1952-1953

Application for research support to

the RESEARCH COMMITTEE OF THE UNIVERSITY OF WISCONSIN

submitted by Joshua Lederberg, Associate Professor of Genetics

January 12, 1952

TITLE AND REASONS FOR STUDY

Bacterial Genetics. One of the most important, but least known aspects of the biology of bacteria is their genetics. In two species so far well studied in this laboratory, Escherichia coli has given accumulated evidence for a sexual cycle resembling that of higher forms; more recently, the related Salmonella typhimurium has exhibited a mechanism of transfer of single or isolated genetic factors that is quite unique, though poorly understood. We can achieve adequate control over microorganisms, whether from a technological, agricultural, or medical point of view only when their genetics is understood, along with their biochemistry, physiology and morphology.

RESULTS OBTAINED TO DATE AND PROCEDURE for projected study.

This project is a continuing one, and it will not be possible to separate future procedures from past results. The accompanying progress report therefore also outlines the directions of work planned for the future.

A. Formal genetics of Escherichia coli. The analysis of formal genetics of Escherichia coli is being continued. Special emphasis is being given to discrepancies between experimental data and the theory of a linear chromosome as the basis of heredity in this bacterium (as it is in higher forms). Single cell studies on segregating diploids suggest that some discrepancies may not be inherent in the actual genetic ratios, but in differential growth of different types. This work is necessarily slow, and definite conclusions will not be available until it is completed.

A study of the genetic effects of radiations on diploid E. coli has been initiated. The most significant finding to date is that single cells treated with UV give progenies with a variety of effects: either the cell (probably the nucleus as well) is not a unit target, or the effects of radiations persist as a biological disturbance despite the apparent growth-recovery of the cell. These effects then later give rise to the variety of changes in different offspring.

The isolation of new strains of E. coli that can be crossed with existing fertile strains was a major subject this year. Earlier estimates were optimistic: of about 1500 strains tested, only 30, or 2% could be crossed. These 30 strains are being studied closely for (a) their linkage patterns, (b) the possibility of compatibility groups and (c) characters of interest for genetic study. So far as can be determined, the new strains resemble the original E. coli K-12 in their fundamental biology and life cycle. However, many of them are culturally and serologically distinct. We are now planning studies on the genetic basis of the natural (as opposed to laboratory-created) differences between the strains. The serological differences, in particular, permit of an immunogenetic analysis . For a variety of reasons, bacteria provide excellent experimental material to study the biological bases of gene-controlled antigenic differences such as are the foundation of blood-typing in man, cattle, and other mammals. In addition, although E. coli is "not pathogenic," the genetic basis of serotypes is of intrinsic interest to the student of infectious diseases. This program was initiated on RF51:430 until definitive support could be secured from the Rockefeller Foundation (effective January 1, 1952). Dr. P. D. Skaar has undertaken its immediate direction, and has completed preliminary experiments and preparation of antiseral reagents.

B. A new mechanism of genetic transmission in bacteria. Salmonella typhimurium (mouse-typhoid; enteric fever group) has been the subject of

genetic study in this laboratory since 1947. During the past year, a reasonably clear picture of its behavior has developed for the first time. The Salmonella bacteria were chosen for this study because they provide good experimental material for studies on virulence, because they are readily cultured in the laboratory and are related to the E. coli familiar to us, and because their serological characters (presumably under genic control) are important in public health bacteriology.

Mr. Norton Zinder undertook this problem in 1948, and is using the experimental data for his Ph.D. dissertation. The plan was to parallel the work with E. coli. Biochemical mutants were made in a large number of strains, and attempts were made to detect crossing by plating mixed cultures on a selective, minimal agar medium. The results obtained were very confusing, as illustrated in previous reports, until it was realized that the recombination mechanism here is quite different from that in E. coli. In E. Coli, all the evidence points to a typical sexual process, wherein recombination results from the fusion, and later segregation, of two intact nuclei. In Salmonella, under certain conditions, the cells release fragments of genetic material into the medium. These fragments may be either single "genes" or small aggregates, but much less than an entire nucleus. Other cells may absorb these fragments. The next steps we can only guess at, but an end result is that a "recombinant" cell is sometimes formed in which the absorbed fragment becomes a part of the genetic mechanism of its new host cell. To distinguish this process from fertilization, which is the union of two essentially equal genotypes, we have designated it as genetic transduction.

Transduction is an infectious process, if regarded in a certain light, and one may ask whether it does not merely depend on the transmission of a virus "disease" from one cell to another. However, a great many characters have been examined, and every one of them is transducible in the same way.

These include many different nutritional requirements, fermentation differences, resistance to streptomycin, and type-diagnostic antigens, the same groups of markers that are inherited as if carried on a chromosome in E. coli. The converse possibility, that genic transduction may throw light on the origin of viruses, should be given close consideration.

From the point of view of adaptive plasticity, transduction is not so efficient as fertilization. In this experiments, only a single factor is transducible to a cell at one time, so that "crosses" of cells differing in many factors result in only a small fraction of all the possible gene combinations.

The Salmonella group has been subjected to very close serological study, and a very large number of antigenic types or "species" are recognized in the diagnostic scheme. These types represent different combinations of somatic antigens (designated by roman numerals) and flagellar antigens (arabic numerals and lower case letters). For example, S. typhimurium is designated as IV, V XII; i; 1, 2, 3, while S. typhi is given as IX, XII; d--. Bacteriologists have often speculated on the evolution of the Salmonella group, and the origin of the different antigenic combinations, but owing to the lack of convincing precedents, recombination was not implicated. By transduction, however, a hybrid of S. typhi x typhimurium has been obtained, with the antigenic formula IX, XII; i--. This hybrid has not previously been described as a Salmonella type. If it had been isolated (and it might well be anticipated to occur in a patient suffering from a double infection) from a carrier or patient, it would certainly have been recognized as a new species. On this precedent, we may predict that Salmonella types have arisen, and will arise again, from the recombination of factors of previously established forms.

Transduction is so different from the hereditary patterns familiar to geneticists that it is difficult to reconcile it with the cytological

observations that suggest the presence of similar nuclear structures in E. coli and in Salmonella. We will have to learn a great deal more about genetic transduction before we can evaluate its significance for our concepts of the nature of the gene and its relationship to the cell.

C. Replica plating and indirect selection. A method has been developed which should be useful in any of a variety of microbiological screening programs, in which a large number of cultures must be tested in a variety of media. This method, replica plating, has already become indispensable in this laboratory for the isolation of nutritional mutants, and testing genetic segregates. In principle, replica-plating is a simple printing process. A sheet of velveteen is fastened to a wooden support. An agar plate carrying the colonies to be screened is then pressed down, transferring an imprint of each colony on the velveteen. Plates of various agar media can then be pressed on the same velvet, and each of them will be imprinted with a replica of the original growth. The advantage of the method is, of course, that upwards of 200 colonies on a plate can be transferred in one operation to a series of other plates with accurate registration of position.

A less routine application of replica plating has been made to the problem of the origin of drug-resistant mutants. Despite considerable evidence in favor of spontaneous mutations followed by biological selection, the question has continued to be mooted whether an antibiotic might not actually induce variations to resistance to it on the part of previously sensitive bacteria. So long as resistant mutants could not generally be detected or isolated without applying the antibiotic or rather unusual environmental conditions, only rather indirect and abstruse evidence could be brought to bear. It is now possible to isolate resistant mutants by indirect selection in which the cells are not exposed to the antibiotic. In principle, spontaneous mutations to resistance to, e.g. streptomycin

are located in a film of growth on plain agar by means of replicas to streptomycin agar. Since such mutations do appear to be inherited, they occur in clones, members of which remain on the plain agar plate after their sibs have been removed via velvet to the selective agar. By using the presumed sites of resistant clones for fresh inocula again to plain agar, a relative concentration of about 100-fold can be achieved for the resistant mutants. Appropriate repititions of the process (four or five times) eventually result in the separation of the mutant clones in well-isolated colonies from which pure cultures can be made. The enrichment line itself has never been exposed to the antibiotic; the sibs are used to locate the resistant mutants. In a sense, indirect selection parallels the use of pedigree data to select roosters or bulls for breeding stock for egg or milk production. The successful application of this method should remove any doubt that mutations to drug-resistance occur in a bacterial culture quite independently of the presence of the drug itself.

D. Genetic studies on actinomycetes. There are both practical and theoretical reasons for comparing the genetics of actinomycetes with that of the simpler bacteria. On the one hand, one might expect to find nuclear processes in a form more readily analyzable, and more closely resembling the filamentous fungi; on the other, the actinomycetes are now among the most important technical microorganisms. A study has been initiated in Streptomyces griseus, and related forms. A number of biochemical mutants have been prepared--these are quite similar to the mutants previously obtained with Neurospora and bacteria--with the help of replica plating. Combinations of different mutants on minimal agar show various effects. Many of the mutants exhibit syntrophism, or mutual feeding of needed metabolites, in an especially dramatic way, but this has been disadvantageous in tending to obscure

genetic interactions. Several combinations of mutants which show little or no syntrophism have given mycelia which continued to grow on minimal medium. The spores, however, give only one or the other parental culture. Although formal proof is so far lacking, there can be little question that we are dealing here with heterokaryons, that is an admixture of genetically different nuclei in a common cytoplasm. It seems likely that heterokaryosis accounts for the often cited observation that a actinomycete culture may remain constant when the mycelium is propagated, but throw many variants when spores are plated. Finally, in a few instances, stable propotrophic cultures have been noted, and these may represent recombinants resulting from a sexual process. If so, sexuality is rather sporadic within this one strain of S. griseus; it must be pointed out that other explanations have not yet been ruled out. A group of other Streptomyces cultures (S. venezuelae, S. coelicolor, S. lavendulae)is being studied in a comparative and combinatorial plan.

4. FINANCIAL SUPPORT requested:

Staff:

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|---|---|---|
| Assistant, 12-month, | Elise Cahn | 1,320 |
| " , 10-month, | To be named | 1,100 |
| " " " | " " " | 1,100 |
| Hourly help: | | 500 |
| | | 4,020 |

5. PERSONNEL:

Most of the present assistants plan to graduate (1 Ph.D., 2 M.S.) this June, and replacement candidates are under consideration. Elise Cahn already has an M.A. (Indiana) and came here last year with an undergraduate GPA 2.20 and two years additional experience. She has worked satisfactorily this past semester, and should ultimately qualify for the Ph.D.