THE UNIVERSITY OF TEXAS
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DEPARTMENT OF ZOOLOGY

January 29, 1952

Dr. Joshua Lederberg
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The University of Wisconsin
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Dear Dr. Lederberg:

In answer to your letter of January 18, 1952 the details of the experiments published in the American Naturalist, 84: 261-274 (1950) follow:

Thirty ml aliquots of nutrient broth (see Hershey, A.D. and Rotman, R., Genetics, 34, 89-106 (1948) for composition) were placed in sterile petri dishes and irradiated with a Hanovia double U - SC-2537 ultraviolet mercury vapor lamp operating at 100 milliamperes. The samples were 10 cms from the lamp during the exposure. The lengths of exposure time were 0, 20, 40, and 60 minutes. Twenty ml aliquots were then pipetted to large sterile aeration tubes. Each of the tubes was then inoculated with approximately 100 thousand organisms per ml from 4 hour log phase cultures of E. coli B or E. coli 58-161. These cultures were then incubated with aeration in a 37°C water bath until there was good turbidity (4 to 6 hours). After incubation each of these cultures was treated as follows:

Twenty tubes containing 2 ml soft (0.7%) agar at 45°C and seeded with approximately 5 X 10^6 phage T1 particles were each inoculated with 0.1 ml of the culture. The tubes were then plated via the soft agar technique and incubated at 37°C overnight. At the same time one to one million and one to 10 million dilutions of the cultures were prepared and plated in triplicate in nutrient agar containing no phage to determine the number of bacteria subjected to the action of the phage. All plates were examined and counted after 24 hours incubation.

A number of the colonies were then picked from the plates of each experiment (one or two colonies from a plate) and checked for resistance. Each colony tested was emulsified in broth then streaked on an agar plate seeded with T1 phage and on one with no phage. At the same time an agar slant was inoculated with the emulsion. Ninety six percent of the colonies tested in this manner proved to be resistant. The false positive slants were discarded and the others stored in the ice box.

To determine if phage-resistant mutants also possessed nutritional deficiencies a small inoculum from each agar slant was emulsified in liquid minimal medium without washing and then streaked onto minimal agar plates and nutrient agar plates. If the organism failed to grow on the minimal medium or if it grew much more slowly than on the nutrient agar plate it

was considered to be a possible biochemical mutant. The agar slants containing phage-resistant biochemical mutants were retained and the others discarded. Many of the mutants were derived from strain 58-161 and whenever such mutants were tested for deficiencies the minimal medium was supplemented with methionine and biotin.

Since both B/1 and B/1,5 mutants are obtained together on complete medium it was suspected that many of the biochemical mutants would be B/1 and require tryptophane, thus mutants not growing on minimal were tested for growth on minimal supplemented with tryptophane (50 gamma/ml). No biochemical mutant grew on this medium. Since this indicated that only B/1,5 mutants were being obtained two more series of T1 resistant mutants were taken from plates after lysis and tested for tryptophane requirement. None of these colonies exhibited such a requirement. The cultures were then tested for their resistance pattern with all 7 phages of the T series by the cross-streak method and all proved to be B/1,5. It was also found that none of the 58-161 mutants required tryptophane. These were not tested to determine if they were resistant to T5 as well as T1 however. A control culture of B/1 tested at the same time as the previously mentioned mutants showed a definite requirement for tryptophane.

The resistant mutants of strain 58-161 which were suspected of having different biochemical requirements from the parent strain were tested on minimal medium supplemented with a vitamin solution, purine and pyrimidine solution, or one of two different "group" amino acid solutions. Washed heavy suspensions of each of the mutants were tested by streaking onto plates of each of the following media:

nutrient agar
minimal agar
minimal agar + biotin + methionine
minimal agar + " " + amino acid solution A.
minimal agar + " + " + amino acid solution B.
minimal agar + " + " + purines and pyrimidines
minimal agar + " + " + vitamines
minimal agar + all supplements.

Using these methods it was found that 19 of 72 virus resistant mutants from untreated media, 12 of 72 from ultraviolet irradiated broth, and 26 of 72 from hydrogen peroxide treated broth exhibited some definite type of stable nutritional deficiency. Of these mutants 11 were found on further testing with single growth factors to have a definite requirement for proline. The remainder show no good growth on any single amino acid, vitamin or purine or pyrimidine but grow as well as the parent strains when yeast extract is added to the medium.

A few of the resistant mutants appeared at first to be vitamin requiring, however these were not stable enough to be certain and on further testing exhibited more and more growth on unsupplemented minimal medium. Therefore they are not included as biochemical mutants but as non-mutants in the above figures.

Each of the positive mutants was checked by the auxanographic method. The results of this test were the same as those of the streak method.

Tests were then carried out on the proline requiring mutants. Three of these would grow only on proline supplemented medium or complete medium. Five would grow on arginine supplemented medium but not nearly as well as on the proline medium. Three of them grew on medium supplemented with either phenylalanine, ornithine, arginine or glutamic acid but not as well as on medium supplemented with proline. All except three of these mutants came from different experiments and from different treatments (i.e. no treatment, treatment with H_2O_2, or treatment with ultraviolet). Two of these three mutants coming from the same experiment (but different plates) were the same (would grow on arginine as well as proline) and one was different (would grow only on proline).

In answer to the questions and suggestions in the last paragraph of your letter I offer the following:

Only one resistant mutant was taken from each plate in the original isolation unless there were distinct colony differences in which case the different colony was taken also. In no case were more than two colonies taken from a plate. It is possible that some of the mutants could come from a single clone since a growth period of 4 to 6 hours was permitted before a culture was tested. This seems unlikely however in view of the differences in the proline mutants and since the mutants finally tested for biochemical requirements came from a total of eleven seperate experiments employing approximately 200 completely seperate cultures.

I have not carried out any genetic tests on any of these mutants. I have intended doing this and also doing some more work on the biochemical aspects. However, last year I was working with Neurospora at Cal Tech. and did not have a chance to do anything with bacteria during this time. On my return to Texas I found that due to stock culture technician difficulties many of the stocks not in immediate use had been allowed to die, including most of mine. I have a couple of Drosophila problems going at present and I am trying to finish up some Neurospora work. For these reasons I haven't gotten back to the bacteria yet. I hope to be able to do this in a couple of months.

I hope that this gives you the information that you need. If there are any other questions about this or if you would like to have any of the cultures please let me know and I will send them if they are still available. Please let me know of any suggestions, criticism, etc. that you may have in regard to these experiments. I can see no reason why our results should differ unless there is a difference in the strains. This seems unlikely since the 58-161 strain used for most of the biochemical mutant work was obtained from you. Also will you please send me copies of your reprints which are available.

Sincerely yours

Felix L. Haas,

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