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Tbc. Research Laboratory,  
411 East 69th St., New York 21, N. Y.

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Dr. Joshua Lederberg,  
Dept. of Genetics,  
The University of Wisconsin,  
College of Agriculture,  
Madison 6, Wisconsin.

Dear Joshua:

Enclosed is a copy of the manuscript that you suggested I publish. Any comments would be appreciated.

Sincerely,

*Bernard D. Davis*

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BDD/hl

*J. Bact. flawed.*

enc.

Non-filtrability of the Agents  
of Genetic Recombination in Escherichia coli

The demonstration of genetic recombination in bacteria (Lederberg, 1947; Tatum and Lederberg, 1947) appears to imply the existence of a sexual mechanism, although direct evidence of cellular fusion has not been produced, presumably because of the low frequency of the phenomenon in the populations studied (ca.  $10^{-6}$ ). Since production of genetic transformation by a soluble material has been established in pneumococci (Griffith, 1928; Avery et al., 1944), and reported in E. coli as well (Boivin, 1947), the possibility of a similar mechanism must be seriously considered for the recombinations discovered by Lederberg.

It is true that the appearance of recombinant progeny involving multiple factors in various combinations is more difficult to reconcile with the hypothesis of a soluble agent than were the original observations (Lederberg and Tatum, 1946) on single genetic factors. Nevertheless, one cannot arbitrarily exclude the possibility of either a complex transforming substance involving a number of genetic units, or a gamete with a full complement of genes but smaller dimensions than the bacterial cell. Previous failure to produce recombination with culture filtrates of E. coli ~~(Tatum and Lederberg, 1947)~~ (Lederberg, 1947) could be due to greater instability in this case than in that of the pneumococcus. It therefore appeared of interest to test further for a filtrable factor, attempting as far as possible to circumvent deterioration by minimizing the delay in transport from donor to recipient cell.

For this purpose a bacterial contraceptive device was fashioned in the form of a U-tube partitioned by an "ultrafine" fritted glass disc (Corning). Its impermeability to bacteria was verified with the organisms under investigation, E. coli K-12 mutants Y-10 (Threonine<sup>-</sup>Leucine<sup>-</sup>Thiamine<sup>-</sup>) and 58-161 (Methionine<sup>-</sup>), kindly furnished by J. Lederberg, were separately inoculated (1 ml. in 10 ml. of medium containing 0.5% yeast extract) in the two sides of the tube. At frequent intervals during cultivation a large portion of the fluid was alternately forced through the disc from one side to the other by application of a vacuum. Simultaneously, cultures were made with a mixture of both inocula in a single tube, as well as with single inocula in separate tubes. After 4 hours all <sup>5</sup> cultures were washed and plated in minimal medium. Conditions of plating were such that at least 90% of the prototroph colonies obtained from the mixed tube had arisen from recombinations occurring in the tube, rather than during subsequent incubation of the mixed plate. This fact had been established by numerous experiments comparing inocula mixed in the tube with inocula of identical size mixed in the plate.

The mixed tube gave 58 prototroph colonies in a 0.05 ml. inoculum; the cultures from each side of the U-tube, and from separate tubes, gave none in volumes up to 0.5 ml. Similar results were obtained in another experiment. It is concluded that recombination by means of a filtrable transforming substance or gamete is unlikely; such a mechanism could be reconciled with these observations only if the material were exceedingly unstable.

Bernard D. Davis,  
U. S. Public Health Service,  
Tuberculosis Research Laboratory,  
Cornell University Medical College,  
New York 21, N. Y.

### References

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