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The Repair of DNA Single-Strand Breaks in *E. coli* K-12 X-Irradiated in the Presence or Absence of Oxygen; the Influence of Repair on Cell Survival

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The repair of DNA single-strand breaks has been studied in *Escherichia coli* K-12 (W3110) after X irradiation in the presence and absence of oxygen. Upon completion in buffer of a very fast repair process (Type I) there are three times as many breaks remaining after irradiation in the presence as compared with the absence of oxygen. These breaks are then rapidly repaired in buffer by the Type II system; a process that is deficient in strains carrying the *polA1* mutation (an amber mutation in the structural gene for DNA polymerase I). After low doses of x rays, the Type II system can repair all of the breaks presented to it. Beyond a threshold dose it repairs a constant proportion (~90%) of the breaks presented to it whether or not oxygen is present during irradiation. Consistent with the apparent insensitivity of the Type II repair process to the presence of oxygen during irradiation, otherwise isogenic pol^+ and pol^- derivatives were found to have the same oxygen enhancement ratio (OER) for cell survival.

After Type II repair is complete, some of the remaining breaks can be repaired by the growth-medium-dependent Type III system. This process can repair only about 2 additional breaks per single-strand genome in this strain, whether irradiation is in the presence or absence of oxygen. Thus, as far as the repair of DNA single-strand breaks is concerned, neither the Type II nor the Type III repair system shows any specificity for breaks produced in the presence or absence of oxygen.

OER values were determined for the *recA* and *recB* mutants which are deficient in Type III repair. While the *recB* derivative showed only a slight reduction in OER, the *recA* derivative had an OER of about 2.0 compared with about 3.4 for the rec^+ parental strain. Since the *recA* mutation would affect Type III repair to the same extent whether the cells were x-irradiated in the presence or absence of oxygen, the reduced OER for the *recA* strain probably reflects the additional involvement of the *recA* gene product in the repair of some other lesion, possibly based damage, that is affected by the presence of oxygen during irradiation.

INTRODUCTION

The repair of radiation-induced DNA single-strand breaks appears to be a process important for bacterial cell survival, since cells which show a reduced capacity to repair such breaks, either because of a mutational defect (*1–3*), altered growth conditions (*4*), or the presence of inhibitory compounds during

(*5*, *6*) or after (*7–10*) irradiation, are also more sensitive to radiation. We have recently suggested (*11*) that the role of oxygen in modifying cellular radiosensitivity is due to differences in the chemical nature of the breaks produced by irradiation in the presence as compared with the absence of oxygen, rather than the quantity in which they are initially produced. The chemical nature of the end groups at the break and the size of the strand interruption determine how many and which enzymes would be necessary to repair the break. Radiation conditions (e.g., anoxia) which enhance the proportion of easily reparable breaks should give improved cell survival by allowing a greater number of breaks to be repaired (*11*).

When the number of DNA single-strand breaks produced in the presence or absence of oxygen was measured under conditions that minimized enyzmatic repair processes, it was observed that the initial yield of DNA single-strand breaks was essentially independent of the presence of oxygen at the time of irradiation (*11*). However, in anoxically irradiated cells a large fraction of this initial number of breaks was very rapidly repaired at 0°C, while after aerobic irradiation, only a small fraction of breaks was repaired under the same conditions. We propose to call this very fast process Type I repair since it is the first to be completed after irradiation. If it is not inhibited, approximately one-third as many breaks remain after its action in cells irradiated under anoxic as compared with aerobic conditions (*11*). Single-strand breaks which are not repaired by the Type I process then become the substrate for two additional repair systems which have already been identified in *E. coli* K-12: a repair system that proceeds rapidly to completion in buffer at room temperature and is primarily dependent upon DNA polymerase I (*3*), which we will call Type II repair; and a growth-medium-dependent repair system that proceeds slowly to completion (20–40 min) at 37°C and requires the presence of functional *rec* genes (*2*)[1], which we will call Type III repair.

It is the purpose of this paper to examine the extent to which DNA breaks remaining after the Type I process are repaired by the Types II and III systems, and whether the number of breaks repaired by each mechanism is influenced by the presence of oxygen during irradiation. The contribution of each repair process toward determining the oxygen effect for cell survival is assessed by examining mutants that are deficient in Type II and Type III repair.

MATERIALS AND METHODS

Bacterial Strains

Most experiments were performed using strain JG139, a thymine-requiring derivative of *E. coli* K-12 W3110 (*pol*$^+$ *rec*$^+$). For the survival data the otherwise isogenic *polA1* derivative JG138 was also used. These strains were kindly provided

[1] The yield of breaks remaining after incubation in buffer for 80 min to complete Type II repair was approximately the same for *rec*$^+$, *recA*, and *recB* cells. When the cells were incubated for 80 min in growth medium instead of buffer, there was additional repair of breaks in *rec*$^+$ cells but not in *recA* cells. The *recB* strain showed a small amount of additional repair in medium. Thus, *recA* and *recB* cells are deficient in growth-medium-dependent Type III repair (D. A. Youngs and K. C. Smith, manuscript in preparation).

by Dr. Julian Gross. The W3110 *recA56* strain (MM450) was obtained from Dr. Marilyn Monk. The procedure described by Hertman and Luria (*12*) was used to isolate *rec*$^+$ (SR207) transductants from this *recA* (SR176) strain. An otherwise isogenic *recB21* (SR208) and *rec*$^+$ (SR209) pair of strains was constructed by P1 transduction of JG139 *thy*$^-$ to *thy*$^+$ and selecting radiation sensitive and radiation resistant *thy*$^+$ recombinants.

Culture Conditions

Cells were grown at 37°C with aeration to log phase ($\sim 2 \times 10^8$ cells/ml) in a glucose salts medium (*13*) supplemented with 2 μg/ml thymine when necessary. For sedimentation experiments, 50 μCi/ml ^{3}H-thymine was included (New England Nuclear, 15–20 Ci/mmole). Cell survival was determined by dilution in phosphate buffered saline (PBS), pH 6.8 (KH_2PO_4 0.165 g, Na_2HPO_4 0.20 g, KCl 0.20 g, NaCl 8.0 g, H_2O to liter) and plating on the above growth medium (supplemented with 10 μg/ml thymine when necessary) solidified with 0.9% Oxoid agar-agar #3.

Irradiation

Cells were collected by Millipore filtration (0.45 μm pore size), washed, resuspended in PBS at room temperature, and allowed to stand at room temperature for 10 minutes before being irradiated at 0°C or room temperature as described previously (*11*).

Sedimentation Experiments

Experiments were performed generally as described previously (*3*) except that fewer cells per gradient were used. Cell suspensions in PBS were adjusted to $OD_{650} \sim 0.02$ (Zeiss PMQII spectrophotometer) either before or after irradiation and 50 μl samples ($\sim 10^6$ cells) were layered onto the Sarkosyl (Geigy, NL30)-NaOH capped gradients.

For the measurement of breaks remaining after Type III repair, a further modification was made. We found that x-irradiated cells became sensitive to handling (filtration and resuspension), especially after post-irradiation incubation in growth medium. This fragility led to variability in the DNA sedimentation profiles, presumably due to cell breakage and shearing of the DNA during handling. Therefore, after irradiation in PBS the cells were centrifuged at room temperature (during the 15-minute period allowed for Type II repair), gently resuspended in growth medium and incubated for 60 min at 37°C. At the end of this period, cells in growth medium were diluted to $OD_{650} \sim 0.02$ and layered directly onto gradients capped with a 0.1 ml layer of 0.5% Sarkosyl in 0.5 *N* NaOH, 10^{-2} *M* EDTA. The EDTA chelates the divalent cations in the growth medium which would otherwise form a complex with the Sarkosyl and cause most of the DNA to sediment rapidly to the bottom of the tube. This modified lysis procedure had no effect on the sedimentation profiles of the DNA from cells in buffer (or PBS) and in later experiments was therefore used for cells suspended in buffer as well.

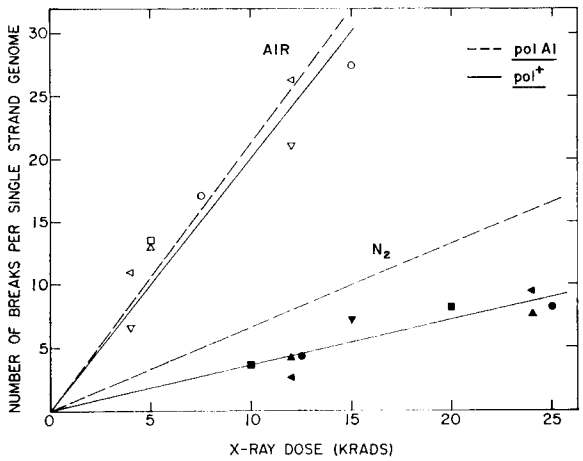

FIG. 1. Production of DNA single-strand breaks in *E. coli* K-12 *pol*$^+$ irradiated at 0°C in PBS and lysed immediately after irradiation (solid lines). Different symbols show data from separate experiments; open symbols in equilibrium with air, closed symbols with N_2. The broken lines show the yields observed in *polA1* cells under the same conditions [ref (*11*)].

RESULTS

Type II Repair

Rapid lysis experiments. We reported previously (*3*) that the same number of single-strand breaks was detected following the rapid lysis of *pol*$^+$ and *polA1* cells irradiated aerobically with 16.3 krads at 0°C. This result was confirmed and extended to various doses as shown in Fig. 1. For cells irradiated aerobically at 0°C and rapidly lysed, essentially the same number of breaks was observed in *pol*$^+$ cells as in *polA1* cells [i.e., the slopes of the two regression lines are not significantly different; 2.02 vs 2.13 breaks per single-strand genome (1.4×10^9 daltons) per krad for *pol*$^+$ and *polA1*, respectively)].

When JG139 (*pol*$^+$) cells were irradiated anoxically at 0°C and lysed immediately after irradiation, the number of breaks observed was only about half of that found previously in *polA1* cells under these conditions (*11*). The slope of the regression line fitting the data from anoxic irradiations (in Fig. 1) is 0.36 breaks per single-strand genome per krad, and differs significantly from the value of 0.66 breaks per single-strand genome per krad for *polA1* cells under similar conditions. This result suggests that the Type II repair of breaks produced under anoxic conditions was less inhibited at 0°C than the Type II repair of aerobic breaks, since approximately one-half of the anoxic breaks were repaired at 0°C within the irradiation period (2–4 minute). The breaks formed under aerobic irradiation conditions were repaired in *pol*$^+$ cells in buffer with a half time of 10 minute at 0°C (data not shown).

In the presence of 10^{-3} *M* sodium cyanide at 0°C, which was previously shown to inhibit DNA polymerase I dependent repair (*3*), the yield of breaks observed in *pol*$^+$ cells in the absence of oxygen (for doses less than 15 krad) increased to the level seen in *polA1* cells irradiated under the same conditions [10^{-3} *M* cyanide

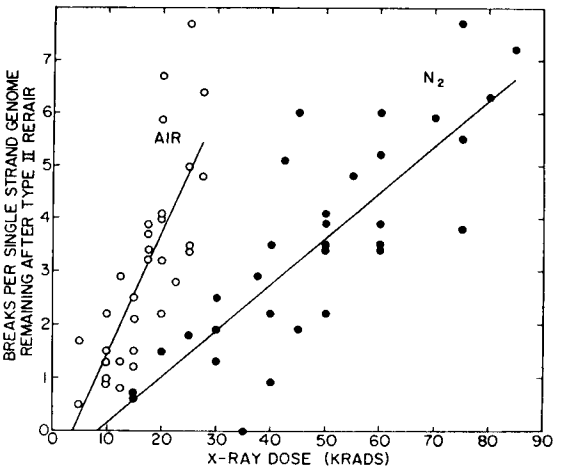

FIG. 2. Number of DNA single-strand breaks remaining after Type II (buffer) repair in JG139 (*pol*$^+$). Cells were irradiated at room temperature in PBS in equilibrium with either air (open symbols) or N_2 (closed symbols) and incubated at room temperature in PBS for 15 min after irradiation. They were then lysed on gradients and analyzed by sedimentation as described in the text.

had no effect on the yield of breaks in *polA1* cells (*11*)]. After higher doses of radiation (15–25 krad) (i.e., longer irradiation times) this concentration of cyanide was not sufficient (cyanide presumably was lost during the gassing pro-

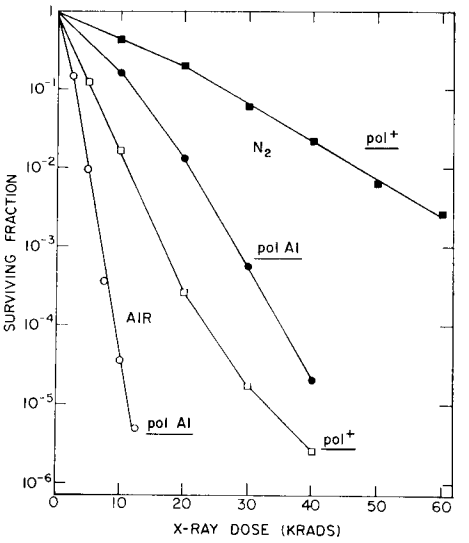

FIG. 3. X-ray survival curves for isogenic *pol*$^+$ and *polA1* derivatives of *E. coli* K-12 irradiated in equilibrium with either air or N_2. Cells were irradiated at room temperature in PBS, diluted in PBS and plated on minimal medium agar. Each point is the mean of several independent determinations. ○, *polA1*-Air; ●, *polA1*-N_2; □, *pol*$^+$-Air; ■, *pol*$^+$-N_2.

TABLE I

X-RAY SENSITIVITY AND OXYGEN ENHANCEMENT RATIOS OF ISOGENIC *polA1* AND *pol*$^+$ DERIVATIVES OF *E. coli* K-12 W3110

Strain	*Dose (krad) giving 1% survival*		*OER*	D_0 *(krad)*[a]		*OER*
	Air	N_2		*Air*	N_2	
JG138 *polA1*	4.9	20.8	4.2	0.9	3.1	3.5
JG139 *pol*$^+$	11.1	46.8	4.2	2.4	9.0	3.7

[a] Dose required to reduce survival by 63% on the exponential part of the survival curve.

cedure) to completely inhibit Type II repair following anoxic irradiation of *pol*$^+$ cells (data not shown).

Number of breaks remaining after Type II repair. Cells were irradiated in PBS at room temperature after equilibration with either air or nitrogen, and then allowed to complete Type II repair during a further 15-min incubation at room temperature in the same buffer without gassing before a sample of cells was lysed directly on the gradient. Results from experiments over a range of radiation doses are shown in Fig. 2; the lines were fitted by linear regression analysis. The line for aerobically irradiated cells has a slope of 0.23 breaks per single-strand genome per krad and intersects the abscissa at 3.7 krad. For anoxically irradiated cells, the slope is 0.087 breaks per single-strand genome per krad, and the intercept is 8.3 krad.

Survival data for polA1 and pol$^+$ *strains.* In order to assess the possible contribution of Type II repair towards determining the size of the oxygen effect for cell survival, data were obtained for the survival of otherwise isogenic *polA1* and *pol*$^+$ derivatives irradiated in equilibrium with either air or nitrogen under the same conditions used for the sedimentation experiments. The results are shown in Fig. 3, and the data are summarized in Table I. Overall, the *polA1* cells are about 2.5 times more sensitive to killing by x-rays than their *pol*$^+$ counterparts under these growth conditions. The protection afforded by anoxia is the same in *pol*$^+$ as in *polA1* cells whether one uses the criterion of D_0 or the dose to give 1% survival.

Type III Repair

Breaks which remain after the completion of Type II repair in buffer may be repaired by the Type III system when *rec*$^+$ cells are subsequently incubated under conditions which permit growth (*2*).[2] These results are shown in Fig. 4; the lines drawn have been fitted by regression analysis. The slopes are 0.21 and 0.083 breaks per single-strand genome per krad for air and nitrogen, respectively, and do not differ significantly from the slopes of the air and nitrogen lines in Fig. 2 for breaks remaining after Type II repair.

These experiments show that at doses below ~10.8 krad in air, or ~34.5 krad in nitrogen, Type III repair rejoined as completely as could be detected

[2] See footnote 1.

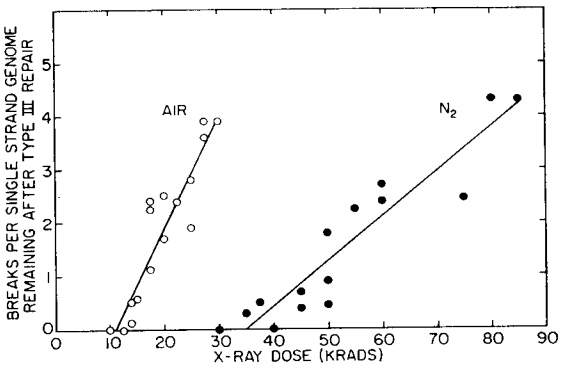

FIG. 4. Number of DNA single-strand breaks remaining after Type III repair in JG139 (*pol*$^+$ *rec*$^+$). Cells which had completed Type II repair in buffer were then incubated for 60 min in minimal medium at 37°C to complete Type III repair before being lysed and analyzed by sedimentation. Details are given in the text.

by sedimentation analysis all the breaks remaining after Types I and II repair. As can be deduced from Fig. 2 and 4, the maximum number of breaks which could be completely repaired after these doses was about 2 per single-strand genome after either aerobic or anoxic irradiation. Above these "critical doses" the number of breaks which were not repaired by Type III increased at about the same rate as the number of breaks not repaired by Type II (i.e., presented to Type III).

Survival data for rec$^-$ *and rec*$^+$ *strains.* In order to assess the possible contribution of Type III repair towards determining the size of the oxygen effect for cell survival, we have measured the OER for *recA* and *recB* strains which are deficient in Type III repair.[3] Cells were irradiated under the same conditions as for the sedimentation experiments. Both of the *rec*$^+$ transductants (partners for the *recA* and *recB* derivatives) had x-ray sensitivities very similar to that of JG139.

TABLE II

RADIATION SENSITIVITY AND OXYGEN ENHANCEMENT RATIOS FOR RECOMBINATION DEFICIENT DERIVATIVES OF *E. coli* K-12 W3110

Relevant allele	D_0 (*krad*)[a]		*OER*
	Air	N_2	
recA56	1.2	2.4	2.0
rec$^+$	2.8	9.6	3.4
recB21	1.4	4.3	3.1
rec$^+$	2.7	9.1	3.4

[a] Dose required to reduce survival by 63% on the exponential part of the survival curve. For all curves, survival was exponential down to $\sim 10^{-2}$ survival, and only the *rec*$^+$ curves for aerobic irradiation deviated from exponential below that survival level.

[3] See footnote 1.

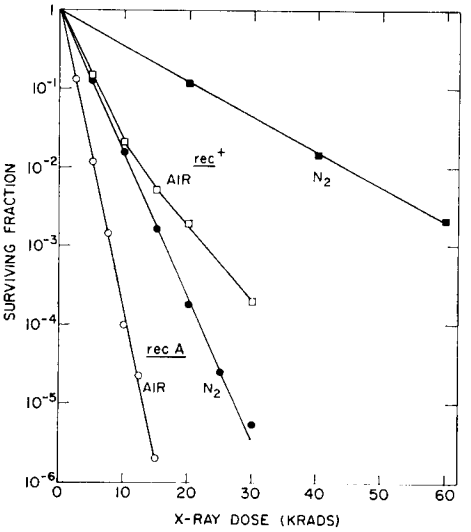

FIG. 5. X-ray survival curves for *E. coli* K-12 *recA56* and a *rec*$^+$ transductant. Cells were irradiated in PBS at room temperature in equilibrium with either air or N_2. They were diluted in PBS and plated on minimal medium agar. ○, *recA56*-Air; ●, *recA56*-N_2; □, *rec*$^+$-Air; ■, *rec*$^+$-N_2. Each point is the mean of two independent determinations.

The *recB21* strain had an OER of 3.1 which is close to the value of 3.4 for its otherwise isogenic *rec*$^+$ partner. However, the OER for the *recA56* strain was 2.0 compared to 3.4 for its *rec*$^+$ partner. The curves for *recA56* and its *rec*$^+$ partner are shown in Fig. 5 and all the data are summarized in Table II.

DISCUSSION

We have presented data on the extent of repair of DNA single-strand breaks by the Type II and Type III systems after various doses of x-irradiation in the presence or absence of oxygen. These two repair systems act on the breaks remaining after the completion of Type I (very fast) repair (*11*).

We have defined Type II repair to include all repair processes which rejoin DNA single-strand breaks in buffer after the completion of Type I repair. In wild-type cells this process is largely dependent upon DNA polymerase I (*3*).[4] The Type II repair of breaks produced under aerobic irradiation conditions is

[4] The *polA1* mutants, which lack DNA polymerase I, are apparently not totally deficient in the buffer repair of x-ray-induced single-strand breaks. If aerobically irradiated *polA1* cells are incubated in buffer at 37°C for 80 min, they *slowly* repair about 75% of the breaks remaining after Type I repair, compared to *pol*$^+$ cells which *rapidly* repair about 90% of the breaks. Preliminary results indicate that this repair process is absent in a *polA1 dnaE* strain at the restrictive temperature, thus implicating DNA polymerase III in this process (D. A. Youngs and K. C. Smith, manuscript in preparation). The rate of buffer repair in *pol*$^+$ cells (i.e., due to DNA polymerases I and III) is about 5 times faster than that observed in *polA1* cells (i.e., due to DNA polymerase III). Thus, the *dnaE* gene-mediated Type II repair can account, at most, for less than ~11% of the total Type II repair occurring in *pol*$^+$ cells, since the two polymerases appear to repair largely the same class of breaks.

more strongly inhibited at 0°C than is the Type II repair of breaks produced under anoxia. This could mean that a greater number of enzymes are required for the repair of breaks produced under aerobic conditions. The Type II repair of breaks produced under anoxia presumably requires at least two enzymes—a DNA polymerase and a DNA ligase. The Type II repair of aerobically produced breaks may therefore require at least one additional enzyme (possibly a phosphatase or nuclease to provide the 3′OH terminus for DNA polymerase).

The slopes of the dashed lines in Fig. 1 [from Ref. (*11*)], for the number of breaks detected in *polA1* cells under rapid lysis conditions, give the yields in the presence and absence of oxygen of those breaks not subject to Type I repair. The slopes of the lines after Type II repair (Fig. 2) give the yields of breaks which cannot be repaired by Type II mechanisms. The difference in slopes of the two pairs of lines is a measure of the extent of Type II repair. After low doses of radiation (<3.7 krad in air or <8.3 krad in N_2) all the breaks remaining after Type I repair are rejoined in buffer at room temperature by the Type II mechanism. At higher radiation doses the slopes of the lines after Type II repair are about 10% of what they were before Type II repair indicating that the Type II mechanism can repair 90% of the breaks presented to it whether the breaks were produced in the presence or absence of oxygen. The linearity of the data, over a considerable dose range, for breaks remaining after Type II repair suggests that the failure of the Type II mechanism to repair all the breaks above a certain dose is not due to saturation of the repair process. These results also suggest that above a threshold dose, a class of breaks arises which is not subject to Type II repair, and which is produced at a rate which is linear with dose above the threshold.

A possibly analogous threshold effect has been observed *in vitro* for radiation-induced loss of the chromophore from DNA in dilute solution (*14*). The bases are thought to be protected from radical attack as long as they remain within the intact double helix.[5] If a similar situation were to occur *in vivo*, the damaged bases might then preclude repair of such a break by the Type II mechanism.

The fact that the survival curves for otherwise isogenic *pol*$^+$ and *polA1* cells have the same oxygen enhancement ratio suggests that repair processes involving DNA polymerase I (i.e., Type II repair) are equally effective for lesions produced in the presence or absence of oxygen. This is consistent with the observation that beyond a threshold dose an equal proportion of single-strand breaks was repaired by the Type II process whether the breaks were produced in air or nitrogen. The greater rate of the Type II repair of breaks produced under anoxia is presumably less significant for cell survival than the final relative proportions of breaks repaired.

The Type III repair process differs from the Type I and Type II processes in that it requires growth medium and can repair only a few of the breaks presented to it. At low doses of radiation the Type III repair process can repair all the breaks presented to it. At higher doses no more than about two breaks per

[5] In confirmation of this hypothesis, J. F. Ward and I. Kuo (Abstract Ec-4, Radiation Research Society Meeting, April 29–May 3, 1973) have recently shown that this threshold effect for the radiation-induced loss of chromophore from DNA is eliminated if a few strand breaks (1–2 per 100 nucleotides) are introduced into the DNA with deoxyribonuclease prior to x-irradiation.

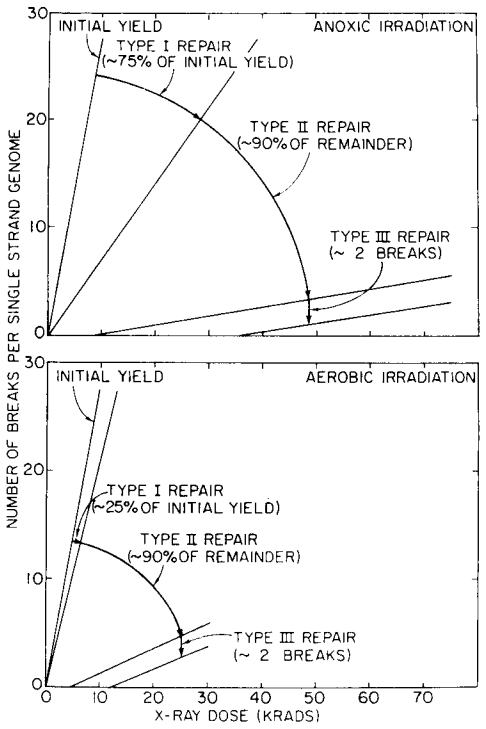

FIG. 6. Summary of the capacity of the Types I, II and III systems for repairing x-ray-induced DNA single-strand breaks. The initial yield was ~2.8 DNA single-strand breaks per single-strand genome (1.4×10^9 daltons) per krad in air or nitrogen (*11*). The very fast Type I system can repair in buffer about 75% of these breaks if they were produced under nitrogen but only 25% if produced in air (*11*). The Type II system then can rapidly repair in buffer about 90% of the remaining breaks whether produced in air or nitrogen (from Fig. 2). The Type III system can repair slowly in growth medium an additional two breaks per single-strand genome whether produced in air or nitrogen (from Fig. 4).

single-strand genome are repaired, irrespective of the number present in the chromosome at that time. These numbers refer strictly to this strain (JG139) under the reported radiation and growth conditions.[6]

The Type III system does not show any preferential repair of breaks produced in the presence or absence of oxygen. However, *recA* strains, which lack the Type III system for rejoining DNA single-strand breaks (*2*),[7] have a lower OER than their wild-type counterparts [this paper and Ref. (*15*)]. The absence of *recA* controlled repair sensitizes anoxically irradiated cells to a greater extent than aerobically irradiated ones. Since this cannot be explained by differential repair of DNA single-strand breaks, we hypothesize that the *recA* gene is also involved in the repair of damage other than single-strand breaks. By analogy

[6] Recent evidence indicates that *E. coli* K-12 strain AB2497, which is somewhat more resistant to x-irradiation than is strain JG139, is capable of more extensive medium dependent repair than is strain JG139 (D. A. Youngs and K. C. Smith, manuscript in preparation).

[7] See footnote 1.

with the *rec* gene-controlled system(s) for the repair of uv-induced DNA base damage (*16*, *17*), this postulated second *recA*-dependent recovery system could involve the repair of x-ray-induced base damage.

The *recB* strain, which is also deficient in Type III repair (*2*),[8] shows an OER only slightly less than that of its otherwise isogenic *rec*$^+$ partner. This suggests that the *recB* gene product is less involved than the *recA* gene product with repair systems other than the Type III repair system.

In summary, we have shown that in *E. coli* K-12, the Type II and Type III repair systems show little or no specificity (in terms of the amount of repair) for DNA single-strand breaks produced by x-irradiation in the presence or absence of oxygen. The OER for survival must be a composite of the differential effects of oxygen and nitrogen on the production and repair of several different types of x-ray-induced damage, of which DNA single-strand breaks are one example. To the extent that unrepaired single-strand breaks contribute to cell death, only the differential ability of the Type I repair system to rejoin single-strand breaks produced in the presence or absence of oxygen can be a factor in determining the OER for cell survival.

The capacities of the Types I, II, and III systems for the repair of DNA single-strand breaks produced under air or nitrogen are summarized in Fig. 6.

ACKNOWLEDGMENTS

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[8] See footnote 1.

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