Overlap of Photoreactivation and Liquid Holding Recovery in Escherichia coli B

Abstract. *It is known that cells of* Escherichia coli *B exposed to 2537-Å ultraviolet radiation will show a higher survival (photoreactivation) if subsequently irradiated at 3650 Å, and that they will also show a higher survival (liquid holding recovery) if subsequently held in a liquid. We find that cells given an optimal recovery treatment of one type show no further recovery if also subjected to the other type of treatment. It is concluded that liquid holding treatment acts only upon photoreactivable damage.*

The 2537-Å ultraviolet killing of microorganisms has been shown to result from absorption mainly in nucleic acid. The most important photoproduct effective in ultraviolet inactivation of transforming DNA seems to be a thymine dimer (*1*), a product that is also formed in irradiated bacteria (*2*). Bacteria exposed to ultraviolet can show a higher survival [photoreactivation (PR); see (*3*)] if subsequently irradiated at 3650 Å, and they can also show a higher survival [liquid holding recovery (LHR); see (*4*)] if subsequently held in a liquid. Setlow and Setlow (*5*) found (i) that the only photoreactivable lesion for inactivation of transforming DNA is the thymine dimer, and (ii) that photoreactivating light reverses the damage in DNA by splitting the dimer.

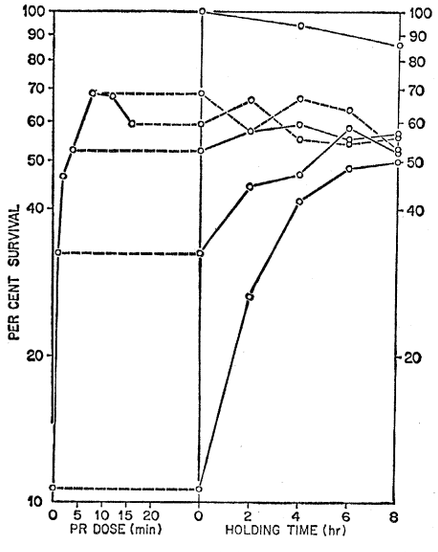

Fig. 1. Liquid holding recovery after photoreactivation of 2537-Å killing in *E. coli* B. Percentage survival is shown on a logarithmic ordinate versus dose of photoreactivating light (left abscissa) or time of holding in phosphate buffer (right abscissa). This is part of a single typical experiment. The zero-time points on the holding curves correspond to 0, 1, 4, 8, and 16 minutes on the photoreactivation curve, as indicated by horizontal broken lines. Control points at the top show loss of viability during holding of unirradiated cells.

Furthermore, recent studies (*6*) show that photoreactivated bacteria contain fewer thymine dimers than those not photoreactivated.

We have attempted to determine the degree of overlap in PR and LHR of killing of *Escherichia coli* B. Extensive overlap would indicate that the effects of the thymine dimer can be negated simply by holding the cells in a liquid medium after irradiation.

Escherichia coli B was grown in Difco nutrient broth for 16 hours (stationary phase). The cells were centrifuged and the pellet was resuspended in 0.067*M* sodium-potassium phosphate buffer (*p*H 7). For colony counts, the cells were diluted in phosphate buffer, plated, and incubated for 16 to 24 hours on Difco nutrient agar.

Ultraviolet was provided by two General Electric 15-watt germicidal lamps. The dose rate, measured with a photovoltaic cell (*7*), was 11 erg mm^{-2} sec^{-1}. Photoreactivating light (3650 ± 100 Å) was obtained from a Hilger quartz monochromator, illuminated by a 1000-watt high-pressure mercury arc lamp (Philips SP-1000). The dose rate, measured with a calibrated thermopile, was kept high (3000 erg mm^{-2} sec^{-1}) to shorten exposure times and thus to minimize liquid holding recovery effects during PR. The PR dose rate was sufficiently constant that dose was proportional to time of exposure. Cells were ultraviolet-irradiated at a concentration of 10^6 cells ml^{-1} in a depression spot plate. After irradiation, they were diluted 1:100 with phosphate buffer and either (i) exposed in a Beckman Corex cuvette (1 cm path) to photoreactivating light, or (ii) held for times up to 8 hours in phosphate buffer at room temperature (23°C). Uniformity of photoreactivation dose was obtained by continuous stirring.

Figure 1 shows (at the left) survival following a fixed dose of ultraviolet and different doses of photoreactivating light and (at the right) survival of the same populations held for times up to 8 hours. Liquid holding recovery from the ultraviolet damage occurs with partially photoreactivated cells, but no LHR takes place with fully photoreactivated cells. We conclude that LHR is operating only on photoreactivable lesions. Furthermore, maximum LHR is obtained by holding for progressively shorter times for more highly photoreactivated cells. This suggests that PR treatment progressively decreases the number of lesions upon which LHR treatment can act and further supports the above conclusion. The drop in the higher curves with holding appears to reflect a similar drop in the controls.

Figure 2 shows (i) survival following a fixed dose of ultraviolet and holding for different times and (ii) survival of the same populations subsequently exposed to photoreactivating light. There is very little, if any, photoreactivation of cells that have fully recovered after holding for 8 hours. This suggests that LHR treatment may be able to operate on all photoreactivable lesions. Figure 2 shows a progressive drop in the level of maximum PR with time of holding. Clearly, much of this drop can be accounted for by the drop in control survival, suggesting that if it were possible to obtain LHR in a much shorter time, a considerably higher recovery might be obtained. However, the drop in maximum PR appears to be more

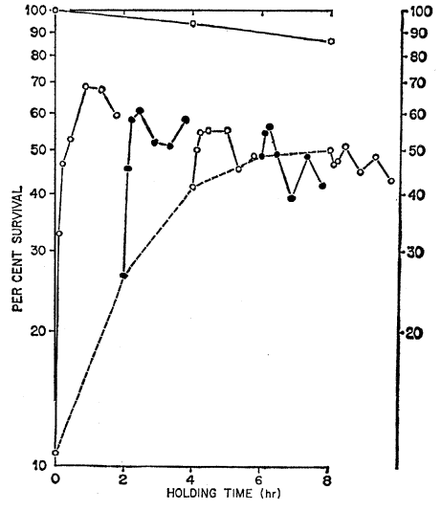

Fig. 2. Photoreactivation (solid lines) after liquid holding recovery (broken line) from 2537-Å killing in *E. coli* B. Percentage survival is shown on a logarithmic ordinate versus time of holding. The photoreactivation points correspond to 0, 1, 2, 4, 8, 12, and 16 minutes of exposure to photoreactivating light. This is part of the same single experiment from which data were taken for Fig. 1. Controls as in Fig. 1.

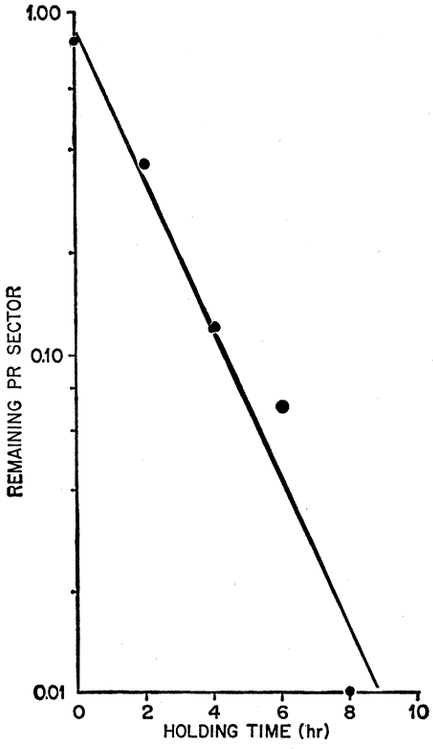

Fig. 3. Loss of photoreactivability in *E. coli* B during holding at 23°C in phosphate buffer. The remaining PR sector after liquid holding for various times is plotted on a logarithmic ordinate versus time of holding. Data derived from Fig. 2.

rapid than the control drop, suggesting that some initially photoreactivable lesions become irreversible (by either holding or PR treatment) during holding. This interpretation is further supported by the observation that after 8 hours of holding, PR treatment cannot significantly raise survival (Fig. 2), whereas the survival of cells receiving various amounts of photoreactivating light at time zero and then held for 8 hours (Fig. 1) is always higher than that obtained with holding alone. We conclude that it is likely (though admittedly on the borderline of significance) that some photoreactivable lesions become irreversible with holding of the cells in phosphate buffer.

Knowing the ultraviolet survival kinetics (virtually exponential in this region), one can calculate (*3*) the photoreactivable sector for any population, which is the fraction of the ultraviolet dose that appears to be eliminated by a full PR treatment. From Fig. 2 one can calculate the sector for holding plus PR treatment (using the highest experimental point on the PR curve) and subtract from it the sector that was eliminated by holding alone. This gives the PR sector remaining after holding. For example, at 2 hours' holding,

$$\text{PR sector remaining} = \frac{\text{Dose}_{UV}(26.5\%\ \text{surv.}) - \text{Dose}_{UV}(60.5\%\ \text{surv.})}{\text{Dose}_{UV}(10.7\%\ \text{surv.})} = 0.36,$$

which means that, after ultraviolet killing to 10.7 percent survival, and holding for 2 hours, 36 percent of the ultraviolet damage can still be photoreactivated. Figure 3 shows that the remaining photoreactivable sector decreases exponentially with time of holding, suggesting a first-order decay of photoreactivable lesions during holding. Most of this decay involves effective repair of the lesion, but a little of it seems to be a transition of the lesion into an irreversible, lethal state.

We conclude that, at these survival levels in these cells, (i) LHR treatment acts only on photoreactivable lesions, and (ii) LHR treatment may be potentially capable of acting on all the photoreactivable lesions. If the only photoreactivable lesion in these cells is the thymine dimer, then LHR treatment must act only on the thymine dimer. By definition, it does this by some "dark" reaction. That such dark reactions exist for ultraviolet damage to cells is strongly suggested by a variety of evidence (see *8*). Metzger (*9*) has shown that a dark reaction leading to "host-cell reactivation" very probably acts on photoreactivable lesions, and it is known that holding in buffer after ultraviolet can induce a division delay upon plating (*10*). It may be that the action of LHR treatment is to induce a division delay that permits more time for host-cell reactivation reactions to act on the ultraviolet lesions.

We also wish to report that *E. coli* B_{s-1} (Hill), a very ultraviolet-sensitive mutant, which is highly photoreactivable (*11*), showed no LHR when irradiated in the stationary phase, held in buffer, and then plated on nutrient agar. Log-phase cells, plated on nutrient agar, showed a small LHR, but only for dark survivals below 1 percent. It is of interest that this organism fails to show other dark reactivations (*8*), which further suggests a link between these reactivations and LHR.

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Pleistocene Wood Rat Middens and Climatic Change in Mohave Desert: A Record of Juniper Woodlands

Abstract. *Leafy twigs and seeds of juniper are abundant in nine ancient* Neotoma *middens discovered in low, arid, desert ranges devoid of junipers, near Frenchman Flat, Nevada. Existing vegetation is creosote bush and other desert shrubs. Twelve radiocarbon dates suggest that the middens were deposited between 7800 to more than 40,000 years ago. Dominance of Utah juniper and absence of pinyon pine in most deposits indicates a local Pleistocene woodland climate more arid than the usual pinyon-juniper climate.*

An area of more than 5000 km² in the vicinity of Frenchman Flat in southern Nevada is occupied by arid basins and ranges which are very sparsely vegetated with a low desert scrub (*1*) except for localized stands of bizarre Joshua trees (*Yucca brevifolia* Engelm.). The mountains immediately surrounding Frenchman Flat basin are relatively low in elevation and do not support coniferous forest or woodland. Even the relatively xerophytic woodland species of juniper and pinyon pine [*Juniperus osteosperma* (Torr.) Little and *Pinus monophylla* Torr. and Frem.] are totally lacking, although they are present on higher ranges such as the Spring Mountains and Shoshone Mountain, some 32 km from the south side of Frenchman Flat.

Therefore, a major climatic change is indicated by the fact that *Juniperus osteosperma* is one of the abundant