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Second Draft

EXPERIMENTS ON SPONTANEOUS AND CHEMICALLY INDUCED MUTATIONS OF BACTERIA GROWING IN THE CHEMOSTAT

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In an earlier paper we reported observations on the spontaneous mutations of bacteria occurring in a device we call the Chemostat.[1] In this device we maintain a bacterial population in the growth phase over an indefinite period of time, and we do this by maintaining the concentration of one of the growth factors --called the controlling growth factor--at a low fixed value, while the concentrations of all the other growth factors are maintained at comparatively high values.

A number of different mutations will occur in a population thus maintained in the Chemostat but in the experiments here reported we are concerned only with the mutation from sensitivity to the bacterial virus T5 to resistance to this virus. In the absence of selection for or against the mutant and if reverse mutations can be neglected, the mutant population in the Chemostat will increase linearly with time, over periods of time short enough to disregard "evolutionary" changes.

By plotting the number of mutants against time we thus obtain a straight line and the slope of this line is proportional to the mutation rate.(1) With tryptophane as the controlling growth factor we found(1) for mutants resistant to T5 that the mutation rate per hour is independent of the growth rate (tested within the range of generation times τ between τ = 2 hours and τ = 12 hours.) The mutation rate was found to be about 1.25×10^{-8} per hour per bacterium. Our subsequent, more accurate measurements indicate that the real value is closer to 1.5×10^{-8}.

It seemed to be of interest to see whether the spontaneous mutation rate de ends on the choice of the controlling growth factor. In Fig. 1 are represented

experiments in which lactate (our carbon source) or ammonium chloride (our nitrogen source) or phosphate was used as the controlling growth factor. As may be seen from the figure, in all three cases the spontaneous mutation rate is appreciably lower than with tryptophane as the controlling growth factor.

The mutation rate appears to be lowest when lactate is the controlling growth factor, i.e. about one-third of the value obtained with tryptophane as the controlling growth factor. Because of this low value and the limited accuracy of our earlier experiments, we were led to believe that with lactate as the controlling growth factor, the number of mutants does not increase at all with time, but stays at a constant level; and we attributed this to selection against the mutant. Our present more accurate results with lactate as the controlling growth factor do not indicate any appreciable selection against the mutant. Such selection, if present, would lead to a curved line instead of the straight line appearing in Fig. 1.

When an amino acid is used as the controlling growth factor, protein synthesis is slowed, and one may ask whether the value of the spontaneous mutation rate obtained with tryptophane as the controlling growth factor is due to the specific choice of tryptophane or whether other amino acids, when chosen as the controlling growth factor, will lead to the same spontaneous mutation rate.

In order to learn something about this point we used a mutant of our strain of coli which requires both arginine and tryptophane as ~~a~~ growth factor. This mutant strain we grew in two Chemostats. Naturally both of these contained both argininless and tryptophane in the nutrient in the storage tank, but one of them contained arginine in excess so that tryptophane was the controlling growth factor, and the other contained tryptophane in excess so that arginine was the controlling growth factor. Figure 2 shows the results of these two experiments. As may be seen from it, the spontaneous mutation rates are about the same in both experiments. ~~(In order to obtain the~~ mutant strain ~~with the double requirement~~

we started with an arginineless strain D84 of the B strain of E coli, kindly supplied to us by Evelyn Witkin, and we picked from it a spontaneous mutant resistant to the bacterial viruses T1 which requires tryptophane as the growth factor.)

Experiments of this sort can be made with other pairs of amino acids and should show whether in general the spontaneous mutation rate is independent of the particular choice of the amino acid which is the controlling growth factor and if this is, indeed, the rule, what exceptions to it there are, if any.

If bacterial mutation arose as the result of an "error" occurring in the act of gene reproduction, one might expect the mutation rate per hour to be inversely proportionate to the generation time rather than to be independent of it. Such independence is, however, well established by our experiments in the case of mutation to T5 resistance. In order to reconcile the error hypothesis of mutations with the experimental facts, one might say that when we slow protein synthesis by using tryptophane as the controlling growth factor, we need not necessarily slow down the rate at which the genes reproduce; the genes might reproduce at an unchanged rate and genes produced in excess might perhaps be discounted. The possibility of this view was pointed out by Luria and also by Hershey (by oral communication) and if this view were correct, it would reconcile the observed constancy of the mutation rate per generation with the error hypothesis of mutants which demands constancy of the mutation rate per gene generation, rather than bacterial generation.

It was thought that if phosphorus or nitrogen were used as the controlling growth factor, the rate of genex synthesis might be slowed down along the rate of protein synthesis and constancy of the mutation rate per gene generation would then show up inasmuch as the mutation rate per hour would no longer be constant but would rather fall proportionately with increasing generation time.

In order to test this point we made experiments with phosphorus as the controlling growth factor using tris(hydorxymethyl) amino methane as buffer in a 1/50th molar concentration. In Fig. 3 are represented two experiments in which the bacteria were growing with different generation times 2.6 hours and 8.3 hours. As may be seen from the figure, the mutation rate per hour is only slightly decreased by a more than three-fold increase in generation time. Thus these experiments do not lend support to the error hypothesis of mutations.

With nitrogen as the controlling growth factor we again found no appreciable decrease of the mutation rate with increasing generation time. If anything, the mutation rate seemed to increase with increasing generation time, but the accuracy of these experiments is not sufficient to say whether this increase is real.

THEOPHYLLINE-INDUCED MUTATIONS

It seemed to be of interest to see whether any compounds which are chemically not very active and which in moderate concentrations will not lead to the killing of bacteria nor appreciably slow their growth rate, will cause mutations in low concentrations. Theophylline has been known to be mutagenic for molds and also for bacteria. If theophylline is added to the nutrient medium to bring its concentration to 150 mgm/l, there is no appreciable killing of bacteria growing in the Chemostat. This concentration of theophylline will, however, raise the rate of mutation from the spontaneous rate of about 1.55 per hour to a rate of about 12 per hour. The measurements carried out at different theophylline concentrations with tryptophane as the controlling growth factor are represented in Figure 4 and in Figure 5 the mutation rate is plotted against the theophylline concentration.

Since we had found that the spontaneous mutation rate is lower when ammonium chloride or phosphorus or lactate were used as the controlling growth factor, it appeared to be of interest to see whether a similar phenomena occurs in the case of theophylline induced mutations. As may be seen from Fig. 6, we obtained with these substances as the controlling growth factor theophylline-induced mutation rate which

is about one-third to one-half of the value of the induced mutation rates obtained with tryptophane as the controlling growth factor.

Figure 7 gives a direct comparison of the theophylline induced mutation rate for the same tryptophane requiring strain once grown with tryptophane as the controlling growth factor and once grown with lactate as the controlling growth factor. Again the mutation rate with lactate as the controlling growth factor is about one-third of the rate obtained with tryptophane as the controlling growth factor. This similarity in the response of the spontaneous mutation rate and the theophylline-induced mutation rate to the choice of the controlling growth factor raises a question of whether the processes leading to spontaneously occurring mutations and those involved in the induction of mutations by theophylline have some one step in common.

Purine Derivatives Other Than Theophylline.

With tryptophane as the controlling growth factor caffeine, paraxanthine, and theobromine, and 8 azaguanine in concentrations of 150 mgm/l have, like theophylline, a marked effect on the mutation rate. This may be seen from the data represented in Figure 8.

The effect of adenine on the mutation rate--with tryptophane as the controlling growth factor--was determined for two different concentrations and the results are represented in Figure 9.

2-6 diamino purine in a concentration of 150 mgm/l did not appear to be mutagenic, at least not within the limits of our experimental error.

Of the purine derivatives which we found to be mutagenic, theophylline, caffeine, paraxanthine, and theobromine are all methyl derivatives with each methyl group attached to one of the nitrogens of the purine ring. Because purines are a constituent of nucleic acid and nucleic acid is a constituent of genes, one might think that the resemblence of mutagenic

purine derivatives to the naturally occurring purines might perhaps have something to do with that mutagenic action. If this were so, one might also expect some pyrimidine derivatives might be mutagenic. We therefore tested for mutagenic action, using tryptophane as the controlling growth factor, the following pyrimidine derivatives: uracil, thymine, 6-methyl uracil, 5-amino uracil, 5 bromo uracil. None of them appeared to be mutagenic within the limits of our experimental error in a concentration of 150 mg/l. Experiments with other pyrimidine derivatives are in progress.

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When any amino acid is used as the controlling growth factor, protein synthesis is slowed, and one may ask whether the value of the spontaneous mutation rate obtained with tryptophane as the controlling growth factor is due to the specific choice of tryptophane or whether other amino acids, when chosen as the controlling growth factor, will lead to the same spontaneous mutation rate.

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In the chemostat we maintain a bacterial population in the growth phase over an indefinite period of time, by maintaining in the growth tube of the chemostat the concentration of one of the growth factors--called the controlling growth factor--at a low fixed value, while the concentrations of all the other growth factors are maintained at comparatively high values. In most of our experiments we used tryptophane as a controlling growth factor for a tryptophane-requiring mutant of the B strain of E coli. A number of different mutations will occur in a population thus maintained in the chemostat and in most of our experiments we are concerned with the observing the mutation from sensitive to the bacterial virus T5 to resistant to this virus. In the absence of selection for or against a mutant and if reverse mutations can be neglected, the mutant population will increase linearly with time over periods of time which are short enough to disregard evolutionary changes in the chemostat. If the experiment is, however, extended over a longer period of time the strain present in the chemostat which grows at the tryptophane concentration at which is automatically maintained in the growth tube of the chemostat at a certain rate might be replaced by a mutant strain which has a high growth rate at that low tryptophane concentration. If the new strain contains the same number of bacterial population as the old strain such a population changeover will not be accompanied by a change in the population density, but will manifest itself if we plot the number of T5-resistant mutants against time, as is shown in Fig. 1. The T5-resistant mutants increase with time in a straight line and if the new strain that is established after the population changeover mutates to T5-resistance at the same rate as the preceding strain from which it arose, there will be no change in the slope of the straight line, but there will be a shift in the straight line at the time of the population changeover, as can be seen in Fig. 1.

If this interpretation of the shift in the straight line ascent of the T5-resistant mutants is correct, then a reconstruction experiment should show that bacteria taken from the chemostat before ~~this~~ such a shift grow more slowly than bacteria taken from the chemostat after ~~this~~ such a shift if when both strains of bacteria are grown together in a chemostat at a low tryptophane concentration. In order to be able to distinguish in such a reconstruction experiment the two strains from each other, ~~it is convenient~~ we to make either one strain resistant to T5 and leave the other sensitive or vice versa. If we grow both strains in a chemostat together and if the strain ~~which we place~~ present before the population ~~xhax~~ changeover is made resistant to T5 and the other strain is senstivie to T5, we should expect the resistant strain to die out in the chemostat ~~according to an~~ exponentially with time.

Conversely, if the strain present in the chemostat after the population changeover is made resistant to T5 and the other strain ~~xxxxxxxx~~ is left sensitive, the we should expect for ~~most~~ low concentrations of the resistant strain the population of the resistant strain to increase exponentially with time.

If in the reconstruction experiments the tryptophane concentration is so low that the growth rate of both strains may be taken as proportionate to the tryptophane concentration, then we ought to have

Fig. 2 shows the result of two such reconstruction experiments in ~~similarly~~ semi logarithmic plot. The slope of the dotted line ~~for~~ is computed from ~~formulated~~ from Equation 1 and it may be seen ~~how well the calculated slope fits the plotted points.~~ that the experimental points fit this slope

If we continue the experiment shown in Fig. 1 out to 450 generations, we observe a number of shifts in the straight line ascent of the T5-resistant mutant and we assume that each such shift indicates a population changeover representing a step in the evolution which our original bacterial strain undergoes while growing in the chemostat.

While the T5-resistant mutant, grown when tryptophane is the controlling growth factor in the chemostat, increase linearly with time, the number of mutants resistant to T4 appear to remain constant after a short initial rise. If the number of mutants remains constant this might be either due to the fact that the mutant grows more slowly when the parent strain under the conditions prevailing in the chemostat, or else it might be due to the fact that the mutation rate is not appreciably different from zero, or else it might be due to the fact that the number of reserve mutations is so large as to compensate. The compensations of any two or all three of these factors is, of course, also a possibility.

When we first observed the fact that T4-resistant mutants remained constant in the chemostat, we tentatively concluded that this was due to selection against a mutant since the strain B/347 is known to be a slow grower. Reconstruction experiments carried out with the chemostat failed, however, to show a sufficiently
in a ppulation composed of a mixture of T4-senstivie and T4-resistant strains of our bacteria, and this lead us to suspect that the number of T4-resistant mutants that remained constant in our experiment because of a high rate of mutation of the resistant strain to sensitivity. In order to test this hypothesis we made an experiment with a device that may be called a phagostat, that permits to keep bacterial viruses growing over an indefinite period of time. The principle of this device is shown in Fig. 4.

We have previously used such a device for continuously growing the bacterial viruses T2r. We obtain a constant type of this virus in the second growth tube if....

For our present purpose we used a synthetic complete medium in both the first growth tube and the second growth tube, kept the value at about 30 min. to prevent the growth of bacteria in the ~~xxdx~~ second growth tube, and using a tryptophane control obtained a population of about _____ of ten different strains of B/4 in the first growth tube. If in the population in the first growth tube there is a high mutation rate to sensitivity to T4, we should then be able to maintain in the second growth tube a T4 titer that will gradually increase with time as the number of T4-sensitive mutants in the first growth tube increases with time.

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