UNIVERSITY OF CALIFORNIA

PARTMENT OF BACTERIOLOGY
ERKELEY 4, CALIFORNIA

April 10, 1950

Dr. Joshua Lederberg
Dept. of Genetics
College of Agriculture
University of Wisconsin
Madison 6, Wisconsin

Dear Dr. Lederberg,

I would like to thank you for your suggestions regarding changes in my application for an American Cancer Society Fellowship. I made every attempt to incorporate your proposals into the application.

I was very much heartened to find that you approve in essence of the program that I have outlined. Out here it is generally believed that an investigation of the role of normal metabolites in spontaneous mutations would lead one on a fool's errand, for "are not spontaneous mutations caused by slips in chromosome duplication", or "doesn't any chemical that causes death cause mutation", etc. I am looking forward to your visit this summer and to spending next year working in your laboratory. If the fellowships fall through, would there be a possibility of obtaining a job in your laboratory? So far I have been refused the N.R.C. and the Merck Fellowships. There still remain the American Cancer Society and the U.S.P.H. Service Fellowships. I have just had an interview with Tatum with regard to the American Cancer Society Fellowship. All I can do now is hope.

I was very happy to learn of the results you obtained with CH_2O. You were correct concerning the negative results I obtained in inducing mutations of coli B to phage resistance. I had not allowed for phenotypic lag. I am in the process of setting up an experiment where growth of the formaldehyde treated cells could take place before phage infection. Have you performed such an experiment? If so, I would be very much interested in hearing your results. A. Doermann mentioned to me last summer at Cal. Tech. that Witkin reported negative results with CH_2O. Are you familiar with this work? Did she also limit herself to so-called "zero-point mutations"?

You asked for some details concerning my experiments with CH_2O. I would have communicated them to you sooner, but I had planned additional experiments, and I thought I would wait until these were completed. The work was begun in the summer of 1948, just after the work of Rapoport became available, and was left too soon, due to the complications that developed, and also to my desire to broaden out along biochemical lines so as to benefit from the storehouse of biochemical techniques and thought available at Cal.

1. A washed suspension of Pseudomonas fluorescens, suspended in phosphate buffer, pH 7, is treated with CH_2O (final concentration 0.1%) for 15 minutes. Phosphate buffer is then added, cells are centrifuged down, supernatant liquid poured off, and cells are

UNIVERSITY OF CALIFORNIA

PARTMENT OF BACTERIOLOGY
BERKELEY 4, CALIFORNIA

resuspended to the original volume. Aliquots are plated on itaconate mineral agar plates to determine the number of mutants, and the viable count is determined by serial dilution and plating on yeast extract agar. A control tube containing a similar suspension of cells is run together with the experimental tube through all the processes, and instead of the addition of CH_2O, a similar volume of distilled water is added. The results of a typical experiment follow:

	Viable count /0.1cc	Viable count /tube 5cc suspension	Per cent survivors	No. mutants /0.1cc	m/1x10^8 cells
Control	2.5 x 10^8	1.26 x 10^{10}	100%	28 17 19 21 15 Av. 25	10
After 15 min. treatment with 0.1cc CH_2O	3.4 x 10^4	1.7 x 10^6	0.014%	225 236 272 255 247 Av. 247	7.3 x 10^4

2. Itaconate mutants isolated after CH_2O treatment are not any more resistant to CH_2O than the untreated wild type.

3. Commercial CH_2O contains many impurities. A pure sample of CH_2O was prepared and tried with similar results.

4. In order to eliminate the possibility that the increased number of mutants may have been the result of additional growth of the parent culture on the itaconate plates, due to traces of CH_2O which were carried over with the cells, the following experiments were conducted:

a. Double washings --- similar results.

b. The mutants were counted by a tube method. This enables one to follow the growth of the parent culture as well as providing additional means of determining the number of mutants present. After CH_2O treatment, the washed suspension is diluted out in itaconate mineral liquid medium and tubed so as to place about one mutant in each tube. Growth of the wild type was followed daily in these tubes by platings on Y.E. and itaconate, and turbid tubes were recorded. Turbidity was demonstrated to be the result of itaconate mutation. A similar procedure was followed with an untreated suspension. This experiment conclusively showed that the CH_2O treated cells (wild type) grew to the same slight extent as the untreated cells and the mutant count by this method checked with the mutant counts as determined by plating.

UNIVERSITY OF CALIFORNIA

PARTMENT OF BACTERIOLOGY
BERKELEY 4, CALIFORNIA

5. An attempt to study the kinetics of the formaldehyde effect revealed the following difficulties:

a. Use of different batches of yeast extract, as well as other media, resulted in large variation in viable count after CH_2O treatment, although there were no differences in viable counts demonstrated with untreated cells:

yeast autolysate agar	3×10^{-4}%	survivors
.5% Y.E. agar	0.3%	"
3% Y.E. agar	2.3%	"
.5% Mineral Asparagine	6.1%	"

b. When a mutant count of CH_2O treated cells is performed if there are 200 colonies from a 10^0 dilution, a 10^{-1} dilution of the same suspension will reveal only 5, or 2, or sometimes 10 colonies. This phenomenon may be due to the decrease in number of living cells on the 10^{-1} plate, which when present, may provide the environment for reactivation of itaconate +, induced, deactivated cells.

c. If one can rely on the 10^0 plate to count the mutants, the absolute number of mutants produced reaches a maximum and then falls off slightly with increased concentrations of CH_2O ← The relative number of mutants increases continually. (and with time)

d. A plot of the log of the per cent survivors against time (0.1% CH_2O at 5, 10, 15, 20 min.) in about 10 experiments reveals a tremendous variation in rates of killing. All experiments had the following results in common. During the first 5 minutes about 99% of the cells were killed and thereafter the remainder were killed at a much slower but constant rate. These results are similar to those of Demerec and Latajet obtained with ultraviolet treatment of resting cultures of coli B.

If cells are treated with varying concentrations of CH_2O (from 0.01% to 0.1%) for 15 minutes, the plot of the log of the per cent survivors against concentration is a straight line.

This account, of course, is, of necessity, brief. If it is at all possible, I would appreciate any and all comments you would care to make concerning these experiments. Hoping to hear from you soon, I remain,

Sincerely yours,

Ellis Englesberg

Ellis Englesberg