RELATIVE STABILITY TO THERMAL DENATURATION OF DEOXYRIBONUCLEIC ACID (DNA) PREPARATIONS CONTAINING BROMODEOXYURIDINE *

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The data to be presented show that bromouracil containing DNA preparations are heat denatured at higher temperatures than normal DNA. The thymidine analog, bromodeoxyuridine, does not inhibit the respiration, glycolysis, protein, RNA, serine, or thymidine synthesis of cells but inhibits competetively the incorporation of thymidine into DNA (Kit, Beck, Graham, and Gross, 1958) and is itself incorporated in place of thymidine into bacterial, bacteriophage, or animal cell DNA (Dunn and Smith, 1957; Djordjevic and Szybalski, 1960; Eidinoff, Cheong, and Rich, 1959; Wacker, Trebst, and Jackerts, 1954; Zamenhof and Griboff, 1954). Cells containing bromodeoxyuridine in their DNA are more susceptible to growth inhibition by radiation than normal cells (Djordjevic and Szybalski, 1960). Bromodeoxyuridine is an established mutagenic agent (Litman and Pardee, 1959). Presumably, it induces errors in DNA replication as a result of which adenine-thymine base pairs are replaced by guanine-cytosine base pairs in the DNA (Freese, 1959).

Strain LM mouse cells and strain B14FAF28 Chinese hamster cells have been cultivated in a medium containing 5, 10 or 25 micrograms per ml. of bromodeoxyuridine. Hsu and Somers (1961) have shown that chromosome breakage is induced after the Chinese hamster cells have been grown for 12 hours or longer in 25 micrograms per ml. of bromodeoxyuridine and a great majority of the breaks are found at one spot, the secondary constriction.

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Chromosome breakage is abundant in LM cells after four to five days of treatment, but after 12 weeks of growth in bromodeoxyuridine the breakage frequency is definitely abated. In confirmation of the experiments of Djordjevic and Szybalski (1960) we have observed in sedimentation equilibrium experiments (Meselson, Stahl, and Vinograd, 1957) that strain LM cells which have been growing for five days in a bromodeoxyuridine medium contain no normal density DNA molecules (ρ^{25° = 1.700 and 1.689 gcm^{-3}) but only molecules whose buoyant densities correspond to hybrid "unifilarly" and heavy "bifilar" substituted DNA. After thirteen weeks or more of growth in bromodeoxyuridine medium, all the DNA is "heavy" (ρ^{25° = 1.73 to 1.77), even though the chromosome breakage frequency is abated and the cells resume normal growth rates. The proportion of dead cells in the bromodeoxyuridine treated population is no greater than that in the untreated population (2-8%).

The sedimentation coefficients, intrinsic viscosities, and extinction coefficients indicate that the bromodeoxyuridine substituted DNA preparations have a high molecular weight and are undenatured (Table 1). The ultraviolet absorption versus temperature curves are, however, abnormal. After 22 hours of growth in bromodeoxyuridine medium, the T_m value is slightly elevated from 88.0° to 88.7° and subsequently, the T_m value increases further to 90° - 91°. In the foregoing experiments, the DNA was dissolved in a buffered solution of 0.15M NaCl. The T_m values of bromodeoxyuridine substituted DNA, however, were greater than normal when the DNA was dissolved in other NaCl solutions. In a 0.075M NaCl solution, the T_m values of normal and bromodeoxyuridine substituted DNA were 82.9° and 86.2°, respectively; whereas, in 0.21M NaCl, the T_m values were 92.1° and 94.8°.

It is improbable that the enhanced T_m value is due to gross changes in the base composition or sequence of the DNA, although some guanine-cytosine substitution (Freese, 1959) and some distortion of the nucleotide sequences (Shapiro and Chargaff, 1960) cannot be excluded. Table 2 shows that the

TABLE 1

Physical Properties of DNA from Tissue Culture Cells Grown in the Presence of 10 μg/ml of Bromodeoxyuridine (BrUDr)

Cell Type	$S_{20,w}$	$[\eta]^{26^\circ}$	$E^{1\%}_{1cm}$
LM	28.4 S	60 dl/g	206
LM (BrUDr)	26.2 S	55 dl/g	207

DNA solutions were dissolved in 0.15M NaCl, 10^{-3}M versenate, 10^{-3}M phosphate, pH 7.

increase in the T_m value occurs after only 1-3 cell divisions and that it may be readily reversed. Cells which had been grown for 70-168 days in bromodeoxyuridine medium were transferred to normal growth medium for 12 to 17 days. The T_m values were only slightly greater than normal (Table 2). Moreover, after density gradient centrifugation in CsCl, the banding of the DNA from the cells which had been grown in bromodeoxyuridine medium and then returned to normal growth medium was indistinguishable from that of the DNA of untreated cells. This finding also eliminates the possibility that DNA substitution by bromodeoxyuridine results in a selective destruction of DNA molecules rich in adenine and thymine.

Possibly, the change in T_m is a more direct consequence of the presence of bromouracil in the DNA. Consistent with this hypothesis are the recent experiments of Baldwin, Inman, and Wake (1960, and personal communications), which show that in solutions of low ionic strength, the T_m value of the enzymatically synthesized adenine-bromouracil polymer is approximately 10° higher than that of the adenine-thymine polymer. At higher ionic strengths, the difference in T_m decreases. The substitution of the bromine atom for the methyl group of thymine might alter the electron distribution in the bromouracil ring so as to strengthen the hydrogen bonding to the purine base of the complementary DNA chain. The T_m value of DNA varies with the cation concentration of the solution. Therefore, the possibility should

TABLE 2

Properties of DNA from LM Cells Grown in the Presence of Bromodeoxyuridine (BrUDr) (10 μg/ml)

Cells	Time in BrUDr	T_m +	2σ +	% Hyperchromicity
LM (normal)	0	88.0°	5.8°	41.3
LM (BrUDr)	22 hours	88.7°	6.0°	40.0
LM (BrUDr)	46 hours	90.0°	5.5°	42.1
LM (BrUDr)	5 days	90.1°	5.8°	42.6
LM (BrUDr)	91 days	89.8°	5.7°	44.7
LM (BrUDr)	158 days	91.3°	5.5°	41.1
LM (BrUDr)*	161 days	91.4°	6.1°	42.1
LM (BrUDr)	70 days, then 12 days in normal growth medium	88.6°	5.4°	42.3
LM (BrUDr)*	168 days, then 17 days in normal growth medium	88.5°	5.7°	39.4

* 25 μg/ml

+ T_m defined as the temperature corresponding to the midpoint of the thermally induced absorbance increase. 2σ defined as the temperature range corresponding to 17-83% of the absorbance increase (Kit, 1960). DNA solutions were dissolved in 0.15M NaCl, 10^{-3}M versenate, 10^{-3}M phosphate, pH 7.

also be considered that the bromouracil modifies the interaction between cations and DNA, thereby increasing the T_m.

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