THE PERSISTENCE THROUGH SEVERAL REPLICATION CYCLES OF MUTATION-PRODUCING PYRIMIDINE DIMERS IN A STRAIN OF *ESCHERICHIA COLI* DEFICIENT IN EXCISION-REPAIR

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Escherichia coli WP2 hcr^- (Hill) is a tryptophan auxotroph of strain B/r which may mutate to prototrophy either by a base substitution at an ochre nonsense triplet in the tryptophan locus or by the induction of ochre suppressors (Bridges, Dennis and Munson, 1967a,b; Osborn and Person, 1967). The ochre suppressors appear to be largely base changes at a site coding the anticodon of a glutamine tRNA (M. Osborn and S. Person, personal communication The strain has no detectable capacity to excise thymine dimers. Such photoreactivable pyrimidine dimers appear to be responsible for over 90% of the mutations (Witkin, 1966), this is true for both true reversions at the ochre triplet and for suppressor mutations (Bridges, Dennis and Munson, 1967b).

We have found that cultures growing exponentially at 37°C in glucose-salts medium supplemented with 10µg/ml tryptophan show no detectable reduction in rate of growth following exposure to 10 ergs mm^{-2} of predominantly 2537 Å UV (Figure 1). Furthermore no inviability can be detected using a plate count assay. Since a dose of 10 ergs mm^{-2} would be expected to produce several dozen thymine dimers in each chromosome (see for example Setlow, Swenson and Carrier, 1963; Wulff, 1963; Boyce et al., 1964; Setlow et al., 1965), it would appear that dimers in moderate numbers may pass

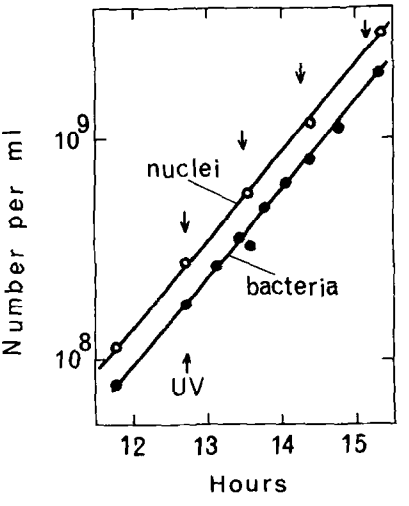
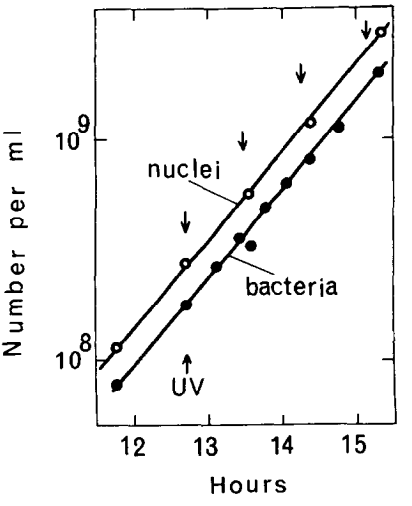


Figure 1. Lack of effect of 10 ergs mm^{-2} UV on rate of multiplication of E.coli WP2 hcr⁻. The culture was diluted by a factor of 2 at the times indicated by the arrows above the curves so as to keep the density between 1 x 10^8/ml and 2 x 10^8/ml. The number of nuclei was obtained by multiplying the bacterial count by the average nuclear number at any given time (see Bridges and Munson, 1965).

through the DNA replication complex with no detectable slowing down of the rate of replication. This conclusion is at variance with the findings of Rupp and Howard-Flanders (1968) on E.coli K12 hcr⁻. From their data a delay after 10 ergs MM^{-2} of about 10 minutes would be expected in the first replication cycle alone. Our experiments show that it could not have been more than half of this in E.coli WP2 hcr⁻.

Perhaps the blocks to replication after somewhat higher doses which have been attributed directly to dimers may rather be due to the chromosomal disorganization following the accumulation of single strand gaps which are produced, at least in E.coli K12, when dimers pass through the replication complex (Rupp and Howard-Flanders, 1968).



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replication after UV (dotted curve, Figure 2a) or if dimers persist indefinitely and produce mutations with a low probability at each replication (e.g. broken curve, Figure 2a, the precise form of which may vary slightly depending upon the number of replication cycles occurring on the plating medium, the segregation pattern, and the time needed for expression). The points may, however, be fitted by a curve calculated on the assumption that dimers are removed from the DNA at a rate proportional to the concentration of a hypothetical enzyme, i.e. at a rate which doubles during each successive generation time, and that dimers may give rise to mutations with a given (low) probability per generation time. The unbroken curve in Figure 2a is the curve calculated in this way which best fits the experimental points. It is assumed that mutations are induced with a probability which decreases with time after UV as shown in Figure 2b. On the above model, the curve in Figure 2b also represents the fraction of dimers capable of giving rise to mutations which remain at any given time.

These results could also be explained if the small fraction of bacteria containing dimers at mutational loci were slow to resume growth after UV. The relative sensitivity of auxotrophs and potential mutants to penicillin was, however, identical at all times, indicating that potentially mutant cells were growing at the same rate as the total population.

In conclusion, a dose of UV (10 ergs mm^{-2}) calculated to produce several dozen pyrimidine dimers per chromosome did not cause any detectable reduction in growth rate of exponentially growing E.coli WP2 hcr^-. As judged by photoreversibility of mutations to prototrophy, dimers at mutable sites persisted for up to about 3.75 generation times after UV and could give rise to mutations

with a low probability in each replication cycle during this period.

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