GENETIC EFFECTS OF HALOGENATED THYMIDINE ANALOGS INCORPORATED DURING THYMIDYLATE SYNTHETASE INHIBITION*

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Received May 19, 1960

Halogenated thymine analogs, 5-chloro-, 5-bromo-, and 5-iodouracil, are incorporated into the deoxyribonucleic acid (DNA) of thymine-deficient bacteria (Zamenhof and Griboff, 1954; Dunn and Smith, 1957). Incorporation into the DNA of prototrophic strains was negligible, but was shown to be increased either by the use of deoxyribosylated analogs or by interference with the endogenous methylation of deoxyuridylic acid by aminopterin or sulfanilamide (Zamenhof et al., 1958; Dunn and Smith, 1957).

The purpose of the present studies was to evaluate the consequences of the incorporation of 5-bromo-(BUDR) and 5-iododeoxyuridine (IUDR) into the DNA of streptomycin (SM) dependent, thymidine-non-requiring strain Sd4 of Escherichia coli, used extensively in our earlier mutagenicity studies (Iyer and Szybalski, 1958).

To create artificially conditions of thymidine deficiency, 5-fluoro-deoxyuridine (FUDR) was employed, an analog known to inhibit irreversibly

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*This research was supported in part by the National Science Foundation.

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thymidylate synthetase (Heidelberger et al., 1957; Cohen et al., 1958). FUDR alone (0.25-0.50 μg/ml) inhibits bacterial growth, an effect which is partially reversed by 50-200 μg BUDR per ml (Fig. 1). Logarithmically growing cells were transferred into SS medium (0.01 g KH_2PO_4; 0.021 g Na_2HPO_4; 0.3 g $MgSO_4.7H_2O$; 3 g NH_4Cl; 3 g Bacto vitamin-free casamino acids; 30 g glycerol; 0.3 ml 1M $CaCl_2$; 1000 ml dist. water) supplemented with 200 mg BUDR or IUDR and 5 mg FUDR, and propagated for two to three generations. Under these conditions BUDR replaced 51% and IUDR 43% of the thymidine in the bacterial DNA. In the absence of FUDR, substitution occurred to the extent of only 15.6% and 19% respectively.

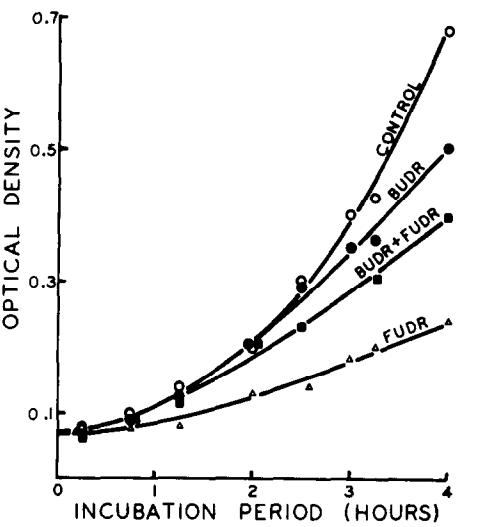

Figure 1. Inhibition of E. coli Sd4 by FUDR (0.5 μg/ml) (Δ) and its partial reversal by BUDR (200 μg BUDR/ml + 0.5 μg FUDR/ml) (■), together with growth curves for BUDR-supplemented (200 μg/ml) (●) and BUDR-free (O) control cultures.

BUDR- or IUDR-labeled cells of E. coli Sd4 do not generally exhibit significantly higher mutation rates toward SM independence, as assessed by methods described earlier (Iyer and Szybalski, 1958), although under some conditions two-fold increases in the frequency of SM-independent mutants were observed among FUDR-, FUDR + BUDR-, or FUDR + IUDR-grown cells. Thus the loci controlling mutation to SM independence in this strain are not susceptible to the mutagenic effect of BUDR or IUDR to the

same extent as certain loci in phage (Freese, 1959) or bacteria (Zamenhof, 1959).

Although no clearly perceptible direct mutagenic effect was observed with BUDR or IUDR, it was interesting to determine whether these analogs affect in any way the ultraviolet light (UV)-initiated mutagenic process. It was reported by Greer and Zamenhof (1957) that 5-bromouracil-grown bacteria become highly sensitive to UV light. The latter effect was

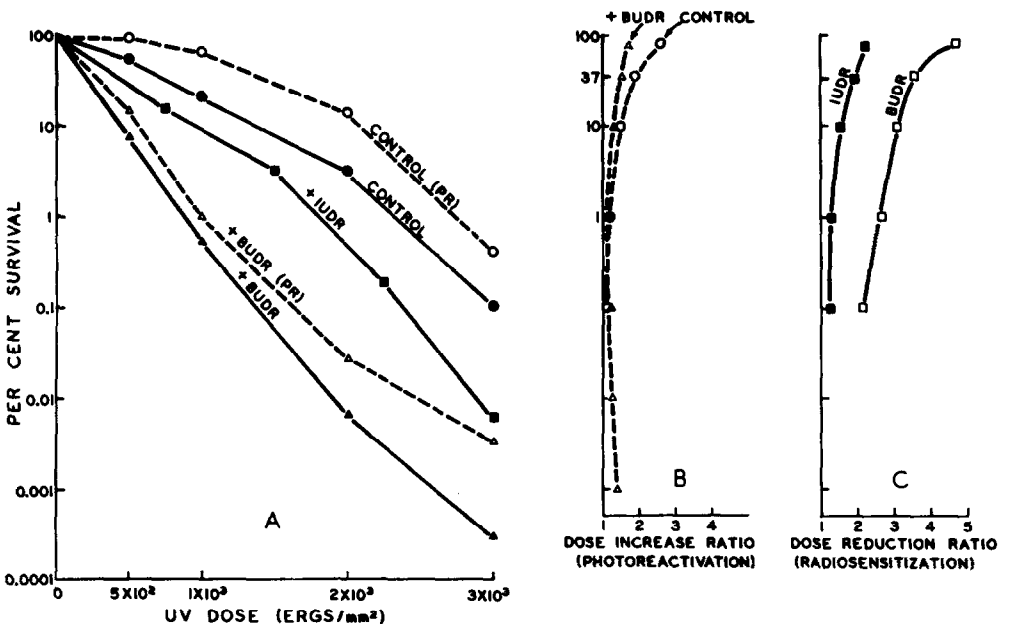

Figure 2. A: UV survival before (solid lines) and after photoreactivation (broken lines) of E. coli Sd4 grown in the absence (control) or in the presence of BUDR or IUDR (200 μg/ml) on a SM (20 μg/ml) and FUDR (0.5 μg/ml) supplemented medium.
B: Graphic comparison of photoreactivability for the control and for the BUDR-grown cultures. Each point represents a UV dose ratio for identical survivals of the photoreactivated and non-reactivated cells, as determined from Figure 2A.
C: UV sensitization of BUDR- and IUDR-grown cells. Each point represents a UV dose ratio for identical survivals of the control and of the BUDR- or IUDR-supplemented cultures.

clearly confirmed with E. coli Sd4 grown in the presence of FUDR and BUDR or IUDR (Fig. 2), and irradiated with a Westinghouse Sterilamp G15T18 (20°C, saline suspension). The resulting radiosensitization, expressed as

the UV dose ratio for identical survivals of normal and analog-grown cells (dose reduction ratio), amounted to as much as 4.7 (50 mol % BUDR substitution) or 2.2 (43 mol % IUDR substitution) (Fig. 2c).

The mutagenicity of UV light was related to its bactericidal effect, with essentially no differences observed between normal and analog-labeled cells (Fig. 3).

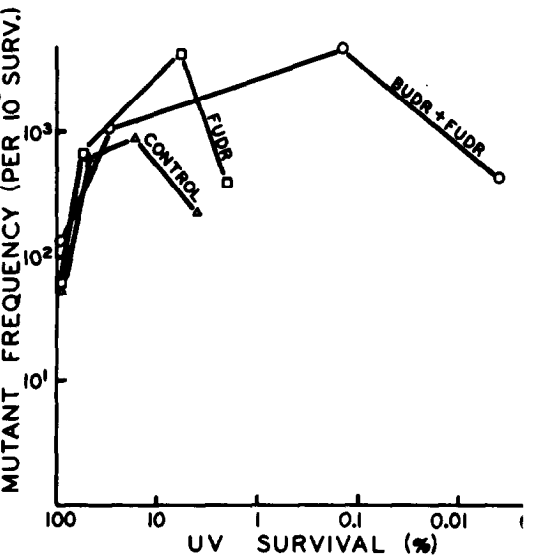

Figure 3. Frequency of UV-induced mutants in E. coli Sd4 cultures grown in the absence or presence of FUDR (0.5 μg/ml) alone or in combination with BUDR (200 μg/ml), and plotted as a function of UV survival.

While attempting to determine the frequency of mutants among photoreactivated cells, it was observed that BUDR-labeled cells are slightly less photoreactivable (cells suspended in saline; 2 fluorescent 20 Watt tubes; 8 cm distance; 2 hr; 37 °C) than normal cells (Fig. 2A, 2B), in agreement with the observations of Greer (1960) and Okun and Stahl (private communication). Neither the normal nor the BUDR-labeled cells were inactivated by white fluorescent light under the conditions employed.

The effect of BUDR and IUDR incorporation on radiation sensitivity prompted us to investigate the effect of other agents known to modify DNA structure. It was found that E. coli Sd4 cells treated with triethylenemelamine (TEM) (2 hr, 37 °C, 10 μg TEM per ml of distilled water, followed by 2 hr growth in

nutrient medium), a powerful bacterial mutagen and an alkylating agent capable of chemically modifying the pyrimidine components of DNA (Lorkiewicz and Szybalski, 1960), become more sensitive to UV light (10-fold lower survival at 3×10^3 ergs/mm^2). Similarly, FUDR (0.5 μg per ml) and another presumptive thymidilate synthetase inhibitor, mitomycin (0.1 μg per ml; gift of Dr. J. Lein, Bristol Laboratories, Syracuse, N. Y.) effect slight increases in the UV sensitivity of cells exposed to these compounds for 2 hr (37°C) in SS medium supplemented with 10% nutrient broth.

It was reported by Greer (1960) that 5-bromouracil-substituted bacteria are more sensitive to the lethal effect of elevated temperatures. The relationship between this phenomenon and the heat lability of the modified DNA molecule was examined. The melting temperature (Marmur and Doty, 1959) was determined for normal and BUDR-labeled DNA (51% BUDR incorporation), extracted from Duponal-lysed cells, deproteinized with chloroform and butanol, and freed of RNA by digestion with RNase followed by dialysis and alcohol precipitation. The measurement, performed in a Beckman DU spectrophotometer equipped with thermospacers (0.15M NaCl, 0.015M sodium citrate), revealed no significant difference between the "melting" temperatures of normal and BUDR-labeled DNA (91.5 and 89.0°C, respectively).

In summary, it was found that the thymidylate synthetase inhibitor, FUDR, greatly increases the incorporation of the thymidine analogs BUDR and IUDR into the DNA of E. coli Sd4. This substitution has essentially no mutagenic effect on the Sd locus, although it renders the cells highly UV-sensitive and less photoreactivable.

Acknowledgments: We are indebted to Dr. A. W. Koziński for helpful discussions and to Miss Clara Biró for the skillful assistance.

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