Second Interim Report to the Natural Sciences Division

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Project: Genetics of Bacteria

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1. Gene enzyme relationships in E. coli.

The studies of the genetic control of lactase which were initiated during the first year of the project (See Interim Report I, 1949) have been continued. The improved colorimetric methods for determining this enzyme (Rep. I) have resulted in a clarification of the nature of the enzyme adaptation illustrated by lactase. It has been well known that E. coli cells do not attack lactose (or various other sugars besides glucose) unless they have been previously exposed to the substrate for some period of time. The development of specific enzymatic capacities under these conditions has been called substrate-dependent enzymatic adaptation. The concept that adaptation is based upon an actual diversion of protein synthesis towards formation of the adaptive enzyme, under the influence of the substrate, is of fundamental importance in these studies, and has been more or less generally accepted, albeit on rather limited evidence. In fact, Deere et. al. reported, some ten to fifteen years ago, that <u>unadapted</u> cells could be activated by vacuum drying, antiseptics, or other treatments which altered the permeability of the cells, and concluded therefrom that adaptation involved only a change in cell permeability which allowed the substrate to gain access to the interior of the cell. Although the methods used by Deere were rather crude, his observations were not controverted, and posed an uncomfortable dilemma. Using our direct colorimetric method for lactase assay, we have been able to confirm Deere's observations. However, adapted cells are also activated in the same ratio as unadapted, so that it can be concluded that adaptation has involved a 50 - 100 fold increase in enzyme content. Unadapted cells ordinarily contain a residual amount of lactase, not readily detected by the older methods, amounting to 1 - 2% of the fully adapted cells. Activation by drying or autolysis causes a 20 - 30 fold augmentation of the apparent activity, which would bring unadapted cells to the same order of activity as the adapted, intact cells.

Unfortunately, certain rather mild treatments, such as standing in M/10 buffer, activate cells to a small, but variable extent. The resulting instability

of assay of assay of intact cells is a troublesome obstacle to kinetic and other studies on the enzyme in such cells, and might well be a factor in other studies of adaptive enzyme formation.

The concept of a complex genetic control of the adaptation mechanism was mentioned in the previous report, (Rep. I). This concept is supported by the behavior of another mutant recently studied. The lactose analogue, "neolactose," or D-altrose- -D-galactoside, is not attacked by wild type E. coli, when tested by routine bacteriological procedures. Apparently neolactose is not an effective stimulus for lactase adaptation, for cells which have been grown on a lactose medium will attack neolactose. After prolonged incubation on neolactose medium, wild type E. coli cells give rise to mutants which will form lactase on this medium, and therefore attack the neolactose. The mutants, however, have turned out to be "constitutive" lactase producers; that is, they will produce this ordinarily adaptive enzyme in large amounts whatever the medium on which they are grown. Their attack of neolactose is made possible not by a new adaptive response to this analogue, but by the fact that the adaptive mechanism has been dispensed with, or probably more accurately, has been triggered intrinsically by a genetic change. The mutation has a specific effect, and does not affect the adaptive responses to any other sugar so far tested including galactose. The interaction of this mutation with other genes affecting lactase formation is under study. The results show, however, that genetic effects may concern any of a variety of phases of enzyme formation, and that it is impossible with the help of present methods to specify a gene as the specificity-model or "template" for an enzyme. They also show that the substrate specificities for adaptation and of the enzyme produced may not be congruent, which would tend to rule out the hypothesis that adaptation is somehow mediated by the combination of the substrate with the small amounts of residual enzyme usually present in unadapted cells.

2. Cytogenetics of E. coli K-12.

The genetic life cycle of E. coli has revealed a number of complexities not yet thoroughly unravelled. At present, a mass of detailed information has been collected, which cannot well be presented until some generalizations have been deduced from it. The diploid phase seems to undergo three types of aberrant behavior: (1) elimination, so that the "diploids" become hemizygous or monogenic for certain blocks of genes, and (2) "double reduction," so that some blocks of genes, originally heterozygous, become homozygous, and (3) probably as a consequence of the eliminations, non-random segregations, viz., that alternative alleles do not occur with equal frequency among the segregants. Although the situation has not yet been clarified, it appears likely that the aberrations can be best interpreted in terms of a chromosomal system like that of higher forms.

Cytological studies have been initiated with the aim of comparing and distinguishing the nuclear morphology of haploid and diploid cells of E. coli. The nuclear components of these types of cells can be distinguished: the diploid cells generally show a more disperse, larger aggregate of nuclear material, containing a larger number of resolvable granules in each aggregate, but with the individual granules smaller than in the haploid. However, we are not yet able to interpret this consistant difference in appearance in terms of their genetic structure.

3. Genetic aspects of bactericide.

It has often been speculated, without proof, that the lethal effects of radiations and other chemical and physical agents on bacteria might have a genetic basis, such as induced lethal mutations. The diploid cultures of E. coli now at our disposal permit an examination of this question. A number of agents have been found which have profound genetic disturbances correlated with bactericidal action, but the disturbances are mostly in the form of haploidization, i.e., the removal of an entire gene set, rather than a lethal mutation (the equivalent of removal of a single gene). This would suggest that the lethal aspects of these agents are more parallel to the so-called physiological or karypathological effects of

radiations on nuclei of higher forms, than to the specific structural (side-?) effects on chromosomes which have engaged the major interest of geneticists. The nature of the haploidization has not yet been thoroughly analysed, but a tabulation of the reagents which do, and do not, resemble radiations in their bactericidal effects may be of interest to the general problem of bactericide. -

Haploidizing bactericides	Non-haploidizing bactericides
Ultraviolet light*	Heat
X-rays *	Methyl green
Nitrogen mustard *	Pyronine Y **
Formaldehyde *	Streptomycin
Hydrogen peroxide*	Iodine
Dimethyl sulfate	Iodoacetamide
Acetic anhydride	Ethyl carbamate**

* Well-established as mutagenic chemicals (for organisms other than bacteria).

**Claimed as possible mutagens for bacteria by other workers.

It may be noted that a common property of the agents listed as haploidizing is their potentiality for inducing substitutions on amino and other reactive groups of proteins or nucleic acids.

4. Selection of genetic recombinants with bacterial growth inhibitors.

In place of a nutritional selection of wild-type recombinants from a mixture of distinct biochemical mutants, it is feasible to select dually resistant recombinants of E. coli from a mixture of cultures each resistant to a distinct antibacterial compound, such as streptomycin and sodium azide. This technique is a useful auxiliary to the nutritional method for E. coli, and should make possible tests for recombination in organisms (like the pathogenic cocci) not readily amenable to nutritional methods.

PUBLICATIONS

The following publications pertain to this project, and have not yet been cited.

1. Aberrant heterozygotes in Escherichia coli. Proc. Nat. Acad. Sci. U.S., 35: 178-84 (1949).
2. Direct utilization of maltose by Escherichia coli. J. Biol. Chem. 179:921-34 (1949).
3. Bacterial variation. Ann. Rev. Microbiol. 3:1-22 (1949).
4. The selection of genetic recombinations with bacterial growth inhibitors. J. Bact. 59:211-215 (1950).

In preparation:

1. The -D-galactosidase of Escherichia coli K-12.
2. Genetic aspects of bactericide.
3. Physiology of the gene: genetic studies on bacteria

In press:

1. The isolation and characterization of biochemical mutants of bacteria. (Methods of Medical Research, Volume III, 1950).
2. Inheritance, Variation and Adaptation. Chapter in Bacterial Physiology. Wilson and Workman (Eds.).