Pattern in the appearance of mutants after DES treatment among non-dividing bacteria*

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The ethylating agent, di-ethyl-sulfate (DES) is mutagenic when applied to non-growing bacteria or to free phage. In bacteria, it has been shown that inhibition of protein synthesis following DES treatment increases the frequency of recovered mutants (Strauss and Okubo, 1960). Freese (1963) suggested that in Strauss and Okubo's experiment, a delayed mutagenic effect might have taken place. This effect was caused by the incubation of the bacteria, subsequent to the treatment, at elevated temperature. Delayed appearance of alkylation-induced mutants was also demonstrated by Green and Kreig (1961) and by Krieg (1963).

In our experiment, 100 ml of an overnight culture of S. typhimurium strain LT2, leu⁻524 (kindly made available by Dr. F. Mukai) were washed and resuspended in 10 ml of T2 phage buffer (ca. 2×10^{10} cells/ml). After adding 0.02 ml of undiluted DES to 5 ml of the concentrated suspension, the latter was incubated for 20 min on a shaker at 37°C, whereupon both control and treated cells were

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washed twice with buffer.

The treated cells were diluted in mimimal medium (0.02% dextros w/v), to ca. 10^5 cells/ml, and 2 ml aliquots were distributed in ca 500 tubes and allowed to stand at 37°C. Cells from the control tube were diluted in the same medium to 10^7 cells/ml (very slight turbidity), and were distributed in 5 ml aliquots to 285 tubes.

Typical results are shown in fig. 1. While the majority of the control tubes remained clear for almost three weeks, some became turbid because of the overgrowth of pre-existing leu^+ mutants. This took place between 30 and 48 hours, after which no new mutants appeared. In the treated series, on the other hand, the number of turbid tubes continued to increase for more than 10 days. Late appearance of mutants was not due to slow growth of pre-existing or induced mutants, as could be shown in reconstruction experiments (table 1). One can calculate, for any given time, the mean number (m) per tube, of mutant cells originally distributed and of subsequent appearance of new

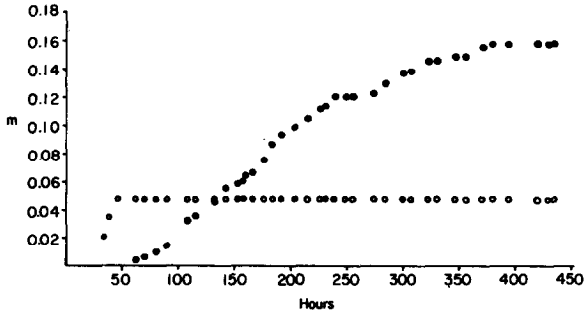

Fig. 1.

m values for the control (open circles) and DES-treated (closed circles) series.

Table 1

Single cells were isolated from tubes which had become turbid at different times, in the main experiment. They were then introduced into tubes containing 2 ml of minimal medium, with or without a background population of ca. 5×10^5 mutant (untreated) cells per ml. The time for overgrowth in each tube was measured.

Origin of introduced mutant and time mutant overgrew before isolation (hours).		Time when first tube became turbid in reconstruction experiment (hours)	
		With background	Without background
CONTROL	39	33,33,33,53	
DES	62	33,33,44	
	81	33	45,45
	108	67,126,36	53,45
	139		
	177	33,33,33,33	
	215		45,53
	286		127,141
	370		53,66

mutants by determining the fraction of clear tubes (P_o) and applying it to the equation:

$$P_o = e^{-m} \qquad (1)$$

(the zero term of the Poisson distribution). Fig. 1 shows the pattern of m values calculated in this experiment.

One can also use the m values found in that way to calculate the chance (μ) of mutation per bacterium per hour (Ryan, 1955). The pattern of μ over the time covered by our experiment is shown in Fig. 2.

Although it seems clear that mutants keep appearing long after DES treatment has been stopped, and that this delayed

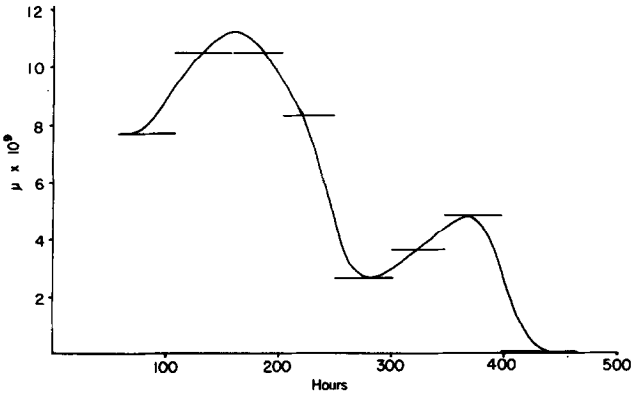

Fig. 2.

μ values, calculated for ca 50 hour periods, for 435 hours.

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appearance is due to delayed mutation rather than to slow growth, at least two points remain unclear: (1) Is the delayed appearance of mutants due to mutations occurring ca. 36 hours (the average time required for one revertant to overgrow) before turbidity appears, or is it due to a very long lag period caused by the treatment and which is followed by normal growth rate (phenotypic delay)? (2) If the first possibility is the correct one, what is the meaning of the deviations in μ, the rate of mutation per bacterium per hour? One hypothesis could be that the different peaks represent the increased rates of appearance of mutants caused by the splitting away of different alkylated bases (or bases alkylated at different N atoms).

During treatment with DES about 50% of the cells are killed. The death rate subsequent to treatment does not differ from that of the control ($k = 6.3 \times 10^{-3}$ and 4.0×10^{-3}, respectively) indicating no apparent delayed death. This may mean that the mechanism of delayed reversion studied in the present experiment is not lethal to any noticeable extent. On the other hand, delayed

death in buffered medium which supported no metabolism was demonstrable with alkylated T4 phage (Bautz and Freese, 1960), E. coli (Strauss, 1961) and various Salmonella strains (unpublished data). The delayed mutation phenomenon, however, can be demonstrated even when metabolism is altogether absent (unpublished data). This could be explained by the following events: alkylation of stationary-phase bacteria results both in breakage of the backbone of the DNA molecule and in shearing off of alkylated bases. The first damage can not be repaired without at least the low level of cell metabolism which occurs when the cells are suspended in the salt-sugar medium although they can not grow there. On the other hand, depurination can be repaired either by base-turnover alone, or it is a reversible event, and can be repaired at any time (e.g., upon plating).

The validity of these hypotheses is being studied by using recombination and mutation as criteria for DNA backbone breakage and base-alkylation respectively and a correlative chemical investigation of DNA fragmentation and the release of alkylated bases is being undertaken. Nonetheless, it is clear that mutants arise in bursts among non-growing, treated cells after the mutagen has been removed.

References

Bautz, E. and Freese, E., Proc. Nat. Acad. Sci. (Wash.) 46:1585 (1960)

Freese, E., in Taylor, J.H., Molecular Genetics (Academic Press) p. 239 (1963)

Green, D.M., and Krieg, D., Proc. Nat. Acad. Sci. (Wash.)47:64 (1961)

Krieg, D., Genetics 48:561 (1963)

Ryan, F. J., Genetics 40:726 (1955)

Strauss, B.S., J. Bacteriol. 83:241 (1962)

Strauss, B.S., and Okubo, S., J. Bacteriol. 79:464 (1960)