Contract No.

December 2, 1953

FINAL REPORT

Submitted by Dr. Leo Szilard

The results which we obtained in our studies have been published in part in a number of papers which are listed below. Our unpublished results are summarized in this final report. Those of our results which have been written up in the form of a paper but not yet published are incorporated in this report by attaching to the report copies of the manuscripts. The published papers are as follows:

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The unpublished manuscripts incorporated in this final report are as follows:

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Howard H. Lee - The Mutation of E. Coli to Resistance to Bacteriophage T6 - to appear in the Archives of Biochemistry and Biophysics.

Our results not contained in the above mentioned papers and manuscripts are summarized below:

In the presence of 150 mg/l of theophylline the mutation rate of the strain B/1t is about 11 per 10^8 bacteria per hour. This rate is reduced to about half if we have present the following concentrations of the three below mentioned anti-

mutagens:

Adenosine	0.4 mg/l
Guanosine	2.0 mg/l
Inosine	2.0 mg/l

About 10 mg of adenosine will completely counteract the mutagenic effect of this concentration of theophylline. It seems to take a higher concentration of these anti-mutagens in order to affect the spontaneous mutations to T_5 resistance. 50 mg per liter of adenosine reduces the spontaneous mutation rate to about one-third.

When B/lt is grown in the presence of 150 mg/l of theophylline (recrystallized) in synthetic medium containing both glucose and lactate, the mutation rate to T_5 resistance is about 14 when grown aerobically, but seems to have a very low value, i.e., less than 1 per 10^8 bacteria per hour when grown anaerobically.

EXPERIMENTS WITH B/r/lt (Experiments of Dr. Aaron Novick)

E. Witkin furnished us with a radiation resistant mutant, B/r, of the B strain. By selecting from this B/r strain a mutant resistant to the virus T_1, which requires tryptophane as a growth factor, we obtained a mutant strain B/r/lt. When grown in the chemostat in a lactate medium with tryptophane as the controlling growth factor this new strain shows a spontaneous mutation rate to T_5 resistance of 4.22 per 10^8 bacteria per hour, which is about three times as high as the rate of our B/lt strain. The spontaneous mutation rate to T_6 resistance is also about threefold and has a value of 1 per 10^8 bacteria per hour.

150 mg/l of theophylline increases the mutation rate to T_5 resistance to about 21 per 10^8 bacteria per hour which is about twice as much as one would obtain for our strain B/lt.

10 mg/l of Adenosine completely counteracts the mutagenic effect of 150 mg/l of theophylline. The value actually observed was 1.4 per 10^8 bacteria per hour which is below the spontaneous mutation rate.

50 mg of Adenosine reduces the spontaneous mutation rate to T_5 resistance to 1.2 per 10^8 bacteria per hour, or to about one-third.

When grown aerobically in synthetic medium containing both lactate and glucose the mutation rate to T_5 resistance is 4.2 per 10^8 bacteria per hour. This is the same as in the lactate medium which does not contain glucose. But when grown anaerobically the spontaneous rate to T_5 resistance was not appreciably different from zero.

When grown anaerobically in the presence of 150 mg/l of theophylline in synthetic medium containing both glucose and lactate the mutation rate to T_5 resistance is again very low and has a value of about 1.

We summarize our results obtained on the mutation rate to T_5 resistance with the strains B/lt and B/r/lt (when grown in the chemostat in simple nutrient medium with tryptophane as the controlling growth factor at generation times of 2 hours or longer) as follows:

These two strains differ in mutation rate to T_5 resistance from each other by a factor of about 3 but they are similar inasmuch as in both strains 2/3 of the mutation rate to T_5 resistance can be suppressed by the presence of 50 mg/l of adenosine and in both strains the mutation rate to T_5 resistance is about 5 times as high as the mutation rate to T_6 resistance. In both strains the mutation rate to T_5 resistance responds strongly to theophylline which is a Purine type mutagen and this effect of theophylline is in both strains fully counteracted by 10 mg/l of adenosine. In these circumstances it is possible to surmise that 2/3 of the spontaneous mutation rate of T_5 resistance might be caused by a purine type mutagen.

Anaerobic growth suppresses both the spontaneous mutation rate to T_5 resistance and the theophylline induced mutation rate to T_5 resistance.

For generation times ranging from 2 to 8 hours both the spontaneous mutation rate to T_5 resistance and to T_6 resistance of the strain B/lt are independent from

the growth rate when this strain is grown with tryptophane as a controlling growth factor.

The mutation rate to T_6 resistance has been studied in our laboratory by Howard H. Lee and a paper written by him entitled "The Mutation of E. Coli to Resistance to Bacteriophage T_6" will appear in the Archives of Biochemistry and Biophysics. A photo-copy of Dr. Lee's paper is enclosed.

EXPERIMENTS IN RICHER MEDIA (Experiments of Dr. Maurey Fox)

B/lt was grown in continuous culture in the absence of any controlling growth factor under conditions in which the turbidity of the culture controls the feeding of fresh nutrient. We shall refer to the apparatus in which bacteria are grown under such conditions as a "breeder." B/lt when grown in such a "breeder" in broth has a mutation rate to T_5 resistance of about 3.7 per 10^8 bacteria per hour and a mutation rate to T_6 resistance of about 1.8 per 10^8 bacteria per hour. Compared to the values obtained in the chemostat with tryptophane as the controlling growth factor and in a simple lactate medium the mutation rate to T_5 resistance is increased more than twofold and the mutation rate to T_6 resistance is increased about sixfold.

Since the purine type mutagens increase the mutation rate to T_5 resistance much more than they do the mutation rate to T_6 resistance, the result cannot be explained by assuming that the bacteria grown in broth are affected by purine type mutagen. Some other mutagenic agent or the physiological condition must be responsible for the high mutation rate to T_6 resistance.

The presence of 150 mg/l of theophylline has no effect on the mutation rate to T_5 resistance when B/lt grows in the "breeder" in broth and it might be that there is enough of purine type antimutagen present either in the broth, or in the bacteria growing in broth, to counteract the mutagenic action of theophylline.

The high mutation rate to T_6 resistance observed is apparently not due to peroxides or other products of aerobic metabolism since it is found that B/lt when grown anaerobically in a "breeder," in broth supplemented with glucose, has a

mutation rate of about 2.3 to T_6 resistance.

The high mutation rates to both T_5 resistance and T_6 resistance observed with bacteria grown in broth in the "breeder" might be due directly to some of the constituents of the broth such as the amino acids, purines, pyrimidines, vitamins or else it might be the direct result of the fast growth rate in broth.

In order to distinguish between these two possibilities B/lt was grown at different generation times ranging from 32 minutes to 280 minutes in casein hydrolysate to which was added tryptophane, vitamins, purines, pyrimidines, glucose, lactate and phosphate, either in "breeders" or in tryptophane controlled chemostats. The T_5 rate for the shortest generation time was about 3.8 falling with increasing generation to about 3.0 for a generation time of 280 minutes. The T_6 rate for short generation times starts at about 2.0; it falls to about 1.0 at a generation time of 100 minutes and remains at 1 up to a generation time of 280 minutes.

EXPERIMENTS ON THE RATE OF AMINO ACID SYNTHESIS (Experiments of Dr. Aaron Novick and Dr. Leo Szilard).

Experiments concerning this topic have been written up in the form of a paper which will appear in Growth under the title of "Experiments with the Chemostat on the Rates of Amino Acid Synthesis in Bacteria." A photo-copy of the manuscript is attached to this report.

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In the presence of 150 mg/l of theophylline the mutation rate of the strain B/lt is about 11 per 10^8 bacteria per hour. This rate is reduced to about half if we have present the following concentrations of the three above mentioned antimutagens:

Adenosine	0.4 mg/l
Guanosine	2.0 mg/l
Inosine	2.0 mg/l

About 10 mg of adenosine will completely counteract the mutagenic effect of this concentration of theophylline. It seems to take a higher concentration of these antimutagens in order to affect the spontaneous mutations to T_5 resistance. 50 mg per liter of adenosine reduces the spontaneous mutation rate to about one-third.

When B/lt is grown in the presence of 150 mg/l of theophylline (recrystallized) in synthetic medium containing both glucose and lactate, the mutation rate to T_5 resistance is about 14 when grown aerobically, but seems to have a very low value, i.e., less than 1 per 10^8 bacteria per hour when grown anaerobically.

EXPERIMENTS WITH B/r/lt (Experiments carried out by Dr. Aaron Novick)

E. Witkin furnished us with a radiation resistant mutant, B/r, of the B strain. By selecting from this B/r strain a mutant resistant to the virus T_1 which requires tryptophane as a growth factor, we obtained a mutant strain B/r/lt. When grown in the chemostat in a lactate medium with tryptophane as the controlling growth factor this new strain showed a spontaneous mutation rate to T_5 resistance of 4.22 per 10^8 bacteria per hour, which is about three times as high as the rate of our B/lt strain. The spontaneous mutation rate to T_5 resistance is also about threefold and has a value of 1 per 10^8 bacteria per hour.

150 mg/l of theophylline increases the mutation rate to T_5 resistance to about 21 per 10^8 bacteria per hour which is about twice as much as one would obtain for our strain B/lt.

10 mg/l of Adenosine completely counteracts the mutagenic effect of 150 mg/l of theophylline. The value actually observed was 1.4 per 10^8 bacteria per hour which is below the spontaneous mutation rate.

50 mg of Adenosine reduces the spontaneous mutation rate to T_5 resistance to 1.2 per 10^8 bacteria per hour, or to about one-third.

When grown aerobically in synthetic medium containing both lactate and glucose the mutation rate to T_5 resistance is 4.2 per 10^8 bacteria per hour. This is the same as in the lactate medium which does not contain glucose. But when grown anaerobically the spontaneous rate to T_5 resistance was not appreciably different from zero.

When grown anaerobically in the presence of 150 mg/l of theophylline in synthetic medium containing both glucose and lactate the mutation rate to T_5 resistance is again very low and has a value of about 1.

We summarize our results obtained on the mutation rate to T_5 resistance with the strains B/1t and B/r/1t when grown in the chemostat in simple nutrient medium with tryptophane as the controlling growth factor at generation times of 2 hours or longer as follows:

These two strains differ in mutation rate to T_5 resistance from each other by a factor of about 3 but they are similar inasmuch as in both strains 2/3 of the mutation rate to T_5 resistance can be suppressed by the presence of 50 mg/l of adenosine and in both strains the mutation rate to T_5 resistance is about 5 times as high as the mutation rate to T_6 resistance. In both strains the mutation rate to T_5 resistance responds strongly to theophylline which is a Purine type mutagen and this effect of theophylline is in both strains fully counteracted by 10 mg/l of adenosine. In these circumstances it is possible to surmise that 2/3 of the spontaneous mutation rate of T_5 resistance might be caused by a purine type mutagen.

Anaerobic growth suppresses both the spontaneous mutation rate to T_5 resistance and the theophylline induced mutation rate to T_5 resistance.

For generation times ranging from 2 to 8 hours both the spontaneous mutation rate to T_5 resistance and to T_6 resistance of the strain B/lt are independent from the growth rate. When this strain is grown with tryptophane as a controlling growth factor, the mutation rate to T_6 resistance has been studied in our laboratory by Howard H. Lee and a paper written by him entitled "The Mutation of E. Coli to Resistance to Bacteriophage T_6" will appear in the Archives of Biochemistry and Biophysics.

EXPERIMENTS CARRIED ON BY DR. MAUREY FOX

B/lt was grown in continuous culture in the absence of any controlling growth factor under conditions in which the turbidity of the culture controls the feeding of fresh nutrient. We shall refer to the apparatus in which bacteria are grown under such conditions as a "breeder." B/lt when grown in such a "breeder" in broth has a mutation rate to T_5 resistance of about 3.7 per 10^8 bacteria per hour and a mutation rate to T_6 resistance of about 1.8 per 10^8 bacteria per hour. Compared to the values obtained in the chemostat with tryptophane as a controlling growth factor and in a simple lactate medium the mutation rate to T_5 resistance is increased more than twofold and the mutation rate of T_6 resistance is increased about sixfold.

Since the purine type mutagens increase the mutation rate of T_5 resistance much more than they do the mutation rate to T_6 resistance, the result cannot be explained by assuming that the bacteria grown in broth are affected by purine type mutagen. Some other mutagenic agent or the physiological condition must be responsible for the high mutation rate to T_6 resistance.

The presence of 150 mg/l of theophylline has no effect on the mutation rate to T_5 resistance when B/lt grows in the "breeder" in broth and it might be that there is enough of purine type antimutagen present either in the broth or in the bacteria

growing in broth to counteract the mutagenic action of theophylline.

The high mutation rate to T_6 resistance observed is apparently not due to peroxides or other products of aerobic metabolism since it is found that B/1t when grown anaerobically in a "breeder" in broth supplemented with glucose has a mutation rate of about 2.3 to T_6 resistance.

The high mutation rates to both T_5 resistance and T_6 resistance observed with bacteria grown in broth in the "breeder" might be due directly to some of the constituents of the broth such as the amino acids, purines, pyrimidines, vitamins or else it might be the direct result of the fast growth rate in broth.

In order to distinguish between these two possibilities B/1t was grown at different generation times ranging from 32 minutes to 280 minutes in casein hydrolysate to which was added tryptophane, vitamins, purines, pyrimidines, glucose, lactate and phosphate , either in "breeders" or in tryptophane controlled chemostats. The T_5 rate for the shortest generation time was about 3.8 falling with increasing generation to about 3.0 for a generation time of 280 minutes. The T_6 rate for short generation times starts at about 2.0; it falls to about 1.0 at a generation time of 100 minutes and remains at 1 up to a generation time of 280 minutes.

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3 copies

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FINAL REPORT - ROUGH DRAFT

In the presence of 150 mg/l of theophylline the mutation rate of the strain B/lt is about 11 per 10^8 bacteria per hour. This rate is reduced to about half if we have present the following concentrations of the three above mentioned antimutagens:

Adenosine	0.4 mg/l
Guanosine	2.0 mg/l
Inosine	2.0 mg/l

It seems to take a higher concentration of the antimutagens in order to suppress the spontaneous mutations to T_5 resistance. For the strain B/lt it takes about 10 to 20 mg/l of Guanosine to reduce the spontaneous mutation rate to T_5 resistance to about half, and even at higher concentrations of Guanosine the mutation rate does not go to zero but drops only to about one third.

When B/lt is grown in the presence of 150 mg/l of theophylline (recrystallized) in synthetic medium containing both glucose and lactate, the mutation rate to T_5 resistance is about 14 when grown aerobically, but seems to have a very low value, i.e., less than 1 per 10^8 bacteria per hour when grown anaerobically.

EXPERIMENTS WITH B/r/lt

Another mutant of the B strain which is resistant to the virus T_1 and which requires tryptophane was obtained from a radiation resistant mutant of the B strain kindly furnished to us by E. Witkin. When grown in the chemostat B/r in a lactate medium with tryptophane as the controlling growth factor this strain showed a spontaneous mutation rate to T_5 resistance of 4.22 per 10^8 bacteria per hour, which is about three times as high as the rate of our B/lt strain is. The spontaneous mutation rate to T_6 resistance is also about threefold and has a value close to 1 per 10^8 bacteria per hour.

150 mg/l of theophylline increases the mutation rate to T_5 resistance to about 21 per 10^8 bacteria per hour which is about twice as much as one would obtain for our strain B/lt.

10 mg/l of Adenosine completely counteracts the mutagenic effect of 150 mg/l of theophylline. The value actually observed was 1.4 per 10^8 bacteria per hour which is below the spontaneous mutation rate.

50 mg of Adenosine reduces the spontaneous mutation rate to T_5 resistance to 1.2 per 10^8 bacteria per hour, or to about one-third.

When grown in synthetic medium containing both lactate and glucose aerobically the mutation rate to T_5 resistance is 4.2 per 10^8 bacteria per hour. This is the same as in the lactate medium which does not contain glucose. But when grown anaerobically the spontaneous rate to T_5 resistance was not appreciably different from zero.

When grown anareobically in the presence of 150 mg/l of theophylline in synthetic medium containing both glucose and lactate the mutation rate to T_5 resistance is again very low and has a value of about 1.

We might now summarize our results obtained on the mutation rate to T_5 resistance with the strains B/lt and B/r/lt when grown in the chemostat in simple nutrient medium with tryptophane as the controlling growth factor at generation times of 2 hours or longer as follows: These two strains differ in mutation rate to T_5 resistance from each other by a factor of about 3 but they are similar inasmuch as in both strains 2/3 of the mutation rate to T_5 resistance can be suppressed by the presence of 50 mg/l of adenosine and in both strains the mutation rate to T_5 resistance is about 5 times as high as the mutation rate to T_6 resistance. In both strains the mutation rate to T_5 resistance responds strongly to theophylline which is a Purine type mutagen and this effect of theophylline is in both strains fully counteracted by 10 mg/l of adenosine. In these circumstances it is possible to assume that 2/3 of the spontaneous mutation rate of T_5 resistance might be caused by a purine type mutagen.

Anaerobic growth suppresses both the spontaneous mutation rate to T_5 resistance and the theophylline induced mutation rate to T_5 resistance.

Independence of the spontaneous mutation rate to T_5 resistance and to T_6 resistance has been well established for the strain B/lt for generation times ranging from 2 hours to 8 hours.

EXPERIMENTS CARRIED ON BY DR. MAUREY FOX

B/lt was grown in continuous culture in the absence of any controlling growth factor under conditions in which the turbidity of the culture controls the feeding of fresh nutrient. We shall refer to the apparatus in which bacteria are grown under such conditions as a "breeder". B/lt when grown in a "breeder" in broth has a mutation rate to T_5 resistance of about 3.7 per 10^8 bacteria per hour and a mutation rate to T_6 resistance of about 1.8 per 10^8 bacteria per hour. Compared to the values obtained in the chemostat with tryptophane as a controlling growth factor in the simple lactate medium the mutation rate to T_5 resistance is increased more than twofold and the mutation rate of T_6 resistance is increased about sixfold.

Since the purine type mutagens increase the mutation rate of T_5 resistance much more than they do the mutation rate to T_6 resistance, the result cannot be explained by assuming that the bacteria grown in broth are affected by purine type mutagen. Some other mutagenic agent or some unknown physiological condition must be responsible for the high mutation rate to T_6 resistance.

The presence of 150 mg/l of theophylline has no affect on the mutation rate to T_5 resistance when B/lt grows in the "breeder" in broth and it might be that there is enough of purine type antimutagen present either in the broth or in the bacteria growing in broth to counteract the mutagenic action of theophylline.

The high mutation rate to T_6 resistance observed is apparently not due to peroxides or other products of aerobic metabolism since it is found that B/lt when grown anaerobically in broth supplemented with glucose in a "breeder" has a mutation rate of about 2.3 to T_6 resistance.

The high mutation rates to both T_5 resistance and T_6 resistance observed with bacteria grown in broth in the "breeder" might either be due directly to some of the amino acids, purines, pirimidines, vitamins, or other constituents of the broth.

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