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Deoxyribonucleic Acid Repair in a Highly Radiation-Resistant Strain of *Salmonella typhimurium*[1]

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Deoxyribonucleic acid repair was studied in gamma-irradiated wild-type *Salmonella typhimurium* and in a radiation-resistant derivative 20 times more resistant than wild type. After exposure to 20 or 50 krad, the wild-type strain (DB21) degraded 30 to 50% of its prelabeled DNA into acid-soluble fragments, whereas the radioresistant strain degraded less than 15% after 4 h of incubation. Post-irradiation synthesis of DNA in the wild-type strain DB21 was reduced after a dose of 20 krad and totally inhibited after exposure to 200 krad. With radiation-resistant strain, D21R6008, on the other hand, DNA synthesis was delayed after a dose of 200 krad but not inhibited. Doses of 20 and 200 krad produced a similar number of single-strand breaks in the DNA of both strains as determined by zone sedimentation analysis in alkaline sucrose gradients. The radiation-resistant strain D21R6008, on the other hand, DNA synthesis was strand breaks in its DNA and repairs these damages more rapidly than wild-type *Salmonella*.

Previously (11) we described the development of a series of gamma radiation-resistant strains of *Salmonella typhimurium* LT2 by a cyclic sequence of irradiation followed by growth. The most resistant strain, designated D21R6008, exhibits D_{10} values (dose necessary to inoculate initial viable cells) exceeding 130 krad which is substantially greater than was previously reported for salmonellae. We suggested that this strain may be capable of hyperefficient repair of irradiated deoxyribonucleic acid (DNA). Radiation-resistant *Escherichia coli* (17) has recently been shown to rejoin single-strand breaks in its DNA with high efficiency (26). In contrast, a report on similar studies of radiation-resistant strains of *Salmonella thompson* (3) concludes that repair of single-strand breaks is not different than in wild type.

In the present study, we examine DNA breakdown and single-strand rejoining activity in our resistant *S. typhimurium* after exposure to gamma radiation. Comparing a resistant strain to wild type we expect that both should be capable of repair; the resistant strain might rejoin strands at a faster rate after low radiation doses and might successfully repair more extensive damage after high doses.

MATERIALS AND METHODS

Bacteria. Strain DB21 is strain 18 (Levine collection) derived from *S. typhimurium* LT2 and cured of known prophages (4). Strain D21R6008 was purified from a radiation-resistant population derived from DB21 after 84 cycles of gamma irradiation followed by growth as described previously (11).

Media. The standard growth media used were Trypticase soy yeast extract (TSY) and EM9, which is M9 mineral salts solution enriched with 0.25% Casamino Acids and 0.2% glucose (11). Cell viability was determined by spreading diluted samples on TSY solidified with 1.5% agar (Difco).

Labeling of cell DNA. Logarithmic-phase cultures were grown at 37 C from a density of 5×10^6 cells/ml to about 5×10^8 cells/ml in EM9 medium containing 250 μg of deoxyadenosine/ml (5) and either 5 μCi of [*methyl*-^3H]thymidine/ml (6.7 Ci/mmol) or 1 μCi of [2-^{14}C]thymine/ml (55.8 mCi/mmol) (New England Nuclear Corp.). The labeled cells were chilled, centrifuged at 6,000× *g* for 5 min, transferred to EM9 medium containing 250 μg of deoxyadenosine/ml and 20 μg of unlabeled thymine/

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ml and held at 37 C. Samples (0.5 ml) were taken at intervals up to 4 h, added to equal volumes of ice-cold 10% tetrachloroacetic acid, held at 0 C for 3 h, and then filtered through membrane filters (24 mm pore size, type HA; Millipore Corp.); the filtrates were retained. Duplicate 0.1-ml samples of each filtrate were added to glass vials containing 10 ml of scintillation fluor, and the ^{14}C radioactivity was counted in a Beckman CPM-100 scintillation counting system. The scintillation fluid was modified from that of Bray (6) and contained: naphthalene, 60 g; 2,5-diphenyloxazole (PPO), 8 g; 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP), 0.2 g; ethylene glycol, 20 ml; and methanol, 100 ml, in 1 liter of dioxane.

Post-irradiation uptake of [^3H]thymidine. Strains DB21 and D21R6008 were grown in EM9 medium shaken at 37 C. When the cultures reached a density of about 4×10^8 to 5×10^8 cells/ml they were chilled, centrifuged, washed, suspended in phosphate buffer, and exposed to ^{60}Co gamma irradiation at 0 C. The irradiated suspensions and unirradiated controls were then resuspended in EM9 medium containing 5 μCi of [*methyl-*^3H]thymidine/ml (6.7 Ci/mmol) and 250 μg of deoxyadenosine per ml and incubated at 37 C. One milliliter samples were taken at intervals for the measurement of [^3H]thymidine incorporation. After a 1:1 dilution in 4% formaldehyde, 0.1-ml quantities were placed on Schleicher and Schuell (No. 895E, 21-mm) filter disks and dried under heat lamps. The dried disks were washed twice in bulk with cold 5% trichloroacetic acid, once in acetone and redried. Radioactivity was measured by placing the disks in glass vials containing 10 ml of scintillation fluor (0.4% PPO, 0.05% dimethyl POPOP, in toluene) and counted.

To verify that the experiments were not biased by the concurrent degradation of preexisting cell DNA (and the associated expansion of DNA precursor pools; references 23, 24), the post-irradiation DNA synthesis-uptake experiments were repeated with cells that were fully prelabeled with [*methyl-*^3H]thymidine prior to irradiation.

Zone sedimentation of DNA in alkaline sucrose gradients. Cells, prelabeled with [^{14}C]thymine or [^3H]thymidine, were suspended at 10^8 cells/ml in phosphate buffer and exposed to 0-, 20-, and 200-krad doses of ^{60}Co gamma rays. Samples were then added to equal volumes of phosphate buffer containing 0.1 M ethylenediaminetetraacetic acid (EDTA) and 0.02 M NaCN and held in ice. To follow repair in growth medium, samples were centrifuged. The cells were suspended in TSY broth, incubated at 37 C for specified times, and finally harvested and resuspended in chilled phosphate buffer containing 0.05 M EDTA and 0.01 M NaCN.

The cells were converted to spheroplasts by procedures based on the lysozyme-EDTA-sucrose method of McGrath and Williams (20). Samples (2.5 ml) of the cell suspensions were washed with tris(hydroxymethyl)aminomethane (Tris)-EDTA buffer (0.1 M Tris-hydrochloride, 0.05 M EDTA, pH 8) and suspended in 2.5 ml of chilled spheroplasting medium (0.5 M sucrose, 0.03 M Tris-hydrochloride, pH 8) and the tubes were held for a further 20 min in ice. Finally, 0.25 ml of a NaCl solution (168 mg/ml) was added (10).

Equal volumes of spheroplast suspensions were carefully mixed so that each mixture contained material from irradiated and unirradiated cells of the same strain but carried opposite radioactivity labels in their DNA. This technique provides internal controls marking the position of unirradiated DNA in the subsequent gradients. A typical mixture contained per 0.1 ml: 12,000 counts/min of ^3H and 3,000 counts/min of ^{14}C in a total of 10^7 spheroplasts.

One-tenth milliliter samples of mixed spheroplasts were slowly added to 0.2-ml quantities of 0.5 M NaOH (containing 0.1% Sarkosyl for DB21 preparations) previously layered on top of 5 to 20% alkaline sucrose gradients. The gradients contained 0.005 M EDTA, 1.0 M NaCl, and were previously adjusted to pH 12.1 by titration with 10 M NaOH. A high-density shelf consisting of 0.3 ml of 80% (wt/vol) iothalamic acid (Angio-Conray; Mallinckrodt) in 20% sucrose was provided at the base of each 4.6-ml linear gradient. The gradients were prepared as described by Botstein (4).

The gradients were held for 30 min before centrifugation in order to release the DNA completely and to bring the system to pH and temperature equilibrium. The gradients were then centrifuged at 35,000 rpm for 90 min at 16 C in a Spinco SW39 rotor. Four-drop fractions were collected from the bottom of the tubes onto Schleicher and Schuell, (895-E) filter paper disks. The disks were dried, washed twice in cold 5% trichloroacetic acid and once in acetone, redried, and added to 10 ml of toluene-based scintillation fluid for counting.

Analysis of sedimentation data. The experimental data were normalized to gradients of unit length as described by Town et al. (29). In our case, the portions of the curves used for calculations are indicated in each figure by vertical arrows. Unlysed material on the high-density shelf or degradative products on the extreme top of gradients were excluded from the analysis. The weight-average sedimentation coefficient(s) was calculated as in Burgi and Hershey (7).

The number-average molecular weight (M_n) for irradiated and unirradiated DNA was calculated as described by Lett et al. (19) and Town et al. (29). For the purpose of comparative calculation we used the Cairns (8) value of 2.9×10^9 for the molecular weight of native DNA and further assumed that exponentially growing bacteria on the average contain 1.4 genomes/cell. Thus, a value of 4.06×10^9 was obtained for the average molecular weight of DNA used in calculating the number of single-strand breaks produced per intact replicating genome.

RESULTS

Survival after gamma irradiation. The survival curves for midlog and stationary-phase cells of strains DB21 and D21R6008 are shown in Fig. 1. Judging from the ratio of the slopes, the D21R6008 cells are about 20 times

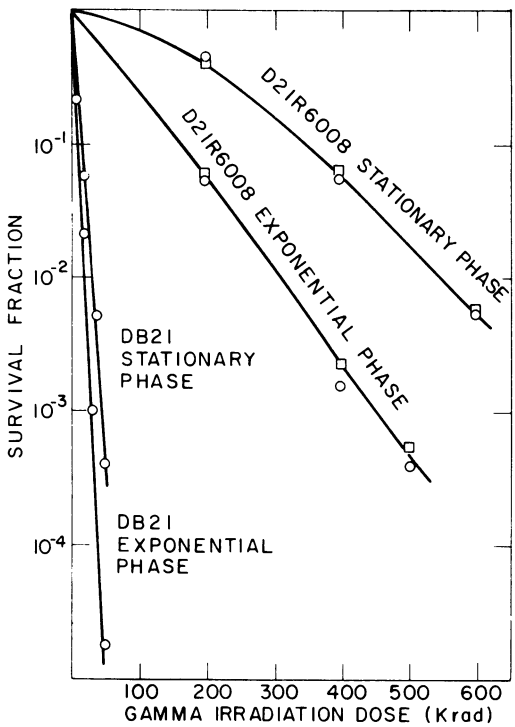

FIG. 1. *Gamma-irradiation survival of radioresistant and parent strains of S. typhimurium LT2. Cultures of D21R6008 and DB21 were grown at 37 C in TSY broth (○) or EM9-glucose (□). Cells were harvested at 3 h (exponential phase) or 15 h (stationary phase).*

more radiation resistant than the wild type in both phases of growth. It is clear that this 20-fold difference applies both to exponential and stationary-phase cultures. Using either strain, stationary-phase cells are about 1.5 times more resistant to gamma irradiation than exponential cells. The variation in radiosensitivity with growth phase observed for both the radioresistant and wild-type strains, is similar to that reported for *E. coli* B/r (21, 25, 29).

Intracellular degradation of DNA. One aspect of DNA repair is the postirradiation breakdown of DNA. When we began to examine this with our strains we made the surprising observation that even in the absence of irradiation, holding of the resistant (D21R6008) strain in nongrowing conditions results in the spontaneous breakdown of cell DNA (Fig. 2). Stationary-phase cultures of D21R6008 show about 25% loss of acid-insoluble radioactivity from DNA in 4 h, whereas similar cultures show no breakdown. Exponential cultures of the resistant strain degrade 4% of this DNA, whereas again the wild type shows no detectable ($<1\%$) degradation.

When exposed to 20- or 50-krad doses, the wild-type strain (DB21) degraded 30 to 50% of its labeled DNA to acid-soluble fragments within 4 h, whereas the radiation-resistant culture D21R6008 degraded less than 15%. Figure 2d shows that 200 krad caused the degradation of over 50% of DB21 DNA in 2 h, whereas equivalent breakdown of the D21R6008 DNA was delayed at least for the first 90 min of incubation.

The addition of 0.2% glucose to the holding medium (data not shown) produced essentially no change in the postirradiation degradation of DNA in either strain.

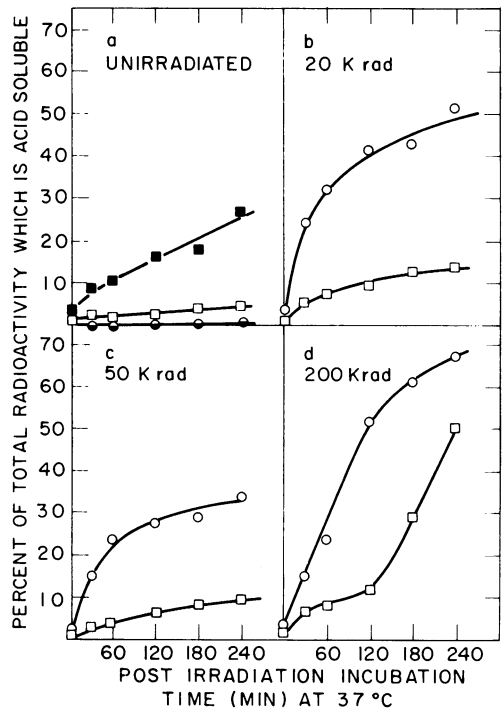

FIG. 2. *Spontaneous and postirradiation loss of radioactivity from the DNA of parent and radioresistant strains of S. typhimurium LT2 during incubation at 37 C in M9-basal salts medium. Cells labeled with ^{14}C-thymine were exposed to different doses of gamma irradiation and incubated for up to 240 min in nonradioactive M9-medium. Released trichloroacetic acid-soluble radioactivity, measured at intervals, is expressed as a percentage of total acid-soluble radioactivity and plotted against incubation time at 37 C after exposure to 0, 20, 50, or 200 krad of gamma rays. The following symbols refer to exponential-phase cultures: (○) DB21; (□) D21R6008. Closed symbols represent spontaneous DNA degradation from stationary-phase cells. One hundred percent radioactivity corresponds approximately to 1,500 counts/min per 100-μliter sample and is the average of three unirradiated zero time samples boiled for 30 min in 10% trichloroacetic acid.*

Postirradiation incorporation of radioactivity into DNA. Data for the incorporation of [^{3}H]thymidine by unlabeled DB21 or D21R6008 cells after 0-, 20-, and 200-krad doses of gamma irradiation are shown in Fig. 3, plotted on linear coordinates. The initial viable cell densities, at time zero, were determined for the unirradiated controls and found to be 4.7×10^8 and 4.3×10^8 cells/ml for strains DB21 and D21R6008, respectively. These counts were considered to be sufficiently close to justify direct comparison of the two sets of data. It should be noted that we have reported previously (11) that, although the mass per viable cell differs in these strains, the DNA content per viable cell is the same.

The postirradiation synthesis of DNA in strain DB21 was apparently reduced after a dose of 20 krads and totally inhibited after 200 krads. In the radiation-resistant D21R6008 cells, however, the uptake of [^{3}H]thymidine after 20 krad continued at the control rate for a period approximately equivalent to one generation time and then exhibited an apparent 40-min delay, before resuming at a normal logarithmic rate. After 200 krad, DNA synthesis was delayed in strain D21R6008 for about 90 min but was still not totally inhibited.

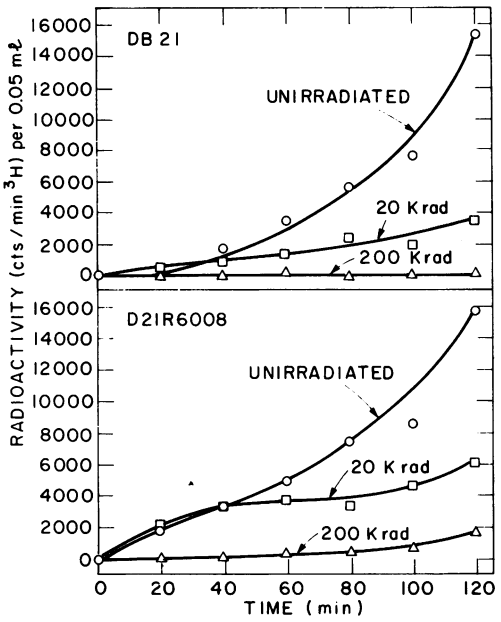

FIG. 3. *Post-irradiation incorporation of [^{3}H]thymidine into unlabeled cells of radioresistant and parent strains to S. typhimurium LT2. Exponentially growing cells were harvested, suspended, and irradiated in 0.067 M phosphate buffer. The irradiated cells were transferred to EM9-glucose medium, containing 5 μCi of [methyl ^{3}H]thymidine/ml and 250 μg of deoxyadenosine/ml to give 5×10^8 cells/ml and were incubated at 37 C. Samples, taken at intervals, were diluted 1:1 with 4% formaldehyde and 0.1-ml quantities dried on filters. The disks were washed twice with 5% trichloroacetic acid, once with acetone, redried, and ^{3}H activity-counted in 10-ml quantities of toluene-based scintillation fluor in a liquid scintillation counter.*

Figure 4 shows postirradiation uptake of [^{3}H]thymidine into cells whose DNA was fully prelabeled. The data are plotted on semilog coordinates and thus the ^{3}H incorporation rates are comparable and independent of initial cell numbers (24). The results suggest the following. (i) For unirradiated cells the rate of DNA synthesis is somewhat lower for D21R6008 than for DB21. (ii) After exposure to 20 krad, [^{3}H]thymidine incorporation was reduced in wild-type cells to a much greater extent than was the case with the resistant strain. This might mean that DNA synthesis in each cell in the two irradiated cultures was partially affected or that a different proportion of the cells in each culture retained normal DNA synthetic capacity. On the latter interpretation we would estimate (by extrapolation) that 68% of the resistant cells continued to incorporate whereas only 10% of the wild-type cells did so. (iii) After exposure to 200 krad, DB21 cells lost approximately 50% of their DNA radioactivity with no appreciable resynthesis, whereas strain D21R6008 resumed net synthesis at a reduced rate after an initial delay of about 40 min.

Repair of gamma-radiation-induced single-strand scissions in DNA. We assayed the production and repair of single-strand scissions in bacterial DNA induced by gamma radiation in alkaline sucrose density gradients using a procedure adapted from McGrath and Williams (20). This experiment was done at two doses: 20 krad (representing about 0.1% survival for DB21 and close to 100% survival for strain D21R6008) and 200 krad (representing about 10% survival for strain D21R6008). In each experiment, differentially labeled ([^{14}C]thymidine) unirradiated cells are mixed with the irradiated samples (labeled with [^{3}H]thymidine) before lysis of the cells. The DNA of the unirradiated cells thus serves as an internal control which monitors all procedures, including spheroplasting, sedimentation, and recovery of the radioactive DNA from the gradients.

The results of this analysis for a 20-krad dose (Fig. 5a, 5d, and Table 1) show first that the extent of single-strand breakage caused by

gamma irradiation is approximately the same in the wild type (DB21) and the resistant (D21R6008) strains. When repair is followed during incubation after irradiation, it is clear from the rest of Fig. 5 that the rate of repair of these single-strand breaks in the DNA is substantially greater in the resistant type (D21R6008) than in the wild type. Eventually, however, both strains finally repair most of the single-strand breaks (40 min for wild type; 15 min for the resistant strain).

After a dose of 200 krad, the results are more complex. The first complication is due to the observation that D21R6008 DNA accumulates some breaks even in the absence of irradiation: this seems to occur during the period of holding in cold phosphate buffer for the 40 min required to deliver 200 krad (Table 2). The second complication is our observation that the number of single-strand breaks we found in the irradiated samples was slightly different for the wild-type and resistant strains. This may reflect some repair by the resistant strain during the processing of the samples.

Nevertheless, Fig. 6 and Table 2 clearly demonstrate that the resistant strain D21R6008 can ultimately repair most of the breaks caused by 200 krad, whereas the wild-type strain D21 shows no evidence whatsoever of repair of single-strand breaks after this large a dose of gamma rays.

DISCUSSION

Ordinarily, research into the genetic mechanisms of bacterial response to radiation damage proceeds through the use of mutants more sensitive to radiation than the wild-type parent (1, 15). We have chosen instead to work with variants (probably mutated at several loci) which are very much more resistant than their wild-type antecedents (11). In their resistance, these strains resemble *Micrococcus radiodurans*, except that no genetically defined radiation-sensitive antecedent to *M. radiodurans* is known.

Our results concerning the response of the abnormally radiation-resistant strain D21R6008 to irradiation can be summarized as follows:

(i) DNA spontaneously breaks down when resistant cells are held in buffer even in the absence of radiation. This phenomenon is reminiscent of the "reckless" phenotype of recombination-deficient (*recA*) mutants of *E. coli* (9) and *S. typhimurium* (32). However, it should be noted that the *rec*$^-$ mutants are not resistant to radiation and D21R6008 has, in preliminary experiments, no alteration in recombination phenotype or in the ultraviolet-inducibility of P22 prophage. The instability of DNA in the resistant strain relative to wild type might indicate hyperactivity of constitutive DNA repair systems or constitutivity of normally inducible DNA repair systems or both. This interpretation must be tempered by the fact that the resistant strain is not a single-step mutant.

(ii) In contrast to the above, after irradiation the resistant strain degrades its DNA substantially less than wild type. Paterson et al. (22) found that, after x-irradiation, DNA degradation in *E. coli polA* 1 is greater than in wild-type cells. Preliminary results (11) hint that the amount of DNA polymerase I per genome may be higher in our resistant strain than in wild type. These experiments also show that the extent of degradation, both in resistant and wild-type strains, is related to dose:

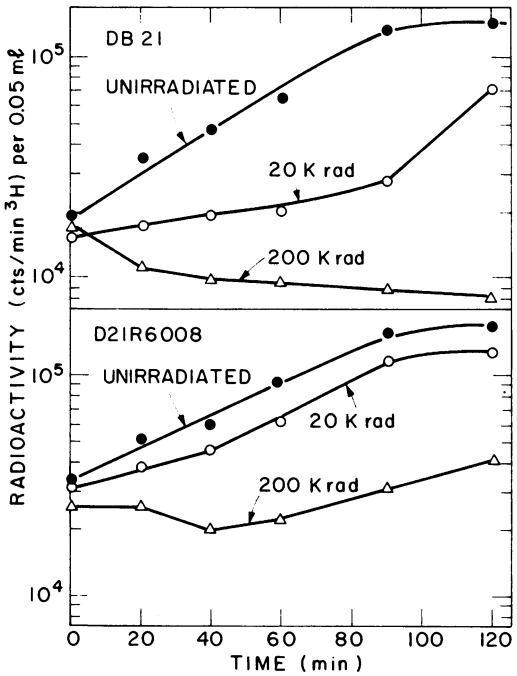

FIG. 4. *Post-irradiation incorporation of [^{3}H]thymidine into prelabeled cells of radioresistant and parent strains of S. typhimurium LT2. Cells were prelabeled by growing for 3 h at 37 C in EM9-glucose medium containing 5 μCi of [methyl ^{3}H]thymidine/ml and 250 μg of deoxyadenosine/ml. The labeled cells were harvested, irradiated, returned to radioactive medium, and the incorporation of ^{3}H label measured as described for Fig. 3. Symbols: (●) unirradiated controls; (○) irradiated at 20 krad; (△) irradiated at 200 krads.*

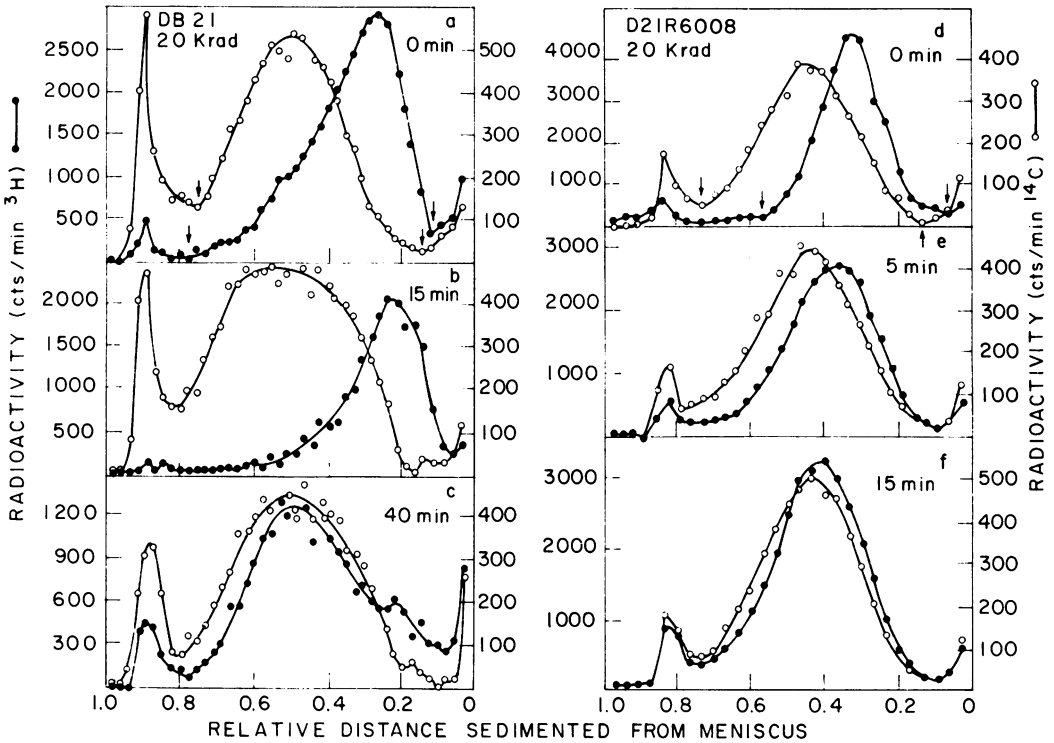

FIG. 5. *Zone sedimentation of DNA in alkaline sucrose gradients irradiated with 20 krad gamma rays. Cells were grown at 37 C to early log phase in EM9-glucose medium containing 250 μg of deoxyadenosine/ml and either 5 μCi of [methyl ³H]thymidine (6.7 c/mmol/ml) or 1 μCi of [¹⁴C]thymine (55.8 mc/mmol) per ml. The labeled cells were then incubated for 30 min in nonradioactive medium, harvested and suspended in 0.067 M phosphate buffer. The [³H]-labeled suspensions were gamma irradiated (20 krad) at 0 C and then samples added to phosphate buffer containing 0.1 M EDTA and 0.02 M NaCN. Repair was followed by centrifuging after irradiation and suspending in TSY broth at 37 C for specified times, harvesting and and suspending in chilled 0.1 M Tris-hydrochloride, pH 8, containing 0.05 M EDTA and 0.01 M NaCN. The [¹⁴C]-labeled cells were also transferred to Tris-EDTA-CN buffer and served as unirradiated controls. The cells were converted to protoplast, mixed, and lysed on top of 5 to 20% alkaline sucrose gradients by procedures described in Materials and Methods. Thus each gradient contained [¹⁴C]-labeled DNA from unirradiated cells and [³H]-labeled DNA from cells which had been irradiated and then incubated for specified times. The gradients were centrifuged for 90 min at 35,000 rpm in a Spinco SW39 rotor at 16 C, collected, and counted as described in Materials and Methods;* (○), *³H radioactivity;* (●), *¹⁴C radioactivity. Sedimentation is from right to left. Arrows indicate portions of curve used in calculations.*

less DNA was degraded after 50 krad than after 20 krad. This effect, previously observed for *E. coli* (27) and *S. typhimurium* (16), has been attributed to a possible inactivation or saturation of degradative enzyme systems. This would imply that the increased breakdown of DNA caused by 200 krad (Fig. 2d) may involve a separate mechanism not detected in the wild type.

In the presence of growth medium (data not shown) less irradiation-induced degradation of DNA occurs; clearly, any observed breakdown of DNA in this circumstance represents the net outcome of competition between degradative and synthetic processes (3). As expected, post-irradiation synthesis of DNA occurs earlier and proceeds at a faster rate in the resistant strain. The two approaches to the study of post-irradiation DNA synthesis, using unlabeled versus prelabeled cells, provide complementary information. The overall result is that DNA synthesis continued or resumed in radiation-resistant cells exposed to doses inhibiting uptake of [³H]thymidine into parental DNA. The incorporation of radioactivity into unlabeled cells, expressed on linear coordinates (Fig. 3), could lead to the conclusion that low doses of irradiation reduced the subsequent rates of DNA synthesis in wild-type cells and temporarily delayed synthesis in the radioresistant strains. However, the experiments with prelabeled cells (Fig. 4) suggest that, with

the exception of DB21, after 200 krad surviving cell fractions (extrapolation to zero time) continued to synthesize DNA at nearly normal rates. Such an interpretation might support the view of Hildebrand and Pollard (14) who believe that ionizing irradiation damage (manifest as DNA degradation) is an all-or-nothing phenomenon within a bacterial population. Our data cannot answer this question. However, the general result, namely that radioresistant populations have substantially more DNA synthetic capacity after irradiation with any dose, is not in question.

The stimulated initial uptake recorded for unlabeled, unirradiated D21R6008 cells could be related to the spontaneous turnover of DNA in these cells relative to the parents even without radiation.

Production and repair of single-strand breaks in DNA. Although single-strand breaks appear to be produced by gamma irradiation in equivalent numbers in the resistant and wildtype strains, repair is much more rapid in the resistant strain. The sedimentation profiles shown in Fig. 5 were used to calculate the average molecular weights of the DNA fragments extracted from the cells; from this we could estimate the number of radiation-induced-strand breaks (Table 1 and 2). Several significant points emerge from these data. The number of strand breakages induced in the DNA by the 20-krad-irradiation dose is approx-

TABLE 1. *Zone sedimentation characteristics of DNA in alkaline gradients after 20 krad of irradiation*

Strains	Irradiation (krad)	Characteristics				
		Avg. distance sedimented D (cm)	Avg. sedimentation[a] coefficient $S_{20,w}$	Wt. avg. mol wt.[b] ($M_w \times 10^7$)	Estimated no. avg.[c] mol wt. ($M_w \times 10^7$) Mn	Estimated radiation-induced single-strand breaks per intact replicating genome
DB 21	0	2.1	130	28	19	0
	20	1.4	83	10	<5	>60
D 21 R 6008	0	2.0	120	25	16	0
	20	1.5	87	11	<5.5	>48

[a] Calculated from the Burgi and Hershey (7) relationship $S_{20,w} = \beta D/\omega^2 t$, where D(cm) is the average distance sedimented after centrifugation at ω(rev per min) for time t. The value of β was 1.09×10^{11} for the conditions used and was determined by sedimentation of ^{3}H-labeled P22 marker DNA as described by Botstein (4).

[b] Calculated from Studier's equation $S_{20,w} = 0.0528\ M^{0.4}$ where M was considered to closely approximate the true weight average molecular weight M_w. (29).

[c] Calculated as described in the Materials and Methods section and as discussed by Lett et al. (19).

TABLE 2. *Zone sedimentation characteristics of DNA in alkaline gradients after 200 krad of irradiation*

Strains	Irradiation (krad)	Characteristics				
		Avg. distance sedimented D (cm)	Avg. sedimentation[a] coefficient $S_{20,w}$	Wt. avg. mol wt.[b] ($M_w \times 10^7$)	Estimated no. avg.[c] mol wt. ($M_w \times 10^7$) Mn	Estimated radiation-induced single-strand breaks per intact replicating genome
DB 21	0	2.1	130	28	19	0
	200	1.1	64	5.0	<2.5	>141
D 21 R 6008	0	1.7	100	16	10	0
	200	1.2	68	6.0	<3.0	>95

[a] Calculated from the Burgi and Hershey (7) relationship $S_{20,w} = \beta D/\omega^2 t$, where D(cm) is the average distance sedimented after centrifugation at ω(rev per min) for time t. The value of β was 1.09×10^{11} for the conditions used and was determined by sedimentation of ^{3}H-labeled P22 marker DNA as described by Botstein (4).

[b] Calculated from Studier's equation $S_{20,w} = 0.0528\ M^{0.4}$ where M was considered to closely approximate the true weight average molecular weight M_w. (29).

[c] Calculated as described in the Materials and Methods section and as discussed by Lett et al. (19).

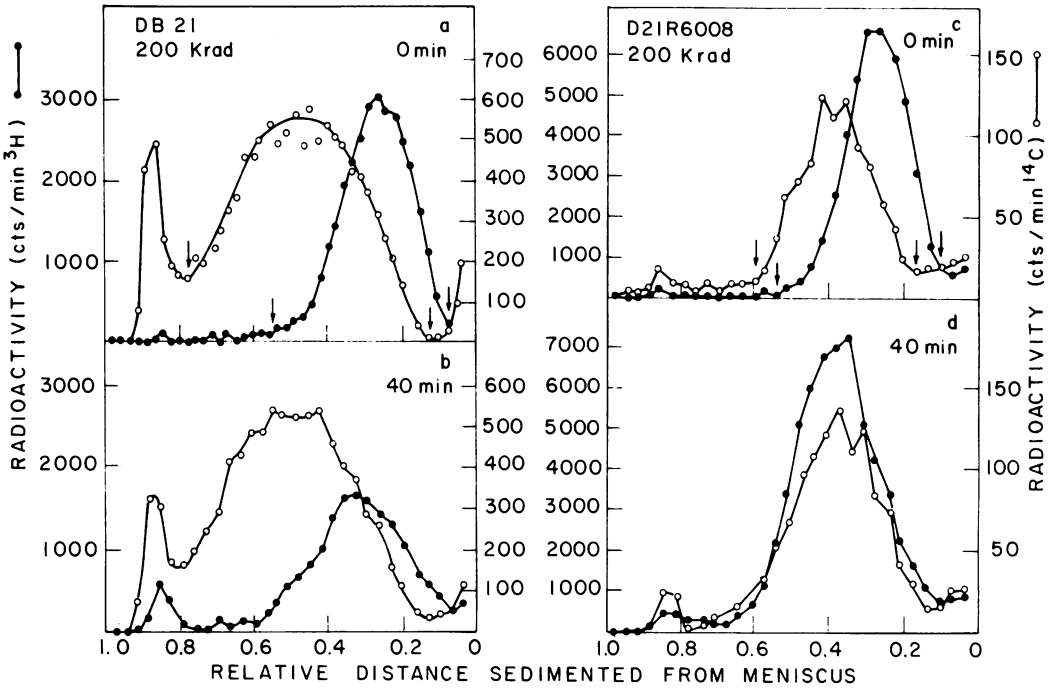

FIG. 6. *Zone sedimentation of DNA in alkaline sucrose gradients irradiated with 200-krad gamma rays. The cells were labeled, irradiated at 200 krad, and lysed on alkaline sucrose gradients as described in the legend to Fig. 5. Arrows indicate portions of curve used in calculations.*

imately the same for each strain and the estimated numbers of strand breaks per intact genome induced by 20 krad are reasonably close to previously reported values (13, 2); at doses of 200 krad considerably fewer breaks than would be expected are found and more breaks are observed in the wild-type strain; finally, the data show that the DNA of unirradiated D21R6008 cells consistently sediments as somewhat smaller pieces than that of wild type.

The observation that a given radiation dose produces the same number of breaks in the DNA of radiation-resistant or parent strains of *S. typhimurium* LT2 is in accord with recent reports for *E. coli* (26) and for *S. thompson* (3). Thus, if single-strand breaks play a role in the lethality of irradiation, the resistance of strain D21R6008 to gamma rays may be a result of its ability to correct these lesions rather than prevent them.

The number of single-strand breaks detected in both strains DB21 and D21R6008 particularly after high irradiation doses (200 krad) is less than expected and requires explanation. The assumptions inherent to the quantitative analysis of sedimentation data inevitably lead to an underestimate of damage (29), and these difficulties are compounded by slow-sedimenting material not related to irradiation (14). It is also possible that extended irradiation exposure times permit the concurrent rejoining of many strand breaks lying close together. Activity of this type, designated "rapid repair," has been identified in *M. radiodurans* (2) and more recently in *E. coli* K-12 with evidence that the *polA* gene (and thus DNA polymerase I) is involved (32). This latter finding might thus be relevant to our present study as suggested above. Recent preliminary results suggest that considerably more extensive strand breakage is detected when cyanide respiratory inhibitors are present during irradiation.

The smaller molecular size of the DNA fragments from unirradiated resistant cells relative to wild type suggests either that the DNA of strain D21R6008 is more susceptible to breakage during extraction or that there are, in fact, more spontaneous single-strand scissions during normal growth. The spontaneous turnover of DNA discussed above may be related to this observation, as may be our previously reported finding of mutator activity (11).

Finally, as commented in Materials and Methods, it was found desirable to omit Sarkosyl from the gradients containing D21R6008

material. The presence of this detergent, which aided lysis and gradient collection in the case of strain DB21, invariably caused the resistant cell DNA preparation to sediment anomalously to the bottom of the gradient tubes. Sarkosyl does not bind with DNA per se but, in the presence of divalent cations, will complex with membrane and phospholipids (31). Although our conditions differ, one might suspect some connection between our observation and the published ones. An increased frequency of association between DNA and membrane has been suggested for *M. radiodurans* (2) and might thus also be a contributory factor to D21R6008 radiation resistance.

In conclusion, we have shown that strain D21R6008, a hyper-radioresistant derivative of *S. typhimurium*, degrades less DNA after exposure to gamma irradiation and displays a marked enhanced ability to repair and, therefore, to tolerate single-strand lesions in its DNA. This is consistent with our previous findings of increased host-cell reactivation of gamma-irradiated prophage. We suggest that these factors account, at least in part, for the observed increases in radiation resistance.

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