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Mutagenesis with light and proflavine in phage T4

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It is known that the absorption by phage particles of a variety of dyes such as methylene blue, toluidine blue and proflavine renders them sensitive to inactivation by visible light (Welsh & Adams, 1954; Yamamoto, 1958; Kaufman & Hiatt, 1959). This process is called photodynamic inactivation. We have carried out experiments with phage T4 which has been sensitized by proflavine in order to test whether irradiation with visible light, as well as causing inactivation, also induces mutation. The mutation studied was the plaque-type change from wild-type (r^+) to rapid-lysis (r). The frequency of r mutants was measured amongst the survivors of photodynamic inactivation and compared with the frequency arising spontaneously and also after treatment with light and proflavine separately.

From Table 1 it can be seen that the irradiation of phage in the absence of proflavine has significantly increased the mutation frequency, although it has caused no inactivation. Nothing is known about the origin of these mutations, although it is possible that they arise from an indirect effect of the light on a minor component in the medium.

Table 1. *Frequency of* r *plaques in an* r^+ *stock after various treatments*

	Arising spontaneously	Light control	Proflavine control	Light and proflavine
Number of r plaques	1	22	24	127
Total number of plaques	10,500	29,500	28,000	42,000
Percentage of r mutants	0·01	0·1	0·1	0·4

Legend: Phages were pre-treated in darkness with 25 μg./ml. proflavine-hemisulphate (British Drug Houses). After 60 min. the phage-dye complexes were diluted 100-fold in buffer to give 10^8 particles/ml. and 5 ml. samples were irradiated for 5 min. in closed petri dishes. The survivors, about 1%, were plated on nutrient agar seeded with *Escherichia coli* strain B. An Hanovia Alpine 10 sun lamp emitting about 50% of its radiation between 3000 Å and 4500 Å was used as the light source. U.V. and heat rays were removed by passing the light through a glass dish containing 5% copper sulphate. All experiments were performed in a darkened room.

Phage particles which have been treated with proflavine and then assayed in the dark also contain a higher frequency of r mutation, again in the absence of any inactivation. Probably this effect is due to the fact that when phages are assayed soon after proflavine treatment, as in these experiments, they inject proflavine into the host bacteria along with their DNA. (We have been able to show that after dilution it takes at least one hour before the proflavine escapes from the head of phage T4.)

The situation in these infected bacteria is then somewhat analogous to that obtained by growing phage-infected cells in the presence of proflavine, which is well-known to induce mutations (Brenner, Benzer & Barnett, 1958).

After photodynamic inactivation the frequency of mutation is approximately twice that which can be accounted for by the controls, showing this treatment does exert its own mutagenic action. In order to obtain some information about the nature of these photodynamic mutants an attempt was made to determine whether they were of the base-analogue type (Freese, 1959) or of the alternative class which is induced by growth in acridines. Twelve *r*II mutants (a sub-group of the *r* mutations which has been extensively studied by Benzer (1955)) were isolated from the survivors of photodynamic inactivation. All of these were found to revert spontaneously.

The reversion rates of these mutants were then measured after growth in the presence of bromodeoxyuridine (BD) and after growth in proflavine.

The results of this test showed that five mutants were induced to revert by BD (base-analogue type), four by acridine (acridine type) and three by neither treatment. As no mutagen is known which can induce both types of mutation, the most likely explanation of this result is that the acridine type mutations are those detected in the control where proflavine was used alone. If this is the case then the photodynamic mutations would be of the base-analogue type. The three mutants which are not reverted by BD are probably of the base-analogue type, since Benzer & Champe (1962) have shown that BD will only revert mutants in which the mutant base pair is guanine-cytosine. This result would agree with work of Simon & Van Vunakis (1962) who have reported that the photodynamic inactivation of free DNA bases in the presence of methylene blue results in the selective destruction of guanine and to a smaller extent thymine. If the photodynamic action of proflavine occurs in the same way, then the removal of guanine would allow replacement by another base and so result in a base-analogue type of mutation.

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