PHENETHYL ALCOHOL AND MESSENGER RNA*

Herbert S. Rosenkranz, Howard S. Carr and Harry M. Rose

Department of Microbiology, College of Physicians and Surgeons

Columbia University, New York, N. Y., 10032

Received July 17, 1964

The bacteriostatic action of phenethyl alcohol (PEA) has been ascribed to its ability to inhibit DNA synthesis (Berrah and Konetzka, 1962). Since most of the other anti-DNA agents studied are bactericidal in nature, the mode of action of PEA was investigated systematically in this laboratory. It was discovered that messenger RNA (m-RNA) function appears to be the biosynthetic site most sensitive to PEA inhibition. This finding is the subject of the present communication.

Pardee and Prestidge (1961) and Nakada and Magasanik (1964) have shown that enzyme induction can be separated into two phases, (a) the synthesis of a specific m-RNA, followed by (b) the synthesis of the specific enzyme. Furthermore, that DNA synthesis is not necessary for the process of enzyme induction can be deduced from the fact that bacteria undergoing thymineless death do not produce DNA but are still capable of synthesizing induced enzymes (Cohen and Barner, 1955; McFall and Magasanik, 1962; Nakada, 1962).

Accordingly, the effect of phenethyl alcohol on enzyme induction was studied. In Figure 1, the levels of alkaline phosphatase produced in the presence of several concentrations of PEA are compared to the turbidities (an index of protein synthesis (Brock and Brock, 1959)) of the cultures. Whereas 0.23% PEA was needed to cause a 50% decrease in "protein" production, only 0.04% PEA was necessary for a similar effect on alkaline phosphatase production. The system of Nakada and Magasanik (1964) was then used to

* This study was supported by the United States Public Health Service (AI-05111), the American Cancer Society, and the Office of Naval Research (Contract Nonr 266 (89)-NR 103-574).

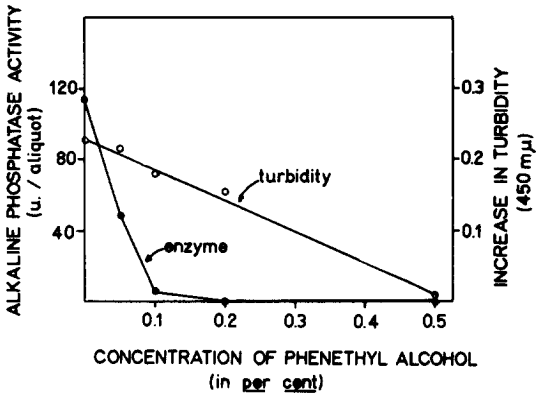

Figure 1: Effect of phenethyl alcohol on the production of alkaline phosphatase. Bacteria (E. coli C600) were brought to the exponential growth phase (2×10^8 cells per ml) in a nutrient medium. The cells were then chilled, harvested by centrifugation, washed with 0.15 M NaCl, and aliquots were distributed into the phosphorus-free medium of Torriani (1960), supplemented with amino acids and thiamine hydrochloride, (Rosenkranz and Bendich, 1964) and containing various amounts of phenethyl alcohol, which had been warmed to 37°C. The cells were incubated at 37°C for 60 minutes, the turbidities were determined and further protein synthesis was halted by the addition of merthiolate. Alkaline phosphatase activity was determined according to the procedure of Echols et al. (1961).

---

study the effects of PEA on various stages of enzyme induction. The data summarized in Table I indicates that the presence of PEA during the initial induction period (i.e., m-RNA synthesis) resulted in an inhibition of the production of β-galactosidase. On the other hand, the addition of PEA after the inductive phase did not cause an inhibition of enzyme synthesis when 0.1% PEA was used; rather, there was an actual increase in β-galactosidase activity upon the addition of 0.1% PEA at t = 3 minutes. This observation was reproducible; its physiological significance is being investigated. When the concentration of PEA at t = 3 minutes was adjusted to 0.2%, there was a 50% inhibition of β-galactosidase, which approximates the inhibition of total proteins by this concentration of PEA (see Figure I).

As anticipated, addition of PEA after the period of induction did not effect production of the enzyme (Table I).

These results suggest that PEA, at levels which are not inhibitory to other processes, interferes with the function or biosynthesis of m-RNA.

Table I

Effect of Phenethyl Alcohol on Various Stages of the Induction of β-galactosidase

Experimental Procedure	mμmoles o-nitrophenol formed in 20 m
Uninduced bacteria	0
Inducer and PEA added at t = 0; not removed	
0 % PEA	30.8
0.1% PEA	7.1
0.2% PEA	3.3
Inducer added at t = 0; not removed. PEA added at t = 3 min.	
0 % PEA	30.8
0.1% PEA	52.8
0.2% PEA	16.3
Inducer and PEA added at t = 0; both removed at t = 3 min.	
0 % PEA	22.4
0.1% PEA	8.7
0.2% PEA	9.5
Inducer added at t = 0 and removed at t = 3 min. PEA added at t = 3 min.	
0 % PEA	22.4
0.1% PEA	25.8
0.2% PEA	19.8

Bacteria (E. coli C600) growing at 30°C in medium HA (Rosenkranz and Bendic 1964) supplemented with glycerol (0.1%) were induced to form β-galactosidase by the addition of methyl-β-D-thiogalactoside (1×10^{-3} M). Phenethyl alcohol was added at the times indicated. The removal of the inducer and PEA was accomplish by collecting and washing the bacteria on filter discs. The bacteria were then resuspended in a pre-warmed (30°C) medium devoid of the inducer and PEA. Enzyme production was stopped at the end of 20 minutes by treating the cells with tolue Enzyme assays were carried out as described by Pardee, Jacob and Monod (1959).

A more extensive report dealing with the effect of phenethyl alcohol on bacterial metabolism and on the properties of macromolecules isolated from such treated bacteria is in preparation.

REFERENCES

Berrah, G., and Konetzka, W. A., J. Bacteriol. 83, 738 (1962).
Brock, T. D., and Brock, M. L., Biochim. Biophys. Acta 33, 274 (1959).
Cohen, S. S., and Barner, H. D., J. Bacteriol. 69, 59 (1955).
Echols, H., Garen, A., Garen, S. and Torriani, A., J. Mol. Biol. 3, 425 (1961).
McFall, E., and Magasanik, B., Biochim. Biophys. Acta 55, 900 (1962).
Nakada, D., Biochim. Biophys. Acta 55, 505 (1962).
Nakada, D., and Magasanik, B., J. Mol. Biol. 8, 105 (1964).
Pardee, A. B., Jacob, F., and Monod, J., J. Mol. Biol. 1, 165 (1959).
Pardee, A. B., and Prestidge, L. S., Biochim. Biophys. Acta 49, 77 (1961).
Rosenkranz, H. S., and Bendich, A. J., Biochim. Biophys. Acta 87, 40 (1964).
Torriani, A., Biochim. Biophys. Acta 38, 460 (1960).

Erratum

Vol. 16, No. 5, pp. 385-390 (1964), in the communication, "Some Products of the Degradation of Blood Group Substances by Alkaline Borohydride," by Kenneth O. Lloyd and Elvin A. Kabat:

Page 388, line 8 from bottom, Compound A 4b yielded 0.95 mole formic acid in 10 hours as would be expected from its proposed structure. The phrase "no formic acid" was a transcription error.