EFFECTS OF TEMPERATURE ON SPONTANEOUS AND INDUCED MUTATIONS IN ESCHERICHIA COLI*

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Communicated by M. Demerec, March 20, 1953

The frequency of spontaneous mutations in Drosophila has been shown to increase, in general, with increasing temperature, and the temperature coefficients reported range from about 2 to 5.[1] These findings played an important part in the early definition of mutation as a definite molecular rearrangement.[2] Very little is known of the response to post-treatment temperature of mutations induced by radiation or chemicals. Two kinds of effects could be investigated in this connection: the effect of temperature on the frequency of induced mutations, and the effect of temperature on the pattern of delayed appearance of induced mutations. In the latter category, Auerbach[3] has described an increase in the frequency of mustard-induced mosaics at low temperatures, which she ascribes to the stabilization by cold of metastable genic configurations induced by the mutagen, leading to extended delay in the shift to the stable mutant condition. Similar effects of temperature on the delayed action of mutagens in Neurospora and Aspergillis found in unpublished experiments by the authors are mentioned by McElroy and Swanson,[4] in support of the concept of mutation via metastable intermediates.

The hereditary change from sensitivity to resistance to bacteriophage in *Escherichia coli* can be followed with a degree of quantitative precision and technical ease that makes it a promising material for an investigation of temperature effects on spontaneous and induced mutation. This report is a preliminary account of such a study.

MATERIAL AND METHODS

Cultures of strain B/r of *Escherichia coli* were grown in a synthetic medium known as "A",[5] with aeration, for 18–24 hours, diluted with saline to an approximate titer of 10^8 cells per ml., and irradiated with 800 ergs per mm.^{2} of ultra-violet light. Irradiation was carried out at room temperature, using a G. E. germicidal lamp, with mechanical agitation during the exposure. Yellow light was used to illuminate all operations following the ultra-violet treatment. After irradiation, aliquots of the treated suspensions were plated on nutrient agar plates and incubated at 37°, 25°, or 16°C. At various intervals of time, sets of six plates were withdrawn from the incubator and chilled rapidly in the freezing compartment of a refrigerator to arrest growth. Two of the six plates were washed with 10 ml. of

saline and the wash fluid was assayed, to determine the total number of bacteria on the plates; the four remaining plates were sprayed with an aerosol of a suspension of bacteriophage T1 having a titer of at least 10^{10} particles per ml., to ascertain the number of phenotypically demonstrable clones of bacteriophage-resistant mutants. The time intervals for withdrawal of plates from the incubator for washing and spraying were based upon previously established growth curves of the irradiated populations at

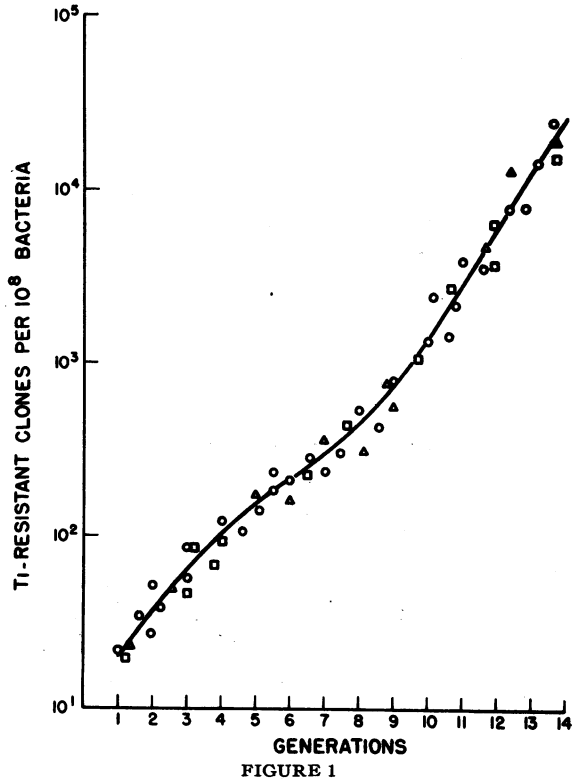

FIGURE 1

Spontaneous mutations to T1 resistance. Circles = incubated at 37°C. Squares = incubated at 25°C. Triangles = incubated at 16°C. Ordinates refer to T1 resistant clones per 10^8 bacteria *initially plated.*

the temperatures used, and were designed to cover the range from 0 to 14 generations. The data obtained from numerous experiments of this type permitted the construction of "expression curves" for the three temperatures studied, in which the number of T1-resistant clones per 10^8 irradiated cells was plotted against the number of post-irradiation generations. These curves are a measure of the pattern and extent of delayed appearance of the induced mutations—the final yield of induced mutations and the

number of generations required to achieve its complete manifestation being easily discerned by the leveling-off point. The necessary correction for spontaneous mutations was determined by similar experiments with unirradiated controls.

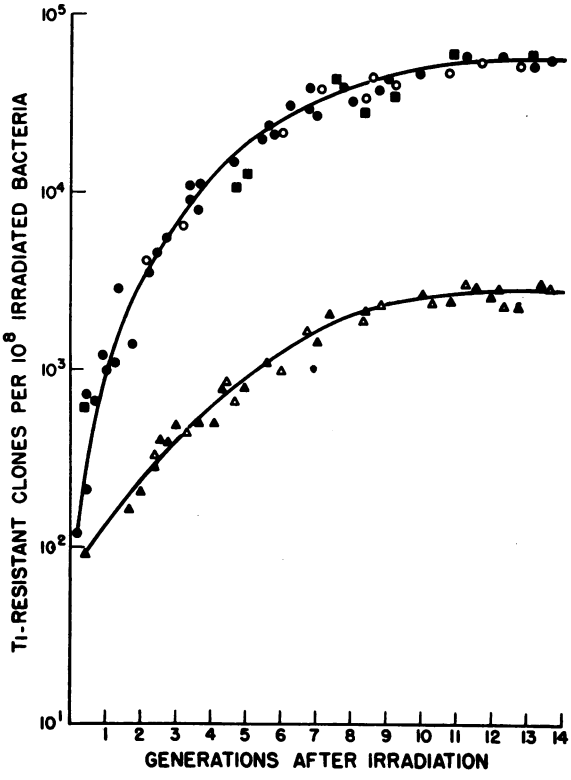

FIGURE 2

Effect of temperature on ultraviolet-induced T1-resistant mutants. Closed circles = incubated at 37° after irradiation. Closed squares = incubated at 25° after irradiation. Closed triangles = incubated at 16° after irradiation. Open circles = first postirradiation generation at 37°, all others at 16°. Open triangles = first postirradiation generation at 16°, all others at 37°. Ultraviolet dose, 800 ergs/mm.^{2}. Survival, 10 per cent.

RESULTS

1. Effect of Temperature on Generation Time.—The rate of growth of untreated and irradiated bacteria on nutrient agar plates was found to have a temperature coefficient of about 3. The generation time is twenty minutes at 37°, one hour at 25°, and three hours and twenty minutes at 16°

The lag phase for untreated cells is one hour and ten minutes at 37°, three hours and a half at 25°, and ten hours and forty minutes at 16°. For irradiated bacteria, the lag phases for the three temperatures are three hours, ten hours, and thirty hours for 37°, 25°, and 16°, respectively.

2. Effect of Temperature on Spontaneous Mutation Rate.—Figure 1 shows the number of T1-resistant clones, per 10^8 unirradiated cells plated, as a function of generations at 37°, 25°, and 16°C. The identity of the three curves shows that the process of spontaneous mutation to T1 resistance, expressed as the probability of mutation per bacterium per unit time, has the same response to temperature as does the generation time (Q_{10} *ca.* 3). The probability of mutation per bacterium *per generation*, which is the conventional way of expressing spontaneous mutation rate in bacteria, thus remains quite constant over the temperature range investigated. The data for spontaneous mutations are plotted in this rather unorthodox fashion so as to show most directly the magnitude of the corrections used in the preparation of expression curves for induced mutations. The same data can be used to calculate mutation rate according to standard formulae.

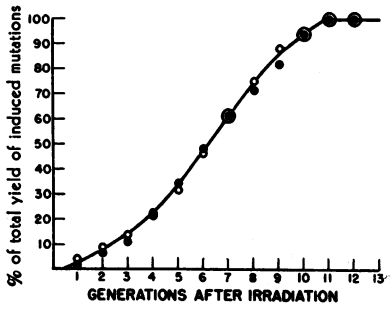

FIGURE 3

Effect of temperature on pattern of delayed appearance of ultraviolet-induced T1-resistant mutants. Open circles = postirradiation growth at 37° C. Closed circles = postirradiation growth at 16° C.

3. Effect of Temperature on Induced Mutations.—The response to temperature of induced mutation to T1 resistance is best shown by expression curves in which the number of detectable mutant clones per 10^8 irradiated cells is plotted against post-irradiation divisions at 37°, 25°, and 16°. These curves are shown in figure 2, already corrected for spontaneous mutations.

(*a*) *Effect of Temperature on the Final Yield of Induced Mutations:* The curve for bacteria incubated at 37° levels off at about 56,000 mutant clones per 10^8 irradiated cells; at 16°, the end-point number is about 2800, or 5 per cent of the yield at 37°. Thus, a profound effect of temperature on the yield of induced mutations is indicated, but the data obtained at 25° show that this effect cannot be expressed as a simple temperature coefficient. Although the data for bacteria incubated at 25° are fairly limited, there seems to be little, if any, reduction of the number of induced mutations at this temperature as compared with the yield at 37°. The temperature effect on the final yield of induced mutations is much greater between 25° and 16° than between 37° and 25°.

(*b*) *Effect of Temperature on the Pattern of Delayed Appearance of In-*

duced Mutants: Figure 3 shows percentages of the final yield of induced mutations as a function of post-irradiation divisions at 37° and at 16°. The identity of the two curves shows that, despite the large differences in final yield of induced mutations at the two temperatures, the span of generations required for a given fraction of the total to manifest itself is not modified by temperature. This supports the conclusion of Demerec and Cahn[6] that the pattern of delayed appearance is a characteristic and stable feature of a particular mutation.

(*c*) *The Temperature-Sensitive Period:* In the experiments described above, the irradiated cells were incubated at 37°, 25°, or 16° throughout the period of post-irradiation growth. Another series of experiments was conducted in which the irradiated bacteria were incubated at 37° for one generation, after which the plates were transferred to 16° for the duration of the experiment. Reciprocal experiments were also made, in which the first division was at 16°, and the remaining divisions took place at 37°. The data of these experiments, plotted also in figure 3, show clearly that the yield of induced mutations depends entirely on the temperature of incubation during the first post-irradiation generation. If the first division proceeds at 16°C., the final yield of mutation is 2800 per 10^8 treated bacteria, whether the remaining divisions take place at 16° or at 37°; similarly, if the temperature during the first division is 37°, the end-point number is 56,000 whether the subsequent divisions are at 37° or at 16°. Although 10–12 generations are required to achieve complete manifestation of the end-point number, it is the temperature during the first division that determines the outcome.

In an attempt to define the temperature-sensitive period more precisely, irradiated cells were exposed to one temperature for various fractions of the time required for the first generation before the plates were transferred to the other temperature. Intermediate yields were obtained, the initial temperature exerting an effect proportional to the fraction of the first generation passed under its influence. At present, these results can be accounted for equally well by two hypotheses concerning the temperature-sensitive period: (1) that the critical period is confined to the actual division process, the intermediate yields being a result of mixtures of cells that have and have not completed the first division; or (2) that the temperature effect is exerted gradually throughout the entire lag phase and first division.

(*d*) *Effect of Temperature after the First Post-irradiation Generation:* It was pointed out above that if the irradiated cells are allowed to pass through the first division at a given temperature, the temperature during the subsequent generations has no further effect on the course of the expression curve. Thus, after the first generation, the rate of appearance of mutant clones as a function of time has the same temperature coefficient as the processes of cell division and spontaneous mutation, about 3, so that the

rate of appearance of mutant clones per generation is not affected by temperature.

(e) *The Dose-Reduction Equivalent of the Temperature Effect:* The expression curve obtained at 16° after a dose of 800 ergs per mm.^{2} can be duplicated at 37° quite exactly when the dose is reduced to 100 ergs per mm.^{2}

DISCUSSION

These experiments have shown that, following treatment with ultra-violet radiation, there is a critical period up to and possibly including the first post-treatment division, during which temperature can influence profoundly the final yield of induced T1-resistant mutants. This observation supports the current view that the mutagenic action of ultra-violet light is indirect, and cannot be regarded as a simple photochemical effect. Beyond this, little can be deduced as to the nature of the genetic events following irradiation. Equally cogent arguments can be mustered in favor of three possible modes of action of ultra-violet light: (1) the production of intracellular mutagens; (2) the production of metastable genic states, and (3) the activation of systems of instability such as those described by McClintock.[7] Further work is required to make possible a critical analysis of these hypotheses. It is of particular importance to determine whether the temperature effects observed here are characteristic of mutational systems other than resistance to phage T1.

Another interesting feature of the results is the identity of the temperature coefficients of three presumably distinct processes—spontaneous mutation as a function of time, cell division, and the delayed appearance of induced mutations per unit time. This observation suggests that spontaneous mutation and the delayed appearance of induced mutation may be similar phenomena, and that both are intimately associated with the mitotic cycle. The separability of the processes of spontaneous mutation and cell division, however, has been demonstrated by studies with the chemostat,[8] and must be taken into account.

SUMMARY

(1) The final yield of ultra-violet-induced T1-resistant mutants in *E. coli* is reduced to about 5% when the incubation temperature following irradiation is 16°C., as compared with the result at 37°. There is little, if any, reduction at 25°. (2) The temperature effect on the number of induced mutants is confined to the period of the first post-treatment division. (3) The temperature coefficients of spontaneous mutation per unit time, cell division, and delayed appearance of induced mutations per unit time are identical (Q_{10} = *ca.* 3). Thus, spontaneous mutation per generation and delayed appearance of induced mutations per generation are constant over

the temperature range investigated. (4) The effect of post-treatment incubation at 16°C. can be duplicated at 37° by reduction of the ultraviolet dose from 800 to 100 ergs per mm.[2]

* This study was supported in part by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

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ON THE EIGENFUNCTIONS AND EIGENVALUES OF THE GENERAL LINEAR ELLIPTIC DIFFERENTIAL OPERATOR

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Communicated by J. von Neumann, March 4, 1953

Let K be a linear elliptic differential operator of order $2m (m \geq 1)$ with suitably differentiable coefficients on the bounded domain D of Euclidean n-space. During the past year, the author presented in several notes in these PROCEEDINGS a general method of solving boundary value problems for K on D.[1] In particular a number of results were obtained concerning the eigenfunctions and eigenvalues of K on D, including the completeness of the eigenfunctions if K is self-adjoint.[2] It is the principal object of this note to outline the proof that completeness persists even if K is not self-adjoint, provided that one admits higher order eigenfunctions into consideration (as in the case of linear transformations of finite dimensional complex vector spaces).[3] The existence of an infinite set of distinct complex eigenvalues for the general linear elliptic operator K follows as a corollary. In addition, it is shown that the eigenfunctions of K are complete in $L^p(D)$ for $p \leq \frac{2n}{n-2m}$ and closed in $L^1(D)$ for $2m > n$ while sharper estimates are given for the relative magnitudes of the real and imaginary parts of the eigenvalues.[4]

Although the discussion of this note is restricted to a single elliptic operator and to the Dirichlet boundary conditions, the method employed