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# Effect of Thymine Starvation on Deoxyribonucleic Acid Repair Systems of *Escherichia coli* K-12

JANET A. ANDERSON AND STEPHEN D. BARBOUR

*Department of Microbiology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106*

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Thymine starvation of *Escherichia coli* K-12 results in greatly increased sensitivity to ultraviolet light (UV). Our studies, using isogenic strains carrying *rec* and *uvr* mutations, have shown the following. (i) Common to all strains tested is a change from multihit to single-hit kinetics of survival to UV after 60 min of thymine starvation. However, the limiting slope of UV survival curves decreases in the *rec*$^+$ *uvr*$^+$ strain and changes very little in several *rec* mutant strains and one *uvrB* mutant strain. Thus, when either the *rec* or *uvr* system is functioning alone, the limiting slopes of the UV survival curves are relatively unaffected by thymine starvation. (ii) Thymine starvation does not significantly inhibit repair processes carried out by either repair system alone; i.e., host cell reactivation of irradiated phage (carried out by the *uvr* system), excision of thymine dimers (*uvr*), or X-ray repair (*rec*). (iii) In a *rec*$^+$ *uvr*$^+$ strain, repair appears to be a synergistic rather than additive function of the two systems. However, after thymine starvation, repair capacity is reduced to about the sum of the repair capacities of the independent systems. (iv) The kinetics of thymineless death are not changed by *rec* and *uvr* mutations. This indicates that the lesions responsible for thymineless death are not repaired by *rec* or *uvr* systems. (v) Withholding thymine from *thy rec*$^+$ *uvr*$^+$ bacteria not undergoing thymineless death has no effect on UV sensitivity. Under these conditions one sees higher than normal UV resistance in the presence or absence of thymine. This is due to increased repair carried out by the *uvr* system. To explain these results we postulate that thymine starvation does not inhibit either the *rec* or *uvr* repair pathway directly. Rather it appears that thymine starvation results in increased UV sensitivity in part by inhibiting a function which normally carries out efficient coordination of *rec* and *uvr* pathways.

Mutations that affect the dark repair of ultraviolet light (UV) damage in *Escherichia coli* K-12 form two main groups, *uvr* and *rec*. *uvr* mutants are unable to carry out excision repair and are incapable of host cell reactivation (Hcr) of irradiated phage (10, 18). Excision repair involves the removal of pyrimidine dimers from deoxyribonucleic acid (DNA) in small acid-soluble pieces (3, 20), followed by repair replication (17) and rejoining of the repaired piece with the original DNA strand (19).

Strains deficient in recombinational ability are also UV sensitive (5, 11). These *rec* mutants are sensitive to ionizing radiation (11, 13, 24) and methyl methane sulfonate (Mount, *personal communication*), but are capable of excising dimers (21) and of Hcr (18). It is not clear whether other mutations that cause increased UV sensitivity, such as deficiencies in DNA polymerase I and DNA ligase, are involved directly in either *uvr* or *rec* pathways (7, 14, 16). A strain that is altered in both the *uvr* and *rec* pathways appears to have lost all capacity for dark repair of UV damage (9, 12).

Thymine starvation of cells that do not carry mutations in any repair pathway greatly increases the UV sensitivity of these cells. Since there is no increase during thymine starvation in the fraction of dimers formed at a constant UV dose, the increase in UV sensitivity is evidently due to a loss in the repair capacity of the cells. To determine which UV repair processes are inhibited or lost during thymine starvation, we have examined the effects of thymine starvation on strains which are altered in one or the other of the dark repair pathways.

## MATERIALS AND METHODS

**Bacterial and phage strains.** Bacterial strains used in these experiments were isogenic *E. coli* K-12 derivatives of strain HF4733, an F⁻, *thy, gal, thi, str*⁺ and endonuclease I-deficient strain. In Table 1 we summarize the origin and properties of strains used. AB 1157 (10) was used in the assay for the presence of UV-inducible phages in our strains. Hcr ability was determined with bacteriophage λ c60, a mutant with a lesion in the c1 repressor gene, obtained from L. Astrachan.

**Media and solutions.** Stock cultures of strains were kept in EM9 medium (4) plus 2 μg of thymidine per ml at 4 C. MM + A19 medium is a mixture of 19 amino acids (25 μg of valine per ml; 50 μg of all others per ml, except histidine) in M9 salts (4) and glucose (0.4%) and was used in amino acid starvation experiments with and without added histidine (50 μg/ml). Phage were diluted before irradiation into a radiation buffer consisting of 0.01 M $K_2HPO_4$, 1 mM $MgSO_4$, and 0.1 mM $CaCl_2$ at *p*H 7.0. In our determinations of Hcr, we diluted a phage suspension with tryptone broth and plated along with cells of the bacterial strain to be tested on 1% tryptone broth plates plus 0.1 M NaCl plus thiamine. For soft agar, 0.65% agar was added to the broth. The presence of UV-inducible phages in our strains was tested for by mixing cells of the strain being tested with an indicator bacterial strain in 0.15 M $CaCl_2$ and 0.3 M $MgSO_4$. This was plated on LC agar (2%) containing 1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.002 M $CaCl_2$, 0.1% glucose, and 0.001% thymidine. LC top agar was the same except that the agar concentration was reduced to 0.7%. Thymidine-*methyl*-$^3H$ (5 mCi/0.0605 mg) was obtained from New England Nuclear Corp. The scintillation fluid has been described (21).

**Growth and thymine starvation of strains.** Overnight cultures of each strain were prepared by diluting the stock culture about 500-fold into EM9 medium plus 2 μg of thymidine per ml and incubating without aeration at 37 C overnight. These overnight cultures were diluted in the morning to give an optical density at 650 nm of about 0.08 and grown with aeration to an optical density of about 0.25. Optical densities were determined on a Gilford spectrophotometer. A sample of the culture was centrifuged in a Sorvall RC 2-B refrigerated centrifuge, head number SS34, at 10,000 rev/min for 1 min. Cells to be irradiated were resuspended in 10 times the original volume of M9 salts. Cells to be starved for thymine were resuspended in 10 times the original volume of EM9 medium and incubated with shaking at 37 C. Samples of the cell suspensions to be irradiated during thymine starvation were removed from this culture, centrifuged as described above, and resuspended in the original volume of M9 salts.

**Irradiation of cells.** A 5-ml amount of cells suspended in M9 salts was placed in a 90 by 15 mm petri dish and irradiated with a 15-w General Electric UV germicidal lamp with dose rates of 0.4 to 2 ergs per mm² per sec. The lower dose rate was achieved by irradiating through several layers of Saran wrap. The dose rate was determined with a Fisher short-wave UV meter. Experiments with UV-irradiated samples were done either in the dark or under yellow light, and the plates were incubated in foil-lined baskets to prevent photoreactivation. The cells were irradiated at a concentration of about $5 \times 10^7$ cells/ml in the excision experiments. At this concentration there is no shielding effect due to cell density. In other experiments, the cell concentration was about $5 \times 10^8$/ml.

Ionizing radiation was given to 2-ml samples of cells suspended in M9 salts ($5 \times 10^8$ cells/ml) in 13 by 100 mm tubes. The suspensions were exposed aerobically to the ionizing radiation of cobalt 60 at a dose rate of 7.5 krads/min.

Viable cell concentrations were determined by diluting the cultures with 0.85% saline and counting colonies that appeared after 24 to 48 hr of incubation on Luria broth (23) plates containing 2% agar and 2 μg of thymidine per ml.

**Host cell reactivation.** Phage λ c60 was suspended in radiation buffer at a concentration of $10^7$ plaque-forming units/ml. A 5-ml amount of this suspension was placed in a 90 by 15 mm petri dish and irradiated by a 15-w UV germicidal lamp at a dose rate of 6 ergs per mm² per sec. Unirradiated control samples and irradiated phage were diluted through 1% tryptone broth plus 0.1 M NaCl. Cells used for the determination of Hcr were grown from a

TABLE 1. *Bacterial strains*

| Strain no. | Relevant genotype | | | | Parent | Doubling time (min) in EM9 medium plus 2 μg of thymidine/ml | Obtained from or derivation |
|---|---|---|---|---|---|---|---|
| | *rec* | *uvr* | *thy* | *his* | | | |
| HF4733 | + | + | − | + | HF4726 × AB3049 | 30 | Howard-Flanders |
| SDB1313 | *A56B21* | + | − | − | SDB1006 (20) | 60 | Trimethoprim selection (22) |
| SDB1314 | *A56* | + | − | − | JC4588 (1) | 60 | Trimethoprim selection (22) |
| SDB1317 | + | *B5* | − | + | HF4733 | 55 | P1 transduction of *uvrB5* from AB2434 (23) |
| SDB1318 | *B21C22* | + | − | − | JC4584 (4) | 60 | Trimethoprim selection (22) |
| SDB1320 | + | *B5* | − | − | SDB1321 | 60 | P1 transduction of *uvrB5* from AB2434 (23) |
| SDB1321 | + | + | − | − | JC4583 (4) | 45 | Trimethoprim selection (22) |

diluted overnight culture in EM9 medium plus 2 μg of thymidine per ml to an optical density at 650 nm of 0.600 ($3 \times 10^8$ cells/ml) (4). A 0.2-ml amount of this culture was added to melted soft agar along with 0.1 ml of various dilutions of irradiated phage and poured on plates. In the test for Hcr ability of cells after thymine starvation, a 3-ml sample of cell culture was centrifuged as described above, resuspended in 30 ml of EM9 medium, and incubated at 37 C for 60 min. At this time the culture was centrifuged and resuspended in 3 ml of EM9 medium. A 0.2-ml amount of this cell suspension plus 0.1 ml of various dilutions of irradiated phage was added to the melted soft agar and poured on plates.

**Excision of thymine dimers.** An overnight culture of cells grown in EM9 medium plus 2 μg of thymidine per ml was diluted 100 times into the same medium to an optical density at 650 nm of about 0.02. A 0.15-ml amount of $^3$H-thymidine was added (1 mCi/ml). The cells were grown to an optical density of 0.250, centrifuged as described above, and washed two times with M9 salts.

The sample to be irradiated and tested for excising ability was resuspended in an equal volume of M9 salts. The remainder was suspended in an equal volume of EM9 without added thymidine. Samples were removed from this culture at various times during the period of thymine starvation, centrifuged, irradiated, and assayed for the excision of thymine dimers (21). After irradiation, glucose, Casamino Acids, and thiamine were added to the cell suspension to concentrations found in EM9 medium. Thymidine at a concentration of 2 μg/ml was added for some experiments, as indicated.

**Test for phage production in strains.** Although the strains used in these experiments have no known prophage, they were tested for UV induction of phage. Cells from an overnight culture were diluted 20 times into EM9 medium plus 2 μg of thymidine per ml and grown to an optical density at 650 nm of about 0.250. The culture was centrifuged as described above, resuspended in M9 salts, and irradiated with UV light. A sample was immediately taken to assay for phage infective centers, and after 60 min 0.1 ml of $CHCL_3$ was added to 10 ml of the suspension which was then incubated with shaking at 37 C for 20 min. The suspension was centrifuged, and 0.1 ml of the supernatant fluid was taken and kept at 37 for 10 min to evaporate the $CHCL_3$. A 0.1-ml portion of a suspension of strain AB1157 ($10^9$ cells/ml) in adsorption medium was added, and the mixture was incubated at 37 C for 20 min to allow phage adsorption. Melted LC top agar was added, and the sample was poured onto LC plates, incubated at 37 C, and examined for plaque formation after 24 hr.

**Amino acid starvation.** Histidine auxotrophs were grown overnight in MM + A19 medium with added histidine (50 μg/ml) and thymidine (2 μg/ml), diluted 1:20 to 1:50 into the same medium, and grown to an optical density at 650 nm of 0.25. The bacteria were centrifuged and resuspended in 10 times the original volume of MM + A19 medium with added thymidine (2 μg/ml), but containing no histidine. Labeled thymidine (2 μCi/ml) was added in certain experiments to measure DNA synthesis during histidine starvation. Samples of cells were added to 1 ml of cold 5% trichloroacetic acid, and the precipitates were collected by filtration on membrane filters (Millipore; 0.45 μm pore size). After washing with two 5-ml portions of 5% trichloroacetic acid and 10 ml of 95% ethanol, the filters were dried and placed in vials containing 10 ml of scintillation fluid [0.1 g of 1,4-bis-2-(5-phenyloxazolyl)-benzene plus 4 g of 2,5-diphenyloxazole per liter of toluene]. Samples were counted in a Packard Tri-Carb liquid scintillation spectrometer.

To determine the UV sensitivity of cells after 100 min of amino acid starvation, a sample of a cell culture that had been incubated in MM + A19 medium plus thymidine (2 μg/ml) for 100 min was centrifuged and resuspended in M9 salts and UV irradiated as described above. Another sample was resuspended in MM + A19, allowed to incubate at 37 C, and tested for viability and UV sensitivity after various periods of simultaneous thymine and amino acid starvation.

## RESULTS

**UV sensitivity of various thymine-requiring strains during thymine starvation.** All strains used showed similar kinetics of thymineless death as shown in Fig. 1. All strains were tested for UV-inducible prophages, and no

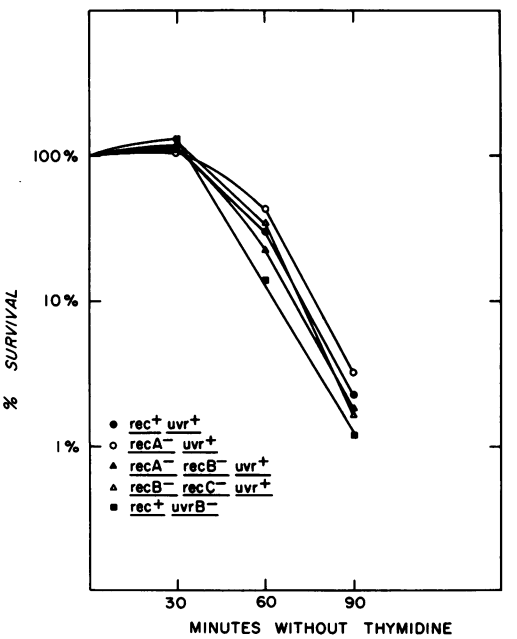


FIG. 1. *Kinetics of thymineless death of various strains. Cells were grown for various times in EM9 medium without added thymidine and plated on Luria agar plates containing thymidine (2 μg/ml). Original cell concentration was about $5 \times 10^8$ cells/ml. Kinetics of thymineless death were the same in those experiments in which the original cell concentration was $5 \times 10^7$ cells/ml. Points are the average values from several (2 to 6) experiments.*

phage could be detected. Changes in UV sensitivity during thymine starvation were observed by determining the fraction of cells of a given strain which survived a fixed dose of UV light after various times of thymine starvation. The dose of irradiation varied with the strains used and was one which gave 10 to 50% survival before thymine starvation. In all strains, the maximum period of UV sensitivity occurred between 40 and 60 min of thymine starvation, after which a more UV-resistant population of cells began to appear in the cells surviving thymineless death. The period of the greatest UV sensitivity was independent of the doubling time of the strains (Table 1).

In these experiments, we observed a large increase in the UV sensitivity of the *rec*$^+$ *uvr*$^+$ strain. The UV sensitivity of strains mutant in either repair pathway was less affected by thymine starvation. To define more clearly the change in sensitivity that occurs during thymine starvation, UV survival curves were determined before and after 60 min of thymine starvation (Fig. 2). Common to all four strains tested is the change from multihit to single-hit kinetics of survival to UV after 60 min of

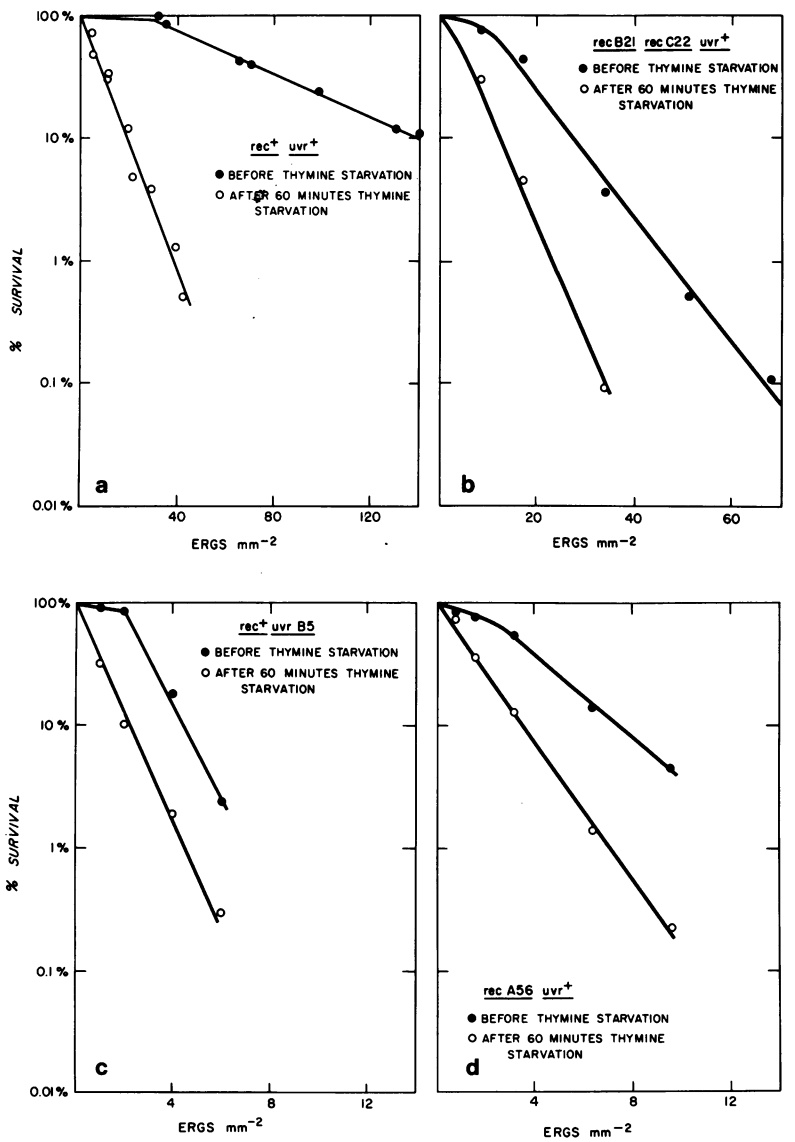


FIG. 2. *UV survival curves before and after 60 min of thymine starvation. a, rec*$^+$ *uvr*$^+$*; b, recB21 recC22 uvr*$^+$*; c, rec*$^+$ *uvrB5; d, recA56 uvr*$^+$.

thymine starvation. The limiting slope at higher UV doses decreases in the *rec*$^+$ *uvr*$^+$ strain but changes very little in the mutant strains after a 60-min period of thymine starvation. In Table 2 the lethal doses ($D_0$ doses) (15) in ergs/log of killing (1/slope) as determined from the straight line portions of the survival curves are compared, and the doses required for 37% survival before and after 60 min of thymine starvation are given. Comparison of the limiting slopes of the survival curves shows that the ability of *rec*$^+$ *uvr*$^+$ cells to repair UV damage decreases sixfold while repair in the mutant strains is relatively little affected by thymine starvation. The effects of thymine starvation on activities that are known to be specific to either pathway were next examined.

**Excision of thymine dimers and host cell reactivation.** Excision of thymine dimers from DNA and Hcr of irradiated phage are processes which are mediated by *uvr* gene products (10, 18). There is a small decrease in Hcr in cells mutant in *rec* genes, but the major portion of phage repair can be attributed to *uvr* gene products (Fig. 3) (11, 18). No excision has been observed in several *uvr* mutants (10, 21), whereas *rec* strains excise at almost the rate of wild-type cells (21). The ability of the *rec*$^+$ *uvr*$^+$ strain to excise thymine dimers did not decrease during the first 90 min of thymine starvation whether or not thymidine was added back after irradiation (Table 3). The capacity of these cells to repair UV damage in irradiated phage did not change after 60 min of thymine starvation (Fig. 3).

**Ionizing radiation.** *rec* cells are especially sensitive to ionizing radiation (13, 24), and *uvr* cells only slightly so (10, 13). Figure 4 shows the effects of ionizing radiation on the cell survival of *rec* and *uvr* mutants and a *rec*$^+$ *uvr*$^+$ strain before and after 60 min of thymine starvation. It can be seen that thymine starvation has very little effect on the sensitivity to ionizing radiation of the *rec*$^+$ *uvr*$^+$ cells.

TABLE 2. *Comparison of UV sensitivity of various strains before and after 60 min of thymine starvation*[a]

| Strain no. | Relevant genotype | | Lethal ($D_0$) dose (ergs/log killing) | | Ratio of lethal doses (0 min/60 min) | 37% Survival (ergs) | |
|---|---|---|---|---|---|---|---|
| | *rec* | *uvr* | 0 min | 60 min | | 0 min | 60 min |
| HF4733 | + | + | 114 | 18 | 6.3 | 75 | 9 |
| SDB1313 | *A56B21* | + | 2.8 | 3.5 | 0.8 | 2.2 | 2.0 |
| SDB1314 | *A56* | + | 6.0 | 3.5 | 1.7 | 4 | 1.6 |
| SDB1317 | + | *B5* | 2.6 | 2.4 | 1.1 | 3.0 | 1.0 |
| SDB1318 | *B21C22* | + | 19.4 | 10.8 | 1.8 | 16.5 | 6.2 |

[a] The results shown in Fig. 2 were used to determine slope and 37% survival. The slope is expressed in the inverse form as ergs required per log of killing in the straight line portions of the UV survival curves (this is called the $D_0$ dose; see reference 15). The 37% survival reflects not only the straight line portion of the survival curves but also the size of the shoulder of the UV survival curves.

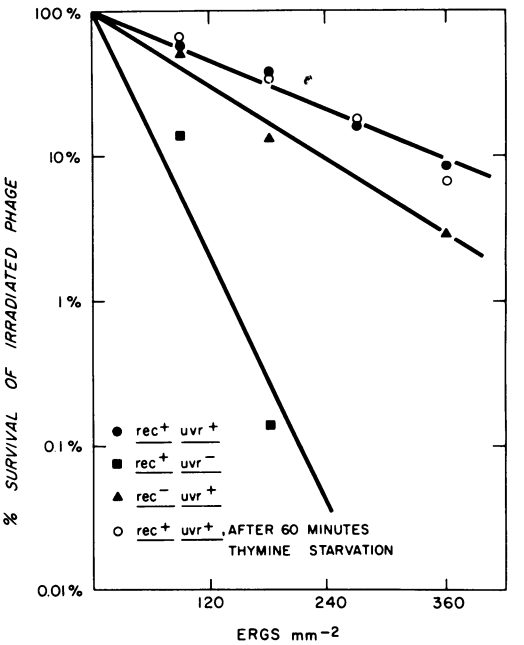


FIG. 3. *Host cell reactivation (Hcr) of irradiated λ c-60 before and after 60 min of thymine starvation in a rec*$^+$ *uvr*$^+$ *strain compared to Hcr in rec*$^+$ *uvrB5 and recA56 uvr*$^+$ *strains without thymine starvation.*

TABLE 3. *Excision in HF4733 (rec*$^+$ *uvr*$^+$*) after a period of thymine starvation*[a]

| Expt no. | Min of thymine starvation | Fraction of radioactivity in dimers (× $10^4$) after UV irradiation | | |
|---|---|---|---|---|
| | | 0 | 30 min | 60 min |
| 1 | 0 | 11.7 | 1.8 | 1.1 |
| | 60 | 7.4 | 1.0 | 0.9 |
| | 90 | 9.2 | 1.2 | 2.0 |
| 2 | 0 | 9.6 | 1.2 | ≤0.5 |
| | 60 | 7.3 | 0.5 | 1.0 |
| | 90 | 7.7 | 0.8 | ≤0.5 |

[a] Cells were irradiated with 200 ergs/mm$^2$. The amount of radioactivity found in the dimer fraction after a period of incubation in M9 salts with added glucose and Casamino Acids is expressed as a fraction of the total radioactivity in acid-insoluble thymine. In experiment 1, thymidine (2 μg/ml) was added after irradiation; in experiment 2, no thymidine was added.

**UV sensitivity after amino acid starvation.** A *rec*$^+$ *uvr*$^+$ *thy his* strain was used to determine the UV sensitivity of *E. coli* after amino acid starvation. This strain had the same kinetics of thymineless death and the same sensitivity to UV irradiation before and after thymine starvation as an isogenic *his*$^+$ strain. After 100 min of histidine starvation, the synthesis of DNA, as measured by the incorporation of labeled thymidine in the cold acid-insoluble fraction, had stopped. Cells that had been prestarved for histidine for 100 min were immune to thymineless death for at least 2 hr after removal of thymidine from the medium. The UV survival curve of a culture that has been prestarved for histidine differs from that of an unstarved culture by the appearance of a large shoulder (Fig. 5). Under these conditions the slope of the UV survival curve at high doses of UV light is not significantly different from that of the unstarved culture. Cells that had been prestarved for histidine for 100 min were then deprived of both histidine and thymidine for 2 hr. The UV sensitivity, as measured by survival after a dose of 360 ergs/mm$^2$, did not change during this 2-hr period of thymine starvation. A UV survival curve, determined after 60 min of thymine starvation was identical to the survival curve determined before thymine starvation (Fig. 5).

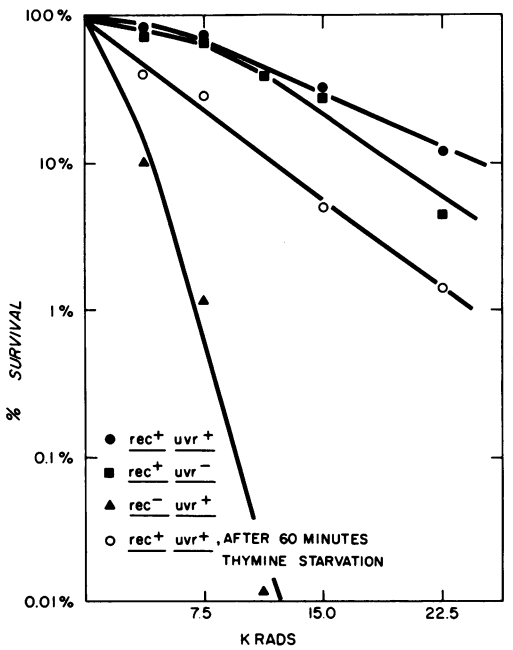


FIG. 4. *Cell survival following ionizing radiation before and after 60 min of thymine starvation of a rec*$^+$ *uvr*$^+$ *strain compared to survival in recA56 recB21 uvr*$^+$ *and rec*$^+$ *uvrB5 strains without thymine starvation.*

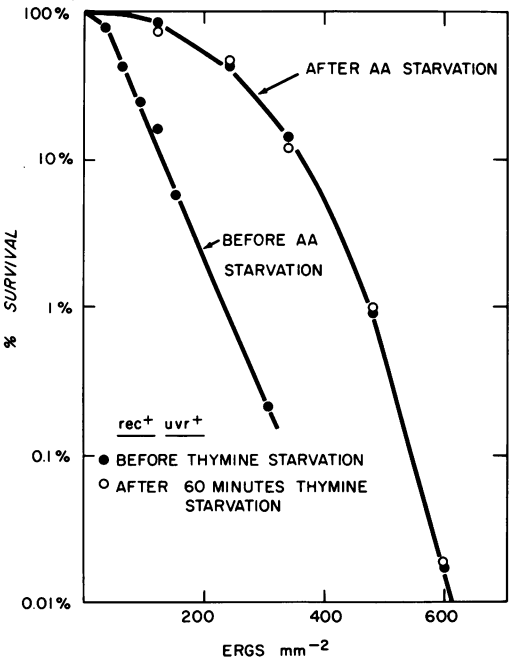


FIG. 5. *UV survival curves before and after 60 min of thymine starvation of rec*$^+$ *uvr*$^+$ *cells (E. coli strain SDB 1321) which were prestarved (100 min) for histidine. Histidine starvation was continued during the 60 min of thymine starvation. Also shown is the survival curve of the same strain without starvation for either histidine or thymine.*

To determine which dark repair system, *rec* or *uvr*, was responsible for the increased UV resistance after amino acid starvation, experiments of this type were also done with *recA recB uvr*$^+$ *his thy* and *rec*$^+$ *uvrB his thy* strains. Prestarvation for an amino acid increases the UV resistance when the strain is *uvr*$^+$ *rec* (Fig. 6A), but not when the strain is *uvr rec*$^+$ (Fig. 6B).

## DISCUSSION

We have observed both a loss of the shoulder and a decrease in the lethal dose (1/slope) in the UV light survival curves of *rec*$^+$ *uvr*$^+$ cells that have been starved for thymine. UV light survival curves of cells that bear mutations in either one of the two dark repair pathways, *rec* or *uvr*, show after a period of thymine starvation a change characterized by a loss of the shoulder, but there is little change in the limiting slopes. *rec*$^+$ *uvr*$^+$ cells which have become immune to thymineless death as a result of prestarvation for a required amino acid show

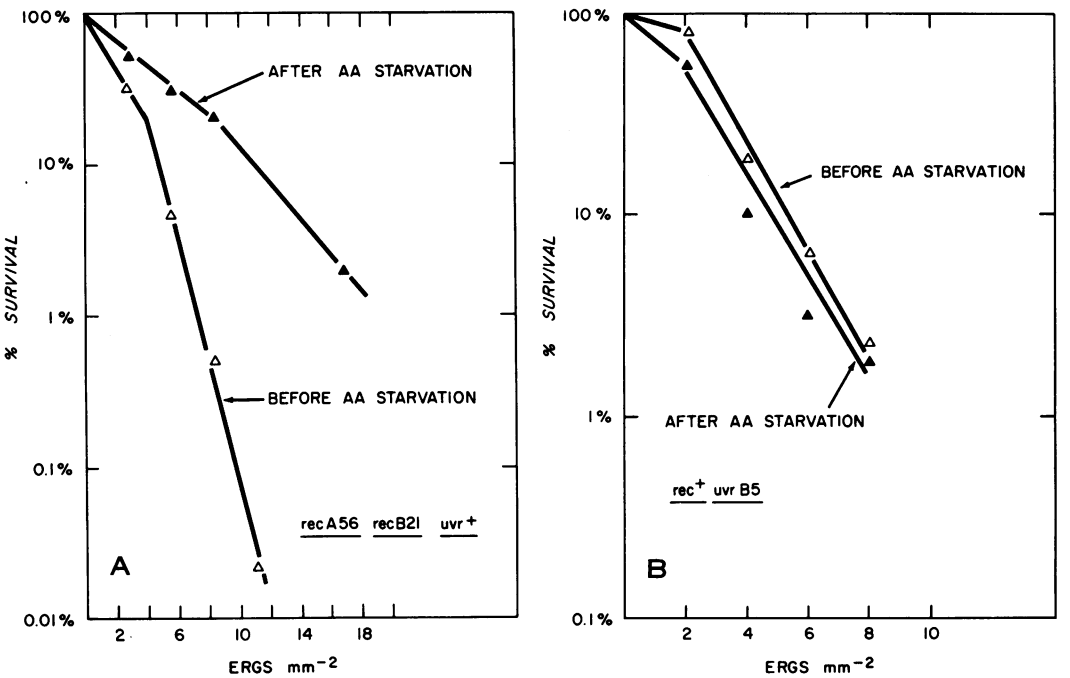


FIG. 6. *UV survival curves before and after 60 min of histidine starvation of recA56 recB21 uvr⁺ (SDB 1313) cells (A) and rec⁺ uvrB5 (SDB 1320) cells (B).*

no change in either the slope or the shoulder of the UV survival curve after a period of thymine starvation (Fig. 5). The presence of a shoulder on a killing curve is attributed to multiple hits (because multiple targets must be inactivated) before the organism dies. This is a widely used model, but there is no proof of correctness of the theory. It is not clear why thymine starvation of all strains tested results in a loss of the shoulder from the UV survival curves. Assuming that the multiple target theory is correct, one possibility is that the prelethal events of thymineless death might influence UV sensitivity. This could occur if these prelethal events inactivate multiple targets, reducing the dose of UV light required to produce a lethal hit. This could have the effect of changing the multihit type of curve to one with single-hit kinetics. Since the mutant strains undergo thymineless death at the same rate as wild-type cells (Fig. 1), it is apparent that the *recA*, *recB recC*, and *uvrB* gene products are not involved in the repair of damage caused by thymine starvation. It appears that the loss of the shoulder observed in the UV survival curves of cells starved of thymine is related to the capacity of the cells to undergo thymineless death (Fig. 5). This is in agreement with the conclusion of Gallant and Suskind (6).

As the dose of UV light is increased, cell death becomes an exponential function of dose. The lethal dose ($D_0$ dose) decreases when mutations that block the repair of UV damage are introduced (Table 2). Thus the lethal dose reflects the repair capacity of a strain. The lethal dose decreases in *rec⁺ uvr⁺* cells which are starved for thymine. Strains that are altered in one of the dark repair pathways show little change in lethal dose after a period of thymine starvation. Evidently, there is some inhibition of repair in *uvr⁺ rec⁺* cells that are starved for thymine not observed in cells in which only one of the dark repair pathways is operating. This suggests that the inhibition affects a function that is operating only when both pathways are carrying out repair. This function might coordinate the two pathways so as to increase efficiency when both are working simultaneously. This possibility is consistent with the observation that the two pathways are synergistic rather than additive in their contribution to survival (9, 18). If our working hypothesis is correct that neither pathway is directly affected by thymine starvation, it predicts that functions that are carried out only by one or the other pathway, in the wild-type cell, should not be affected by thymine starvation. We have examined the effect of thymine starvation on

repair of ionizing radiation (carried out by the *rec* system), Hcr (carried out by *uvr* only), and excision (carried out by *uvr* only). Little or no effect of thymine starvation has been observed on these functions. This is in striking agreement with the predictions of our working hypothesis.

From the experiments with cells that have been prestarved for an amino acid, it is evident that merely withholding thymine from a thymine auxotroph does not lead to the inhibition of repair of UV damage. The large shoulder observed in the UV survival curves of cells prestarved for amino acids has been attributed to the physiological state of the chromosome, i.e., the absence of replication forks (2, 8). It has been postulated that in this state the major portion of repair is carried out by excision repair (2). Consistent with this report, we have found (Fig. 5, 6) that, though amino acid starvation enhances UV survival of *rec*$^+$ *uvr*$^+$ and *recA recB uvr*$^+$ strains, it does not enhance survival of a *rec*$^+$ *uvrB* strain. The repair that occurs in this state is not inhibited by thymine starvation. This may be because the repair is largely due to one system (*uvr*) operating alone.

We conclude that thymine starvation results in increased UV sensitivity in part because it inhibits a function that efficiently coordinates the operation of the *rec* and *uvr* pathways.

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