INCORPORATION OF TRITIUM INTO IRRADIATED NUCLEOTIDES

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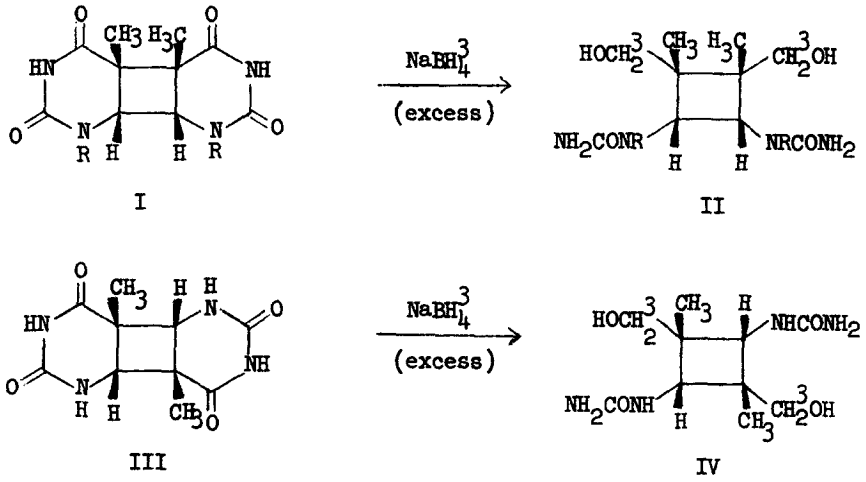
Photodimers I and III of thymine (R = H) and thymidine (R = deoxyribose)



I II III IV

are smoothly hydrogenolyzed to cyclobutane derivatives (II and IV) by sodium borohydride in aqueous solution at room temperature (Kunieda and Witkop, 1967). We have now applied this reaction to the hydrogenolysis of irradiated nucleotides with sodium borotritide.

In the control experiments non-irradiated polyadenilic and polyuridylic acids were incubated with sodium borotritide at room temperature and 60° for 5 to 24 hr (Table I). All the samples which were repeatedly flash-evaporated with the addition of distilled water initially retained considerable radioactivity. It was necessary to digest the samples 3-4 times with 2.0 N

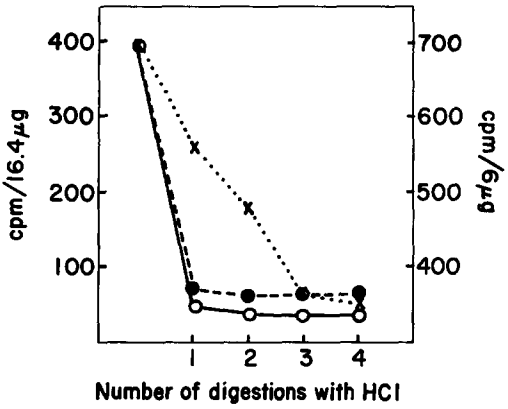


Fig. 1.-Relative residual activities (left ordinate) of (unirradiated) samples of poly A (-o-o-) and poly U (-●-●-) reach constant values after 3-4 digestions with 2 N HCl and evaporation. The constant radioactivity (·x·x·) of hydrogenolyzed cis-syn-thymine-dimer (right ordinate) is more than ten times as high.

hydrochloric acid overnight and then to evaporate in order to arrive at acceptable background values (Fig. 1). The residual tritium activity was fairly independent of the conditions of incubation of nucleotides with $NaBH_4^3$ (Table I) and appears to be a non-volatile impurity of the borotritide (cf. Cerutti, Holt and Miller, 1968). When thymidine, cis-syn-thymidine photodimer (I) and cis-anti-thymidine dimer (III) were treated with $NaBT_4$ in 50% aqueous

Table I

Residual Background Activity of Samples of Poly (U) and Poly (A) After Incubation with $NaBH_4^3$ under Various Conditions and After Four Digestions with 0.1 N HCl and Repeated Flash-evaporations.

The figures are cpm/16.4 µg (cf. Fig. 1).

	Poly (A)			Poly (U)		
	20° 24 hr	60° 5 hr	60° 24 hr	20° 24 hr	60° 5 hr	60° 24 hr
H_2O	56	55	64	96	82	84
50% Dioxan	57	59	45	62	70	70

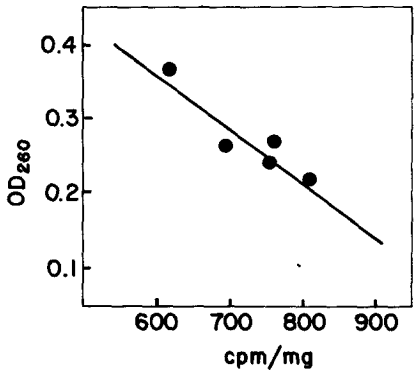


Fig. 2.-Incorporation of tritium into irradiated poly-U as a function of photodimerization as determined by decrease in optical density at λ_{max} 260 mμ.

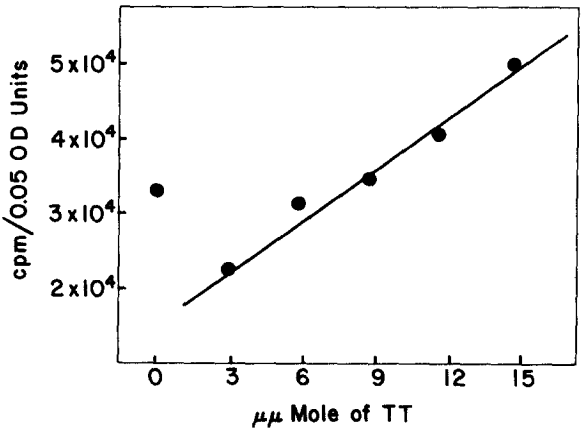


Fig. 3.-Incorporation of tritium into irradiated DNA (E. coli). The high control value is probably due to a nonvolatile impurity in commercial borotritide.

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dioxan at 50° overnight, and digested and evaporated three times with hydrochloric acid, the residual activities were 180 cpm/μmole for thymidine as the control substance, and more than 30 times as high, namely, 6400 and 5900 cpm/μmole, respectively, for the ureido alcohols II and IV.

On the strength of this encouraging result irradiated samples of polyuridylic acid and DNA from E. coli were hydrogenolyzed by $NaBT_4$ in 50% dioxan at 50-60° overnight. The irradiated samples of poly (U) attained constant activity after three acid treatments followed by evaporation. When radio-

activity was plotted versus concentration of uracil dimers ($\widehat{UU}$) an essentially linear relationship resulted (Fig. 2). When the irradiated samples of DNA were plotted in a similar fashion the introduction of tritium correlated with the time of irradiation (Fig. 3).

These preliminary results suggest the hydrogenolysis of irradiated nucleotides and nucleic acids by sodium borotritide as a feasible method for marking and modifying the areas of photodimerization with tritium (cf. Witkop, 1968). In addition, with better control of residual background activity in unirradiated samples, the method should lend itself to a quantitation of photodimerizations and to differentiation between cyclobutane and other dimers (Stafford and Donellan, 1968; Wang and Varghese, 1968).

REFERENCES

Cerutti, P., Holt, J. W., and Miller, N., J. Mol. Biol., 34, 505 (1968).
Kunieda, T., and Witkop, B., J. Am. Chem. Soc., 89, 4232 (1967).
Stafford, R. S., and Donellan, J. E., Proc. Nat. Acad. Sci., 59, 822 (1968).
Wang, S. Y., and Varghese, A. J., Science, 160, 186 (1968).
Witkop, B., Photochem. Photobiol., 7, 813 (1968).