REACTIVATION OF ULTRAVIOLET-IRRADIATED BACTERIOPHAGE BY MULTIPLE INFECTION

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For a number of years, we have employed radiation in our laboratory as a microsurgical tool to analyze properties and growth of bacteriophages. In general, radiation appears to suppress reproductive ability of phage at a faster rate than other phage properties, a fact utilized in dissociating the latter properties from the former one. Also, radiation was used to follow the phage in the course of its intracellular growth (Luria and Latarjet, '47). In all this work, loss of reproductive ability ("inactivation") was considered as an irreversible change affecting the phage particle as a whole. What is observed, however, is actually the loss of ability to initiate production of active phage *under given conditions.* Results obtained in our laboratory in the past two years indicate that these conditions have to be defined more precisely before the term "inactivation" can be used in a meaningful sense. Moreover, it may be possible to localize the changes involved in inactivation more precisely than in the virus particle as a whole. The new finding is that active virus can be produced from particles that would ordinarily be considerd inactive in a number of clearly definable situations.

The first case to be discussed in this paper is that of multiplicity reactivation (Luria, '47); photoreactivation will be discussed by Dulbecco. Ultraviolet-irradiated coli phages (T2, T4, T5, T6) are still adsorbed by sensitive bacteria at approximately normal rate (irradiated phage T1 is less well adsorbed). The active titer, measured by plaque count after

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mixing with a very large excess of bacteria (single infection) diminishes logarithmically with the dose. The irradiated particles can then be divided into two classes: (a) those that give rise to production of active phage following single infection (active particles); (b) those that upon single infection do not give rise to active phage (inactive particles). It was found that, for all the phages listed, active phage may be produced by bacteria infected by more than one of the "inactive" particles. Active phage of a given type, for example T2, can also be produced if one "inactive" particle is taken up by a bacterium along with an active particle of a related phage (T4 or T6), not of an unrelated phage.

The phages T2, T4, and T6 are those for which recombination of genetic characters upon mixed infection have been demonstrated (Delbrück and Bailey, '46; Hershey and Rotman, '48, '49). These recombinations can most easily be interpreted in terms of recombination of discrete material determinants. An appealing working hypothesis to explain reactivation was, then, that active phage was produced from inactive particles by genetic recombination. The "inactive" particles would be those in which radiation had inactivated certain determinants. This would be those replaceable by homologous active determinants ("units") supplied by other particles infecting the same bacterium.

A number of simplifying assumptions permitted quantitation of this hypothesis in such a way as to yield predictions on how the probability of active phage formation in a bacterium depends on the number of infecting inactive particles and on the dose of ultraviolet light they had received ("simple theory"). The assumptions consisted mainly in postulating (a) a fixed number of damageable and replaceable units per phage particle, (b) identification of the average number of damaged units per particle with the natural logarithm of the survival ratio (number of "hits"), and (c) a minimum requirement for active phage production of at least one undamaged copy of each unit among the infecting particles

taken as a group. An additional nonessential assumption is that of equal ultraviolet sensitivity for all units.

These assumptions led to an expression of the probability of active phage production that could be tabulated numerically and plotted in curves. A number of such curves (Luria and Dulbecco, '49) are shown and compared with the experimental values of the frequency of active phage production. The general trends of theoretical and experimental values are similar. There is, however, a systematic deviation, in that the experimental frequency lags behind the theoretical probability as the number of infecting particles increases. This might be interpreted as suggesting a low degree of cooperation among several particles in the same bacterium. The striking fact is that the frequency of reactivation per multiple infected bacterium still remains near unity with particles that have received a fairly large number of "hits." If the working hypothesis of localized damage and recombination of undamaged units is correct, then the high frequency of reactivation can only be interpreted as the result of an extremely efficient mechanism of recombination, which can bring together all undamaged units.

As one such mechanism, I suggested ('47) independent replication of the active units followed by reassembly of active particles. This hypothesis agrees with the accumulating evidence for an early period after phage infection, in which active particles are either not present or at least not recoverable from the infected bacterium (Doermann, '48). No evidence is available for replication of parts of inactive particles in bacteria, in which reactivation does not take place. That deep changes take place in these bacteria, however, is shown by interference phenomena, suppression of bacterial growth, and cytological changes (Luria, Human, and Robinow, unpublished).

It may be added that the frequency of reactivation as a function of the dose of radiation is characteristic for each phage. From the "simple theory" we can calculate a minimum number of discrete, damageable, and transferable units

per particle (defined according to our assumption). These numbers vary from 25 to 30 for T2 and T6 to around 15 for T4, possibly 10 for T5 and less than 4 or 5 for T1. It must be understood clearly that these values are meaningful only if the hypotheses of the simple theory are justified, which remains to be confirmed.

Since the whole theory hinges on the concept of damage in discrete units of genetic material, one attempt at confirmation consisted of experiments using genetically marked strains of phage T2 (Dulbecco and Luria, to be published). Bacteria were infected with one particle of an active strain and one particle of an irradiated strain, the two strains differing in a pair of alternative characters. Each bacterium thus infected should liberate a mixture of the two infecting types. Failure to liberate the type corresponding to the irradiated parent could indicate inactivation and loss of the locus corresponding to the marker character. The proportion of bacteria liberating a mixture of the two types was determined and a loss was found for several loci. The frequency with which a locus was recovered decreased more or less logarithmically with increasing doses of radiation.

Some questions on the interpretation of these results were raised, however, by experiments in which the yields from individual bacteria infected with one active and one irradiated particle differing in one pair of characters was analyzed. It was found that the type corresponding to the irradiated parent, when present, constitutes a small minority of the yield from a mixed-infected bacterium. This finding contradicts the hypothesis of complete independence of replication and reassembly of the hypothetical units; units derived from the active parent appear to be favored. It also casts doubt on the interpretation of the "loss" of a locus in these experiments as resulting from its specific inactivation, since a locus might be missing from the yield without necessarily being directly damaged.

The lack of certain proof for a localization of the ultraviolet-produced damage in any specific genetic determinant

of the phage particle leaves the interpretation of the phenomenon of multiplicity reactivation open. The occurrence of photoreactivation, discussed by Dulbecco, without contradicting the hypothesis of localized damage in discrete determinants, suggested the need for caution in interpreting multiplicity reactivation, since physiological mechanisms of repair may be involved. In an attempt to ascertain the role of genetic recombination in multiplicity reactivation, a group of experiments was done by Dulbecco using mixed infection with one irradiated and one active particle differing by two genetic characters. The mixed yields from such bacteria always contained more recombinant types than would be expected if the irradiated particle had to be repaired as a whole before taking part in recombination. This indicates that recombination actually is a primary factor in reactivation; there is a greater recovery of parts of an irradiated particle than of the particle as a whole.

[Evidence obtained while this paper was in press (Dulbecco, '52) has made the hypothesis of reactivation by genetic recombination inadequate and has led to its abandonment.]

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