GENETIC TRANSFORMATION STUDIES. II. RADIATION SENSITIVITY OF HALOGEN LABELED DNA*

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The replacement of the methyl group in the 5-position of the thymine molecule by halogen atoms (Cl, Br, I) of similar van der Waals radius produces a biologically functional analog which can substitute for thymine in the deoxyribonucleic acid (DNA) molecule. Mammalian and bacterial cells, on the one hand (Djordjevic and Szybalski, 1960; Greer, 1960; Lorkiewicz and Szybalski, 1960), and phages on the other hand (Kozinski and Szybalski, 1959; Litman and Pardee, 1960), become highly sensitive to the lethal effects of ultraviolet light (UV) as the result of such a substitution. Since 5-bromodeoxyuridine (BUdR) replaces thymidine, a DNA component, it became important to test directly whether purified BUdR-labeled DNA is radiosensitized to a similar extent as the intact cells from which it is extracted. This experiment became feasible, when it was shown by Szybalski et al. (1960) that it is possible to separate and purify by centrifugation in the CsCl gradient a BUdR-labeled DNA from Bacillus subtilis cells, the biological activity of which can be quantitatively assayed since it retains full transforming capacity.

EXPERIMENTAL. - A BUdR-labeled culture of B. subtilis (wild-type) was prepared in the manner described earlier (Szybalski et. al, 1960), employing a minimal medium (glucose-4g; $MgSO_4.7H_2O$-0.2g; citric acid. H_2O-2g; K_2HPO_4-10g; $NaNH_4HPO_4.4H_2O$-3.5g; water up to 1L; pH-7) (Vogel and Bonner, 1956), supplemented with 200 µg/ml of BUdR, 4µg/ml of 5-fluorodeoxyuridine (FUdR), and 50 µg/ml of deoxycytidine (CdR). The latter ingredient, which was not previously employed in the medium designed on rational biochemical grounds by Lorkiewicz and Szybalski (1960), reversed the partially inhibitory effect of BUdR without affecting BUdR incorporation. Since at least part of the toxic effects

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of thymidine analogs could be ascribed to inhibition of the reductive conversion of cytidine diphosphate to deoxycytidine diphosphate by the phosphorylated form of BUdR, in analogy with the thymidylate inhibition of this reaction reported by Reichard et al. (1960), it is logical that CdR addition should restore the supply of deoxycytidylates for DNA synthesis.

The UV sensitivities of the BUdR-labeled and BUdR-free transforming DNA were determined either by irradiation of (1) extracted DNA or of (2) intracellular DNA (irradiation of whole cells followed by extraction of DNA), and assaying the surviving transforming activity for the indole marker, employing an indole-receptor strain under conditions outlined by Szybalski et al. (1960). To create comparable conditions for extracellular irradiation of DNA, the optical density of the DNA solution (260 mμ) was adjusted to the same level ($O.D._{260}=4.5$) as that of the cell suspension. Both were irradiated under two sets of conditions: in 60 mm Petri dishes on an orbital shaker or in 10 cm long quartz capillaries (I.D. = 1 mm); the shape of the survival curves was essentially the same whichever irradiation method was employed. The samples for measuring cell survival (Fig. 1) were obtained from the same cell pool which served for extraction and assay of intracellularly irradiated transforming principle. The extracted DNA was irradiated and its activity assayed after various degrees of purification: as (1) a lysozyme-sodium lauryl sulfate lysate, alcohol precipitated, redissolved in 0.15N NaCl, and deproteinized, (2) a RNase-treated and dialysed preparation, and (3) a pooled fraction banding in the CsCl gradient, at the densities of 1.740 to 1.748 (BUdR-labeled) or 1.700 to 1.705 (control) as illustrated in the previous paper of this series (Szybalski et al, 1960).

RESULTS AND DISCUSSION. - It is evident in Figure 1 that BUdR incorporation into cell DNA sensitizes to a similar degree both intact cells and the DNA extracted from these cells and purified by CsCl-gradient centrifugation to remove any unlabeled DNA. This observation confirms two notions: (1) exposure of cells to BUdR sensitizes exclusively, or highly preferentially, their DNA component, and (2) DNA is principal target of the lethal radiation effects. Should the latter not be the case, the radiosensitization of DNA by BUdR labeling, as measured by assaying its transforming activity, would not be reflected by a similar degree of sensitization for whole BUdR-labeled cells, as illustrated in Figure 1. Analogous experiments employing X-radiation lead to essentially identical conclusions, and seem to provide conclusive evidence that DNA is the most radiosensitive of the indispensable cell components. This is not in

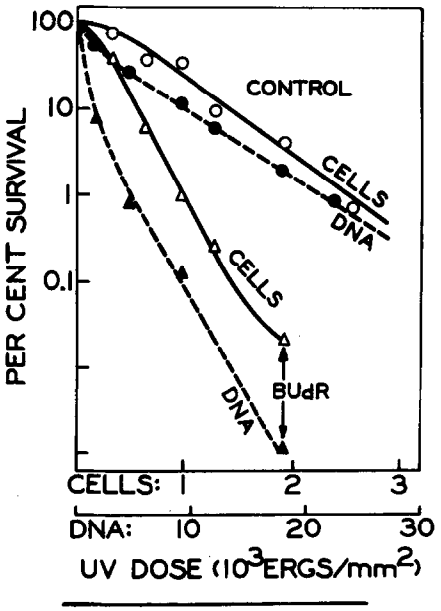

Figure 1. UV survival (colony-forming ability) of B. subtilis wild-type cells (solid lines) grown for 3.5 hrs. in the presence (BUdR) or in the absence (control) of BUdR (200 μg/ml) and FUdR (4 μg/ml), washed with saline, and irradiated at 4°C in a 2 mm layer of stirred suspension ($O.D._{260}$ = 4.5) employing a Westinghouse Sterilamp G15T18 (16 cm distance); and UV inactivation of transforming DNA (broken lines) isolated from the above described BUdR-free (control) and BUdR-labeled (BUdR) cells, deproteinized, RNase treated, purified by CsCl-gradient centrifugation, irradiated in quartz capillaries at $O.D._{260}$ = 0.26, and measured as ability to transform indole requiring (ind^-) receptor cells to prototrophy. The actual UV dose delivered to the cells or DNA (Figures 1 and 2) was calculated according to Morowitz (1950).

disagreement with the observation on the high sensitivity of new enzyme synthesis to X-radiation (Bollum et al., 1960), since the presence of undamaged DNA might have to be postulated to account for new protein synthesis.

To compare the sensitivity of transforming DNA under conditions of intracellular and extracellular irradiation, the cell suspension was divided into two equal portions and irradiated under conditions of identical UV absorbancies either as extracted DNA (first aliquot) or as a whole cell suspension (second aliquot). DNA was extracted from the latter immediately after irradiation, and the transforming activity was assayed. The intracellularly (in vivo) irradiated DNA seems to be only slightly more sensitive than extracellularly (in vitro) irradiated DNA (Fig. 2), less so than reported for Pneumococcus by Ellison and Beiser (1960). It is not quite certain, however, whether the actual UV doses delivered to the DNA, in vitro and in vivo, were identical. The radiosensitizing effect of incorporated BUdR was practically identical under both conditions (Fig. 2). The small difference in the shape of the UV-survival curves for the CsCl-gradient purified (Fig. 1) and for the unfractionated (Fig. 2) BUdR-labeled DNA, at higher UV doses, could be ascribed to the inhomogeneity of the latter as to BUdR labeling.

In summary, it might be concluded that halogenation of DNA radiosensitizes intact cells to a similar extent as transforming DNA

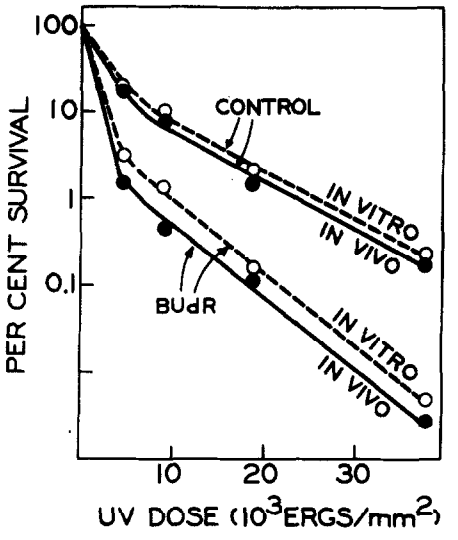

Figure 2. UV inactivation of transforming DNA isolated under standardized conditions (lysozyme + Na.lauryl sulfate cell lysis, alcohol precipitation, single $CHCl_3$ deproteinization) from BUdR-free and BUdR-labeled cells (referred to in Figure 1), irradiated either prior to extraction from the cells (in vivo) or after extraction (in vitro). Both cell suspensions and extracted nucleic acid solutions were adjusted $O.D._{260} = 4.5$ and irradiated in 2 mm layers as described in Figure 1.

extracted from the same cells. This observation constitutes evidence that DNA is the most radiosensitive cell component and thus the principal target of lethal radiation effects. This conclusion, especially as related to the molecular basis of X-ray effects, will be the subject of a future publication.

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