Action of Phenethyl Alcohol on the Synthesis of Macromolecules in *Escherichia coli*

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**ABSTRACT**

PREVOST, C. (University of California, Berkeley), and V. MOSES. Action of phenethyl alcohol on the synthesis of macromolecules in *Escherichia coli*. J. Bacteriol. 91:1446-1452. 1966.—A kinetic study of the effects of various concentrations of phenethyl alcohol on the synthesis of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), protein, and β-galactosidase in *Escherichia coli* has confirmed that RNA synthesis, rather than DNA synthesis, is first and most affected by phenethyl alcohol. The presence of inducer did not protect β-galactosidase synthesis from inhibition by phenethyl alcohol. Little preferential inhibition of β-galactosidase synthesis was observed; this is in contrast to the severe catabolite repression which results from partial inhibition of total protein synthesis caused by chloramphenicol or starvation for a required amino acid. We found no evidence that messenger RNA synthesis was inhibited to a greater extent than total RNA synthesis.

The bacteriostatic action of phenethyl alcohol (6) was first ascribed in *Escherichia coli* to a specific and reversible effect on bacterial deoxyribonucleic acid (DNA) synthesis (1). Sporulation and germination of *Bacillus megaterium*, however, were inhibited by phenethyl alcohol at a concentration which did not inhibit DNA synthesis (15). Although phenethyl alcohol inhibited the growth of DNA phages (4), it was also inhibitory for the growth of ribonucleic acid (RNA) phages (11). This suggested that the primary site of action may not necessarily be the inhibition of DNA synthesis. A recent paper indicated that a lower concentration of phenethyl alcohol is required to give 50% inhibition of the incorporation of radiophosphorus into RNA than into DNA (14). On the basis of a preferential inhibition of the synthesis of alkaline phosphatase compared with that of total protein, and some measurements of "mRNA" of induced β-galactosidase, Rosenkranz, Carr, and Rose (13) suggested that the synthesis of messenger RNA was the principal point of attack by phenethyl alcohol.

We have measured the rates of protein, RNA, and DNA synthesis in *E. coli* before and after the addition of various concentrations of phenethyl alcohol, and indeed find that RNA synthesis, but not necessarily messenger RNA synthesis, is the process showing greatest inhibition.

**MATERIALS AND METHODS**

*Bacterium E. coli* Cavalli, a derivative of *E. coli* K-12, was obtained from Aileen Simmons of the Molecular Biology Department, University of California, Berkeley. It is auxotrophic for methionine and thymine.

**Medium and growth.** The bacteria were grown aerobically with stirring at 37°C in M-63 medium (12) supplemented with 0.2% glycerol, 50 µg/ml of methionine, and 2.0 µg/ml of thymine. In experiments where uracil incorporation was measured, the cells were usually grown in the presence of 70 µg/ml of uracil; the cells preferentially used the externally added uracil (8). Growth was followed by measuring turbidity at 650 mAU in a Beckman DK-2 spectrophotometer. The doubling time was about 60 min.

**Incorporation of labeled precursors.** Labeled precursors (New England Nuclear Corp., Boston, Mass.) of the following specific radioactivities were added to the cultures at the beginning of the experimental period to measure the rates of labeling of macromolecules: methyl-[H]thymine, 300 µc/µmole; uracil-[2-14C], 1.4 µc/µmole, methyl-[3H]methionine, 1.4 µc/µmole. Samples (0.25 ml) from the culture of growing cells were added to 0.25 ml of 10% trichloroacetic acid and left at 0°C for at least 30 min. The whole 0.5-ml sample was transferred to a prewetted Millipore filter (HAWP 025; 00, 0.45 µ) and washed 10 times with 1 ml of distilled water. The Millipore filter was dissolved in 18 ml of scintillation fluid (3). The trichloroacetic acid-insoluble fraction includes proteins, DNA, and RNA. It was shown by two-dimensional paper chromatography (phenol-water, 72:28, w/w, followed by n-butanol-propionic acid-
Effect of phenethyl alcohol on the synthesis of induced β-galactosidase. IPTG was added at time zero to four parallel cultures of exponentially growing cells. Phenethyl alcohol was added 32 min later: △, 0% (v/v); ○, 0.10%; ●, 0.15%; △, 0.25%. At zero-time the culture contained 80 μg of bacterial protein per ml, and the doubling time was 68 min.

Enzyme induction and assay. Isopropyl-thio-β-D-galactopyranoside (IPTG), a gratuitous inducer of the lactose operon, was added to the bacterial cultures at a final concentration of 5 × 10^-4 M. Galactosidase activity was assayed by measuring the rate of hydrolysis of o-nitrophenyl-β-D-galactopyranoside as described by Kepes (2). One unit of enzyme activity is defined as the amount which catalyzes the hydrolysis of 1 mmole of substrate per min per ml at 37°C and pH 7.4.

Protein. Total protein was measured chemically (1) and by following the incorporation of methionine-C14.

RESULTS

Effect on turbidity, and the synthesis of protein and induced β-galactosidase. The time course of the inhibitory effect on the synthesis of induced β-galactosidase by increasing concentrations of phenethyl alcohol is illustrated in Fig. 1. The differential rate of β-galactosidase synthesis (β-galactosidase versus metionine-C14 incorporation) is definitely not depressed at lower concentrations of phenethyl alcohol but is even slightly stimulated (Fig. 2A). Higher concentrations of phenethyl alcohol cause some degree of preferential inhibition which increases with time (Fig. 2B, 2C).

Phenethyl alcohol, at a concentration of 0.30%, was sufficient to cause an almost immediate cessation of protein synthesis and increase in turbidity (Fig. 3). At a slightly lower concentra-
FIG. 3. Effect of phenethyl alcohol (0.30%) on turbidity and total protein.

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FIG. 4. Effect of phenethyl alcohol (0.28%) on total protein and induced β-galactosidase synthesis. At the time of adding phenethyl alcohol, the culture contained 175 μg of bacterial protein per ml, and the doubling time was 74 min.

At the time of adding phenethyl alcohol, the culture contained about 110 μg of bacterial protein per ml, and the doubling time was 75 min.

FIG. 5. Inducibility of β-galactosidase after the addition of phenethyl alcohol (0.28%). Phenethyl alcohol was added to a culture of exponentially growing cells. At various times, samples of the culture were removed and β-galactosidase synthesis was induced with IPTG. β-Galactosidase activity was then followed for the next 25 min. Induction started at the following times in relation to addition of phenethyl alcohol: Δ, minus 20 min; ○, plus 10 min; ●, plus 70 min; □, plus 130 min; •, plus 190 min. At the time of adding phenethyl alcohol, the culture contained 175 μg of bacterial protein per ml, and the doubling time was 74 min.

FIG. 6. Effect of phenethyl alcohol (0.30%) on thymine incorporation in exponentially growing cells of a thymine auxotroph. Culture A was grown ab initio in presence of thymine-H⁺. Culture B was grown in a parallel flask with thymine-H⁺, and thymine-H³ was added 5 hr after the addition of phenethyl alcohol. At the time of adding phenethyl alcohol, the cultures contained about 110 μg of bacterial protein per ml, and the doubling time was 75 min.
ACTION OF PHENETHYL ALCOHOL ON MACROMOLECULES

Fig. 7. Effect of phenethyl alcohol (0.30%) on thymine and uracil incorporation. Thymine-H³ and uracil-2-C¹⁴ were added simultaneously with phenethyl alcohol to exponentially growing cells. At the time of adding phenethyl alcohol, the culture contained about 116 µg of bacterial protein per ml, and the doubling time was 61 min.

Fig. 8. Effect of phenethyl alcohol (0.30%) on the rate of labeling of RNA with uracil-2-C¹⁴. The cells were grown in the absence of uracil. At various intervals, 0.25-ml samples were added to 0.025 ml of uracil-2-C¹⁴ (15.8 µmole; 31.6 µc/µmole), incubated for 2 min at 37 C, and killed by the addition of 0.25 ml of 10% trichloroacetic acid at 0 C. At the time of adding phenethyl alcohol, the culture contained 100 µg of bacterial protein per ml, and the doubling time was 72 min.

Fig. 9. Effect of phenethyl alcohol on thymine-H³ and uracil-2-C¹⁴ incorporation in exponentially growing cells: (A) 0.25% phenethyl alcohol; (B) 0.30% phenethyl alcohol. At the time of adding phenethyl alcohol, the cultures contained 140 and 112 µg of bacterial protein per ml, and the doubling times were 79 and 62 min, respectively.

20 to 40 min, then recovered somewhat during the next 2 hr before being completely inhibited thereafter (Fig. 4). At no time, at these high concentrations of phenethyl alcohol, was there any significant uncoupling between general protein synthesis and induced β-galactosidase synthesis. β-Galactosidase synthesis was induced at various times after the addition of phenethyl alcohol (Fig. 5). It was found that the rate of β-galactosidase synthesis was inhibited to the greatest extent 10 min after the addition of phenethyl alcohol and to the least extent 60 min later, during the recovery period. In an experiment...
similar to the one reported in Fig. 5, IPTG was added 3 hr after phenethyl alcohol (0.30%) and, although protein synthesis was inhibited by about 98%, the differential rate of β-galactosidase synthesis was not reduced by more than 25%.

Effect on nucleic acid synthesis. DNA synthesis was inhibited after about 1 hr by 0.30% phenethyl alcohol (Fig. 6). The small extent of incorporation of thymine-H³ added 5 hr after 0.30% phenethyl alcohol revealed that only a small portion of the DNA was turning over at that time. The synthesis of DNA and RNA were compared (Fig. 7) by simultaneously adding thymine-H³, uracil-C¹⁴, and phenethyl alcohol (0.30%). DNA synthesis continued at an undiminished rate for at least 1 hr before slowing down. The amount of DNA made during that period corresponded to 40 to 60% of the amount present at the time of addition of phenethyl alcohol. In contrast to the observation made with thymine, the incorporation of uracil was relatively low. The rate of uracil incorporation decreased by 90% within 20 min after the addition of phenethyl alcohol (Fig. 8). The cells were nevertheless able to incorporate uracil during a 2-min pulse at a low rate for at least 3 hr. Continuous measurement of the incorporation of uracil-C¹⁴ added 30 min before phenethyl alcohol showed that not only was uracil incorporation inhibited within a few minutes, but RNA also underwent a progressive breakdown (Fig. 9B). Similar measurements at 0.25% phenethyl alcohol again showed that RNA synthesis was more inhibited than DNA synthesis (Fig. 9A). After an initial and complete inhibition, which lasted approximately 20 min, RNA synthesis recovered to only 10% of the rate immediately preceding the addition of phenethyl alcohol. By comparison, the rate of DNA synthesis decreased approximately 40% during the 1st hr, and the rate of protein synthesis was even less affected.

**Discussion**

Phenethyl alcohol has been widely used as a specific inhibitor of nucleic acid synthesis. There is no general agreement, however, as to the actual site of action or even on the relative extent of inhibition of the biosynthesis of the various macromolecules. The results reported in the present paper do not support the contention of Berrah and Konetzka (1) that DNA synthesis is selectively inhibited by phenethyl alcohol.

Treick and Konetzka (18) followed the kinetics of the inhibition of DNA synthesis as well as that of RNA synthesis by measuring the incorporation of labeled thymine and uracil after the addition of phenethyl alcohol to cells growing in a rich medium. In their experiments, uracil incorporation continued unimpaired for up to 4 hr in the presence of 0.32% phenethyl alcohol, whereas DNA synthesis ceased within 1 hr. They did not indicate, however, the rate of uracil-C¹⁴ incorporation in the absence of phenethyl alcohol. We have obtained similar kinetic results as far as the inhibition of DNA synthesis is concerned, but have been unable to confirm the response of RNA synthesis to phenethyl alcohol which they observed. In our experiments, with cells grown on glycerol-salts medium, we found that this concentration of phenethyl alcohol not only stopped total RNA synthesis, but breakdown of the previously formed RNA occurred (Fig. 9B). The rate of uracil uptake with phenethyl alcohol reported by Treick and Konetzka (18) may have been a very small fraction of the rate at which uracil was taken up prior to the addition of the inhibitor. If this were the case, their findings would agree with the observations reported in this communication (Fig. 7 and 8). Our results concerning the inhibition of RNA synthesis are in agreement with those of Rosenkranz et al. (14).

Maašé and Hanawalt (7) found that inhibition of the synthesis of RNA and protein by the removal of uracil and arginine from *E. coli* 15 T⁻U⁻A⁻, which requires these substances for growth, prevented the initiation of a new cycle of DNA replication while permitting the completion of the cycle then in progress. In view of this observation, it is to be expected that phenethyl alcohol, which caused a severe inhibition of RNA and protein synthesis, would ultimately have a similar effect on the synthesis of DNA.

Rosenkranz et al. (13) demonstrated that low concentrations of phenethyl alcohol inhibit the synthesis of alkaline phosphatase to a much greater extent than the synthesis of proteins in general. A study by them of the effects of phenethyl alcohol on the induction of β-galactosidase seemed to indicate that, at levels which were not inhibitory to other processes, phenethyl alcohol interfered with the function or biosynthesis of β-galactosidase-specific messenger RNA. They have therefore proposed that phenethyl alcohol selectively inhibits the synthesis of messenger RNA. This explanation, however, is not consistent with the results reported in the present communication.

It has been estimated that 3 to 4% of all of the RNA in *E. coli* has a messenger function (5). If all messenger RNA is labile, then a total inhibition of messenger RNA synthesis, which would rapidly be reflected in a total cessation of protein synthesis, would appear as only a 4%
inhibition of total RNA synthesis. However, in experiments reported above, the partial reduction in protein synthesis has always been associated with a much greater inhibition of RNA synthesis than 4%. Were messenger RNA molecules for the bulk of the cellular protein not short-lived (9), complete inhibition of messenger RNA synthesis would not immediately have affected the synthesis of the total protein. Messenger RNA for β-galactosidase, however, is known to be very labile (2), so that we would expect a rapid preferential inhibition of β-galactosidase synthesis in the presence of phenethyl alcohol; such preferential inhibition has not been observed.

In contrast to the earlier findings with alkaline phosphatase and β-galactosidase (13), we found only a comparatively slight preferential inhibition of β-galactosidase synthesis (Fig. 2). A comparison of our findings with those of Rosenkranz et al. (13) is shown in Table 1. Thus, if the large inhibitory effects on β-galactosidase synthesis, observed by Rosenkranz et al. in the first few minutes after phenethyl alcohol addition, were due as they suggest to preferential inhibition of the specific messenger RNA, such an inhibition must be a very transitory phenomenon from which the cells recovered rapidly (see Fig. 1).

The presence of inducer did not protect β-galactosidase synthesis from phenethyl alcohol. Induction of β-galactosidase synthesis at various times after the addition of phenethyl alcohol (Fig. 5) followed the same pattern of inhibition and recovery as β-galactosidase synthesis induced before the addition of phenethyl alcohol (Fig. 4).

Although our kinetic experiments do not demonstrate that phenethyl alcohol is more inhibitory for the synthesis of messenger RNA than for other types of RNA, it is nevertheless obvious that a marked inhibition of RNA synthesis in general must eventually result in a lack of ribosomal and transfer RNA necessary for the translation of messenger RNA, and consequently cause a decrease in the rate of protein synthesis. We have no immediate explanation for the period of preferential inhibition of β-galactosidase at low concentrations of phenethyl alcohol (Fig. 2A).

The small degree of catabolite repression with phenethyl alcohol is rather unusual. It has frequently been observed in the past that, when protein synthesis is partially inhibited, the differential rate of β-galactosidase synthesis falls as a consequence of catabolite repression. For instance, Sypherd, Strauss, and Trefers (17) found that when protein synthesis was inhibited by 40 to 50%, with chloramphenicol the differential rate of β-galactosidase synthesis fell by 68%.

<table>
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<tr>
<th>Determination</th>
<th>Phenethyl alcohol conc (v/v)</th>
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<tr>
<td></td>
<td>0.10%</td>
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<tr>
<td>Rosenkranz et al.*</td>
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<tr>
<td>Protein (turbidity) (per cent of control)</td>
<td>73</td>
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<tr>
<td>Alkaline phosphatase (per cent of control)</td>
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<td>Alkaline phosphatase/protein</td>
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<td>Protein† (per cent of control)</td>
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<td>β-Galactosidase (per cent of control)</td>
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<tr>
<td>β-Galactosidase/protein</td>
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* The values were computed from the results presented in Fig. 1 from Rosenkranz, Carr, and Rose (13).
† These results were calculated from the experiment described in Fig. 1 and 2. The estimate of protein synthesis was the same whether turbidity or methionine-C14 incorporation was used.

Nakada and Magasanik (10) have noted that "cells cannot be induced in a medium containing glycerol as well as chloramphenicol; as in all cases of inhibition of protein synthesis without the removal of the source of catabolites, this effect may be ascribed to catabolite repression." Inhibition by phenethyl alcohol does not seem to follow such a rule, since, in the presence of glycerol, inhibition of protein synthesis by as much as 98% only resulted in a 25% reduction of the differential rate of induced β-galactosidase synthesis. We might even conclude from this that one of the modes of action of phenethyl alcohol is interference with the operation of catabolite repression.

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


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