THE EFFECT OF VIRUS INFECTION ON THE UTILIZATION OF TRYPTOPHAN BY ESCHERICHIA COLI

RUTH N. RAFF AND SEYMOUR S. COHEN

The Children's Hospital of Philadelphia (Department of Pediatrics) and the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

Received for publication April 18, 1950

Several techniques have been utilized in this laboratory for the study of the nutritional requirements for virus synthesis in Escherichia coli infected by bacteriophage. For instance, it was observed that when E. coli strain B grown in nutrient broth was resuspended in a simple medium (F) and infected with T2r+ bacteriophage, there was a marked decrease in the amount of virus produced, as well as an increase in the latent period before virus liberation. These effects were overcome when the F medium was supplemented with amino acids, purines, and pyrimidines (Fowler and Cohen, 1948). When L-histidine, L-isoleucine, L-glutamic acid, L-leucine, L-phenylalanine, L-valine, or L-tryptophan was omitted singly from the complex medium, the burst size of the infected cells was decreased (Cohen and Fowler, 1948). When L-tryptophan was added to unsupplemented F, a stimulation of virus production did not result (Fowler and Cohen, 1948). As a result of other types of analysis and the reversal of the inhibition caused by 5-methyltryptophan by tryptophan, this amino acid has been shown to be a specific requirement in virus synthesis in the E. coli–T2 system (Cohen and Fowler, 1947). Tryptophan also acts as an adsorption cofactor in the T4 and T6 strains of phage studied in this laboratory (Anderson, 1945).

A study of this amino acid, therefore, presented an interesting starting point in a closer examination of the requirements for individual amino acids of bacteriophage–E. coli systems as revealed by their depletion from a medium during the infection process. Preliminary observations concerning the utilization of various amino acids in a synthetic medium in which E. coli B was infected with T2r+ phage have been conducted in this laboratory through the use of single dimensional paper chromatography (Cohen, 1949b). The usefulness of this technique to determine the fate of amino acid constituents of a defined medium, before and after growth of bacteria, had been suggested by Linggood and Woiwod (1948, 1949). However, our results using this technique were inadequate in that reliable quantitative data could not be easily obtained, and the method of microbiological assay of amino acids appeared more readily applicable.

MATERIALS AND METHODS

Medium. The medium used for the assay of tryptophan with Escherichia coli strain B/1 and for the preparation of E. coli B consisted of mineral medium M

1 This research was conducted under Office of Naval Research Contract N6ori-188, Task Order 1, NR 136-055.
(Cohen, 1949a; Cohen and Arbogast, in press) and glucose (G), supplemented with 0.05 per cent Difco vitamin-free "casamino acid" and was designated as CM + G. One-hundred-ml quantities were autoclaved for 10 minutes at 15 pounds pressure, and 100 mg of sterile glucose were then added. To sterile, metal-capped, matched Klett-Summerson tubes cleaned according to the method described by Toennies and Gallant (1947) were pipetted 5 ml of the completed medium. In the preparation of the standard curve (from 0 to 10 μg per 5.5 ml of medium) CM + G was prepared at twice the final concentration, and 2.5-ml quantities were added to 1 ml of standard solutions of L-tryptophan (Winthrop Chemical Company) in H₂O, 1.5 ml of distilled H₂O, and 0.5 ml of unsupplemented M + G. The amino acid mixture containing 200 μg per ml of each amino acid of L-configuration and 400 μg per ml of the racemic mixtures consisted of DL-phenylalanine, DL-methionine, DL-leucine, DL-glutamic acid, L-arginine, L-histidine, L-hydroxyproline (Nutritional Biochemical Corporation); DL-serine, DL-valine, DL-isoleucine, DL-threonine, DL-alanine, DL-norleucine, L-cystine, DL-aspartic acid, L-lysine (Merck and Company); glycine (Eastman Kodak Company); L-proline (Bios Laboratory); L-tyrosine (Fischer Scientific Company); and L-tryptophan (Winthrop Chemical Company).

**Bacteriophage preparations.** Purified concentrates of T2r+, T2r, T4r+, T4r, T6r+, and T6r in 0.85 per cent saline were prepared according to a method described elsewhere (Cohen and Arbogast, in press).

**Preparation of E. coli B.** E. coli B, subcultured from stock slants on nutrient broth agar (Cohen, 1947) to 10 ml of CM + G, was incubated with aeration at 37 C overnight. Two ml of the culture were introduced to flasks containing 200 ml of the same medium, and the culture was aerated at 37 C. The bacteria, harvested by centrifugation when the cell concentration was 2.4 × 10⁸ per ml (as estimated by the optical density of the culture), were washed twice with M and finally resuspended in M so that the final concentration was 1 × 10⁸ per ml. Aliquots of this suspension were then added to the experimental tubes containing the amino acid mixture, phage, and glucose so that the final concentration of each amino acid was 10 μg per ml; the phage, 5 × 10⁸ per ml; glucose, 1 mg per ml; and the bacteria, 1 × 10⁸ per ml, thus establishing a 5-fold multiplicity of phage to bacteria. Samples withdrawn from the experimental tubes were heated and centrifuged 20 to 25 minutes at 5,000 rpm. The supernatants were assayed for residual tryptophan.

**Preparation of E. coli B/1.** The assay organism, a mutant of E. coli B resistant to phage T1 requires tryptophan for growth. It was routinely prepared by inoculating from stock slants on nutrient broth to 10 ml of CM + G supplemented with 100 μg L-tryptophan. After aeration at 37 C overnight, 0.1 ml of the culture was introduced into 10 ml of the same medium, incubated, and aerated at 37 C until growth reached 1 to 2 × 10⁶ cells per ml, determined turbidimetrically. Five ml of culture were centrifuged, washed twice with M, and resuspended in 5 ml of M. Aliquots of 0.05 ml B/1 suspension were added to assay tubes, which were incubated at 37 C overnight. The optical densities of the resulting cultures were read in a Klett-Summerson photoelectric colorimeter fitted with a 420 filter.
EXPERIMENTAL PROCEDURES

Medium for assay. The assay medium CM + G was devised in an attempt to increase the amount of growth of the tryptophan-requiring mutant B/1 in stationary cultures. M + G supplemented with amounts of tryptophan in the range of 0 to 10 μg per 5.0 ml of medium, to be determined in the course of our studies, did not appear adequate, since the turbidity of cultures containing 10 μg did not exceed a reading of 48 after 16 hours at 37 C. In addition, the growth of the organism in aerated cultures containing 10 μg of tryptophan per 5.0 ml of mineral medium was slow and the final density after 6 hours was half that obtained with E. coli B in M + G not supplemented with tryptophan. However, when M + G was enriched with acid-hydrolyzed casein and varying amounts of tryptophan, the growth of E. coli B/1 after 17 hours at 37 C in-

![Figure 1](http://jb.asm.org/)

*Figure 1. Growth of E. coli B in CM + G supplemented with either amino acid mixture (○) or tryptophan (×).*

creased more than 2-fold, with the most sensitive part of the curve falling between 0 to 6 μg tryptophan per 5 ml of medium. CM + G without added tryptophan did not support the growth of the organism, nor did the addition of other amino acids from 0 to 10 μg per 5.5 ml of medium increase the amount of growth over that found in CM + G enriched with tryptophan alone. This is illustrated in figure 1.

Preparation of samples for assay. When E. coli B was grown in CM + G, washed, and suspended in M at a concentration of 10⁶ per ml, then heated for 2 minutes in a boiling water bath, the supernatant fluid was freed of all viable organisms, since no growth resulted when aliquots of the supernatant fluid were incubated in CM + G. In addition, under these conditions there was no detectable liberation of tryptophan from the bacteria since 1 ml of the same supernatant added to 5 ml of CM + G did not support the growth of B/1. To test
the recovery of tryptophan from the medium, varying amounts of tryptophan were added to culture tubes containing suspensions of heat-killed organisms at a concentration of $10^6$ per ml and then heated for an additional 2 minutes. After centrifugation, the supernatants were reassayed and were found to contain the added tryptophan (table 1). These control observations have been routinely substantiated since the recovery of added tryptophan at 0 time of incubation of our washed B suspended in M, glucose, and 10 $\mu$g of each of the amino acids has always been 100 $\pm$ 5 per cent.

Tryptophanase activity of E. coli B in CM + G. It was important to establish that any disappearance of tryptophan from the medium was due to its assimila-

<table>
<thead>
<tr>
<th>TRYPTOPHAN/6 ML OF MEDIUM</th>
<th>COLORIMETER READINGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heated cultures + tryptophan; centrifuged</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>42.5</td>
</tr>
<tr>
<td>4</td>
<td>67</td>
</tr>
<tr>
<td>6</td>
<td>81</td>
</tr>
<tr>
<td>8</td>
<td>96</td>
</tr>
<tr>
<td>10</td>
<td>104</td>
</tr>
</tbody>
</table>

This indicated that the organism may contain a very slight tryptophanase activity. This would agree with results obtained by Happold and Hoyle (1936) which demonstrated that tryptophanase was not formed in E. coli in the presence of glucose although it may be formed in a glucose-free synthetic medium in the absence of added tryptophan.
When E. coli B, prepared in an identical manner, is resuspended in a medium consisting of 10 μg tryptophan per ml of M + glucose, there is an immediate, marked loss of the amino acid from the medium (figure 2). Thus the cells that we shall infect are almost devoid of tryptophanase and use tryptophan only in the presence of a carbon source permitting growth.

The depletion of tryptophan from a medium by virus-infected cells. The protocol of a typical experiment comparing tryptophan disappearance in cultures of phage-infected and noninfected B is outlined in table 2. Bacteria were routinely infected in these experiments with 5 phage particles per cell.

Figure 2. Depletion of tryptophan from various media containing E. coli B. Aliquots were assayed for residual tryptophan with E. coli B/1.

RESULTS

E. coli infected with T2r and T2r+. As can be seen in figure 3, when tryptophan recovery obtained from the average of triplicate assays of the same sample is plotted against time, cultures of B infected with T2r or T2r+ did not effect so rapid a disappearance of tryptophan from the medium as did the uninfected cells. In addition, there appeared to be a slight reproducible difference in the rate and ultimate amount of tryptophan loss from the medium containing cells infected with T2r as compared to the rate and amount of loss from the medium with cells infected with T2r+. The depletion by T2r+-infected cells appeared slightly greater.

E. coli infected with T4r and T4r+. The addition of T4r or T4r+ phage to a
**TABLE 2**

Schedule of experiment on tryptophan utilization by virus-infected and noninfected bacteria

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>OPERATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>-180</td>
<td>2 ml of 24-hr CM + G culture inoculated into 200 ml CM + G. Incubated with aeration at 37°C until colorimeter reading = 64 (2.4 × 10⁸ cells/ml). 160 ml centrifuged at 5,000 rpm for 10 min, washed twice with M, and resuspended in 38 ml M. Reading of final suspension B = 263.</td>
</tr>
<tr>
<td>0</td>
<td>Tube 1. To 1.3 ml of 0.85 per cent NaCl containing 114 µg of amino acids and 11.4 mg glucose were added 10.2 ml of B. 2 ml withdrawn immediately, added to sterile tube, immersed in boiling water bath for 2 minutes. Remainder in tube 1 aerated in 37°C water bath.</td>
</tr>
<tr>
<td>1</td>
<td>Tube 2. 5 × 10¹⁰ T2r phage particles added to 114 µg of amino acids, 11.4 mg glucose, and saline to make a final volume 1.3 ml.</td>
</tr>
<tr>
<td>2</td>
<td>10.2 ml of B added to tube 2; 2 ml withdrawn immediately, added to sterile tube, immersed in boiling water bath 2 minutes. Tube 2 aerated in 37°C water bath.</td>
</tr>
<tr>
<td>3</td>
<td>Tube 3. 5 × 10¹⁰ T2r+ phage particles added to 114 µg of amino acids, 11.4 mg glucose, and saline to make a final volume of 1.3 ml.</td>
</tr>
<tr>
<td>4</td>
<td>10.2 ml of B added to tube 3; 2 ml withdrawn immediately, added to sterile tube, immersed in boiling water bath 2 minutes. Tube 3 aerated in 37°C water bath.</td>
</tr>
<tr>
<td>5</td>
<td>2 ml withdrawn tube 1, added to sterile tube, and heated 2 minutes in boiling water bath.</td>
</tr>
<tr>
<td>10</td>
<td>2 ml withdrawn tube 2, added to sterile tube, and heated 2 minutes in boiling water bath.</td>
</tr>
<tr>
<td>15</td>
<td>2 ml withdrawn tube 3, added to sterile tube, and heated 2 minutes in boiling water bath.</td>
</tr>
</tbody>
</table>

All aliquots were centrifuged 20 to 25 minutes at 5,000 rpm, 0.5 ml of supernatant were added to triplicate tubes containing 5 ml of CM + G. Inoculated with 0.05 ml B/1. Standard curves on known tryptophan concentrations were run in duplicate.

Suspension of B, in duplicate experiments, resulted in a more rapid disappearance of tryptophan from the medium. This is illustrated in figure 4. Again tryptophan loss appeared to be greater when the cells were infected with the r+ strain.

*E. coli* infected with *T6r* and *T6r*. As may be observed in figure 5, cells infected...
Figure 3. Depletion of tryptophan from a medium supplemented with amino acids by *E. coli* B infected with either T2r or T2r+ strain phage.

Figure 4. Depletion of tryptophan from a medium supplemented with amino acids by *E. coli* B infected with either T4r or T4r+ strain phage.
Figure 5. Depletion of tryptophan from a medium supplemented with amino acids by E. coli B infected with either T6r or T6r+ strain phage.

Figure 6. Depletion of tryptophan from a medium supplemented with amino acids by E. coli B infected with T2r+, T4r+, or T6r+ strain phage.
with a T6 strain were slower than uninfected B in removing tryptophan from the medium. In general, cells infected with T6 were also slower in depleting tryptophan than other infected cells.

Because of the apparent difference in effect of each strain of phage on the recovery of tryptophan from the medium, the need for comparative studies among the r and r+ strains added to the same preparation of bacteria was indicated. In figure 6 the utilization of tryptophan by B infected with T2r+, T4r+, or T6r+ is illustrated. In two experiments T2r+-infected cells removed the most

![Figure 7. Depletion of tryptophan from a medium supplemented with amino acids by E. coli B infected with T2r, T4r, or T6r strain phage.](image)

tryptophan, and T6r+-infected cells were far slower than cells infected with T2r+ or T4r+.

In similar experiments with T2r, T4r, and T6r (figure 7), the loss of tryptophan from the medium containing T2r-infected cells was greatest, whereas the presence of T6r-infected B resulted in the least disappearance of that amino acid.

*E. coli infected with irradiated or nonirradiated T2r+.* It was of interest to investigate the effect on tryptophan utilization of bacteria exposed to irradiated T2r+. A preparation of T2r+ was exposed to ultraviolet irradiation. The calculated number of hits determined from the percentage of survivors was 3.39. The depletion of tryptophan from the medium was then determined for suspensions
of noninfected bacteria, or bacteria infected with irradiated or nonirradiated phage. The results of one experiment may be found in figure 8. Although $5 \times 10^{10}$ particles were added to B in either case, the infection of bacteria with irradiated T2r$^+$ resulted in a more rapid and complete depletion of tryptophan from the medium than the presence of uninfected cells, whereas infection with nonirradiated T2r$^+$ resulted in a decrease of tryptophan utilization.

**Figure 8.** Depletion of tryptophan from a medium supplemented with amino acids by *E. coli* B infected with irradiated or nonirradiated T2r$^+$.

**DISCUSSION**

It is apparent that this technique may be useful in the study of nutritional requirements of virus-infected cells. The disappearance of any amino acid found in a defined medium could be followed in a similar manner with the use of an appropriate assay organism. Conceivably this technique is also adaptable to the utilization of amino acids in tissue cultures, virus-infected or not.

Of interest has been the finding that cells infected with T6 strains were quite slow in the utilization of tryptophan. This may indicate that T6 contains less tryptophan than either T2 or T4. Since the T6 strains appear to have adsorption cofactor (tryptophan) requirements of the same order as these T4 strains, this difference in inducing cells to use tryptophan does not appear to be related to
that function. However, T2 strains have no cofactor requirement, and the more rapid utilization of the amino acid by cells infected with T2 may indeed be related to the necessity of building tryptophan into the external surface of the phage. It would clearly be of interest to correlate the physiological data presented above with analysis of the tryptophan contents of these phages. It has been shown that the assimilation pattern of virus-infected cells appears similar to that of normal cells with respect to phosphorus and carbon utilization (Cohen, 1949b; Cohen and Arborgast, in press). Indeed, the rates of synthesis of protein and nucleic acid were essentially identical in a comparison of r and r+ strains. In a comparison of cells infected with T2r, T4r, and T6r, the maximal rates of nucleic acid synthesis were identical. In this study, however, it has been shown that the removal from the medium of a relatively complex building block (tryptophan) essential for virus synthesis does vary with the virus being synthesized. This difference from the earlier studies presumably depends on the complexity of the substance removed from the medium and the number of operations that must be performed to fit the substance into the virus structure.

A certain amount of variability was noted from one experiment to the next. It is now considered desirable in the course of each experiment to correlate the loss of amino acid from the environment of the host cell with the results of another type of function of virus synthesis, such as deoxyribose nucleic acid synthesis or phage formation.

**SUMMARY**

*Escherichia coli* B/1, a tryptophan-requiring mutant of *E. coli* B has been used in the microbiological assay of low concentrations of L-tryptophan.

A method has been devised for measuring the depletion of tryptophan from a medium incubated with normal and virus-infected bacteria.

*E. coli* B grown in a medium containing acid-hydrolyzed casein and glucose develops little or no tryptophanase but is capable of the immediate utilization of tryptophan under conditions suitable for growth.

*E. coli* B infected with phage, suspended in a defined medium containing 20 amino acids, rapidly removed L-tryptophan from the medium.

The uptake of tryptophan was a function of the virus strain used, the rates of depletion by virus-infected cells being in the order T2 > T4 > T6.

**REFERENCES**


