August 31, 1956

Dear Dr. Dienes:

Thank you for your letter of the 29th, and the ms. I am looking forward to meeting Dr. Sharp: would you ask him to call me at home (CEdar-3-2968) when he arrives, or let us know where we can pick him up?

Since I wrote to you before, I have been learning more and more, as much from reading and re-reading your papers as from new experiments. It is obvious to me now that protoplasts are the initial large bodies, the starting point of the L growth cycle. (I wish I had as clear a grasp of this when I wrote that paper!)

I have also been able to achieve essentially quantitative yields of L colonies, both from E. coli K-12 and from Proteus 52/xthuxkatkarx when bacteria are inoculated into soft nutrient agar + penicillin + Mg + sucrose. The sucrose is quite essential for this efficiency; it doubtless serves the same function as your 3.5% NaCl, though I think more effectively. However, I have had very limited success so far in propagating the L-colonies further & still have to work this out. I have cause to underline lines 9-12, page 539 of your 1949 paper on Proteus! However, in my own faltering way, I am learning some of your tricks-- hoping, of course, to be able to extend them to my own favorite strains.

Unfortunately, I have not been able to obtain a floating growth even with several trials on Proteus 52; it seems quite possible that the strain has become less apt during storage here, and I would therefore renew my request for the bacterial culture and flor a floating growth. It seems likely, as I am sure you have in mind, that the floating growth is a mass which furnishes itself the requisiste physical conditions for preliferation.

I have madeca few observations on slide cultures; these suggest that the mode of proliferation of the initial L colony is by <u>budding</u> of the protoplast. Are there any <u>direct microscopical</u> observations of the role of minute granules in proliferation? In retrospect, Klieneberger's observations are not entirely convincing (I mean her filtration experiment). Even the coarsest filter removed most of the viable count! The typical size of the reproductive units there may have been very little smaller than that of the bacteria (which are rather small themselves).

Anyhow, there will be many things for Dr. Sharp and me to talk about; I hope it will not be too long before I can pay my respects to you again in person.

Yours sincerely,

Joshua Lederberg