# INDUCTION OF h-MUTATIONS IN THE EXTRACELLULAR PHAGE T2 BY γ-IRRADIATION

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The extracellular induction of mutations in viruses and phages by chemical agents has been reported by a number of authors (Loveless, 1958; Gierer and Mundry, 1958; Tessman, 1959), using TMV and phages T2, T4 and ϕX 174. The induction of mutations in bacteriophages treated in vitro with ultraviolet irradiation has also been demonstrated. Tessman (1956) observed a 2.5-fold increase of mutants of phage T1 upon UV irradiation. This effect might have been due to sampling errors and the author did not consider it as statistically very significant. Krieg (1958, 1959) obtained reverse mutations from rII to $r^+$ in the T4 bacteriophage. In Krieg's experiments, however, the multiplicity of infection was higher than one particle for a bacterial cell, so that some complications might have been present. Mutations induced by ultraviolet light in free bacteriophages were later observed with several bacteriophages (Kaplan et al., 1961 ; Krivisky, 1959 ; Folsome, 1961, 1962).

Failure to produce mutations in the free phage T2 by UV and ionizing radiation was reported (Krivisky, 1961). In this phage, two types of host-range mutations of this phage are known, h and ht, whose determinants have been mapped in the phage genetic map. Both these mutations cause changes in the structure of the proteins of the receptor-apparatus of the tails (Franklin, 1961). In this paper we report experiments on induction of host-range mutations in extracellular phage T2 by γ-rays.

## MATERIALS AND METHODS.

Escherichia coli B, sensitive to T2 and T2h, from S. Benzer, and E. coli B/2, resistant to T2, but lysed by T2h, from V. Chuboukoff of this laboratory were used. The experiments were divided into three series. In the first series the medium was prepared with beef-peptone-broth with 100 mg% of amino-nitrogen. In the second series the medium was prepared with Hottingers hydrolysate (130 mg% of amino-nitrogen). The pH was adjusted to 7.0. In the third series of experiments we used the medium of Hershey and Rotman (1949). In the first and the second series of experiments both strains of E. coli were grown on agar slants for 24 hours. Each slant was then washed with 5 ml of broth. This suspension contained about $2 \times 10^9$ bacteria/ml. In the third series of experiments we worked with a 4-hr culture of bacteria grown in broth with shaking. The concentration was the same as stated above. We employed a phage concentrate containing $3 \times 10^{13}$ particles/ml. The suspension used for irradiation contained $10^9$ - $10^{10}$ particles/ml. To eliminate indirect effects of $\gamma$-irradiation the mixtures were irradiated in broth. As the source of irradiation $Co^{60}$ was used. The Gratia technique was used for plating. The surviving phages were measured by counting the number of plaques formed on E. coli B. The number of mutants was determined in the following manner. Mixtures of phage and E. coli B were incubated in a water bath at 37°C for 5 minutes to allow adsorption. One ml samples of the adsorption mixtures were added to 2.5 ml of molten agar (0.7 %) at 47°C, containing 0.2 ml of E. coli B/2. These samples were then poured on agar plates and incubated for 8-10 hours. The mutant plaques were counted and individual plaques of h-mutants were stabbed by sterile Pasteur pipettes, transferred to 3 ml of broth, and diluted. Similar aliquots of phage (1 ml) from the dilution were seeded on E. coli B and on B/2. Counts were made after 18 hr. Incubation at 37°C.

## R E S U L T S .

In all the experiments the number of mutants was higher than the spontaneous background. Two types of plaques were revealed after plating on E. coli B/2 : round plaques with a clear center and turbid halo (h-mutants) and plaques of considerably smaller size, rounded, turbid, without a halo, sometimes in the form of stars (ht-mutants). It is known (Hershey and Davidson, 1951) that the efficiency of plating of h-mutants on E. coli B and E. coli B/2 is similar. The mutants ht form plaques 100 times more frequently on E. coli B than on B/2. From some thousand mutants, we selected about 30 h and 10 ht mutants for investigation of plating efficiency. Statistically treated results of our experiments indicate an efficiency of plating of the h-mutants about 15 times higher than the efficiency of plating of the ht-mutants ($0.087 \pm 0.015$ for h-mutants and $0.005 \pm 0.003$ for the ht-mutants). But the efficiency of plating of h-mutants on E. coli B and B/2 in our experiments was considerably less than reported by Hershey and Davidson (1951). Passages of h and ht mutants from one plaque to the other demonstrated their stability. All data in this

paper relate only to the h-mutant type.

In the region of the dose curve from the beginning to a dose 140 kr there is a linear dependence between the dose and the number of mutants. The survival curve in these dose ranges is exponential. Statistical treatment of the results of our investigations revealed the significance of the differences between the frequency of mutants and in the experimental and the control samples for all doses of irradiation. The significance levels were determined. The differences for 20 kr were significant at a confidence level of 0.01, for the other doses at 0.001. The data on survival and mutability (relative frequency of mutants) are summarized in Table I.

TABLE I

| Dose (Kr) | survival per cent survival | mutability frequency of mutants ($\times 10^{-9}$) | Increase of mutants |
|---|---|---|---|
| 1 | 2 | 3 | 4 |
| Control | 100 | $14.0 \pm 3.0$ | |
| 20 | $65.08 \pm 2.33$ | $31.7 \pm 8.1$ | $2.3 \pm 1.0$ |
| 60 | $34.81 \pm 1.58$ | $57.9 \pm 12.6$ | $4.1 \pm 1.8$ |
| 100 | $18.18 \pm 1.10$ | $92.4 \pm 15.3$ | $6.6 \pm 2.5$ |
| 140 | $9.27 \pm 0.62$ | $115.4 \pm 26.7$ | $8.2 \pm 3.5$ |
| 200 | $1.0 \pm 0.08$ | $189.1 \pm 60.2$ | $13.5 \pm 7.1$ |

The numbers of mutants in relation to the survival and the standard error are presented in the third column. In the fourth column we have presented values, indicating the factors by which experimental mutability is higher than the mutability of controls. The errors in this colum were computed by the equation (Iveronova, 1953) :

$$\frac{\bar{X}_1 \pm \Delta X_1}{\bar{X}_2 \pm \Delta X_2} = \frac{\bar{X}_1}{\bar{X}_2} \pm \frac{\bar{X}_1}{\bar{X}_2}\left(\frac{\Delta X_1}{\bar{X}_1} + \frac{\Delta X_2}{\bar{X}_2}\right)$$

where $\Delta X_1$ is the standard error in the numerator ; $\Delta X_2$ is the standard error in the denominator.

## DISCUSSION.

Phages and viruses are convenient models for radiogenetical investigations. Geneticists and biophysicists are attracted by the simplicity of the molecular organization of bacteriophages. The phages are not metabolizing extracellulary and form large populations ; it is possible to investigate very rare mutations. The study of mutations induced in vitro may help to understand the primary mechanisms of injury of the genetical apparatus and the significance of DNA replication for mutation production. The h-mutants of phage T2 are localized in a very small site of the chromosome between the loci $ht_2$ and $i_1$. It is known that h-mutants change the phage tail proteins and thereby the host range. The frequency of spontaneous mutations at this locus is very low ($10^{-8}$ $10^{-9}$). Thus, a small increase over the spontaneous background can be demonstrated. The increases observed in our experiments cannot be due to selection of preformed mutants, as the absolute number of mutant plaques counted in the experimental samples was often higher than the number of mutant plaques in the controls. The effects observed must be the result of true induction of mutations under conditions that permitted the direct action of radiation.

In our experiment the efficiency of plating of the h mutants was nearly 10 times lower than in experiments of other authors. This may reflect differences in the properties of the cultures of E. coli B/2. The ratio of efficiency of plating of h and ht mutants was similar in our experiments to that found by other investigations (0.1 - 0.01).

As induction of mutation on resting phages is demonstrated for more and more phages and a greater variety of characters, it seems likely that all types of mutations may be obtained by irradiation of phage particles. Experiments are in progress on the induction of mutations in phage T2 by UV-irradiation.

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