JOURNAL OF BACTERIOLOGY, Mar. 1971, p. 976-983
Copyright © 1971 American Society for Microbiology
Vol. 105, No. 3
Printed in U.S.A.

Repair of Irradiated Transforming Deoxyribonucleic Acid in Wild Type and a Radiation-Sensitive Mutant of *Micrococcus radiodurans*

B. E. B. MOSELEY AND ALICE MATTINGLY
Department of General Microbiology, University of Edinburgh, College of Agriculture, Edinburgh 9, Scotland

Received for publication 21 September 1970

The survival of biological activity in irradiated transforming deoxyribonucleic acid (DNA) has been assayed in the wild type and a radiation-sensitive mutant of *Micrococcus radiodurans*. The frequency of transformation with unirradiated DNA was lower in the mutant to about the same extent as the mutant's increased sensitivity to radiation. However, in both the wild type and the mutant, the irradiated DNA that was incorporated into the bacterial genome was repaired to the same extent as determined by the loss of transforming activity with increasing radiation dose. This applied to DNA irradiated either with ionizing or ultraviolet (UV) radiation. The rate of inactivation of biological activity after UV radiation was the same in any of the DNA preparations tested. For ionizing radiation, the rate of inactivation varied up to 40-fold, depending on the DNA preparation used, but for any one preparation was the same whether assayed in the wild type or the radiation-sensitive mutant. When recipient bacteria were irradiated with ionizing or UV radiation immediately before transformation, the frequency of transformation with unirradiated DNA fell, rapidly and exponentially in the case of the sensitive mutant but in a more complicated fashion in the wild type. The repair of DNA irradiated with ionizing radiation was approximately the same whether assayed in unirradiated or irradiated hosts. Thus, irradiation of the host reduced the integration of DNA but not its repair.

Wild-type *Micrococcus radiodurans* possesses the most efficient dark repair mechanism of radiation damage to deoxyribonucleic acid (DNA) in vegetative bacteria thus far investigated (1, 2). This bacterium is capable of undergoing genetic transformation and is able to repair ultraviolet (UV)-irradiated transforming DNA with the same high efficiency that it repairs its own DNA (12).

This study has now been extended to the repair of transforming DNA irradiated with both UV and γ rays in the wild-type and in a radiation-sensitive mutant of *M. radiodurans*.

MATERIALS AND METHODS

Bacteria. Three strains of *M. radiodurans* were used: the wild type which was sensitive to streptomycin, a mutant resistant to 200 μg of streptomycin per ml, and the radiation-sensitive strain UV17 (9).

Media. TGY medium for growth contained tryptone (Difco), 5 g; glucose, 1 g; yeast extract (Difco), 3 g; and distilled water to 1 liter. TGY agar was made by solidifying this medium with 15 g of agar per liter. The 0.067 M phosphate buffer (pH 7.0) contained 4.73 g of Na_2HPO_4 and 4.5 g of KH_2PO_4 per liter of distilled water.

Survival curves of bacteria. Samples from an 18-hr culture of *M. radiodurans* were added to flasks containing 20 ml of TGY medium to give a turbidity reading of 0.08 on a nephelometer (Evans Electroselenium Ltd.) with an orange filter, having an approximate wavelength 590 nm (about 2.5×10^7 viable units/ml). The flasks were swirled at 30 C until the turbidity reached 0.50 to 0.55 (1.4×10^8 viable units/ml). The bacteria were centrifuged, washed, and resuspended in chilled 0.067 M phosphate buffer at a concentration of 10^8 viable units/ml. For UV irradiation, 5-ml samples of the washed bacterial suspension were irradiated in petri dishes (9 cm in diameter) at a distance of 40 cm from a Hanovia germicidal lamp (model 12). The incident dose rate was 22.5 ergs per mm^2 per sec. The suspension was agitated during irradiation by means of a magnetic stirrer. At intervals, 0.1-ml samples were removed and suitably diluted into chilled TGY, and 0.1-ml quantities were spread on TGY plates. Colonies de-

rived from surviving cells were counted after incubation at 30 C for 2 days.

Gamma irradiation was carried out in a ^{60}Co source at a dose rate of 10 krad/min. Three-milliliter volumes of the washed bacterial suspension were irradiated, oxygen being bubbled during the irradiation. Samples were removed and viability was measured in the same way as for that after UV irradiation.

Preparation of transforming DNA. A streptomycin-resistant strain of *M. radiodurans* was grown to saturation in TGY, washed in SSC (0.15 M NaCl and 0.015 M sodium citrate), and resuspended in SSC at a concentration of about 10^{10} viable units/ml. The bacteria were gently shaken for 18 hr at 37 C with 2 mg of lysozyme per ml (EC 3.2.1.17), centrifuged, resuspended in 0.1 SSC at a concentration of about 2×10^{10} to 3×10^{10} viable units/ml, and lysed by the addition of 20% sodium lauryl sulfate to a final concentration of 2%. The DNA was purified by the method of Marmur (8) and was diluted in 0.067 M phosphate buffer. The concentration of DNA was calculated from the absorption of a suitably diluted sample at 260 nm.

Preparation of radioactive M. radiodurans DNA. Radioactive DNA was made in the same manner. A streptomycin-resistant strain of *M. radiodurans* was grown to saturation in 250 ml of TGY containing 2 mCi of ^{3}H-thymidine (specific activity 14 Ci/mM). The specific activity of the purified transforming DNA was 1.25×10^{4} counts per min per μg of DNA.

Preparation of a crude extract of wild type M. radiodurans. This was used for enhancing the frequency of transformation in *M. radiodurans* (12). Wild-type bacteria were grown to saturation in TGY, washed, and resuspended in 0.067 M phosphate buffer at a concentration of 2×10^{10} viable units/ml. This suspension was passed twice through a French press (pressure, 6,000 psi). Viable cells and cell wall debris were removed by centrifugation, and the resulting extract was autoclaved at 15 psi of steam for 15 min.

Procedure for transformation. Streptomycin-sensitive strains, i.e., wild type or UV17, were grown in TGY at 30 C to a concentration of approximately 3×10^{8} viable units/ml. To 0.2-ml quantities of these cultures in test tubes were added 0.05 ml of transforming DNA (concentrations given above) or 0.05 ml of 0.067 M phosphate buffer and 0.05 ml of crude extract of the wild-type bacteria, and the resulting cultures were shaken gently at 30 C for 3 hr. After appropriate dilution with chilled TGY, 1-ml samples were plated in 10 ml of TGY agar which was melted and cooled to about 40 C before being mixed with the bacteria. The plates were incubated for about 8 hr at 30 C and then overlaid with 10 ml of the same melted agar medium containing 200 μg of streptomycin per ml. Colonies derived from transformed bacteria were counted after incubation at 30 C for at least 4 days.

Irradiation of transforming DNA. For UV irradiation, the transforming DNA was diluted to 0.05 mg/ml, and 1 ml was irradiated in a petri dish 4.5 cm in diameter at a distance of 40 cm from the germicidal lamp. Gamma irradiation was carried out in a ^{60}Co source at a dose rate of 10 krad/min. The concentrations of DNA used are described above.

Irradiation of bacteria used as recipients in transformation. *M. radiodurans* was grown as for transformation experiments, washed, and resuspended in chilled 0.067 M phosphate buffer and irradiated either with UV or γ rays as described above. Quantities (0.5 ml) were removed at various times from the irradiated suspensions, centrifuged, and resuspended in 0.5-ml quantities of chilled TGY. These suspensions were then used for transformation in the normal way.

Measurement of DNA uptake by irradiated bacteria. Bacteria were grown, irradiated, and resuspended in chilled TGY as described above, and 0.3 ml of these suspensions were gently shaken at 30 C for 3 hr with 7 μg of tritiated DNA from *M. radiodurans*. Deoxyribonuclease (EC 3.1.4.6), 0.1 ml of 0.5 mg/ml, was added to each culture for an additional 30 min, and the cells were collected by centrifugation. They were washed four times with 0.067 M phosphate buffer and then lysed with hot 98% formic acid in scintillation vials. The formic acid was boiled off, and 1 ml of distilled water was added while the vials were still hot. Dioxan scintillation fluid was added after the vials cooled, and the radioactivity in each vial was measured. The radioactivity in the supernatant fluid from the first centrifugation was also measured. The efficiency of counting was 20%.

RESULTS

Radiation inactivation of bacteria. The dose-response curves of the wild type and the radiation-sensitive mutant UV17 of *M. radiodurans* to UV and ionizing radiation are plotted in Fig. 1 (a and b). The shapes of the curves may be defined in terms of their intercept number (the dose obtained by extrapolating the exponential part of the curve to unit survival) and the $1/e$ value (the dose required to decrease the colony count of a culture by 63% on the exponential part of the survival curve and thus required to give an average of one lethal event per bacterium). These values (Table 1) were slightly different from those previously reported (9), but the bacteria were irradiated in log-phase growth as opposed to the resting phase. The sensitive mutant was about five times as sensitive to radiation as the wild type.

Radiation inactivation of transforming DNA. The survival of transforming activity as a function of UV dose to the transforming DNA is shown in Fig. 2. Both wild-type *M. radiodurans* and UV17 were used as recipients, and the survival of transforming activity was the same, although there was a lower frequency of transformation in the case of UV17. Nevertheless, the slopes of surviving activity as a function of dose were the same, the $1/e$ dose being 116,000 ergs/mm^2.

The resistance of transforming DNA to γ radiation was extremely variable. Figure 3 shows the survival of transforming activity in two different preparations of DNA assayed in wild-type and UV17 strains of *M. radiodurans*. The survival of transforming activity in a preparation of

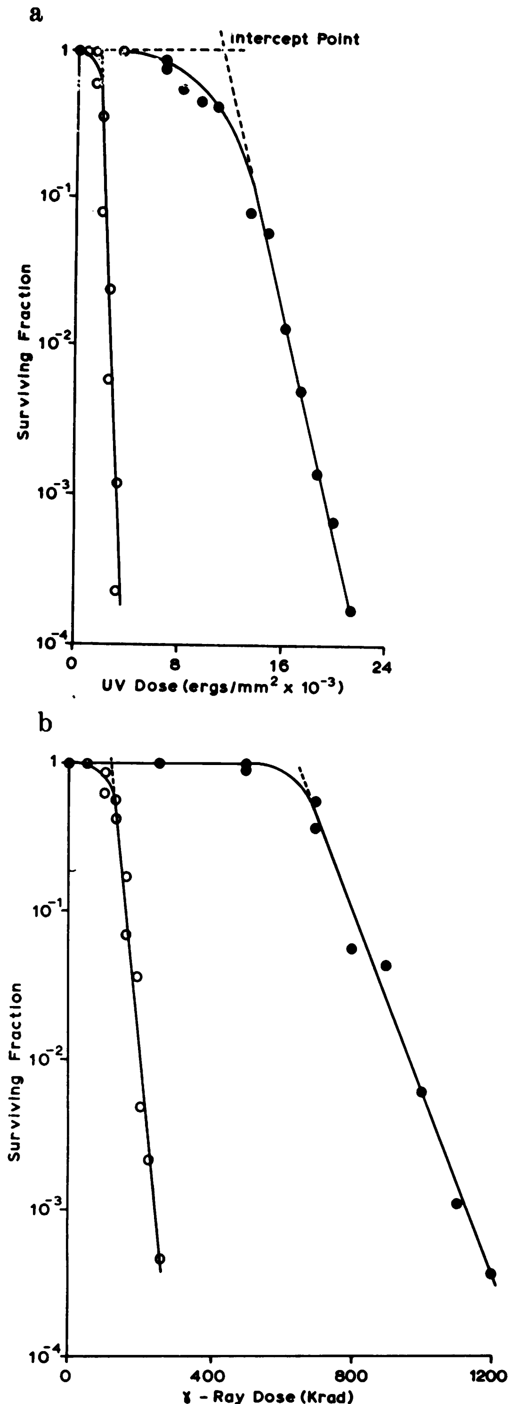

FIG. 1. (*a*) *UV irradiation survival curves of Micrococcus radiodurans wild type* (●) *and the mutant UV17* (O). *The exponential portion of the survival curve has been extrapolated to unit survival to indicate the derivation of the intercept value used in Table 1.* (*b*) *Gamma irradiation survival curves of M. radiodurans wild type* (●) *and UV17* (O).

DNA was the same whether assayed in wild type or UV17, but the slope of the survival curve varied from one preparation of DNA to another. For example, the $1/e$ values for the two DNA preparations shown in Fig. 3 are 3.6 and 33 krad, a ratio of 1:9 in terms of resistance, and, in another DNA preparation assayed only in wild type, the $1/e$ value was 155 krad, a 40-fold increase in resistance compared with the most sensitive batch.

The survival of transforming activity did not become much more sensitive to radiation during the various steps in the purification of the DNA. Such survival in one batch of DNA was assayed at each of the steps from the first precipitation of DNA through the ribonuclease step to the isopropanol precipitation, and the survival slopes did not vary greatly. Thus, the step which is perhaps most important in giving quite different sensitivities of transforming activities was the lysis of the bacteria which is not a well-defined step in *M. radiodurans*. However, different batches of DNA which showed very great differences in the survival of transforming activity after γ radiation gave identical survival when assayed after UV irradiation, the $1/e$ value being 116,000 ergs/mm^2.

Survival of transforming activity in irradiated recipient bacteria. Suspensions of wild-type and UV17 *M. radiodurans* were irradiated with various doses of UV or γ radiation, and their capacity to be transformed was measured as a function of dose (Fig. 4 and 5). The doses given were quite small and on the survival curve of the bacteria (Fig. 1) show 100% survival. However, when held in liquid media, irradiated *M. radiodurans* shows a loss in viability which is not shown when the cells are plated immediately (11). In these experiments, 100% viability was maintained at as high as 900 and 6,500 ergs of UV radiation per mm^2 and at 45 and 180 krads of γ radiation for UV17 and wild-type *M. radiodurans*, respectively. In Fig. 4 and 5, the frequency of transformation is plotted against dose to the recipient bacteria.

Both qualitatively and quantitatively the survival of transforming activity differed in the two recipients. In UV17, transformation fell off exponentially to almost zero. The $1/e$ dose for the loss of transformability was 225 ergs/mm^2 for UV radiation and 15 krad for γ irradiation. These are the $1/e$ values for inactivation on the exponential part of the survival curve. In the wild type, transformation decreased initially at the same rate and then became refractory at about 20% of the initial frequency.

Repair of irradiated transforming DNA in irradiated recipient bacteria. The biological activity of transforming DNA irradiated with increasing

TABLE 1. *Analysis of the dose-response curves shown in Fig. 1*[a]

Strain	UV radiation dose-response curves				γ Radiation dose-response curves			
	Ergs/ 1/e mm²	Ratio	Intercept	Ratio	1/e krad	Ratio	Intercept	Ratio
Wild type	1,220	1	11,000	1	64	1	640	1
UV17	225	0.18	1,630	0.15	15	0.23	127	0.20

[a] The 1/e value is the dose required to decrease the viability of a culture by 63% on the exponential part of the survival curve and is the dose required to give an average of one lethal event per bacterium. The intercept value, in terms of dose, is obtained by extrapolating the exponential part of the curve to unit survival. The ratios are obtained from the 1/e and intercept values divided by those of wild type.

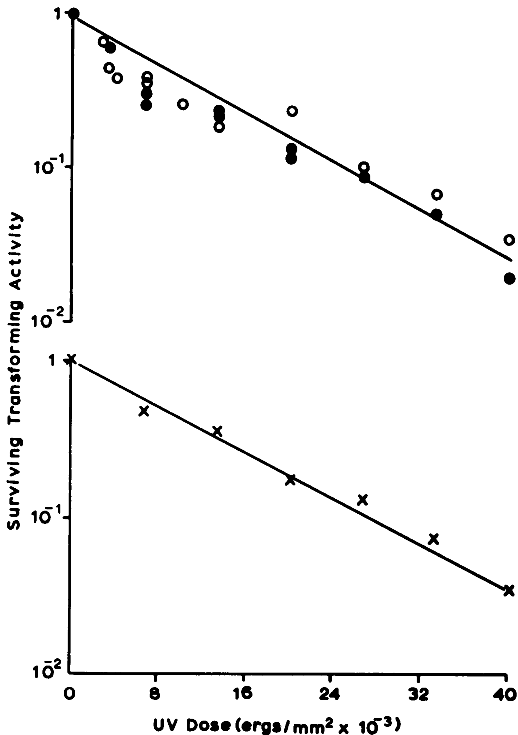

FIG. 2. *Survival of transforming activity in UV-irradiated transforming DNA. The upper slope represents the survival of transforming activity in one preparation of DNA (preparation 1) assayed on Micrococcus radiodurans wild type (●) and the radiation-sensitive mutant UV17 (O). The transformation frequency at zero irradiation was 10^{-5} in the wild type and 4.0×10^{-6} in UV17. The lower slope (×) is the survival of transforming activity in a second preparation of transforming DNA (preparation 2) assayed in the wild type (cf. Fig. 4). The transformation at zero dose was 8.0×10^{-6}.*

doses of γ radiation was assayed in unirradiated bacteria and in bacteria irradiated with 1,800 ergs of UV radiation per mm² (Fig. 6) and 65 krads of γ radiation (Fig. 7). Although the transformation frequency fell when the recipient bacteria were irradiated, the repair of DNA was not affected, as determined by the slope of inactivation of biological activity against radiation dose. With UV-irradiated bacteria, the loss of biological activity was the same in irradiated and unirradiated cells, and when the cells were γ-irradiated it appeared that the survival of biological activity was slightly more resistant in the irradiated bacteria.

The survival of UV-irradiated transforming DNA in irradiated hosts could not be assayed because the drop in transformation frequency caused by both irradiating the recipient bacteria and diluting the DNA sufficiently to give it a suitable dose of UV radiation prevented large enough numbers of transformants from being obtained to provide significant results.

Uptake of DNA into unirradiated and irradiated cells of wild-type and UV17 M. radiodurans. The uptake of DNA was measured in unirradiated and irradiated bacteria. Doses were chosen which reduced the frequency of transfor-

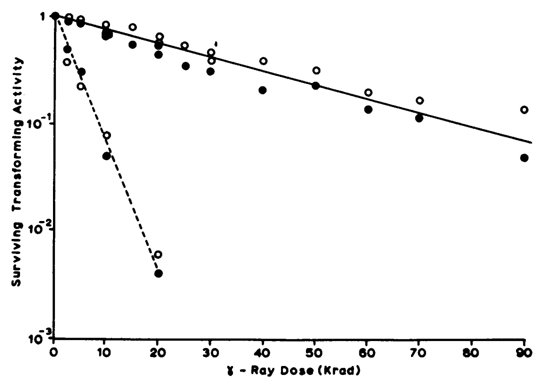

FIG. 3. *Survival of transforming activity in γ-irradiated transforming DNA. The DNA was irradiated at a concentration of 1.5 mg/ml and 0.05 ml was added to each assay tube. The continuous line represents survival of transforming activity of preparation 1 DNA assayed in Micrococcus radiodurans wild type (●) and UV17 (O). The transformation frequency at zero dose was 1.2×10^{-4} in wild type and 4.0×10^{-5} in UV17. The dashed line is the survival of transforming activity in preparation 2 DNA assayed in wild type (●) and UV17 (O). The transformation frequency at zero dose was 2.0×10^{-5} in wild type and 4.0×10^{-6} in UV17.*

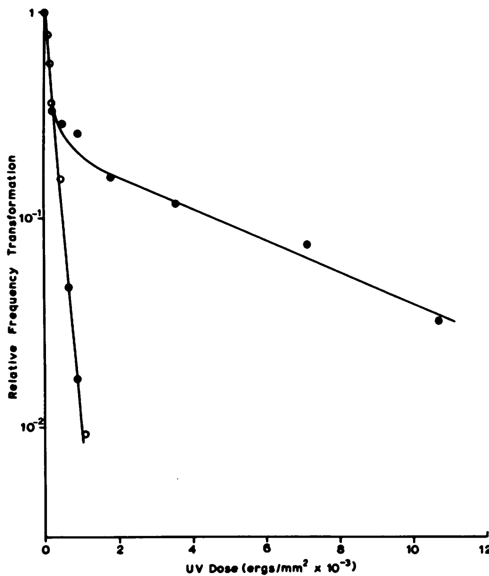

FIG. 4. *Effect of UV-irradiating the recipient bacteria on the frequency of transformation in Micrococcus radiodurans wild type* (●) *and UV17* (O). *The frequency of transformation at zero dose was* 5×10^{-5} *for the wild type and* 9.0×10^{-6} *for UV17.*

mation to about 10% of that in the corresponding unirradiated bacteria. As a control, unirradiated cells were heated at 70 C for 10 min. This killed the bacteria but did not lyse them. The amounts of radioactive DNA taken up by bacteria under transformation conditions are shown in Table 2. The amount of DNA taken up was not reduced significantly as a result of irradiation and could not account quantitatively for the 90% decrease in transformation.

DISCUSSION

Radiation-sensitive mutants give various responses when irradiated, transforming DNA is assayed in them. For example, UV-sensitive mutants of *Haemophilus influenzae* show a reduced capacity to repair UV-irradiated transforming DNA compared with wild type, although the increase in sensitivity does not correlate with the increase in sensitivity of the mutant hosts (15). Reiter and Strauss (14) and Okubo and Romig (13) found that mutants of *Bacillus subtilis* which were six to eight times more sensitive to UV than the wild type showed only a two- to threefold difference in their capacity to repair transforming DNA. They concluded that competent bacteria (i.e., those in a state required to take up DNA) differ quantitatively from noncompetent bacteria in their capacity to repair UV-induced lesions in DNA, a difference associated with the physiological states of competence and noncompetence. Another UV-sensitive mutant of *B. subtilis* was not different from the wild type in its capacity to repair UV-irradiated transforming DNA, and no differences were observed in the sensitivity of cells during the competent stage (7).

In the case of wild-type *M. radiodurans*, it has already been established that the capacity for repair of UV-irradiated transforming DNA is high, since it is impossible to enhance survival of the irradiated DNA by preincubating it with highly purified photoreactivating enzyme (12). Of course, in UV irradiation of bacteria damage is caused to both DNA strands, whereas in transformation damage is probably confined to one

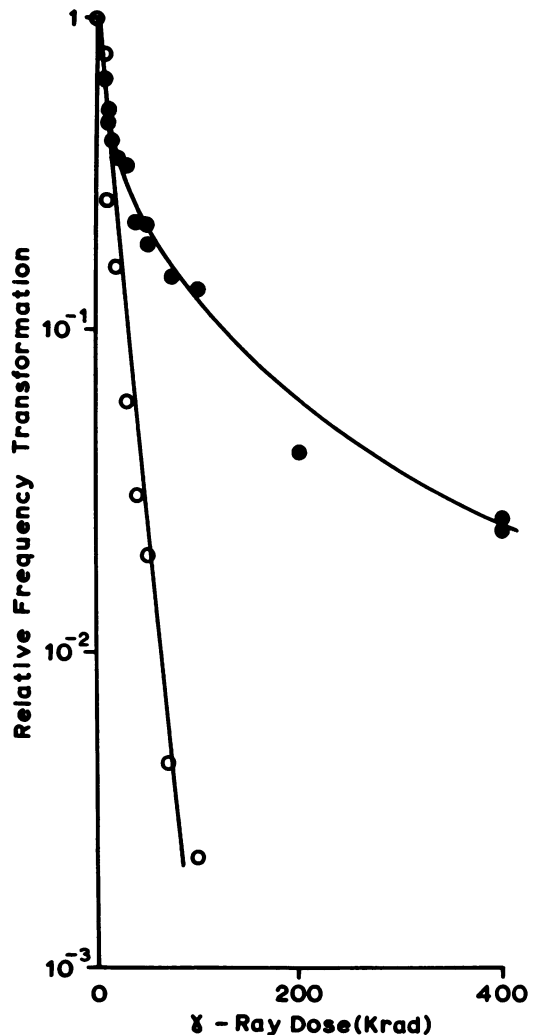

FIG. 5. *Effect of γ-irradiating the recipient bacteria on the frequency of transformation in Micrococcus radiodurans wild type* (●) *and UV17* (O). *The frequency of transformation at zero dose was* 3.0×10^{-5} *for the wild type and* 2.5×10^{-6} *for UV17.*

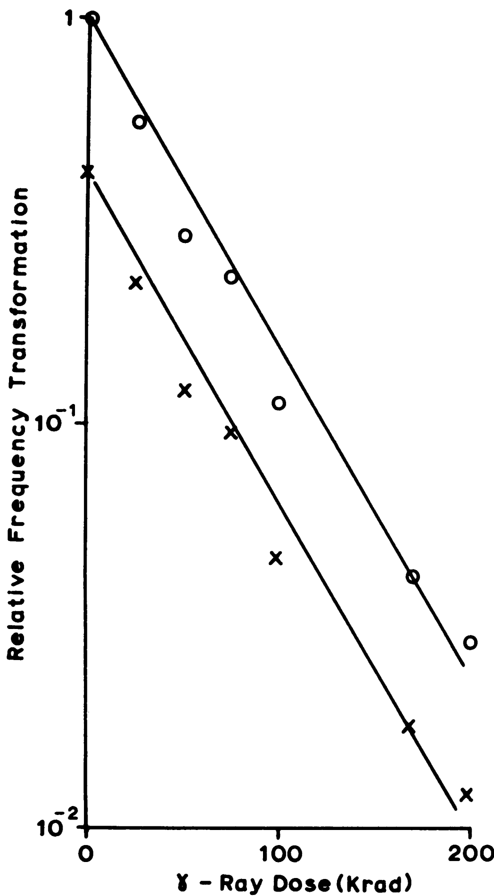

FIG. 6. *Survival of transforming activity in γ-irradiated transforming DNA assayed in unirradiated (O) and UV-irradiated (×) cells of wild-type Micrococcus radiodurans. The frequency of transformation with both unirradiated cells and DNA was 3.0×10^{-5}. As a result of irradiating the recipient cells with a dose of 1,800 ergs of UV radiation per mm^2, the frequency dropped to 41% that of the unirradiated cells.*

strand since only one of the strands of transforming DNA is incorporated into the host genome (3). It may be assumed that since repair of irradiated transforming DNA is the same in the wild type and the UV-sensitive mutant of M. radiodurans that both strains have the same capacity for the repair of damage in one strand of DNA when the complementary strand is undamaged, but the wild-type is able to cope with damage to both strands much more efficiently. This may be the case, because the wild type has a more closely coordinated repair sequence and prevents repair regions from overlapping in the two DNA strands (11).

As previously pointed out (12), UV inactivation of the biological activity of the DNA does not fit a square-root plot as observed, for example, for *H. influenzae* DNA. The frequency of transformation in *M. radiodurans* is quite low, but the data presented would suggest that the inactivation is almost exponential. Agents which cause single-strand breaks in DNA such as deoxyribonuclease and X rays usually give such exponential plots, whereas agents which produce base damage only, such as chemical mutagens or UV radiation, yield curves which fit the square-root plot (5). This would suggest that the repair of base damage in UV-irradiated transforming DNA is complete in *M. radiodurans* and that such inactivation as occurs is due to strand breakage.

The irradiation sensitivity of the mutant UV17 is shown by irradiating it before using it as a recipient in transformation. In this case, transforming activity is lost at quite small doses of radiation in the sensitive mutant but not in the wild type. This is not due to reduced uptake of DNA caused by irradiation, since irradiated and

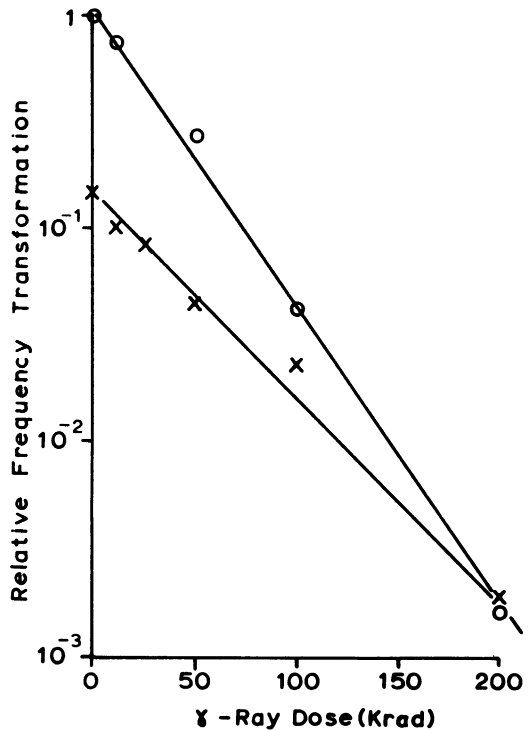

FIG. 7. *Survival of transforming activity in γ-irradiated transforming DNA assayed in unirradiated (O) and γ-irradiated (×) cells of wild-type Micrococcus radiodurans. The frequency of transformation with both unirradiated cells and DNA was 2.5×10^{-5}. As a result of irradiating the recipient cells with a dose of 65 krad of γ radiation, the frequency dropped to 14% of that of unirradiated cells.*

TABLE 2. *Uptake of ³H-labeled Mecrococcus radiodurans DNA by unirradiated and irradiated strains of M. radiodurans*[a]

Strain of *M. radiodurans*	Treatment of bacteria	Counts per min of bacteria	Counts per min per 0.1 ml of supernatant
Wild type	Heated at 70 C for 10 min	43	31,500
	Unirradiated	1,266	33,000
	UV-irradiated	1,373	30,000
	γ-Irradiated	2,627	36,000
UV17	Heated at 70 C for 10 min	43	22,000
	Unirradiated	1,027	41,000
	UV-irradiated	577	32,500
	γ-Irradiated	2,035	39,000

[a] Bacteria were grown as though for a transformation experiment. The irradiated cells were given doses of UV or γ radiation which reduced the frequency of transformation to approximately 10% of that of unirradiated recipient bacteria. Bacteria heated to 70 C for 10 min were killed but not lysed. The bacteria were incubated, under transformation conditions, with ³H-DNA and then treated with deoxyribonuclease, washed several times, and hydrolyzed; the amount of radioactivity in the bacteria and the medium was assayed.

unirradiated cultures of both strains take up similar quantities of DNA at doses which reduce the transforming frequency to about 10% in the irradiated cultures. Thus, the reduction in transformation is connected with events which follow uptake of DNA and probably are connected with integration of DNA. One other feature which distinguishes the sensitive mutant from the wild type is that the frequency of transformation under identical conditions is about fivefold less than in the wild type, about the same extent as its increased sensitivity to UV radiation. Whether this is a characteristic of all UV-sensitive mutants of *M. radiodurans* is not clear. In the studies by Setlow et al. (15) and Mahler (7), the frequencies of transformation in transformable UV-sensitive mutants of *H. influenza* and *B. subtilis* were the same. However several independently isolated radiation-sensitive mutants of *M. radiodurans* all show a reduced frequency of transformation. Temperature-sensitive mutants isolated by using similar methods, on the other hand, give normal transformation frequencies, so that the reduced frequency is not a result of the mutagen treatment used in the isolation of the mutants. Thus, the enzyme step in repair, the loss of which causes UV sensitivity in the mutant, would also appear to reduce, by about the same amount, the integration of transforming DNA into the host genome although not affecting its subsequent repair.

Mutant UV17 seems to be equivalent to the *exr*⁻ mutants of *Escherichia coli*. As described by Witkin (16), mutations at the *exr* or *lex* locus cause increased sensitivity to X rays, to nitrosoguanidine, and to UV light and have no demonstrable effect on the ability of Hcr^+ (uvr^+) strains to effect excision repair. Both *exr* and *lex* mutants exhibit reduced recombination ability to an extent that is commensurate with the reduction in UV resistance, and they also show increased DNA degradation after UV radiation. All of these criteria apply to mutant UV17 of *M. radiodurans* (9-12).

These characteristics caused Witkin (16) to suggest that *exr* and *lex* mutations reduce UV resistance by reducing the efficiency of postreplication recombinational repair (4). This process is being investigated in *M. radiodurans*.

ACKNOWLEDGMENT

This investigation was supported by a grant from the Medical Research Council of Great Britain.

LITERATURE CITED

1. Anderson, A. W., H. C. Nordan, R. F. Cain, G. Parrish, and D. Duggan. 1956. Studies on a radioresistant micrococcus. Isolation, morphology, cultural characteristics, and resistance to gamma radiation. Food Technol. **10**: 575-578.
2. Duggan, D. E., A. W. Anderson, P. R. Elliker, and R. F. Cain. 1959. Ultraviolet exposure studies on a gamma radiation resistant micrococcus isolated from food. Food Res. **24**:376-382.
3. Fox, M. S., and M. K. Allen. 1964. On the mechanism of deoxyribonucleate integration in pneumococcal transformation. Proc. Nat. Acad. Sci. U.S.A. **52**:412-419.
4. Howard-Flanders, P. 1968. DNA repair. Annu. Rev. Biochem. **37**:175-200.
5. Hutchison, F., and H. B. Hales. 1970. Mechanism of the sensitization of bacterial transforming DNA to ultraviolet light by the incorporation of 5-bromouracil. J. Mol. Biol. **50**:59-69.
6. Lacks, S. A. 1962. Molecular fate of DNA in genetic transformation of *Pneumococcus*. J. Mol. Biol. **5**:119-131.
7. Mahler, I. 1965. Characteristics of an ultraviolet irradiation sensitive strain of *Bacillus subtilis*. Biochem. Biophys. Res. Commun. **21**:384-391.
8. Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3**:208-218.
9. Moseley, B. E. B. 1967. The isolation and some properties of radiation sensitive mutants of *Micrococcus radiodurans*. J. Gen. Microbiol. **49**:293-300.
10. Moseley, B. E. B. 1967. The repair of DNA in *Micrococcus radiodurans* following ultraviolet radiation. J. Gen. Microbiol. **48**:vi.
11. Moseley, B. E. B. 1969. Repair of ultraviolet radiation damage in sensitive mutants of *Micrococcus radiodurans*. J. Bacteriol. **97**:647-652.
12. Moseley, B. E. B., and J. K. Setlow. 1968. Transformation in *Micrococcus radiodurans* and the ultraviolet sensitivity of its transforming DNA. Proc. Nat. Acad. Sci. U.S.A. **61**:176-183.
13. Okubo, S., and W. R. Romig. 1965. Comparison of ultravi-

olet sensitivity of *Bacillus subtilis* bacteriophage SPO2 and its infectious DNA. J. Mol. Biol. **14**:130-142.

14. Reiter, H., and B. Strauss. 1965. Repair of damage induced by a monofunctional alkylating agent in a transformable ultraviolet-sensitive strain of *Bacillus subtilis*. J. Mol. Biol. 179-194.
15. Setlow, J. K., D. C. Brown, M. E. Boling, A. Mattingly, and M. P. Gordon. 1968. Repair of deoxyribonucleic acid in *Haemophilus influenzae*. I. X-ray sensitivity of ultraviolet-sensitive mutants and their behavior as hosts to ultraviolet-irradiated bacteriophage and transforming deoxyribonucleic acid. J. Bacteriol. **95**:546-558.
16. Witkin, E. M. 1969. Ultraviolet mutation and DNA repair. Annu. Rev. Microbiol. **23**:487-514.