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Production and Repair of Radiochemical Damage in *Escherichia coli* Deoxyribonucleic Acid; Its Modification by Culture Conditions and Relation to Survival

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Late log-phase *Escherichia coli* B/r cells are 1.6 times more sensitive to killing by X rays than are stationary-phase cells when grown in Brain Heart Infusion (BHI) + glucose. The number of single-chain breaks formed per krad is the same for log- and stationary-phase cells. Stationary-phase cells show a somewhat greater ability to repair single-chain breaks (especially after high doses of X rays) than do log-phase cells. The rapidity and extent of postirradiation deoxyribonucleic acid (DNA) degradation are greater in log-phase cells than in stationary-phase cells. The enhanced viability exhibited by stationary-phase cells thus appears to correlate both with enhanced single-chain break repair and the reduced degradation of DNA. Cells grown to stationary phase in peptone medium (PO cells) are 3.4 times more sensitive to killing by X rays than cells grown to stationary phase in peptone medium supplemented with glucose and phosphate buffer (PG cells). The yield of single-strand breaks is the same for both types of cells (but the absolute yield is about two times higher than in the cells grown in BHI + glucose). The kinetics for the repair of single-chain breaks are the same for both types of cells for about 30 min. After this time period, further repair ceases in the PO cells but continues in the PG cells, provided that glucose is present in the medium. Postirradiation DNA degradation is both more rapid and more extensive in PO cells than in PG cells whether or not glucose is present in the postirradiation incubation medium. The survival of stationary-phase *E. coli* B/r grown in PO or PG medium is likewise unaffected by the presence of glucose in the plating medium, and thus correlates better with the lower DNA degradation seen in the PG cells than with the increased strand rejoining, since this latter process requires the presence of glucose.

There is good evidence that deoxyribonucleic acid (DNA) is an important target molecule in radiation-induced cell death [see review by Kaplan (9)]. McGrath and Williams (13) demonstrated that X irradiation produced single-strand breaks in *Escherichia coli* DNA, as determined by its reduced sedimentation rate under alkaline conditions. They showed that the same number of lesions was produced in both of the strains B/r and B_{s-1}, but that the strains differed in their ability to repair the lesions. In *E. coli* B/r there were few single-strand breaks remaining after 40 min of reincubation, whereas in strain B_{s-1} no repair was seen during this time. They showed that approximately one single-strand break was produced in each single-strand genome by the D_{37} dose for B_{s-1} and that, in the absence of repair, these lesions would account both conceptually and quantitatively for X ray-induced cell death in this strain. In strains which are capable of repairing these breaks, they are assumed to be of little significance.

A similar picture emerges from radiation studies on recombination-deficient mutants (5, 20) of *E. coli* K-12. Mutations at any of the three loci, *recA*, *recB*, or *recC*, cause a considerable increase in X-ray sensitivity, and the ability of the cells to repair X ray-induced single-chain breaks is much reduced, if not totally abolished (10). These results suggest that single-strand breaks in DNA may be significant lesions in cells which possess a reduced capacity to repair these lesions.

The above studies have correlated changes in repair capacity of cells differing from one another by mutations at one (or a few) loci with changes

in sensitivity to X rays. It seemed desirable also to study the basis of variations in radiosensitivity within a single strain. Such a study might either confirm the significance of single-strand breaks in DNA, as discussed above, or might alternatively reveal other significant lesions. The X-ray sensitivity of *E. coli* B/r can be varied quite widely by different preirradiation culture conditions (14–16)

We have therefore examined both the yield and repair of X ray-induced single-chain breaks in DNA and the postirradiation degradation of DNA in *E. coli* B/r grown under these different conditions.

MATERIALS AND METHODS

Cells and culture media. Cells of *E. coli* B/r *thy* (obtained originally from D. Freifelder) were used in all the experiments.

The liquid growth medium for the experiments comparing log- versus stationary-phase cultures was Brain Heart Infusion (BHI) medium (Difco). In early experiments this was sterilized by autoclaving and then supplemented with 5 μg of thymine per ml. At this time it was difficult to obtain reproducible results on the timing and magnitude of the cyclic variations in X-ray sensitivity. A variability in the absorbance of the BHI at 650 nm was also noted and ascribed to the autoclaving procedure. Subsequent batches of media were therefore sterilized by membrane filtration (Millipore Corp.), and 1% glucose was added to further reduce variability. The addition of glucose led to an increase in stationary-phase culture mass, so the thymine concentration in the filtered BHI plus 1% glucose medium was increased to 10 μg/ml. This medium will be referred to as supplemented BHI. All liquid cultures were incubated at 37 C in a gyratory water bath (New Brunswick Scientific) at 240 rev/min, the ratio of culture volume to flask volume being kept at 1:5 to ensure uniform aeration conditions. For stationary-phase cultures, a small part of a single colony was inoculated into 5 to 10 ml of medium, and the culture was incubated for 16 to 18 hr at 37 C. Log-phase cultures were started from such stationary-phase cultures with a starting inoculum of $\sim 10^6$ cells/ml. Late log phase was achieved after approximately 5 to 6 hr of incubation at 37 C, having a titer of 5×10^8 to 3×10^9 cells/ml. For determination of cell viability, 0.1-ml samples were spread on either BHI-agar (liquid BHI solidified with 0.9% Oxoid agar-agar no. 3) or yeast extract-nutrient broth (YE-NB) agar (0.75% yeast extract, 2.3% nutrient agar; Difco). The plates were incubated at 37 C for 18 to 24 hr before counting colonies.

In the experiments comparing stationary-phase cells grown in the presence or absence of glucose, peptone medium, 1% peptone (Difco) containing 10 μg of thymine per ml, was used either alone (PO medium) or supplemented with 1% glucose and 0.01 M phosphate buffer, *p*H 6.8 (PG medium). The peptone plates contained the liquid peptone media solidified with 0.9% Oxoid agar-agar no. 3.

Labeling procedures. For the alkaline sedimentation and DNA degradation experiments, cellular DNA was labeled by totally replacing the thymine supplement in the growth medium with thymine-2-^{14}C (Schwarz Bio-Research, Inc.; 49 mCi/mmole) to the same concentration (i.e., 10 μg/ml, 4 μCi/ml).

Irradiation conditions. The cultures were centrifuged, washed, and suspended in 0.1 M phosphate buffer (*p*H 6.8). The irradiations were carried out by using a twin-tube, 50 kVp, X-ray unit, designed and built by Loevinger and Huisman (12). The output of the set is 8 to 10 krad/min, as determined by $FeSO_4$ dosimetry, and depends on the geometry of the sample and the type of added filtration. [At low photon energies (<50 keV), the energy absorbed by a solution is more dependent on the atomic number of its solutes than at higher energies, due to the greater contribution of the photoelectric effect ($\sim Z^3/E^3$). The dose received by the ferrous sulfate solution (largely H_2SO_4) must thus be corrected to energy absorbed in the biological sample or appropriate irradiation medium by using the relevant mass energy absorption coefficient. Relative to water (1.00), phosphate buffer (DTM minimal medium) is about 1.07 and the Fricke dosimeter is about 1.13. In this paper the Fricke doses have been corrected to energy absorbed in phosphate buffer by multiplying by 0.95 (see reference 12).] In most of the experiments, filtration was increased to 0.3-mm Al. Cells were irradiated in buffer at room temperature and air equilibrium. In some of the later experiments requiring higher doses, air was bubbled through the suspension before and during irradiation. When using the bubbling irradiation vessel, filtration was reduced to 0.2-mm Al, to maintain a comparable dose rate.

Sedimentation studies. For experiments not involving postirradiation incubation, the cells were chilled immediately after irradiation by transferring the suspension to a tube standing in ice. The cells were then filtered, washed and resuspended in ice cold tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride buffer (0.05 M, *p*H 8.1 at 0 C); they were then held on ice pending further manipulations. When repair kinetics were being studied, a zero-time sample was taken and treated as described above. The remaining irradiated cell suspension was filtered, and the filter membrane was immediately dropped into warm culture medium which was shaken vigorously so as to suspend the cells. After various times of reincubation, samples were filtered, washed, and suspended in ice-cold Tris.

Linear alkaline sucrose gradients [5 to 20% (w/v) sucrose in 0.1 N NaOH] were made in sets of three by using a Buchler Gradient Maker with a peristaltic pump. These gradients were overlaid with a 0.1-ml cap of 0.5% (w/v) Sarkosyl (Geigy NL30) in 0.5 N NaOH. The cells were then treated for 5 to 10 minutes with a mixture of lysozyme-ethylenediaminetetraacetic acid (EDTA) at 0 C (lysozyme, 40 μg/ml; EDTA, 0.002 M; and cells, 10^9/ml; in Tris at *p*H 8.1). After this treatment, 10- to 20-μliter samples, containing 5×10^6 to 10^7 cells, were layered onto each gradient by using a wide-bore capillary pipette. The cap of each gradient was gently stirred with a pin. On average the gradients then stood for about 30 min while other gradients were layered and the centrifuge was loaded. The gradients were then centrifuged for 2 hr at 30,000 rev/min and 20 C in an SW50.1 rotor on a Beckman L2 65B centrifuge.

After the run, each tube was pierced by passing a needle through the bottom, and five-drop fractions

(approximately 0.12 ml) were collected from the bottom of the tube onto filter discs (Whatman 3MM paper). After drying, the discs were washed twice in 5% trichloroacetic acid, and once each in ethanol and acetone. When dry, each disc was placed in a glass vial with 5 ml of counting fluid [4 g of 2,5-diphenyloxazole, 0.1 g of 1,5-bis-2-(5-phenyloxazolyl)-benzene per liter of toluene], and assayed for radioactivity in a Nuclear-Chicago liquid scintillation counter. The hole in the centrifuge tube was sealed, and the tube was rinsed with 0.5 ml of NaOH. A sample of this rinse was spotted onto a disc and processed with the rest of the discs from that gradient.

DNA degradation studies. Cells prelabeled with ^{14}C-thymine were irradiated in phosphate buffer. Immediately after the last irradiation, 1 ml of each sample of cells was added to 9 ml of warm nonradioactive growth medium and incubated at 37 C in a gyratory water bath. Samples (0.1 ml) were taken at various times from each flask and spotted onto paper discs and dried under a heat lamp. At the end of the experiment, the discs were washed, dried, and counted as described in the previous section.

Approximately 7 to 10 min elapsed between the start of the irradiation and taking the first sample. Because considerable degradation takes place in buffer at room temperature, the 100% value for the irradiated samples was assumed to be the same as that for the first sample taken from the unirradiated culture. Time zero was set at the beginning of irradiation.

Analysis of the sedimentation data. The data from a typical sedimentation experiment show the distribution of DNA in terms of W and S, where W is the relative weight of DNA having a particular sedimentation coefficient S. For convenience, all the experimental data have been normalized to gradients of unit length with the meniscus as origin. The activity in each fraction is expressed as a percentage of the total radioactivity found on the gradient. For purposes of comparison, one of the simplest parameters that can be derived for each gradient is its first moment, defined as $D = \Sigma f_i d_i$, where f_i is the fraction of radioactivity in the i^{th} fraction, which is at a distance of d_i from the meniscus. This is equal to the weight-average sedimentation coefficient, after multiplication by the appropriate constants; and for symmetrical distributions (i.e., those resulting from random strand breakage) it is also the point beyond which half of the labeled material sediments (19).

In this paper we will express radiation damage as the number of breaks per single-strand genome. There are several approximations involved in our method for deriving this value. Most of the approximations (and shortcomings) of such calculations have been discussed in some detail in a recent publication by Lett et al. (11). We shall briefly reiterate some of them in our derivation.

When a population of polymer molecules (e.g., DNA) is irradiated, a number of breaks are produced which change the size distribution of the population. If the number-average molecular weight of the population before irradiation is M_{n1}, and after irradiation is M_{n2}, then the number of breaks introduced per number-average piece by the radiation is given by (M_{n1}/M_{n2})-1. The number of breaks produced per single-strand genome is then found by multiplying this value by the number of number-average pieces per single-strand genome. That is, the number of breaks per single-strand genome = $M_{ss}/M_{n1}\ [(M_{n1}/M_{n2})\text{-}1] = M_{ss}\ [(1/M_{n2}) - (1/M_{n1})]$, where M_{ss} is the molecular weight of the intact single-strand genome.

By using Studier's empirical relationship between sedimentation coefficient and molecular weight (1, 17) and marker DNA of known molecular weight, molecular weights can be ascribed to each fraction of the gradient. Hence, the number-average and weight-average molecular weights can be calculated. The number-average calculation is extremely sensitive to material near the top of the gradient and was found difficult to use objectively (see also reference 11).

The ratio M_{n1}/M_{n2} can be approximated as follows: $(D_1/D_2)^{1/0.38} = (S_1/S_2)^{1/0.38} = M_1/M_2 \simeq M_{w1}/M_{w2} \simeq M_{n1}/M_{n2}$, where D is the first moment of each distribution; S is the weight-average sedimentation coefficient; M is a weight-average molecular weight, derived from S by using Studier's relationship. It is not equal exactly to the true weight-average molecular weight M_w. M_n is the number-average molecular weight, which for truly random breakage satisfies $2M_n = M_w$. This appears to be the case for all irradiated populations, but not all unirradiated populations (see also reference 11).

The ratio M_{ss}/M_{n1} is approximately six according to McGrath and Williams (13). Our own data tend to support this value, although the use by McGrath and Williams of their weight-average molecular weight to deduce this value suggests that it probably should be somewhat higher.

The data are thus plotted as the number of breaks per single-strand genome = $6\ [(D_1/D_2)^{2.63} - 1]$. Although the data calculated in this manner may be in error by greater than a factor of two in terms of absolute values, interexperiment comparisons based on these calculations will be relatively accurate, since many of the errors involved are systematic.

RESULTS

Cells grown in supplemented BHI: survival after different preirradiation growth conditions. For cells grown in supplemented BHI, the period of maximum sensitivity occurs in late log phase (5×10^8 to 3×10^9 cells/ml). It lasts for 1 to 2 hr and is not affected by the cell manipulations required to prepare the cells for irradiation and sedimentation.

Figure 1 shows the survival curves for late log- and stationary-phase cells. As judged by slope ratio, the late log-phase cells are about 1.6 times more sensitive than the stationary-phase cells. The same survival was seen on YE-NB or BHI-agar plates.

Production and repair of DNA single-strand breaks. In Fig. 2, the rate of production of single-strand breaks is shown for log- and stationary-phase cells. The cell genomes appear to be equally sensitive to the production of single-strand breaks at both phases of the growth cycle. The data points at high doses suggest that the

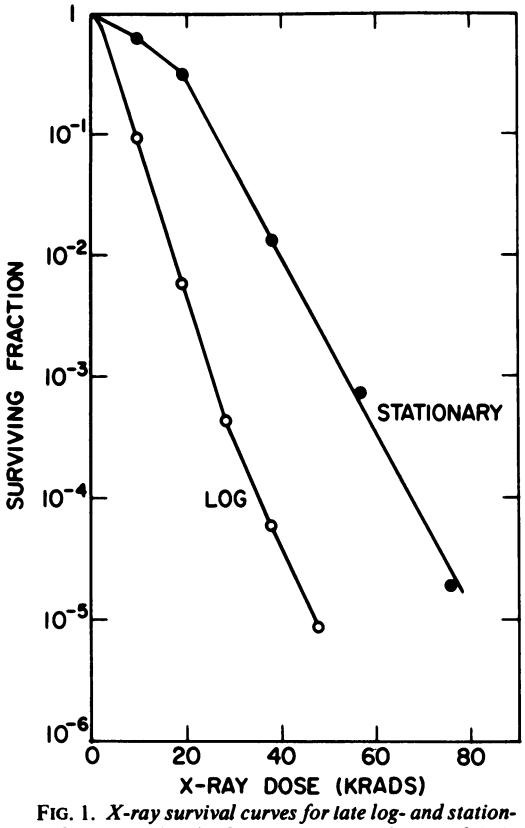

FIG. 1. *X-ray survival curves for late log- and stationary-phase E. coli B/r thy grown in supplemented BHI and plated on YE-NB agar. As determined from the slopes of the exponential portions of each curve (first three decades for late log-phase cells; decades two through five for stationary-phase cells), late log-phase cells are 1.6 times more sensitive than stationary-phase cells.*

number of single-strand breaks increases linearly with dose. The preponderance of points below the line at low doses probably reflects the insensitivity of the system to demonstrate a small number of additional breaks in an already polydisperse population. However, the possibility of a threshold effect cannot be excluded.

At lower doses (16.2 krad) both types of cells are capable of repairing single-strand breaks as completely as can be detected by sedimentation experiments. This repair is complete within a period of about 45 min (*data not shown*). After higher doses (28.5 krad), however, the stationary-phase cells show a somewhat greater ability to repair single-strand breaks (Fig. 3).

DNA degradation during postirradiation incubation. The effect of several doses of radiation on DNA degradation by log- and stationary-phase cells is shown in Fig. 4. In the log-phase cells, degradation begins immediately after irradiation. Although the 10-min points suggest some early degradation by stationary-phase cells, this is slight, and the major part of the degradation does not begin until about 30 min after irradiation. It is both slower and less extensive than the degradation in log-phase cells.

Cells grown to stationary phase in peptone media: survival after different preirradiation growth conditions. The radiosensitivity of stationary-phase cells grown in peptone-based medium depends strongly on whether glucose and phosphate buffer are added to the medium (Fig. 5). This difference in sensitivity is considerably larger than that between log- and stationary-phase cells grown in supplemented BHI (slope ratio of 3.4 compared with 1.6). The relative radiosensitivity of the PO and PG cells (cells grown to stationary phase in PO and PG media, respectively) was the same whether the cells were plated on YE-NB, PG agar, or PO agar but the absolute survival was slightly higher on YE-NB than on PO or PG agar. It was also noted that the plating efficiency of the PO cells was about 50% higher on the PG agar than on PO agar, whereas that of the PG cells was unaffected by the plating medium.

Because this difference in radiosensitivity between PG and PO cells was both larger and more reproducible than that between stationary-phase cells grown in BHI with and without glucose, we chose to extend the observations on the production and repair of single-strand breaks by using this system.

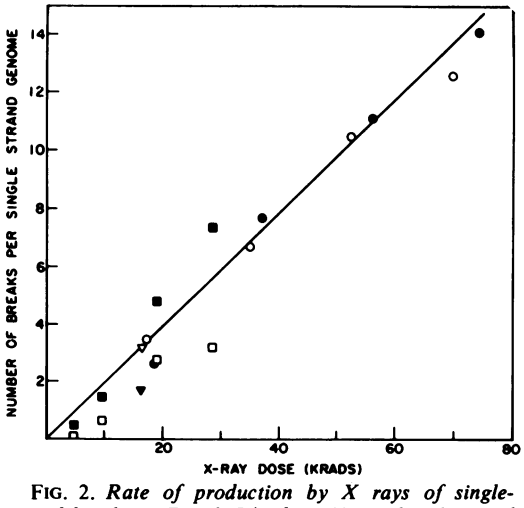

FIG. 2. *Rate of production by X rays of single-strand breaks in E. coli B/r thy DNA in late log- and stationary-phase cells, grown in supplemented BHI. The different symbols show data from independent experiments; solid symbols—stationary phase, open symbols—late log phase.*

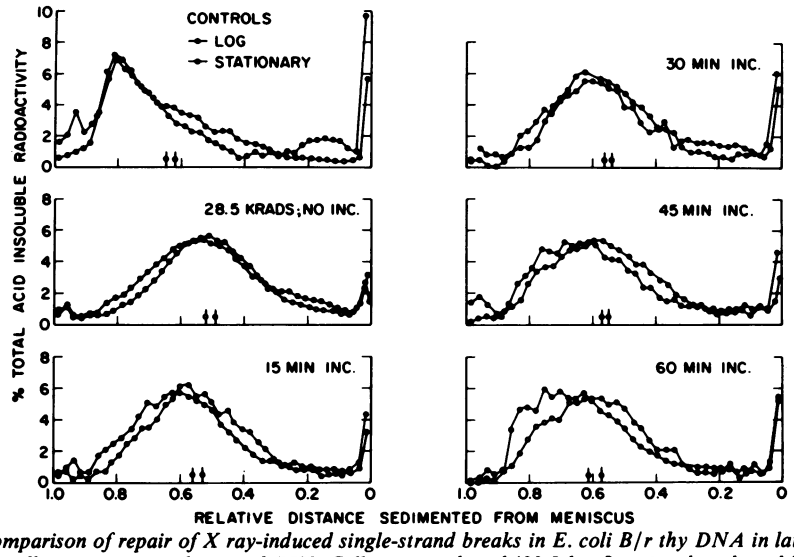

FIG. 3. *Comparison of repair of X ray-induced single-strand breaks in E. coli B/r thy DNA in late log- and stationary-phase cells grown in supplemented BHI. Cells are irradiated (28.5 krad), reincubated, and lysed on top of alkaline sucrose gradients as described in text. The vertical bars show the position of the first moment of each sedimentation pattern.*

Production and repair of DNA single-strand breaks. For the two doses used in this study (16.2 and 38.0 krad), the initial yield of single-strand breaks was the same in both types of cell, although the absolute yield of breaks was higher in the peptone-grown cells than in the cells grown in supplemented BHI. (After 38.0 krad, there were about 17 breaks per single-strand genome in peptone-grown cells compared with about 8 in cells grown in supplemented BHI.) This may reflect a difference in the DNA milieu in cells grown in different media.

Repair kinetics were studied after 16.2 and 38.0 krad in both types of peptone-grown cells. Figure 6 shows the patterns of repair after 38.0 krad since the difference in repair is more apparent after this dose. At early times in the postirradiation incubation, repair proceeds at the same rate in both types of cell. By 30 min, no further repair is seen in the cells grown in peptone only (PO cells). Further incubation of these cells leads to a small but consistent decrease in molecular weight. The small decrease in molecular weight which occurs suggests that this is an exonucleolytic type of degradation. In the cells grown in medium containing peptone + glucose + phosphate buffer (PG cells), continued incubation after 30 min allowed a further increase in molecular weight to take place. The high-molecular-weight edge of the profile continued to increase in sedimentation coefficient, whereas the position of the low-molecular-weight edge did not change. Thus, at this stage of the incubation, only a fraction of the molecules in the population undergo further repair, although this fraction is clearly much larger than the surviving fraction after this dose ($\sim 10^{-2}$). The results suggest that, after this dose of radiation, a fraction of the PG cells are capable of extensive DNA repair, whereas in the PO cells ($\sim 10^{-5}$ survival), no repaired material is seen under the peak region of the unirradiated DNA.

The above experiments were carried out with the cells reincubated in the same medium as used for preirradiation growth. To investigate whether the difference in repair was in any way due to the composition of the incubation medium, reciprocal experiments were run (*data not shown*) in which PO cells were reincubated in PG medium after irradiation, and PG cells in PO medium. The PO cells showed the same repair kinetics when incubated in either PO or PG medium. In contrast, when the irradiated PG cells were reincubated in PO medium, the DNA repair seen was almost the same as that seen in PO cells. Repair proceeded no further than shown in Fig. 6b. The time course of this repair was the same as that shown in Fig. 6. Continued incubation from 30 to 60 min did not lead to the rejoining seen in Fig. 6c. In contrast to the PO cells, however, no decrease in molecular weight of the partially repaired material was seen in this time.

DNA degradation during postirradiation incubation. Figure 7 shows the loss of trichloroacetic acid-insoluble radioactivity from PO and PG cells during postirradiation incubation. After a

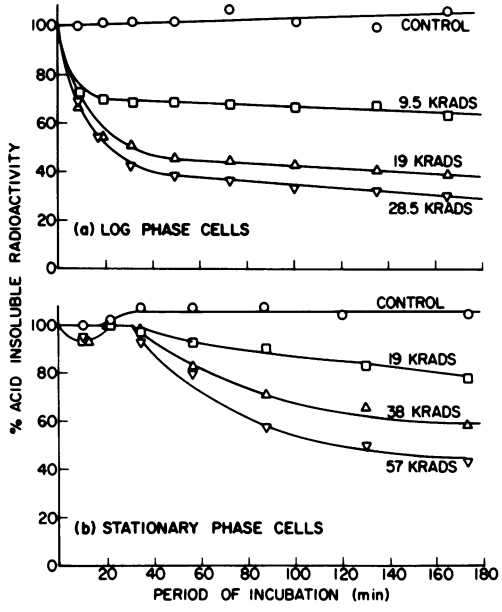

FIG. 4. *DNA degradation during postirradiation incubation in log- and stationary-phase cells of E. coli B/r thy grown in supplemented BHI. The fraction of radioactivity remaining insoluble in trichloroacetic acid is plotted as a function of time during the postirradiation incubation period.*

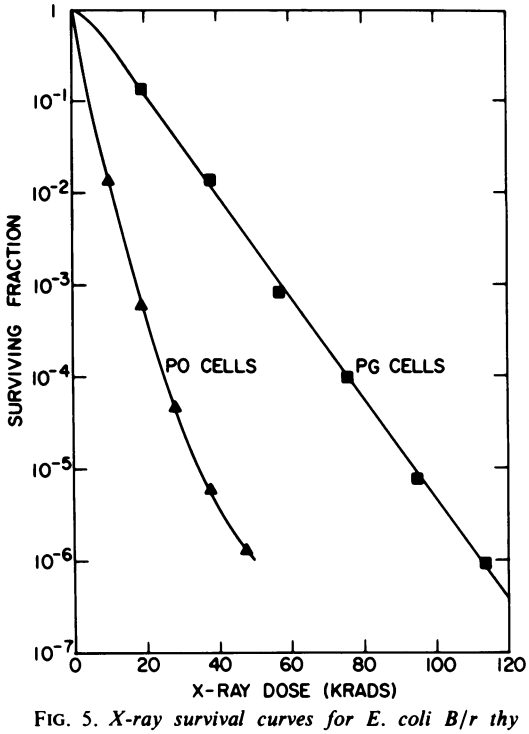

FIG. 5. *X-ray survival curves for E. coli B/r thy grown to stationary phase in peptone media. PO cells were grown in 1% peptone and 10 μg of thymine per ml; PG cells were grown in the same medium supplemented with 1% glucose and 0.01 M phosphate buffer (pH 6.8). Comparing the initial slope (first three decades) of the PO curve with the slope of the PG curve, PO cells are about 3.4 times as sensitive to X rays as PG cells.*

given dose of radiation, degradation was both more rapid and more extensive in PO than in PG cells. In addition, there was a lag of about 30 min before degradation began in the PG cells, whereas in the PO cells degradation began immediately. The same degradation was seen in both types of cells whether the postirradiation incubation was carried out in either PO or PG medium.

DISCUSSION

BHI-grown cells. The differences in the sensitivity of *E. coli* B/r to killing by X rays at different phases of the growth cycle and in stationary phase after growth in different media have been reported previously (14–16). Ginsberg and Webster (7) showed that the production of single-strand breaks in *E. coli* B/r was the same in log- and stationary-phase cells after 20 krad. We have confirmed this observation over a wide range of doses. Thus, the difference in X-ray sensitivity of log- and stationary-phase cells cannot be due to a difference in the efficiency of production of single-chain breaks.

At lower doses of X rays (16.2 krad), log- and stationary-phase cells appear to be equally competent at repairing single-strand breaks in their DNA. However, the fragmented nature of the isolated unirradiated genome makes it difficult to detect the presence of one unrepaired single-strand break in the genome, which might represent the critical differential between the repair capabilities of log- and stationary-phase cells. At higher doses (28.5 krad), however, the rejoining process in stationary-phase cells is more prolonged than in log-phase cells and leads to a greater proportion of high-molecular-weight material. The major difference that we detect between the response of log- and stationary-phase cells to irradiation is their degradation of DNA. The log-phase cells degrade their DNA both more rapidly and more extensively after a given dose of radiation. This confirms the work of Trgovcevic and Kucan (18), who demonstrated that in several derivatives of *E. coli* B, irradiated at different phases of growth, there was a close correlation between the extent of degradation and loss of colony-forming ability. For these strains, and several other *E. coli* strains that did not show a variation in X-ray sensitivity with growth phase, these authors demonstrated a striking correlation between X-ray sensitivity (loss of colony-forming ability) and the extent of DNA degradation.

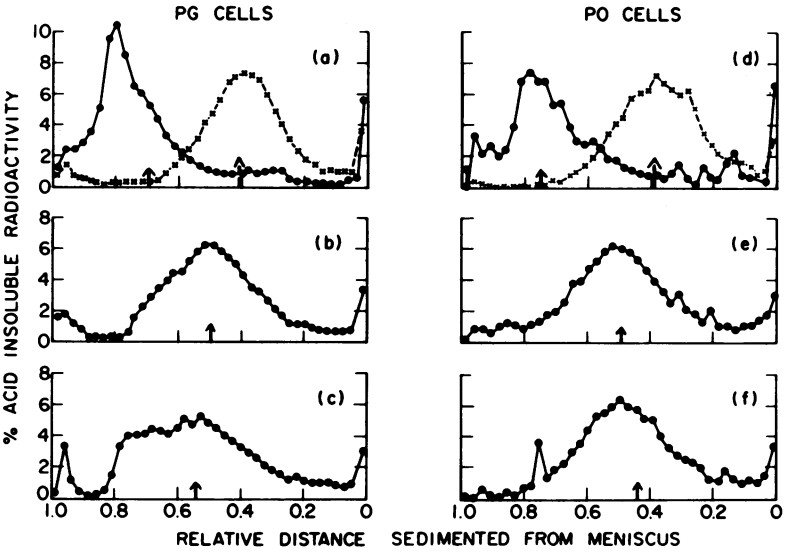

FIG. 6. *Comparison of repair of X ray-induced single-strand breaks in E. coli B/r thy DNA. Cells were grown to stationary phase in peptone media with (PG cells) and without (PO cells) the glucose-phosphate supplement. (a–c) PG cells, (d–f) PO cells. (a and d) Control (solid line) and 38.0 krad, no reincubation (broken line); (b and e) 38.0 krad, 30-min reincubation; (c and f) 38.0 krad, 60-min reincubation. The vertical arrows show the position of the first moments of each sedimentation pattern.*

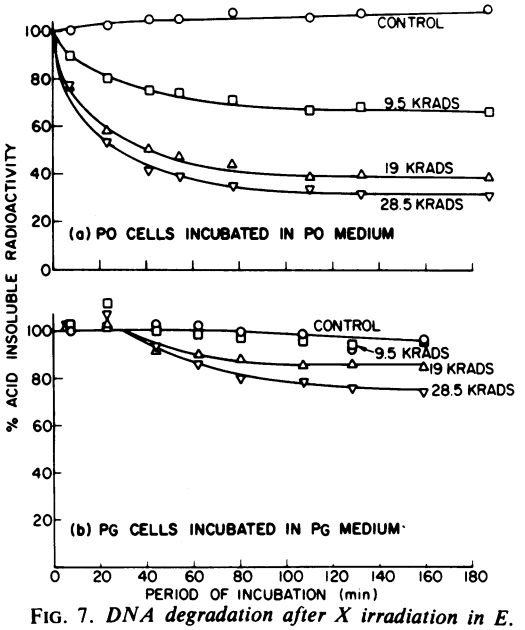

FIG. 7. *DNA degradation after X irradiation in E. coli B/r thy. Cells were grown to stationary phase in peptone media with (PG cells) and without (PO cells) the glucose-phosphate supplement. The fraction of the radioactivity remaining insoluble in trichloroacetic acid is plotted as a function of time during the postirradiation incubation period. (a) PO cells reincubated in PO medium, (b) PG cells reincubated in PG medium.*

One might postulate that the difference between log- and stationary-phase cells which determines their radiosensitivity lies in the relationship between the repair and degradation systems in these cells. An alteration in this relationship could affect the proportion of cells which are able to reconstruct an intact genome before a lethal amount of degradation has taken place, i.e., degradation may be one of the determinants of X-ray lethality (e.g., see reference 8).

An alternative explanation is that degradation is not primarily responsible for cell death. If such degradation requires a DNA single-strand break as a starting point, the extent of degradation may be a measure of the number of such breaks remaining in a population after all lesions capable of being repaired have been repaired. The different degradation kinetics seen in log- and stationary-phase cells (after low doses of X rays) might thus reflect a difference in the number of unrepaired single-strand breaks which was too small to be detected by alkaline sucrose sedimentation. After higher doses of radiation, however, a difference was detected by sedimentation.

Peptone-grown cells. The development of resistance in stationary-phase cells grown in peptone in the presence of various amounts of glucose and buffer was investigated in some detail by Stapleton and Engel (15). The addition of glucose to the growth medium causes a change in *p*H of the medium during growth. The time course of this change depends on the amounts of both glucose and phosphate in the medium. By varying these, Stapleton and Engel (15) found a strong correlation between radiosensitivity and the final *p*H of the culture. If the growing cultures were

titrated with NaOH to maintain neutrality, radioresistance did not develop. However, once resistance had been reached in stationary phase, it was not possible to reverse this by incubation in buffer at a different *p*H.

In a later study, Stapleton and Fisher (16) concluded that "the shift to a lower *p*H (... which occurs in late logarithmic and early stationary-phase cultures grown in glucose-broth ...) seems to dissociate cell division from macromolecular synthesis, since shortly after the low *p*H is reached the cells stop dividing and become large and contain more RNA, DNA and protein than do cells grown in L-broth without glucose." They found that on inoculation into fresh broth, the resistant cells synthesize protein without a delay, whereas in the sensitive cells protein synthesis does not reach its maximal rate until about 45 min after inoculation, during which time new pools of 30 and 50*S* ribosomal particles are being synthesized. DNA and ribonucleic acid (RNA) synthesis kinetics are about the same in resistant and sensitive cells. After irradiation, DNA synthesis proceeds at about 80% of the control synthesis rate in resistant cells. Irradiated sensitive cells synthesize little DNA in this 90-min period. In resistant cells RNA and protein synthesis progress at about 80% of the control rate. In sensitive cells RNA synthesis is depressed by the same amount as in resistant cells, but protein synthesis is strongly affected, suffering either a reduction in rate or an extension of the lag period preceding the initiation of new synthesis.

In this study, we have observed that when reincubated in their own growth medium, the PG cells show more extensive DNA strand rejoining than the PO cells. Glucose is required by the PG cells for the expression of this difference in strand rejoining, but has no effect on the rejoining capabilities of the PO cells when added after irradiation. Glucose thus influences the strand-break repair mechanism both before and after irradiation. However, the data do not permit a distinction to be made between an increase in the capacity of a repair system by preirradiation growth in glucose or the synthesis of a new repair system that requires glucose for its function.

The PO cells are 3.4 times more sensitive to radiation than the PG cells when assayed for colony-forming ability on complex medium (YE-NB or peptone agar). However, survival of both types of cells is unaffected by the presence of glucose in the plating medium.

PG cells show a lag of about 40 min before postirradiation DNA degradation begins. In PO cells degradation begins immediately after irradiation and is both more rapid and more extensive after a given dose of radiation. The degradation kinetics are not affected by the presence of glucose, except possibly for a slightly longer lag period when PG cells are incubated in PO medium.

The facts that glucose does not affect the DNA degradation kinetics in either PO or PG cells and is not required after irradiation for the expression of radiation resistance in the PG cells suggest that it is DNA degradation which determines cell viability under these experimental conditions. The additional strand rejoining of which the PG cells are capable does not take place in glucose-less conditions where viability remains high. It is possible that the increased strand rejoining seen in the PG cells is a reflection of the much reduced degradation in these cells. It is not clear how the ability of the PG cells to reinitiate synthesis more rapidly than the PO cells (16) may contribute to the higher survival unless this renewed synthesis causes the lag in DNA degradation in the resistant PG cells.

Genetic factors involved in the "log-stationary" and "glucose" effects. The cyclic variation in X-ray sensitivity of the type investigated here, first reported for *E. coli* B/r by Stapleton (14), is also shown by *E. coli* B and B_{s-2} (18), but not by *E. coli* B_{s-1} (7). Thus, it would seem that in the B series, the "log-stationary" effect is shown only in Hcr^+ cells. *E. coli* K-12 *lon* (AB1899) has a pattern of cyclic variation in X-ray sensitivity which differs markedly from that of its *lon*$^+$ progenitor (AB1157) but closely resembles that of the B series (2). It is interesting in this context to note that all of the B series are reputedly *lon*, the familiar expressions of this defect being suppressed in B/r (4). Data are not available on the effect of *uvr* or *rec* mutations on the cyclic variation of X-ray sensitivity seen in K-12 *lon* strains.

The glucose effect is shown by *E. coli* B/r but not by strain B_{s-1} (16). In *E. coli* K-12 the integrity of the *rec* genes [Friesen et al. (6); *our unpublished data*] and the *lon* gene (2) are required for the glucose effect to be expressed. Although as sensitive as the *rec* strains to X irradiation, the DNA polymerase deficient strain *polA1* (3) still shows the glucose effect (*unpublished data*). Since mutations in the *rec* genes of *E. coli* K-12 appear to affect both the rejoining of DNA strand-breaks (10) and the glucose effect (on stationary phase cells), this suggests that a fully functional rejoining system (or a normally controlled degradation system, or both) is required for the expression of the glucose effect on viability after X-irradiation.

General conclusions. In both the log-stationary and glucose effects, the more resistant cells show more strand rejoining and less DNA degradation than the sensitive cells. In the log-stationary ef-

fect, the differences in strand rejoining are only apparent after high X-ray doses (~30 krad), whereas the degradation differences are seen after all doses used.

In the glucose effect, differences in both rejoining and degradation are seen after lower doses. Since the *rec* genes in K-12 affect both these functions it is not possible to say which is the more important in the glucose effect. The results presented above suggest that, in *E. coli* B/r, the differences in DNA degradation may be more important than those in strand rejoining.

Thus, in both effects, the differences in degradation may in themselves be the determinant of loss of viability and also of the reduced amount of strand rejoining. Alternatively, the differences in degradation may reflect a difference in the number of unrepaired single-chain breaks, which was too small to be detected by sedimentation analysis after low doses of X rays, but is nevertheless the true determinant of the difference in viability.

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