Research in Microbial Genetics, 1947-1957
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A. Main lines of research.

1. BACTERIAL SEXUALITY. The discovery of sexual reproduction in Escherichia coli is the starting point of a direction of microbiological research, which has helped to restore the bacteria to a definite place in the scheme of terrestrial life. The mating process, originally inferred from cross-breeding experiments, has been observed under the microscope and consists of a cell-to-cell pairing or conjugation, during which genetic material passes from one partner to the other. An elaborate system of mating types controls which combinations are sexually compatible.

2. RECOMBINATION ANALYSIS. This system of cross-breeding allows a detailed analysis of the genetic control of specific physiological processes. For example, the ability to form an enzyme, lactase, is controlled by a considerable number of different genetic loci, some of which have been found to influence different aspects of enzyme synthesis. Some of these loci are found in very compact clusters and they interact in such a way as to indicate regional organization of the bacterial chromosome in relation to the physiological actions of the genes. Mutations for drug resistance are likewise found to relate to changes of specific loci.

3. LYSOGENICITY. It has been known for many years that certain bacterial strains might harbor viruses in a latent form, a relationship called 'lysogenicity'. Recombinational analysis of lysogenic strains of E. coli has shown the intimacy of the relationship between the provirus and the bacterial host, within whose chromosome it occupies a definite site. This finding has bolstered the speculation that a virus consists of a potentially autonomous segment of the host's chromosome--the generality of this statement can, of course, be questioned.

4. TRANSDUCTION. This is another mechanism of recombination, whereby small fragments of the heredity of one strain can be transmitted to another. In Salmonella, these fragments are carried by phage, being incorporated more or less adventitiously during the growth of the virus on the first host. In addition to its intrinsic interest as an unsuspected feature of virus-cell interactions, transduction also affords another tool for genetic analysis. In E. coli, another type of transduction has been found in which the fragment is directly linked to the provirus. The interaction between the input fragment and the chromosome of the recipient cells has been studied in detail, and found to parallel the events of crossing-over in hybrid zygotes which have received a full complement from both parents.

5. IMMUNOGENETICS OF SALMONELLA. The pattern of naturally occurring antigenic combinations in these bacteria has been a long-standing puzzle of considerable theoretical and epidemiological interest. By transductional techniques, it has been possible to reconstruct new types, and to explain the existing types as the recombinations of various antigenic factors. In addition, the phenomenon of phase variation has been clarified as an alternation in the local functional "state" of an antigen-determining gene. (This dimension of genic variation can be contrasted with mutational changes of specificity, and offers new grist for the model-building mills of theoretical embryology).

6. ABORTIVE TRANSDUCTION AND LINEAR INHERITANCE. Another consequ nce of transduction, besides the development of genetically stable recombinant types, is a clone in which the new trait is exhibited by only one or a few cells. When these cells divide, the trait is passed on to only <u>one</u> of the progeny, thus creating a single line of descent. This pattern of linear inheritance (which also has some developmental analogies in stem-cells) can be explained by the transmission of a non-replicating gene-product, or perhaps a damaged gene itself.

7. PHYSIOLOGICAL GENETICS OF GALACTOSE ENZYMES: POSITION EFFECT. Transduction in E. coli by the phage lambda uniquely concerns a group of genes affecting the metabolism of galactose. Kalckar and Kurahashi have shown that a number of these mutants are deficient either in a) the enzyme galactokinase, or b) the enzyme uridine-di-phosphate-glucose transferase. (These mutants have precisely the same biochemical defect as humans suffering from the hereditary disease, "galactosemia.") By transductional methods, we have found that the mutants within group a) show a cis-trans position effect, that is heterogenotic cells which have the constitution +-/-+ are unable to utilize galactose, while cells of the constitution ++/-- can. The same holds for the mutants in group b. However, although both groups of mutants are closely linked to each other, a mutant from a) does not show the position effect with a mutant from b). That is, the +-/-+ combination here will produce both enzymes. These results are explicit support for a unique, coherent functional segment of a chromosome concerned with a single enzyme. However, there are some apparent exceptions to the rule which may give still further insight into these relationships.

8. ORIGIN OF DRUG-RESISTANT STRAINS OF BACTERIA. There has been much controversy over this question, some authorities holding that antibacterial chemicals might react directly with the hereditary framework of the bacteria to produce resistant mutants; on the other hand, much indirect evidence pointed to the sporadic occurrence of such mutants, independently of the drug, whose function is simply to provoke the selective outgrowth of the rare resistant forms. By various technical innovations, it became possible to isolate resistant mutant clones without ever exposing them directly to the drug. That spontaneous mutation can account for at least some examples of drug resistance is therefore now beyond question, though there remains some discussion as to the possible operation of the alternative mechanism in a few

unsettled cases.

9. PROTOPLASTS, L-FORMS, AND THE MECHANISM OF ACTION OF PENICILLIN. Many puzzling observations (some quite ancient) on atypical growth forms of bacteria have been coordinated into a simple working hypothesis, based on observations on the action of penicillin on E. coli. The well-known fact that penicillin is lethal only to growing bacteria, which then lyse, has been the basis of the very useful 'penicillin-method' for the isolation of growth-factor dependent mutants. Recently, it was found that this lysis could be forestalled by maintaining the treated cells in a medium containing hypertonic levels of sucrose (or certain other solutes). Instead of lysing, the treated cells expand, and the intracellular protoplast sheds the limiting cell wall, becoming a spherical globule. These protoplasts remain viable so long as they are kept in the sucrose, and will regenerate walls to revert to normal bacillary shape if the penicillin is removed. If they are placed in water or ordinary dilute medium, however, the protoplasts lyse and, of course, lose their viability as a result. Therefore, the bactericidal action of penicillin may be explained as an inhibition of cell-wall synthesis, while protoplasmic synthesis continues until the wall has burst.

In penicillin-sucrose broth, the protoplasts increase in substance, but fail to multiply, simply becoming larger and larger spheres. In agar medium, however, the expanding protoplast is confined by the agar meshwork, and therefore forms blebs and processes at the points of least resistance. These blebs enlarge and eventually round up, the reiteration of the process giving a colony of protoplasts of various sizes: this is, in fact, the L-type growth of previous authors. As a rule, the protoplastic colonies resume bacillary form, even after many serial passages, when planted into medium without penicillin. However, from time to time, mutations have occurred which imposed a genetic block to some element of cell-wall synthesis, for example in the biosynthesis of diaminopimelic acid. These mutants correspond to the

genetically stable 'L-forms' of other workers. The subject has formerly been dominated by life-cyclic or adaptive, rather than mechanistic hypotheses.

These protoplasts have become useful objects of biochemical study, e.g. of protein and nucleic acid synthesis, in the hands of other workers. My own interest in the problem was motivated in part by the hope that wall-less protoplasts would be more receptive to the penetration of genetically active nucleic acids. This hope is still unfulfilled, but remains the subject of continued experiment.

Selected References

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C. As important a contribution as findings of fact is the training of graduate students and research fellows. Some of those, who have worked in the laboratory, and are now pursuing their own research programs in microbial genetics are:

Dr. N.D. Zinder Rockefeller Institute for Medical Research New York

Dr. M.L. Morse Dept. Biophysics, U. of Colo. Medical Center Denver

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