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Anti-mutagens

WHILE studying the mutagenic action of various purine derivatives on bacteria, we came across a new phenomenon : we found that certain nucleosides can act as anti-mutagens.

Following the discovery[1] that caffeine—a purine derivative—increases the mutation-rate in fungi and in bacteria, we began a quantitative study of the mutagenic action of purine derivatives. Such a study has been made possible by the use of a constant flow device, called the 'Chemostat'[2-4], which maintains a stationary bacterial population growing at a fixed rate that can be set at will. The concentration of the bacterial population maintained in the growth tube of the 'Chemostat' is determined by the input concentration of one of the required nutrients, called the controlling growth factor, and the growth-rate is fixed by the rate at which fresh nutrient flows into the growth tube.

A variety of different mutations will occur at different rates in such an otherwise stationary population, and if one plots the concentration of one particular type of mutant against time, one should obtain a straight line which rises with a slope that is determined by the mutation-rate. This holds for each type of mutant which grows at the same rate as the parent strain, that is, if there is no selection for or against the mutant. If there is selection against a mutant, the concentration of that mutant will remain stationary after an initial rise.

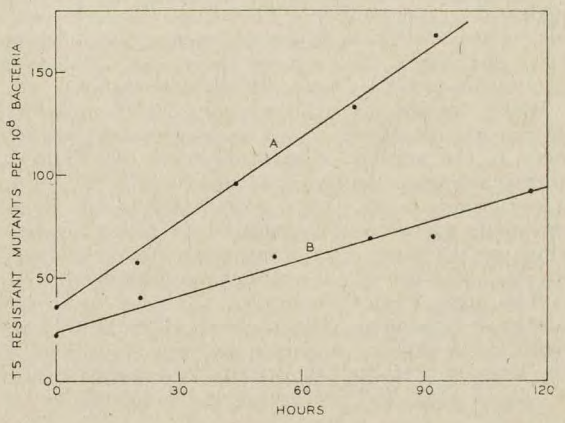

Fig. 1

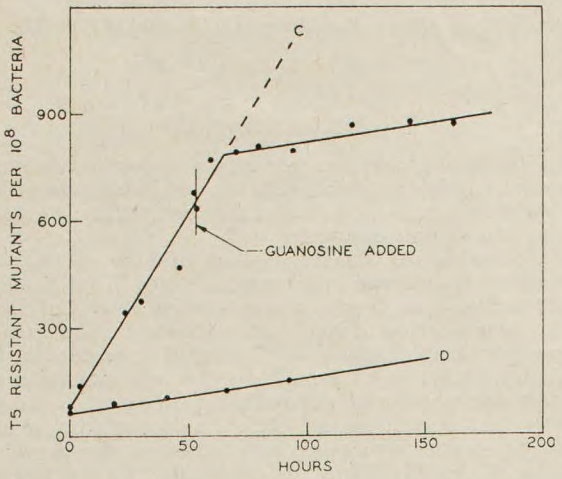

Fig. 2

In the experiments to be reported here, we used a strain of *E. coli* (*B*/1*t*) requiring tryptophane, and used tryptophane as the controlling growth-factor. This organism is sensitive to the bacteriophage *T*5. Mutants resistant to *T*5 present at any given time in the population growing in the 'Chemostat' can be scored by colony count simply by adding a small quantity of the virus *T*5 to an aliquot at the time of plating; in the presence of the virus, only the resistant mutants will grow out into colonies.

As we reported earlier[3], mutation to *T*5 resistance occurs at a constant rate independent of the rate at which the bacteria grow, that is, independent of the generation-time of the bacteria. By plotting against time the number of *T*5-resistant mutants present in the growth tube of the 'Chemostat', one obtains curve *A* of Fig. 1. The slope of this straight line gives a mutation-rate $\mu = 1{\cdot}4 \times 10^{-8}$/bacterium/hr.

When the nutrient medium contains theophylline (a dimethylxanthine) in a concentration of 150 mgm./l., the number of mutants rises very quickly, corresponding to the straight line *C* in Fig. 2, giving a mutation-rate of $10{\cdot}7 \times 10^{-8}$/bacterium/hr. This represents a seven-fold increase in the mutation-rate, which we attribute to the mutagenic action of theophylline. However, if the nutrient contains, in addition to 150 mgm./l. of theophylline, 50 mgm./l. of the nucleoside guanosine, the number of mutants rises much more slowly, as shown by line *D* in Fig. 2. The slope of this line corresponds to a mutation-rate of about 1×10^{-8}/bacterium/hr., indicating that guanosine, in the concentration used, completely counteracts the mutagenic action of the theophylline.

In the experiment mentioned earlier, which is described in the upper curve in Fig. 2, the bacterial population is first grown (at a generation-time of 3·2 hr.) in the presence of 150 mgm./l. of theophylline with no guanosine present. After 53 hr., guanosine is added to give a concentration of 150 mgm./l. For the first 53 hr. and for a short time thereafter, the number of mutants follows the straight line *C*, which gives a mutation-rate of $10{\cdot}7 \times 10^{-8}$/bacterium/hr.; but afterwards the number of mutants follows another straight line which gives a mutation-rate of less than $1{\cdot}5 \times 10^{-8}$/bacterium/hr. The two straight lines intersect, not at the time when the guanosine is added, but about 12 hr. later.

In order to explain this 12-hr. delay in the fall of the mutation-rate after adding guanosine, we do not have to assume that it takes that time for the guanosine to counteract the mutagenic effect of theophylline, but may attribute the delay to the fact that mutations are not immediately expressed in the phenotype of the bacteria. When the guanosine is added, the mutations induced by theophylline may very well cease to occur; but the mutations induced prior to the addition of guanosine continue to be expressed phenotypically for a period of about 12 hr.

The results shown in Fig. 2 have to be interpreted as an actual reduction of the mutation-rate by guanosine; that is, they cannot be attributed to a selection against the bulk of *T*5-resistant mutants resulting from the presence of guanosine. It is easy to show that if such a selection were responsible for the low mutation-rate shown by line *D* in Fig. 2, then in the upper curve in Fig. 2 the number of mutants resistant to *T*5 should fall steeply after adding guanosine at the 53rd hour.

The concentration of guanosine needed to counteract the mutagenic effect of 150 mgm./l. of theophylline is quite low. For a concentration of about 2 mgm./l. of guanosine, the rate of mutation induced by theophylline falls to one-half.

The other normally occurring purine ribosides were examined for anti-mutagenic action at concentrations of 5 mgm./l. At this concentration adenosine and inosine are strongly anti-mutagenic against theophylline, whereas xanthosine has no such activity. In contrast to inosine itself, its components, that is, the free purine hypoxanthine and the free sugar ribose, are not anti-mutagenic even at concentrations of several hundred milligrams per litre.

A concentration of 500 mgm./l. of guanosine gives

practically complete suppression of the mutagenic action of the following purine derivatives (at concentrations of 150 mgm./l.) : theophylline, caffeine, theobromine, paraxanthine, and 8-azaguanine. But tetramethyluric acid and benzimidazole retain more than half their mutagenic effect.

One may ask what effect guanosine has on the spontaneously occurring mutations. As can be seen from line *B* in Fig. 1, 50 mgm./l. of guanosine gives a mutation-rate of $0{\cdot}6 \times 10^{-8}$/bacterium/hr., that is, one-half to one-third as much as the spontaneous mutation-rate derived from curve *A*. This shows that guanosine in the concentration used reduces the mutation-rate to *T*5 resistance appreciably below the spontaneous mutation-rate.

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