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5-Bromouracil Utilization by *Bacillus subtilis*

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The replacement of thymine (T) in deoxyribonucleic acid (DNA) by 5-bromouracil (BU) (F. Weygand, A. Wacker, and H. Dellweg, Z. Naturforsch. **7b**:19, 1952) is an aid in density separation of types of DNA, and in studies on radiobiology and mutagenesis. The usefulness of BU is, however, limited by its toxic effects. Extensive substitution in DNA of T by BU is lethal for most *Escherichia coli* and *Bacillus subtilis* strains. To decrease this toxicity, Oishi and Sueoka (Proc. Natl. Acad. Sci. U.S. **54**:483, 1965) suggested adding T to BU-containing media. Because of an apparent heterogeneity among a population of competent *B. subtilis* (E. W. Nester and B. A. D. Stocker, J. Bacteriol. **86**:785, 1963; W. F. Bodmer, J. Mol. Biol. **14**:534, 1965), it was necessary, before using this approach for our studies, to determine whether *B. subtilis* would preferentially utilize thymidine (TdR) as compared with 5-bromodeoxyuridine (BUdR). If there were a preference, then growth of *B. subtilis thy*$^-$ strains in a medium containing a mixture of T and BU could greatly complicate the interpretation of small density differences in the DNA isolated from such a population of cells.

TABLE 1. *Buoyant densities of DNA isolated from Bacillus subtilis cultures containing various mixtures of thymidine (dT) and 5-bromodeoxyuridine (dBU), or thymine (T) and 5-bromouracil (BU)*

Sample	dT/dBU (A–E) or T/BU (1-3) in medium	Buoyant density of bifilarly labeled DNA (g cm^{-3})	
		Expected	Observed
A	50/0	1.703	1.703
B	25/25	1.753	1.709
C	10/40	1.783	1.735
D	5/45	1.793	1.769
E	0/50	1.803	1.803
1	50/0	1.703	1.703
2	25/25	1.753	1.707
3	10/40	1.783	1.719

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The assay for BU incorporation involved CsCl density gradient equilibrium centrifugation (pycnography) of DNA extracted from *B. subtilis* cultured with various mixtures of T and BU, or TdR and BUdR. The increase in buoyant density of the DNA is a measure of the extent of BU substitution for T (R. L. Baldwin and E. M. Shooter, J. Mol. Biol. **7**:511, 1963). The ratio of T to BU in the DNA, when compared with the corresponding ratio in the culture medium, reflects the overall preference of the cells for the two bases.

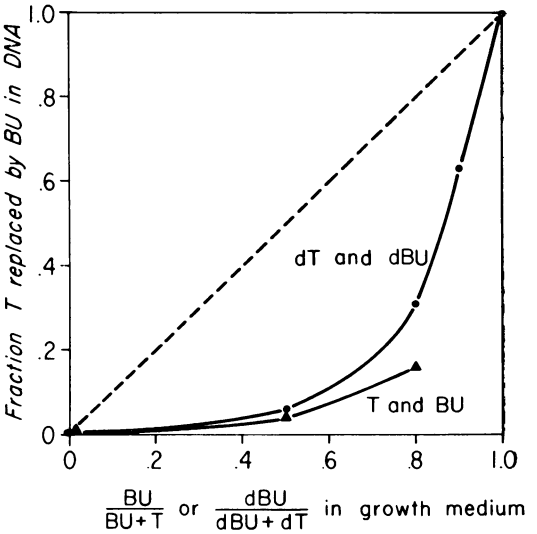

FIG. 1. *Fraction BU (BUdR) in growth medium versus fraction T replaced by BU in DNA. Data are taken from Table 1. The dashed line represents the expected curve if BU (BUdR) and T (TdR) were incorporated at random.*

Materials and methods have been described (W. F. Bodmer, J. Mol. Biol. **14**:534, 1965). An overnight culture of SB747 (*try*$_2^-$ *his*$_2^-$ *thy*$^-$), a thymine-requiring mutant of *B. subtilis* 168 (J. L. Farmer and F. Rothman, J. Bacteriol. **89**:262, 1965), was resuspended up to 2 × 10^7 cells/ml in Spizizen minimal medium containing glucose (0.5%), casein hydrolysate (0.02%), DL-tyrosine, DL-tryptophan, and DL-histidine

(each at 20 μg/ml), and *p*-hydroxybenzoic acid and *p*-aminobenzoic acid (each at 0.2 μg/ml). To portions of the culture, mixtures of BUdR and TdR, or BU and T, were added to a total concentration of 50 μg/ml. Cells were harvested after approximately three optical density doublings; DNA was extracted by the method of J. Marmur (J. Mol. Biol. **3**:208, 1961), and the density of the bifilarly BU-labeled DNA was determined (model E analytical ultracentrifuge). Densities were calculated from the equation $\frac{d\rho}{dr} = \frac{\omega^2 r}{\beta}$ (J. B. Ifft, D. H. Voet, and J. R. Vinograd, J. Phys. Chem. **65**:1138, 1961), with dAT ($\rho = 1.678$ g cm^{-3}) used as a density marker.

The densities of the DNA preparations are given in Table 1, and are considerably less than would be expected if BUdR and TdR (T and BU) were utilized randomly by SB747. In Fig. 1, the ratio of the components in the medium is plotted versus the fraction T replaced by BU in the DNA, illustrating the marked preference for T or TdR.

It should be noted that the preference for T compared with BU is greater than the preference for TdR compared with BUdR. Possibly this is a consequence of the increased number of opportunities for selectivity during the utilization of the free bases in the synthesis of DNA.

Similar conclusions with respect to preferential utilization of T compared with BU were implicit in the experiments of Weygand, Wacker, and Dellweg (Z. Naturforsch. **7b**:19, 1952) and have recently been extended by Hackett and Hanawalt (Biochim. Biophys. Acta **123**:356, 1966) using *E. coli* TAU-bar. Presumably this process is another aspect of a general mechanism of selective utilization of natural metabolites compared with their analogues (E. S. Kempner and D. B. Cowie, Biochim. Biophys. Acta **42**:401, 1960).

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