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Modification of DNA Repair and Survival of X-Irradiated *pol*, *rec*, and *exr* Mutants of *Escherichia coli* K-12 by 2,4-Dinitrophenol[1]

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2,4-Dinitrophenol (DNP) (3×10^{-3} *M*), when added to growth medium for 90 min of postirradiation incubation, markedly potentiated the x-ray induced killing of *E. coli* K-12 "wild-type," *polA1* and *uvrB5* cells. The *recA13*, *recB21*, and *exrA* mutants, which are deficient in the slow, growth-medium dependent (Type III) repair of x-ray-induced DNA single-strand breaks, did not show radiation sensitization by DNP. In the *polA1* background, DNP treatment was as effective as an *exrA* mutation in sensitizing *polA1* cells to killing by x-rays.

Alkaline sucrose gradient studies showed that DNP inhibited the Type III repair of single-strand breaks. This inhibition was largely irreversible.

INTRODUCTION

The x-ray-induced single-strand breaks in the DNA of *Escherichia coli* have been shown to be repaired by three distinct systems (*1*) (Table I). The Type I and Type II repair processes can occur during incubation in buffer while Type III repair requires incubation in growth medium. When the Type III repair of DNA single-strand breaks is absent due to a mutational defect (*2*), or chemically inhibited by impure hydroxyurea (*3*), acriflavine (*4*), chloramphenicol (*5*) or quinacrine (*6*), enhanced killing is observed after x-irradiation, suggesting that such repair contributes to the restoration of viability of x-irradiated *E. coli* K-12 cells.

It has been reported that low concentrations of 2,4-dinitrophenol (DNP) inhibit a buffer repair system for DNA single-strand breaks in mammalian cells (*7*). This inhibition is rapidly reversible and survival studies show no sensitization but rather a small protection with this DNP treatment (*8*, *9*). The addition of low concentrations of DNP to the medium on which *E. coli* B cells are plated results

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TABLE I

SYSTEMS FOR THE REPAIR OF X-RAY-INDUCED DNA SINGLE-STRAND BREAKS[a]

Designation	*Characteristics*
Type I	Very fast (<2 min at 0°C); occurs in buffer; does not require DNA polymerase I; repairs ~75% of the x-ray-induced breaks produced under anoxic conditions but only ~25% of the breaks produced under aerobic conditions.
Type II	Fast ($T_{\frac{1}{2}}$ for aerobic X-irradiation ~10 min at 0°C, ~1 min at 37°C); occurs in buffer; is largely deficient in strains which lack DNA polymerase I; repairs ~90% of the breaks remaining after Type I repair; whether produced under anoxic or aerobic conditions.
Type III	Slow (40–60 min at 37°C); requires complete growth medium; controlled by *rec* and *exr* genes; does not require DNA polymerase I; repairs approximately two breaks per single-strand genome in addition to the breaks repaired by Type II repair whether produced under anoxic or aerobic conditions.

[a] Compiled from Town, Smith, and Kaplan (*1*) and Youngs and Smith (*11*).

in higher survival after X-irradiation (*10*). In contrast, using *E. coli* K-12, higher concentrations of DNP, and different treatment procedures, we have observed a marked enhancement by DNP of the killing of x-irradiated cells of both "wild-type," *polA* and *uvrB* strains and a largely irreversible inhibition of the Type III repair of DNA single-strand breaks. Cells carrying the *recA*, *recB*, or *exrA* mutations, strains genetically deficient in this repair process (*2*, *11*, *12*), were not sensitized or protected by post-irradiation treatment with DNP.

MATERIALS AND METHODS

The bacterial strains used were all derivatives of *E. coli* K-12 (cf. Table II). The growth media used were supplemented minimal medium (SMM) and

TABLE II

Escherichia coli K-12 DERIVATIVES USED

Designation	*Mating type*	*Relevant genotype*	*Other markers*	*Ref. or source*
AB2497	F^-	"wild-type"	*arg his leu pro thi thr thy ara gal lac mtl xyl* $T6^r$ λ^s Str^r	(*20*)
AB2487	F^-	*recA13*	*arg his leu pro thi thr thy ara gal lac mtl xyl* $T6^r$ λ^s Str^r	(*20*)
SR78	F^-	*recB21*	*arg his leu pro thi thr thy ara gal lac mtl xyl* $T6^r$ λ^s Str^r	(*2*)
SR188	F^-	*exrA*	*thy rha lac* Str^r	D. A. Youngs
JG138	F^-	*polA1*	*thy rha lac* Str^r	J. Gross

Abbreviations: (*21*). The symbols *arg, his, leu, pro, thi, thr, thy* denote requirements for arginine histidine, leucine, proline, thiamin, threonine and thymine, respectively; *ara, gal, lac, mtl, rha* and *xyl*, the inability to utilize arabinose, galactose, mannitol, rhamnose and xylose, respectively; T6, λ and Str, response to the phages T6 and λ, and to the antibiotic streptomycin (^{r} indicates resistance, ^{s}, sensitivity); *rec* denotes genes affecting genetic recombination and uv and x-ray sensitivity; *exr* denotes genes affecting uv and x-ray sensitivity; *pol* denotes genes affecting the synthesis of DNA polymerase I and uv and x-ray sensitivity.

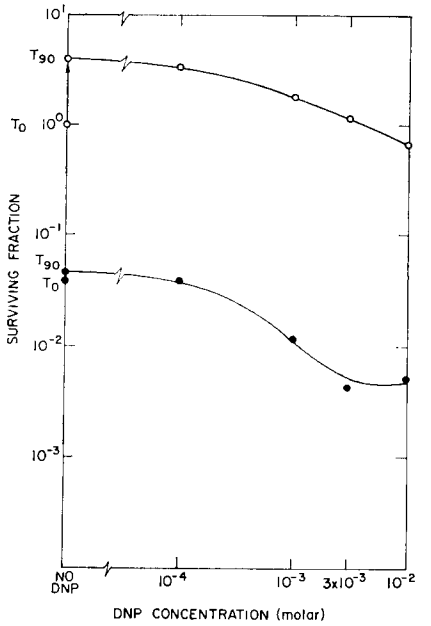

FIG. 1. Treatment of x-irradiated (20 krads) and unirradiated cells of *E. coli* K-12 wild-type (AB2497) for 90 min with different concentrations of DNP. For the cells incubated without DNP, the cell number at the beginning and at the end of the 90 min incubation period are shown by T_0 and T_{90}, respectively. Unirradiated cells (○); irradiated cells (●).

supplemented minimal medium agar (SMM-agar) which were previously described (*2*, *13*).

2,4-Dinitrophenol (DNP) was obtained from Fisher Scientific Co. Fresh solutions were prepared at room temperature for every experiment.

For the survival studies, overnight stationary phase cultures were diluted in fresh SMM to about 10^7 cells/ml and grown for 3 to 4 hr at 37°C in a Gyrotory water bath (New Brunswick Scientific) to exponential phase (1 to 1.5 × 10^8 cells/ml). The cells were collected on a Millipore filter (0.45 μm) and resuspended at 2 to 3 × 10^8 cells/ml in fresh SMM.

Five milliliter samples were placed in plastic Petri dishes (60 mm diameter) and irradiated at room temperature with a twin-tube beryllium window x-ray unit (50 kV, 50 and 48 mA, 0.3 mm Al filtration) (*14*) with a dose rate of 8.2 krads/min as measured with ferrous sulfate dosimetry.

Immediately after irradiation the cells were diluted either with an equal volume of SMM or an equal volume of SMM containing 6 × 10^{-3} *M* DNP. The cells were reincubated at 37°C for different periods of time, and then plated on SMM-agar after dilution in 0.067 *M* phosphate buffer (pH 7.0). Colonies were counted after 48–72 hr of incubation at 37°C.

Alkaline Sucrose Gradients

The repair of x-ray-induced single-strand breaks was followed by the technique of McGrath and Williams (*15*), as modified by Town, Smith and Kaplan (*16*).

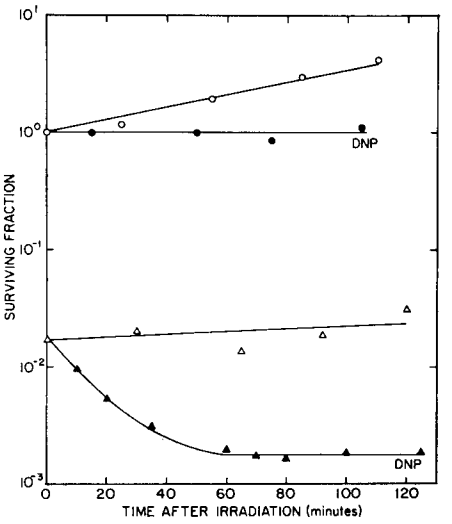

FIG. 2. The kinetics of the sensitization of *E. coli* K-12 wild-type cells (AB2497) to X-irradiation (20 krads) by 3×10^{-3} *M* DNP. Cells were incubated for 120 min postirradiation with or without DNP. Unirradiated cells (○); unirradiated cells treated with DNP (●); irradiated cells (△); irradiated cells treated with DNP (▲).

The first moment was used as a convenient index of sedimentation behavior (*17*).

An overnight stationary-phase culture was diluted to about 10^7 cells/ml in fresh SMM containing 50 μCi/ml thymine-methyl-^{3}H (New England Nuclear, 20 Ci/mmole) in a total thymine concentration of 2 μg/ml. The culture was incubated for approximately 3 hr until it reached a density of about 1.2×10^8 cells/ml. The cells were collected, irradiated and reincubated in nonradioactive medium in the same manner as for the survival experiments. Samples were taken immediately after irradiation and after 90 min of incubation in SMM with or without DNP. A portion of the DNP treated cells was filtered, washed and resuspended in fresh SMM and incubated for an additional 60 min in the absence of DNP.

All samples were filtered, washed and resuspended in 0.067 *M* phosphate buffer, and 0.05 ml (5×10^5 cells) was layered onto 0.1 ml of 0.5% Sarkosyl NL30 (Geigy) in 0.5 *N* NaOH on top of 4.8 ml linear gradients of 5–20% (w/v) sucrose in 0.1 *N* NaOH. After standing for 1 hr, the gradients were spun at 30,000 rpm for 105 min at 20°C (using a Beckman SW50.1 rotor), fractionated and processed as described by Kapp and Smith (*2*).

All the survival and sedimentation experiments were repeated at least three times, and the data of representative experiments are presented.

RESULTS

The growth of nonirradiated cells was nearly completely stopped during a 90 min incubation in growth medium containing 3×10^{-3} *M* DNP. When higher

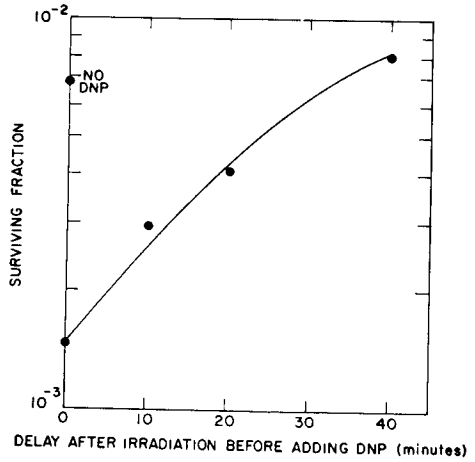

FIG. 3. Effect of delay in adding 3×10^{-3} *M* DNP on the survival of x-irradiated *E. coli* K-12 wild-type cells. Irradiated (20 krads) cells were incubated in SMM for various lengths of time before the DNP was added and incubation was continued for 90 min before diluting and plating. The survival level of non-DNP treated cells is indicated on the ordinate.

concentrations were used, cell killing appeared (~30% decrease in cell number at 10^{-2} *M* DNP) (Fig. 1).

Irradiated cells (20 krads) were much more sensitive to DNP treatment. Incubation for 90 min with 10^{-3} *M* DNP killed about 70% of the cells (Fig. 1). The maximum effect seemed to be reached at 3×10^{-3} *M* where about 90% of the irradiated cells were killed by the DNP treatment. When the DNP treatment of irradiated cells was done in buffer no sensitizing effect was seen (results not shown).

The kinetics of the sensitization of x-irradiated cells (20 krads) by DNP treatment are shown in Fig. 2. Cell survival was a function of the length of DNP treatment and decreased rapidly for up to 60 min after irradiation. Treatment for longer times had no additional effect.

When the irradiated cells were incubated in growth medium for increasing lengths of time before DNP treatment, the sensitizing effect diminished rapidly. A delay of only 10 min between irradiation and the DNP treatment resulted in a 50% decrease in the sensitizing effect of DNP. After a delay of 40 min, the DNP treatment no longer had an effect on the survival of the irradiated cells (Fig. 3). In contrast, however, keeping the cells in buffer during this delay period did not reduce the effectiveness of the subsequent DNP treatment (results not shown).

The x-ray-survival curves for wild-type and *recA* cells with and without a 90 min DNP (3×10^{-3} *M*) treatment are given in Fig. 4. The wild-type cells had a D_0 of 4.5 krads in the absence of DNP and 3.0 krads after treatment with DNP. This is a dose reduction factor of 1.5. In marked contrast, the survival of *recA* cells (Fig. 4) and of *exrA* and *recB* cells (Fig. 5a and b), which are deficient in the slow rejoining of DNA single-strand breaks (*2, 11, 12*), was uninfluenced by the

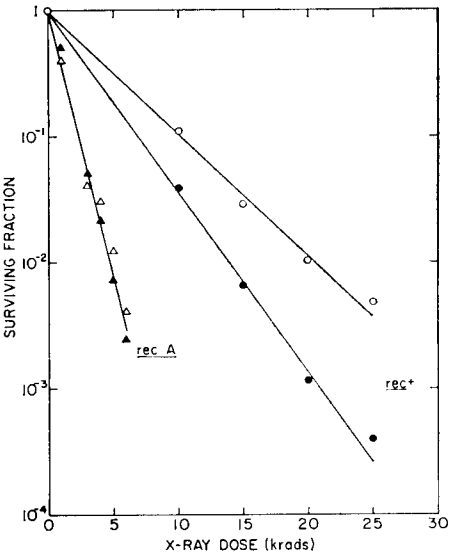

FIG. 4. X-ray-survival curves for *E. coli* K-12 wild-type (AB2497) cells and *recA13* (AB2487) cells with and without DNP treatment. Cells were plated immediately after irradiation or after 90 min of incubation with 3×10^{-3} *M* DNP. Wild-type cells (○); wild-type cells treated with DNP (●); *recA* cells (△); *recA* cells treated with DNP (▲).

DNP treatment. On the other hand, the *polA1* strain which is capable of Type III repair (*11*), showed enhanced cell killing when incubated in DNP after irradiation. The D_0 of the *polA1* cells was reduced by a factor of 1.4 by the DNP treatment (Fig. 5c). A similar sensitization (i.e., a slope reduction factor of 1.5) was found after DNP treatment of x-irradiated *uvrB* cells (results not shown).

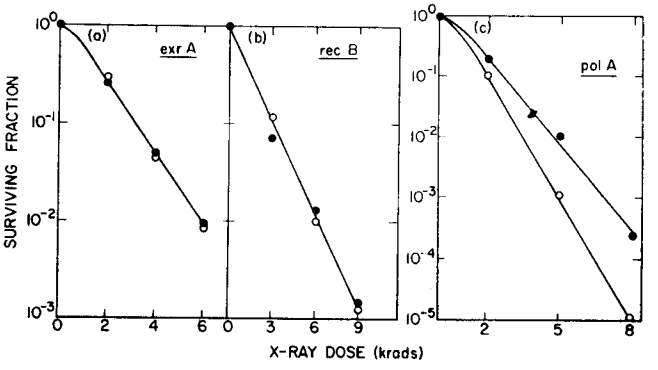

FIG. 5. Survival curves of *E. coli* K-12 *exrA*, *recB* and *polA1* cells with and without postirradiation treatment with 3×10^{-3} *M* DNP. Cells were plated immediately after irradiation and after 90 min of incubation with DNP. (a) *exrA*; (b) *recB21*; (c) *polA1*. Irradiated cells (●); irradiated cells treated with DNP (○).

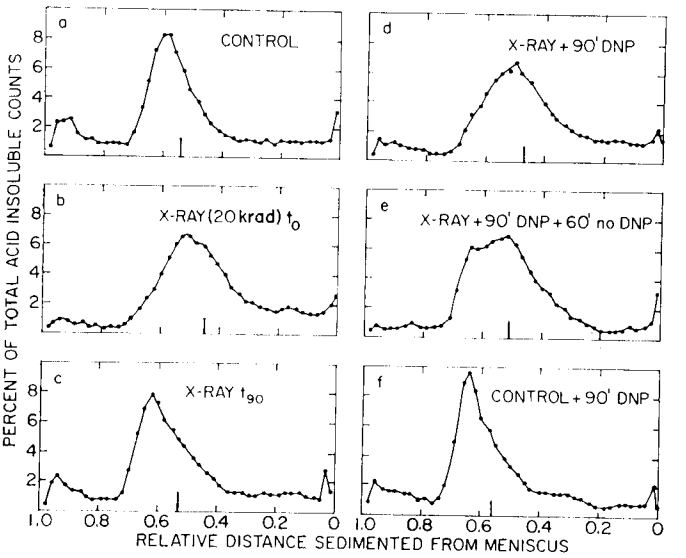

FIG. 6. Influence of DNP on the Type III repair of DNA single-strand breaks. Cells prelabeled with ^{3}H-thymine were lysed on top of alkaline sucrose gradients (5–20% in 0.1 *N* NaOH). Sedimentation was in a SW50.1 rotor for 105 min at 30,000 rpm at 20°C. The direction of sedimentation is from right to left. (a) Control; (b) x-ray 20 krads t_0; (c) x-ray (20 krads) incubated for 90 min in SMM; (d) x-ray (20 krads) incubated for 90 min in SMM with 3×10^{-3} *M* DNP; (e) x-ray (20 krads) incubated for 90 min in SMM with 3×10^{-3} *M* DNP, followed by 60 min in SMM; (f) control, incubated for 90 min with 3×10^{-3} *M* DNP. The first moment is denoted by the vertical bar under each DNA peak.

Repair of Single-Strand Breaks

The influence of DNP on the repair of single-strand breaks is shown in Fig. 6. Incubation for 90 min in growth medium allowed the cells to repair all the radiation-induced single-strand breaks (Fig. 6a, b and c). When DNP was added to the medium, however, there was a complete inhibition of the Type III repair system as exemplified by the fact that no shift in the first moment of the gradient was observed after incubation (Fig. 6d).

Washing out the DNP and reincubating the cells in growth medium for 60 min did not allow these cells to complete their repair. Although some DNA of higher molecular weight appeared, a significant amount remained in the low molecular weight range, thus broadening the peak (Fig. 6e). Further incubation up to 2 hr after washing out the DNP did not yield any additional repair (data not shown). The DNP treatment of nonirradiated cells did not induce any lowering of the DNA molecular weight (Fig. 6f) as indicated by the position of the first moment of the gradient.

The incubation of x-irradiated wild-type cells in buffer containing 3×10^{-3} *M* DNP did not affect the buffer repair of DNA single-strand breaks (Types I and II). With 10^{-2} *M* DNP, the rate of Type II repair was decreased but this inhibition was rapidly reversible (results not shown).

DISCUSSION

DNP at 3×10^{-3} *M* was bacteriostatic to unirradiated *E. coli* K-12 wild-type cells over the 90–105 min treatment used in these studies (Figs. 1 and 2). Treatment of x-irradiated cells with this concentration of DNP resulted in sharply decreased survival (Figs. 1 and 2). Concentrations of DNP greater than 3×10^{-3} *M* did not further increase the sensitization of x-irradiated cells but became lethal to unirradiated cells (30% killing at 10^{-2} *M*). Incubation with DNP for about 60 min at 37°C in complete growth medium was required for maximal sensitization of x-irradiated cells (Fig. 2). No sensitization to x-rays was seen when cells were treated with DNP in buffer.

When wild-type cells were incubated at 37°C in growth medium for various times before adding DNP, the cells became progressively more resistant to the sensitizing effect of DNP (Fig. 3). After a delay of about 40 min [a time interval sufficient for completion of Type III repair (*2*)], the cells were no longer sensitized by DNP. If the irradiated cells were incubated in buffer before treatment with DNP in growth medium, the cells did not lose their sensitivity to DNP. These data show that DNP sensitizes x-irradiated cells by interfering with a slow repair process which is growth medium-dependent.

Type III repair of DNA single-strand breaks is deficient in *recA* or *recB* cells [(*2*); Youngs and Smith, unpublished observations], and in an *exrA* strain (*11*, *12*). None of these strains was sensitized by DNP (Figs. 4 and 5), whereas all of the strains tested which are Type III repair proficient (wild-type, *polA*, *uvrB*) were sensitized by DNP. The survival data thus show that DNP sensitizes by interfering with a relatively slow repair mechanism which requires growth medium and is dependent on the *recA*, *recB* and *exrA* gene products. Type III repair of DNA single-strand breaks is the only known repair mechanism for x-ray-induced damage which satisfies all of these conditions.

The alkaline gradient experiments show that DNP inhibits the growth medium-dependent Type III repair of DNA single-strand breaks. This inhibition is partially reversible, as indicated by a small shift in the first moment after 60 min of further incubation in the absence of DNP (Fig. 6e). When irradiated cells were treated with 3×10^{-3} *M* DNP in buffer, no sensitization occurred and the Type II (buffer) repair of DNA single-strand breaks took place to the normal extent (data not shown).

A closely related compound, trinitrophenol (picric acid), does not uncouple oxidative phosphorylation but does inhibit the growth of bacteria (*18*). We have observed that trinitrophenol at 3×10^{-3} *M*, a concentration which produced an inhibition of growth comparable to that of 10^{-3} *M* DNP, had no sensitizing effect on x-irradiated bacteria. This may be interpreted as indirect evidence that the uncoupling of oxidative phosphorylation is implicated in the mechanism by which DNP sensitizes *E. coli* K-12 cells to x-irradiation. Consistent with this hypothesis is the observation that petite mutants of yeast, deficient in their capacity for respiration, are more sensitive than normal cells to x-rays (*19*).

The data of Moss *et al.* (*7*) and Dalrymple, Sanders and Baker (*8*), which show that a reversible inhibition of the repair of DNA single-strand breaks with DNP

does not sensitize mammalian cells to killing by x-rays, are consistent with our observation that the reversible inhibition of buffer repair (Type II repair) by 10^{-2} *M*DNP does not affect the survival of irradiated cells. The higher survival of *E. coli* B cells after x-irradiation when plated on medium containing DNP (*10*) is probably due to a different mechanism since at the concentrations used (2×10^{-4} *M*), we found that DNA single-strand break repair was not affected (results not shown).

It is of interest to compare the quantitative extent of the DNP effect on the survival of x-irradiated cells with that of certain mutations. The *polA* mutant (JG138) possesses a Type III repair system, but this repair process can be genetically blocked by an *exrA* mutation (*11*, *12*). The sensitization resulting from the introduction of an *exrA* mutation into *polA* cells is a factor of 1.3 (*13*). A similar degree of sensitization of *polA* cells (factor of 1.4) was achieved with 3×10^{-3} *M* DNP (this paper). Thus, in the *polA* background, the DNP treatment was as effective as an *exrA* mutation in sensitizing *polA* cells to killing by x-rays.

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