DARK REPAIR OF ACRIDINE DYE-SENSITIZED PHOTOEFFECTS IN E. COLI CELLS AND BACTERIOPHAGE.

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Bacteria and phages can be inactivated by visible light in the presence of sensitizing dyes and oxygen. The lesions, which are mainly due to effects in nucleic acid, are quite different from UV lesions not only in the physical-chemical changes brought about (e.g. FREIFELDER and URETZ, 1966), but also with respect to the biological effects produced: they are neither photo-enzymatically repairable nor multiplicity-reactivable (WELSH and ADAMS, 1954). While recent work with E. coli and yeast cells suggest that the photodynamic lesions are also unaffected by enzymatic processes known to achieve extensive dark repair of UV lesions (e.g. URETZ, 1964; FREIFELDER and URETZ, 1966; GEISSLER, 1966, 1967; PATRICK et al., 1964), other investigations indicated the occurrence of dark repair of photodynamic damage in E. coli and Serratia marcescens (RUPP, 1966; BRENDEL and KAPLAN, 1967). The results reported in this paper show that damage inflicted to bacterial or phage DNA by white fluorescent light in the presence of acridine orange or acriflavine is repaired at varying extents by both of the two dark repair systems operating on UV damage. However, the photodynamic lesions are not affected by two phage-controlled dark repair systems existing in T4.

MATERIALS AND METHODS

Bacteria. We used the E. coli K12 derivatives AB 1157 ("wild type"), AB 2437 (uvrA-6), AB 2463 (rec-13), and AB 2480 (uvrA-6, rec-13), described by HOWARD-FLANDERS et al. (1966a,b), and the B derivatives B/r mal^+ (ARBER and

LATASTE-DOROLLE, 1961), and B_{s-1} (HILL, 1958). Cultures were grown overnight in 0.8% Nutrient Broth (Difco) with 0.5% NaCl in an incubator shaker at 37°C so that the cells were in stationary phase. They were centrifuged, washed, resuspended and diluted in a mineral salts buffer consisting of 7 g $Na_2HPO_4 \cdot 12\ H_2O$, 3 g KH_2PO_4 and 4 g NaCl, dissolved in 1000 ml deionized water, to which 4 ml of 0.5 M $MgSO_4$ solution was added after autoclaving.

Phage. We used λvir h (obtained from J. WEIGLE, Pasadena, Calif.), T1, T4D, as well as the mutants T4v_1 and T4 v_1x (HARM, 1963), all grown and stored in M9 medium. The same buffer as described before was used to dilute the phage.

Dye-sensitized photo-inactivation. Acridine orange (purified from commercially obtained substance by Dr. W.C. Cramer, Univ. of Calif., San Diego, and kindly given to me by Dr. M.H. Patrick) was added at a concentration of 2×10^{-5} M to bacterial suspensions of less than 5×10^7 cells/ml or at a concentration of 2×10^{-6} M to phage suspensions of less than 3×10^7 particles/ml. In our experience, the high ionic strength used in our cell suspensions, requiring relatively long exposure times to reach a certain survival level, improves the accuracy and reproducibility of the results. Acriflavine hydrochloride (Allied Chemical Corp.) was applied at a concentration of 10 μg/ml. The mixtures were kept dark for 20 minutes at 37°C before being exposed at a few centimeters distance to the light of 3 closely spaced 15 Watt G.E. "Daylight" fluorescent lamps at 37°C. All other manipulations were carried out under red light from G.E. "Red" fluorescent lamps.

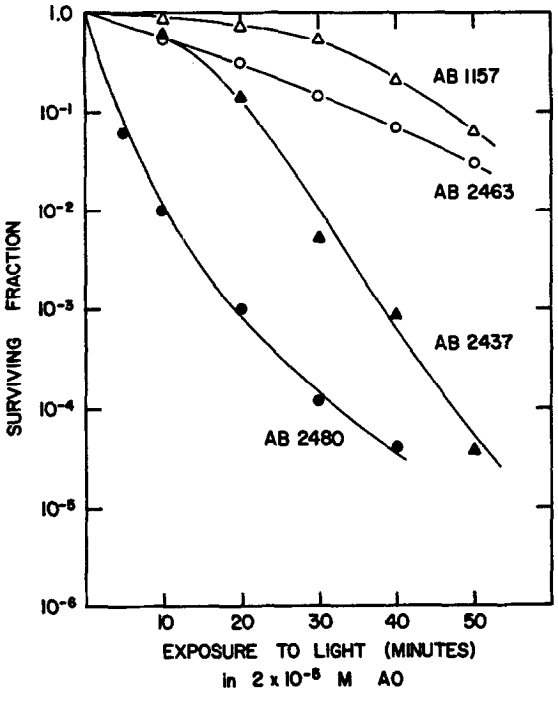
Plating and incubation. Bacteria: 0.4 ml-samples of appropriately diluted bacterial suspensions were pipetted into empty petri dishes, and approximately 20 ml nutrient agar (consisting of 3.7% Brain Heart Infusion, dehydrated (Difco) and 1% Bacto-Agar (Difco)), kept liquid at 44°C, was poured onto the sample in a manner that distributed the cells evenly. After agar hardening, plates were incubated dark at 37°C. Phage: 0.1 ml-samples of diluted phage suspensions were mixed with 0.3 ml plating bacteria (see below) and 2.5 ml

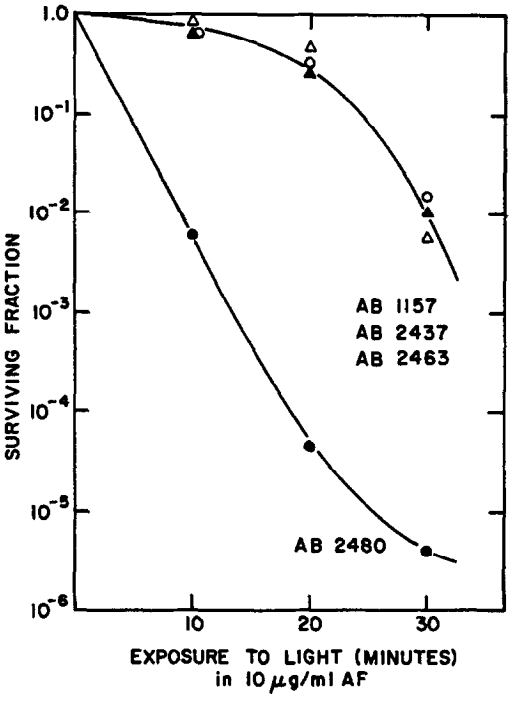
"soft agar" (consisting of 3.7% Brain Heart Infusion, dehydrated (Difco) and 0.65% Bacto-Agar (Difco)), kept liquid at 44°C. The mixture was poured on plates with a solid tryptone agar layer (1% Bacto-Tryptone (Difco), 0.5% NaCl, and 1% Bacto-Agar (Difco)). The plating bacteria for T1 and T4 were grown in Nutrient Broth to approximately 2 x 10^8 cells/ml. The plating bacteria for λ were grown to 10^9/ml, centrifuged, resuspended in 0.01 M $MgSO_4$, starved for 1 hour at 37°C, and after addition of glycerol at 15% v/v they were frozen at -70°C until being used. To obtain good plaques in the case of λ, the mixture of phage and plating bacteria was allowed to stand for 15 minutes at 37°C before adding the soft agar and pouring.

EXPERIMENTAL

K12 Derivatives. With respect to UV damage, the four K12 derivatives AB 1157 (*uvr*$^+$*rec*$^+$), AB 2463 (*rec*$^-$), AB 2437 (*uvr*$^-$) and AB 2480 (*uvr*$^-$*rec*$^-$) differ in 2 dark repair systems, the double mutant strain AB 2480 being the most sensitive (HOWARD-FLANDERS and BOYCE, 1966a). Fig. 1 shows the survival of these 4 strains after exposure to various doses of light in the presence of $2x10^{-5}$ M acridine orange (AO). The results suggest that each of the two dark repair systems also repairs a fraction of the photodynamic lesions, because the "wild type" strain is most resistant, the double mutant is most sensitive, and the two single mutants are intermediate in sensitivity. Control experiments showed very little, if any, inactivation when cells of the four strains were kept for 90 minutes at 37°C either dark in the presence of $2x10^{-5}$ M AO, or in light after diluting the dye suspension 1:10^3 in buffer.

In the presence of 10 μg/ml acriflavine (AF), the survival pattern is different from that obtained in the presence of AO: the wild type and the two single mutant strains give virtually identical survival curves, only AB 2480 is much more sensitive (Fig. 2). This suggests that either of the two dark repair systems is capable of repairing the photodynamic lesions (as in the case of AO), but that there is complete overlap in their actions so that it





Figs. 1 and 2. Survival of AB 1157 (△), AB 2437 (▲), AB 2463 (○), and AB 2480 (●), as a function of exposure to light in the presence of 2×10^{-5} M AO (Fig. 1) or 10 μg/ml acriflavine (Fig. 2). Each value is the geometric mean of 3 experiments.

is irrelevant whether one or both systems are operating. The reason for the somewhat different repairability of AF- and AO-sensitized effects is not known.

Because of the supposed lack of dark repair in photodynamically inactivated AB 2480 cells attempts were made to photoreactivate them after a 1000-fold dilution in buffer. However, photoreactivation was found for neither AO nor for AF treated cells.

B/r and B_{s-1}. Similar experiments as with the K12 derivatives were carried out with strains B/r and B_{s-1}, which differ in at least two mutations affecting the UV sensitivity (MATTERN et al., 1966; GREENBERG, 1967). As shown in Fig. 3, B_{s-1} is more sensitive than B/r to light in the presence of either

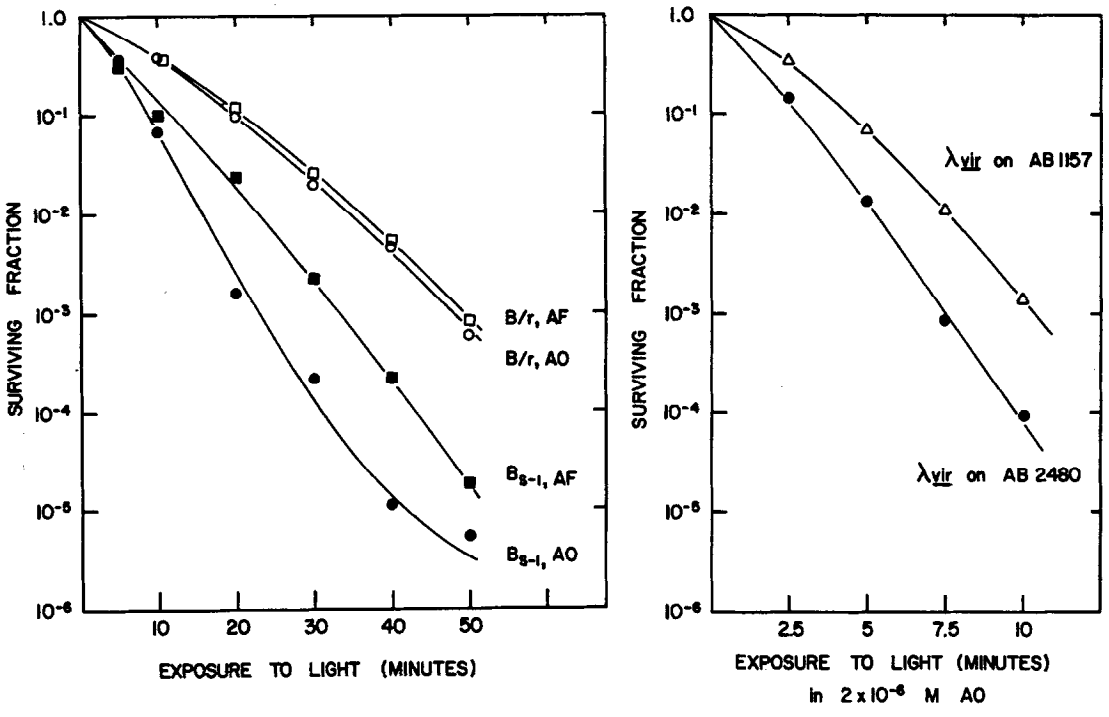


Fig. 3. Survival of B/r (open symbols) and B_{s-1} (closed symbols) as a function of exposure to light in the presence of 2×10^{-5} M AO (○, ●) or 10 μg/ml acriflavine (□, ■). Each value is the geometric mean of 2 to 5 experiments.

Fig. 4. Survival of phage λvir as a function of extracellular exposure to light in the presence of 2×10^{-6} M AO, plated on AB 1157 (△) or AB 2480 (●). Geometric mean of 3 experiments.

AO or AF, suggesting that at least one UV repair system can also repair photodynamic lesions.* The finding that the difference between B/r and B_{s-1} in sensitivity to photodynamic damage is considerably smaller than that between strains AB 1157 and AB 2480 is paralleled by the fact that also the difference in UV sensitivity is smaller. As in the case of K12 cells, neither dark exposure of B/r or B_{s-1} cells to the dyes, nor light exposure of dye-sensitized

*This is in contrast to earlier results by URETZ (1964). However, URETZ (pers. comm.) informed me that he has rechecked the photodynamic sensitivity of B_{s-1} and B/r, using his original methods, and has found some difference between these two strains.

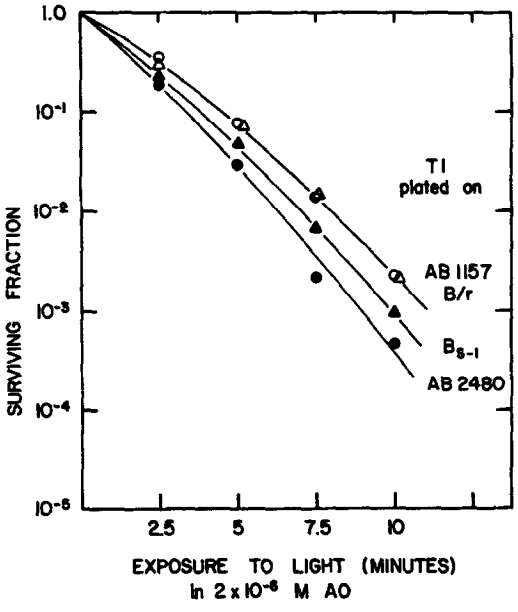


Fig. 5. Survival of phage T1 as a function of extracellular exposure to light in the presence of 2×10^{-6} M AO, plated on B/r (○), AB 1157 (△), B_{s-1} (▲) or AB 2480 (●). Geometric mean of 2 to 3 experiments.

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cells after $1:10^3$ dilution into buffer resulted in any significant loss of viable cells.

Phages λ and T1. Extracellular phages λvir and T1 were exposed to light in the presence of 2×10^{-6} M AO and were then permitted to infect bacterial strains of differential repair potentialities. The results are shown in Figs. 4 and 5. Since there can be no difference in the extent of damage in the infecting phage in each case, the differences in phage survival must be ascribed to dark repair in the host cells. For both phage types the observed repair effects are much less extensive than those for the bacterial cells, but this is paralled by the fact that dark repair of UV-irradiated phage is also less extensive than that of UV-irradiated bacterial cells.

Phage T4. T4, which after UV irradiation is not affected by the two kinds of bacterial dark repair, is also not affected after extracellular photodynamic inactivation. However, if cells of either AB 1157 or AB 2480 are in-

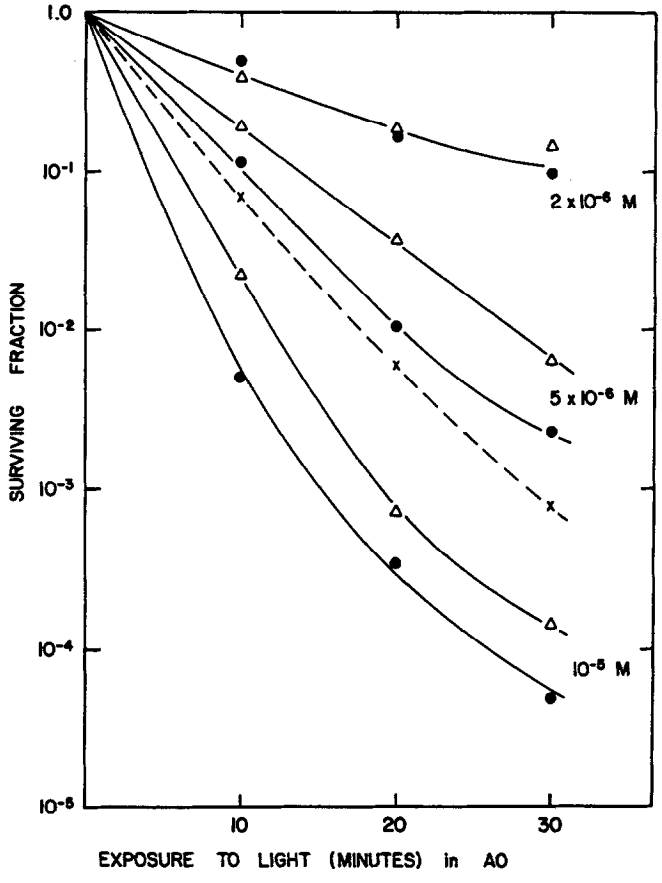

Fig. 6. Survival of phage T4D as a function of intracellular exposure to light. Infected AB 1157 cells (△) or AB 2480 cells (●) were suspended for 20 minutes in dark at various AO concentrations before being exposed to light. Geometric mean of 2 experiments. The survival curve for extracellular exposure to light in the presence of 2×10^{-6} M AO is represented by the dashed line.

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fected with T4 in the presence of 0.002 M KCN (to prevent intracellular growth of phage), and are exposed to light after suspension for at least 20 minutes in AO, slight differences in phage survival are found (Fig. 6). This might indicate slight differences in the intracellular dye concentrations for the two strains, which, however, would be by far too small to account for the large differences in the survival curves between these strains (cf. Fig. 1). In Fig. 6 the survival curve of extracellular phage T4 at 2×10^{-6} M AO is shown

for comparison. It is roughly by a factor of 3 steeper than the survival curve of intracellular T4 at the same dye concentration. Since it is not likely that intracellular T4 after infection in the presence of KCN is intrinsically less sensitive than extracellular T4, the difference might be due to reduced capacity of the host cells which were sensitized and exposed to light together with the phage (cf. CRAMER and URETZ, 1966). Another possible reason could be a lower effective dye concentration for intracellular DNA (due to different ionic strength inside the cell), compared to DNA of extracellular phage.

The survival curve for photodynamic treatment of extracellular T4 is not significantly different from that of T4v or T4vx, lacking 1 or 2 phage-controlled dark repair systems, respectively (HARM, 1963, 1964). So far, these repair systems are found to operate on UV damage only.

CONCLUSIONS

E. coli strains differing in their potentialities to repair UV damage differ accordingly in their potentialities to repair photodynamic lesions in their own DNA or in DNA of infecting phage. However, the extent at which such damage is repaired is far less than in the case of UV damage. It is not clear why in some of the published work no indications for repair were found; possibly the repairability of photodynamic effects depends on factors such as ionic strength or spectral composition of the inactivating light.

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