POLYAMINES AND INHIBITION OF RNA SYNTHESIS IN E. COLI BY LEVORPHANOL

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Levorphanol has been shown to inhibit selectively the synthesis of ribosomal RNA in E. coli (Simon and Van Praag, 1964a). RNA synthesis in crude E. coli extracts or with purified RNA polymerase was not inhibited by this drug (Simon and Van Praag, 1964b). This has led to the hypothesis that levorphanol may produce its inhibition indirectly by affecting the regulation of RNA synthesis. Recently evidence has been obtained suggesting the involvment of the polyamines, spermidine and putrescine, in the control of RNA synthesis of E. coli (Raina and Cohen, 1966). We wish to report a number of experiments indicating that spermidine reverses the inhibition of RNA synthesis by levorphanol and that levorphanol, in fact, influences the polyamine content and metabolism of E. coli.

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Reversal of levorphanol inhibition by spermidine

Incorporation of C^{14}-uracil into the acid-precipitable fraction of E. coli K-13 was measured after simultaneous addition of levorphanol and polyamines (Figure 1). It can be seen that spermidine protects against inhibition by levorphanol. Some protection against 1.35mM levorphanol is afforded by

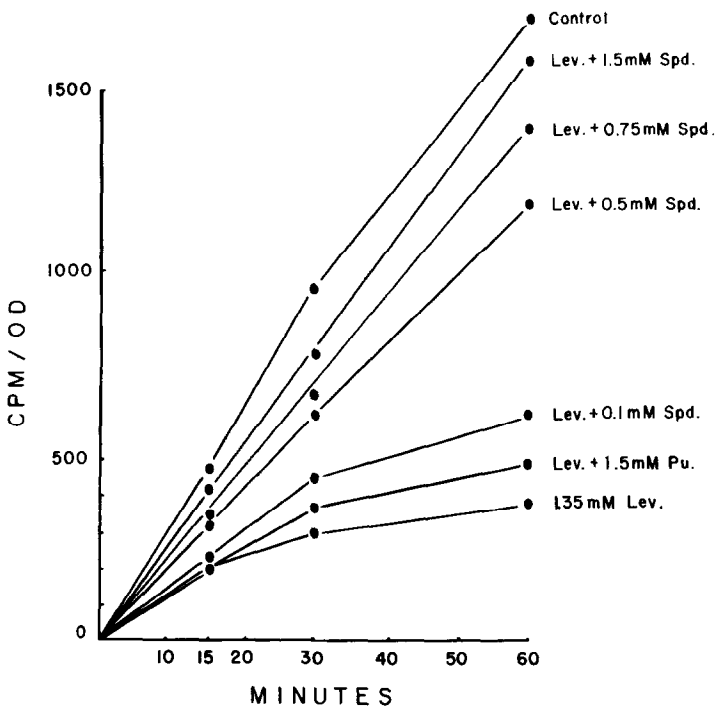

FIGURE 1: Effect of polyamines on inhibition of RNA synthesis by levorphanol. C^{14}-uracil (7.2µg/ml, .06µc/ml) was added to a culture of E.coli K-13 in exponential growth 5 min. after addition of levorphanol and polyamine. Samples were removed into 1M perchoric acid at indicated intervals, filtered through millipore membranes and counted as described previously (Simon and Van Praag, 1964b). The optical density at 550mµ was measured in a Lumetron colorimeter. Spd=spermidine, Pu-putrescine, Lev=levorphanol.

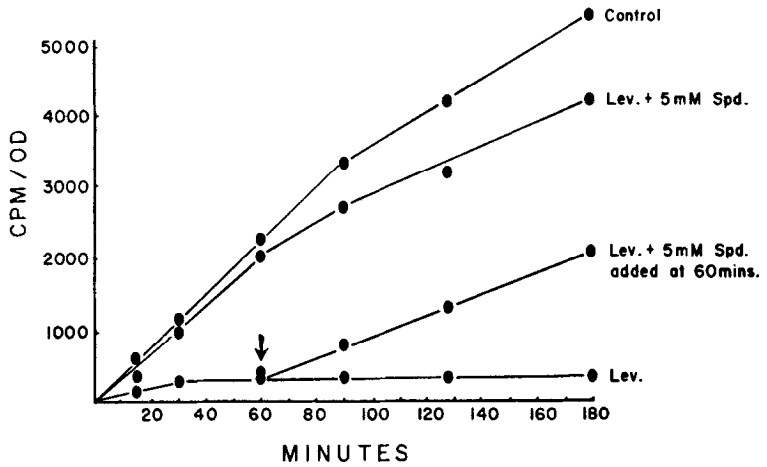

FIGURE 2: Reversal of levorphanol inhibition of RNA synthesis by spermidine in E.coli K-13.

0.1mM spermidine. The degree of protection increases with spermidine concentration and becomes almost complete at 1.5-5mM, with some variability from experiment to experiment. On the other hand, little or no protection is seen in the presence of putrescine even in the range of 1.5-5mM.

The addition of spermidine after levorphanol has been allowed to inhibit RNA synthesis also causes reversal of inhibition (Figure 2). This reversal is somewhat less than the protection seen when the polyamine is added together with levorphanol. Reversal of inhibition of RNA synthesis allows the bacteria to resume growth as illustrated by increases in turbidity and in viable counts. Spermidine or putrescine alone in the concentrations used here had no effect on either RNA synthesis or bacterial growth. Spermidine reversal of levorphanol inhibition permitted the synthesis of normal ribosomal components (50S and 30S), as revealed by sucrose gradient

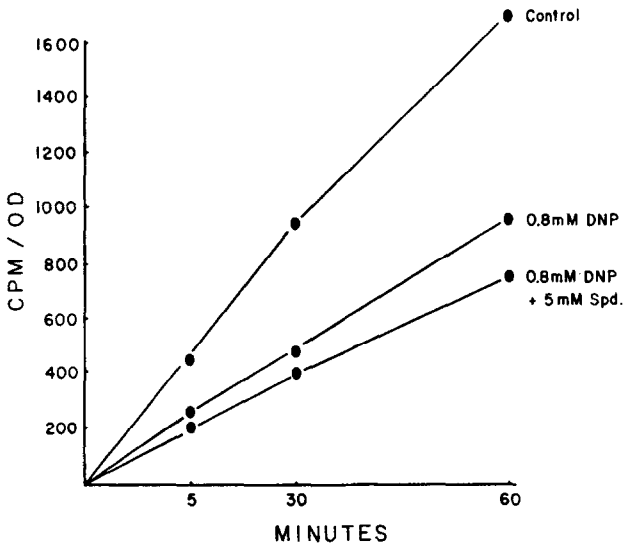

FIGURE 3: Lack of protection by spermidine against inhibition of RNA synthesis by 2,4-dinitrophenol in E.coli K-13.

centrifugation of extracts of bacteria incorporating C^{14}-uracil. Results similar to those presented here for E. coli K-13 have been obtained in strains 15 TAU and 58-161.

Selective inhibition of ribosomal RNA synthesis has also been shown for 2,4-dinitrophenol (DNP) (Simon, Van Praag and Aronson, 1966). As shown in Figure 3, the inhibition of RNA synthesis by DNP (0.8mM) is not prevented by the addition of spermidine even at a concentration of 5mM.

Effect of levorphanol on cellular content of polyamines

The results of an experiment designed to determine the effect of levorphanol on the cell content and accumulation of polyamines in E. coli 15 TAU are presented in Figure 4. The results are expressed as percent of initial cellular content. The actual initial values in mμmoles are listed in the legend. While the control culture showed an increase in cellular putres-

cine content of 130% over the zero time value during the 150-minute period of observation, cells treated with levorphanol had lost most of their putrescine. In these cells the cellular putrescine dropped to less than 30% of its initial value within 45 minutes after addition of levorphanol and had fallen to 10% of its initial amount at 90 minutes. The curves at the right of Figure 4 show that net synthesis of RNA is inhibited 85-90% by levorphanol throughout the period of observation.

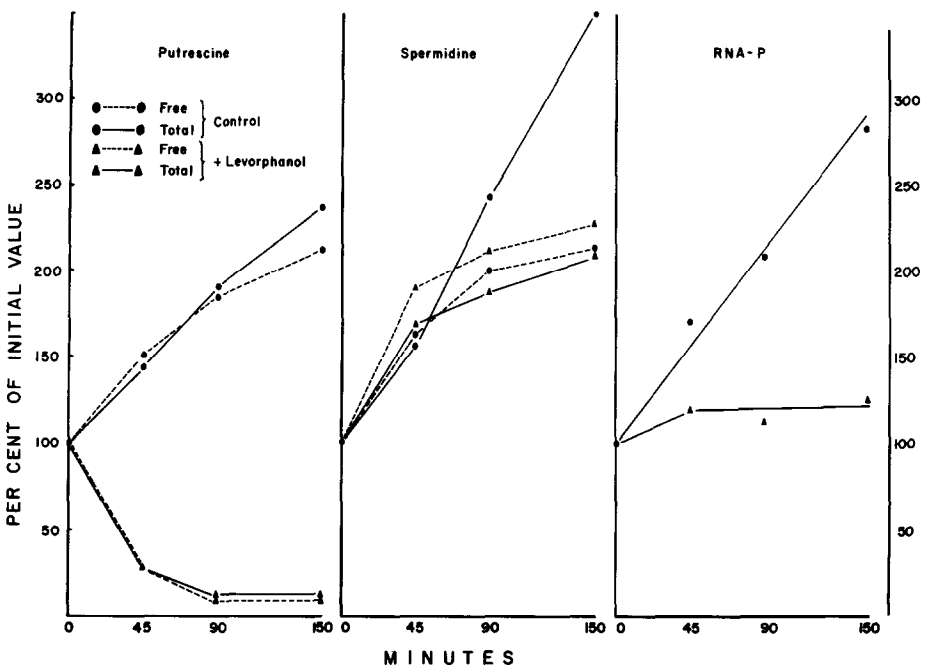

FIGURE 4: Effect of levorphanol on accumulation of putrescine, spermidine, and RNA-P in E.coli 15 TAU. Results are expressed as percent of initial content. Aliquots (150 ml) of the cultures were removed at the indicated intervals and analyzed for polyamines and RNA as described by Raina and Cohen (1966). The actual zero time values for cellular polyamines and RNA-P in mumoles were: putrescine free: 593, total: 641; spermidine free: 203, total: 282; RNA-P: 8100.

The spermidine content of the control culture increases throughout the observation period. After 45 minutes, most of the increment is found as monoacetylspermidine. The spermidine content of the culture treated with levorphanol increases to approximately the same extent as the control in the first 45 minutes but then levels off, so that there is little increase in either free or acetylated spermidine thereafter. In an identical experiment using strain K-13 the arrest of spermidine accumulation after 45 minutes was complete.

Discussion

Addition of spermidine, a naturally occurring polyamine, results in a very marked protection against the inhibitory effect of levorphanol. It is of considerable interest that the spermidine precursor, putrescine, produces little, if any, such protection. It is not yet possible to state whether the observed effect of spermidine is on the entry of levorphanol into the cell or whether it is related to the observed slowing down of spermidine accumulation in the levorphanol-treated cells. Spermidine has no effect on the inhibition of RNA synthesis by 2,4-dinitrophenol. This is similar to the findings with magnesium, which reversed inhibition by levorphanol but not by DNP (Simon, Van Praag and Aronson, 1966).

The observation that the presence of levorphanol causes marked changes in the polyamine content of treated cells is further evidence suggesting a relationship between polyamines and the action of levorphanol. The decrease in cellular putrescine probably reflects leakage into the medium and may

be due to an alteration in cell membrane permeability caused by the drug. Further studies are in progress to determine whether a causal relationship exists between the effects of levorphanol on polyamines and its inhibition of RNA synthesis.

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