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Project: Genetics of Bacteria

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Three modes of hereditary transmission are now recognized to occur in bacteria. The first is simply the production of like by like in the fission or vegetative growth of bacterial cells. The second is embodied in "sexual" reproduction, whereby two cells can exchange or recombine their hereditary particles, giving rise to new genotypes distinct from either parent. The third is infective transmission, whereby a hereditary character of one microbial line may be transmitted via the medium to another. Of these three modes, the first two are of course found in nearly all forms of life; the third (apart from the behavior of parasitic viruses) is a new principle which has been firmly established only in recent years (1) through the investigation of transformations of type in pneumococcus. Sexual reproduction is an even newer tool in the hands of the bacterial geneticist. The author's recent investigations of this phenomenon are reported in the reprints attached to this memorandum.

The research program which is planned and under way in this laboratory can be categorized under two headings. No. 1 is an application of the tool of breeding analysis in bacteria to a problem in gene action--the relationship between gene mutations and changes in enzymatic specificity, which is supposed to represent the primary function of the gene. No. 2 is an investigation of infective transmissions in a unique strain of E. coli, wherein A. Boivin has reported the transformation of a rough variant of one type (C2-R) to the smooth form of a type (C1-S) from which the active extract is prepared.

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1. See McCarty, M., Taylor, H., Avery, O. T. Biochemical studies of environmental factors essential in transformation of pneumococcal types. Cold Spr. Harb. Symp. Quant. Biol. 11: 177-183 (1946).

Subtitle #1. Genetic control of bacterial enzymes

Although the general theory that the primary function of genes is the elaboration of enzymes is now generally accepted, there has been little exploration of the genetic control of specific enzyme systems (1). What work has been done has led to the generalization that there is a one-to-one correspondence between genes and enzymes, from which it has been inferred (2) that the specificity of an enzyme is conferred upon an inactive precursor in a single step by a single gene.

Bacteria have long been favorable materials for enzymatic research; now that genetic procedures are also feasible in E. coli, this organism becomes well-suited for the experimental study of gene-enzyme relationships. Carbohydrate splitting enzymes were selected for this study because of the ease with which mutants lacking particular enzymes can be detected, using indicator media.

About 200 mutants deficient in the splitting of lactose have been isolated so far. Genetic and chemical tests are still under way, but it can be stated that mutations at no less than eight different genetic loci can lead to the loss of lactose-splitting activity. To a limited extent so far, mutations at different loci can be distinguished by their effect on the enzymatic splitting of synthetic analogues of lactose such as methyl-B-D-galactoside. Likewise, at least four genetic loci involved in maltose-splitting enzymes have been identified.

"Progressive" mutations, in the direction of increased enzymatic capacities, have been looked for also. Thus far, in spite of extensive attempts, only two have been found, one capable of utilizing the glucoside salicin, and one capable of utilizing the straight-chain component of starch, amylose. In view of the work previously described, part of the difficulty in adapting E. coli to new substrates may lie in the possibility that several gene changes are required concurrently.

Preliminary chemical studies suggest that only a single enzyme is involved in the splitting of lactose to hexose sugars, but it is premature to deny the universal validity of the 1:1 theory. However, to impute eight, and possibly more, enzymatic steps to the reaction is a strain on the imagination. Some modification of the one-to-one generalization may be anticipated. Clarification of the relationships will require an extensive program of chemical and genetic analysis.

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1. Beadle, G. W. Biochemical Genetics. Chem. Rev. 37: 15-96. (1945)

2. Bonner, D. M. Biochemical Mutations in Neurospora. Cold Spr. Harb. Symp. Quant. Biol. 11: 14-24. (1946)

Dr. K. P. Link of the Department of Biochemistry is kindly assisting in the preparation of some of the synthetic substrates to be used in this study.

Subtitle #2. Genetic transformations in *E. coli*

Dr. Boivin has kindly made available strains C1 and C2 mentioned in the preface. Since the C2R - C1S transformation is difficult to apply quantitatively, and provides only a single marker, we have endeavored first to obtain a series of mutants of both these strains. By the use of strains carrying several transformable characters, such problems as the linkage or association of distinct transforming substances, and the possibility of separating the chemical of specific transforming agents can be attacked.

Several mutants have been obtained in the transformable type C2, mostly nutritional mutants, requiring various amino acids for growth, including arginine, leucine, lysine, threonine, tryptophane, isoleucine, and valine. One strain has been isolated which appears to require a hitherto unidentified substance. Tests for the induced reversion to normal of these mutants under the influence of extracts of C1 are under way as of this date. A C2 mutant which was anaerogenic (unable to produce CO_2 gas in the course of sugar fermentation) was however shown to be converted to the wild, aerogenic type by such extracts. To date this has been our first confirmation of Boivin's conclusions.

Mutants have also been obtained in C1, and will be used to determine whether mutant characters can also be transferred in this type of hereditary transmission.

The geneticist's primary interest in these phenomena resides in the gene-like behavior of transforming extracts. Not the least of our efforts must therefore be in the direction of purification, separation and chemical characterization of these agents.

This work was initiated in collaboration with Prof. E. L. Tatum at Yale University, with whom day-to-day exchange of information and strains is continuing.