December 20, 1952

Dr. Norton Zinder
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New York 21, N.Y.

Dear Norton:

This is an answer to yours of the 21st, which only just arrived. The Christmas disorganization of the mails, and my own flood of letters to you (Dec. 14, Dec. 24, and postcard) is likely to wreck orderly communication between us. I am answering immediately, hoping you will get this before you have had to answer mine of the 24th, so that we can go back to an orderly progression. I promise not to burden you with any more until I have had your reply to this one.

I am sorry if you feel you are in an awkward position re Bruce. It simply illustrates the problem of a 3-way collaboration; I could imagine other unfortunate incidents that would be much worse if we felt that mutual clearance was necessary before A even talked to B. The suggestion that Bruce assume sole authorship was only a trial balloon, in the hope that it might simplify his problem of authorship. Time is getting on, and this paper should have been in press well before now. Your interest in the matter is in no danger, and not even any tentative action would be taken before consulting you. Someone had to speak to someone first. If you feel differently about how this paper should be written, you need have no embarrassment about it. Although we must all have a part in it, the decision (in my opinion) is preeminently Bruce's. Had we been in closer communication ourselves, you would have heard about it sooner. Still I am personally regretful if you have felt any trouble about this, but am sure there has been no irreparable damage. I still feel that a 3-way authorship is too complex; if you want to work out a dual authorship with Bruce, it is all right with me. I have no objection to the use of my "backcross" data on the linked transduction if Bruce regards them as essential to the present purpose.

To turn to a more scientific question, the "backcrosses" did not require additional phages. Fortunately, many of the transductions in this system are still sensitive to the transducing phage, so that it was possible to use PLT22B again, after sensitive progeny were discovered in each combination.

I don't quite follow your reservation about Fla- allelism? Do you mean that two non-motile stocks each carrying the same Fla- might each carry modifiers that restore motility to any other non-motile stock? This would be equivalent to suggesting that each non-motile is based on a unique constellation of factors, the replacement of any one of which restores motility. I suppose that this is still a formal possibility which could be verfied only if we could select easily for Fla- $\times$ Fla+. For a more complete analysis, it would be better to work with a group of mutants all derived, presumably by single mutational steps, from the same wild type strain. However, I do not think this affects the validity of allelism tests. It might alter the interpretation of "Fla_x-"; on the simplest version, the genetic backgrounds of the different O stocks are more or less the same; to make it more complicated, Fla_x- might give flagella in the residual geno-

type Y, but not in X. Still for A—x B to give a new form (motile), they must carry at least one non-allelic factor, and this is tacitly named Fla_{-}, although, as you say, there might be more than one Fla_b factor. Expressed this way, your notion parallels the now rejected hypothesis to explain the H_1-Fla_1 linkage by two alternative, non-linked factors.

It is fairly likely, from what your letter reports, that your variant differs somewhat from my 22V. It produces perfectly clear plaques (later some granularity) on LT-2. I wasn't sure what you meant by recovery of infective centers in the third sentence of this paragraph. Do you mean that bacteria recover to give colonies, Poisson referring to the calculated fraction of multiply infected bacteria, or that they give plaques?, (Poisson referring to calculated ratio of infecting particles to infective centers)? I just couldn't understand your last two sentences in this paragraph at all, can you? Typhimurium grows more rapidly than what?

Our UV conditions must have been different: I used undiluted broth lysates, and must have had a sufficiently thick layer to have had considerable absorption by the broth. Subsequent runs have given steeper killing curves in diluted broth. The main point is not the absolute dosage comparisons, but the survival of FA when phage (plaques) has been greatly reduced, so that phage could be thus separated artificially from transduction. I have not done the complete curve; especially as the distinction of 1- and 2-hit is pretty delicate. Did you not carry your irradiations to higher doses? With the high doses, it is almost impossible to avoid multiple infection in the assays (calculated on the basis of total particles of phage). However, I did get linear dilution response, and could not find any difference as between calculated multiplicities of about 1 and 10. Still, the surviving plaques might represent some of the multiply infected centers, though there should have been a substantial increase with 10 as compared to 1. The main point is that transductions and plaques could be counted on the same plate, and the former were non-lysogenic.

A brilliant (?) experiment that didn't work: UV'd PLT22 does not protect against 22V. [I had thought there might be recombination between inactivated PLT22 and active 22V, protecting against the latter, and inducing lysogenicity. This might then have been a model for lysogenization in general.]

Transduction frequency of 1:50,000 should make it possible to test dual transductions for independence, though you may still have to use a selective setup (diauxotrophs). This is not entirely uninteresting. If you find the ratio of duals/singles to be 1:200,000 instead of 1:50,000 you could argue rather reasonably that the nucleus, not the cell is the unit of transduction.

We have the same unpromising experience with lwoffing LT22 and LT2(22). SW-666 or 543 infected with PLT-22B works much better, but this phage has a low eop and transductive efficiency back on typhimurium.

I did understand your delay experiment this time (hallelujah!). The result seems very neat, but perplexing? I don't think you can correlate the tracks with this: they represent the abortive transductions, and much of the persistence may well be purely phenotypic. We don't know anything about delay or segregation in the initiation of swarms. Why should there be a difference in lag? I can't understand your Xyl result at all. Aren't your Xyl+'s growing ? It looks as if these had not been transinduced initially at all, but why such a high final count? What do you make of it.

I don't envy you the complexities of your virulence problem. There must be a number of ways to do the statistical analysis of heterogeneity. One, probably inefficient, would be to cut your population arbitrarily in half, and compare the sum of the variances of your two halves with the whole. When you say you suspect bimodality, you are suggesting only that the sample is more highly dispersed than you would have expected, but I cannot see any standard variance that you could use to justify your expectation. What you can do, however, is to assume that you have two populations, centered at the two modes, and proportional to the square (?) of the modal values. You can then show that you can fit your data to the sum of these two populations with a much lower variance than to the whole. I don't think there is any way of showing that your samples are taken from an anormally distributed population, except perhaps by comparing moments of successive orders. I don't know the significance tests for this, but Fisher has worked them out. Have you entirely exhausted the possibilities of an in vitro system? Hmmm How about mouse-serum (! sic) broth, considering serums from normal as well as challenged animals? Your job is to make a testtube a mouse.

This letter is written on the plan of your own, but I think needs no P.S., after the deluge of this and mine of the 24th.

Sincerely,

Joshua Lederberg

P.S. I did slip. Columbia College Fund has asked me to be a local "chairman", i.e., to make phone calls to local alumni to remind them of their responsibilities to the Fund. They still have your name listed locally. I'll have this corrected, but meanwhile will discharge my task by this sentence.

PPS. Do you think it would be appropriate for you to act as a sort of courier in distributing reprints to your colleagues at Rockefeller? I thought it might give you an excuse to communicate with them (or is this not such a problem)! It would be a favor to me, but if dropping the papers into mailboxes or what not would inconvenience you, or seem at all undignified in the local contexts, please say noo This remark does not, of course, apply to your paper.