December 1, 1951

Annual Progress Report and Proposal for Contract Extension
to
Atomic Energy Commission
Contract No. AT (11-1)-64 Project 10
CYTOGENETIC EFFECTS OF RADIATIONS ON BACTERIA

Department of Genetics, University of Wisconsin
Project Leader: Joshua Lederberg, Ph.D., Associate Professor of Genetics

PROGRESS REPORT

The general purpose of this investigation is to analyse the effects of radiations on diploid cells of bacteria (Escherichia coli). This is potentially favorable material for detecting various types of genetic and cytological changes which may be involved in radiation damage to cells. The present work was entirely preliminary, and designed to establish whether the study should be continued. The scale of the work to date has been extremely modest, expenditures under the contract having been confined essentially to part-payment of the salary of one graduate student research assistant during the past five months. Although much of this time was necessarily spent in adapting and learning of certain techniques, the project leader feels that the results point to the desirability of continuing the project on a modest scale.

For reasons of technical convenience, the preliminary experiments have been conducted principally with ultra-violet light. Entirely comparable results have been obtained with X-rays, and it may be pointed out that very marked effects have been detected with doses of 1000 roentgens, at which there is very little reduction in viability. It should be possible to detect genetic effects at dosages of the order of 100 roentgens. Although this is not an unusually small dose for experiments with higher organisms, most microbiological experiments have required doses measured in the tens or hundreds of kiloroentgens.

The most prominent effect of ultra-violet treatment of diploid cells is to induce segregation at a high rate. The result is that a treated cell gives rise to a colony which consists mostly of haploids, but usually retains a diploid residue at the center.

This is quite the contrary of what would be expected if recessive lethal mutations played a large part in radiation damage. In the diploid cell lethal changes would be masked in the heterozygous condition, but should prevent the development of haploid cells carrying the lethal. Thus, recessive lethals should effectively <u>prevent</u> segregation, whereas irradiation seems to <u>induce</u> segregation, even at very low doses. At first, the residual diploid component of colonies from treated cells was overlooked, and it was thought that the unit target of radiation damage could be defined as the haploid-chromosome-set (as has since been suggested by other workers). The picture is greatly complicated, however, by the fact that single treated cells appear to give diverse progeny: induced haploids, and residual diploids. It was then found that the residual diploids were also not all alike: many of them have undergone changes similar to those involved in somatic crossing-over in Drosophila. This is reflected in the occurrence of new diploid types that are now homozygous for some of the factors for which they were previously heterozygous. This type of "diploid

segregation" has been observed in untreated cultures, but only rarely, whereas a large fraction, perhaps the majority, of residual diploids are so affected.

The complexity of the genetic changes is paralleled by the cytological findings. Figures 1 and 2 show microcolonies from control and ultra-violet treated cells.

A good deal of radiation microbiology work so far carried out has assumed that single cells behave as units to radiation response: for example the effects of ultra-violet and X-rays in causing delay in growth have been interpreted on the basis of the progeny, taken as a whole, of single cells. Newcombe and Scott have pointed out the difficulties that may arise from failing to take into account the discrepancies in recovery times of different cells. These are multiplied by the finding that the early progeny of a single treated cell may then grow at widely different rates, and give different types of genetic change. Much of the complexity in the analysis of apparent delay in the mutagenic effects of radiations may result from this variable response.

The proper analysis of these complex genetic effects requires the separation of single treated cells under the microscope, followed by a pedigree or microcolony analysis. Only in this way can the distribution of effects in the progeny be separated from selective growth of different type of clones. The micromanipulatory technique necessary for these experiments has just been learned in this laboratory, and has been applied to microcolonies of untreated (control) diploids. Previous suspicions that selective growth of certain segregant types caused a serious perturbation of segregation ratios have been confirmed.

The discovery by Lwoff and Gutmann that radiations would activate latent intracellular viruses of bacteria has suggested another modality of radiation effects. As most of our diploid strains are lysogenic, the activation effect might have been suspect as the basis of the genetic and cytological changes. However, preliminary experiments with nonlysogenic lines have given the same picture of responses to ultraviolet light.

The genetic effects of ultraviolet light and X-rays are closely simulated by a variety of chemicals, especially hydrogen peroxide and formaldehyde. Of these, however, only the ultra-violet effect is subject to photo-reactivation with intense visible light.

PROPOSALS FOR FURTHER WORK

It is proposed to continue and develop the investigations initiated in this project. The single cell isolations with the micromanipulator will be continued to give a clearer picture of the sequence and kinds of genetic alterations following treatment with radiations. A further search for lethal mutations should be made; no evidence of their occurrence in this material has yet been found. The cytological and genetic effects of X-rays should be compared in detail with those of ultraviolet light, hydrogen peroxide, formaldehyde, and other mutagens. Finally, the responses of cells with and without latent viruses should be compared to determine under what conditions the activation of latent virus is important in bactericide, and to what extent this activation can be related to the other cytogenetic effects of the radiations.

PERSONNEL

Project Assistant: Ethelyn R. Lively, M.S. 1950. Has conducted cytological work, and more recently, single-cell isolation with micro-manipulator.

Project Assistant to be appointed: Melvin L. Morse, M.S., formerly Junior Biologist, Oak Ridge National Laboratory.

PROPOSED BUDGET
(March 1, 1952 to February 28, 1953)

Salary (M. L. Morse, 1/2-time) Project Assistant	$ 1320
Supplies, expendable (glassware, reagents)	500
Travel (to use facilities not available at this campus)	200
	2020
Overhead, at 8%	162
Total	$ 2182

The expected duration of the project, if continued, is to July 1954. Depending on the progress of the work, future requests may be increased to a level of $4-5000 per annum if a greater concentration of effort becomes advisable.

The principal contribution of the University of Wisconsin will be the salary of the Project Leader, and the facilities of a microbiological research laboratory. The University is conducting a program of research in the genetics of microorganisms at this laboratory, many phases of which interlock with radiation problems. The budget for the laboratory (including salaries) is approximately $25,000 per year. Approximately half these funds are secured from outside the University, principally from the Rockefeller Foundation, the National Institutes of Health, and the Chemical Corps. These funds are earmarked in very general terms for research in bacterial genetics.

RESPONSIBILITIES OF PROJECT LEADER

The statement previously filed still applies, except that the laboratory staff has increased to include four to five graduate research assistants, in place of three; two postdoctoral research associates in place of one, and a fulltime technical assistant. Approximately 15 percent of the Project Leader's time will be related directly to this project, although almost all of the bacterial genetic research conducted in this laboratory is more or less indirectly connected with it.