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Effect of Quinacrine on X-Ray Sensitivity and the Repair of Damaged DNA in *Escherichia coli* K-12[1]

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FUKS, Z., AND SMITH, K. C. Effect of Quinacrine on X-ray Sensitivity and the Repair of Damaged DNA in *Escherichia coli* K-12. *Radiat. Res.* **48,** 63–73 (1971).

Quinacrine (0.15 m*M*), when added to the postirradiation incubation medium of *E. coli* K-12 *rec*$^+$ cells, markedly potentiated the killing induced by x-irradiation. Alkaline sucrose gradient studies showed that quinacrine was a potent inhibitor of the repair of DNA single-chain breaks in *rec*$^+$ cells and that this inhibition was largely irreversible. The *recA13* mutant (deficient in the slow repair of x-ray induced single-chain breaks in DNA) did not show radiation sensitization by quinacrine. The possible use of quinacrine as a radiation potentiator in humans is discussed.

INTRODUCTION

Recent investigations of x-ray-induced damage in bacteria have elucidated the role of DNA single-chain breaks in the radiosensitivity of *E. coli* (*1*).[2] While double-strand breaks in DNA appear to cause irreversible lethal damage (*3*), single-strand breaks can readily be repaired by many wild-type, radioresistant strains of *E. coli* (*1–5*). Mutants of *E. coli* that are deficient in genetic recombination (*rec*) are also deficient in one mechanism for the repair of single-chain breaks (*1*, *5*). For these repair deficient mutants, DNA single-strand breaks are probably lethal (*1*, *3*).

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[2] Recently it has been shown that there is a system in *E. coli* for the fast repair of x-ray-induced single-chain breaks in DNA (*2*). This system requires DNA polymerase (*pol*) and repairs about 85% of the chain breaks in a few minutes in buffer. The recombinational (*rec*) repair system repairs the remaining single-chain breaks in about 40 minutes in growth medium (*1*). The x-ray sensitivities of *rec* and *pol* mutants are about the same (*1*, *2*). In the present work, DNA polymerase dependent repair was largely complete before the quinacrine was added. Therefore, we are only concerned here with the effect of quinacrine on the *rec* repair system.

The discovery of genetically controlled mechanisms for the repair of radiation damage has suggested a new class of radiosensitizers—chemicals that can be added after irradiation and which prevent the repair of DNA and thus convert potentially repairable damage into lethal lesions. Numerous chemicals have been shown to potentiate the killing of x-irradiated cells: actinomycin D (*6–8*), acriflavine (*8*)[3], ethidium bromide (*8*), caffeine (*8–10*), Lucanthone (*11*), chloroquine (*10*), hydroxyurea (*12*) and an unidentified impurity present in certain commercial preparations of hydroxyurea (*13*). The suggestion has frequently been made that these agents may sensitize by virture of inhibiting repair but except for the case of the "hydroxyurea contaminant" (*13*) and acriflavine,[3] the prevention of DNA single-strand break repair was not proven directly.

Quinacrine (Atabrin), well known for its wide use in the therapy of malaria, belongs to a group of intercalating agents known as amino acridines which readily bind to DNA (*14–16*). In this report we describe the inhibitory effect of quinacrine on the *rec* gene controlled repair of single-strand breaks in DNA and its effect on the radiosensitivity of certain strains of *E. coli*.

MATERIALS AND METHODS

The bacterial strains employed were AB2497 (*rec*$^+$) and AB2487 (*recA13*) which are thymine requiring multiple auxotrophs of *E. coli* K-12. The mutant *recA13* is deficient in genetic recombination and is x-ray sensitive (*1*, *17*). Growth media consisted of liquid supplemented minimal medium (SMM) and supplemented minimal medium-agar (SMM-agar) as described previously (*1*, *18*). Aqueous solutions of quinacrine hydrochloride (Sigma Chemical, Inc.) were made up just prior to their use.

Overnight stationary-phase cultures were diluted 1:50 in fresh SMM and grown at 37°C in a Gyrotory water bath (New Brunswick Scientific) to exponential growth phase (5×10^7 cells/ml). Bacteria were then harvested by centrifugation in a Sorvall centrifuge (Model SS-1) for 5 minutes at 3000 rpm at room temperature and resuspended in nonsupplemented salts medium. Two-milliliter samples were placed in sterile plastic Petri dishes (35 mm diameter), exposed to air, and were irradiated at room temperature using the twin-tube beryllium window x-ray unit (50 kV, 50 and 48 mA, 0.3 mm Al filtration) developed by Loevinger and Huisman (*19*). The dose rate was 9.8 krads/min. Immediately after irradiation, the medium was supplemented with glucose, amino acids, thymine, thiamine, and when indicated, with quinacrine. Unless otherwise stated, they were then reincubated at 37°C in the Gyrotory water bath for an additional 60 minutes.

Survival was measured by the ability of single bacteria to form colonies. For this purpose, cells were diluted in double distilled water and plated on SMM-agar. Colonies were counted after 48–72 hours of incubation at 37°C.

[3] D. S. Kapp, Production and repair of x-ray-induced strand breaks in DNA: *in vitro* and *in vivo* studies. Thesis, Stanford University (1970).

The ability of the cells to repair x-ray-induced single-strand breaks was studied by a modification of the McGrath and Williams (*4*) method for sedimenting DNA in alkaline sucrose gradients. An overnight culture of AB2497 (*rec*$^+$) was diluted to 3×10^7 cells/ml and thymine–methyl-^{3}H [Lot No. 560-023, New England Nuclear, 13.6 Ci/mmole] was added to a concentration of 0.34 μg/ml (40 μCi/ml) (total thymine, 2.34 μg/ml). The culture was grown to $\sim 1.1 \times 10^8$ cells/ml, filtered, washed, and resuspended in complete growth medium (nonradioactive). The cells at 3.2×10^8 cells/ml were irradiated at room temperature in air with a dose of 20 krads. A control and irradiated sample were stored in ice. One irradiated sample was incubated for 60 minutes at 37°C and then stored in ice. Quinacrine was added to a final concentration of 75 μg/ml (0.15 m*M*) to two other irradiated samples in complete growth medium. At the end of 60 minutes at 37°C one sample was stored in ice and the other sample was filtered and washed and resuspended in normal growth medium (without quinacrine) and incubated for 60 minutes at 37°C. All the samples were then centrifuged, resuspended in cold 0.05 *M* Tris buffer at pH 8.1. The cells were adjusted by dilution to give 3.4×10^8 cells/ml and converted to spheroplasts (*1*). Twenty microliters of the spheroplasts (equivalent to $\sim 4 \times 10^6$ cells) was added to a 0.1-ml layer of 0.2% Sarkosyl NL30(Geigy) in 0.5 *N* NaOH (*20*) on top of a 4.8-ml gradient of 5–20% sucrose in 0.1 *N* NaOH. The samples were spun at 30,000 rpm for 105 minutes at 20°C, collected, and assayed as described previously (*1*).

RESULTS

Effect on Viability of Postirradiation Treatment with Quinacrine

The addition of quinacrine to the postirradiation incubation mixture of *rec*$^+$ cells caused a marked enhancement of the x-irradiation killing effect (Fig. 1).

This potentiation of killing may be conveniently described in terms of the Survival Reduction Factor (SRF) which, is the decrease in colony-forming units obtained as a result of treating the irradiated cells with the drug. This value, corrected for drug toxicity to the unirradiated controls, was computed as:

$$\text{SRF} = \frac{CD/C}{ID/I},$$

where C is the number of colony-forming units at zero-time in the unirradiated controls; CD is the number of colony-forming units in unirradiated controls treated for a given time with the drug; I is the number of colony-forming units immediately after irradiation, and ID is the number of colony-forming units after incubation of irradiated cells with the drug for a given time.

An SRF of about 4 was obtained with nontoxic concentrations of quinacrine but this value rose quickly as toxicity was reached in the unirradiated cells (Fig. 1). Nearly maximal potentiation of x-ray killing was achieved with 0.15 m*M* quinacrine; more concentrated quinacrine solutions did not add very much to the potentiation effect but increased the toxicity to the unirradiated controls. Therefore, in sub-

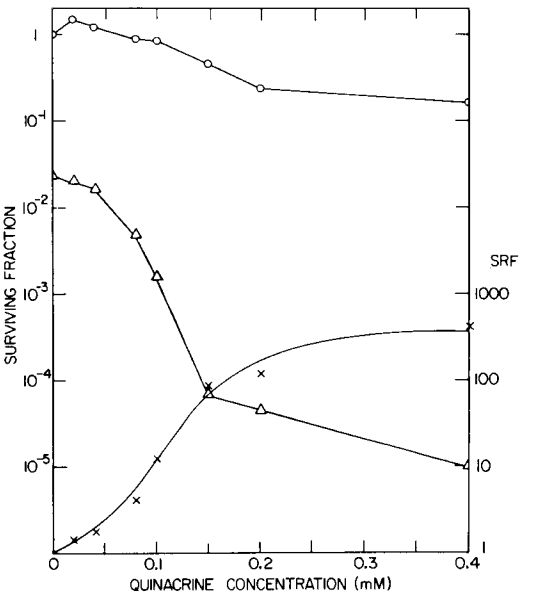

FIG. 1. Postirradiation treatment of *E. coli* K-12 *rec*$^+$ (AB2497) with quinacrine. Cells in exponential growth were x-irradiated (20 krads) and were incubated thereafter with different concentrations of quinacrine for 60 minutes at 37°C: Unirradiated controls treated with quinacrine, ○; irradiated cells treated with quinacrine, △; Survival Reduction Factor (SRF) calculated from above data (see text), ×.

sequent experiments 0.15 m*M* quinacrine was used, a concentration that killed about 50% of the control cells and gave an SRF value of about 100 for x-irradiated *rec*$^+$ bacteria killed to a survival level of 10^{-2}.

Figure 2 presents the kinetics of the development of the potentiation of x-ray induced killing of *E. coli* K-12 *rec*$^+$ by 0.15 m*M* quinacrine. Nearly maximal values of SRF were obtained after 60 minutes of postirradiation incubation. Incubation for longer than 60 minutes resulted in little increase in SRF values, but during this period the killing of the unirradiated controls by the drug doubled. A standard time of 60 minutes was therefore used for further experiments.

The toxic effect of quinacrine on x-irradiated cells was dependent on the temperature of the postirradiation incubation mixture. Table I shows that incubating the cells at 0°C prevented the toxic manifestations of quinacrine on unirradiated cells as well as on irradiated cells.

The timing of the addition of quinacrine after x-irradiation was critically important in eliciting the potentiation of the radiation effect (Fig. 3). A 10-min delay at 37°C in the addition of quinacrine after x-irradiation resulted in the loss of about 95% of its potentiation effect (SRF value reduced from 82 to 6). If quinacrine was

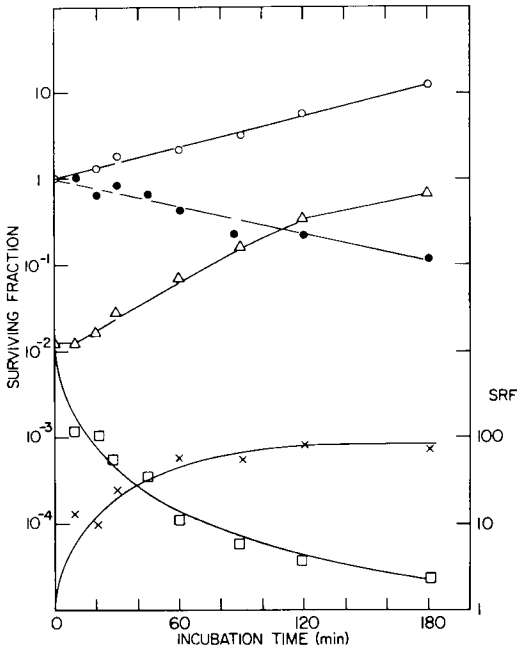

FIG. 2. The kinetics of the potentiation of x-ray-induced killing by quinacrine. *E. coli* K-12 *rec*$^+$ (AB2497) cells in exponential growth phase were x-irradiated (20 krads), and quinacrine was added to final concentration of 0.15 m*M*. The cultures were incubated at 37°C and samples were taken for colony counts at various times: Unirradiated control, ○; unirradiated control incubated with quinacrine, ●; 20 krads irradiated control, △; 20 krads irradiated and incubated postirradiation with quinacrine, □; Survival Reduction Factor calculated from the above data, ×.

TABLE I

EFFECT OF POSTIRRADIATION INCUBATION TEMPERATURE ON QUINACRINE POTENTIATION OF X-RAY KILLING OF *E. coli* K-12 *rec*$^+$ (AB2497)

	Temperature (°C) of post irradiation incubation	*Number of colony-forming units*	*Surviving fraction*
Unirradiated controls, zero-time	—	6.1×10^7	1
Unirradiated controls, incubated 60 min in SMM	37	1.1×10^8	1.6
	0	5.0×10^7	0.73
Unirradiated, incubated 60 min with 0.15 m*M* quinacrine in SMM	37	2.3×10^7	0.3
	0	5.0×10^7	0.73
Irradiated (20 krad), zero-time	—	7.9×10^5	1.1×10^{-2}
Irradiated (20 krad), 60-min post-irradiation incubation in SMM	37	1.0×10^6	1.4×10^{-2}
	0	7.6×10^5	1.1×10^{-2}
Irradiated (20 krad), 60 min post-irradiation incubation with 0.15 m*M* quinacrine in SMM	37	1.85×10^3	2.7×10^{-5}
	0	7.41×10^5	1.1×10^{-2}

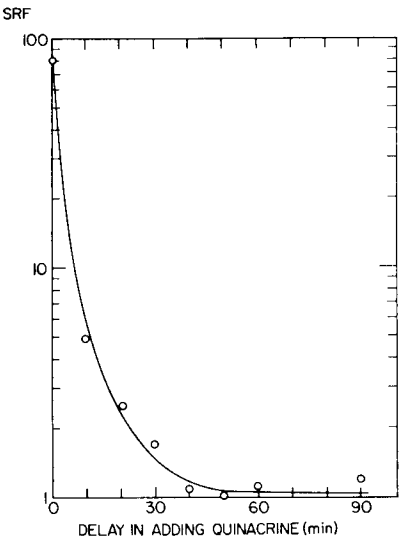

FIG. 3. Effect of the delay in addition of quinacrine to x-irradiated *E. coli* K-12 *rec*$^+$ (AB2497) on the Survival Reduction Factor. After x-irradiation (20 krads) the cells were grown at 37°C for various times before adding quinacrine to a concentration of 0.15 m*M*. The cells were then further incubated for 60 minutes and plated. The Survival Reduction Factor (see text) is plotted vs the time of delay of addition of quinacrine.

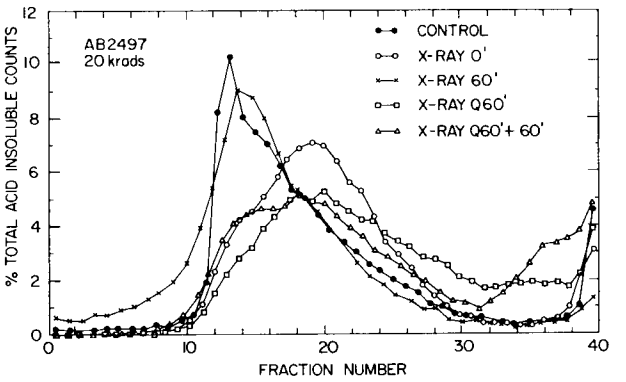

FIG. 4. Sedimentation patterns of DNA from x-irradiated *E. coli* K-12 AB2497 (*rec*$^+$): Unirradiated, ●; 20 krads, no reincubation, ○; 20 krads, 60 min reincubation, ×; 20 krads, 60 min incubation with added quinacrine (0.15 m*M*), □; 20 krads, 60 min incubation with added quinacrine plus 60 min incubation without quinacrine, △. The cells whose DNA was prelabelled with [^{3}H] thymidine were converted to spheroplasts and lysed on top of alkaline sucrose gradients (5–20% sucrose in 0.1 *N* NaOH). Sedimentation was performed in an SW 50.1 rotor for 105 minutes at 30,000 rpm at 20°C. The direction of sedimentation is from right to left.

added 40 minutes (or later) after x-irradiation no potentiation could be demonstrated.

Inhibition of the Repair of Single-Strand Breaks by Quinacrine

Figure 4 demonstrates that although *rec*$^+$ cells can efficiently repair DNA single-chain breaks they were prevented from doing so in the presence of 0.15 m*M* quinacrine. Washing the cells and allowing an additional 60 minutes of growth in the absence of added quinacrine resulted only in a slight amount of repair. This suggests that the inhibition of repair of x-ray induced DNA single-chain breaks by quinacrine is largely irreversible.

Comparison of the Effect of Quinacrine on the Viability of X-irradiated rec$^+$ *and recA13 Cells*

Figure 5 presents the survival curves for x-irradiated *rec*$^+$ cells and *recA13* cells with and without postirradiation incubation with 0.15 m*M* quinacrine. The *rec*$^+$

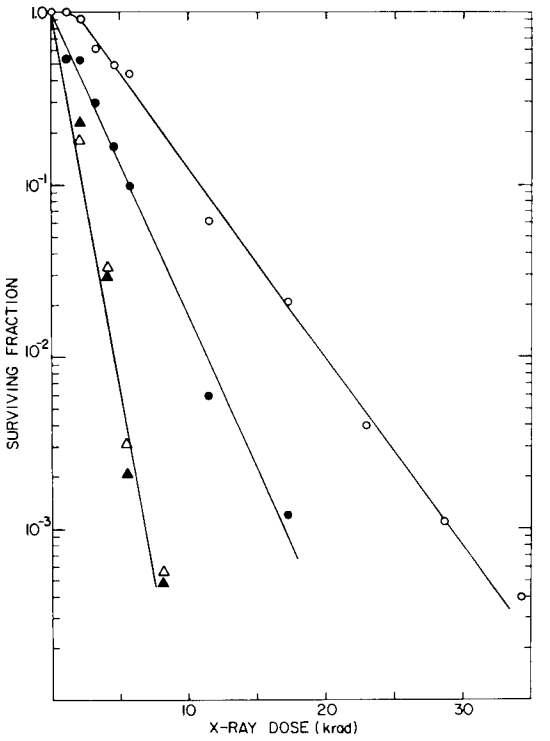

FIG. 5. X-Ray survival curves for *rec*$^+$ and *rec*$^-$ derivatives of *E. coli* K-12 with and without a 60-minute postirradiation treatment with quinacrine: *rec*$^+$, ○; *rec*$^+$ treated with 0.15 m*M* quinacrine, ●; *recA13*, △; *recA13* treated with 0.15 m*M* quinacrine, ▲.

(AB2497) cells have a survival curve with a D_0 value of 3.8 krads and a shoulder region with an extrapolation number of two.[4] Sixty minutes postirradiation incubation in quinacrine caused the disappearance of the shoulder and a decrease of D_0 to 2.6 krads. The recombination deficient *recA13* cells are also deficient in the repair of single-strand breaks in DNA (*1*) and have a D_0 value of 1.1 krads. Treatment with quinacrine did not sensitize these repair-deficient cells to killing by x-rays and the D_0 remained 1.1 krads.

DISCUSSION

The addition of quinacrine (0.15 m*M*) to the postirradiation incubation mixture potentiates the x-ray-induced killing of *E. coli* K-12 *rec*$^+$. It does not potentiate the killing of *recA13* (Fig. 5), a strain that is deficient in the slow repair of x-ray-induced DNA single-chain breaks (*1*). The potentiation of killing of the *rec*$^+$ strain by quinacrine is probably due to its inhibition (which is largely irreversible) of the repair of x-ray-induced DNA single-chain breaks (Fig. 4). Similar conclusions have been reached from experiments using 5 μg/ml of acriflavine.[3] The molecular basis of quinacrine's potentiating action is demonstrated indirectly (Fig. 3) by the fact that this potentiation is prevented when there is a delay of 40 minutes (or more) in the addition of quinacrine after irradiation. Forty minutes is the time needed for the cells to complete the *rec* gene controlled repair of DNA single-chain breaks (*1*).

The inhibition of repair by quinacrine does not appear to be complete since the survival curve of *rec*$^+$ cells treated with quinacrine, although shifted towards the survival curve of *recA13*, does not coincide with the latter (Fig. 5).

The action of quinacrine on x-irradiated cells is largely irreversible, as judged both from the fact that washing the cells after treatment with the drug did not prevent its adverse effect on viability (Fig. 5) or on the repair of DNA single-chain breaks (Fig. 4).

Quinacrine (0.15 m*M*) also potentiates the killing of UV-irradiated "wild-type" cells of *E. coli* K-12.[5] It inhibits the *recA* gene-controlled repair of daughter-strand gaps produced when UV-induced base damage (in the parental strands) is bypassed during DNA synthesis but this inhibition appears to be completely reversible when the cells are washed and resuspended in normal medium.[5] Thus, two repair systems controlled by the *recA* gene, the repair of x-ray induced parental-strand DNA chain breaks and the repair of daughter-strand gaps produced when base damage is bypassed during DNA synthesis, are both inhibited by quinacrine. However, the

[4] When *E. coli* K-12 (AB2497) *rec*$^+$*uvr*$^+$ is x-irradiated in air in supplemented minimal medium its survival curve has no shoulder and a D_{37} of 5.7 krads (*1*). The survival curve of AB2487 (*recA13*) is unaffected by whether it is irradiated in minimal medium minus glucose and nutritional supplements (present report) or in supplemented minimal medium (glucose plus nutritional supplements) (see Ref. (*1*)).

[5] Z. Fuks and K. C. Smith, Effect of quinacrine on survival and DNA repair in ultraviolet-irradiated *Escherichia coli* K-12 (manuscript in preparation).

inhibition of the former system is largely irreversible while the inhibition of the latter appears completely reversible.

A similar selective inhibition of these two *recA* gene-controlled repair functions has been observed with impure hydroxyurea. It also potentiates the lethal effects of both UV- and x-irradiation. Impure hydroxyurea irreversibly inhibited the repair of x-ray-induced DNA single-chain breaks in *E. coli* K-12 *rec*$^+$*uvr*$^+$ (*13*) but showed very little inhibitory effect on the gap-filling step of *rec* repair in UV-irradiated cells. What little inhibition there was in these UV-irradiated cells in the presence of the drug appeared completely reversible when the drug was removed.[6]

The production of the quinacrine effect appears to be dependent upon active metabolism since incubation of cells in the presence of quinacrine at 0°C had no effect on cell viability. Quinacrine has been shown to inhibit the action of DNA polymerase (*14–16*). This is probably not the basis for its effect on viability since pure hydroxyurea, a potent inhibitor of DNA synthesis, does not potentiate the effects of x-irradiation on bacteria (but impure hydroxyurea does) (*13*).

Acridine derivatives have been shown to stabilize the helical structure of DNA such that its melting temperature is increased by as much as 13°C (*21*). Recombinational events probably require an unfolding of the helical structure of DNA to allow for sister strand exchanges (*22*, *23*). It is possible that quinacrine may function to stabilize the DNA and thus prevent recombinational repair. Since the inhibition is largely irreversible one may postulate that the quinacrine binds irreversibly to the broken chain ends or by physically blocking repair for a given period of time allows for other irreversible events to take place in the cell.

In view of quinacrine's effectiveness as a radiation sensitizer in bacteria it seems of value to discuss the potentialities of quinacrine as a radiosensitizer in the human. Quinacrine has a wide history of use in the treatment of malaria. Moreover, there are data that demonstrate a selective uptake of quinacrine by some experimental tumors in rats (*24*) and mice (*25*). Thus, quinacrine may have two essential properties of a radiosensitizing agent for use in the human—low toxicity to normal tissues and possible selective accumulation in tumor cells.[7]

ACKNOWLEDGMENT

The able assistance of Mrs. Elaine Suffolk in performing the alkaline sucrose gradient experiments is gratefully acknowledged.

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[6] J. Wilcockson, M. W. Gray and K. C. Smith, Lethal synergism between hydroxyurea and UV-irradiation in *Escherichia coli* (manuscript in preparation).

[7] Quinacrine has been shown to potentiate the x-ray-induced killing of Chinese hamster cells and to inhibit the repair of single-chain breaks in DNA (N. Voiculetz, K. C. Smith and H. S. Kaplan, manuscript to be submitted to *Cancer Research*).

REFERENCES

1. D. S. Kapp and K. C. Smith, 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. *J. Bacteriol.* **103,** 49–54 (1970).
2. C. D. Town, K. C. Smith, and H. S. Kaplan, DNA polymerase required for rapid repair of X-ray-induced DNA strand breaks *in vivo*. *Science* **172,** 851–854 (1971).
3. H. S. Kaplan, DNA-strand scission and loss of viability after X-irradiation of normal and sensitized bacterial cells. *Proc. Nat. Acad. Sci. U.S.* **55,** 1442–1446 (1966).
4. R. A. McGrath and R. W. Williams, Reconstruction *in vivo* of irradiated *Escherichia coli* deoxyribonucleic acid; the rejoining of broken pieces. *Nature* (*London*) **212,** 534–535 (1966).
5. M. Morimyo, H. Zen-ichiro and K. Suzuki, Appearance of low molecular weight DNA in a *rec*$^-$ mutant of *Escherichia coli* K-12 irradiated with X-rays. *J. Radiat. Res.* **9,** 19–25 (1968).
6. M. M. Elkind, H. Sutton-Gilbert, W. B. Moses and C. Kamper, Sublethal and lethal radiation damage. *Nature* (*London*) **214,** 1088–1092 (1967).
7. L. J. Grady and E. C. Pollard, DNA degradation by ionizing radiation in *Bacillus subtilis*. Synergistic effect of actinomycin D. *Biochim. Biophys. Acta* **145,** 837–839 (1967).
8. C. F. Arlett, The influence of post-irradiation conditions on the survival of Chinese hamster cells after gamma-irradiation. *Int. J. Radiat. Biol.* **17,** 515–526 (1970).
9. R. A. Deering, Postirradiation caffeine sensitization of gamma-irradiated resistant and sensitive strains of *D. discoideum*. *Biophysical Soc. Abstr.*, p. 263a (1970).
10. D. Gaudin and K. L. Yielding, Response of a "resistant" plasmocytoma to alkylating agents and X-ray in combination with "excision repair" inhibitors caffeine and chloroquine. *Proc. Soc. Exp. Biol. Med.* **131,** 1413–1416 (1969).
11. R. Bases, Enhancement of X-ray damage in HeLa cells by exposure to Lucanthone (Miracil D) following radiation. *Cancer Res.* **30,** 2007–2011 (1970).
12. R. A. Phillips and L. J. Tolmach, Repair of potentially lethal damage in X-irradiated HeLa cells. *Radiat. Res.* **29,** 413–432 (1966).
13. D. S. Kapp and K. C. Smith, The chemical inhibition of the repair of single-strand breaks in DNA: Postirradiation sensitization to X-rays. *Int. J. Radiat. Biol.* **19,** 225–262 (1971).
14. R. L. O'Brien, J. G. Olenick and F. E. Hahn, Reactions of quinine, chloroquine and quinacrine with DNA and their effects on the DNA and RNA polymerase reactions. *Proc. Nat. Acad. Sci. U.S.* **55,** 1511–1517 (1966).
15. J. Ciak and F. E. Hahn, Quinacrine (Atabrin): mode of action. *Science* **156,** 655–656 (1967).
16. K. Van Dyke, C. Lantz and C. Szustikiewicz, Quinacrine: mechanisms of antimalarial action. *Science* **169,** 492–493 (1970).
17. P. Howard-Flanders and L. Theriot, Mutants of *Escherichia coli* K-12 defective in DNA repair and in genetic recombination. *Genetics* **53,** 1137–1150 (1966).
18. A. K. Ganesan and K. C. Smith, Dark recovery processes in *Escherichia coli* irradiated with ultraviolet light. I. Effect of *rec*$^-$ mutations on liquid holding recovery. *J. Bacteriol.* **96,** 365–373 (1968).
19. R. Loevinger and P. Huisman, A twin-tube 50-kvp beryllium-window X-ray unit for microbial radiobiology. *Radiat. Res.* **24,** 357–367 (1965).
20. D.M. Ginsberg and H. K. Webster, Chemical protection against single-strand breaks in DNA of gamma-irradiated *E. coli*. *Radiat. Res.* **39,** 421–435 (1969).
21. L. S. Lerman, Acridine mutagens and DNA structure. *J. Cell Comp. Physiol.* **64,** Sup. 1, 1–18 (1964).
22. P. Howard-Flanders, W. Dean Rupp, Brian M. Wilkins and R. S. Cole, DNA replication

and recombination after UV irradiation. *Cold Spring Harbor Symp. Quant. Biol.* **33,** 195–207 (1968).

23. A. J. Clark, Toward a metabolic interpretation of genetic recombination of *E. coli* and its phages. *Annu. Rev. Microbiol.* **25,** 437–464 (1971).

24. N. B. Ackerman, D. K. Haldorsen, D. L. Wallace, A. J. Madsen and A. S. McFee, Aminoacridine uptake by experimental tumors. *J. Amer. Med. Ass.* **191,** 115–116 (1965).

25. L. J. Anghileri, Uptake of iodine-131-labeled Atabrine by Ehrlich ascites tumor and by sarcoma S-180 BALB. *Nature* (*London*) **211,** 878 (1966).