In contrast to the case in animals, the dictyotene stage observed in mosses is not accompanied by an appreciable growth of the spore mother cells, nor does it persist for a relatively long time in the meiotic sequence. Obviously too little information is available, at present, to state the functional significance of this stage in the meiotic events of mosses.

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Inhibition of Bacterial Growth by Drugs of the Morphine Series

Abstract. *The growth of* Escherichia coli *is reversibly inhibited by drugs of the morphine series. The order of inhibitory effectiveness for the drugs tested was levallorphan > levorphanol > dextrorphan > nalorphine > morphine. The synthetic analgesic, levorphanol, was studied in greater detail. Its effectiveness was found to be strongly dependent on the* p*H of the medium. Raising the* p*H of the medium provides a higher concentration of the neutral free base which is thought to diffuse across cell membranes more readily. However, considerations other than the rate of entry of drug into the cells must be of importance since an already established growth inhibition is promptly reversed upon lowering the* p*H of the medium. Two mutants of* Escherichia coli *with altered sensitivity to levorphanol were isolated.*

An investigation of the effects of narcotic analgesic drugs on single cells was initiated in the hope of elucidating the biochemical action of these compounds. Recent studies on the effects of morphine and related compounds on human cells in tissue culture (*1*) stimulated us to investigate the action of these compounds on bacteria. It has been reported that unicellular organisms are unaffected by high concentrations of morphine in the growth medium (*2*), and this finding was confirmed by us with cultures of *Escherichia coli*. However, when closely related drugs, known to be more toxic than morphine to animals, as well as to cells in culture, were examined, bacterial growth was found to be inhibited. The present report deals with the inhibition of bacterial growth, particularly that of *E. coli*, by levorphanol tartrate and other drugs closely related to morphine in structure. The isolation of two mutants of this organism, which differ in their sensitivity to levorphanol, is also described.

The addition of levorphanol tartrate (*3*) at a concentration of $3 \times 10^{-3}M$ to *E. coli*, strain W, growing logarithmically in a minimal medium, resulted in complete inhibition of growth (Fig. 1) (*4*). The fact that the culture showed no decline in absorbancy at a wavelength of 490 mμ in the presence of the drug indicates that there was no detectable cell lysis. Viable cell counts made by spreading suitable dilutions of aliquots of the cultures on nutrient agar plates showed that the growth inhibition was reversed when the drug was removed from the medium, and that little or no cell death had occurred. Identical results were obtained when this strain was grown in enriched media, such as nutrient or peptone broth, provided the *p*H was kept near neutrality by the addition of a buffer. Under the same conditions morphine had no effect at its limit of solubility; nalorphine showed inhibition at a concentration of about $10^{-2}M$; while dextrorphan, the enantiomorph of levorphanol, was 20 to 30 percent less effective than levorphanol. Levallorphan, the *N*-allyl analogue of levorphanol, was somewhat more inhibitory than levorphanol. Thus, for the drugs tested, the general order of effectiveness in inhibiting bacterial growth parallels their order of toxicity in intact animals (*5*) and in human cells in tissue culture. Efforts to demonstrate antagonistic effects of the *N*-allyl derivatives against their parent compounds have so far been unsuccessful.

The *p*H of the medium has a marked effect on growth inhibition by levorphanol. Below *p*H 6 no inhibition was demonstrable. The effective dose is 3 to $4 \times 10^{-3}M$ when the *p*H of the medium is near neutrality, and inhibition decreases steadily as the *p*H is raised. At *p*H 8.5, the highest *p*H at which good growth of *E. coli* was obtained, multiplication was inhibited completely by $1 \times 10^{-3}M$ levorphanol, and some inhibition of growth was observed at concentrations as low as $1 \times 10^{-4}M$.

In a variety of systems molecules cross cell membranes more readily in the neutral than in the charged state. Raising the *p*H could, therefore, provide a higher concentration of unprotonated base for diffusion into the cells. On the basis of this hypothesis one would predict that once an inhibitory dose had entered the cells it would be effective regardless of the *p*H of the medium. This prediction is not borne out. It can be seen from Fig. 2 that a culture incubated at *p*H 8.2 with an inhibitory dose of levorphanol long enough to be inhibited, and presumably long enough for the drug to have reached its site of action, resumed growth promptly when the *p*H was lowered to 6.5. Thus the simple hypothesis that the effectiveness of the

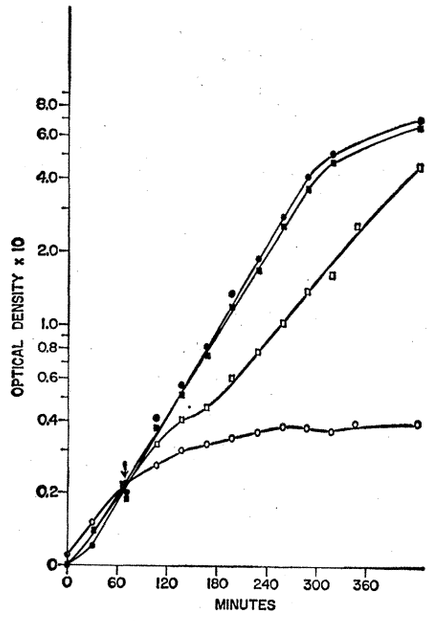

Fig. 1. Inhibition of *E. coli*, strain W and mutant S-6 by levorphanol at *p*H 6.7. Strain W and mutant strain S-6, which exhibits some resistance to the action of levorphanol at neutral *p*H, were grown in minimal medium (*8*) with 0.5 percent sodium lactate as the carbon source. Levorphanol tartrate was added at the time indicated by the arrow. Closed circles, strain W control; closed squares, mutant S-6 control; open circles, strain W + $2.7 \times 10^{-3}M$ levorphanol tartrate; open squares, mutant S-6 + $2.7 \times 10^{-3}M$ levorphanol tartrate. Growth was followed by optical density measurements at 490 mμ in a Lumetron colorimeter.

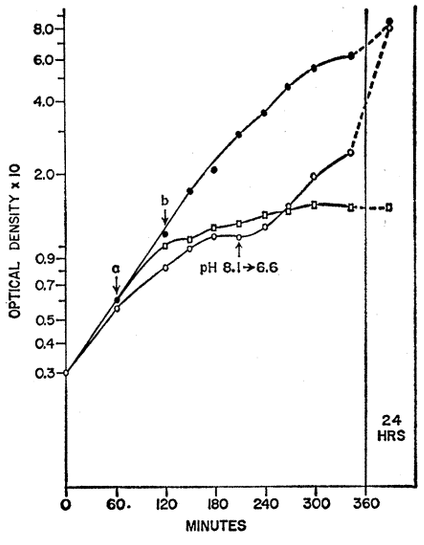

Fig. 2. Reversal of growth inhibition of *E. coli*, strain W by levorphanol when the *p*H of the medium was lowered. Cells were grown at *p*H 8.1 in a minimal medium buffered with 0.05*M* triethanolamine; sodium lactate (0.5 percent) was the carbon source. Levorphanol was added at arrows *a* and *b*, with a final concentration of $2 \times 10^{-3}M$. Closed circles, control culture at *p*H 8.1; open squares, culture with $2 \times 10^{-3}M$ levorphanol at *p*H 8.1; open circles, culture with $2 \times 10^{-3}M$ levorphanol at an initial *p*H of 8.1, which was decreased from 8.1 to 6.6 at the time indicated. Identical results were obtained in another experiment in which cell growth was determined by viable cell counts.

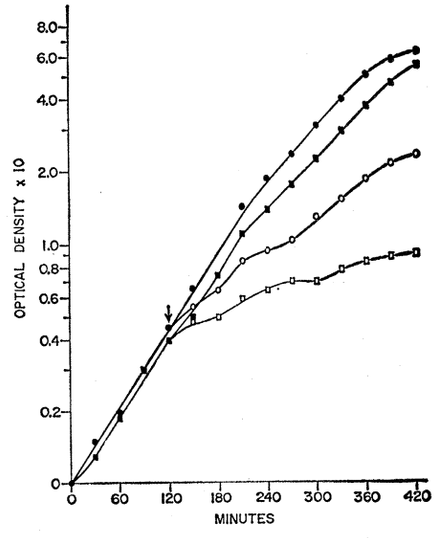

Fig. 3. Inhibition of *E. coli*, strain W and mutant S-6 by levorphanol at *p*H 8.2. Cells were grown in minimal medium buffered with 0.05*M* triethanolamine. Closed circles, strain W control; open circles, strain W with $1 \times 10^{-3}M$ levorphanol; closed squares, mutant S-6 control; open squares, mutant S-6 with $1 \times 10^{-3}M$ levorphanol.

drugs is increased at high *p*H because of the increased concentration of more permeable neutral molecules must be modified. At least three modifications suggest themselves. (i) If levorphanol is inactivated upon entering the cell, its effectiveness would depend on constant replenishment from the medium, which would be slowed down or cut off when the *p*H of the medium is lowered. (ii) The effective concentration or binding of levorphanol at the active site, in the cell or on its surface, may be altered by changes in *p*H. The latter could be accomplished by changes in ionization of either the drug or the receptor molecule. (iii) In addition to the effect of *p*H on the rate of entry of levorphanol into the cells, *p*H may also have an effect on the intracellular steady-state concentration of the drug, provided the steady state is reached rapidly. This has been found in some other cell systems, such as the human erythrocyte (*6*), in which there exists a *p*H differential between cells and medium which decreases with a fall in *p*H. This causes the cell-to-medium concentration ratio of a weak base to be higher at a higher *p*H. Lowering the *p*H of the medium might then result in an efflux of drug from the cells in order to approach its new and lower intracellular steady-state concentration, which might be insufficient to cause growth inhibition.

Other strains of *E. coli*, such as K-12 and 15, were found to react similarly to levorphanol as strain W, but with slight variations in sensitivity. *Bacillus subtilis* was somewhat less sensitive than *E. coli* at neutral *p*H but showed comparable inhibition at *p*H 8. An unencapsulated strain of pneumococcus derived from *Diplococcus pneumoniae*, type II was also tested. When this strain was grown in neopeptone, beef heart infusion broth at *p*H 7.3 to 7.8, growth was inhibited by $4.5 \times 10^{-4}M$ levorphanol, while at $1.3 \times 10^{-3}M$ and above the organisms appeared to be killed. This is in contrast to the largely reversible growth inhibition obtained in tests with *E. coli*, even at a relatively high drug concentration.

Attempts were made to isolate drug-resistant mutants of *E. coli* as a possible experimental model for the phenomenon of tolerance observed in higher animals and man. By incubating large inocula of bacteria (10^6 cells per ml) in the presence of inhibitory doses of levorphanol at neutral *p*H, two mutants, S-3 and S-6, which exhibited some degree of resistance to levorphanol, were isolated. This is illustrated in Fig. 1 for one of the mutants (S-6). With $2.7 \times 10^{-3}M$ levorphanol, which completely inhibited the growth of the wild type, the mutant showed only a slight decrease in growth rate. An interesting difference between the mutants was discovered when they were tested at *p*H 8 or above. One mutant, S-3, exhibited some resistance to levorphanol at all *p*H values. The other mutant, S-6, while resistant in the neutral range, proved to be consistently more sensitive to the drug than the wild type at or above a *p*H of 8, as shown in Fig. 3. Mutant S-3 was also found to be cross-resistant to the effects of dextrorphan while mutant S-6, in the *p*H range in which it is resistant to levorphanol, exhibited little or no cross-resistance to its enantiomorph.

It is of interest that microorganisms can be affected by drugs related to morphine. Growth inhibition was not previously demonstrated because it requires the more effective synthetic analogues of morphine, such as levorphanol, dextrorphan and levallorphan, and because these drugs become relatively effective only at alkaline *p*H values.

Work on the biochemistry of the growth inhibition is currently in progress and a preliminary communication (*7*) has appeared.

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