October 8, 1953

Dear Dr. Hungate:

This is rather like trying to make a medical diagnosis by mail, but it appears to me that the most vulnerable point of your procedure has to do with "post-incubation" [after mutagenic treatment, prior to plating]. (See page 8 of the mimeographed version of "Isolation and characterization of bacterial mutants"). In addition, your conditions of treatment might conceivably be selecting against auxotrophs.

Morphologically typical bacteria (no less than long forms) behave as if they are multinucleate, and in fact usually display 2 to 4 nuclei per cell in stained preparations.

I cannot conceive that you are mis-scoring auxotrophs, but if you are still worried about this, I will send a culture of Y-87 (methionine-dependent, lactose-negative). The incidence of auxotropic mutants, even after the most effective mutagenic treatment, may still be one percent or considerably less. I would strongly recommend that you screen your colonies (post-incubation) by means of the replica-plating technique.

On the whole, mutations to auxotrophy do not make for a very handy system of measuring mutagenic effects. If you can work at P_{32} levels which are not too toxic, I would suggest Novick and Szilard's methodology as far and away the most reliable for quantitative measurements of mutation (See Cold Spr. Harbor, 1951; Nature, 170: 926, 1952). No other system is as free from incorrigible errors. Novick is spending the year at the Pasteur Institute, if you would be interested to communicate with him. If I am not mistaken, he may once have conducted a few casual experiments with P32.

If you must use auxotrophic mutations, I would suggest that the best means of collecting quantitative data is through the penicillin method, using a marked auxotroph (like Y-87) as a standard. Cf. Lieb, Genetics 36:466 , 1953. [Her conclusion as to the absence of phenotypic delay must be qualified very strongly by her own reservations].

Yours sincerely,

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