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R.b.e. of 50 kVp X-rays and 660 keV γ-rays (^{137}Cs) with respect to the production of DNA damage, repair and cell-killing in *Escherichia coli* K-12

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We have compared the efficiency of cell-killing, DNA single-strand breakage and double-strand breakage in an *Escherichia coli* K-12 wild-type strain after irradiation with soft X-rays (50 kVp) and hard γ-rays (660 keV) under aerobic conditions. Irradiation with 50 kVp X-rays resulted in 1·47 times more cell-killing than was observed with ^{137}Cs γ-rays based on a comparison of D_0 values evaluated from the survival curves. DNA sedimentation studies showed that, although 50 kVp X-rays were 1·93 times more effective than ^{137}Cs γ-rays in producing DNA double-strand breaks, there was no significant difference between the two qualities of radiation with respect to the initial number of single-strand breaks produced. When the cells were irradiated and allowed to repair maximally in minimal medium, 1·57 times more unrepaired DNA single-strand breaks remained per krad after irradiation with 50 kVp X-rays than with ^{137}Cs γ-rays. The increased yield of DNA double-strand breaks resulting from 50 kVp X-irradiation may account for most of these additional unrepaired single-strand breaks, since single- and double-strand breaks are indistinguishable on alkaline sucrose gradients. These results suggest that the greater r.b.e. of 50 kVp X-rays may be related to an increased effectiveness for producing DNA double-strand breaks compared with the higher energy ^{137}Cs γ-rays.

1. Introduction

The efficiency by which ionizing radiation produces a biological effect has been shown to be a function of the quality of the radiation. For example, numerous studies have shown that 200 kVp X-rays are more effective than hard γ-rays in producing a radiobiological effect. Sinclair, Gunter and Cole (1959) have shown that 200 kVp X-rays are 1·17 times more effective than ^{60}Co γ-rays in inactivating *Saccharomyces cerevisiae*. Similar r.b.e. values were obtained by Sinclair and co-workers, using other biological systems and end-points (Sinclair and Blackwell 1962, Sinclair, Blackwell and Humphrey 1962). Other investigators have made similar observations using mammalian cells in culture with changes in D_0 or $LD_{50/30}$ as end-points (Hendry 1972, Malone, Porter and Hendry 1974). Although the determination of r.b.e. for therapeutic X-rays and hard γ-rays has been intensively studied, no determinations of molecular changes in DNA have accompanied the biological end-points.

The most likely mechanism to explain why photons of lower energy are more effective than high-energy photons probably lies in the greater ionization density (or LET) of the low-energy secondary electrons which they produce. Using microdosimetry, Rossi (1964) has shown that low-energy secondary electrons arising from hard γ-ray interactions can deposit as much energy in a small volume as a proton produced by a high-LET neutron. Soft X-rays, which produce relatively more low-energy electrons than do hard γ-rays, should have

a significant component of high-LET secondary electrons and should exert an effect on DNA which is comparable, at least in a qualitative sense, to those effects seen by using high-LET particles. In this regard, most studies concerning the molecular changes brought about by high-LET radiation have dealt with accelerated particles interacting with free DNA or bateriophage (Hutchinson 1965, Christensen, Tobias and Taylor 1972, Neary, Simpson-Gildemeister and Peacocke 1970, Neary, Horgan, Bance and Stretch 1972). These studies have shown that, as the LET of an accelerated particle increases, that particle has a greater probability of introducing a double-strand break into DNA than a less heavily-ionizing particle or sparsely-ionizing hard γ-rays. Some recent experiments (M. A. Ritter, personal communication), using Chinese hamster cells, demonstrated that the repairability of DNA strand breaks, as measured on alkaline sucrose gradients, decreased as the particle LET increased. Unfortunately, because of the limitations of alkaline sucrose gradients, it was not possible to determine whether the repairability of single and/or double-strand breaks decreased with increasing LET, since both lesions have been shown to be repaired in Chinese hamster cells (Corry and Cole 1973, Veatch and Okada 1969).

Here we present data which show that 50 kVp X-rays have a significant r.b.e. with respect to ^{137}Cs γ-rays, using both a biological end-point (survival) and a molecular end-point (DNA strand breakage).

2. Materials and methods

2.1. *Bacterial strains and growth conditions*

Three strains of *E. coli* K-12 derived from W3110 were used. Two were originally obtained from J. D. Gross: JG138 *polA*1 *rha lac str thyA thyR* and JG139 *pol*$^+$ *rha lac str thyA thyR*. A second *polA*1 strain was used in some studies: DY100 *polAl lac str metE thyA thyR* (Youngs and Smith 1973).

A minimal salts medium (Ganesan and Smith 1968) was supplemented with thiamine (0·5 μg/ml) and thymine (10 μg/ml) for overnight cultures (and exponentially-growing cultures used only for survival experiments) and 10^{-3} M methionine when required. The overnight cultures were diluted 1 : 50 into fresh medium supplemented with ^{3}H-methyl-thymine (New England Nuclear Corp., >12 Ci/mmol) at 200 μCi/ml for cells to be used for the determination of DNA double-strand breakage and 100 μCi/ml for cells used for the determination of single-strand breakage. Unlabelled thymine at 2 μg/ml was added to labelled cultures. Cells were grown at 37°C for several generations in exponential phase to a density of $\sim 2 \times 10^8$ cells/ml.

2.2. *Irradiation conditions*

Cells were collected by filtration on to membrane filters (0·45 μm pore size, Millipore Corp.) and resuspended at room temperature in either 0·05 M tris(hydroxymethyl)amino-methane (tris) buffer, pH 7·6 or DTM buffer (minimal salts medium without glucose or supplements). Results obtained with either buffer were similar. Cells were resuspended to a density of $\sim 4 \times 10^7$ cells/ml for sedimentation experiments and $\sim 2 \times 10^8$ cells/ml for survival assays.

Irradiations were performed on cell suspensions that were aerated for 5 min before and during irradiation. Samples were maintained at 0° to 4°C or room temperature ($\sim$23°C) during irradiation as desired.

X-irradiation was performed using the twin-tube 50 kVp X-ray unit described by Loevinger and Huisman (1965). The X-ray tubes were operated at 48 and 50 mA with 0·25 mm Al added filtration. The vessel containing the cell suspension was constructed of Plexiglas. The dose-rate was determined by ferrous sulphate dosimetry, using a *G* value of 14·6 (Geisselsoder and Karzmark 1969) and a correction for the mass absorption coefficient of a biological sample as compared with the dosimeter solution (Loevinger and Huisman 1965). The dose-rate thus determined was 7·21 krad/min.

Gamma-irradiation was performed using an 8000 Ci ^{137}Cs source (J. L. Shepard and Associates), emitting a γ-photopeak with an energy of 660 keV. The irradiation vessel was a water-jacketed glass tube. Dosimetry was undertaken by the ferrous sulphate method described above, using a *G* value of 15·6 (Shalek and Smith 1969) with no mass absorption correction: the dose-rate was 5·66 krad/min. As a check on the chemical dosimetry, thermoluminescent (LiF) dosimetry and readings from a PTW ionization chamber both gave dose-rates of 5·5 krad/min. Dose-rates determined by the ferrous sulphate method were considered to be accurate and were used in the experiments reported in this paper.

2.3. *Survival curves*

For survival measurements, irradiated cells were diluted in 0·067 M phosphate buffer pH 7·0 and plated on minimal medium plates supplemented with 10 μg/ml thymine (1·6 per cent Difco Noble agar). The plates were incubated 48–72 hours at 37°C to allow for colony formation.

2.4. *Sedimentation experiments*

Irradiated cells were transformed into spheroplasts and lysed on neutral sucrose gradients to determine the production of DNA double-strand breaks. Details of the composition of the sucrose gradients and sedimentation conditions have appeared elsewhere (Bonura, Town, Smith and Kaplan 1975, Bonura and Smith 1975). Single-strand breaks were measured by the alkaline sucrose gradient method described by Town, Smith and Kaplan (1971).

Number average molecular weights were calculated directly from the sedimentation profiles when possible. However, sedimentation profiles for cells irradiated with doses > 24 krad and allowed to repair (figure 4) contained low-molecular-weight material, which resulted in an underestimate for M_n. For these experiments the weight average molecular weight was calculated, as it is a measurement less sensitive to material in the trailing portion of the profile (Ehmann and Lett 1973). The relation, $2/M_w = 1/M_n$ is assumed in figure 4.

3. Results

3.1. *Survival curves*

Figure 1 shows survival curves for the wild-type strain JG139 irradiated with 50 kVp X-rays and 660 keV γ-rays under aerobic conditions. The D_0 values calculated from the exponential portions of the survival curves were $3{\cdot}37 \pm 0{\cdot}1$ krad for 660 keV γ-rays and $2{\cdot}29 \pm 0{\cdot}065$ krad for 50 kVp X-rays. Thus, the relative biological effectiveness (r.b.e.) for 50 kVp X-rays with respect to 660 keV γ-rays is $1{\cdot}47 \pm 0{\cdot}06$. We have observed a similar r.b.e. using the DY100 and JG138 *polA*1 strains and also for a *recB* strain (data not shown).

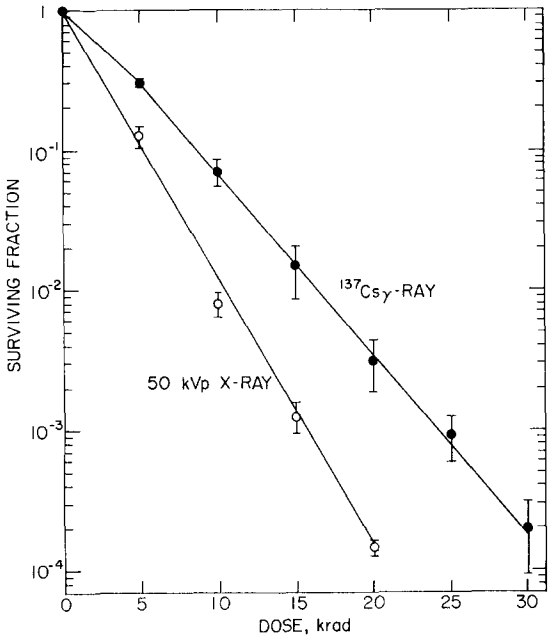

Figure 1. Survival curves for the *E. coli* K-12 wild-type strain (JG139) irradiated under aerobic conditions at room temperature in 0·05 M tris buffer pH 7·6 with either 50 kVp X-rays (○—○), or 660 keV ^{137}Cs γ-rays (●—●). Lines fitted to data by least-squares regression analysis. Points represent the average of 3 (○—○) or 4 (●—●) experiments with associated standard deviation.

3.2. *DNA sedimentation studies*

We measured the rate at which DNA double-strand breaks (DSB) are produced by both types of radiation by irradiating JG139, converting the cells to spheroplasts, and lysing them on neutral sucrose gradients. The reciprocal number average molecular weight (which is proportional to the number of double-strand breaks) was plotted as a function of dose (figure 2). The data shown for the 50 kVp X-irradiation was taken from a previous paper (Bonura *et al.* 1975). The slopes of the regressed lines were $0{\cdot}195 \pm 0{\cdot}01 \times 10^{-9}$ krad^{-1} for 50 kVp X-irradiation and $0{\cdot}101 \pm 0{\cdot}007 \times 10^{-9}$ krad^{-1} for 660 keV γ-irradiation and were used in determining the number of double-strand breaks (table). The r.b.e. for DNA double-strand breakage by 50 kVp X-rays is $1{\cdot}93 \pm 0{\cdot}17$ with respect to 660 keV γ-rays. Since DNA double-strand breaks do not appear to be rejoined by *E. coli* K-12 under these conditions (Kaplan 1966, Bonura *et al.* 1975), the values given here may be considered to be the final yield of DNA double-strand breaks.

To arrive at an estimate of single-strand breakage† under conditions in which repair is minimized, we used two strains containing the *polA*1 mutation (JG138 and DY100). These strains have been shown to be partly defective in the rapid repair of X-ray-induced single-strand breaks which can occur in buffer (Type II repair) (Town *et al.* 1971). To further minimize repair the

† Single-strand breaks is a category used to describe those breaks measured on alkaline sucrose gradients which include double-strand breaks, true single-strand breaks, and alkali labile bonds.

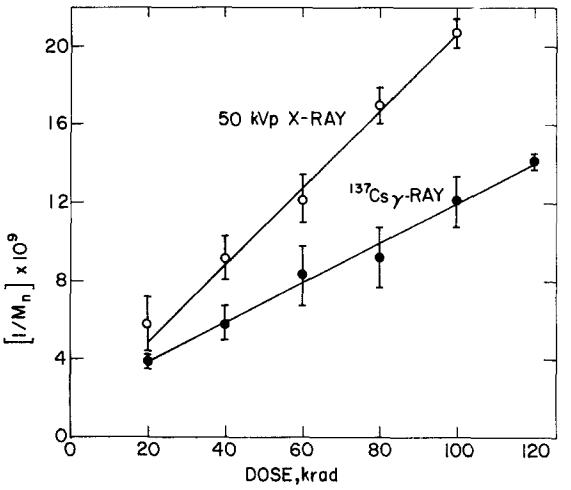

Figure 2. Production of DNA double-strand breaks in irradiated JG139 wild-type cells. The reciprocal number of average molecular weight is shown as a function of dose for DNA liberated from spheroplasts on neutral sucrose gradients. Cells irradiated at 23°C in 0·05 M tris buffer pH 7·6 under aerobic conditions with either 50 kVp X-rays (○—○) or ^{137}Cs γ-rays (●—●). Points represent the average of 5 (○—○) or 3 (●—●) experiments with associated standard deviation. Lines fitted by least-squares regression analysis.

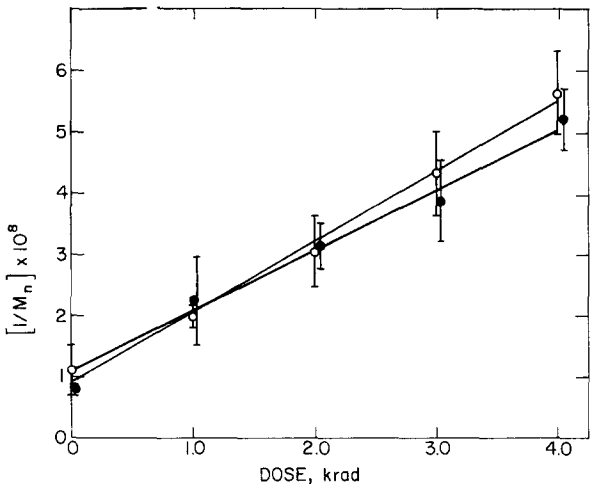

Figure 3. Production of DNA single-strand breaks in *polA*1 cells. Reciprocal number average molecular weight as a function of dose for DNA from *polA*1 cells (JG138 or DY100) lysed on alkaline sucrose gradients immediately after irradiation with 50 kVp X-rays (○—○) or ^{137}Cs γ-rays (●—●). Irradiation at 0°C under aerobic conditions, in DTM buffer (minimal medium without glucose or supplements). Points represent the average of 6 (○—○) or 4 (●—●) experiments with associated standard deviation.

	^{137}Cs γ-ray (A)	50 kVp X-ray (B)	R.b.e. (Ratio B/A)
D_0	3·370 ± 0·10	2·290 ± 0·065	1·47†
SSB	1·410 ± 0·13	2·220 ± 0·160	1·57
DSB	0·285 ± 0·02	0·550 ± 0·030	1·93
SSB–2DSB	0·840 ± 0·14	1·120 ± 0·170	1·33
Total DNA breaks remaining unrepaired [(SSB–2DSB) + DSB]	1·13	1·67	1·48

† Ratio of A/B.

Yields of DNA double-strand breaks (DSB) and single-strand breaks (SSB) in JG139 wild-type *E. coli* cells. Single-strand breaks were measured after complete repair in minimal medium at 37°C, double-strand breaks were measured immediately after irradiation. All units are breaks (single or double) per genome ($2{\cdot}8 \times 10^9$ daltons) per krad. The D_0 values (in krad) are taken from figure 1.

cells were kept at 0° to 4°C during irradiation. The slopes of the regressed lines for DNA single-strand breakage (figure 3) are $1{\cdot}15 \pm 0{\cdot}07 \times 10^{-8}$ krad^{-1} for 50 kVp X-irradiation and $0{\cdot}97 \pm 0{\cdot}17 \times 10^{-8}$ krad^{-1} for 660 keV γ-irradiation. These slopes are not statistically different ($p > 0{\cdot}05$).

A measure of the final yield of unrepairable single-strand breaks was obtained by irradiating JG139, the wild-type strain, and allowing the cells to repair completely in minimal medium at 37°C for 80 min before lysis on alkaline sucrose gradients (figure 4). More unrepairable damage resulted from irradiation with 50 kVp

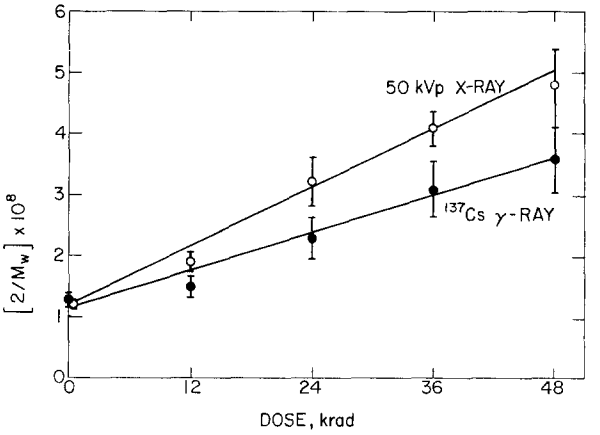

Figure 4. Repair of DNA single-strand breaks in JG139 wild-type cells. The reciprocal number average molecular weight is shown as a function of dose after maximal repair. Cells irradiated with 50 kVp X-rays (○—○) or 600 keV ^{137}Cs γ-rays (●—●) at 23°C in DTM buffer, and incubated in minimal medium at 37°C for 80 min to complete repair before being lysed on alkaline sucrose gradients. Points represent average of five experiments with associated standard deviation.

X-rays than from irradiation with ^{137}Cs γ-rays. The number of DNA single-strand breaks remaining unrepaired was calculated from the slopes of the lines and are shown in the table. The ratio of the final yields of unrepaired single-strand breaks was 1·57.

The yield of unrepaired DNA single-strand breaks shown in the table includes DNA double-strand breaks. Since a DNA double-strand break is measured as two single-strand breaks on alkaline sucrose gradients and assuming DNA double-strand breaks are not repairable (Kaplan 1966, Bonura *et al.* 1975), then the number of single-strand breaks excluding those arising from double-strand breaks can be calculated as SSB–2DSB. As shown in the table, there appeared to be 1·33 times more DNA single-strand breaks remaining unrepaired after irradiation with 50 kVp X-rays than after ^{137}Cs γ-rays even, after the double-strand break component was subtracted. Because of the large errors involved in the estimates of single- and double-strand breakage, however, this difference is not statistically significant ($p > 0{\cdot}05$). The number of strand breaks remaining unrepaired is simply the sum of the yield of DNA double-strand breaks and the yield of single-strand breaks after correction for the contribution of double-strand breaks (i.e. (SSB–2DSB) + DSB). The r.b.e. for 50 kVp X-rays in terms of the number of DNA strand breaks remaining unrepaired (1·48) is in close agreement with the value obtained using the survival parameters ($1{\cdot}47 \pm 0{\cdot}06$).

4. Discussion

Many observations concerning the r.b.e. of various energies of X- and γ-radiation have been made with regard to cell survival. However, to our knowledge, none of these studies has described associated macromolecular changes such as DNA strand breakage. In the present study we have compared the r.b.e. of 50 kVp X-rays with 660 keV γ-rays in terms of survival, and DNA single- and double-strand breakage.

The r.b.e. of 50 kVp X-rays in terms of cell-killing was 1·47 with respect to 660 keV γ-rays (figure 1). Sinclair *et al.* (1959) reported an r.b.e. of approximately 1·17 for 200 kVp X-rays with respect to 22 MVp X-rays and ^{60}Co γ-rays. Thus, the very low-energy 50 kVp X-rays which have been routinely used in this laboratory probably have a significant r.b.e. even with respect to 200 kVp X-rays, although we have not tested this possibility.

The r.b.e. of 50 kVp X-rays relative to 660 keV γ-rays was the same when either survival or the total number of unrepaired DNA strand breaks (corrected single plus double) was evaluated (table). Most of the enhanced killing by 50 kVp X-rays can be attributed to an enhanced effectiveness in producing DNA double-strand breaks (table), a lesion that has often been shown to be dependent on LET.

The range of LETs for 50 kVp X-rays is approximately 12 to 200 MeV-cm^2/g, with a mean of approximately 70 MeV-cm^2/g (ICRU 1970). The ^{137}Cs γ-rays show a similar range of about 2 to 200 MeV-cm^2/g, however, the average LET is approximately 2 MeV-cm^2/g. Thus, 50 kVp X-rays will produce a greater fraction of high-LET secondary electrons than will ^{137}Cs γ-rays.

There have been studies of the change in r.b.e. as a function of LET for DNA double-strand breakage in bacteriophage DNA preparations (Christensen

et al. 1972, Neary *et al.* 1970, 1972). The DNA preparations, irradiated with accelerated particles under conditions which minimize indirect action, showed significant increases in the rate of double-strand breakage only for LET values > 100 MeV-cm^2/g.

In contrast, our data for DNA double-strand breakage show that 50 kVp X-rays (with an average electron LET of 70 MeV-cm^2/g) have an r.b.e. of 1·93 relative to ^{137}Cs γ-rays. There may be several reasons to account for the quantitative discrepancy between our results and those obtained with accelerated particles and bacteriophage DNA. Firstly, the comparison of average LET values for X-irradiation with those calculated for particulate irradiation may be misleading. The LET spectrum associated with charged particles is much narrower than that associated with secondary electrons resulting from X-irradiation. The presence of a significant fraction of high-LET secondary electrons after X-irradiation might exert a strong biological effect without appreciably influencing the value of the average LET. Secondly, our experiments are complicated by indirect effects which were largely eliminated in the experiments on bacteriophage DNA described above. Thirdly, the relation between r.b.e. and LET is dependent on the size of the sensitive target (Neary, Preston and Savage 1967), which for *E. coli* DNA irradiated *in situ*, may be different from bacteriophage DNA in solution.

Munson, Neary, Bridges and Preston (1967) studied changes in the sensitivity of *E. coli* as a function of irradiation by ionizing particles of various LETs. These authors formulated an hypothesis for cell-killing involving the production of two types of damage by ionizing radiation. Type I damage is proposed to be damage localized in one DNA strand, is not necessarily unrepairable, and is independent of LET. The second type of damage (Type II) varies with LET, is considered irreparable and is presumably a DNA double-strand break arising as a single event. These authors suggest that in a strain proficient in the repair of Type I lesions, sensitivity should increase with increasing LET, because of the increasing probability of forming a Type II lesion. Our data give experimental support for the increase in Type II lesions (DNA double-strand breaks) with increasing LET. It is not possible to ascertain whether or not the 1·33-fold difference in the yields of unrepaired DNA single-strand breaks (table) is real, because of the considerable uncertainties of these estimates. The data do indicate that the difference in unrepaired DNA single-strand breaks for the two radiation qualities is far less striking than the difference in DNA double-strand breakage, an observation consistent with the predictions made by Munson *et al.* (1967).

Acknowledgments

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Nous avons comparé chez une souche sauvage d'*Escherichia coli* K-12 le pouvoir létal des rayons de types X (50 kVp) et γ (660 KeV) de même que l'efficacité de ces radiations d'énergies différentes à produire des bris mono- et bicaténaires dans l'ADN bactérien en présence d'oxygène. Les valeurs Do déterminées à partir des courbes de survie obtenues après irradiation indiquent que les rayons X sont 1,47 fois plus efficaces que les rayons γ provenant d'une source de Cs^{137} pour tuer les cellules. Si on se base maintenant sur les études de sédimentation de l'ADN, il semble qu'aucune différence significative ne distingue ces deux qualités de radiations quant au nombre de bris monocaténaires produits initialement. Notons toutefois que les rayons X 50 kVp induisent 1,93 fois plus de cassures bicaténaires dans l'ADN que les rayons γ Cs^{137}; et que dans les conditions optimales de réparation en milieu minimum, les bactéries exposées à ce premier type de radiations présentent 1,57 fois plus de bris monocaténaires non réparés dans leur ADN que celles irradiées avec les rayons γ Cs^{137}. L'excédent de cassures bicaténaires observé précédemment après exposition aux rayons X 50 kVp est probablement à l'origine de la plupart de ces bris monocaténaires supplémentaires, les deux types de dommages de l'ADN ne pouvant être distingués l'un de l'autre sur gradient alcalin de sucrose. Dans ces circonstances, il semble donc que l'efficacité biologique relative (r.b.e.) plus élevée des rayons X 50 kVp par rapport aux rayons γ Cs^{137} est attributable à leur plus grande facilité à produire des cassures bicaténaires dans l'ADN.

Es wurde die Effizienz für Zelltötung sowie die Bildung von Einzel- und Doppelstrangbrüchen in einem *Escherichia coli* K–12 Wildtyp nach Bestrahlung unter aeroben Bedingungen mit weichen Röntgen- (50 kVp) und harten Gammastrahlen (660 keV) verglichen. Die Bestrahlung mit 50 kVp-Röntgenstrahlen führte zu einer um einen Faktor 1,47 erhöhten Abtötung, verglichen mit ^{137}Cs-Gammastrahlen, wenn man die D_0-Werte der Überlebenskurven als Grundlage der Berechnung benutzt. DNS-Sedimentationsstudien zeigten keine signifikanten Unterschiede im Hinblick aud die Zahl der Einzelstrangbrüche unmittelbar nach Exposition, obwohl 50 kVp-Röntgenstrahlen um einen Faktor 1,93 wirksamer waren als ^{137}Cs-gammastrahlen in Bezug auf die Bildung von Doppelstrangbrüchen. Wenn die Zellen nach Bestrahlung bis zur maximalen Reparatur in Minimalmedium gehalten wurden, verblieben 1,57 mal mehr unreparierte Einzelstrangbrüche pro krad in der DNS nach Einwirkung von 50 kVp-Röntgenstrahlen als bei den ^{137}Cs-Gammastrahlen. Die höhere Ausbeute an Doppelstrangbrüchen nach Bestrahlung mit 50 kVp-Röntgenstrahlen ist wahrscheinlich hauptsächlich für diese zusätzlichen unreparierten Einzelstrangbrüche verantwortlich, da beide Brucharten in alkoholischen Saccharosegradienten nicht zu unterscheiden sind. Die Ergebnisse lassen vermuten, daß die größere RBW von 50 kVp-Röntgenstrahlen in Beziehung steht zu der mit ^{137}Cs-Gammastrahlen erhöhten Bildungsausbeute an Doppelstrangbrüchen.

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