DARK- AND K-REACTIVATION
IN UV IRRADIATED \textit{ESCHERICHIA COLI}

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Introduction

UV sensitivities (initial slopes) of the colony forming ability of different strains of \textit{E. coli} in the dark vary by a factor of at least 1000 (Hill and Simson, 1964). They can be varied by environmental conditions (Zelle and Hollaender, 1954). It is in part known (Jagger, Wise and Stafford, 1964; van de Putte, Westenbroek and Rörsch, 1963), and for the rest it will be shown, that such diverse treatments as heat reactivation (HR)\(^1\) (Stein and Meutzner, 1950), pantoyl lactone plating (van de Putte et al., 1963), pregrowing the bacteria, liquid holding (LH) (Jagger et al., 1964; Roberts and Aldous, 1949), and photoprotection (Jagger et al., 1963) influence UV sensitivity only by changing the efficiency of two reactivating mechanisms that can overcome in specific ways part of the lesions that are otherwise lethal to the cell.

One of them is found in all wild strains and is called dark reactivation (DR), or host cell reactivation because it works on phage DNA too (Garen and Zinder, 1955; Sauermann, 1962a; Setlow and Carrier, 1964; Boyce and Howard-Flanders, 1964). Mutants without DR have been isolated in the laboratory from several strains: Bs-1 (Hill et al., 1964); Bs\(^{-}\) (Rörsch, Edelman, V. D. Kamp and Cohen, 1962); K12hr\(^{-}\) (Harm, 1963); K12uvt\(^{+}\) (Boyce et al., 1964). DR seems to work enzymatically (Sauermann, 1962a) by excising DNA lesions (Setlow et al., 1964; Boyce et al., 1964) and replacing them by new DNA (Pettijohn and Hanawalt, 1964).

Another reactivating mechanism (KR, see below) is found in strain K12 but not in B and BB. It does not work on phage DNA and can be lost (fil\(^{+}\), lon\(^{-}\)) or gained (fil\(^{-}\), lon\(^{+}\), /r) by mutation (Rörsch et al., 1962; Howard-Flanders, Simson and Theriot, 1964a; Witkin, 1947). However, it can be re-established by several post-irradiation treatments such as plating and incubation at 45°C in place of 37°C or on media containing pantoyl lactone or by chloramphenicol treatment (Harm and Stein, 1952; Rörsch et al., 1962; Alper and Gillies, 1960). Until a rational name is found for all these cases it may be called K-reactivation (KR) because it occurs naturally in strain K12. KR and DR are largely independent (van de Putte et al., 1963; Howard-Flanders et al., 1964a).

In this paper observations are presented on both these reactivations and their interrelationship. They lead to the conclusion that KR consists of two components, a regulatory and a reactivating one, the effect of the former being a prerequisite for the latter to work. The regulatory component is the one missing in strains

\(^{1}\)Abbreviations used: DR, dark reactivation; DRF, dose reduction factor; HR, heat reactivation; KR, K-reactivation; LH, liquid holding.
B and BB, but its effect can be mimicked by heat or pantoyl lactone. KR has an additional effect on DR, preventing dark reactivable lesions from being actively lethalized, as occurs in growing cultures, and in this respect it can be replaced by liquid holding or photoprotection.

Materials and Methods

**Strains.** All strains used are described in the literature: HfrH, 222 B/r [called B/r in the text], 220 BB [BB], Bs-1 △ [Bs-1], K12 hcr− (Jacob and Wollman, 1961; Arber and Latasle-Dorolle, 1961; Kees, Metzger and Serviere, 1965; Harm, 1963).

**Media.** Tryptone medium LT: 10 g Bacto tryptone, Difco; 5 g NaCl; 1 L H2O. LT plates: LT medium plus 1.5% Bacto agar, Difco. PL plates: LT plates plus 12 g/l DL-pantoyl lactone, Calbiochem (0.09 M).

**Bacteria.** Bacteria were grown at 37°C with aeration in LT medium. Resting bacteria were harvested from overnight cultures, growing ones from exponential cultures 90 minutes after dilution 1:100 into fresh LT medium of overnight cultures. They were centrifuged, resuspended and diluted to titer between 106 and 5 × 107 in 0.02 M MgSO4, and sometimes aerated (Jagger et al., 1964) for 30 minutes (growing) or 120 minutes (resting bacteria), which did not markedly affect the results.

**UV irradiation.** Cell suspensions were irradiated with stirring under a low pressure mercury vapor lamp Osram HNS 12 which was calibrated by comparison of the T4 JR UV survival curve with that obtained by Wulf (1963) to yield 3.8 erg/mm² sec. All experiments were performed in dim yellow light.

**Liquid holding (LH).** UV irradiated cells were kept in 0.02 M MgSO4 for 5 hours at room temperature.

**Heat reactivation (HR).** UV irradiated bacteria were diluted and plated at 45°C—47°C, kept there for 5 hours and further incubated at 37°C.

Results

It is a well known fact (Zelle et al., 1954; Rörsch et al., 1962), that E. coli B is more UV sensitive by a factor of ten if grown to the exponential phase before UV irradiation than in the resting state of overnight cultures (see Fig. 1b for strain BB; whenever experiments reported for strain BB have been repeated using E. coli B, the results were essentially the same). Since the DNA target does not grossly change in UV absorption during growth, the difference must be the result of different action of secondary, e.g. reactivating processes.

This was tested for DR by inactivation in the resting and growing state of strain Bs-1 (no DR, no KR) which differs from BB by the absence of DR. The results of Fig. 1a show that in both states this strain is equally sensitive as measured by the initial slope (see also Rörsch et al., 1962; the shift of the flat part of the survival curve towards higher survival will be discussed later). Thus in strain BB the high sensitivity of growing cultures is caused by reduced efficiency of DR as compared to resting cells.

Liquid holding (LH) which produces a marked growth-division delay (Jagger et al., 1964), restores in growing BB cells the low UV sensitivity of resting cells (Fig. 1b) but does not influence the initial slope of Bs-1 (Fig. 1a). Its effect therefore is due to DR enhancement. The same has been found for photoprotection (Jagger et al., 1964). Conceivably in fast growing cells there is just no time for complete DR, but LH or photoprotection provide it (compare also Alper and Gilles, 1960). However, other models are possible. Resting BB cells show some further LH effect (Fig. 1d; see Discussion).