APPLICATION FOR RESEARCH SUPPORT

To the Research Committee

Submitted by J. Lederberg, Department of Genetics

1950-1951

TITLE AND REASONS FOR STUDY

Bacterial Genetics. The mechanism of genetic recombination remains one of the crucial problems of bacterial genetics. The controlled hybridization of bacteria requires the most detailed information from genetic and cytological studies on Escherichia coli K-12, the bacterial strain in which gene recombination has been most clearly demonstrated.

PROCEDURE

A. Cytological and genetic comparisons of haploid and diploid cultures.

In a current project, exceptional diploid cultures of E. coli are being compared cytologically with the typical haploid strains. The diploids can be characterised as such by their genetic behavior, namely that single cells give rise, during subsequent fissions, to "segregant" cultures showing various combinations of the characters of the ultimate parents. Cytologically, the diploids show a more complex and disperse nuclear structure than the haploids. The observations may ultimately lead to the simplest, obvious interpretation: that the nuclear units, or "chromosomes" are relatively reduplicated in the diploid. However, these studies must be considerably extended before any conclusions can be confirmed.

The primary difficulty, of course, lies in the extremely small size of the bacteria nuclei, so that they can only barely be

resolved with the light microscope. To avoid subjective errors of interpretation, especially since there is considerable variation in nuclear morphology within haploid cultures, photomicrographic records should be used as far as possible. Although Dr. Ris, of the Department of Zoology, has cooperated very generously thusfar in making available his facilities, no suitable photomicrographic equipment is available in the Department of Genetics, where it should be located if any extensive use is to be made of it for this project. It is requested, therefore, that support for this project be continued with a grant for a) renewal of an assistantship for Miss B. R. Lively, who is currently working on the cytological problem, and b) microscopic and photomicrographic equipment.

B. Genetic mechanisms of bactericidal action.

The bactericidal action of radiations, and to some extent of other agents, has been accounted for by many workers as due to the induction of lethal mutations, like those which have been studied in Drosophila (cf. Lea, Actions of Radiations on Living Cells). Although uni-cellular organisms such as bacteria are especially suitable for study of lethal radiological effects, it has not been possible to rationalize the known genetic with the lethal effects of radiations, bedause haploid cells are directly affected by lethal mutations, and the killed cells are unavailable for further study. Lethal mutations in diploid organisms should, however, have no immediate effect if they are recessive in character, because the lethal mutation in one chromosome will be protected by the unmutated homologue in the other chromosome. However, lethal mutations can usually be detected by further genetic analysis.

Surprisingly, preliminary experiments with ultra-violet light

have indicated an entirely distinct mechanism of action. Even with highly lethal doses of UV, few or no recessive lethal mutations are found. Nor can the results be accounted for by so-called dominant lethal mutations. Instead, the killing appears to be accountable for by effects on individual chromosomes or nuclei as units; that is, diploid cells are made effectively haploid under the influence of UV. Killing is possibly the succeeding stage in the series diploid-haploid-death. Such a sequence would also account for the sigmoid type of dose-response curve found with UV.

It is hoped to continue these studies, to ascertain the units and kinetics of bactericidal action not only of UV but of ionizing radiations (X-rays, gamma rays), as well as of mutagenic chemicals (e.g., nitrogen mustard), other disinfectants, and heat. No special equipment is required for this study, but request is made for a second research assistant to work on this and other problems.

C. Other problems.

The detailed study of the genetic behavior, in crosses, of a number of different characters of E. coli K-12 is being continued. Until we have a clear picture of the genetic organization, in terms of linkage maps and the mechanism of segregation, our understanding of hybridization in this bacterium remains incomplete. There is strong evidence for linear linkage, like that of higher forms of life, but there are also anomalies not yet satisfactorily explained. This holds in particular for the segregation ratios of diploid heterozygotes, which, according to the simple model, should be 1: 1 for each character pair, but which is as peturbed as 13: 1 in some instances. The only approach to this perplexity is simply the sustained study of the linkage behavior of a great many characters in crosses.

The validity of other projects in progress, for example, the genetic control of bacterial enzymes, depends in part on the success of our efforts at rectifying the gene system.

A number of colleagues have felt that the scope of genetic recombination, and presumably of sexuality in bacteria, should not be limited to a single strain of a single species. Recombination is being looked for, therefore, in other cultures, and finally has been demonstrated in other cultures, first by L. Cavalli (Cambridge, England), and then again here. Advantage is being taken of the inter-crossability of these three bacteria (all E. coli) to study the genetic basis of natural varietal differences.

FINANCIAL SUPPORT

Microscopic and micrographic equipment	$ 550.
Research Assistants, 12 mo., [2] at 1150.	2300.
Hourly help	200.
TOTAL	3050.

PERSONNEL

Project Leader: Joshua Lederberg, Assistant Professor of Genetics.

Assistant : Ethelyn R. Lively, Graduate Student, began residence Sept. 1949; Is pursuing her research very satisfactorily

Assistant : To be selected from current applications.