*THYMINELESS DEATH AND ITS RELATION TO UV SENSITIVITY IN ESCHERICHIA COLI**

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Various agents that act selectively on DNA have been shown to kill bacteria. While mitomycin treatment,[1-3] ultraviolet irradiation,[4-7] and thymineless death[8-11] have different primary effects on DNA, the bacterial survival characteristics exhibit distinct similarities. It has been noted that *E. coli* K12 highly sensitive to ultraviolet (UV) irradiation are also highly sensitive to mitomycin[12] and that a synergistic relationship exists between UV irradiation and thymineless death in *E. coli* B3.[13] Similarly, recombination-deficient mutants of *E. coli* K12 are ultrasensitive to UV irradiation,[16] and most recently it has been demonstrated[18] that a transformation-deficient strain of *B. subtilis* also displays a high sensitivity to UV irradiation and mitomycin. These and other parallel observations[11] have led to the proposal [7, 14-18] that the basic mechanism in all these processes involves repair of DNA.

Although this hypothesis of DNA repair is certainly an attractive proposal, there is little direct evidence that such a single mechanism would account for the action of all these treatments. There has been no definitive study of the various modes of DNA inactivation on the same organism and, moreover, the kinetics of thymineless death[8, 9] are not in agreement with the kinetics observed in the other lethal processes. For example, no so-called ultrasensitivity to thymineless death has been reported. It was therefore decided to examine thymine-requiring mutants of bacteria known to have a high sensitivity to UV irradiation[4, 16] and then to compare the various survival characteristics. It was found that *E. coli* B exhibited a higher sensitivity to thymineless death, UV irradiation, and mitomycin treatment than did the resistant strain *E. coli* B/r. Contrary to this finding, while ultra-UV-sensitive mutants of *E. coli* K12 showed an increased sensitivity to mitomycin treatment, they were no more sensitive to thymineless death than were the normal wild-type K12 strains.

Materials and Methods.—Thymine-requiring strains of *E. coli* were used throughout this study. A recombination-deficient strain (JC1569, Rec^-) and its immediate normal progenitor strain (JC1557, Rec^+) of K12 were obtained from Dr. A. J. Clark. A thymine-requiring derivative (AB2500) of the UV-sensitive *uvrA-6* (AB1886) strain was obtained from Dr. P. Howard-Flanders. Strain B has been used in the authors' laboratory for several years and was obtained originally from Dr. S. Luria; strain B/r was obtained from Dr. H. Boyer. Thymine-requiring derivatives of the *E. coli* strains were isolated utilizing the aminopterin selection technique;[19, 20] this procedure yielded mutants that required 20 μg/ml of thymine for optimal growth. These bacterial strains were grown aerobically in a glucose-phosphate-salts minimal medium at 37°C with required supplements. Changes of medium were achieved by 1000-fold dilution, and samples were then taken at intervals and assayed on Difco-tryptone agar plates.

UV irradiation was performed in minimal-salts medium without glucose at 25°C using a 30-watt germicidal lamp (G.E.)[21] backed by a polished reflector and mounted 75 cm from a designated target plane. (This apparatus was used with the kind permission of the Department of Biophysics.) The intensity of this apparatus for wavelengths below 3200 Å was 60 erg/mm²/sec, and 85% of the emitted energy had a wavelength of 2537 Å.[21] To obtain a convenient dose rate for the bacteria irradiated, the source was attenuated 100-fold by neutral light filters consisting

of two brass screens of fine mesh and a single layer of Saran wrap. The cell suspensions were irradiated in an open Petri dish with shaking and samples taken at various times. Difco-tryptone assay plates were incubated in the dark at 30°C to avoid heat and photoreactivation.[22]

The bacterial strains were treated with mitomycin C (MC) (obtained from Sigma Chemicals) in glucose-salts minimal medium with required supplements for 25 min at 37°C. The treatment was stopped by a 30-fold dilution with minimal-salts medium, and the samples were then plated and incubated overnight in the dark at 37°C.

Results—*E. coli* B and B/r thymine-requiring strains displayed the expected[4] response to UV irradiation. In Figure 1, it can be seen that *E. coli* B is readily distinguishable from B/r on the basis of its UV sensitivity. A similar difference was obtained on treating these strains with mitomycin C: *E. coli* B was about 30 times more sensitive to MC than was strain B/r (Fig. 2). This result agrees with previous data[12] on the comparative sensitivity to MC of UV-sensitive and UV-resistant *E. coli* K12 strains. The UV and MC inactivation curves both proceeded with a lag in the resistant bacteria, but were exponential in the sensitive strain. The survival curves of bacteria undergoing thymineless death also exhibit a lag prior to the onset of cell death.[8, 9] Figure 3 illustrates the typical thymineless death curve of *E. coli* B/r in which the viable cell number remains unchanged for about 50 min and then falls at an exponential rate. *E. coli* B, on the other hand, behaved in a new and unexpected manner by responding without delay to thymine deprivation with death occurring at an exponential rate. Thus, *E. coli* B displayed a marked sensitivity to UV irradiation, MC treatment, and thymineless death relative to *E. coli* B/r. To be certain that *E. coli* B was not more susceptible to death

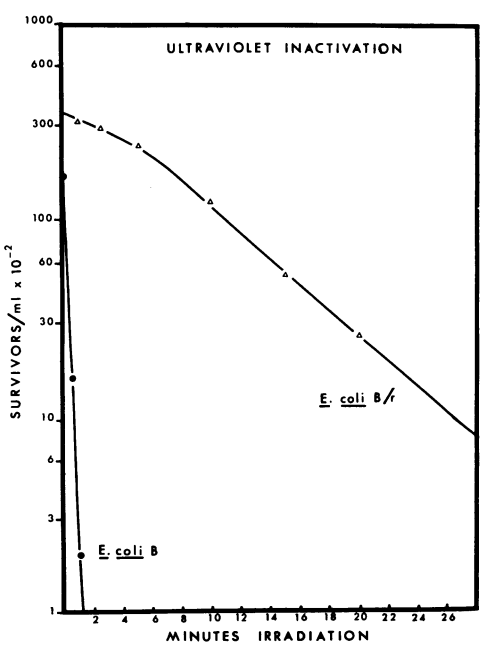

FIG. 1.—Comparison of the UV sensitivity of *E. coli* B and B/r. The *E. coli* B studied appears to be about 40 times more sensitive to UV irradiation than B/r with respect to colony formation.[4, 23, 24]

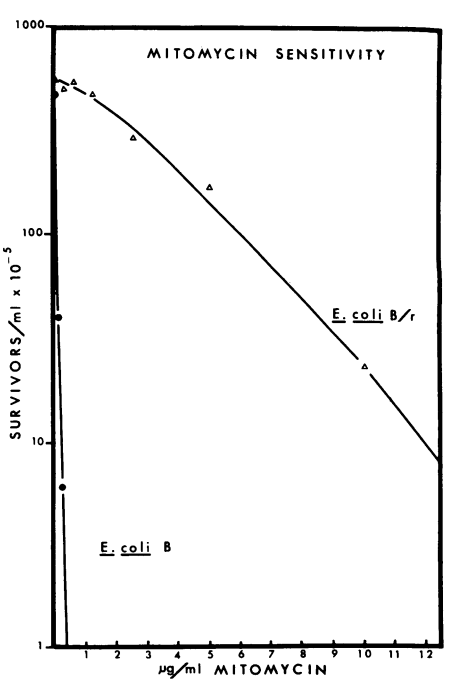

FIG. 2.—Mitomycin C sensitivity of *E. coli* B and B/r. The relative difference observed in MC sensitivity was similar to that obtained with UV irradiation.

by any means, strains B and B/r were subjected to heat inactivation at 55°C. After a delay period of about 3 1/2 min, both strains were inactivated rapidly and no meaningful differences were detected. These observations on killing by UV, MC, and thymine starvation tend to support the hypothesis that the ultimate effects of such seemingly different primary actions on DNA are related. A defect in DNA repair could well be the specific trait that distinguishes *E. coli* B from B/r.

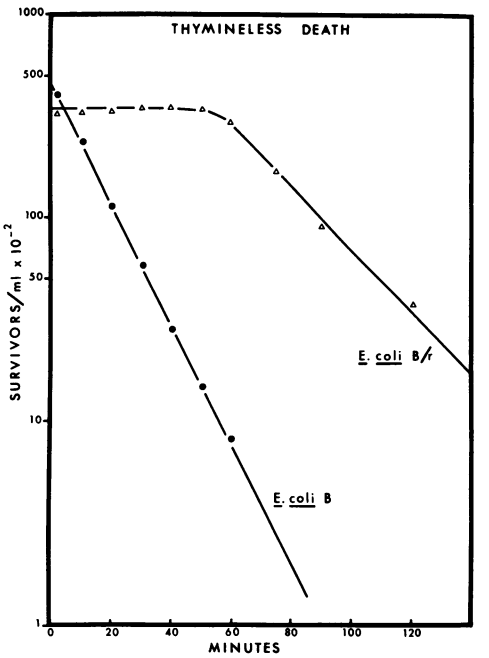

FIG. 3.—Effect of thymine deprivation on *E. coli* B and B/r. *E. coli* B lost the ability to form colonies immediately and did so at about twice the ultimate exponential death rate of B/r.

From the preceding results it was anticipated that mutants of *E. coli* K12 ultrasensitive to UV irradiation would also be ultrasensitive to thymineless death. As indicated previously, strains of *E. coli* K12 unable to undergo genetic recombination are also ultrasensitive to UV.[16] Thymine-requiring strains of recombination-deficient (Rec$^-$) and wild-type (Rec$^+$) *E. coli* K12 were therefore examined for their sensitivity to the various DNA treatments. Figure 4 illustrates the expected[16] response to UV irradiation. As with *E. coli* B, the Rec$^-$ strain was also found to be highly sensitive to MC (Fig. 5), in agreement with prior results on other UV-sensitive strains of *E. coli* K12.[12] Upon examining thymineless death sensitivity, however, it was found that Rec$^-$ and Rec$^+$ strains did not differ in their response to thymine deprivation (Fig. 6). Exponential death proceeded only after a delay of about 50 min, a behavior similar to that obtained with *E. coli* 15 T$^-$,[8, 9] B13,[13] and B/r. It is possible that *E. coli* K12 might differ physiologically from *E. coli* B in either the amount or utilization of intracellular thymine and might thereby mask any unusual sensitivity to thymine starvation. This possibility can be excluded on the basis that if such were the case, (1) viable cell number should increase during the delay period, (2) when exponential death does occur, the rate should be faster in the Rec$^-$ than in the Rec$^+$ strain, and (3) new DNA should be synthesized during the delay period. The first two events did not occur here and, in experiments to be reported elsewhere, nitrogen-15 was not incorporated into DNA during the delay period.

It is also possible that the Rec$^-$ strain of *E. coli* K12 was not the proper type of UV-sensitive mutant. Although the Rec$^-$ strain is ultrasensitive to UV,[16] it is nonetheless able to excise thymine dimers.[17] The ultrasensitive *uvrA* strains examined by Howard-Flanders,[15, 17] however, appear to be of a different type in that they are unable to excise thymine dimers and are competent in genetic recombination. Examination of one of these *uvrA* mutants showed that it responds to thymine deprivation in a manner similar to the Rec$^-$ strain (Fig. 7). Thus, *E. coli*

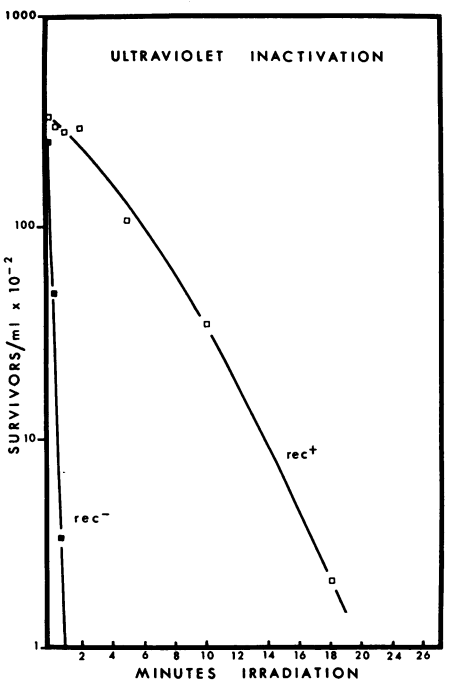

FIG. 4.—Comparison of the UV sensitivity of *E. coli* K12 Rec⁻ and Rec⁺. These results are similar to those obtained previously.[16]

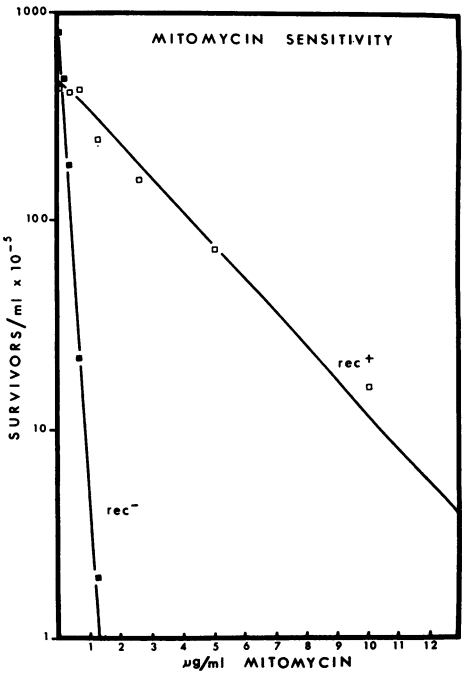

FIG. 5.—Mitomycin C sensitivity of *E. coli* K12 Rec⁻ and Rec⁺. The ultrasensitivity of the Rec⁻ strain is similar to that found with another type[12] of ultra-UV-sensitive *E. coli* K12.

K12 strains that are ultrasensitive to UV and MC appear to have the same susceptibility to thymineless death as do all the resistant strains investigated.

Discussion.—The phenomenon of thymineless death has been re-examined with respect to UV sensitivity, mitomycin sensitivity, and recombination deficiency. The results indicated that while certain parallels existed, not all responses were correlated. The information obtained with *E. coli* B was consistent. In all cases, *E. coli* B was ultrasensitive as compared with B/r. In *E. coli* K12, however, no correlation between sensitivity to UV or MC and sensitivity to thymineless death was observed. This result was true regardless of whether the UV-sensitive K12 mutants were defective in thymine dimer excision or in genetic recombination. The failure of these experiments to demonstrate a consistent pattern of sensitivity to thymineless death in all UV- and MC-sensitive strains of *E. coli* contradicts the hypothesis that the ultrasensitivity of *E. coli* B to all three treatments is due to a single defect in DNA repair. The observation that two different UV-sensitive mutants of *E. coli* K12 fail to exhibit a heightened sensitivity to thymineless death suggests that these bacteria employ an independent mechanism to repair lesions produced by thymine deprivation. A possible explanation for the unique behavior of *E. coli* B would then be that in addition to being unable to repair UV- or MC-damaged DNA, this strain has a further defect that renders it incapable of repairing lesions induced by thymine deprivation. On the basis of genetic evidence,[15, 16] it appears that both the Rec⁻ and *uvrA-6* mutants of *E. coli* K12 arose

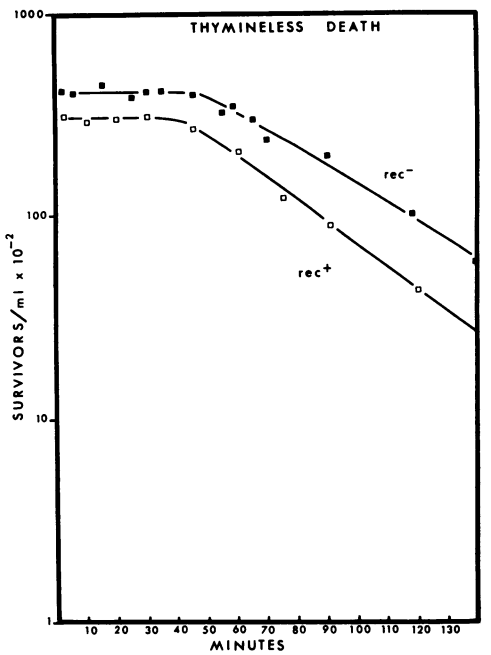

FIG. 6.—Effect of thymine deprivation on *E. coli* K12 Rec⁻ and Rec⁺. The same response to thymine deprivation in the Rec⁻ was obtained also after filtration on a Millipore filter followed by a 1000-fold dilution into thymineless medium.

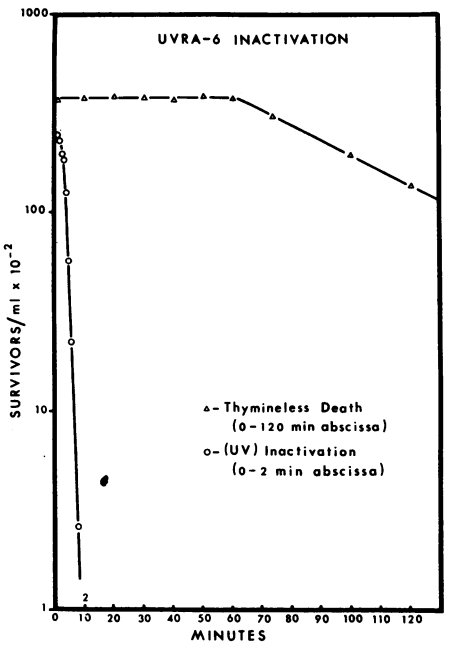

FIG. 7.—UV inactivation and thymineless death of *E. coli* K12 *uvrA-6*. The thymineless death curve was essentially the same in this strain as for the Rec⁻ and Rec⁺ strains.

by one-step mutations at single loci. On the other hand, it is not clear how many mutational steps separate *E. coli* B from B/r; quite possibly they differ by a number of traits, some of which affect sensitivity to UV and MC, while others affect sensitivity to thymineless death. Whether the *E. coli* B strain reported here is unique in this regard is currently under investigation.

Summary.—Thymineless death in various strains of *E. coli* has been examined and compared with sensitivity to UV irradiation and to mitomycin (MC) treatment. It was found that *E. coli* B, a strain ultrasensitive to UV and MC, displayed a new and unexpected sensitivity to thymine deprivation. Instead of the usual delayed response to thymine starvation, death commenced immediately and at an exponential rate about twice that ultimately obtained in B/r. On the other hand, thymineless death in UV- and MC-ultrasensitive strains of *E. coli* K12 proceeded with the usual lag of about 50 min before the onset of cell death. From these results it was concluded that a single defect in DNA repair cannot satisfactorily account for both ultrasensitivity to thymineless death and ultrasensitivity to either UV or MC.

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[1] Shiba, S., A. Terawaki, T. Taguchi, and J. Kawamata, *Nature*, **183**, 1056 (1959).

[2] Sekiguchi, M., and Y. Takagi, *Biochim. Biophys. Acta*, **41**, 434 (1960).

[3] Mennigmann, H., and W. Szybalski, *Biochem. Biophys. Res. Commun.*, **9**, 398 (1962).

[4] Witkin, E. M., *Genetics*, **32**, 221 (1947).

[5] Wacker, A., *Progr. Nucl. Acid Res.*, **1**, 369 (1963).
[6] Setlow, R. B., and J. K. Setlow, these PROCEEDINGS, **48**, 1250 (1962).
[7] Setlow, R. B., and W. L. Carrier, these PROCEEDINGS, **51**, 226 (1964).
[8] Barner, H. E., and S. S. Cohen, *J. Bacteriol.*, **68**, 80 (1954).
[9] Maaløe, O., and P. C. Hanawalt, *J. Mol. Biol.*, **3**, 144 (1961).
[10] Pritchard, R. H., and K. G. Lark, *J. Mol. Biol.*, **9**, 288 (1964).
[11] Pauling, C., and P. Hanawalt, these PROCEEDINGS, **54**, 1728 (1965).
[12] Boyce, R. P., and P. Howard-Flanders, *Z. Vererbungslehre*, **95**, 345 (1964).
[13] Gallant, J., and S. R. Suskind, *J. Bacteriol.*, **82**, 187 (1961).
[14] Pettijohn, D., and P. C. Hanawalt, *J. Mol. Biol.*, **9**, 395 (1964).
[15] Boyce, R. P., and P. Howard-Flanders, these PROCEEDINGS, **51**, 293 (1964).
[16] Clark, A. J., and A. D. Margulies, these PROCEEDINGS, **53**, 451 (1965).
[17] Howard-Flanders, P., and L. Theriot, personal communication.
[18] Okuba, S., and W. R. Romig, *J. Mol. Biol.*, **15**, 440 (1966).
[19] Okada, T., K. Yanagisawa, and F. J. Ryan, *Nature*, **188**, 340 (1960).
[20] Stacey, K. A., and E. Simson, *J. Bacteriol.*, **90**, 554 (1965).
[21] Lee, H. H., and T. T. Puck, *Radiation Res.*, **12**, 340 (1960).
[22] Anderson, E. H., *J. Bacteriol.*, **4**, 389 (1951).
[23] Ellison, S. A., R. R. Feiner, and R. F. Hill, *Virology*, **11**, 294 (1960).

[24] Judging from the degree of sensitivity to UV irradiation, it is not likely that the *E. coli* B examined here is identical with the B examined by others.[7, 23] It is more likely that our strain is similar to the ultra-UV-sensitive strains B_{s-1} and B_{s-11} which are defective in their ability to excise thymine dimers.[7]