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Thymineless Death in *Escherichia coli* 15T⁻ and Recombinants of 15T⁻ and *Escherichia coli* K-12

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Thymineless death was examined in *Escherichia coli* 15T⁻ and recombinants of 15T⁻ and *E. coli* K-12. Those strains that were very sensitive to thymine deprivation were also very sensitive to a variety of inducing agents (mitomycin C, ultraviolet light, hydroxyurea, and nalidixic acid). Those strains that were relatively resistant to thymineless death were also relatively resistant to the inducing agents. After exposure to thymineless death and the inducing agents, sensitive strains lysed, produced colicin, and had phage particles in their lysates. These strains also showed an increase in the 6-methyladenine content of their deoxyribonucleic acid (DNA) and an increase in the DNA methylase activity of their crude extracts under these conditions. None of these effects was noted in the strains relatively resistant to thymineless death and the inducing agents. These data indicate that there are two types of thymineless death. One is represented by the strains that are very sensitive to thymine deprivation and other inducing agents and is secondary to the induction of phage ψ. The strains more resistant to thymine deprivation and the other inducing agents undergo a non-phage-mediated thymineless death. The mechanism of this latter process is currently under study.

When transient thymine deprivation is imposed on the logarithmically growing cells of the thymine auxotroph *Escherichia coli* 15T⁻, cell viability decreases exponentially (1). This killing effect has been termed thymineless death. The induction of a temperate phage during thymineless death has been shown to be a factor in this lethal process (9). It is not completely clear, however, whether thymine deprivation alone without prophage induction can lead to cell death.

Ishibashi and Hirota have derived a strain of 15T⁻ that is relatively resistant to thymineless death (JG 151; reference 7). They have also isolated a number of recombinants of 15T⁻ and *E. coli* K-12 (JG 179, JG 150, JG 166, JG 185) with varying susceptibility to thymineless death. Other derivatives of 15T⁻ with varying susceptibility to thymineless death have been isolated in this laboratory.

This report deals with an examination of the kinetics of thymineless death in all of these strains. It establishes a correlation between increased sensitivity to thymine deprivation and the occurrence of phage induction. Those strains that have increased resistance to thymine deprivation are also more resistant to antibacterial treatments that lead to phage induction in lysogenic bacteria. These findings support the conclusion that there are two types of thymineless death. The type manifested by increased susceptibility to thymine deprivation and a variety of inducing agents is secondary to phage induction. The more resistant killing pattern of thymineless death is secondary to the lethal effect of thymine deprivation alone, without prophage induction.

MATERIALS AND METHODS

Microorganisms. The strain of *E. coli* 15T⁻ employed is a thymine auxotroph with a low thymine requirement (2.5 μg/ml) and most closely corresponds to the strain 70 V3-462 described by Breitman and Bradford (2). JG 151 is a strain derived from surviving cells of 15T⁻ after thymine deprivation. JG 185, JG 166, JG 150, and JG 179 are recombinants of 15T⁻ and *E. coli* K-12 and were kindly provided by Y. Hirota. *E. coli* $15T_R^-$ is a mitomycin C-resistant thymine auxotroph derived in this laboratory (9). All of these strains have the same thymine requirement for growth as the parent 15T⁻. All of the T⁺ strains are spontaneous prototrophic revertants of the original thymine auxotrophs.

Media. The organisms were grown in Salts A medium (3), and thymine was added to give a final concentration of 2.5 μg/ml. Amino acids and uracil were added when required by the organisms.

Chemicals. Mitomycin C was purchased from Nutritional Biochemical Corp. (Cleveland, Ohio) and phenethyl alcohol from Eastman-Kodak. Hydroxyurea was kindly provided by E. R. Squibb and Co. and nalidixic acid by Winthrop Laboratories. Penicillin G was purchased from E. R. Squibb and Co., and novobiocin (Albamycin) was kindly provided by Upjohn. [^{14}C-methyl]S-adenosyl-L-methionine with a specific activity of approximately 30 millicuries/millimole was obtained from Tracerlab.

Growth and harvesting of organisms. The bacteria were grown in Salts A medium at 37 C with vigorous shaking until mid-log phase (2×10^8 cells/ml). The appropriate antibacterial agents were then added at the following concentrations: mitomycin C, 2 μg/ml; hydroxyurea, 8 μg/ml; nalidixic acid, 40 μg/ml; phenethyl alcohol, 3 mg/ml; novobiocin, 500 μg/ml; and penicillin, 100 units/ml. Ultraviolet (UV) irradiation of the culture was done in the dark for 20 to 80 sec with a Sylvania germicidal G 15T8 UV lamp at a distance of 50 cm from the organisms. The bacteria were swirled in open petri dishes during the irradiation. In the thymine deprivation experiments, the organisms were made thymineless by filtration and resuspension in thymineless medium. Colony counts were done on A C Medium (Difco).

Assays. Deoxyribonucleic (DNA) methylase activity was measured by using the method of Fujimoto et al. (6). DNA was extracted by either the Marmur (8) or the Schmidt-Thannhauser (12) procedure. The DNA obtained from the various strains was either depurinated (14) or hydrolyzed with formic acid (16). The base content was then determined by quantitative descending two-dimensional paper chromatography (15).

Phage harvest and colicin determination. Bacteria lysates were prepared as previously described (9). The lysates were then examined for phage by electron microscopy with the use of ammonium molybdate negative staining, and for colicin activity, by the use of one drop of concentrated lysate on a lawn of *E. coli* 15T$^-$.

RESULTS

The effect of thymine deprivation on the thymine auxotrophs tested is shown in Fig. 1. *E. coli* 15T$^-$, JG 179 and JG 150 are very sensitive to thymine deprivation whereas JG 185, JG 166, and JG 151 are relatively resistant. *E. coli* 15T$_R^-$ does not grow in the absence of thymine, but no apparent killing occurs in this strain under thymineless conditions. All of these organisms maintain the stringent thymine requirement for growth of the parent 15T$^-$.

A similar pattern of survival curves is obtained when these strains are exposed to mitomycin C (Fig. 2). UV light, hydroxyurea, and nalidixic acid also give similar results. JG 151 is atypical because it is very sensitive to mitomycin C and UV light but resistant to thymine deprivation,

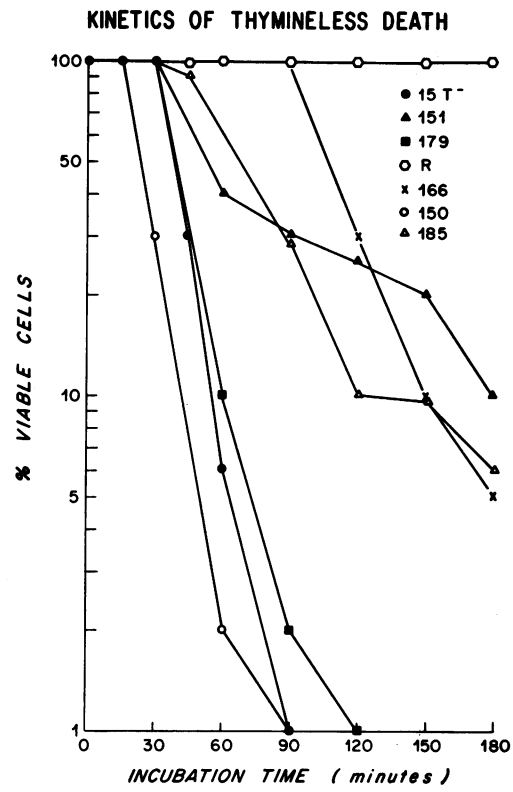

FIG. 1. *Kinetics of thymineless death.*

hydroxyurea, and nalidixic acid. All of these agents have been shown to induce phage ψ in *E. coli* 15T$^-$ (9).

The strains that are very sensitive to thymineless death and the other inducing agents show a 5- to 10-fold increase in the 6-methyadenine content of the extractable DNA in the culture after exposure to these inducing agents. The DNA methylase activity of their crude extracts is also increased by 10- to 100-fold after this exposure (Table 1). Examination of the culture supernatant fluids of the sensitive strains after treatment with the various agents shows phage particles (Fig. 3) and colicin activity. The more resistant organisms show none of these changes.

The data on JG 151 (Table 1) suggests that this strain is not lysogenic and that its mitomycin C and UV sensitivity is not based on the induction of prophage.

Phenethyl alcohol, novobiocin, and penicillin do not induce phage ψ in 15T$^-$ (9). All of the strains tested show equal sensitivity to these noninducing agents. This is illustrated by the data on phenethyl alcohol (Fig. 4).

Spontaneous revertants to thymine independence were isolated from these strains and exposed

to all of the agents used on the thymine auxotrophs. Exactly the same results were obtained as with the thymine auxotrophs; the killing effect of mitomycin C, UV light, nalidixic acid, and hydroxyurea appears dependent on whether these strains are lysogenic.

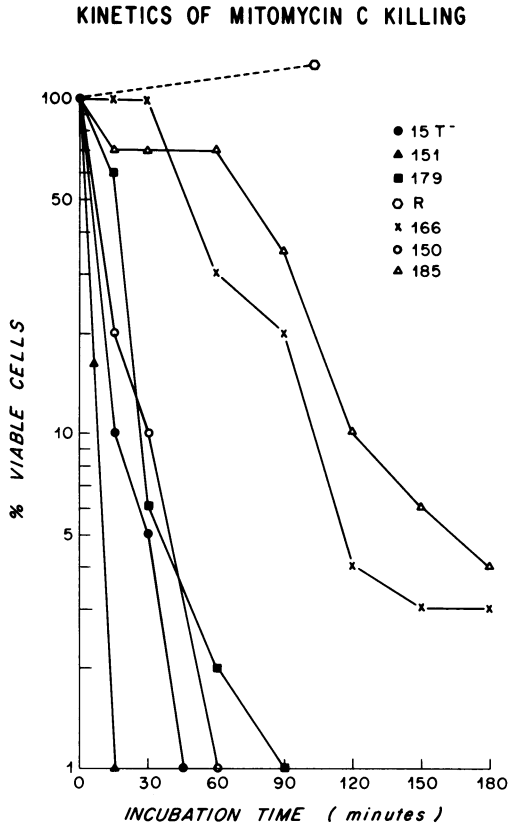

FIG. 2. *Kinetics of mitomycin C killing.*

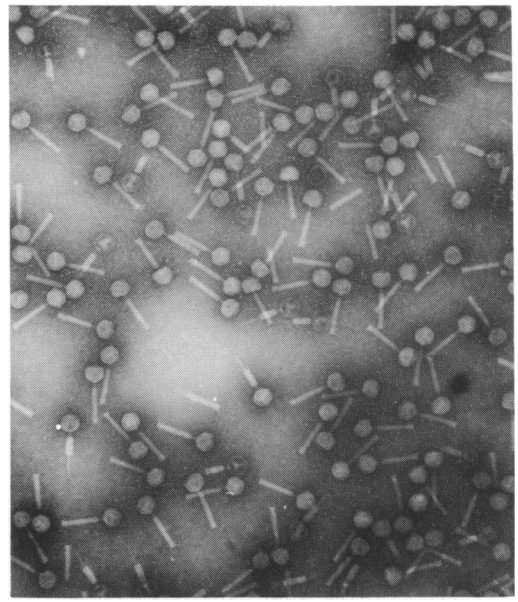

FIG. 3. *Electron micrograph of phage particles.*

DISCUSSION

The data presented in this report suggest that there are two types of thymineless death. One is represented by the strains that are very sensitive to thymine deprivation and the other inducing agents; the increased killing is associated with the induction of phage ψ. The strains more resistant to thymine deprivation and the other inducing agents show no evidence of phage induction during the killing process; they appear to be nonlysogenic. *E. coli* $15T_R^-$ is not killed by thymine deprivation or by mitomycin C. Presumably this strain is resistant to the non-phage-mediated thymineless death. An alternate explanation is

TABLE 1. *Summary of the effects of inducing agents on Escherichia coli 15T⁻, derivatives of 15T⁻, and recombinants of 15T⁻ and E. coli K-12*

Bacteria	Increase in 6-methyladenine content[a]	Increase in DNA methylase activity[b]	Phage	Colicin activity	Sensitivity to thymineless death
Escherichia coli 15T⁻	10×	100×	Many phage	Present	Sensitive
JG 179	5×	10×	Moderate number of phage	Present	Sensitive
JG 150	5×	10×	Moderate number of phage	Present	Sensitive
JG 151	None	None	Absent	Absent	Relatively resistant
JG 166	None	None	Absent	Absent	Relatively resistant
JG 185	None	None	Absent	Absent	Relatively resistant
E. coli $15T_R^-$	None	None	Absent	Absent	Completely resistant

[a] Ratio of 6-methyladenine to adenine.
[b] Expressed as nanometers per milligram of protein.

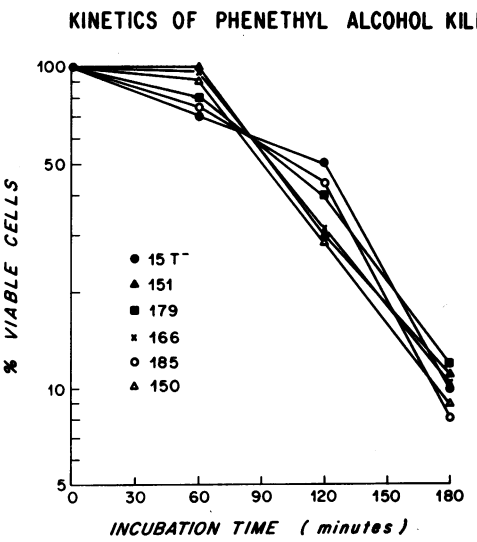

FIG. 4. *Kinetics of phenethyl alcohol killing.*

that $15T_R^-$ is the only nonlysogenic strain and the relatively resistant strains carry defective phage. There is no evidence for the latter hypothesis.

The fact that the T^+ revertants have the same pattern of sensitivity to the inducing agents supports the conclusion that the differences in survival rates are dependent on the presence of prophage in the strains. The changes in methylation, the colicin elaboration, and the phage particles seen in the electron micrographs further support this conclusion.

The concept that thymineless death may be caused by the induction of a bacteriophage has received much support (11). Endo et al. (4) showed that colicin 15 activity is associated with phage particles, and Mennigmann (10) was able to induce phage particles in $15T^-$ with UV light. Moreover, it has also been shown that, when $15T^-$ does not produce colicin, or is nonlysogenic, it is relatively resistant to thymine deprivation (13). This report confirms both of these observations. The fact that two mechanisms of thymineless death exist makes it obvious that, when thymineless death is being studied, the organism being examined must be characterized as to whether it is lysogenic or nonlysogenic.

The process by which thymine deprivation induces prophage is unknown. The mechanism of the non-phage-mediated thymineless death is also unknown. Recently, (5) it has been suggested that failure to repair DNA strand breakage might be involved in this process. Further studies of DNA repair during thymineless death with the use of the more resistant strains are currently in progress.

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