September 27, 1966

Dear Dr. Dimes:

First let me thank you for directing not only the various cultures, but also Dr. Sharp, to the lab. here. I had a most enjoyable and informative discussion with him.

We are finally learning how to grow the L colonies of E. coli K-12. The experience with Proteus was invaluable for this purpose. Then we managed to handle another coli strain, and finally K-12 itself. There were a number of ridiculous, empirical variables: the exact recipe for the nutrient broth base, the freshness of the Mg+penicillin solutions, and worst of all the batch of Difco agar. Finally, we found a lot of New Zealand agar which is the best of all.

Since writing that note for PNAS, which should be in print at any moment I have been finding that the use of penicillin + a protective (hypertonic) medium has been anticipated several times for the production of "protoplasts". The only distinction I can claim for the data of that note are 1) the use of E. coli, 2) the semantics, which was not entirely clear to me then, and 3) the quantitative and enzymological features. [There is, for example, a report by Bonifas, Schw. Ztschr.Path. Bakt.,17,525 "Influence de la pression osmotique surele maintien en milieu liquide penicilline, d'une souche de Proteus sous sa Forme L", which we must both have missed until recently — my lead to it was the rather good account of Liebermeister & Kellenberger, quoted in the enclosure].

I have also had many discussions, including with Dr. Sharp, concerning the implication for mechanism of penicillin action. His data on the carbohydrate content of stabilized L-forms are very impressive, but I gather you and he do not necessarily agree with my proposal. The mechanism of stabilization of L-forms is a provocative problem, and a feature which has no direct explanation from this proposal, but I feel it is a separate problem. If we can get such stable L's from the coli mutants, it should be possible to define their genetic basis. I had hoped that the implications from the one sentence in the discussion in the PNAS note would be sufficiently clear, but evidently they are not to a number of people I have talked with— and there is still so much nonsense about penicillin floating around, all based on studies of disrupted cells! At any rate, I am planning to submit the enclosed as a note for J. Bact.

With best regards,

Yrs. sincerely,

Joshua Lederberg