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CYTOGENETIC EFFECTS OF RADIATIONS ON BACTERIA

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This project was originally formulated to study the cytogenetic consequences of irradiation of haploid and diploid bacteria. During its early stages, we encountered an unexpected phenomenon which has appeared so fundamental to all of the studies in this laboratory that the original project has been diverted, for the time being. It is intended, ultimately, to return to the original problem but we do not at present have sufficient personnel or facilities to carry both. When an occasion suitable for resumption presents itself, it is intended to renew this proposal for research support from the AEC, but in the interval other support is being sought.

In the previous report it was mentioned that our test organism, the bacterium Escherichia coli strain K-12 was lysogenic, carrying a symbiotic bacteriophage, "lambda." The genetic relationships of lambda and strain K-12 have been the subject of other investigations from this laboratory (Lederberg and Lederberg, 1953). It had been discovered by Lwoff that one of the modalities of radiation-killing of various bacteria concerns the activation of symbiotic phages, resulting in the lysis of the host bacterium. In order to substantiate the analysis of similarities and differences in radiation responses of haploid and diploid bacteria it was essential to study the role of the "Lwoff effect": induction of the

latent phage. A preliminary study showed that, under certain conditions, the presence of symbiotic lambda did, in fact, potentiate the sensitivity of K-12 bacteria to ultraviolet light. A by-product of these investigations was the production in the induced lysates of high titers of lambda (over 10^{10} per ml) such as had not previously been attainable by more familiar methods.

Concurrent studies in this laboratory (Zinder and Lederberg, 1952) have shown that another bacterium, Salmonella typhimurium, displays a mechanism of genetic transfer quite distinct from the apparently sexual recombination process in E. coli K-12. In Salmonella, certain lines of bacteriophage are capable of transferring individual genetic determinants from one genetically marked strain to another. Repeated attempts have been made to demonstrate a similar transduction process in E. coli, either as an alternative explanation of, or as an auxiliary to the sexual mechanism. These experiments, involving a variety of cell-free preparations, extracts, and lysates had been consistently unsuccessful. The availability of high-titered stocks of lambda led, however, to a casual re-examination of the possibility of a transduction in E. Coli. The first stocks tested included a number which gave negative results, but also one, known to carry Gal- and Lac- (galactose and lactose-negative) mutations. When plated with lambda from induced lysates of lysogenic wild type bacteria, the Gal- Lac- stock was noticed to produce numerous papillae on EMB lactose agar. Further study showed that the papillae were, in fact Gal+ Lac- (cf. Lederberg, 1952), and subsequent experiments have been carried out on EMB galactose agar. Tests of several other mutant stocks of strain K-12 have uncovered no character other than galactose fermentation that is subject to transduction by lambda, thus sharply differentiating this system from transduction in Salmonella, which may involve any single trait, and from recombination in K-12 itself which typically involves

numerous linked factors, en bloc. Previous work had also shown that sexual recombination in E. coli K-12 was indifferent to the presence of lambda in either or both of the parents (Lederberg, Cavalli and Lederberg, 1952). Conversely, it has since been shown that the transduction of Gal+ proceeds readily between sexually incompatible strains (i.e., two cultures both F-).

To summarize, the transduction of the Gal+ factor is detected by the augmentation in the number of galactose-positive papillae evoked from various Gal- strains on EMB galactose agar. Unfortunately, most of the available Gal- cultures are capable of reverting spontaneously, so that control platings invariably show a number of papillae. The apparent augmentation by lambda was therefore scrutinized with some suspicion that a selective rather than transductive effect might be operating, and it was some time before the balance of the evidence weighed in favor of the latter. In a typical experiment, control platings might show 50 papillae per plate, while 10^9 added lambda particles would evoke 500 papillae. This effect was consistently observed in replications of the experiment, and disappeared if the lambda was previously boiled. It was observed whether the Gal- indicator culture was sensitive or resistant-lysogenic for lambda, but not when the indicator was immune, i.e., did not adsorb the phage. Finally, lambda secured from Gal- cultures lacked the effect.

These observations indicating that lambda could, in fact, transduce the Gal+ factor were supported by a further study of the induced papillae. Whereas the papillae arising by spontaneous reversion were invariably stable Gal+, most of the papillae on the experimental plates gave rise to cultures which were unstable Gal+, having a sectored appearance on EMB galactose, and continuing to split off Gal- types after repeated single-colony isolations. In a few instances stable Gal+ types have also been noted to arise from the unstable cultures, but the facts here

are still not well-established. It is also not clear whether all the transduction Gal+ are unstable. The number of initially stable Gal+ papillae on experimental plates is generally greater than on controls, so that it is likely that some of the transductions are initially stable, while others are unstable. In the absence of further evidence to confirm the numerical differences this point remains unsettled.

Further experiments have been designed to study the scope of transduction from a genetic viewpoint. Remarkably, there are at least three or four distinct Gal- mutations, at different loci ("Gal_1," "Gal_2" etc.), each subject to transduction, while no other marker has been so far. However, it is quite likely, from the results of crossing experiments, that these Gal factors form a fairly closely linked cluster, perhaps comparable to the "pseudoalleles" now so prominent in the genetics of higher forms. The different Gal- types can be distinguished in two ways: their interaction in transduction experiments, and their recombination to give Gal+ in crosses. The two criteria, after certain technical problems were cleared up, have given consistent results. This has enabled a study to be made of the apparent segregation process involved in the unstable Gal+ behavior. For example, Gal_2^- Gal_4^+ --x Gal_2^+ Gal_4^- has given unstable galactose-positives which split off predominantly Gal_4^-, but also some Gal_2^-. (The symbol --x denotes transduction by lambda from the left-hand to the right-hand term). It is concluded from this experiment that the unstable positives have a genotypic formula somewhat similar to:

$$\frac{Gal_2^+ \quad Gal_4^-}{\cdots \quad \cdots} \cdots$$
$$\cdots \quad Gal_2^- \quad Gal_4^+$$

in which the short lower line represents a small "chromosome" fragment transduced by lambda. An important question remaining to be settled is how such fragments come to be incorporated in the stable genotype of the cell, e.g., in the establishment of stable Gal_2^- "segregants." The same question applies to transduction in Salmonella, but we do not have the advantages there of the unstable intermediate state, nor of analysis by sexual recombination.

Proof that the transducing element in lysates is the phage itself is afforded by the correlation between ability to adsorb lambda, and susceptibility to transduction among various indicator stocks and similar rates of absorption of the two activities on indicator bacteria. In addition, there is a close correlation between the lysogenization of sensitive and the transduction of Gal+ to Gal- cells. However, only about 1 phage particle per million capable of lysogenizing also transduces Gal+. Current studies are in progress on a system showing a much higher efficiency.

Preliminary studies have shown an anomalous effect of ultra-violet light on the transducing efficiency of lambda. Small doses, which reduce the plaque-forming titre of a lambda preparation by no more than 40% potentiate the transducing activity by 10-fold. Higher doses will then ultimately reduce transduction, but not so rapidly as infectivity. No explanation for this behavior is offered at present.

The experiments summarized in this report are principally the work of Mr. M. L. Morse, in collaboration with E. M. Lederberg and the undersigned.

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