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Repair of Radiation-Induced Damage in *Escherichia coli*

II. Effect of *rec* and *uvr* Mutations on Radiosensitivity, and Repair of X-Ray-Induced Single-Strand Breaks in Deoxyribonucleic Acid[1]

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Strains of *Escherichia coli* K-12 mutant in the genes controlling excision repair (*uvr*) and genetic recombination (*rec*) have been studied with reference to their radiosensitivity and their ability to repair X-ray-induced single-strand breaks in deoxyribonucleic acid (DNA). Mutations in the *rec* genes appreciably increase the radiosensitivity of *E. coli* K-12, whereas *uvr* mutations produce little if any increase in radiosensitivity. For a given dose of X-rays, the yield of single-strand breaks has been shown by alkaline sucrose gradient studies to be largely independent of the presence of *rec* or *uvr* mutations. The *rec*$^+$ cells (including those carrying the *uvrB5* mutation) could efficiently rejoin X-ray-induced single-strand breaks in DNA, whereas *recA56* mutants could not repair these breaks to any great extent. The *recB21* and *recC22* mutants showed some indication of repair capacity. From these studies, it is concluded that a correlation exists between the inability to repair single-strand breaks and the radiosensitivity of the *rec* mutants of *E. coli* K-12. This suggests that unrepaired single-strand breaks may be lethal lesions in *E. coli*.

Mutations in the genes controlling excision repair (*uvr*) and genetic recombination (*rec*) have been shown to alter the radiation sensitivity of *Escherichia coli* K-12 (6, 8-11). Double-chain breaks in deoxyribonucleic acid (DNA) induced by X-irradiation appear to be lethal lesions in a radioresistant strain of *E. coli* (12). Radioresistant strains, such as *E. coli* B/r, repair single-strand breaks induced by X-irradiation, whereas more radiosensitive strains, such as *E. coli* B_{s-1}, lack the ability to repair these breaks (14). It has been suggested that single-strand breaks may be lethal to those cells unable to repair these lesions (12, 14). We attempted to test the generality of this hypothesis by employing eight derivatives of *E. coli* K-12 which are mutant in *uvr* and *rec* genes. We find that a correlation does exist between the radiosensitivity of these mutants and their ability to repair single-strand breaks induced by X-irradiation. [The first paper in this series (17) investigates the effect of *rec* mutations on postreplication repair of damage due to ultraviolet (UV) radiation. At the doses of UV-radiation used, *recA* strains are unable to perform this type of repair, whereas *rec*$^+$, *recB*, and *recC* strains could perform this function (irrespective of the presence of *uvr* mutations).]

[1] A preliminary account of this work was presented at the 14th Annual Meeting of the Biophysical Society, Baltimore, Md., February 1970.

MATERIALS AND METHODS

Bacterial strains. The bacteria utilized are thymine-requiring multiple auxotrophs of *E. coli* K-12 mutant in *uvr* or *rec* genes (Table 1).

Media. The liquid minimal medium (MM) and supplemented minimal medium agar (SMM-agar) have been previously described (7). Supplemented MM (SMM) was MM to which the following additions were made: required L-amino acids to a final concentration of 10^{-3} M, 2 μg of thymine per ml, and 0.5 μg of thiamine hydrochloride per ml.

Survival curves. The bacteria were innoculated from stationary-phase overnight cultures into prewarmed SMM and incubated at 37 C in a Gyrotory water bath. Cells in exponential growth (10^7 to 5×10^7 cells/ml) were irradiated in their growth medium in air. Immediately after irradiation, the cells were diluted in double-distilled water and plated on SMM-agar. Colonies were counted after 1 to 3 days of incubation at 37 C.

Irradiations. Cell suspensions (2 ml in SMM; 10^7 to 5×10^7 cells/ml) exposed to air were irradiated with X-rays (50 ma and 48 ma, 0.3-mm Al filters) using the twin-tube 50 KVP beryllium window X-ray unit developed by Loevinger and Huisman (13). The

TABLE 1. *Escherichia coli K-12 derivatives used*[a]

Designation	Mating type	Relevant genotype	Other markers	Reference	D_{37} (krads)
AB2487	F⁻	*recA13*	*thr leu arg his thi pro thy ara lac gal mtl xyl str*ʳ *T6*ʳ λˢ	10	1.1
AB2497	F⁻		*thr leu arg his thi pro thy ara lac gal mtl xyl str*ʳ *T6*ʳ λˢ	10	5.7
AB2499	F⁻	*uvrB5*	*thr leu arg his thi pro thy ara lac gal mtl xyl str*ʳ *T6*ʳ λˢ	11	5.7
SR72	F⁻	*recA56 uvrB5*	*thr leu arg his thi pro thy (ara) lac (gal) (mtl) (xyl) str*ʳ *T6*ʳ λˢ		1.0
SR74	Hfr	*recA56*	*thr ilv thi thy (spm*ʳ*)*		1.4
SR78	F⁻	*recB21*	*thr leu arg his thi pro thy (ara) (lac) (gal) (mtl) (xyl) str*ʳ *T6*ʳ λˢ		1.7
SR88	F⁻	*recC22*	*thr leu arg his thi pro thy (ara) (lac) (gal) (mtl) (xyl) str*ʳ *T6*ʳ λˢ		1.7
SR111	F⁻	*recA13 recB21*	*thr leu arg his thi pro thy ara lac gal mtl xyl str*ʳ *T6*ʳ λˢ	21	0.8

[a] Abbreviations (3, 4, 19): the symbols *arg*, *his*, *ilv*, *leu*, *pro*, *thi*, *thr*, *thy* denote requirements for arginine, histidine, isoleucine and valine, leucine, proline, thiamine, threonine, and thymine, respectively; *ara*, *gal*, *lac*, *mtl* and *xyl*, the inability to utilize arabinose, galactose, lactose, mannitol, and xylose, respectively; *T6*, λ, *spm*, and *str*, response to the phages T6 and λ and to the antibiotics spectinomycin and streptomycin (ʳ indicates resistance, and ˢ, sensitivity); *rec* denotes genes affecting genetic recombination and ultraviolet (UV) sensitivity; *uvr* designates genes affecting host-cell reactivation and UV sensitivity. Markers in parentheses have not been tested, but are inferred from the characteristics of the parent strains. D_{37} is the dose of radiation at which 37% of bacteria survive to form colonies (calculated from Fig. 1).

mean dose rate for the 2-ml suspension was 9.8 krad/min. For 3.5-ml cell suspensions irradiated as above, the mean dose rate was 8.8 krad/min. All irradiations were carried out with the samples in plastic petri dishes (35 mm diameter).

Alkaline sucrose gradient studies. The ability of the various bacterial strains to repair X-ray-induced single-strand breaks was studied by using the method of McGrath and Williams (14) for sedimenting DNA in alkaline sucrose gradients. Cells were labeled in their DNA by inoculating stationary-phase cells into prewarmed SMM containing tritiated thymine (17.4 c/mmole, 25 μc/ml of media; New England Nuclear Corp., Boston, Mass.). The size of the inoculum was adjusted to permit three to four generations of growth in the label-containing media before harvesting the cells in exponential growth (at 10^7 to 5×10^7 cells/ml). The cells were rapidly filtered (type HA membrane filter, 0.45 μm; Millipore Corp., Bedford, Mass.), washed, and resuspended in prewarmed, unlabeled MM without glucose. Samples (3.5 ml) were then irradiated in air at room temperature. They were then either immediately stored in ice or, after the addition of glucose and the MM supplement, were reincubated at 37 C for the desired period of time and then transferred to chilled centrifuge tubes. All samples were pelleted and washed twice in cold 0.05 M tris-(hydroxymethyl)aminomethane (Tris) (*p*H 8.1). The cells were then resuspended in 0.5 ml of 0.05 M Tris (*p*H 8.1) and 0.3-ml samples were employed for the production of spheroplasts (16). Samples (20 μliters) of the spheroplast suspension (≃10^6 spheroplasts) were mixed into a 0.2-ml layer of 0.5 N NaOH on top of a 4.6-ml gradient of 5 to 20% sucrose (w/v) in 0.1 N NaOH. The gradient tubes were centrifuged in the SW 50.1 rotor for 105 min at 30,000 rev/min at 20 C in an ultracentrifuge (Beckman model L2-65B). After centrifugation approximately 37 fractions of 5 drops each were collected and counted by the procedure of Kaplan (12). Data are plotted as per cent of total acid-insoluble radioactivity recovered from the gradient against fraction number.

RESULTS

Survival curves. X-ray survival curves of the eight strains tested are shown in Fig. 1. The D_{37} values (dose of radiation at which 37% of bacteria survive to form colonies) for the various strains are given in Table 1. The *rec* mutations had a considerable effect on the radiosensitivity of the bacteria (three- to seven fold increase), whereas the *uvr* mutations had little, if any, effect. The *recA13 recB21* mutant was only slightly more sensitive than the *recA13* mutant from which it was derived.

Intrinsic sensitivity. The number of single-strand breaks in bacterial DNA after irradiation (22.0 krad) was essentially the same for all strains tested. By using the first moment of each alkaline sucrose gradient sedimentation curve as a convenient index of sedimentation behavior (12), no significant difference was observed between the radioresistant strains (*rec*⁺, *uvrB5 rec*⁺) and the relatively radiosensitive strains (*recA56*, *recB21*, *recC22*, and *recA13 recB21*) after an

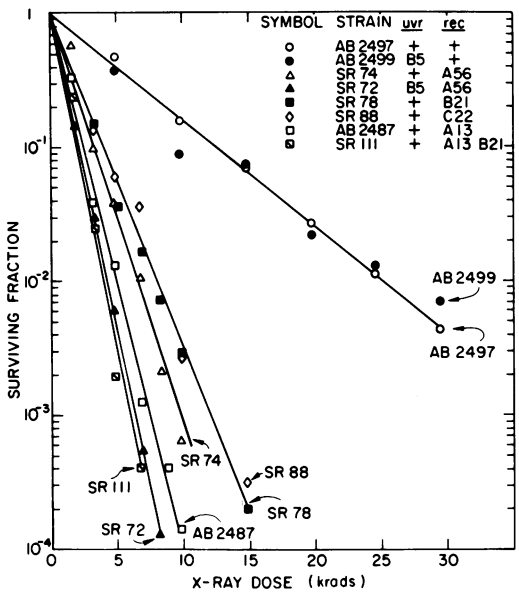

FIG. 1. *X-ray sensitivities of uvr and rec mutants of E. coli K-12. Cells in exponential growth were irradiated in SMM, diluted in double-distilled water, plated on SMM-agar, and incubated from 24–72 hr at 37 C.*

irradiation dose of 22.0 krad. The average position for the first moment (as a fraction of the distance from the meniscus) in the irradiated samples before reincubation was 0.47 ± 0.04 for the resistant strains, whereas, for the relatively radiosensitive strains, a value of 0.45 ± 0.04 was obtained.

Repair of single-strand breaks. The relative abilities of the various strains to repair the single-strand breaks produced in their DNA by X-irradiation can be inferred from the sedimentation profiles in Fig. 2 and 4 to 7. By comparing the sedimentation profile of unirradiated *rec*$^+$ cells (Fig. 2A) with that of irradiated cells (Fig. 2B), it can be seen that irradiation produced a decrease in the molecular weight of the bacterial DNA. A comparison of the sedimentation profiles obtained after 20, 30, and 60 min postirradiation incubation (Fig. 2C–E) shows that reincubation resulted in a progressive shift in the sedimentation profiles toward that of the unirradiated cells. We interpret this restoration to reflect the functioning of a repair process that rejoins broken pieces of DNA with bonds stable in alkali.

The inherent insensitivity of the alkaline sucrose gradient method for detecting small amounts of single-strand breakage, day-to-day variation between runs, and the difficulty of obtaining reproducible control peaks for the unirradiated *rec*$^+$ cells have all contributed to our inability to quantitate satisfactorily the rate of repair of single-strand breaks for the various mutants. However, if the sedimentation data for five experiments on the *uvr*$^+$ *rec*$^+$ strain are pooled, a qualitative picture of the repair kinetics can be obtained by comparing the increase in molecular weight of the DNA as the irradiated bacteria were permitted to reincubate. It can be seen (Fig. 3) that the rejoining process is essentially completed in 40 to 60 min for irradiated *rec*$^+$ cells reincubated in SMM.

Figure 4 illustrates that the extent of repair that occurs in the *uvrB5 rec*$^+$ mutant cannot be distinguished from that which occurs in the *uvr*$^+$ *rec*$^+$ strain. A mutation at the *uvr* gene, therefore, has little effect upon the repair of X-ray-induced single-strand breaks in DNA.

In contrast, the *recA56* mutant appears unable to rejoin the X-ray-induced single-strand breaks to any great extent, even after a postirradiation incubation period of 70 min (Fig. 5). However, the results are complicated by a decrease in the total number of acid-insoluble counts on the gradient with increasing incubation time (from 2.63×10^4 to 6.77×10^3 counts/min in 70 min) and the appearance of an additional low-molecular-weight peak near the meniscus. These complications are a consequence of the extensive DNA degradation that occurs in *recA* derivatives after both UV and X-irradiation (2, 15). To circumvent this problem of degradation, a *recA13 recB21*

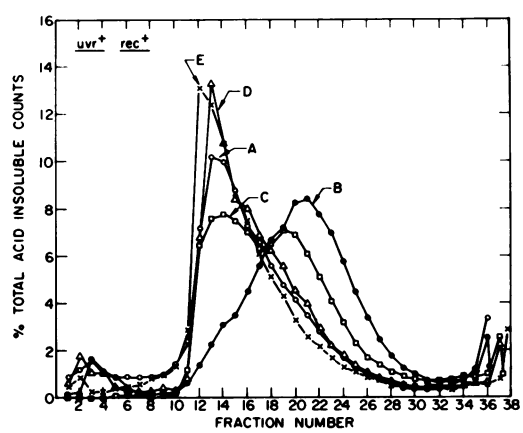

FIG. 2. *Sedimentation patterns of DNA from irradiated E. coli K-12 AB2497 (uvr*$^+$ *rec*$^+$*). (A) Unirradiated; (B) 22 Kr, no reincubation; (C) 22 Kr, 20-min reincubation; (D) 22 Kr, 30-min reincubation; (E) 22 Kr, 60-min reincubation. Cells were converted to spheroplasts and lysed on top of the alkaline sucrose gradients (5 to 20% sucrose in 0.1 N NaOH). Sedimentation was performed in an SW 50.1 rotor for 105 min at 30,000 rev/min at 20 C. The direction of sedimentation is from right to left.*

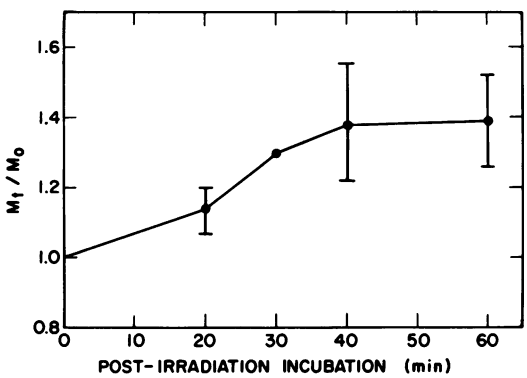

FIG. 3. *Kinetics of repair of single-strand breaks for the uvr+ rec+ (AB2497) derivative of E. coli K-12. The increase in the molecular weight of the bacterial DNA upon postirradiation incubation was calculated from the formula of Burgi and Hershey (1): $D_t/D_0 = (M_t/M_0)^a$, where D_t, D_0 represent the sedimentation distances and M_t, M_0 the molecular weights of the DNA of the respective samples. The subscripts indicate the time (in minutes) of postirradiation incubation. The value of 0.40, determined by Studier (18), was employed for the exponent a. The D values were approximated by use of the normalized distance from the meniscus to the location of the first moment of each sedimentation profile. The data represent a composite of five separate alkaline sucrose gradient sedimentation studies on the uvr+ rec+ (AB2497) derivative performed as described in the legend to Fig. 2. The vertical bars represent the standard deviations from the mean values* (●).

double mutant was employed. This double mutant shows the low level of DNA degradation after UV irradiation characteristic of strains carrying *recB21* alone (20). A similar low level of degradation of DNA by this double mutant was found after X-irradiation. The sedimentation profiles obtained (Fig. 6) show that, like the *recA56* mutant, this *recA13 recB21* derivative exhibits only a small capacity for the repair of X-ray-induced single-strand breaks.

Sedimentation profiles of the *recC22* derivative show some repair of single-strand breaks (Fig. 7). However, the shift in the sedimentation patterns for the reincubated cells is not as substantial as that of the *rec*+ cells, and an appreciable amount of the DNA remains in the nonrepaired low-molecular-weight peak. A similar result was obtained for the *recB21* mutant (*not shown*). Whatever rejoining occurs in the *recC22* and *recB21* derivatives takes place during the first 30 min of incubation.

DISCUSSION

Survival curves. The X-ray survival curves reported here agree qualitatively with those which have been reported in the literature (6, 9). Mutations in the *uvr* genes have little, if any, effect on X-ray sensitivity, whereas *recA* mutations have a considerable effect. The *recB21* and *recC22* mutants are less sensitive than the *recA* mutants. The double-mutant strain, *recA13 recB21*, was found to be only slightly more sensitive to X-irradiation than the *recA13* mutant.

Production and repair of single-strand breaks. The results of the alkaline sucrose gradient studies presented here indicate that for a given dose of X-rays the yield of single-strand breaks

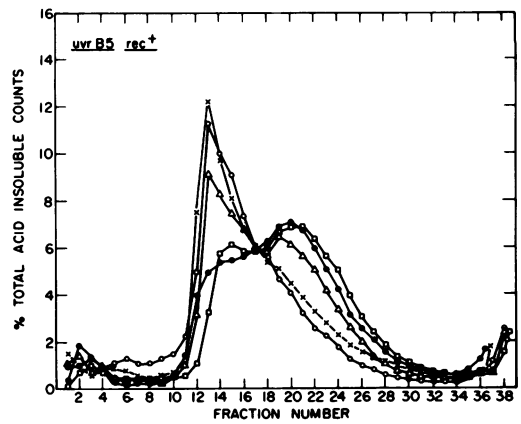

FIG. 4. *Sedimentation patterns of DNA from irradiated E. coli K-12 AB2499 (uvrB5 rec+). (○) Unirradiated; (●) 22 Kr, no reincubation; (□) 22 Kr, 15-min reincubation; (△) 22 Kr, 30-min reincubation; (×) 22 Kr, 60-min reincubation. Lysis and sedimentation performed as in Fig. 2.*

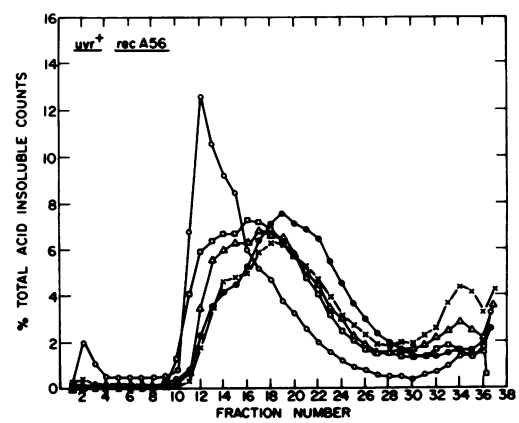

FIG. 5. *Sedimentation patterns of DNA from irradiated E. coli K-12 SR74 (uvr+ recA56). (○) Unirradiated; (●) 22 Kr, no reincubation; (□) 22 Kr, 20-min reincubation; (△) 22 Kr, 40-min reincubation; (×) 22 Kr, 70-min reincubation. Lysis and sedimentation were performed as in Fig. 2.*

is essentially independent of the presence of *rec* or *uvr* mutations. The difference in the radiosensitivities of these strains may therefore reflect the ability of the mutant strains to repair X-ray-induced lesions. We have examined the ability of these strains to rejoin single-strand breaks produced in their DNA by X-irradiation. The *rec*$^+$ cells (including those carrying the *uvrB5* mutation) could rejoin single-strand breaks, whereas *recA56* mutants showed little capacity to repair these breaks at the X-ray dose employed (22 krad). Similar findings have been reported for other *rec*$^+$ (JC1557) and *recA* (JC1569b) derivatives of *E. coli* K-12 after lower doses of irradiation (7.5 krad; reference 15). The lack of any appreciable capacity of the *recA* mutants to repair single-strand breaks correlates well with their high radiosensitivities.

Further evidence supporting the hypotheses that *recA* mutants cannot repair single-strand breaks to any great extent and that unrepaired single-strand breaks may lead to cell death has been obtained from investigations involving the postirradiation treatment of *rec*$^+$ and *recA* derivatives with hydroxyurea, which proved later to have several unidentified contaminants. Active samples of the drug produced a potentiation of X-ray-induced killing in *rec*$^+$ strains and, from alkaline sucrose gradient studies, appeared to irreversibly inhibit the repair of X-ray-induced single-chain breaks. If *rec*$^+$ cells were incubated after irradiation for a period previously shown to be sufficient to restore the sedimentation properties of their DNA to those of DNA from unirradiated cells, the sensitivity to the drug was lost. *RecA* cells were not subject to potentiation

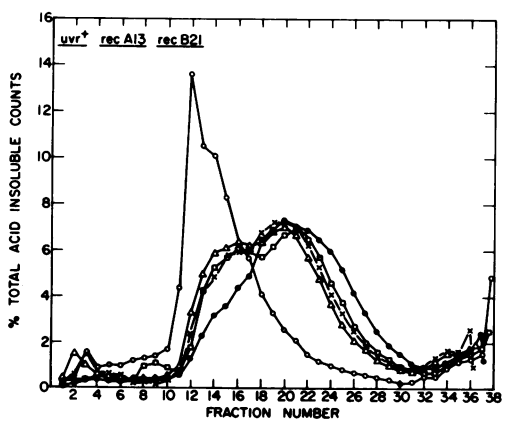

FIG. 6. *Sedimentation patterns of DNA from irradiated E. coli K-12 SR111 (uvr*$^+$ *recA13 recB21). (○) Unirradiated; (●) 22 Kr, no reincubation; (□) 22 Kr, 20-min reincubation; (△) 22 Kr, 40-min reincubation; and (×) 22 Kr, 60-min reincubation.*

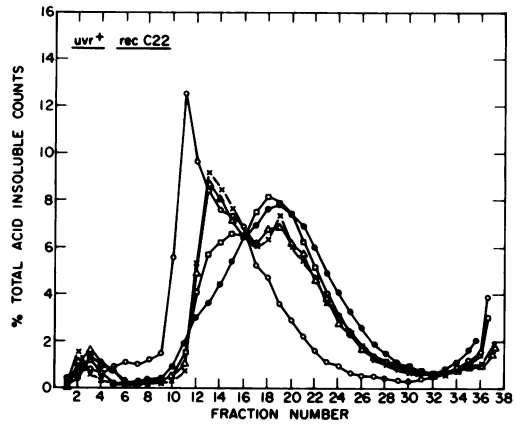

FIG. 7. *Sedimentation patterns of DNA from irradiated E. coli K-12 SR88 (uvr*$^+$ *recC22). (○) Unirradiated; (●) 22 Kr, no reincubation; (□) 22 Kr, 10-min reincubation; (△) 22 Kr, 30 min reincubation; (×) 22 Kr, 60-min reincubation.*

by active drug samples administered immediately after irradiation nor did postirradiation incubation before drug treatment alter this (D. S. Kapp and K. C. Smith, Nature, *submitted for publication*).

All active samples of hydroxyurea proved to be chromatographically heterogeneous but not all heterogeneous samples were active and no apparently homogenous sample was active. A report from this laboratory on the capacities of a number of different "hydroxyurea" samples to potentiate radiation-induced killing in *rec*$^+$ strains and the degree to which the inhibition of single-chain break repair is reversible on subsequent removal of the drug is in preparation.

Our sucrose gradient results indicate that the *recB21* and *recC22* mutants show some slight capacity for the repair of X-irradiation-induced single-strand breaks. This correlates with the survival data indicating that, although they are considerably more radiosensitive than the *rec*$^+$ strains, they are somewhat more radioresistant than the *recA* mutants. Studies on the sensitization of *recA13* and *recC22* mutants to X-rays by oxygen and triacetoneamine-*N*-oxyl (TAN) have also suggested that a possible difference exists in ability of these mutants to repair X-ray-induced damage (5).

The finding that *uvrB5 rec*$^+$ cells show little, if any, difference from *uvr*$^+$ *rec*$^+$ cells in their ability to repair single-strand breaks is in agreement with the similar radiosensitivities of these strains. It therefore appears that the repair of single-strand breaks can occur in the absence of the excision enzymes controlled by the *uvr* genes.

In summary, the results of this investigation

support the hypothesis that unrepaired single-strand breaks may be lethal lesions in *E. coli* (12, 14, 15).

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LITERATURE CITED

1. Burgi, E., and A. D. Hershey. 1963. Sedimentation rate as a measure of molecular weight of DNA. Biophys. J. **3**:309–321.
2. Clark, A. J., M. Chamberlin, R. P Boyce, and P. Howard-Flanders. 1966. Abnormal metabolic response to ultraviolet light of a recombination deficient mutant of *Escherichia coli* K-12. J. Mol. Biol. **19**:442–454.
3. Curtiss, R., III. 1968. UV-induced genetic recombination in a partially diploid strain of *Escherichia coli*. Genetics **58**:9–54.
4. Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**:61–76.
5. Emmerson, P. T. 1968. Sensitization of anoxic recombination-deficient mutants of *Escherichia coli* K-12 to X-rays by triacetoneamine N-oxyl. Radiat. Res. **36**:410–417.
6. Emmerson, P. T. 1968. Recombination deficient mutants of *Escherichia coli* K-12 that map between thyA and argA. Genetics **60**:19–30.
7. Ganesan, A. K., and K. C. Smith. 1968. Dark recovery processes in *Escherichia coli* irradiated with ultraviolet light. I. Effect of *rec*$^-$ mutations on liquid holding recovery. J. Bacteriol. **96**:365–373.
8. Howard-Flanders, P. 1968. DNA repair. Annu. Rev. Biochem. **37**:175–200.
9. Howard-Flanders, P., and R. P. Boyce. 1966. DNA repair and genetic recombination: studies on mutants of *Escherichia coli* defective in these processes. Radiat. Res. Suppl. **6**:156–184.
10. Howard-Flanders, P., and L. Theriot. 1966. Mutants of *Escherichia coli* K-12 defective in DNA repair and in genetic recombination. Genetics **53**:1137–1150.
11. Howard-Flanders, P., R. P. Boyce, and L. Theriot. 1966. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics **53**:1119–1136.
12. Kaplan, H. S. 1966. DNA-strand scission and loss of viability after X-irradiation of normal and sensitized bacteria. Proc. Nat. Acad. Sci. U.S.A. **55**:1442–1446.
13. Loevinger, R., and P. Huisman. 1965. A twin-tube 50-Kvp beryllium-window X-ray unit for microbial radiobiology. Radiat. Res. **24**:357–367.
14. McGrath, R. A., and R. W. Williams. 1966. Reconstruction *in vivo* of irradiated *Escherichia coli* deoxyribonucleic acid; the rejoining of broken pieces. Nature **212**:534–535.
15. Morimyo, M., H. Zen-ichiro, and K. Suzuki. 1968. Appearance of low molecular weight DNA in a *rec*$^-$ mutant of *Escherichia coli* K-12 irradiated with X-rays. J. Radiat. Res. (Japan) **9**(1):19–25.
16. Rupp, W. D., and P. Howard-Flanders. 1968. Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. J. Mol. Biol. **31**:291–304.
17. Smith, K. C., and D. H. C. Meun. 1970. Repair of radiation induced damage in *Escherichia coli*. I. Effect of *REC* mutations on post-replication repair of damage due to ultraviolet radiation. J. Mol. Biol., *in press*.
18. Studier, F. W. 1965. Sedimentation studies of the size and shape of DNA. J. Mol. Biol. **11**:373–390.
19. Taylor, A. L., and C. H. Trotter. 1967. Revised linkage map of *Escherichia coli*. Bacteriol. Rev. **31**:332–353.
20. Willets, N. S., and A. J. Clark. 1969. Characteristics of some multiply recombination-deficient strains of *Escherichia coli*. J. Bacteriol. **100**:231–239.