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Changes in survival of *Escherichia coli* K–12 cells as a function of the medium in which they are X-irradiated: A *rec* and *exr* gene-dependent phenomenon

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Escherichia coli K–12 wild-type cells suspended in growth medium during X-irradiation have a higher survival than cells irradiated in buffer, whether irradiated in air or nitrogen. To determine if this response was due to differences in radiochemistry during irradiation or to differences in the amount of repair, we studied the influence of the irradiation medium on the survival of several repair-deficient mutants (*recA*, *recB*, *exrA*, *uvrB*, *polA*1 *and* *polA*1 *exrA*) and on the repair of radiation-induced DNA single-strand breaks in a wild-type strain.

Our studies showed that the effect of the irradiation medium on survival was dependent on the presence of functional *recA*, *recB*, and *exrA* genes. Sedimentation studies, however, did not show a difference in the final amount of repair of DNA single-strand breaks after X-irradiation in buffer or growth medium. Thus, the increased X-ray sensitivity resulting from irradiation in buffer appears to be due to the inhibition of some *rec* and *exr* gene-dependent repair process other than the repair of DNA single-strand breaks.

1. Introduction

It was previously observed (Fuks and Smith 1971) that *E. coli* K–12 wild-type cells X-irradiated in buffer showed a lower survival than cells irradiated in growth medium. If this were the result of changes in radiochemistry during irradiation, resulting in the production of different amounts of radiation-induced lesions, this effect should also be seen in repair-deficient mutants. If, however, the effect were due to an interference with repair, no effects should be observed in a strain which is deficient in the repair process affected.

We have determined the survival of several repair deficient strains after X-irradiation in buffer and growth medium and have also investigated the effect of these irradiation conditions on the repair of X-ray-induced DNA single-strand breaks in a wild-type strain.

2. Materials and methods

2.1. *Survival curves*

All strains used were derivatives of *E. coli* K–12 (see the table). The growth medium used was supplemented minimal medium (SMM) (Ganesan and Smith 1968). DTM buffer (Kaplan, Smith and Tomlin 1962) was SMM without glucose or the required supplements. The assay of colony-forming ability

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Escherichia coli K–12 derivatives used (abbreviations are as used by Taylor (1970))

Designation	Mating type	Relevant genotype	Other markers	Reference or source
AB2497	F⁻	wild-type	*arg his leu pro thi thr thy ara gal lac mtl xyl tsx str*	Howard-Flanders and Theriot (1966)
AB2487	F⁻	*recA*13	*arg his leu pro thi thr thy ara gal lac mtl xyl tsx str*	Howard-Flanders and Theriot (1966)
SR78	F⁻	*recB*21	*arg his leu pro thi thr thy ara gal lac mtl xyl tsx str*	Kapp and Smith (1970)
AB2499	F⁻	*uvrB*5	*arg his leu pro thi thr thy ara gal lac mtl xyl tsx str*	Howard-Flanders, Boyce and Theriot (1966)
JG138	F⁻	*polA*1	*thy rha lac str*	J. Gross
SR188	F⁻	*exrA*	*thy rha lac str*	D. A. Youngs
DY101	F⁻	*polA exrA*1	*thy met lac str*	D. A. Youngs
DY98	F⁻	wild-type	*thy met lac str*	D. A. Youngs

was done on SMM medium solidified with 0·9 per cent Oxiod agar–agar No. 3.

Overnight cultures were diluted 1 : 100 into fresh SMM and grown to log phase (about three cell doublings). Cells were collected on a Millipore filter (0·45 μm) and resuspended either in fresh SMM or in DTM buffer. A sample of 5 ml of cells was irradiated at room temperature with a twin-tube beryllium window X-ray unit (50 kVp, 50 and 48 mA) (Loevinger and Huisman 1965) while being bubbled with air or nitrogen for 5 min before and during irradiation. The dose-rate was 8·8 krads/min as measured with ferrous sulphate dosimetry (Fricke and Hart 1966).

The cells that were kept in SMM were irradiated immediately, the cells that were transferred to DTM buffer were irradiated after 30 min to standardize the time between transfer and irradiation. Samples were diluted in phosphate buffer (0·067*M*, pH 7·0) and plated on SMM-agar to assay for colony-forming ability.

2.2 *Sedimentation studies*

An overnight culture was diluted into fresh SMM medium containing 200 μCi of ^{3}H-thymine (New England Nuclear; $\sim$18 Ci/mmol) or 8 μCi of ^{14}C-thymidine (Amersham Searle Corporation; 520 mCi/mmol) per ml. The total thymine or thymidine concentrations were 2 and 3·7 μg/ml, respectively. Log phase cells were irradiated in SMM or DTM buffer as described above. Samples in SMM were added to an equal volume of SMM and samples in DTM buffer were added to an equal volume of SMM with twice the normal concentration of glucose and supplements and then incubated for 80 min at 37°C. The cells labelled with ^{3}H or ^{14}C, were mixed at the desired ratio and $\sim 10^{6}$ cells were layered onto a 0·1 ml cap of 0·5 per cent Sarkosyl (Geigy NL30) and 0·01 *M* EDTA in 0·5 N NaOH (Town, Smith and Kaplan 1973) on top of 4·8 ml linear gradients of 5–20 per cent (weight to volume) sucrose in 0·1 N NaOH. After standing for 40 min, the gradients were centrifuged at 30 000 r.p.m. for 105 min at 20°C in a SW50·1 rotor. The procedures for processing the gradients and analysing the data have been described (Kapp and Smith 1970). The first moment was used as a sedimentation index to calculate the number of single-strand breaks (Town, Smith and Kaplan 1971).

3. Results

E. coli K–12 wild-type cells (AB2497) showed a higher survival when irradiated in SMM than when irradiated in DTM buffer. The slopes of the survival curves differed by a factor of 1·3. This was true for cells irradiated in air (figure 1 (A)) or in nitrogen (figure 1 (B)). The same effect was seen in DY98, a wild-type strain with the same genetic background as the *polA*1 and *exrA* strains used (data not shown).

The *recA*, *recB* (figure 2 (A)) and *exrA* (figure 2 (B)) strains showed identical survival curves whether irradiated in SMM or DTM buffer. The *polA*1 (figure 3) and *uvrB* (results not shown) strains were sensitized to the same extent as wild-type cells by irradiation in DTM buffer. The introduction of an *exrA* mutation into a *polA*1 strain (i.e. the *polA*1 *exrA* strain) eliminated the difference in survival seen in *polA*1 cells irradiated in SMM versus DTM (figure 3).

The sedimentation studies (figure 4) showed that there was no difference in the final extent of repair of DNA single-strand breaks in a wild-type strain

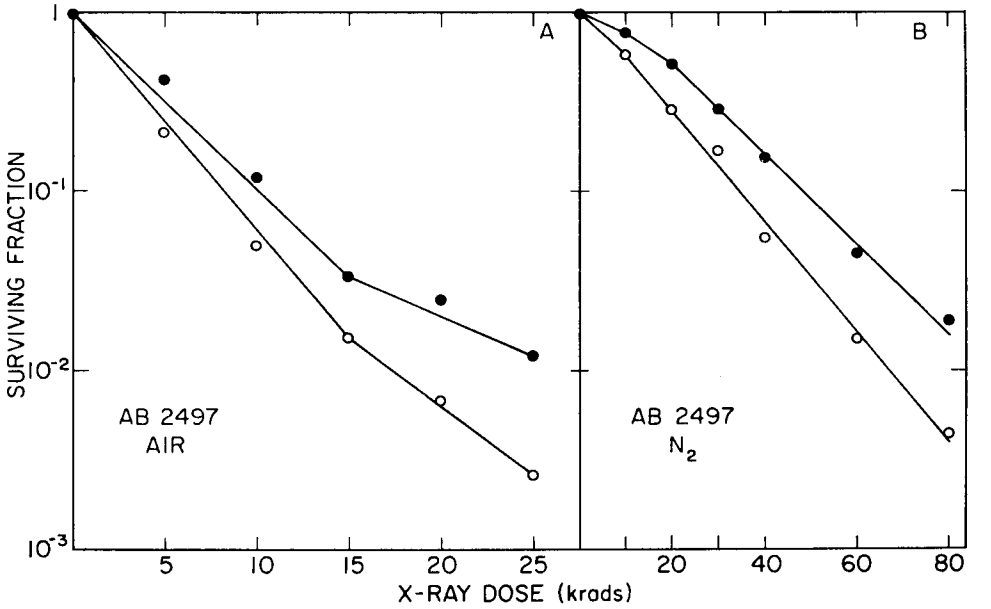

Figure 1. Survival of *E. coli* K–12 wild-type cells (AB2497) after X-irradiation in medium or buffer. Exponentially-growing cells were resuspended in fresh growth medium (●) or DTM buffer (○) and irradiated in equilibrium with air (A) or nitrogen (B).

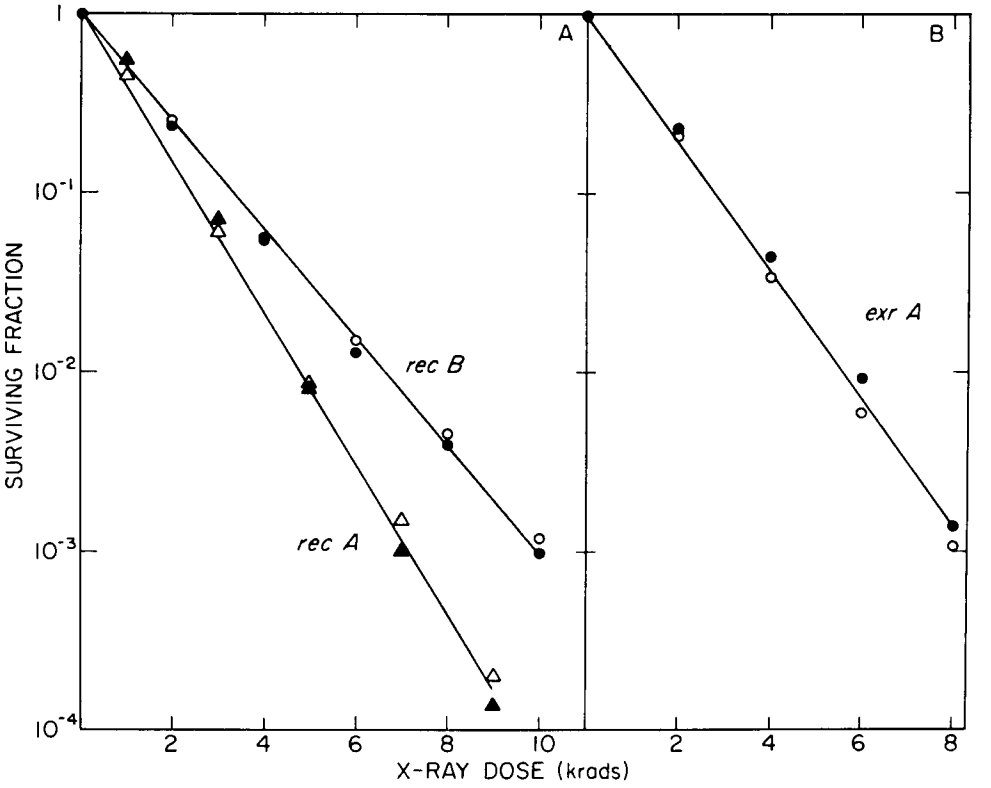

Figure 2. Survival of *rec* and *exr* strains after X-irradiation in medium (closed symbols) or buffer (open symbols). The conditions are described in figure 1. (A) ▲, △ *recA*13 (AB2487); ●, ○ *recB*21 (SR78); (B) *exrA* (SR188).

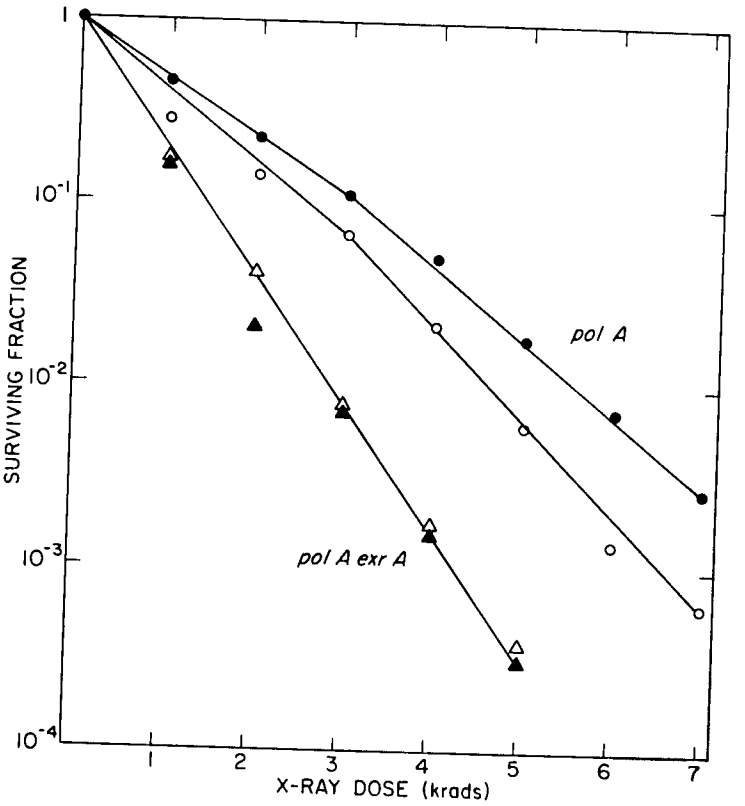

Figure 3. Survival of *polA1* and *polA1 exrA* strains after X-irradiation in medium (closed symbols) or buffer (open symbols). The conditions are described in figure 1. Strains are: ●, ○ *polA1* (JG138) and ▲, △ *polA1 exrA* (DY101).

(AB2497) for doses between 25 and 60 krads whether the cells were irradiated in buffer or growth medium.

4. Discussion

The difference in survival due to X-irradiation in medium or buffer seen in wild-type (figure 1), *polA1* (figure 3) and *uvrB* (data not shown) strains was absent in the *recA*, *recB*, *exrA* (figure 2) and *polA1 exrA* strains (figure 3). Thus, the phenomenon seems to be due to the inhibition of a repair process dependent on *rec* and *exr* gene functions rather than to differences in radiochemistry caused by the two irradiation media. The fact that the same difference in survival was observed whether the cells were irradiated in air or nitrogen suggests that the repair process which is affected by irradiation in buffer does not distinguish between lesions which maybe preferentially produced by X-irradiation in air or nitrogen.

The *rec* and *exr* strains are much more sensitive to X-rays then wild-type cells (Howard-Flanders and Theriot 1966, Mattern, Zwenk and Rörsch 1966). One of the repair processes which is lacking in these strains is the slow, growth medium-dependent repair of DNA single-strand breaks (Kapp and Smith 1970, Sedgwick and Bridges 1972, Youngs and Smith 1973 a). There is also evidence

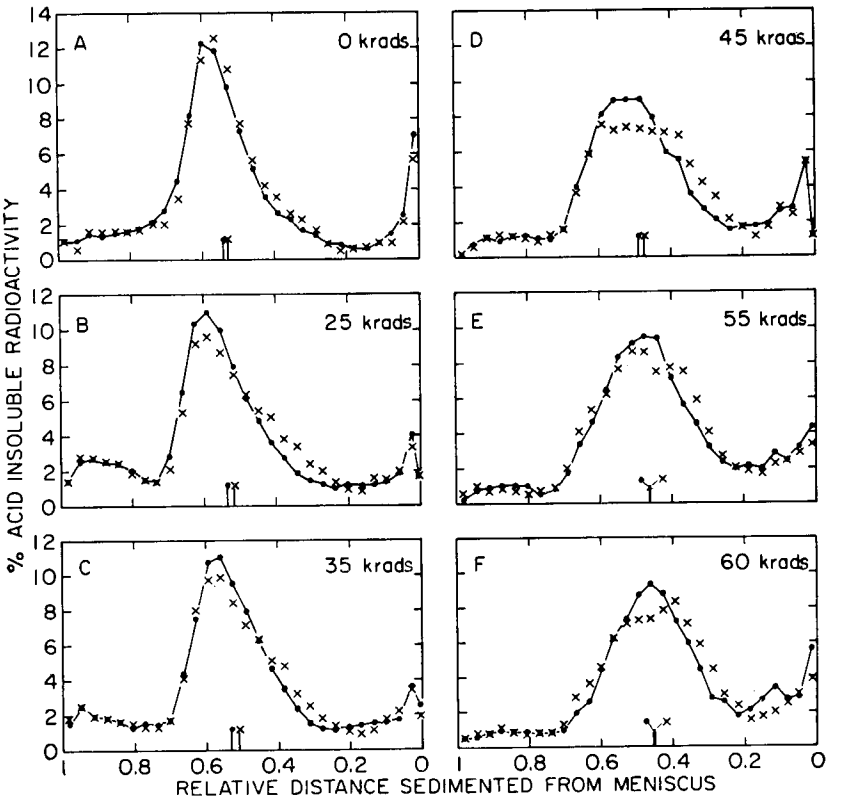

Figure 4. Repair of X-ray-induced DNA single-strand breaks after X-irradiation in SMM or DTM buffer. Cells were labeled with ^{3}H-thymine and irradiated in DTM buffer (x) or with ^{14}C-thymidine and irradiated in SMM (●). After irradiation all samples were incubated for 80 min in SMM. Cells labelled with ^{14}C or ^{3}H were then mixed and centrifuged together. The number of DNA single-strand breaks remaining per single-strand genome were: after irradiation in SMM (B) 0·2, (C) 0·4, (D) 1·9, (E) 3·0, (F) 3·6; and after irradiation in DTM (B) 0·4, (C) 0·7, (D) 2·1, (E) 2·7, (F) 3·2,. The first moment is denoted by the vertical bar under each DNA peak.

that other repair processes are lacking in the *rec* (Town *et al.* 1973) and *exr* strains (Youngs and Smith 1973 a), the functions of which are not yet defined.

The extent of repair of DNA single-strand breaks after irradiation in buffer or medium was checked but no evidence for a differential amount of this type of repair was found. Thus, it seems that the lower survival of cells irradiated in buffer is due to the impairment of a *rec* and *exr* gene-dependent repair process that is not involved in the repair of DNA single-strand breaks. Since *rec* (Cooper and Hanawalt 1972) and *exr* (Youngs and Smith 1973 b, Van der Schueren and Smith 1973) gene products are involved in the repair of U.V.-induced base damage, they may also be involved in the repair of X-ray-induced base damage.

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Les cellules *E. coli* K–12 souche sauvage ont une meilleure survie quand elles sont irradiées dans un milieu de croissance, que lorsque l'irradiation se fait en tampon. Ce phénomène est observé aussi bien après irradiation en conditions aérobiques qu'anaérobiques.

En vue de déterminer si cette différence est due à des changements dans la radiochimie pendant l'irradiation, ou dans le taux de restauration, nous avons étudié l'influence du milieu d'irradiation sur la survie de plusieurs mutants, ayant un faible pouvoir de resaturation (*recA*, *recB*, *exrA*, *polA*1 et *polA*1 *exrA*) ainsi que sur la réparation de cassures d'une chaîne de l'ADN dans cette souche sauvage.

Cette étude nous montre que l'effet du milieu d'irradiation sur la survie dépend de la présence de gènes fonctionnels *recA*, *recB* et *exrA*.

Cependant, lors des études faites sur la sédimentation, nous n'avons trouvé aucune différence dans le taux final de réparation de cassures d'une chaîne de l'ADN, aussi bien après irradiation en milieu tampon qu'en milieu de croissance.

On peut donc conclure que la plus grande sensibilité des cellules *E. coli* K–12 irradiées en milieu tampon, est due à l'inhibition d'un procédé de réparation, déterminé par les gènes *rec* et *exr*, mais qui diffère du système de resaturation de cassures d'une chaîne de l'ADN.

E. coli K–12 Wildtyp-Zellen, die während der Bestrahlung in Wuchsmedium suspendiert waren, haben eine höhere Überlebensrate als Zellen, die in Puffer bestrahlt wurden; das gilt für die Bestrahlung in Luft oder Stickstoff. Um herauszufinden, ob dieses Verhalten an Unterschieden in der Strahlenchemie während der Bestrahlung oder an Unterschieden im Ausmaß der Raparatur lag, studierten wir den Einfluß des Bestrahlungsmediums auf das Überleben von verschiedenen reparatur-defekten Mutanten (*recA*, *recB*, *exrA*, *uvrB*, *polA*1 and *polA*1 *exrA*) und auf die Reparatur von strahlungsinduzierten DNA Einzelstrang-Brüchen im Wildtyp-Stamm.

Unsere Studien zeigten, daß der Effekt des Bestrahlungsmediums für das Überleben abhängig war von der Funktion der *recA*, *recB*, und *exrA* Gene. Sedimentation zeigte jedoch keinen Unterschied im Ausmaß der Reparatur von DNA Einzelstrang-Brüchen nach Röntgenbestrahlung in Puffer oder Wuchsmedium. Somit scheint die erhöhte Röntgenstrahlen-Empfindlichkeit bei der Bestrahlung in Puffer von der Hemmung eines *rec* und *exr* gen-abhängigen Reparatur-Prozesses herzurühren, der von der Reparatur von DNA-Einzelstrang-Brüchen verschieden ist.

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