CARNEGIE INSTITUTION OF WASHINGTON
DEPARTMENT OF GENETICS
COLD SPRING HARBOR, LONG ISLAND, N. Y.

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Dr. Joshua Lederberg
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Dear Joshua:

Thanks so much for your letter -- I am genuinely excited by your results, and wanted to lose no time in telling you so. I think you are putting bacterial genetics on a sound basis, and my hat is off to you.

I would like to let you know something about how I have handled the agglutionation problem. In my earlier work with acriflavine, I of course ran into it, and found some ways of getting around it. More recently, I have managed to overcome it completely, I think. In using heavy cell suspensions and fast-killing concentrations of acriflavine, agglutination is likely to produce just the kind of complications you mention. With lower concentrations, while agglutination occurs, there is a gradual unclumping, and 24-hour exposures give fairly homogeneous suspensions. Tests with mixtures showed no complications under these conditions. I made some measurements of the rate of infection by phage of acriflavine survivors, however, and found a significant slow-down, which naturally worried me. All this made me spend considerable time working out a method of verifying the indications of mutagenicity of acriflavine. I am planning to publish results of this work soon.

Essentially, the method is as follows: About 10^7 bacteria are inoculated into 5 ml. of 0.01% acriflavine in broth, in each of 50-100 tubes. There is no detectable agglutination with suspensions of this density, and if there is some that escapes detection, subsequent operations nullify it. After 4 hours of incubation, when survival is about 0.1%, sodium nucleate is added to each of the tubes to give a concentration of about 0.2%. The nucleate instantly knocks out the acriflavine, and the survivors begin normal division after a slightly prolonged lag. The tubes are then incubated 24 hours, and assayed to determine the no. of T1-resistant mutants per 10^8 bacteria. What you have is the growth of 10^4 survivors of acriflavine treatment in broth containing nucleate-inactivated acriflavine. The final growth is entirely homogeneous, and infection-rate by phage entirely normal. Controls consist of 50-100 tubes containing both acriflavine and nucleate, inoculated with 10^4 untreated bacteria, incubated 24 hours and assayed in the same way. The controls grow exactly as they would in broth alone, and give a frequency distribution of mutants comparable to that given by a similar series of broth cultures. The distribution in the

experimental series, however, is strikingly different -- most of the cultures have enormous numbers of mutants, and I have some evidence that both zero points and delayed mutations contribute to the final yield. Advantages of this method are a) agglutination is completely eliminated, and 2) the final test with phage is on bacteria that have gone through an essentially normal culture cycle after treatment, and normal infection rate is assured. Thus, I have little doubt at present that acriflavine is truly mutagenic.

Incidentally, Braun has found that agglutination with acriflavine is a good indication of rough-smooth differences, and B/r, which is relatively smooth, gives much less of it than rougher strains like B. If K-12 is rough, you will get much more agglutination than we get with B/r.

I am no longer working with chemical mutagens, having been caught up in the problem of delayed mutations and segregation, but if you have any compounds that you would like tested, I would be glad to give them a whirl. I think, though, that this would have to be slow and careful work, as each compound presents its own special hazards in mutagenicity tests, and to my mind, requires a great deal of work to arrive at any certain answer. I have a good deal of confidence in acriflavine now, but it took a great deal of work to get it.

As far as urethan is concerned, Bryson spent a long time on it, and other carbamates. He found enormous selective survival of phage-resistant mutants, and was therefore unable to conclude anything about its mutagenicity in this system. Latarjet published results with it (Comptes Rendus - CXLIII, June 1949, p. 776). He apparently got large mutagenic effects, and mentioned specifically that he did not observe the selective effects that Bryson got -- which is curious indeed, since they used exactly the same material and methods, as far as I could tell.

Hope you'll send something on your work in for the next MGB -- the April issue will be quite good, I think, and very international.

With best regards,

Sincerely

Evelyn

Evelyn M. Witkin

I forgot to mention that nucleate added to a heavily agglutinated suspension of bacteria in acriflavine will produce instant unclumping.