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Dr. Joshua Lederberg
Professor of Genetics
Department of Genetics
Standord University
Stanford, California, U.S.A.

Dear Doctor Lederberg:

Enclosed herewith I send you a preliminary note regarding my recent research. Since this is my first excursion into the domain of classical bacterial genetics, you must not be surprised to find signs indicating my lack of experience. I, therefore, would greatly appreciate your valuable advice and severe criticism. I realize some of the shortcomings of this work: as for instance, I am not quite happy about the insufficient number of selected markers. However, I have not been able to establish new ones, especially with BCG. Modification of the known techniques to some extent became necessary in view of the particular pattern of growth of mycobacteria.

What appears to be of interest to me is that I recently succeeded in conferring the streptomycin resistance to M. phlei via a cell-free filtrate of the resistant BCG strain (both in the presence and in the absence of streptomycin). First estimations of the frequency of occurrence show that about 1-10 out of 10^8 M. phlei cells are affected by this change. I should like to mention that the new strains thus produced yield about twice as many colonies on agar media in the presence of streptomycin as in the absence of this antibiotic. This, however, happens only if platings are made immediately after the appearance of growth on a selective medium. Approximately 48 hours later (i.e. eight to twelve generations) the reversal of this ratio can be observed.

Serial transfer to a non-selective medium will also result in decrease of the number of organisms maintaining the transferred streptomycin-resistance. I wonder if this might account for what you call the "heterogenote" stage, or do other mechanisms play a role in it? The similarity of behaviour of resulting streptomycin-resistant clones suggests a possible similarity in the mechanism of transfer of this single trait when either intact cells or cell-free filtrates are carrying the genetic information, even though the frequency of transmission is different. Another interesting point is that while the streptomycin-resistance of the BCG strain was acquired in four or five steps, the highly increased streptomycin-resistance of the new organism immediately emerged after isolation in both cell-to-cell contact or cultivation in a filtrate. Some of the facts presented here bear a resemblance to that of the interspecies induction of streptomycin-resistance in the genus Hemophilus as shown by Leidy, Hahn and Alexander. Although at present I do not think that the mechanism by which the streptomycin-resistance is transmitted among mycobacterial species is a DNA-transduction, I plan to examine in the near future the effect of DNAse on the whole phenomenon. It is worth mentioning that so far no transfar of characters could be achieved by a cell-free filtrate of M. phlei. Uni-directional transfer seems also to be operating in this case.

Also enclosed are three plates, as follows:

Plate I: Typical multiplication of BCG. 72-hour process. Generation time is minimally 18 hours.

Plate II: Usual elongation of "cocci" of M. phlei and their subsequent division when transferred to a fresh medium. 46-hour process. Generation time: 5 - 8 hours.

Plate III: aims to give a hint at the possible mode of conjugation of individuals belonging to M. phlei and BCG strain respectively. 18-day process.

In addition to the distinct cellular morphology of M. phlei ("cocci" at the beginning which later elongate to form rods and divide like rods) and M. tuberculosis (longer rods), growth at room temperature was chosen as marker character. After conjugation has taken place (fig. 6), temperature was set at 37°C. Shortly after this, a small coccoid form resembling the morphology of the M. phlei organism appeared at the opposite end of the conjugating BCG rod. A new cytogenetic approach employing additional marker characters became necessary in order to elucidate the genetic significance of the emergence

of such forms. Do they actually result from the conguga-tion process or, as is seen in other instances, are they merely representing the re-organization into rods of amorphous mycobacterial masses? Such questions and other controversial points raised by the above continuous observation of the conjugation process have to be cleared.

I am anxious to know your opinion about this work, which is still in a very early stage.

Very sincerely yours,

Stephen Juhasz, M.D.

SJ:MB