase action differs from its incorporation following deamination, or when it occurs as an original substrate.

Fig. 4 shows the activity of the two systems on substrates which disappeared without detectable conversion. Thus hypoxanthine and thymine disappeared in the infected system at a greater extent than in the

![Fig. 4. Utilization of substrates not detectably converted in a normal (O) and infected (X) culture.](http://www.jbc.org/)

Disappearance of substrate begins at 10 minutes and is complete at 20 minutes in the normal system, whereas in the infected system, the substrate remains at 40 per cent at 40 minutes.
normal one, whereas the disappearance of guanine was less in the infected system. Of an initial concentration of 0.12 μM of 4-amino-5-imidazole-carboxamide per ml., 4 per cent disappeared in the normal system and 7 per cent in the infected one.

DISCUSSION

The results presented in this paper indicate that E. coli B has diverse enzymatic activities on many substrates involved in nucleic acid metabolism. Such substrates influence neither the rate nor extent of DNA synthesis in either system when added separately to the medium.

A fundamental difference of infected organisms is a marked lag in enzymatic activities involved in the degradation of purine and pyrimidine substrates from the medium during the first 20 to 30 minutes after infection. The nature of this effect is obscure, although the time interval corresponds to a period when host DNA is being degraded for transfer to virus progeny (10), and this lag may represent a diversion or inhibition of these enzymes during this process. Following the initial lag, there is a more rapid rate or extent of disappearance with several substrates, indicating altered reaction kinetics in the metabolism of infected cells. These substrates include adenine, adenosine, isoguanine, thymine, thymidine, and thymidylic acid.

Coupled with this, a general lag in degradative enzymatic activities appears, as evidenced in deamination, in nucleoside phosphorylase and phosphatase activity, in the disappearance of cleaved desoxypentose phosphate, and in the disappearance of the remaining substrates tested. It is difficult to assess the proportionate increment of each enzymatic activity due to an increase in bacterial numbers, since, under growth conditions, a multiplying normal and a non-multiplying infected system are being compared. However, in view of the altered reaction kinetics with the aforementioned substrates, and the greatly increased synthesis of DNA in the infected system, these lags may have significance above that occasioned by the difference in the two systems.

The incorporation of guanine from the medium into Tsr+ virus during replication in the presence of ammonium ion has been reported (6). Results of the present study indicate that adenine, as well as its deamination product, hypoxanthine, is much more effectively utilized by an infected cell than is guanine. It is of interest to note that the riboside of adenine is not an intermediate in the incorporation of the base, as judged by the greater accumulation of hypoxanthine as a result of deamination.

The disappearance of cytosine and its derivatives, and other substrates in a system synthesizing virus nucleic acid either low or devoid of these substrates, is not inconsistent with the view that these substrates may be
interconverted into metabolically active components eventually used in this synthesis. In the case of cytosine, the possibility has not been excluded that an active derivative of it may be found in virus nucleic acid.

The authors are indebted to Dr. S. S. Cohen for many helpful suggestions during the course of this investigation. Generous samples of 4-amino-5-imidazolecarboxamide were provided by Dr. E. Schultze of Sharp and Dohme, Inc., isoguanine by Dr. A. Bendich and Dr. G. B. Brown of the Sloan-Kettering Institute for Cancer Research, hypoxanthine desoxyriboside and thymidine by Dr. L. A. Manson and Dr. J. O. Lampen of Western Reserve University, and thymidine, thymidylic and desoxyadenylic acids by Dr. Waldo E. Cohn of the Oak Ridge National Laboratory.

**SUMMARY**

1. The enzymatic activities of *Escherichia coli* B on purine and pyrimidine substrates has been determined with resting cells, cell-free extracts, and during growth in salts-glucose medium. The activities found were deamination of adenine, adenosine, adenosine-3'-phosphate, cytosine, cytidine, cytidylic acid, isoguanine, and 2,6-diaminopurine; nucleosidase activity for inosine, guanosine, uridine, thymidine, and hypoxanthine desoxyriboside; and nucleotidase activity for inosinic, guanylic, uridylic, and thymidylic acids. Resting cells infected with T4+ virus showed all these enzymatic activities, the differences being quantitative.

2. A culture infected with concentrated T4+ virus showed the following differences from a normal growing culture: (a) there was a marked decrease in degradative enzymatic activity during the first 20 to 30 minutes following infection; (b) following this, an increased rate or extent of disappearance of substrate was noted with adenine, adenosine, isoguanine, thymine, thymidine, and thymidylic acid; (c) a general lag in degradative enzymatic activities occurred, the most marked being in the deamination of cytosine and its derivatives, cleavage by nucleoside phosphorylase or phosphatase of thymidine, hypoxanthine desoxyriboside, uridine, thymidylic and uridylic acids, and the disappearance of desoxypentose liberated from the desoxyribosides. Of substrates not detectably converted, hypoxanthine disappeared faster in the infected than in the normal system; guanine was slower.

3. The rate and extent of DNA synthesis were unaffected by single purines or pyrimidines in either system.

**BIBLIOGRAPHY**

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