INHIBITION OF SPORULATION AND GERMINATION OF BACILLUS MEGATERIUM BY PHENETHYL ALCOHOL*

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The selective growth inhibition of bacteria by beta-phenethyl alcohol (PEA) was shown to be due to a selective and reversible inhibition of the synthesis of DNA (Berrah and Konetzka, 1962). Further evidence for this explanation of the inhibition was given when it was demonstrated that PEA had a striking effect on the replication of the bacteriophage T2 on E. coli H (Konetzka and Berrah, 1962). Recent reports indicate that PEA inhibition of DNA synthesis demonstrate that DNA replication is necessary for transfer of the bacterial chromosome in bacterial recombination (Bouck and Adelberg, 1963) and for the genetic recombination of bacteriophage (Folsome, 1963).

This communication reports the inhibition of sporulation and germination of B. megaterium at substantially lower concentrations of PEA than that required to inhibit growth and under conditions where no demonstrable inhibition of DNA synthesis is occurring suggesting that PEA may be acting at another site or may have more than one mechanism of inhibition.

For sporulation studies a 24 hr half-strength nutrient broth culture of B. megaterium was washed twice with distilled water and suspended in saline to a desired optical density reading. One half ml of the saline suspension served as an inoculum for 50 ml chemically

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defined sucrose medium (Slepecky and Foster, 1959) in 250 ml Erlenmyer flasks equipped with Klett tube side arms for turbidimetric growth determinations. Flasks were incubated at 30 C on a reciprocal shaker operating at 78 four inch strokes per min. One tenth ml aliquots were removed at various time intervals for direct counts in a Petroff-Hauser chamber and observed in a dark contrast phase contrast microscope. Such growth conditions gave greater than 90% sporulation after 65 hr incubation (Table 1). Addition of PEA to the growth

Table 1

Effect of PEA on the sporulation of B. megaterium

% PEA	Hour of Peak Growth	% Growth Inhibition*	% Spores at 65 hr
0	15	—	93
0.10	15	18	65
0.15	15	24	30
0.20	20	20	2
0.25	20	14	0

*% Growth Inhibition: Klett reading in control culture minus Klett reading in inhibitor culture/ Klett reading in control X 100

cultures at zero time led to a marked inhibition of sporulation without considerable growth inhibition. Complete inhibition of sporulation was achieved at 0.25% PEA concentration; no spores appeared even after extended incubation to 120 hr. Beyond 0.30% PEA elongated cell forms similar to that described for E. coli by Berrah and Konetzka (1962) appeared. Marked growth inhibition was found at 0.40% PEA and complete cessation of growth at 0.50% PEA. Placement of washed suspensions from sporulation inhibited cultures into fresh medium resulted in sporulation

indicating that the inhibition is reversible.

DNA determinations (Burton, 1956) of 0.25% PEA exposed cultures wherein the whole culture, previous procedure scaled down to 10 ml total culture, represented various points on the growth curve indicated no inhibition of the synthesis of DNA although sporulation was inhibited 100%. This is in agreement with Coulston and Konetzka (private communication) who have found 0.38% PEA necessary for DNA synthesis inhibition in B. megaterium under the same conditions.

For germination studies spores were obtained from control cultures as described previously for the sporulation studies. The optical

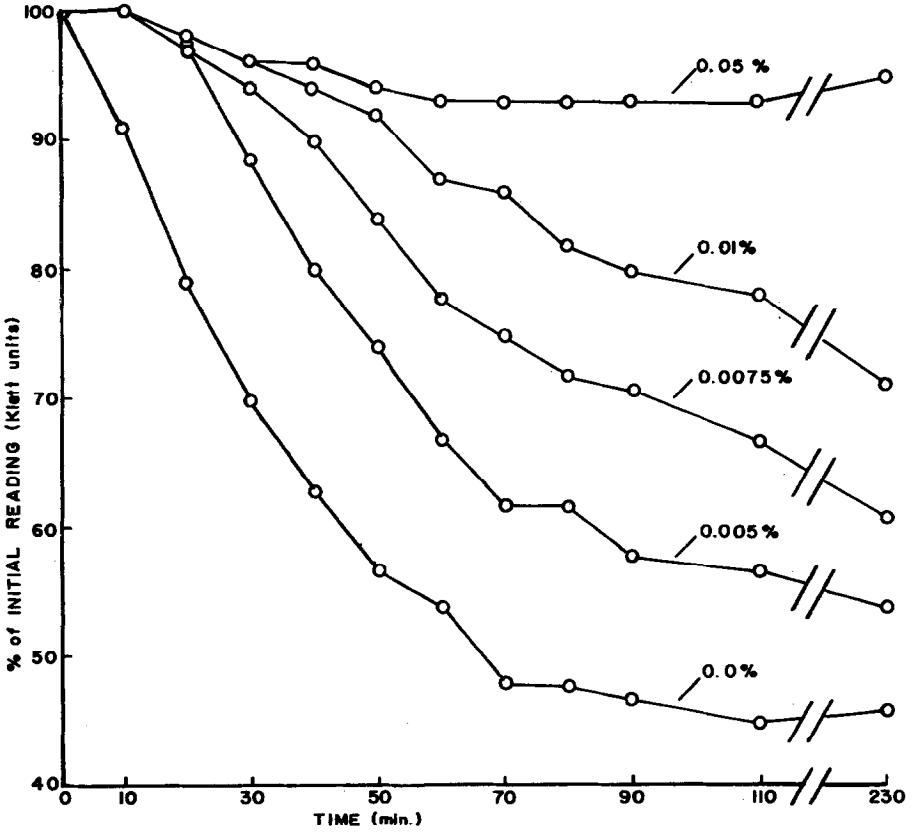

Figure 1. Effect of various concentration of PEA on suspensions of spores of B. megaterium. Spores were preheated in water 60 min at 60 C and suspended in 0.066 M phosphate buffer, pH 7.0, containing 100 micrograms per ml L-alanine; 100 micrograms per ml inosine; and the indicated concentration of PEA. The temperature of the experiment was 37 C. The ordinate is expressed as percentage of the initial reading measured in Klett-Summerson photometer units. The green filter, no. 54, was used.

density method of measuring germination (Powell, 1950; Rede and Foster, 1962) was used. Complete inhibition of germination was obtained with 0.05% PEA (fig. 1). Spores inhibited from germinating by 0.05% PEA and higher concentrations (e.g. 0.1% PEA) could germinate in fresh germination mixture without PEA after washing indicating reversibility of PEA inhibition as in the sporulation system. Addition of the inhibiting concentration of PEA (0.05%) at various times (fig. 2) gave inhibition of germination after a short lag period even when added after 10 minutes. That DNA synthesis is not occurring during the early phases

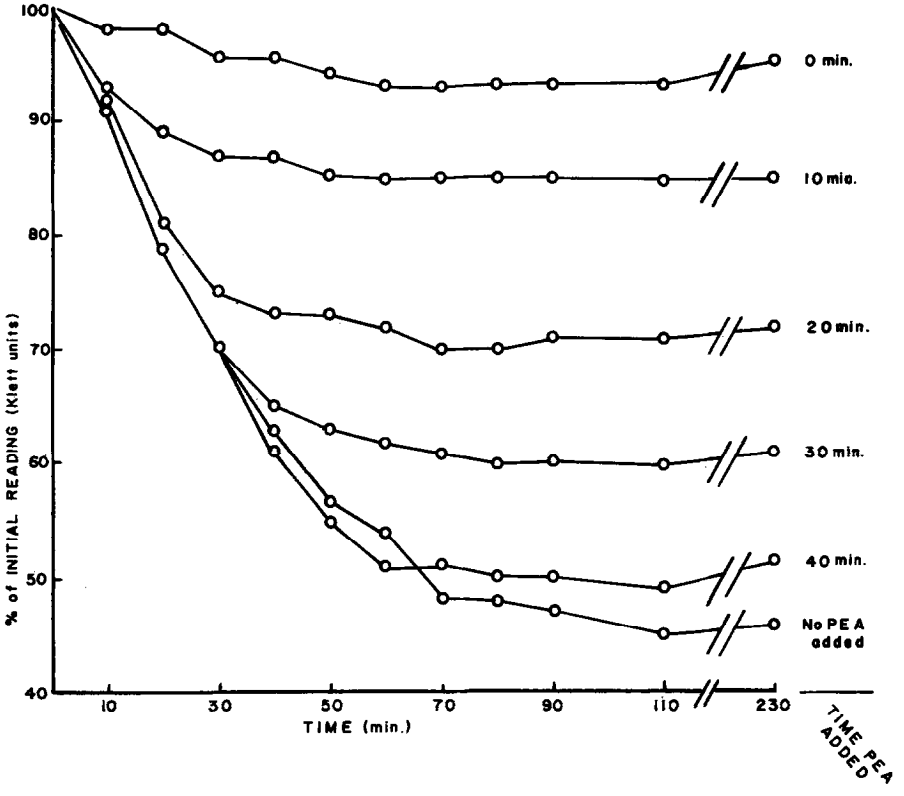

Figure 2. Reduction in optical density of suspensions of spores of B. megaterium to which 0.05% PEA was added at the times indicated. Conditions of the experiment were identical to that given in fig. 1.

of germination studied here and begins much later prior to cell division is well documented (Fitz-James, 1955; Stuy, 1958; Woese and Forro, 1960). It must be concluded that, as is the case with the PEA inhibition of

sporulation, the inhibition of germination by PEA must be occurring at some site other than the synthesis of DNA. While data indicating an alternative mechanism for the PEA inhibition reported here are not available it is suggested that PEA may be preventing the formation of stable informational RNA reported to be required for spore formation (del Valle and Aronson, 1962) and the formation of new RNA during the early stages of germination (Woese and Forro, 1960). This hypothesis is currently under investigation.

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