THE UNIVERSITY OF WISCONSIN

Department of Genetics,

November 16, 1947.

Dear Evelyn,

Here is your MS. Your accompanying letter was no less interesting. While reading it in preparation for the seminar yesterday AM some of the following questions came up:

1) The cells used. Is it possible that resting bacteria accumulate spontaneous genetic changes which are not manifested until the conditions of certain toxic treatments are met? If most coli cells are dikaryotic, for example, mutations in one nucleus would not be expressed until segregation of the nuclei or suppression of one of them took place. Your choice of low background pools might bear on this also. Do you get the same quantitative response to mutagens on samples from the same pool tested on successive days?

2)The assay. Firstly, the previously put question of the empirical validation of the assay method by recovery experiments. Secondly, I can't help but feel slightly concerned about whether there is enough phage on the plates to be sure of total, immediate lysis of all sensitive cells. Particularly is this so in tests of populations which for the most part contain dead bacteria, which have therefore been concentrated. The killed bacteria presumably are capable of adsorbing phage irreversibly, and there may be autolytic products as well which can inactivate phage. The best way I can think of to check this point is to test a series of dilutions of the same treated suspension and demonstrate that you assay the same number of mutants per unit bacteria independently of the concentration at which they are tested. I notice that you use 10^9 phage per plate as compared with 10^8 used by Demerec and Fano. Satisfy it.

3)The selection experiments. I notice that you use B/r/1 obtained as spontaneous mutants, on the basis that they are presumably the variation on which selective or competitive killing could operate. This is fine, but do you not think it also advisable to test for selection by a given agent a representative sample of the mutants obtained under the action of that agent?

In relation to your NaCl results, have you noticed any adaptive responses of coli similar to those described by Doudoroff, JGP 1941?

Since my previous letter I've started to work in my improvised lab. Some of the recent expts may interest you. Up to now, I have picked up a number of lactose-negative (Lac-) mutants in post-incubated, irradiated or mustarded populations by plating the bacteria in suitable dilution on Eosin-Methylene Blue plates. The mutants show up as pink, non-acid producing colonies. However, when 10^8 bacteria are spread on each plate, and then irradiated so as to leave ca. 10^2 survivors (pS~ 6) between 10^{-3} and 10^{-4} mutants are found, but not as entire colonies. Rather, one usually finds sectored colonies in which the Lac- may compose from 1/4 to 3/4 of the colony, the remainder being Lac+. The components of the colony are easily separated and prove to be quite stable. If this finding is related to delayed effect, as I think it must be, it tends to exclude phenotypic delay, I am inclined to favor a segregation mechanism, and to explain the length or rather to introduce "genotypic" delay as a fairly certain factor. There still might be phenotypic lag for V_1.

of time over which it seems to take place as due in part to a variable lag in some treated, particularly mutant cells. I have noticed that the mutants sometimes come up more slowly than most of the population, and the decreasing yield with very high doses of UV in particular suggest that nascent mutants may be more fragile.

I would appreciate very much hearing your comments on these questions. Esther and I are happy here. Give our best to the gang.

Sincerely,

Joshua