EVIDENCE FOR TWO MECHANISMS OF PHOTOREACTIVATION IN *ESCHERICHIA COLI* B

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ABSTRACT *Escherichia coli* B phr⁻, which is not photoreactivable under certain conditions, has been shown to exhibit photoreactivation of killing in the logarithmic growth phase at 3341 A. Dependence of the reaction upon (*a*) wavelength, (*b*) dose, and (*c*) dose rate of the reactivating radiation, as well as upon (*d*) temperature during reactivation treatment, is very similar to that of photoprotection. We conclude that this photoreactivation is similar in mechanism to photoprotection, believed to be an indirect repair process, the initial step of which is non-enzymatic and leads to a growth-division delay. We therefore call the present phenomenon "indirect photoreactivation." Similar studies suggest that indirect photoreactivation of killing occurs also in the parent strain, *E. coli* B (Harm). It has often been supposed that all photoreactivation results from a photoenzymatic reaction similar to that found to operate *in vitro* on transforming DNA. Our data provide the first evidence for two distinct types of photoreactivation of cell killing, one of which appears not to involve photoenzymes. These experiments also show that photoprotection results from intracellular events that can be induced by treatment after, as well as before, far ultraviolet irradiation.

INTRODUCTION

Survival after far ultraviolet (UV) irradiation (2300-3000 A) can be increased greatly in most cells by a *post-treatment* with near ultraviolet (3000-3800 A) or violet and blue (3800-5000 A) radiation. This phenomenon has been called "photoreactivation" (see Jagger, 1958, and Rupert, 1964, for reviews). A similar effect is obtained with some cells by a *pretreatment* with near ultraviolet radiation only. This has been called "photoprotection" (see Jagger, 1964, for review). Action spectra for these effects are shown in Fig. 1.

A variety of evidence suggests that photoreactivation in *E. coli* B is primarily a direct effect, involving an enzymatic reaction that occurs close in time and space to photon absorption. Photoprotection in *E. coli* B, however, appears to be an indirect effect, in which an initial photochemical reaction, probably not involving

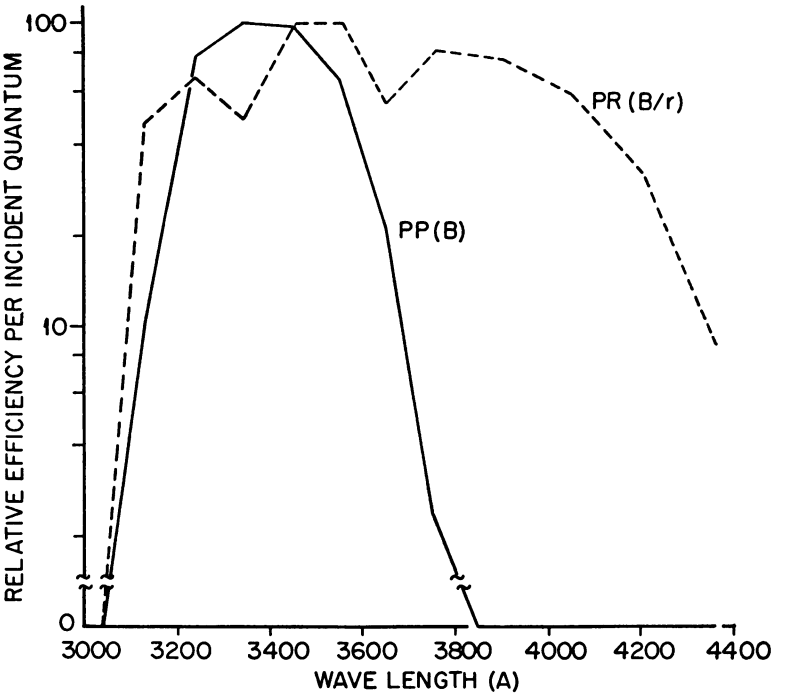

FIGURE 1 Action spectra for photoprotection from killing in *E. coli* B (solid line) and for photoreactivation of killing in *E. coli* B/r (broken line). (Jagger and Stafford, 1962; the broken line is from data of Jagger and Latarjet, 1956).

enzymes, begins a chain of events (some of which may be enzymatic) that ultimately lead to repair of the UV damage. These presumed differences in mechanism are reflected in the observations that the amount of photoreactivation per unit dose of reactivating radiation is strongly dependent upon temperature and radiation dose rate during photoreactivation treatment, while the amount of photoprotection per unit dose of protecting radiation is almost independent of temperature and dose rate during the photoprotection treatment.

It has been suggested (Jagger *et al.*, 1964) that the essential step in the chain of events following photoprotection treatment is the induction of a growth-division delay that occurs after the cells have been plated. This delay presumably permits more time for intracellular recovery processes to take place. If this postulate is correct, it should not matter whether the treatment leading to growth-division delay is applied just before or just after UV irradiation. Thus, in photoprotectable cells, some of the increased survival caused by a post-UV treatment with near ultraviolet radiation could be a result, not of the expected direct and enzymatic reaction of photoreactivation, but rather of the indirect and initially non-enzymatic reaction involved in photoprotection.

Since most strains of *E. coli* show the usual direct photoreactivation, an indirect component might be difficult to detect. Recently, however, Harm and Hillebrandt (1962) isolated a strain, B phr⁻, that shows no photoreactivation of killing of stationary cells upon post-UV illumination with white fluorescent light (which contains little near ultraviolet). It might be possible with this organism to obtain a recovery by post-UV illumination in the near ultraviolet that would be similar in mechanism to the reaction involved in photoprotection. We find such a recovery in this mutant, and present evidence for its existence also in the parent strain.

To avoid unwieldy terminology, we shall in this paper refer to irradiation at 3341 and 4047 A as "illumination," even though 3341 A radiation is not visible light.

EXPERIMENTAL PROCEDURES

E. coli B phr⁻ and the parent strain *E. coli* B (Harm) were obtained from Dr. Walter Harm, University of Cologne. The cells were grown overnight in Difco nutrient broth with shaking at 36°C. They were then diluted 1:100 with fresh medium and grown for 2 hours to produce exponential cultures. These cultures were then spun down and resuspended in M/15 sodium-potassium phosphate buffer (pH 6.8), which was used for all later dilutions. After treatment, the cells were diluted, plated on Difco Nutrient Agar, and incubated overnight at 36°C.

After beginning these experiments, it was found that if these exponential cells were starved in phosphate buffer for 1 hour at 36°C, they showed a lower "liquid-holding recovery" [recovery of irradiated cells by a postirradiation holding in liquid (Roberts and Aldous, 1949)]. Because this recovery was considered an undesired complication, roughly the last one-third of the experiments was done with such starved cells. That these were still essentially log phase cells was indicated by their UV sensitivity and their multiplication upon introduction of nutrient. Their response to illumination treatments was not distinguishable from that of unstarved cells.

Cells were inactivated by exposure to far UV (2537 A) radiation from two General Electric 15-watt germicidal lamps. During irradiation, the cells were in phosphate buffer in depression spot plates at room temperature (23°C) and at a concentration of 10^5 cells ml^{-1}. The dose rate was about 10 erg mm^{-2} $sec.^{-1}$, and the time required for inactivation to 1 per cent survival was about 20 seconds.

Cells were "illuminated" by exposure to radiation (3341 and 4047 A) from a Hilger crystal quartz monochromator (Perry, 1932), using Philips 500-watt (SP-500) or 1000-watt (SP-1000) high pressure, water-cooled mercury arc lamps. Band widths never exceeded 200 A and were usually 100 A. Dose rates were measured with a calibrated thermopile and DC breaker amplifier, and varied from 42 to 1700 erg mm^{-2} $sec.^{-1}$. These dose rates were confirmed by potassium ferrioxalate actinometry (Hatchard and Parker, 1956). The highest dose rate (1700 erg mm^{-2}) was obtained with a continuous beam. Lower dose rates were obtained by placing in the beam a rotating sector that produced light flashes at intervals of 0.05 sec. Dark periods therefore had a maximum duration of 0.05 sec., which is 1/700 of the time constant estimated by Bowen (1953) for the dark reaction in photoreactivation of phage in *E. coli* at 37°C (this time constant is even greater at room temperature). Subsequent experiments using only continuous light have confirmed the results obtained with the rotating sector. Cells were illuminated at 10^5

or 10^6 cells ml^{-1} in phosphate buffer in rectangular Corex cuvettes (optical path 1 cm) and were stirred magnetically.

"Illuminations" (3341 and 4047 A) were done at room temperature (23°C) except in the temperature studies, in which they were done at 5 and 32°C. The cells were cooled slowly (about 5 minutes) from room temperature to 5°C, since cooling shocks can be very damaging to log phase *E. coli* B (Meynell, 1958). Temperatures were maintained by a copper container that fitted the irradiation cuvette closely on three sides and that could be filled with warm water or ice water. To avoid undesired photoreactivation, experiments were performed in a room illuminated by General Electric "gold" fluorescent lamps, which emit light only above 5000 A.

Growth delays were measured by continuous turbidimetry in a Bonet-Maury biospectrophotometer (Maison Jouan, Boulevard St. Germain, Paris) using white light and 30-mm cuvettes. Cultures were started in the log phase, at about 3×10^7 cells ml^{-1}, and were followed into stationary phase.

RESULTS

Pre-UV and Post-UV Illumination at Different Wavelengths. Fig. 2 shows the effects of pre-UV illumination on survival of strain B phr⁻ and its parent strain

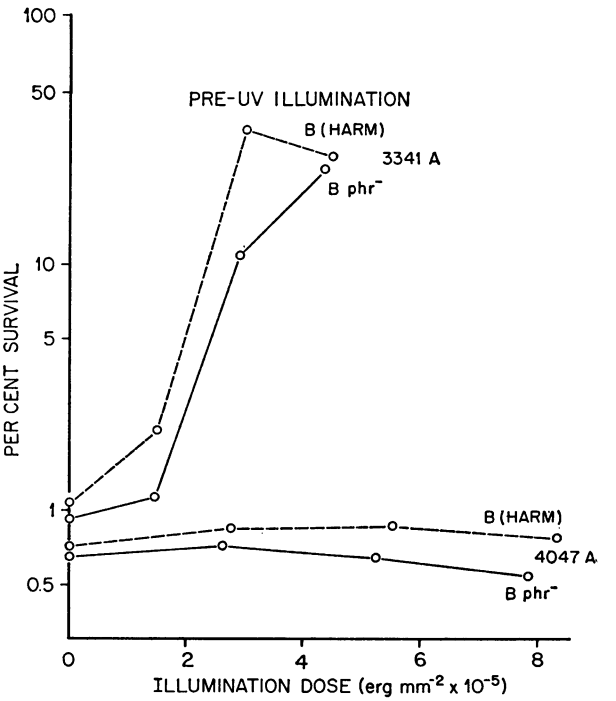

FIGURE 2 Effect of pre-UV illumination at 3341 and 4047 A on survival of *E. coli* B (Harm) (broken lines) and *E. coli* B phr⁻ (solid lines) irradiated with UV (2537 A) to about 1 per cent dark survival. Per cent survival on a logarithmic scale is plotted against illumination dose. Dose rate of illumination was 160 erg mm^{-2} $sec.^{-1}$. Data are from a typical single experiment.

B (Harm). These data show that the efficiency of photoprotection in the phr⁻ strain is almost as high as in the parent, and also that the wavelength dependence for photoprotection in both strains must be roughly similar to that reported for *E. coli* B, namely a large effect at 3341 A and no effect at 4047 A (Fig. 1).

Fig. 3 shows the effects of post-UV illumination on survival of the two strains.

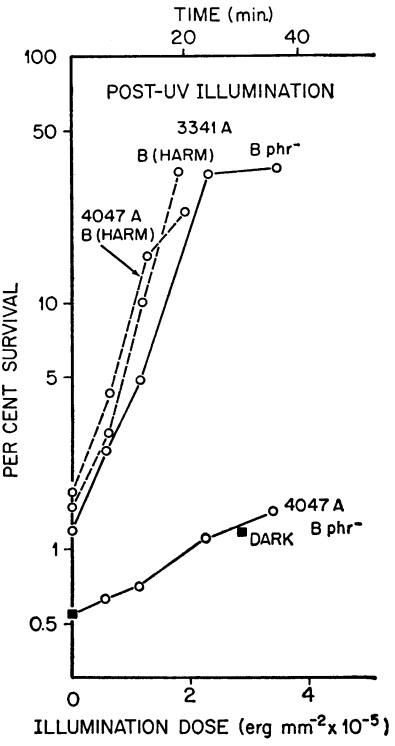

FIGURE 3 Effect of post-UV illumination. The square symbols represent liquid-holding recovery in the dark over the time (upper abscissa) required to treat B phr⁻ at 4047 A. Otherwise similar to Fig. 2.

The illumination dose rate was kept low (160 erg mm^{-2} $sec.^{-1}$) to avoid saturation of any limiting enzymatic reaction. The phr⁻ strain shows a large post-UV illumination recovery at 3341 A. Thus, the question arises of whether this should be considered a "nonphotoreactivable" mutant.

Fig. 3 also shows that 4047 and 3341 A produce roughly similar responses in the parent strain, in accord with the action spectrum for photoreactivation in *E. coli* B/r (Fig. 1). The mutant strain, however, shows a large response only at 3341 A. Many experiments show that the small effect at 4047 A can be completely accounted for by liquid-holding recovery (see Experimental Procedures). Thus, the wavelength dependence for post-UV illumination recovery in the mutant is roughly similar to that for photoprotection and altogether dissimilar to that for photoreactivation in other *E. coli* strains.

It was not possible to eliminate liquid-holding recovery by increasing dose rate (and thus shortening time of exposure) or by decreasing temperature, because these alterations also decrease enzymatic photoreactivation. We did find, however, that holding recovery was lower in starved cells (see Experimental Procedures), and these were used in later experiments. Since, in stationary phase *E. coli* B, photoreactivation treatment operates on the same damage as liquid-holding treatment (Castellani *et al.*, 1964), the curves in Fig. 3 showing strong illumination recovery probably involve only a very small component due to holding recovery, for the number of lesions available for holding recovery would decrease rapidly during photoreactivation.

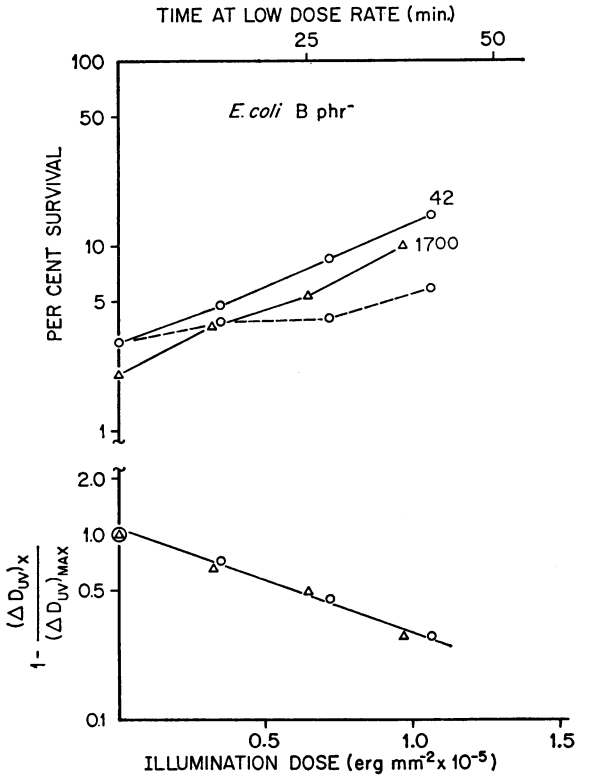

FIGURE 4 Effect of dose rate at 3341 A on post-UV illumination recovery in *E. coli* B phr⁻. *Upper graph* shows per cent survival on a logarithmic scale *versus* illumination dose on lower abscissa. Numbers on curves are dose rates in erg mm^{-2} $sec.^{-1}$. Broken line shows liquid-holding recovery during the longer exposure (lower dose rate) and relates to upper abscissa. Data from a typical single experiment. *Lower graph* shows the fraction of the reversible part of the UV dose remaining unreversed (see text), on a logarithmic scale, *versus* illumination dose. Points are derived from the curves on upper graph (triangles, high dose rate; circles, low dose rate). Slope of curve on lower graph is a measure of recovery efficiency.

A variety of experiments at different survival levels demonstrate that the recovery obtained with pre-UV illumination of the mutant shows roughly the same dose response as the recovery obtained with post-UV illumination, except that pre-treated cells show evidence of a slight threshold (low recovery at low illumination dose) not apparent in post-treated cells (*e.g.*, compare Figs. 2 and 3 at 3341 A).

In summary, the data of Figs. 2 and 3 suggest that the post-UV illumination recovery in the mutant might be largely accounted for by a reaction similar to that operating in photoprotection. "Photoreactivation" in this strain is therefore probably an indirect effect.

Differences in response of the parent strain, B (Harm), to pre-UV and post-UV illumination at 3341 A are essentially the same as observed for B phr⁻. This suggests that a large part of the "photoreactivation" at 3341 A in the parent strain might also be similar to the process involved in photoprotection.

Why do we observe in the mutant a large post-UV illumination effect that was not observed by Harm and Hillebrandt? They used a white fluorescent lamp, with presumably very low emission below 3650 A, and which would therefore be quite ineffective in photoprotection or in the post-UV illumination recovery of the mutant. These authors state, however, that they found photoprotection in the mutant with the same dose and source that yielded no post-UV illumination recovery. The explanation probably lies in differences in the metabolic state of the cells, since (*a*) they gave post-UV illumination to cells on agar, while we used cells in liquid, (*b*) their illumination times (90 minutes) were considerably longer than ours (about 30 minutes), and (*c*) they used stationary cells, which we have found show a very low post-UV illumination recovery.

Dose Rate Dependence. Attempts were made to study the nature of the post-UV illumination recovery by examining the effect of dose rate of the illumination on the amount of recovery per unit dose. Fig. 4 shows the course of recovery in the mutant strain at two extreme dose rates at 3341 A.

A measure of the amount of recovery per unit illumination dose (recovery efficiency) can be obtained from a plot of the logarithm of

$$1 - \frac{(\Delta D_{uv})_x}{(\Delta D_{uv})_{max}} \qquad (1)$$

where $(\Delta D_{uv})_x$ is the amount by which the UV dose is effectively reduced by an illumination dose x, and $(\Delta D_{uv})_{max}$ is the amount by which the UV dose is effectively reduced by an illumination dose that gives maximum recovery. The fraction in this expression is the fraction of the entire reversible part of the UV dose that is reversed with illumination dose x. Values of $(\Delta D_{uv})_{max}$ were estimated from survival curves and data similar to those shown in Figs. 2 and 3. They were found to correspond to dose-reduction factors (see Jagger, 1958) of 0.11 ± 0.03 for either pretreatments or post-treatments of either strain. Plots of Equation 1 are shown at

the bottom of Fig. 4. The negative of the slope of such a curve is a measure of the recovery efficiency.

It may be noted that the method used here for estimation of the reaction rate (Equation 1) is not the usual one (see Jagger, 1958). Actually, however, there is probably better theoretical justification for the use of this method, which we hope to discuss in detail elsewhere. In the meantime, the practical justification is that Equation 1 provides much straighter lines than the usual one, permitting all of the data to contribute to the determination of reaction rate. Either method, of course, gives a reaction rate that is independent of dark survival level.

Fig. 4 shows that post-UV illumination of the mutant at 3341 A shows essentially no dependence upon dose rate, the ratio of the recovery efficiencies at 42 erg mm^{-2} $sec.^{-1}$ and at 1700 erg mm^{-2} $sec.^{-1}$ being 1.0 in this experiment, and averaging 1.13 in all experiments.

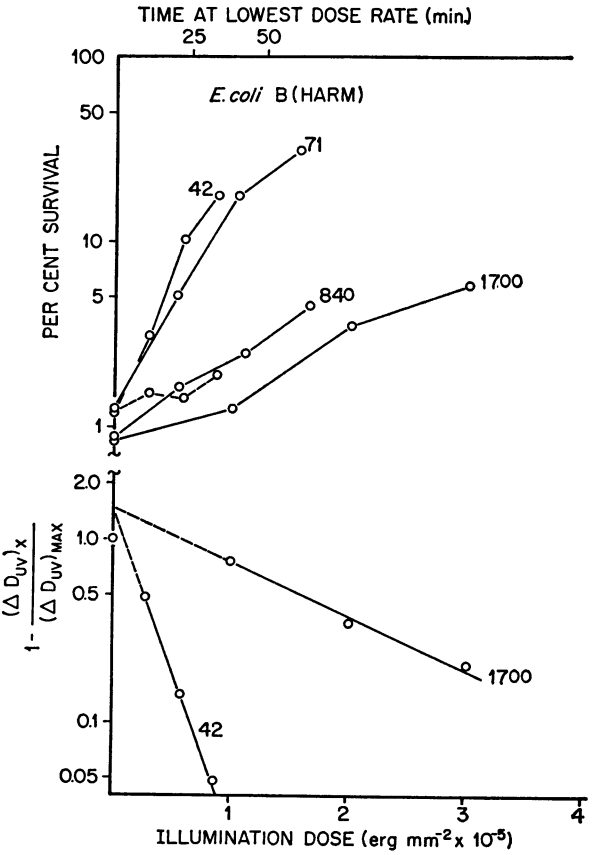

FIGURE 5 Effect of dose rate at 4047 A on post-UV illumination recovery in *E. coli* B (Harm). Otherwise similar to Fig. 4. Note difference in scale of abscissa.

Fig. 5 shows, as expected for enzymatic photoreactivation, a strong dependence upon dose rate at 4047 A for the parent strain, the recovery efficiency at 42 erg mm^{-2} $sec.^{-1}$ being 6.0 times that at 1700 erg mm^{-2} $sec.^{-1}$. (Correction for the maximum possible contribution of liquid-holding recovery to the low dose-rate curve reduces this ratio to 5.4.)

Fig. 6, however, shows a relatively weak dependence upon dose rate at 3341 A for the parent strain, the recovery efficiency at 42 erg mm^{-2} $sec.^{-1}$ being only 1.31 times that at 1700 erg mm^{-2} $sec.^{-1}$ (the average for all experiments was 1.56).

These data suggest that the post-UV illumination recovery in the mutant, and a large part of the recovery at 3341 A in the parent strain, is due to a process similar to that occurring in photoprotection.

Temperature Dependence. In a further effort to explore the nature of the post-UV illumination recovery, studies were made of the reaction at 5 and 32°C.

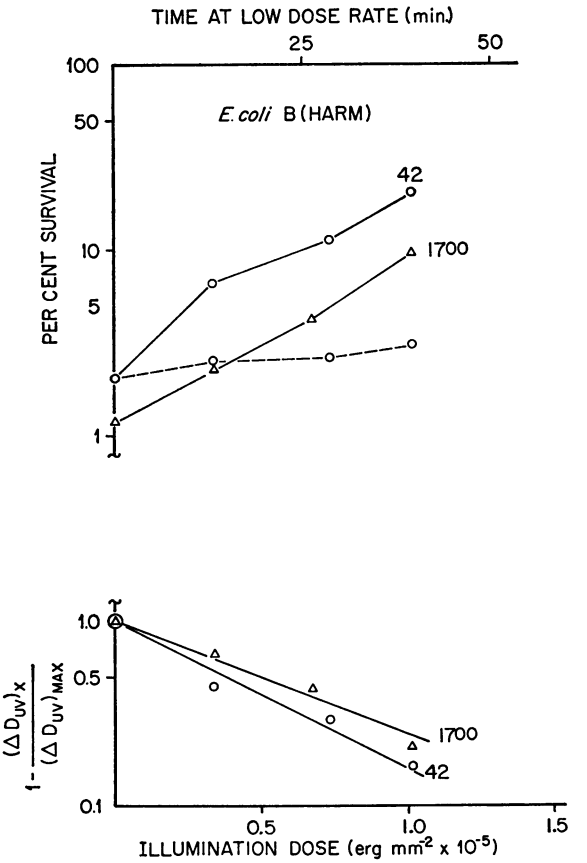

FIGURE 6 Effect of dose rate at 3341 A on post-UV illumination recovery in *E. coli* B (Harm). Otherwise similar to Fig. 4.

Recovery efficiencies were determined in a manner similar to that illustrated in Figs. 4 and 5. Because of the relatively low dose rate used (160 erg mm^{-2} $sec.^{-1}$), there was some liquid-holding recovery at 32°C, but in most experiments this was slight and no correction was made for it. Ratios of the recovery efficiencies at 32°C to those at 5°C are shown in Table I, where the "expected" values conform to

TABLE I

EFFECT OF TEMPERATURE ON RECOVERY EFFICIENCY*

	Preillumination	Postillumination	
	3341 A	3341 A	4047 A
E. coli B (Harm)	1.5	2.2	5.4
E. coli B phr⁻	1.3	1.8	
"Expected"‡	1.2	7.1	

*The numbers shown are the ratios of the recovery efficiencies (see text) at 32°C to those at 5°C. Each number is from data of 3 experiments.
‡Values obtained on stationary cells with broad radiation bands that cover the entire effective spectrum, from data of Jagger (1960).

what one would expect for the presumably photochemical (1.2) or largely enzymatic (7.1) reactions which are displayed by broad-band induction of photoprotection and photoreactivation, respectively. Post-UV illumination at 4047 A in the parent yields a recovery efficiency ratio that is close to expectation for enzymatic photoreactivation, and which is about 3.6 times that obtained in the same strain with pre-UV illumination at 3341 A. However, the ratios for post-UV illumination at 3341 A are much lower for both strains, being only 1.4 times the ratios obtained with pre-UV illumination. These data again suggest that a large fraction of the post-UV illumination reaction at 3341 A in either parent or mutant is similar to that involved in photoprotection.

The ratios obtained for pre-UV illumination are slightly higher than the "expected" value, which is derived from data on stationary cells. If the discrepancy is significant, it may reflect a slightly different behavior of photoprotection in log phase cells. Also, the ratio at 4047 A is not as high as the "expected" value for a broad radiation band. This suggests that longer wavelengths may have temperature coefficients much larger than that at 4047 A.

Induction of Growth Delay. If the post-UV illumination recovery at 3341 A in B phr⁻ and most of the recovery at 3341 A in B (Harm) are due to a process similar to that of photoprotection, then this wavelength should induce growth delays in both strains. Using continuous turbidimetry, we have found that illumination (300,000 erg mm^{-2}) of either strain in phosphate buffer at 3341 A

induces a subsequent large growth delay (about 100 minutes) in nutrient broth, while equal doses at 4047 A have no effect upon growth.

DISCUSSION

In this discussion, we shall use the terminology outlined in the Appendix.

Extracts from photoreactivable cells will, in the presence of wavelengths effective for the photoreactivation (PR) of these cells, cause the PR of transforming DNA (Rupert *et al.*, 1958). The active fraction of these extracts has been shown to include at least one enzyme. Furthermore, this *in vitro* PR shows a strong dependence upon temperature during PR and upon dose rate of the reactivating rediation (Rupert, 1962), behavior that is also characteristic of the PR of cells (Jagger, 1958).

Thus it is clear that this enzymatic PR is associated with strong temperature and dose-rate dependence, but the reason is not really known. The dose-rate dependence could result from an excess of substrate over enzyme or from a very transient enzyme-substrate complex (see Rupert, 1962), but, if this were so, one would expect this dose-rate dependence to be a function of UV dose, and no such behavior has yet been reported. Also, for a photochemical reaction, one would not expect a temperature dependence unless only about 1/200 of the photon energy (an amount corresponding to thermal energies) was being utilized, and it therefore seems more likely that the temperature dependence is associated with some other step in the reaction than that of photon absorption.

Nevertheless, the *empirical* evidence cited above means that, when one observes strong temperature and dose-rate dependence in the PR of a cell, it is likely that the major process involved is an enzymatic one, similar to the *in vitro* PR of transforming DNA. For such reasons, our observations lead us to conclude that PR at 4047 A in log phase B (Harm) probably involves chiefly direct photoenzymatic repair of DNA.

Photoprotection (PP) in *E. coli* B shows no dependence upon dose-rate changes over a range of 64 and it shows only a small temperature coefficient (Jagger, 1964). This behavior is consistent with the initial event being photochemical and not involving enzymes, although it does not constitute proof of this. The work of Kashket and Brodie (1962; 1963) led Jagger *et al.* (1964) to suggest that this initial event is the direct photochemical destruction of quinones. Loss of these quinones presumably then leads to impairment of respiratory processes, and this in turn causes a delay in the growth and division of the cell after it has been irradiated with UV. This delay presumably permits time for "dark" enzymes to act on the UV damage.

All the above *empirical* evidence means that when one observes little or no temperature or dose-rate dependence in PR, it is not likely that direct photoenzymatic repair of DNA is involved, but rather some initially non-enzymatic process, such as that proposed for PP. In our particular system, the additional evidence

that the wavelength dependence of PR in B phr⁻ is roughly similar to that of PP, plus the fact that 3341 A induces a large growth delay, whereas 4047 A does not, makes it very likely that the process involved in PR of B phr⁻ is the same as that operating in PP of *E. coli* B.

We conclude that PR at 3341 A in log phase B phr⁻, and most of the PR at 3341 A in log phase B (Harm), is an indirect process, similar to that operating in PP, and probably initially non-enzymatic.

One consequence of this finding is that any organism that shows PP may have an initially non-enzymatic component in its PR. In such organisms, the difference between the PR action spectra at high and low temperatures, or at high and low dose rates, then becomes more nearly the true spectrum for the direct enzymatic reaction, and is a curve that one might hope to match to the absorption spectrum of the chromophore for direct enzymatic PR.

Our experiments suggest that the efficiency of direct enzymatic PR in *E. coli* is considerably lower at 3341 A than at 4047 A, and that only in photoprotectable cells would the efficiency be high at 3341 A. The action spectrum for PR in *E. coli* B/r shows high efficiency at 3341 A (Fig. 1) but B/r has been found by several workers (see Jagger, 1964) to show no PP. However, these reports of lack of PP in B/r apparently concerned only stationary cells. Witkin (1963) has recently reported PP of B/r WU 36 in the late lag phase, and we have since found (unpublished observations) that B/r (Oak Ridge National Laboratory) shows no PP in stationary phase, but a large PP in log phase. Since the action spectrum of Jagger and Latarjet (Fig. 1) was done on starved log phase cells, we suppose that their spectrum included some indirect PR at 3341 A, which would explain the high efficiency at this wavelength.

Setlow and Boling (1963) show that the efficiency of PR of transforming DNA in yeast extract drops rapidly at wavelengths shorter than 3500 A and averages roughly 60 per cent as high at 3341 A as at 4047 A. Since there can be no reaction *in vitro* that would involve a growth-division delay, we suppose that this system involves only photoenzymatic direct repair, and hence expect the rate to be lower at 3341 A than at 4047 A.

We appreciate the kindness of Dr. Walter Harm in sending us cultures of the strains B (Harm) and B phr⁻ shortly after his discovery of the mutant. We are grateful to Dr. Jane K. Setlow for helpful discussions concerning definitions.

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APPENDIX

Several effects have now been found that render existing definitions of photoreactivation and photoprotection (see Jagger, 1958) inadequate. Halldal (1961) has shown that far-UV–induced reduction of delayed motility in a green alga can be partially cancelled by

subsequent irradiation at *shorter* wavelengths. Setlow and Setlow (1962) have shown that biological damage induced by UV at 2800 A can be partially reversed by subsequent irradiation at 2400 A (thymine-dimer photoreversal), although this effect so far has been observed only in transforming DNA and not in cells. Finally, the effect reported in this paper (resulting from what we may call "photo-induced growth-division delay") clearly differs from the direct photoenzymatic repair of transforming DNA in cell extracts, observed by Rupert *et al.* (1958) and believed to be the major mechanism of photoreactivation in cells (Jagger, 1958).

We propose a minimal redefinition of terms, in line with our conviction that the terms "photoreactivation" and "photoprotection" should be (*a*) *operational,* (*b*) restricted to damage that can be *assayed biologically,* and, (*c*) as *general* as possible. More specific terminology (*e.g.* "thymine-dimer photoreversal") is required for a description of the particular molecular processes involved.

Photoreactivation (PR) is the reduction in response to far ultraviolet (roughly below 3000 A) irradiation of a biological system resulting from a *concomitant or post-treatment* with non-ionizing (photoreactivating) radiation.

Direct PR is PR in which the molecular site of action of the far ultraviolet radiation is immediately and directly altered by the energy of the photoreactivating photon.

Indirect PR is PR in which the molecular site of action of the far ultraviolet radiation is not immediately and directly altered by the energy of the photoreactivating photon.

Photoprotection (PP), *direct PP,* and *indirect PP* are defined as above, except for substitution of "PP" and "photoprotecting" for "PR" and "photoreactivating," and of "pretreatment" for "concomitant or post-treatment."

The definition of PR given above, and the usage of the terms "direct" and "indirect," differ from those earlier proposed by Jagger (1958) simply because present knowledge permits the formulation of much better definitions. It is felt that the present definitions are sufficiently general that they are not likely to require alteration in the near future. It will be noted that indirect PR includes PR in which there is *no alteration* of the molecular site of action of the far ultraviolet ("by-pass" or "stimulation" mechanisms).

Within these definitions, the *Halldal effect* is a "photoreactivation," but whether direct or indirect is not yet known. *Photoenzymatic repair* of transforming DNA or of cells by *longer* wavelengths (the usual PR) and *thymine-dimer photoreversal* of transforming DNA by *shorter* wavelengths both result in "direct PR." The effect discussed in this paper (resulting from *photo-induced growth-division delay*) is apparently "indirect PR" if caused by a post-treatment and "indirect PP" if caused by a pretreatment.

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