RECOMBINATION IN BACT. COLI K 12: UNI-DIRECTIONAL TRANSFER OF GENETIC MATERIAL.

Five years ago Lederberg and Tatum[1] demonstrated that when two mutant strains of Bact. coli K 12 having double and complementary growth factor requirements were plated together in a basal synthetic medium, colonies appeared in the ratio of one to every 10^6 or 10^7 cells seeded, although colonies did not arise by back mutation when the mutants were plated separately. These colonies were prototrophic and similar to the wild type in their independence of added growth factors. Not only did they breed true, but it was also shown that if each mutant was further denoted by complementary differences in characters not selected by the basal medium (such as fermentative capacity for various carbohydrates and sensitivity or resistance to specific virus infection), the pattern of these characters in prototroph cultures was usually different from that in either mutant. It was clear, therefore, that prototroph colonies arose from the transfer of genetic material between the mutants and not from complementary exchange of growth factors. Unlike type transformation in Pneumococcus or the "directed mutation" of similar mechanism in Bact. coli, growth of one K 12 mutant in culture filtrates of the other failed to stimulate prototroph formation. This, in conjunction with recent evidence for the occasional occurrence of diploid heterozygous prototrophs[2], strongly support the view that genetic recombination is mediated by the

classical mechanism of sexual conjugation. Attempts to reproduce the phenomenon by similar techniques in other strains of Bact. coli and in Salm. typhimurium have failed, though Cavalli and Heslot[3] succeeded in out-crossing K 12 mutants with a naturally nutritionally exacting strain of Bact. acidi lactici.

In the following experiments, Bact. coli K 12 mutants 58-161, requiring biotin and methionine, and W 677, requiring leucine, threonine and aneurin, were employed. The aneurin requirement of W 677 was neutralised by the addition of this substance to the basal medium. An attempt was made to investigate the dynamics of recombination by spreading a mixture of 58-161 and a streptomycin-resistant mutant of W 677 (W 677/S^r) on a series of plates of basal medium, and then adding streptomycin to each plate in turn at different intervals after seeding. It was anticipated that the streptomycin would rapidly block the recombination mechanism by inactivating 58-161, while allowing resistant prototroph cells formed prior to its addition to develop into colonies. It was found in practice that the number of prototroph colonies did not differ greatly whether streptomycin was incorporated in the basal medium before plating, or was added up to four hours later. Since similar results were obtained when the mutants were mixed for the first time during plating, the occurrence of recombination in mixtures before contact with streptomycin was excluded. Either prototrophs arose before the action of streptomycin on the

sensitive mutant became effective, or else those functions of the cell affected by streptomycin were not involved in recombination.

Logarithmic phase broth cultures of 58-161 were treated with either 1000 or 2000 µg./ml. streptomycin, under conditions optimal for bactericidal effect, for from 4 to 18 hours. After washing and concentration in saline, volumes of suspension turbimetrically equivalent to from 1.5×10^8 to 4.0×10^8 cells (i.e. double the inoculum used in recombination experiments) frequently proved sterile and rarely yielded more than 100 colonies on nutrient agar after 72 hours at 37°C. Nevertheless such suspensions (58-161/S^t) invariably stimulated prototroph formation when mixed with W 677/S^r on basal medium containing 200 µg./ml. streptomycin. The recombination rate, however, was usually appreciably less, and showed much more marked variation between experiments than when viable, untreated 58-161 suspensions were used. Whenever streptomycin treatment failed to produce sterility, control reconstruction experiments showed that about 1000 times as many untreated 58-161 cells as those which had survived treatment failed to yield prototroph colonies when mixed with W 677/S^r suspension under similar conditions.

Mixtures of streptomycin-treated W 677 (W 677/S^t) and 58-161/S^r, on the other hand, invariably failed to produce prototrophs although comparable recombination rates were given by the mixtures (W 677/S^r_s + 58-161) and (58-161/S^r_s + W677). In these experiments

streptomycin was not incorporated in the basal medium since previous analysis of proven prototrophs had shown that about 95 per cent carried the S^r or S^s character of W 677. The clearcut distinction between 58-161/S^t and W 677/S^t in ability to participate in recombination was shown to be independent both of the presence of the S^r character in the complementary mutant and of the basal medium environment. Thus (58-161/S^t + W 677) produced prototroph colonies on every occasion whether cultured directly on basal medium or initially on nutrient agar. The mixture (W 677/S^t + 58-161), however, failed to do so repeatedly on basal medium and, in a single experiment, when seeded on nutrient agar.

These findings require a revision of the current concept of recombination. It is unlikely that sensitive cells which have been acted upon for 18 hours by very high concentrations of streptomycin are still able to participate in conjugation in the continued presence of the drug. Moreover, if conjugation under these conditions was possible for 58-161/S^t it might also reasonably be assumed for W 677/S^t which is equally sensitive to streptomycin. Yet suspensions of the latter are inactive to recombination. It is simpler to suppose that recombination is mediated by genetic elements which are extruded by the viable cell but remain adherent to the cell wall and which, like viruses, are unaffected by streptomycin. Thus the cell could be regarded

merely as a passive carrier for its genetic elements after their expulsion, a function which could be performed equally well by dead or by living cells. The incompetence of W 677/8^{t} becomes intelligible only if we suppose that the role of W 677 is the vital one of accepting genes and incorporating them into its genetic structure. It is possible that W 677 is also a gene donator but, if this is so, then 58-161 is inherently incapable of accepting them.

It has recently been shown that symbiotic bacterial viruses can transfer hereditary characters to heterologous cells which they infect[4]. It is also known that K 12 carries a symbiotic virus which is liberated from the cells in appreciable numbers only after UV irradiation. This virus can infect and lyse mature cultures of the K 12 mutant "S" although young cultures of "S" display the peculiar property of resisting lysis while still absorbing the virus[5]. The existence of a latent virus in 58-161 has been unmasked by X-radiation (personal observation). It seem possible that such a virus might be the agent of genetic transfer. The apparent restriction of the recombination phenomenon to one strain of Bact.coli, its relatively low rate and the inability of culture filtrates to induce it, the stimulation of recombination by doses of UV light too low to increase the mutation rate[6], the competence of streptomycin-"sterilised" cultures of 58-161 and the one-way transfer of the

genetic agent are all in conformity with such a concept. Work is now in progress to determine whether filtrates of 58-161, from which latent virus has been released by UV irradiation, can induce prototroph formation in W 677.

Department of Bacteriology,
Postgraduate Medical School of London.

W. Hayes.

6th November, 1951.

1. Lederberg, J. and Tatum, E.L., (Nature), 158, 558 (1946).
2. Lederberg, J., Proc.Natl.Acad.Sci.U.S., 35, 178 (1949).
3. Cavalli, L.L. and Heslot, H., Nature, 164, 1057 (1949).
4. Felix, A. and Anderson, E.S., Nature, 167, 603, (1951).
5. Weigle, J.J. and Delbrück, M., J. Bact., 62, 301(1951).
6. Haas, F., Wyss, O. and Stone, W.S., Proc.Natl.Acad.Sci.U.S., 34, 229, (1948).