WASHINGTON UNIVERSITY
SCHOOL OF MEDICINE
SAINT LOUIS

THE EDWARD MALLINCKRODT
DEPARTMENT OF PHARMACOLOGY
EUCLID AVENUE AND KINGSHIGHWAY

March 15, 1958

Dr. Joshua Lederberg
Department of Genetics
University of Wisconsin
Madison, Wisconsin

Dear Josh:

We have had some very interesting results from E. coli strains which you sent us. We have made penicillin protoplasts with each of the five strains. None of them accumulates nuceotides. However, they all leak ultraviolet absorbing material into the medium so that at the present time the interpretation of the results is very difficult. I wonder if the function of sucrose and magnesium in protoplast formation isn't partly to prevent the leakage of materials at too rapid a rate. This leakage in E.coli under the influence of penicillin had been previously obersved by Binkley, who identified the ultraviolet absorbing material as uracil.† I want to try other stabilizing substances on these protoplasts since it may be possible to prevent this leakage.

Somewhat more interesting is the nucleotide accumulation in the DAP⁻ protoplasts. With one strain we observed nothing, but in the other strain, there seemed to be a small accumulation of nucleotide. This would correspond to the accumulation of nucleotides in staphylococci in lysine-deficient medium which I mentioned to you. We isolated a compound from a small scale run with DAP⁻ protoplasts and in general it has chromatographic and electrophoretic properties similar to the staphylococcal compound. To go any further, we have to have a very much larger quantity of material which we are in the process of trying to get now. It would be very worthwhile for us to have additional DAP⁻ strains, particularly the organism used by Bauman and Davis, and by Meadow and Work, and I would very much appreciate it if you could send additional strains to me as soon as convenient for you. It may be that one of them will accumulate a larger quantity of nucleotides.

† author Brown Briefly about a year ago — not a very good paper but the important fact is there.

We have rather large scale preparations of extracts from various types of coli protoplasts from the above experiments, and if any of these would be interesting to you, I would be very glad to send them.

Best regards,

Sincerely,

Jack

Jack L. Strominger

JLS:pkh

Re penicillin and DAP⁻ protoplasts look very different. I've never seen good DAP⁻ protoplasts. What the strains I have give is many large bodies and bizarre shaped forms (still rod-like) (even though at the same time lysis is complete in the absence of sucrose in the medium). If these "protoplasts" from the DAP⁻ sucrose Mg++ medium are centrifuged and resuspended in water, they do not lyse. The same strains DAP+ and plus penicillin, sucrose, Mg++ give beautiful round protoplasts. (will lyse in water.) It is not surprising that these penicillin protoplasts leak since their volume looks 10-20 x the bacilles - and the cytoplasmic membrane must be very stretched.

The experiment of Tweeo and

Pardee (Jan JBC) is very poor experimental design. With C_{14}-glucose, the maximum decrease in incorporation into walls which they might expect would be 5 or 10% - well beyond the limits of accuracy of their method - and see the last paragraph of their discussion. I'm perfectly willing to consider other hypotheses, but these experiments lend no support to them. Last summer we showed that C_{14}-lysine incorporation into Staph walls is 50%-70% inhibited by penicillin (no inhibition into soluble protein). Similar experiments with Coli are very important and we'll be very interested to see the results, as soon as we have time to do them / but using a specific label is essential.

A lot of our experiments, including the isotopic ones, would be greatly aided by D-alanine and/or D-glutamic acid requiring mutants. Any chance of getting them?

Thanks for the photographs. I used one of them in the paper.