MUTATIONS INDUCED BY ULTRAVIOLET LIGHT WITHOUT ATTENDANT LETHALITY

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Procedures have been developed in this laboratory which allow the quantitation of the mutagenic effects of ultraviolet light on bacterial populations, unobscured by concomitant lethal effects. For example, large numbers of mutations can be induced in *Escherichia coli* strain B/r at the locus controlling high levels of streptomycin resistance by doses of ultraviolet (UV) so small that there is no measurable decrease in the number of viable cells following exposure to the radiation. With these procedures direct and sensitive measurements can be made of induced mutational events, and the subsequent fate of the mutants in the population can be followed. The estimation of the extent of photoreversal of the mutagenic effects is not complicated by photoreactivation. Other pre- and post-irradiation treatments that influence mutations are more readily analyzed from data where no corrections must be made for the effects of "dead cells." The methods appear to be especially valuable for securing information on the vexing problem of delayed phenotypic expression. In this paper we have attempted to delineate several applications of the new procedure for securing data on mutations and the mutagenic process.

MATERIALS AND METHODS

Brain Heart Infusion medium (BHI) (Difco) was employed in all phases of the following investigation, since it has been shown to have a beneficial effect on the recovery of induced streptomycin resistant types (Rubin, 1954). Dihydrostreptomycin sulfate (E. R. Squibb and Sons) was employed in a concentration of 1 mg per ml of medium. Soft agar (0.75 per cent) was used to support membrane filters.[1]

Preparation of replicate log phase bacterial populations positionally fixed on membrane filters. BHI broth cultures of *E. coli* strain B/r were placed on a reciprocal shaker and incubated at 35 C. When the culture had grown to 2×10^8 cells per ml, as indicated by turbidity measurements, it was placed in a refrigerator for 30 min to arrest cell division. One ml aliquots of the cold culture were impinged on membrane filters and the latter were transferred to refrigerated soft BHI plates by means of sterile forceps. The refrigeration technique was adopted since the preparation of each membrane required 1 min, and from 20 to 50 membranes were needed in each experiment. Preliminary experiments showed that comparatively few mutations could be induced by noncidal doses of UV in populations consisting of cells taken from the stationary phase of growth; only log phase cells were employed in these studies. The number of mutations induced by a given noncidal dose of UV increased with the population size; 2×10^8 cells per membrane was selected since the approximately 100 mutational events induced by the noncidal UV doses was a convenient number for counting.

Ultraviolet irradiation and post irradiation procedures. A 15 watt G.E. germicidal bulb served as the UV source. At 15 in, the output between 2450 and 2800 A was 4 ergs per mm^2 per sec, as measured by a Hanovia Ultraviolet Meter. The populations were irradiated by placing the cold plates under the UV lamp and removing the petri dish lids for the desired length of time. Growth was initiated by transferring the membranes to prewarmed soft BHI plates. After varying periods of incubation at 37 C, the membranes were transferred to soft BHI plates containing streptomycin and incubated until resistant clones could be scored. Nonirradiated control populations were incubated for the same lengths of time to obtain the number of spontaneous mutants. Population sizes could be determined at any given time since the growth could be removed quantitatively by shaking a replicate membrane in a 125-ml Erlenmeyer flask containing 20 ml of suitable

[1] 50 mm "Bac-T-Flex" filters obtained from the Carl Schleicher and Schuell Co., Keene, N. H.

diluting fluid. Viable plate counts were made of appropriate dilutions of the resulting suspension.

Photoreversal procedures. The light source consisted of a battery of four 30 watt fluorescent bulbs mounted 7 in from the table top. Populations were prepared and exposed to UV in the usual manner. Five min illumination with white light on cold plates maximally reversed the mutagenic effects of an exposure to 4 ergs per mm² of UV. The acquisition of photostability was measured by applying the photoreversal technique to replicate populations which had been maintained at 37 C on BHI plates for varying intervals of time following UV treatment.

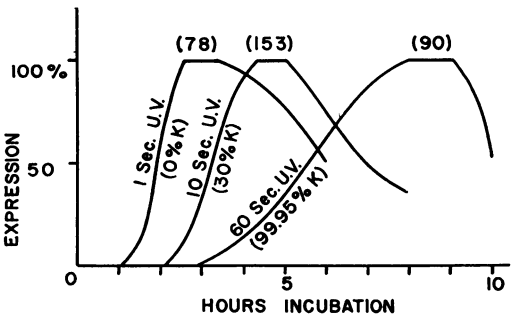

Figure 1. Dose dependence of the patterns for delayed expression of mutants. (Figures in parentheses give actual maximum numbers of mutants appearing at the indicated radiation dose.)

RESULTS AND DISCUSSION

The results of a typical experiment showing the mutagenic effect of the noncidal dose (4 ergs per mm²) are presented by the first curve in figure 1. As is customary in experiments involving high amounts of UV, no streptomycin resistant mutants beyond the spontaneous level appear unless the organisms are allowed to undergo a period of growth prior to screening. The number of induced mutants which become phenotypically expressed increases sharply on incubation, with a maximum (in this case 78) being noted after 2½ hr. Viable counts made at the beginning and end of this interval indicated that the bulk of the population had undergone 4 generations of growth. Incubation beyond 2½ to 3 hr resulted in a decline in the number of recoverable mutants, possibly due to population effects as described by Newcombe (1953).

The second curve in figure 1 describes the pattern of delayed appearance of mutants induced with 10 sec of UV (40 ergs per mm²). This dose killed 30 per cent of the bacterial population, and a maximal number of induced mutants (153) were phenotypically expressed after 4 to 5 hr incubation.

The third curve in figure 1 describes the pattern of delayed appearance of mutants induced with 60 sec of UV (240 ergs per mm²). In this case, 99.95 per cent of the population was inactivated and 8 hr incubation was required to complete the delayed expression of the mutants. Viable counts made immediately after irradiation and at the end of 8 hr incubation indicated that the bulk of the population had undergone some 19 generations of growth.

For this experiment nonirradiated, but otherwise identical, control populations gave rise to 2 resistant clones after 2½ hr incubation and 5 following 4 hr. The number of spontaneous mutants was negligible when compared with the total number of mutant clones arising in the irradiated populations; however, to give the actual induction effect these can be subtracted from the total mutant score. The low spontaneous mutation rate of this locus (Matney, 1955) greatly facilitates the quantitation of the mutagenic effect.

Comparison of the three patterns of delayed appearance in figure 1 indicates a marked dose dependency. Such a finding indicates that the average number of cell divisions made by an irradiated population before maximal expression is achieved may not be a reliable measure to characterize a mutational event. Newcombe (1953) has suggested a correlation between the ultraviolet dose and the extent of the delay before phenotypic expression of T1 resistance. The data of Demerec and Cahn (1953) do not indicate such a relationship for the expression of certain auxotrophic mutants.

There is little doubt that the largest number of a given type of mutation is induced in bacterial populations by doses of UV which also cause extensive killing. However, from an investigative point of view, greater precision can be obtained under conditions which measure only one UV effect (mutagenic) rather than two effects simultaneously (mutagenic and lethal).

The membrane filters did not contribute to the mutagenic effect of the ultraviolet light. The same results were obtained when *E. coli* strain B/r was grown and irradiated in M-9 minimal

broth, with subsequent incubation and screening being done with BHI layer plates. Comparable numbers of high level streptomycin resistant mutants were induced by noncidal doses of UV, and mutants induced by larger amounts of UV required longer incubation periods for maximal expression. The membrane filters provided an even surface for irradiation, as well as a convenient vehicle for the transport of positionally fixed populations from one environment to another.

The streptomycin resistant clones that developed on the membrane filters were readily separable into large and small colony types as shown in figure 2. The small type was found to be either partially or completely streptomycin dependent, whereas the large type was independent of the antibiotic for further growth. With the systems used here, conditions have not been found that consistently favor the induction of one type over the other. In figure 1 the results include both types of mutants, since in positionally fixed populations any failure of the dependent types to grow on the streptomycin-free medium after phenotypic expression does not affect the mutant assay.

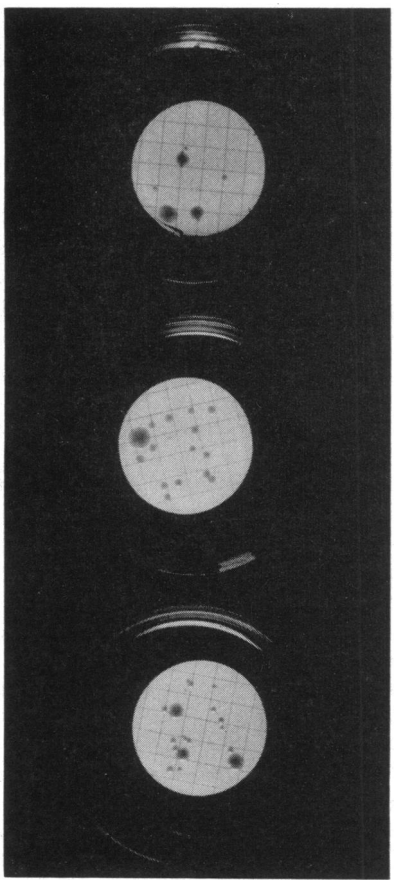

Figure 2. Streptomycin resistant and dependent colonies on membrane filters.

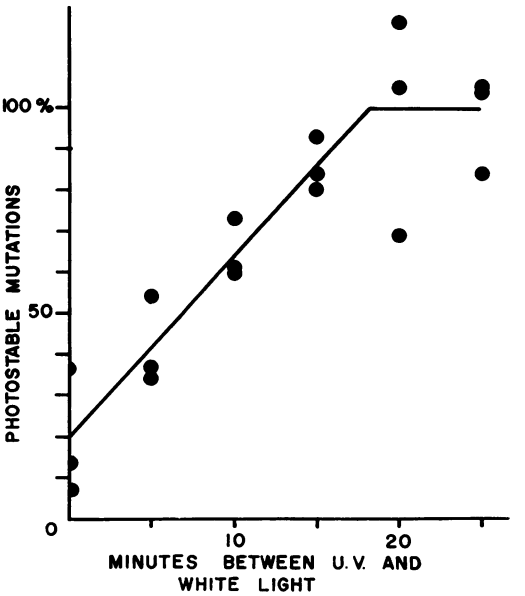

Figure 3. Acquisition of photostability in mutants induced by noncidal doses of UV.

Figure 3 presents results which indicate that 80 per cent of the mutational events initiated by noncidal doses of UV can be photoreversed, providing that the populations are exposed to white light immediately after the ultraviolet treatment. Photoreversibility was lost rapidly if the ultraviolet irradiated populations were incubated at 37 C for short periods of time prior to the exposure to white light. The establishment of complete photostability required some 20 min and suggests a gene change of permanent nature (Newcombe, 1955). If we accept this period as the mutational lag, most mutations become irreversible early in the first post-UV division period. These data illustrate the inherent advantage of the noncidal UV dose, in that the estimation of the extent of photoreversal of the mutagenic action is not complicated by photoreactivation of a "dead" fraction of the bacterial population.

Noncidal doses of UV have also been used to induce high level streptomycin resistance in *E.*

coli strains K-12 and W, as well as in *Bacillus cereus*. T2 phage resistance has been induced in *E. coli* strain B/r by noncidal doses of UV, but the higher spontaneous mutation rate of the locus involved hinders accurate quantitation of the mutagenic effect. The small UV dose (4 ergs per mm^2) used with the above strains was found to kill 99 per cent of *E. coli* strain B populations.

SUMMARY

Mutations may be induced in bacterial populations by doses of ultraviolet light so small that there is no measurable decrease in the number of viable cells following irradiation.

Mutations to streptomycin resistance induced by noncidal doses of ultraviolet can be photoreversed only for a short period of time following ultraviolet treatment.

The delay in the phenotypic expression of mutants proved markedly dependent upon the dose of radiation.

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