Enhancement by Adenine of the Inhibition of Salmonella typhimurium by Ethionine

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ABSTRACT

SMITH, ROBERT C. (Auburn University, Auburn, Ala.), and W. D. SALMON. Enhancement by adenine of the inhibition of Salmonella typhimurium by ethionine. J. Bacteriol. 89:1494-1498. 1965.—The inhibition by ethionine of the growth of Salmonella typhimurium was increased by adenine and several other purines. This effect was counteracted by the addition of methionine to the cultures. There was no preferential inhibition of nucleic acid or protein synthesis by adenine plus ethionine. Adenine stimulated the uptake of radioactive ethionine by the cells. Adenine may produce the effects observed by increasing the rate at which ethionine enters the cell and reaches its primary site of antibacterial action.

The inhibition of the growth of bacteria by ethionine, and the ability of methionine to overcome this inhibition, were first observed by Harris and Kohn (1941). Several mechanisms have been suggested to explain the growth inhibition produced by ethionine. It may compete with methionine and be activated to S-adenosylmethionine, which could either interfere in transmethylation reactions directly or undergo transmethylation reactions to form ethyl analogues of normally methylated metabolites. S-adenosylmethionine could act as a trap for adenine. Ethionine may also be incorporated into protein to form abnormal proteins containing ethionine in place of methionine. Recent reviews of ethionine have been provided by Farber (1963) and Stekol (1963).

Several reports have recently appeared on the use of adenine and adenosine triphosphate (ATP) to counteract the inhibitory effects of ethionine in rats. Villa-Trevino and Farber (1962) found that the inhibition of protein synthesis in the liver of ethionine-treated rats was prevented by injecting the rats with ATP or adenine. These two compounds also counteracted the decrease in hepatic ATP concentration induced by ethionine injection (Villa-Trevino, Shull, and Farber, 1963), and prevented the induction of fatty liver in rats treated with ethionine (Farber, Lombardi, and Castillo, 1963). ATP also counteracted ethionine-induced inhibition of elongation of Avicennia coleoptiles (Norris, 1964). Since adenine and ATP, like methionine, are effective in overcoming many of the effects produced by ethionine, we have investigated the effect of adenine on the growth of Salmonella typhimurium inhibited by ethionine.

MATERIALS AND METHODS

Organism and growth conditions. The wild-type strain of S. typhimurium and tris(hydroxymethyl)aminomethane (Tris)-glucose medium, employed in previous studies (Smith and Maasje, 1964), were used throughout the experiments. L-Ethionine and adenine were added at the concentrations indicated. The bacteria were incubated in Erlenmeyer flasks shaken in a water bath (37°C). Growth was followed by optical density readings (450 μm, 1-cm light path) against a Tris medium blank in a Beckman DU spectrophotometer. Exponential growth was reached and maintained for at least three generations before an experiment was begun.

Chemical determinations. Standard procedures were used for the determination of ribonucleic acid (RNA; Brown, 1946), deoxyribonucleic acid (DNA; Burton, 1956), and protein (Lowry et al., 1951) content of cells that had been extracted for 16 hr at 2°C with 5% trichloroacetic acid.

Ethionine-ethyl-1-C14 experiments. Portions of an exponentially growing bacterial culture were added to fresh medium (37°C) containing 8 μg/ml of L-ethionine and about 0.05 μc/ml of ethionine-ethyl-1-C14. Samples (2 ml) of the culture were removed and harvested by filtration on a membrane filter (Millipore Filter Corp., Bedford, Mass.) and washed three times with 5-ml portions of Tris medium. The filters were dried and counted as described previously (Smith and Salmon, 1965).

Source of chemicals. The amino acid analogues, purines, and pyrimidines were purchased from Calbiochem.
RESULTS

The effect of ethionine on the growth of the bacteria is shown in Fig. 1. The inhibition of growth produced by ethionine at concentrations of 200, 400, and 800 µg/ml was similar. At higher concentrations of ethionine, the inhibition of growth became progressively greater. The effect of 10 µg/ml of adenine on the growth of cultures incubated in medium containing a relatively low amount of ethionine (200 µg/ml) or a relatively high amount of ethionine (1,600 µg/ml) is shown in Fig. 2. At both concentrations of ethionine, the growth of the cultures containing adenine plus ethionine was markedly slower than growth of the cultures containing ethionine alone. The growth of the culture containing 200 µg/ml of ethionine and 10 µg/ml of adenine was comparable with that of a culture containing 1,600 µg/ml of ethionine. The addition to the medium of adenine alone at this concentration had no effect on the growth of the bacteria.

The effect of the concentration of adenine on the growth of bacteria incubated in medium containing 200 µg/ml of ethionine plus adenine is shown in Fig. 3. As the concentration of adenine increased, there was a progressively greater inhibition of growth. Since both 100 and 200 µg/ml of adenine gave the same response, this is the maximal inhibition that can be obtained with adenine. At these latter concentrations there was a slight decrease in growth rate produced by adenine alone. Similar observations have been reported by other workers (Brooke and Magasanik, 1954; Demain, 1964; Moyed, 1964; Neidhardt, 1963). Adenosine, inosine, hypoxanthine, and deoxyinosine were also found to enhance the inhibition induced by ethionine. Guanine, cytosine, uracil, thymine, and xanthine had no effect on the growth of cultures incubated in medium containing 200 µg/ml of ethionine. Adenine also enhanced the inhibition produced by norleucine in cultures of *S. typhimurium*, but adenine had no effect on the growth of cultures inhibited by p-fluorophenylalanine (Warren and Smith, unpublished data). The effect of adenine on the growth of bacteria inhibition by either ethionine or norleucine was completely counter-

![Fig. 1. Optical densities of cultures of *Salmonella typhimurium* incubated with L-ethionine at the following levels (µg/ml): ◆, 0; O, 200; ▲, 400; △, 800; ■, 1,200; ◇, 1,600; +, 2,000.](http://jb.asm.org/)

![Fig. 2. Optical densities of cultures of *Salmonella typhimurium* incubated with: ◆, 200 µg/ml of L-ethionine; ▲, 200 µg/ml of L-ethionine plus 10 µg/ml of adenine; △, 1,600 µg/ml of L-ethionine; ■, 1,600 µg/ml of L-ethionine plus 10 µg/ml of adenine. Growth of *S. typhimurium* in Tris-glucose medium: ◆.](http://jb.asm.org/)
**Discussion**

Although adenine can counteract many of the effects produced by the injection of ethionine into rats (Farber et al., 1964), the results show that adenine enhances the inhibition by ethionine of bacterial growth. This increase in growth inhibition does not appear to be due to preferential inhibition of nucleic acid or protein synthesis. Adenine was found to stimulate the incorporation of radioactivity from ethionine-ethyl-1-C\(^{14}\) into

![Graph](https://via.placeholder.com/150)

**FIG. 3.** Optical densities of cultures of Salmonella typhimurium incubated with 200 \(\mu\)g/ml of L-ethionine and adenine at the following levels \((\mu\)g/ml\): ○, 0; △, 1; Δ, 10; ■, 100; □, 200. Growth of *S. typhimurium* in Tris-glucose medium: ●.

The effect of adenine on the RNA, DNA, and protein composition of cultures inhibited by ethionine is summarized in Table 1. There was apparently no preferential inhibition of any of these three components.

Adenine enhanced the uptake of radioactivity of ethionine-ethyl-1-C\(^{14}\) by the bacteria (Fig. 5). The uptake of radioactive material is plotted against the optical density of the culture. As the concentration of adenine in the medium was increased, the incorporation of radioactivity into the cells became greater. Ethionine, even at the low concentration of 8 \(\mu\)g/ml, produced a slight inhibition of growth, which was increased by adenine. After 3 hr, a control culture had an optical density of 0.429; a culture with ethionine had an optical density of 0.381; and a culture with ethionine plus adenine (10 \(\mu\)g/ml) had an optical density of 0.338.

**Table 1. Effect of adenine on RNA, DNA, protein, and optical density of cultures inhibited by ethionine**

<table>
<thead>
<tr>
<th>Component</th>
<th>Culture A (control)</th>
<th>Culture B (ethionine)</th>
<th>Culture C (ethionine + adenine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA</td>
<td>26.9</td>
<td>12.8</td>
<td>11.8</td>
</tr>
<tr>
<td>DNA</td>
<td>4.3</td>
<td>2.4</td>
<td>2.3</td>
</tr>
<tr>
<td>Protein</td>
<td>95.2</td>
<td>55.6</td>
<td>47.8</td>
</tr>
<tr>
<td>Optical density</td>
<td>0.508</td>
<td>0.300</td>
<td>0.306</td>
</tr>
</tbody>
</table>

* Bacteria were incubated for 3 hr in Tris-glucose medium containing: no addition, culture A; 1,600 \(\mu\)g/ml of ethionine, culture B; and 200 \(\mu\)g/ml of ethionine plus 100 \(\mu\)g/ml of adenine, culture C. Results are expressed as micrograms per milliliter of culture.
Fig. 5. Incorporation of radioactive ethionine into the cold trichloroacetic acid-insoluble fraction of Salmonella typhimurium. The bacteria were incubated with adenine at the following levels (μg/ml): ● 0; ○ 10; ▲ 20; △ 30.

The bacteria may produce the effects observed on ethionine-inhibited cells by increasing the rate at which ethionine enters the cell and reaches its primary site of antibacterial action.

The results reported by Nagano et al. (1952) have also been suggested for the basis of resistance of mutants of Saccharomyces cerevisiae to ethionine (Sorsoli, Spence, and Parks, 1964). There are other mechanisms that can also explain inhibition by normal purines of a guanineless mutant of Bacillus subtilis. Permeability has also been suggested for the mechanism of resistance of mutants of Bacillus subtilis. These mechanisms may also explain inhibition of growth by normal purines and resistance to amino acid analogues (Fangman and Neidhardt, 1964a, b; Lewis, 1963; Martin and Moo-Penn, 1963; Moayed, 1964; Rowbury and Woods, 1961).

In contrast with findings reported in this paper, Travassos, Curly, and Hutner (1964) reported that adenine plus uracil can reverse ethionine inhibition produced in Candida slooffii. They suggested that ethionine may interfere with nucleic acid synthesis. Since their results were reported in a preliminary form, it is not possible to decide whether the differences between their work and the results reported here are due to the microorganisms used, the media used, or to the experimental design. It should be noted that they used a yeast that required methionine and that their medium contained 0.0003% methionine.

Acknowledgment

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Literature Cited


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