

## NOTES

### NONFILTRABILITY OF THE AGENTS OF GENETIC RECOMBINATION IN *ESCHERICHIA COLI*

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Genetic recombination in *Escherichia coli* (Lederberg: Genetics, **32**, 505, 1947; Tatum and Lederberg: J. Bact., **53**, 673, 1947) appears to imply a sexual mechanism, but the evidence is incomplete since cellular fusion has not been demonstrated directly. Although the low frequency of recombination (ca.  $10^{-6}$ ) can account for this failure, a mechanism similar to the soluble pneumococcus transforming substance (Avery *et al.*: J. Exptl. Med., **79**, 137, 1944) must also be considered. Though this hypothesis became less attractive following the demonstration of recombinant progeny involving multiple genetic factors in various combinations, one cannot rigorously exclude 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. Since the failure of culture filtrates to produce recombination (Lederberg) could be due to unusual lability of the agent, further tests on filtrates were undertaken, deterioration being minimized by avoiding delay in transport from donor to recipient cell.

A U-tube, 25 mm in diameter, partitioned by an "ultrafine" fritted glass disk (Corning), was shown to be impermeable to the organisms under investigation. K-12 mutants Y-10 (Thr<sup>-</sup>, Leu<sup>-</sup>, Thi<sup>-</sup>) and 58-161 (Meth<sup>-</sup>), kindly furnished by J. Lederberg, were separately inoculated (1 ml of turbid culture in 10 ml of medium containing 0.5 per cent yeast extract) in the two sides of the tube. During incubation a vacuum was applied alternately to each side, approximately half the total fluid being forced through the disk in 3 cycles per hour. Accumulation of bacteria on the disk was partly prevented by shaking with glass beads. Similar cultures with a mixture of both inocula, as well as single inocula, were simultaneously incubated in test tubes.

After 4 hours all 5 cultures had reached heavy turbidity (ca.  $3 \times 10^9$  cells per ml). They were washed and pour-plated in minimal medium, which would yield only recombinant prototroph colonies. The mixed tube gave 58 colonies from a 0.05-ml inoculum; the cultures from each side of the U-tube, and from separate tubes, gave none in volumes of 0.05 to 0.5 ml. Similar results were obtained in a second experiment.

Previous experience had shown that over 90 per cent of the colonies obtained under these conditions arose from recombinations occurring in the mixed tube, rather than subsequently in the plate. This experiment should therefore have detected, in the largest inoculum, U-tube recombination in 1/500 the frequency

of the mixed tube. Since none was observed, recombination via a filtrable substance seems unlikely; such a mechanism could be reconciled with these observations only if the material were exceedingly unstable.

### DETECTION OF ROUGH DISSOCIANTS OF PASTEURELLA PESTIS WITH TETRAZOLIUM CHLORIDE<sup>1</sup>

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Lederberg (J. Bact., **56**, 695, 1948) reported the use of triphenyl tetrazolium chloride to detect fermentative variants of *Escherichia coli* K-12. We have found that smooth and rough colonies of *Pasteurella pestis* could be identified easily when grown on tryptose (Difco) agar containing this compound. On many routine and modified laboratory media that did not contain tetrazolium, differentiation was accomplished only with considerable difficulty.

A 1 per cent aqueous solution of tetrazolium was sterilized by autoclaving and added aseptically to melted tryptose agar so that the final concentration of the dye in the medium was 0.005 per cent. Approximately 20-ml volumes of the medium were then apportioned to petri plates, which were dried at 37 C for 24 hours. These were seeded thinly with both smooth and rough forms of a virulent and an avirulent strain of *P. pestis*. After incubation for 4 days at 30 C, typical smooth colonies were approximately 2 mm in diameter and round, and had a sharply defined carmine red center. Rough forms were irregular in shape and diffused pink in color. Although these differences could be detected by the unaided eye, best results were obtained when a dissecting microscope was used to study the colonies.

Intermediate type colonies were observed on this medium with very low, but as yet undetermined, frequency. On subculturing, these appeared as either smooth or rough.

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