

COLLEGE OF TECHNOLOGY
MANCHESTER 1

February 24, 1949.

Dr. Joshua Lederberg,
Asst. Professor of Genetics,
The University of Wisconsin College of Agriculture.

Dear Professor Lederberg,

Thank you for your letter of the 9th.
I am afraid I have no methods of the type you suggest. My own ideas are along the lines of modifications of the "layer plate" technique, either by use of cellophane membranes or by allowing active substances to diffuse upwards into the inoculated agar. With fast spreading moulds these techniques have manifest disadvantages, and though in theory they sound satisfying enough I have not had time to experiment much with them. On the basis of my experience I would suggest (for P. notatum or Asp. nidulans or veger) the growth of the plate of agar until mycelium just appear. The removal either by heat (a hot point or rod) of the grown colonies and then the raising of the plate by interposition of sterile flameless steel gauze or filter paper to allow liquid containing the factor to be run underneath, or alternatively the whole agar layer could be bodily

placed upon a prepared agar-growth-substance gel.

I have, as published, used the filtration technique of Fries. This appears to depend on the power of germination of the organism in the absence of the deficient factor. Usually the spores will be able to germinate on their reserves to a sufficient extent to make them "particle size", considered from the point of view of filtration, comparable with that of the normal. Again one would expect their heat or alcohol-susceptibility to be much the same.

I have tried no mass-selection in repeated subcultivation, via mass spore-suspension-inocula, in presence of the substance whose deficient mutants are under study. This appears to me to have possibilities as a mode of bacterial study, but with would be the difficulties in distinguishing ^{truly} identical mutants from similar mutants (i.e. members of the same & different clones) would render any study of mutation frequencies liable to error. However this might prove a useful way of obtaining large numbers of mutants if the mass spore technique was accompanied by ^{successive} mass exposure to mutagen.

As my work is more concerned with the chemical synthetic mechanisms (a paper will shortly appear in *Biochimica et Biophysica Acta*) I am engaged now upon quantitative work stemming from the original findings. So far these appear to be confirmed although the picture may not be quite so simple as there represented. I will let you have the reprint, if I manage to get any.

Thanking you for your very kind interest and hoping that my observations will be at least a little use to you,
Yours sincerely,
Donald Hockenbury.