INDUCTION OF RADIORESISTANCE IN *ESCHERICHIA COLI*

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ABSTRACT The effect of prior treatment by inducing agents on the radioresistance of cells of *Escherichia coli* has been studied. In order to separate the induction process from the radiation-damage process, cells were first treated with inducing agents such as ultraviolet light, ionizing radiation, or nalidixic acid, allowed to become induced by incubation for 50 min and then given rifampin to prevent further induction. They were then tested for radiation sensitivity. It was found that all strains tested except *recA*$^-$, *lex*$^-$, and *recB*$^-$ showed very apparent protection. Induction by UV had the most effect and by nalidixic acid the least. The time course of development of protection was observed in one case: it is 50% established in 15 min. The absence of effect in *recA*$^-$ and *lex*$^-$ is explainable by the fact that these cells cannot be induced, for example, for prophage or the inducible inhibitor of post-irradiation DNA degradation. We suggest that the inducible inhibitor of postirradiation DNA degradation is one factor in a recovery system possessed by *E. coli* cells.

INTRODUCTION

The phenomenon of radiation induction of factors involved with cellular response to radiation has been the subject of considerable study recently. In addition to the radiation induction of prophage, induction of filament formation (Witkin, 1967), reactivation of irradiated phage (Weigle, 1953; George et al., 1974), an error-prone repair system related to mutation (Witkin and George, 1973; Witkin, 1974), and the inducible inhibition of postirradiation DNA degradation (Grady and Pollard, 1968; Pollard and Randall, 1973; Marsden et al., 1974; Pollard et al., 1974; Pollard et al., 1975) have been observed.

It is not known whether all these various responses to ultraviolet light and ionizing radiation (UV and ior[1]) are due to the induction of one kind of protein molecule or whether there are multiple phenomena. Evidence seems to favor the latter. We were led to undertake the work described here during the course of studies on the inducible inhibition of postirradiation DNA degradation. The findings on this inducible system parallel those on others and because much of the work is recent and therefore possibly unfamiliar, a short statement of the findings may be useful.

[1] An abbreviation for ionizing radiation (X-rays, gamma rays, fast electrons, accelerated ions, alpha particles, etc.) is needed. It is suggested, in view of the preemption of "ir" by infrared, that "ior" is suitable.

An early observation of relevance to the present work was made by Miletić et al. (1961). They observed that in the presence of chloramphenicol the amount of post-irradiation DNA degradation was increased, suggesting an involvement of protein synthesis. A second observation by Miletić and co-workers (1964) was to the effect that a second dose of ior produced markedly less degradation than the first. This suggests that the first dose modifies the cells against the second. The work of Grady and Pollard (1968) confirmed these findings and also showed that in two strains of *E. coli,* one having a defective prophage and one cured of that factor, the degradation process was different. In the cured strain the degradation continued longer after irradiation and was unaffected by chloramphenicol, nor did any changes in growth conditions have any effect, as observed with the uncured strain. The suggestion was made that radiation acts to cause the initiation of DNA degradation and *also* to induce a factor controlling the amount of degradation. In this work it seemed likely that the induction process involved a defective prophage, known to be present in the strain used. Later work, mentioned below, suggests that the prophage may be a factor, but it is not essential.

Using preliminary treatment with UV together with a subsequent prevention of transcription with rifampin, Pollard and Randall (1973) established the time course of the induction of the inhibition and also some of the strains in which it could be found. It appeared to be much less likely that the inhibition is due to the induction of a prophage, at least in some cases. The technique of preliminary induction by UV was then used by Marsden et al. (1974) to show that postirradiation DNA degradation of both host DNA and infecting phage DNA can be inhibited in strains which are wild type and *uvr*$^-$ but not in strains which are *recA*$^-$ and *exr*$^-$ (*lex*$^-$). The suggestion was made that the *exr* gene is involved with the inhibition. The necessity for protein synthesis was further shown by Pollard et al. (1974) using the technique of amino acid deprivation in amino acid-requiring strains, one of which contains a defective prophage. Simultaneously the observation of the induction of endolysin (concomitant with a defective prophage) and of inhibition was made. Since strains in which endolysin production was marginal still showed good inhibition it was suggested that the defective prophage is not necessary and that the inhibitor is induced independently of the prophage. This is further confirmed by work by Pollard et al. (1975) where strains containing λ-prophage and those without it are compared. The conclusion reached is that the inhibitor is a property with characteristics similar to phage induction but not the same as phage induction. In addition, it has been found that induction renders the repair of single-strand breaks more apparent (Tolun et al., 1974).

It would seem plausible, therefore, that the induction of the inhibitor of postirradiation DNA degradation should confer some resistance on the induced cells. Trgovčević and Rupp (1974) have shown that the induction of the *red* and *gam* genes in λ-prophage confers resistance on strain K12λ. However, attempts in our laboratory to demonstrate this kind of resistance in strains of cells inducible for the inhibitor of postirradiation DNA degradation proved generally equivocal. Pollard and Randall (1973) found that cells which can be induced had the property of increased resistance

if grown on rich medium, where presumably the increased number of genomes confers an increased survival potential if DNA degradation is prevented. On the other hand, Pollard et al. (1974) found very little protection in cells which had been induced by UV. In these experiments one choice of strain (15T⁻-555-7) was probably unfortunate as it carries a defective prophage which, upon induction, lyses cells. Induction will therefore kill by causing lysis. The findings with strains of B/r cannot be explained in this way. The results of this paper suggest a reason.

The phenomenon of induction of all these factors requires an inducing agent such as UV, ior, nalidixic acid, mitomycin C, or thymine starvation. It is also necessary for protein synthesis to take place after the inducing event. Pollard and Randall (1973) found that although rifampin potentiates postirradiation DNA degradation, the inhibition of such degradation was very effective if after the induction was initiated the cells were incubated for 50 min, before rifampin was given for 10 min, and the cells then challenged for degradability by ior. It seemed reasonable that if the effect of induction on sensitivity were to be studied, the use of rifampin to prevent the induction action of the second dose of radiation should make the protective action clearer. We have followed this procedure with results which are definite: in strains which are capable of radiation induction, and which also have the machinery for postirradiation DNA degradation, there is considerable protection by induction, whether it be by UV, nalidixic acid, or ior; in cells not capable of induction, namely those deficient in the *recA* and *exr* (or *lex*) genes, there is no protection at all. Nor is there significant protection in a *recB*⁻ strain, which is deficient in exonuclease V, the major enzyme involved in postirradiation DNA degradation (Emmerson, 1968; Youngs and Bernstein, 1973).

Failure to observe protection in previous work (Pollard et al., 1974) we therefore attribute to the presence of two factors, the damaging action of the ior, and the concomitant induction of the protective response. Previous induction by UV may not show much effect. Subsequent experiments have shown that previous UV irradiation followed by incubation without the use of rifampin does protect in some circumstances in some strains. One example of this is reported here. Preventing subsequent induction with rifampin makes the effect of pretreatment clearer.

MATERIALS AND METHODS

Bacterial Strains

In Table I are listed the various strains used, their relevant genotypes and their sources.

Growth Conditions

Cells were grown with aeration in Roberts' C-minimal medium (3 g NH_4Cl, 6 g Na_2HPO_4, 3 g KH_2PO_4, 3 g NaCl, 124 mg $MgCl_2 \cdot 6\ H_2O$, 80 mg Na_2SO_4 per liter) with 5 g glucose/liter and a supplement of 2 g/liter casamino acids; this medium is referred to as "casaC." At 37°C the doubling time was about 30 min. Where necessary, supplements were added: thiamine at 20 μg/ml final concentration for the AB strains, and thymine at 20 μg/ml final concentration for strains W3110 and P3478. For some experiments Brain Heart Infusion (BHI) (Difco Labora-

TABLE I

STRAINS OF CELLS USED, WITH RELEVANT INFORMATION

Strain	Relevant genotype	Growth requirements	Source
AB1157	Wild type	arg*, his*, pro*, thr*, leu*, thiamine	Dr. R. Boyce
AB2463	recA$^-$	Same	Same
AB2470	recB$^-$	Same	Same
AB2494	lex$^-$	Same	Same
W3110	polA$_1^+$	thy	Dr. D. Billen
P3478	polA$_1^-$	thy	Same
WU36-10	uvr$^+$, B/r	tyr*, leu*	Dr. E. M. Witkin (via Dr. S. Person)
WU36-10-89	uvr$^-$, B/r	tyr*, leu*	Same

* Requirements supplied by casamino acid supplement.

tories, Detroit, Mich.) at 37 g/liter was used; the doubling time approximated 16 min in this medium.

Irradiation Methods

The UV source was a G15T8 germicidal light (Sylvania) wrapped with insulation tape to diminish the yield when necessary. Three such bulbs were used, giving dose rates of 9.6, 6.8, and 0.5 ergs/mm^2-s as measured by a "Jagger" meter (Jagger, 1961) and calibrated against a Bureau of Standards standard lamp by Dr. R. A. Deering of these laboratories.

Three means for delivering ior were used. The first is a single rod, containing ^{60}Co, surrounded by a sample holder suitable for test tubes. The dose rate at the distance used was 2 krads/min, measured by ferrous sulfate dosimetry. The second is a ^{60}Co Gammacell 200 (Atomic Energy of Canada, Ltd., Ottawa, Canada) delivering 6.5 krad/min, as determined by ferrous sulfate dosimetry. The third is a Picker 50 kV X-ray machine (Picker Corp., Cleveland, Ohio), calibrated by exposing the same culture to the X-rays in a small petri dish and on the same occasion to the Gammacell. The X-ray exposure at 4 cm from the target gave the same effect as 7.8 krads/min in the Gammacell. The cells were either equilibrated with air or bubbled with oxygen during irradiation.

Cultures were grown to the required number of cells per milliliter, usually about 2×10^8, and chilled on ice. The pretreatment was then given to the chilled cells. In the case of cells grown on casaC the absorption of the UV light was insufficient to require correction. Cells grown in BHI were centrifuged and resuspended in unsupplemented C-minimal medium. They were then resuspended in the appropriate medium after treatment. After the induction treatment the cells were bubbled with air at 37°C for the required time (usually 50 min), given

rifampin at 50–100 μg/ml final concentration, incubated with bubbling for 10 min and then irradiated. In the experiments where the inducing event was ior and the irradiation itself was UV, the rifampin could not be added to the irradiation dish since it strongly absorbs UV. It was added, at 15 μg/ml, to the first dilution tubes in the plating process. More than 10 min was allowed to elapse before further dilution. In all experiments involving UV at all the work was carried out in yellow light.

Inducing Procedure

The appropriate dose for induction varies considerably with the strain and the agent. Previous studies (Pollard and Randall, 1973; Pollard and Keller, submitted for publication) have shown that for UV induction there is a sharp maximum, which is around 150 ergs/mm^2 for wild-type strains and around 20–40 ergs/mm^2 for *uvr* and *polA* strains. The inducing dose was varied to fit the strain.

For ior there is also a sharp rise in induction with dose, but the maximum is not nearly so pronounced. Doses between 4 and 12 krads were used, the lower doses for the *uvr*$^-$ cells and the higher for the wild type. Nalidixic acid was used at final concentrations of 50 μg/ml for wild type and 15 μg/ml for *uvr*$^-$. The cells were exposed to nalidixic acid for 5 min at 37°C and then centrifuged and resuspended in the same medium. Unpublished work in this laboratory by Kathleen Keller shows that nalidixic acid readily induces the inhibitor of postirradiation DNA degradation.

Plating Technique

The dilution blanks were of C-minimal salts, with no carbon source. They were chilled to refrigerator temperature (7° C). On occasion sterile distilled water was used, but with this fluid the plating results proved to be more liable to fluctuate; in particular the unirradiated samples showed more variability.

½ ml samples of the appropriate dilution were pipetted onto the bottom of fresh sterile plastic petri dishes, and 12 ml of nutrient agar at 50° C was poured onto the sample, gently swirled, and left to harden. The 12 ml was secured with a tip-bottle automatic dispenser (California Laboratory Equipment Co., St. Emeryville, Calif.). The great majority of the colonies appear as small spheroids of about ½ mm diameter in the body of the agar. There are a few on the top which appear as normal surface colonies and a few in the air space on the bottom which have a pale appearance. These show the familiar tendency to run and give multiple colonies. The usual procedure was to ignore the bottom colonies (about 1%). By counting sectors of known area ratio, consistent counts of as many as 5,000/plate can be made. Normally each irradiation sample was plated at three dilutions, covering a range of 100. Duplicate plates were included at the dilution expected to give about 300 colonies per plate. The technique has been briefly described by Pollard and Randall (1973).

RESULTS

Fig. 1 shows the results obtained with strain AB1157. The ratio of the colony-forming ability (CFA) to that of the unirradiated sample is plotted for the three situations: no predose of UV, no rifampin; no predose but with 100 μg/ml rifampin for 10 min before exposure to gamma radiation and a predose of 200 ergs/mm^2 followed by incubation for 60 min at 37° C, 100 μg/ml rifampin for 10 min and then gamma irradiation.

It can be seen that rifampin sensitizes the cells considerably. This sensitization is removed completely by the predose of UV followed by incubation and a resistant be-

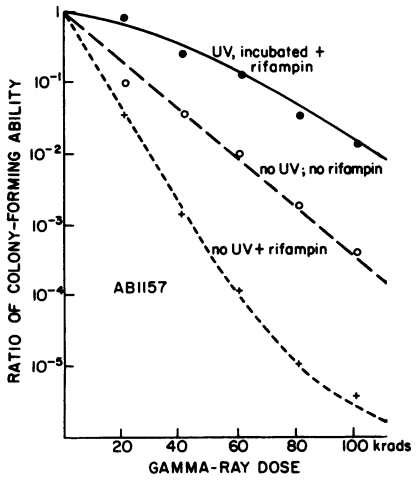

FIGURE 1

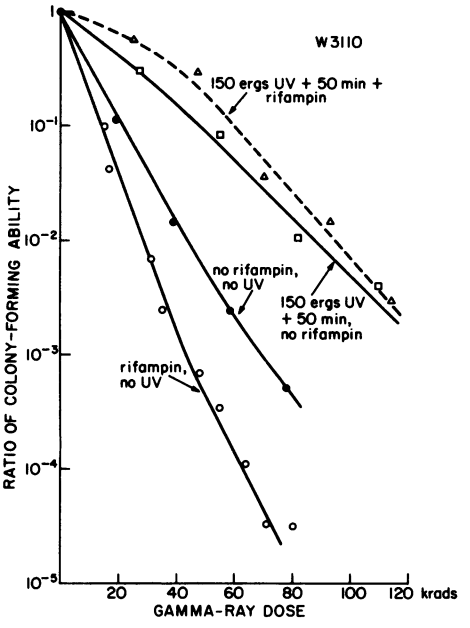

FIGURE 2

FIGURE 1 Colony-forming ability (CFA) remaining after various gamma-ray doses under the conditions shown. Incubation is for 60 min at 37°C and rifampin at 100 μg/ml is given 10 min before gamma rays. The predose of UV was 200 ergs/mm^2. Induction by UV clearly provides protection.

FIGURE 2 Colony-forming ability for strain W3110 under the conditions shown. The inducing dose of UV (150 ergs/mm^2) causes protection even in the absence of rifampin, but the effect is greater if rifampin is used.

havior with a shoulder and a less negative slope appears. AB1157 shows inhibited postirradiation DNA degradation which is increased by rifampin treatment and decreased by predoses of UV (Pollard et al., 1975).

In Fig. 2 we show similar data for strain W3110. It can be seen that in the absence of rifampin the inducing dose produces some protection. In the presence of rifampin the change introduced is more striking because the transition is from more sensitive to even more resistant. W3110 shows inhibited postirradiation DNA degradation increased by rifampin treatment and decreased by predoses of UV (Pollard and Randall, 1973).

Since the concept of these studies is that an inducible factor confers protection, it is of interest to observe the time course of development of protection. At zero time after induction there should be no protection. In Fig. 3 we show the results of giving a uniform predose of 150 ergs/mm^2 of UV to cells of W3110 and allowing different incubation times before adding rifampin prior to irradiation with the doses shown. It can be seen that if rifampin is added immediately after giving UV, or 5 min after, there is no protection, but rather a slight increase in sensitivity, which may not be statistically significant. By 15 min there is marked development of protection and at 35 min the

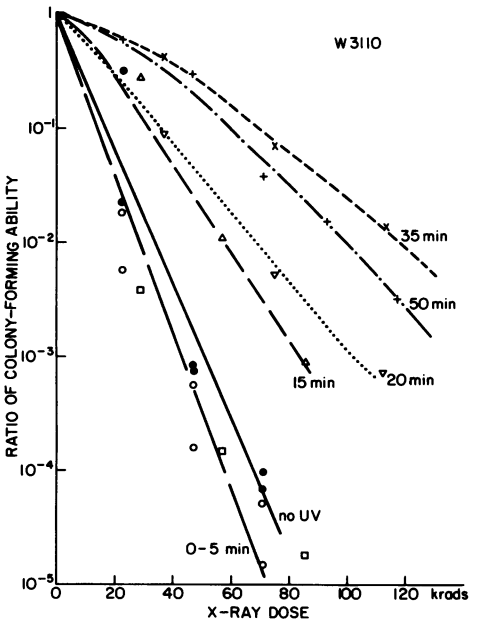

FIGURE 3

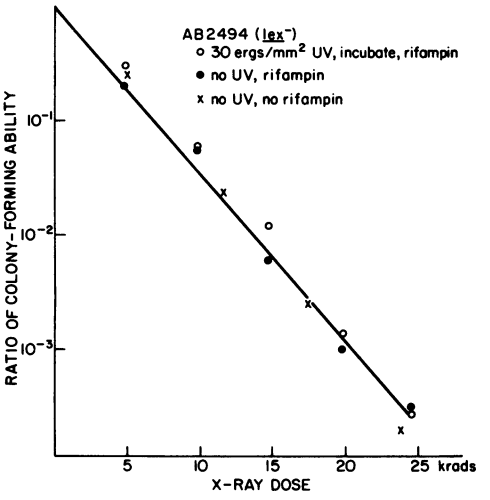

FIGURE 4

FIGURE 3 The time course of development of protection after the inducing dose of 150 ergs/mm^2 of UV. At 5 min there is still a slight sensitization, but by 15 min the protection is present and it is complete between 35 and 50 min.

FIGURE 4 CFA plots for AB2494 (*lex*$^-$), a strain that is not inducible. There is no protection.

cells are fully protected. The slight drop in protection at 50 min we attribute to the outgrowth of the fraction of uninduced cells which are not protected. The time at which there is 50% expression of the change in sensitivity is 15 min, which compares with 14 min found for 50% expression of degradation inhibition by Pollard et al. (1974).

Results with five mutant strains are shown in Figs. 4–8. Fig. 4 refers to AB2494 (*lex*$^-$). There is no sign of protection; no induction can occur. Postirradiation DNA degradation is extensive; no inducible inhibition is apparent (Pollard et al., 1975). Fig. 5 refers to AB2470 (*recB*$^-$). This cell lacks exonuclease V. Pollard and Keller (submitted) have shown that there is very little postirradiation DNA degradation and that pretreatment with UV gives no inhibition. There is a very slight protection in these experiments, possibly statistically significant. The data points are for two experiments performed 2 mo apart. Fig. 6 refers to AB2463 (*recA*$^-$). For these three mutant strains, in contrast to other strains, there was no difference in X-ray survival whether rifampin was used or not. Here for the *recA*$^-$ strain there is a slight potentiation by the predose of UV. Ultraviolet light alone produces considerable DNA degradation. Possibly the potentiation is related to this fact. Postirradiation DNA degradation has been observed to be extensive; no inducible inhibition is apparent (Pollard et al., 1975).

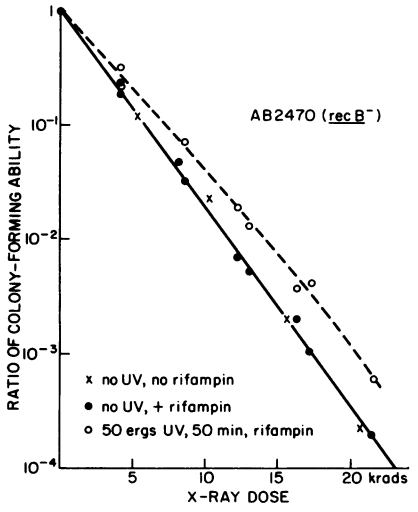

FIGURE 5

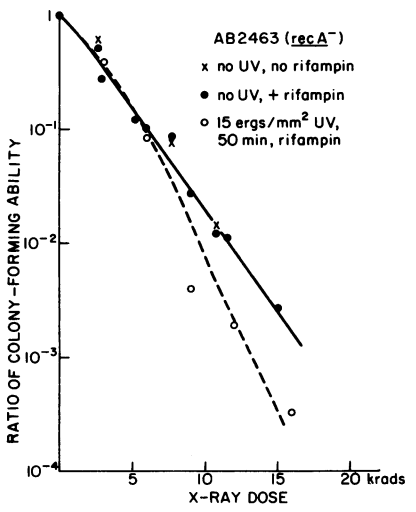

FIGURE 6

FIGURE 5 CFA plots for strain AB2470 (*recB*⁻). This strain lacks exonuclease V but is nevertheless inducible for prophage if it is present. There is slight protection after giving 50 ergs/mm^2 UV, suggesting that while the majority of the inducible effect acts on the exonuclease V, some additional factor is also induced.

FIGURE 6 CFA plots for strain AB2463 (*recA*⁻). This strain is not inducible. It shows no protection. There may, indeed, be some increased sensitivity.

Fig. 7 refers to P3478 (*polA*⁻). It can be seen that there is protection. This strain is of interest in that the dose of UV to induce is low and if larger doses are given the observed protection is reduced. This strain shows extensive postirradiation DNA degradation which, however, is reduced by pretreatment with UV.

Fig. 8 shows the effect on two strains of B/r, WU36-10 (*uvr*⁺) and WU36-10-89 (*uvr*⁻). Both strains show protection. The inducing dose of UV is very different in the two cases as can be seen from the figure; it is only 15 ergs/mm^2 for the *uvr*⁻ cell. Both these strains show post-irradiation DNA degradation which is modified by preirradiation with UV (Pollard, unpublished observations).

In Fig. 9 we show that the induction process does not necessarily protect only against ior damage. Here cells of strain WU36-10 (*uvr*⁺) were given 7 krads of preirradiation in the cobalt source, allowed to incubate at 37° C for 60 min and then irradiated for the UV doses indicated. They were then diluted into C-minimal salts containing 15 μg/ml of rifampin. They were held in this condition for 10 min and then further diluted and plated. The control received no gamma radiation and was treated the same. It is quite apparent that there is protection by the predose. The same was found for the *uvr*⁻ strain with about one-half the degree of protection.

In Fig. 10 the effect of preliminary induction with nalidixic acid is shown. The strains are WU36-10 (*uvr*⁺) and WU36-10-89 (*uvr*⁻). Nalidixic acid was given for 5 min at 50 μg/ml for the *uvr*⁺ and 15 μg/ml for the *uvr*⁻. These latter cells are sensitive

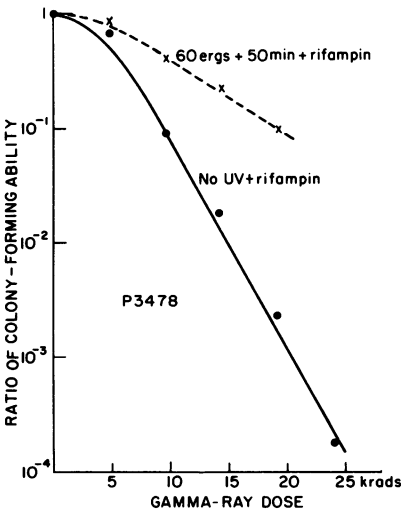

FIGURE 7

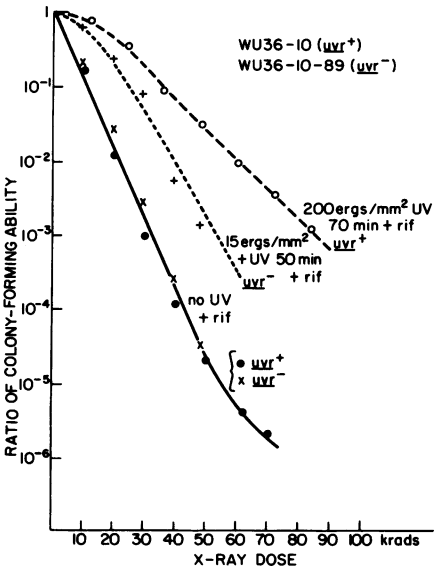

FIGURE 8

FIGURE 7 CFA plots for P3478 (*polA*$^-$). There is clear protection at the low dose of 60 ergs/mm^2 UV used.

FIGURE 8 CFA plots for two strains of B/r, WU36-10 which is *uvr*$^+$ and WU36-10-89 which is *uvr*$^-$. Protection is observed in both cases. The inducing dose in the *uvr*$^-$ is quite low.

to nalidixic acid: 50 μg/ml caused a factor of 10 killing; 15 μg/ml caused no loss. It can be seen that the induction process has caused protection. Because the complete dose response curve for nalidixic acid induction has not yet been studied, the fact that *uvr*$^+$ cells show more protection should not be overly stressed.

To be able to tabulate the data, since more than 50 survival of colony-forming ability curves have been taken, we need some formula which has parameters descriptive of the results. One such has been proposed by Green and Burki (1974). In the form useful to us this can be written:

$$\text{Fraction surviving}, f = \frac{\rho + 1}{\rho + e^{D/D_1}},$$

where D is the dose in krads, D_1 a sensitivity parameter—the smaller D_1 is the more sensitive the cell strain—and ρ is a parameter that measures the ability of the cell strain to recover after receiving radiation. A positive value of ρ indicates the presence of a recovery factor; negative values of ρ suggest that the strain may actually deteriorate in place of recovering after radiation, as could occur, for example, if DNA degradation were initiated by radiation and caused cell death before plating.

This formula fits the data reasonably well and it has only two parameters. Accordingly, we have tabulated the values of D_1 and ρ for the various strains and conditions.

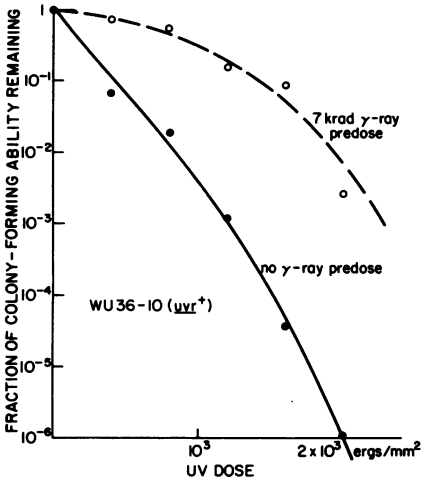

FIGURE 9

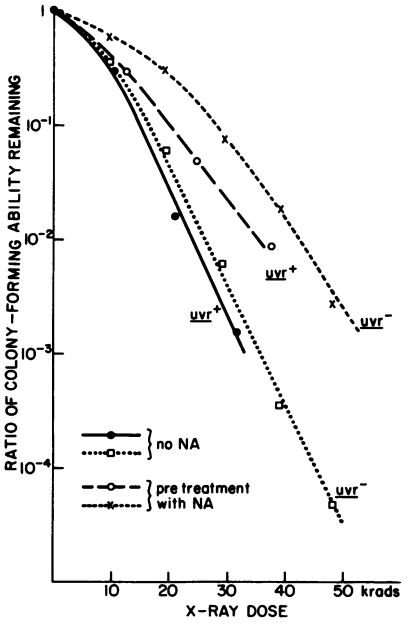

FIGURE 10

FIGURE 9 The effect of inducing with ior. CFA plots are shown for WU36-10 for no gamma ray predose and with 7 krads predose and incubation in the normal way. The agent causing the reduction of CFA in this case is UV. Rifampin was given briefly just after UV. There is good protection.

FIGURE 10 Protection by induction with nalidixic acid (NA). CFA plots are shown for the *uvr*$^+$ and *uvr*$^-$ strains. For the former 50 μg/ml NA was given for 5 min to induce; for the latter 15 μg/ml were used. Incubation and 10-min exposure to rifampin were as usual. Protection is seen in both cases.

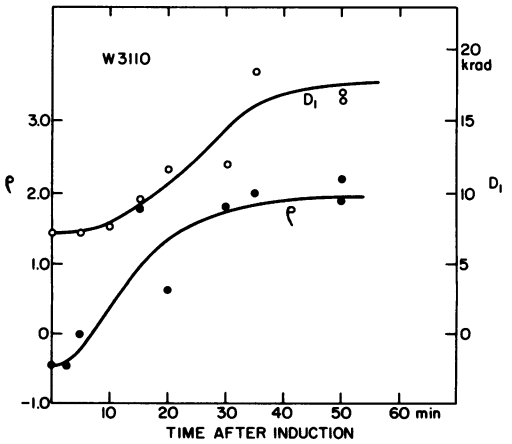

FIGURE 11 The values of the two parameters of the Green-Burki (1974) formula plotted as a function of the time after induction. There is an increase in both the sensitivity factor which goes from 7 to 18 krads and also in the recovery factor which goes from a slightly negative value to a positive value of about 2.0. The time for the recovery factor to reach 50% of its final value is 15 min; for the sensitivity it is later, 25 min.

TABLE II

PARAMETERS D_1 AND ρ*

Strain	Inducing agent	Incubation time	Rifampin added	D_1	ρ
		min		*krads*	
W3110	None	None	None	9.0	0.0
	None	None	Yes	6.0	0.0
	150 ergs UV	50	None	18.0	0.5
	150 ergs UV	50	Yes	16.6	2.2
	None	None	Yes	6.0	0.0
	None	None	Yes	6.0	0.4
	160 ergs UV	50	Yes	18.0	6.0
	10 krads γ-ray	50	Yes	14.4	6.0
	None	None	Yes	7.2	0.0
	None	None	Yes	7.5	0.0
P3478	None	None	Yes	2.3	4.2
polA$^-$	60 ergs UV	50	Yes	6.0	2.2
AB1157	None	None	None	12.0	0.0
	None	None	Yes	5.5	0.0
	200 ergs UV	50	Yes	20.0	2.3
AB2463	None	None	None	2.6	0.6
recA$^-$	None	None	Yes	2.6	0.6
	15 ergs UV	50	Yes	1.8	1.2
AB2494	None	None	None	2.8	0.0
lex$^-$	None	None	Yes	2.8	0.0
	30 ergs UV	50	Yes	2.8	0.0
AB2470	None	None	None	2.4	0.0
recB$^-$	None	None	Yes	2.4	0.0
	50 ergs UV	50	Yes	2.4	0.9
WU36-10	None	None	Yes	4.5	0.0
uvr$^+$	150 ergs UV	50	Yes	10.0	3.5
	10 krads γ-ray	50	Yes	6.5	0.0
	NA	50	Yes	6.2	1.5
	No NA	None	Yes	3.8	1.8
BHI-grown	None	None	Yes	15.0	0.0
	150 ergs UV	50	Yes	26.0	4.9
WU36-10-89	None	None	Yes	4.6	1.8
uvr$^-$	15 ergs UV	50	Yes	5.2	4.0
	NA	50	Yes	7.1	2.5
BHI-grown	None	None	Yes	6.0	0.0
	20 ergs UV	50	Yes	13.0	2.1

NA, nalidixic acid; BHI, Brain Heart Infusion (Difco).

*From applying the Green-Burki (1974) formula to survival curves under the conditions stated. Note that the main objective in this work was to compare different conditions. Thus the absolute values of the parameters should not be used in precise interpretation. In several cases duplicate experiments are included, and the values obtained give an idea of the consistency to be expected.

In the case of the three experiments shown in Fig. 1, the no-pretreatment case gives D_1 as 12 krads and $\rho = 0$; with rifampin and no UV D_1 becomes 5.5 krads and ρ is again 0; with pre-UV and rifampin D_1 rises to 17.5 krads and $\rho = 3.5$ indicating positive recovery. The curve for the upper line in Fig. 1 follows the formula for these values.

The variation of the values of these two parameters as the time of incubation after UV varies is shown in Fig. 11. The data were taken on W3110 as described in Fig. 3. Fig. 11 includes additional data. It can be seen that both D_1 and ρ increase as the incubation time increases, up to a maximum. There is a little difference in the time course, which may be significant. D_1 increases somewhat later than does ρ. Considerable further data is given in compact form in Table II. It can be seen that predoses of ior protect against ior itself. Also there is an interesting modification of the behavior of the cells in BHI. Whereas in casaC the two strains of WU36-10, *uvr*$^+$ and *uvr*$^-$, are of nearly equal X-ray sensitivity, the situation is drastically changed in BHI. Here the *uvr*$^+$ cell shows much more resistance, whereas the *uvr*$^-$ strain is almost unchanged. After induction the *uvr*$^+$ is still less sensitive and has a large recovery factor, the largest recorded. Another interesting finding is that the noninducible strains do not show a sensitization in the presence of rifampin.

DISCUSSION

The work reported here bears a clear relation to the more specialized observations of Trgovčević and Rupp (1974). Working with a temperature-inducible lysogen of K-12 they found that a short exposure to heat caused partial induction which did not greatly affect the cell survival. The partly induced cells were then challenged with X-irradiation and resistance was shown to develop, Trgovčević and Rupp were able to give a clear demonstration that the λ_{red} gene is a factor in overcoming the radiation sensitivity introduced by the *recB*$^-$ mutation of the cells. They also showed that the λ_{gam} gene, an inhibitor of exonuclease V, is a factor in conferring radiation resistance on the wild-type cells.

Our work involves the induction of genes which are not on the λ-genome and the cells are not at risk due to the induction of a lytic phage. Nevertheless the conclusion is very similar: the induction of a factor or factors which influence the general cellular response to radiation also influences the survival of cells when irradiated. The fact that the resistance shows more clearly when cells are treated with rifampin is most probably due to two major processes. The first is that rifampin is a potentiator of DNA degradation (Pollard and Weller, 1969) and so causes increased sensitivity in those cells where DNA degradation is a factor in reducing cell survival. The second is that the reduction of cellular protein synthesis due to rifampin will reduce the amount of enzymatic repair protein in the cell and thus put the cell at a disadvantage if competition between degradation processes and repair processes is a factor in survival.

The experimental findings suggest that the inhibition of the exonuclease V activity in rifampin-treated cells does confer survival value; thus the inducible inhibitor is one more system in the whole complex of postirradiation recovery.

It is of interest that in both these experiments and those of Trgovčević and Rupp, strains that presumably use the exonuclease V system of enzymes to advantage (because they have better survival when exposed to X-rays than do cells which are *recB*$^-$ and lack the action of exonuclease V) can nevertheless be protected by inhibiting the action of exonuclease V. The explanation that appeals to use is that in the normal

functioning of the recombination system, which confers survival value, it is necessary to remove some DNA by degradation and also to stop that process, presumably by inhibition. Under the conditions of our work the rifampin prevents the induction of inhibition and sensitizes the cells. Preinduction of the inhibition process provides the recombination system with the needed inhibitor. It then functions to increase radioresistance.

It is also likely that the event of preinduction causes more alterations than simply the presence of the inhibitor of exonuclease V. This is supported by the observation of protection against UV by gamma-ray induction of the cells. UV-produced lesions do not seem to invoke the same extent of DNA degradation as ior and it might be supposed that post-UV DNA degradation is not a great factor in cell killing; nevertheless protection is seen.

As a pro tem unifying hypothesis the following is offered. Induction of cells that are not *recA* or *lex* minus by UV, ior or nalidixic acid causes the added synthesis of RNA and proteins which are part of a radiation recovery system. One of these proteins has been termed the inducible inhibitor of postirradiation DNA degradation. These proteins act to give the repair systems in the cell more time to restore an intact genome before the cell has become unbalanced and so they confer protection. At the same time, as in the experiments of Trgovčević and Rupp (1974), the agent causing induction is damaging to the cell and therefore the protection may be found only under conditions where the radiation-induced molecules are effective and the induction produces minimal killing.

Growth on rich medium increases the amount of DNA in the cell (Cooper and Helmstetter, 1968; Kubitschek and Freedman, 1971). This gives the cell more genetic material to use in recombination and also more inducible genes from which to synthesize the new protective proteins. Thus, so long as the inducing dose is kept small, there should be maximal protection in cells grown under rapid growth conditions. Results shown in Table II support this idea.

During the course of writing up this work we became aware of the work of Kendric C. Smith and K. G. Martignoni. In their experiments the agent used to confer protection is X-irradiation and what we consider to be the "challenge dose" is either UV or X-irradiation. While there are differences in specifics, the general conclusion that protection can be conferred is also shown in their work which was done independently of ours.

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