

Growth involves the integrated synthesis of a series of substances:

a, b, .... The steps in the synthesis of these substances are controlled by a set of genes, essentially homologous to the substances, which we shall designate as A, B, .... or more specifically, A+, B+, ~~###~~ ..... We use the + terminology because it is possible to modify the activity of specific genes so that the synthetic step under their control is blocked. Such genes are designated A-, B-, .... This is demonstrable in Neurospora, where the occurrence of A- rather than A+ is associated with the inability to synthesize a, and the consequent appearance of a as a nutritional requirement of the strain. Such strains are known as biochemical mutants, since the modification of the homologous gene is a mutation. The mutation of a particular 'gene' has been achieved only in the pneumococcus transformations. In Neurospora, gene mutations are randomly induced by radiation, and detected by the alteration in growth factor requirements in the resulting strain.

By similar treatments, it is possible to isolate strains of Bacteria (E. coli/in particular) with similar alterations in nutritional requirements. Because of the evident analogy with Neurospora, we believe these alterations to be based on gene mutations, and it is the purpose of this investigation to use these characters in investigating the genetics of bacteria. On the basis of the Neurospora work we can make partial deductions of the genotype on the ~~basis~~ basis of the nutritional behavior of a strain. If a substance k is not required by a particular strain, it is K+. However there may also be present K- 'genes' masked by the activity of K+. We proceed now to our experiments.

There are two modes of approach, in general: the study of the appearance of K- strains after various treatments, particular with respect to the time of such appearance which affords evidence of the segregation of K- from K+; the other the appearance of new types in mixed cultures. While we are using both approaches, it is developments in the ~~mix~~ latter which have proven so exciting as to be the basis of this request for extension. The main result is that there appear,

under certain conditions in mixed cultures of [A-, B-, C+, D+, ...] and [A+, B+, C-, D-, +....] cells of wild type or prototroph constitution, i.e. [A+, B+, C+, D+, ...].

The interpretation of this result depends on the results of certain other experiments which occupied most of my first three months on this project. Firstly it was necessary to use double mutants [K-, L-, +++] rather than only single mutants [K-L+ +++] because it had previously been found (Ryan and Lederberg unpubl.) that the change from K- to K+ occurs in general with a measurable frequency of the order of  $10^{-8}$ . However, the mutation of different genes K- and L- to their + analogues is independent and should occur <sup>coincidentally</sup> with an expected (but unmeasurable frequency of the order of  $10^{-16}$ ). It was necessary to demonstrate this independence, and in my experiments I have found no instance of the occurrence of a wild type ( [K+L+] ) in cultures inoculated with [K-L-].

Secondly, it was found during the last three months that different mutants can interact through the medium, so that a mixed population might grow on minimal medium: If K+L- and K-L+ are inoculated together, the former manufactures and releases into the medium sufficient k for the latter to grow, and conversely. Analysis of the populations after such 'syntrophic' growth demonstrated this phenomenon independently of any genetic interaction that might have taken place. A similar situation applies to the combined growth of a pair of double mutants. If k is proline (so that K- is proline-less) it was found that K+ produces considerable quantities of proline and releases it into the medium during early growth but it is evidently reabsorbed in the later phases of the growth cycle, an indication that there exists a continual interchange between cells and medium.

In view of the occurrence of syntrophism, assurance would be required that the prototrophs (A+B+C+....) mentioned above did not merely represent cells temporarily stuck together. The stability of these prototrophs was tested, and confirmed, even when most of the cells had been killed by ultraviolet. Under these conditions one would expect many cell pairs to have been represented

by only a single mutant survivor; none were found under ideal testing conditions covering thousands of cells. Further evidence that these prototrophs are genetically unique is provided by the results of certain experiments still in progress wherein bacteriophage susceptibility was used as a marker. We found, to our surprise and fortune that the *E. coli* strain we have been using is susceptible to virus T-1 which has been the subject of investigation by the Cold Spring Harbour Group. Designating resistance and susceptibility as T<sub>r</sub> and T<sub>s</sub>, the former obtained in various strains by mutation and selection, the following 'crosses' were undertaken : [A-B-C+D+T<sub>r</sub>] + [A+B+C-D-T<sub>s</sub>] a wild type susceptible strain could readily be isolated. [A+B+C+D+T<sub>s</sub>]. If cells had merely been stuck together one should have been left with a large proportion of resistant mutant cells after application of the virus.

Spontaneous mutation has been eliminated as the source of these monotonously + strains. We have looked for these because they are patently the easiest to find in large populations, and their rate of occurrence in our best experiments has not exceeded  $10^{-6}$ . To complete the proof that gene recombination is the basis of these results it will be necessary to isolate ~~gene~~ gene recombinant types ( such as [A+B-C+D-] and other permutations of +,-) from A-B- and C-D-..'crosses'. There is a little evidence that they may occur. The possibilities of the detailed genetic mechanisms - occurrence of linkage groups, haploidy or polyploidy, the life cycle, etc. are too manifold to be adequately investigated in one summer. I could, I think, accomplish a great deal in another year, and if these suppositions turn out correctly, open to investigation a broad field obscured hitherto by the lack of basic knowledge of microbial inheritance.