Kinetic Isotope Studies on the Inactivation of Transforming Deoxyribonucleic Acid, Tobacco Mosaic Virus, and Its Nucleic Acid by Ultraviolet Light*

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ABSTRACT: The effect of solvent on the inactivation of transforming deoxyribonucleic acid (DNA), tobacco mosaic virus, and its nucleic acid by ultraviolet irradiation at 253.7 mμ was investigated. The rate of inactivation of tobacco mosaic virus ribonucleic acid in heavy water was slower than in light water. The ratio of the rate constants, K_{H_2O}/K_{D_2O}, is about 1.9. Ribonucleic acid irradiated in either light or heavy water photoreactivated to the same extent. Irradiation of transforming DNA in either light or heavy water did not affect the rate of inactivation. Based on the observations of the irradiations of pyrimidine derivatives in light and heavy water, it is suggested that the addition of a water molecule to the 5,6 double bond of the pyrimidine ring may be the cause of inactivation of tobacco mosaic virus ribonucleic acid. In the case of transforming DNA, the photochemical reaction leading to inactivation does not appear to be directly caused by reaction with solvent but probably by the photodimerization of the pyrimidine residues. Irradiation of intact tobacco mosaic virus in either light or heavy water exhibited no kinetic isotope effect. Thus inactivation of intact virus may result from a mechanism different from that of the free nucleic acid.

The effects of ultraviolet irradiation on bacteria, viruses, viral nucleic acids, and transforming DNA have been the subject of extensive investigations in recent years. Irradiation of these materials at wavelength 253.7 mμ causes a dose-dependent inactivation of biological activity. Soon after the discovery by Beukers and Berends (1960) that thymine can be dimerized by ultraviolet irradiation in frozen solution, thymine dimer was isolated from ultraviolet-irradiated DNA (Beukers *et al.*, 1960). This suggests that formation of thymine dimer is possibly one of the chemical consequences leading to damage in DNA. The biological importance of this lesion has been further demonstrated by Setlow and Setlow (1962) and Wulff and Rupert (1962). The effect of ultraviolet irradiation on RNA is still obscure. In 1949, Sinsheimer and Hastings reported the reversible photochemical alteration of uracil and cytidylic acid. Moore (1958) subsequently showed that this reaction involved the reversible hydration of the 5,6 double bond of uracil to yield 6-hydroxy-5-hydrouracil. It is possible that both ultraviolet-induced dimerization and hydration of the pyrimidines may contribute to the damage in RNA.

The present paper deals with experiments designed to determine the chemical effects leading to the inactivation of TMV and TMV-RNA by ultraviolet light. The effect of ultraviolet irradiation on transforming DNA was also investigated using similar experimental conditions.

Materials and Methods

Preparation of DNA. Transforming DNA was prepared from the prototrophic strain WB746 of *Bacillus subtilis* according to the procedure of Marmur (1961). After deproteinization with chloroform–octanol, the preparation was treated with 50 μg/ml of RNase, which had been boiled to destroy any DNase. Following further deproteinization, the sample was precipitated in 95% ethanol and stored in 0.14 M NaCl and 0.01 M sodium citrate at −20°.

Preparation of Virus. U-1 and U-2 strains of TMV were grown in Turkish tobacco plants for about 2 weeks, after which the leaves were harvested and frozen at −20°. Virus was obtained and purified from these frozen leaves by differential centrifugation (Knight, 1962). The virus were stored at 4° in water.

Preparation of Infectious TMV-RNA (U-1). Infectious TMV-RNA of U-1 strain was prepared from the virus by the standard phenol extraction method of Haschemeyer *et al.* (1959). The RNA was stored in water at either −20 or −80°.

Ultraviolet Irradiation of Transforming DNA, TMV, and TMV-RNA. Irradiations were carried out with a Mineralight V-41 low-pressure mercury vapor lamp

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equipped with a Corning filter no. 9863 (Ultra-Violet Products Inc., San Gabriel, Calif.). The principal wavelength emitted is at 253.7 mμ. The energy of emission measured at a distance of 43 cm from lamp source was 131 ergs/mm² per min. Dosimetry was performed by a photocell calibrated using potassium ferrioxalate as an actinometer (Hatchard and Parker, 1956).

Samples (1.5–2.0 ml) of transforming DNA, virus, or viral nucleic acid were irradiated with shaking at 4° in open Petri dishes (5 cm in diameter) at a distance of 43 cm from the light source. Transforming DNA was irradiated at a concentration of 5.5 μg/ml. U-1 and U-2 TMV were irradiated at concentrations of 1.0 and 1.6 μg/ml, respectively, and U-1 TMV-RNA at concentrations of 20–50 μg/ml made up in either water or deuterium oxide (purchased from Bio-Rad Laboratories, Richmond, Calif.). At different time intervals, appropriate aliquots of the irradiated solutions were withdrawn for assay. In all experiments, transforming DNA, virus, or viral nucleic acid dissolved in water and in deuterium oxide were irradiated simultaneously.

Ultraviolet Irradiation of Model Compounds. Thymine was purchased from California Corp. for Biochemical Research, Los Angeles, Calif. CMP[1] was obtained from Pabst Laboratories, Milwaukee, Wis. Polyuridylic acid and polycytidylic acid which were obtained from Miles Chemical Co., Elkhart, Ind., were the gifts of Dr. E. W. Davie of this department. $(pT)_4$ was the gift of Dr. P. T. Gilham, Purdue University, Ind.

Thymine, CMP, and poly C were irradiated at concentrations to give an initial optical density at the principal maximum of 0.8–1.0. Irradiations were carried out with a Chromato-Vue C-81 short-wave lamp equipped with filters (Ultra-Violet Products Inc., San Gabriel, Calif.). The principal wavelength emitted is at 253.7 mμ. The energy of emission at a distance of 10 cm from the lamp source was 4.6×10^3 ergs/mm² per min.

Irradiation of poly U and $(pT)_4$ was carried out with a Mineralight V-41 low-pressure mercury lamp equipped with a Corning filter no. 9863 (similar results were obtained when a Chromato-Vue short-wave lamp was used). The energy output measured at a distance of 15 cm from the lamp source was 8.3×10^2 ergs/mm² per min. Poly U was irradiated at a concentration of 0.5 mg/ml. $(pT)_4$ was irradiated at concentrations to give an initial optical density of 0.8–1.4 at the principal maximum.

In all experiments, 2.0–2.5 ml of H_2O and D_2O samples were irradiated simultaneously with shaking at 4° in closed quartz dishes (4.5 cm in diameter) fitted with glass stoppers to prevent excessive evaporation during the course of irradiation. At different time intervals, the irradiated samples were removed and the changes in absorbance were followed by using a Beckman DU spectrophotometer. In all irradiations, the D_2O concentration in the D_2O samples was 98 mole % or greater.

Transformation Procedure. B. subtilis strain 168 (try_2^-) was used as the recipient strain. The cells were made competent and frozen in this stage according to the procedure of Romig (1962). They were stored at −125° until use. The DNA was added at a final concentration of 0.55 μg/ml, a limiting dose under the conditions of the assay. Transformants were plated in triplicate on minimal medium, supplemented with 20 μg/ml of L-proline, to stimulate growth. Appropriate contamination and reversion controls were run with each experiment.

Infectivity Assays for TMV and TMV-RNA. Infectivity tests of virus or viral nucleic acids were performed by assaying in the local lesion host, *Nicotiana tabaccum* var. *Xanthi*, n.c. (Fraenkel-Conrat, 1959). Nonirradiated control and irradiated samples were compared on opposite halves of the same leaf and the survival of irradiated samples was expressed as the percentage of the control taking into account any dilution factor.

Assays for the Infectivity of Viruses Were Performed under Room Lighting. Infectivity assays for viral nucleic acid under conditions of nonphotoreactivation were performed under red safety lights (Kodak Safelight Filter, Wratten Series 6B, Eastman Kodak Co., Rochester, N. Y.). The plants were kept in the dark for at least 3 hr before transferring to the plant growth room.

Photoreactivation. In order to test for photoreactivation of irradiated viral nucleic acid, two sets of plants were used. One set of plants was inoculated under room lighting and transferred immediately after inoculation to intense (640 ft-candles) artificial lighting (Ken-Rad fluorescent lights, 40 w, cool white). The other set of plants was inoculated under red safety lights and left in the dark. After 3 hr, both sets of plants were transferred to the plant growth room and kept until lesions were fully developed.

Results

Effects of Solvent on the Photolysis of Model Compounds. A. THYMINE. Irradiation of thymine either in H_2O or in D_2O up to 2 hr under the conditions described did not show measurable changes in optical density. The result is in agreement with the report that thymine in aqueous solution is relatively inert to ultraviolet irradiation at 253.7 mμ (Wacker, 1963).

B. TETRATHYMIDYLIC ACID. Figure 1 shows the log of changes in absorbance of $(pT)_4$ at 270 mμ as a function of time of irradiation with a 253.7-mμ source. Ultraviolet irradiations of pTpT, polythymidylic acid (Deering and Setlow, 1962), and TpT (Johns *et al.*, 1964) lead predominantly to formation of dimers between adjacent thymine residues. The absorption of thymine dimer at 270 mμ is negligible (Setlow, 1966a).

[1] Abbreviations used in this work: oligo T, oligothymidylic acid; $(pT)_4$, tetrathymidylic acid; pTpT, dithymidylic acid; TpT, thymidylyl-(3′,5′)-thymidine; CMP, cytidine 5′-phosphate; Up, uridine 3′-phosphate.

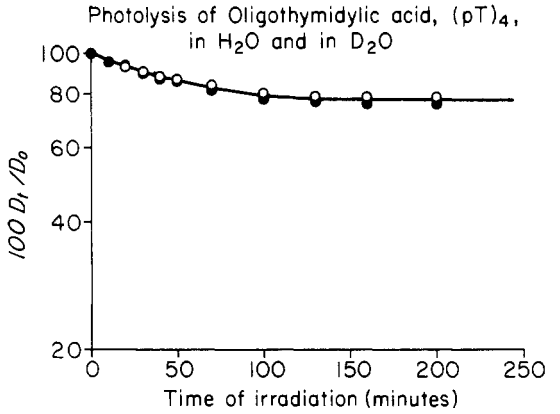


FIGURE 1: A semilog plot showing the change in the absorbance of $(pT)_4$ at 270 mμ as a function of the time of 253.7-mμ radiation. The lamp intensity was 8.3×10^2 ergs/mm^2 per min. D_0, initial absorbance; D_t, absorbance after time t of irradiation. (O——O), in H_2O; (●——●), in D_2O.

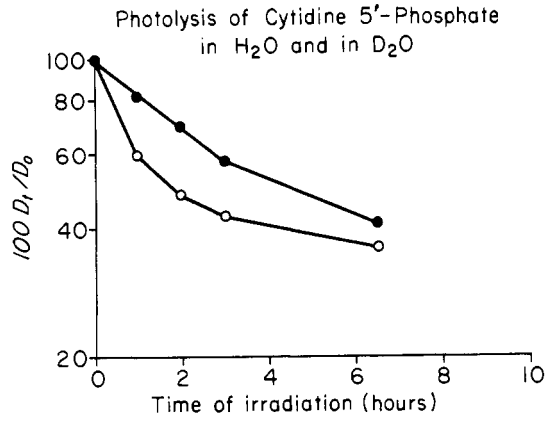


FIGURE 2: A semilog plot showing the change in the absorbance of cytidine 5'-phosphate at 270 mμ as a function of the time of 253.7-mμ radiation. The lamp output energy was 4.6×10^3 ergs/mm^2 per min. D_0, initial absorbance; D_t, absorbance after time t of irradiation. (O——O), in H_2O; (●——●), in D_2O.

Therefore, the change of ultraviolet extinction at this wavelength is a measure of the amount of undimerized material present at different time intervals of irradiations. The results shown in Figure 1 indicate that a steady state is reached where the formation and breakage of dimers occurred to the same extent. The steady-state fraction of dimers is approximately 24%. This value is considerably less than that reported by Deering and Setlow (1962) for pTpT and poly T. They reported a value of 33% for pTpT and 39% for poly T using 254-mμ radiation. However, Johns *et al.* (1964) found a value of 27% for TpT. The discrepancy is probably due to the ultraviolet source used, since slight contamination of the ultraviolet source used in either laboratory with wavelengths other than 254 mμ may affect the fraction of dimers at the steady state. Irradiation of $(pT)_4$ in either H_2O or D_2O did not affect the rate of change in absorbance. This is expected if dimers were the predominant photoproducts formed. It is conceivable that solvent molecules are not involved in the dimerization of adjacent thymine residues; therefore no kinetic isotope effect may be observed.

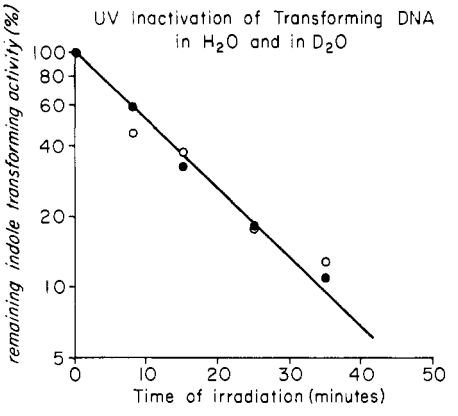


FIGURE 3: A semilog plot showing the effect of 253.7-mμ radiation on the transforming activity of *B. subtilis* DNA. DNA isolated from prototrophic strain WB746 of *B. subtilis* was irradiated in H_2O and in D_2O at a concentration of 5.5 μg/ml and then assayed using strain 168 (try_2^-) requiring indole for growth. The final D_2O concentration was about 98 mole %. (O——O), in H_2O; (●——●), in D_2O.

C. CYTIDINE 5'-PHOSPHATE. A typical plot of log of changes in absorbance of CMP *vs.* time of irradiations (Figure 2) shows that photolysis of CMP occurred faster in H_2O than in D_2O. This agrees with the observation on cytosine and cytidine (Wierzchowski and Shugar, 1957). The kinetic isotope effect for CMP was 3.1 calculated from the initial linear portion of the curves. This value was obtained from averaging several experiments. The CMP photoproduct reverses rapidly to CMP at room temperature. Based on the recovery of the absorbance at 270 mμ, complete reversibility was observed when an irradiated sample was kept overnight at room temperature. The reverse reaction occurred faster in H_2O than in D_2O. The curves in Figure 2 show a reduction in the rates of photolysis as irradiation progresses. This may be a reflection of the reverse reaction. The results indicate that photolysis of CMP leads to the uptake of a water molecule at the 5,6 double bond of the pyrimidine ring (see a review by McLaren and Shugar, 1964).

D. POLY C AND POLY U. Irradiation of poly C in H_2O and in D_2O showed a kinetic isotope effect of 1.2 at the initial stage of photolysis. Adjacent cytosine residues in poly C are sterically favorable for dimerization. Ultraviolet irradiation of cytidylyl-(3',5')-cytidine was shown to result both in hydrate and dimer formation (Freeman *et al.*, 1965). It is conceivable that poly

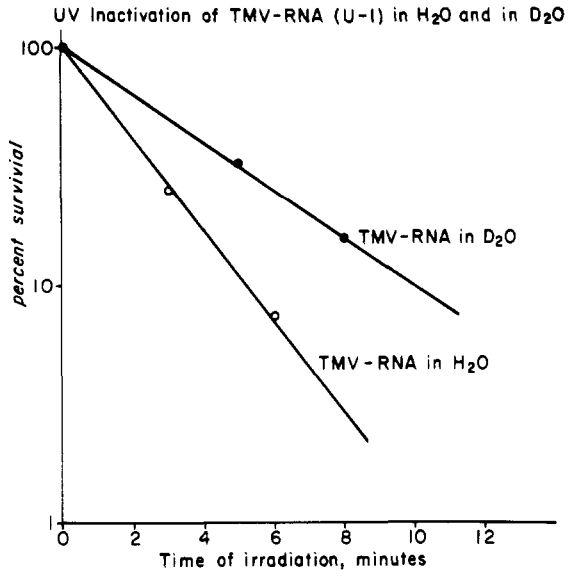


FIGURE 4: Inactivation of TMV-RNA (U-1) in H_2O and in D_2O by ultraviolet irradiation at 253.7-mμ wavelength. The final D_2O concentration was about 99 mole %. Assay was performed under red safety lights.

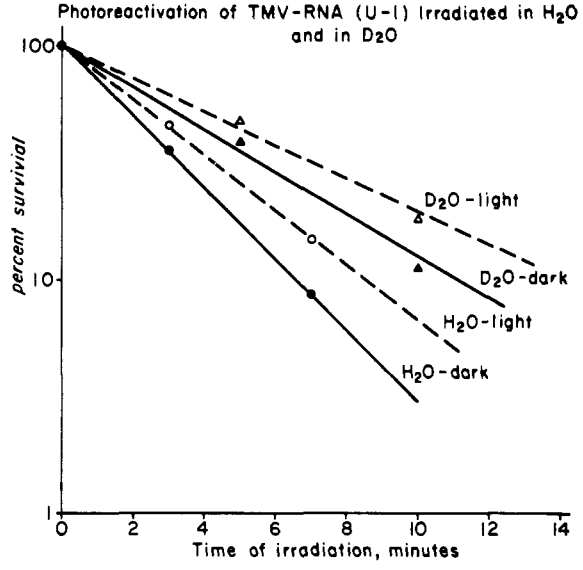


FIGURE 5: Photoreactivation of TMV-RNA (U-1) irradiated in H_2O and in D_2O at 253.7-mμ wavelength. The final D_2O concentration was about 98 mole %.

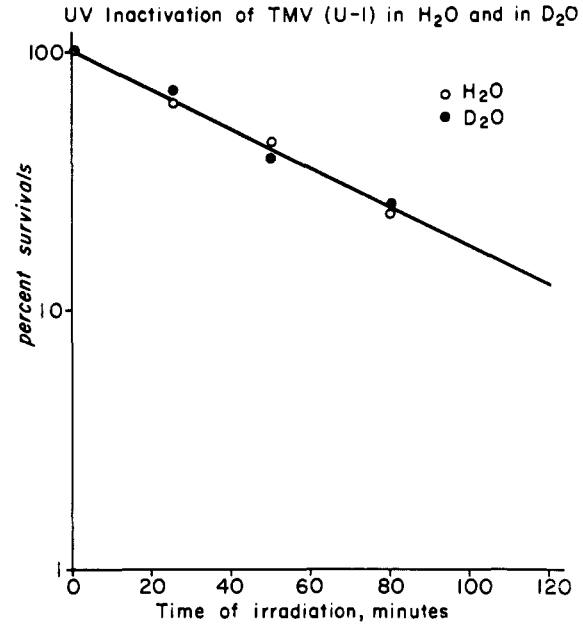


FIGURE 6: Inactivation of U-1 TMV in H_2O and in D_2O by ultraviolet irradiation at 253.7-mμ wavelength. The final D_2O concentration was about 99 mole %. (O——O), in H_2O; (●——●), in D_2O.

C may undergo a similar reaction. This may explain the small kinetic isotope effect observed, since dimerization of adjacent cytosine residues, similar to that of thymine residues in poly T, should not exhibit any isotope effect. Prolonged irradiation of poly C should lead mainly to photohydration similar to what was observed in the irradiation of poly U. The kinetic isotope effect should increase at the later stage of photolysis and reach a value comparable to that of CMP. However, due to the lability of the hydrate photoproduct of cytosine residues, it is difficult to carry out too long a period of irradiation since the reverse reaction tends to mask the forward reaction. Qualitatively, our results obtained for poly U were similar to those observed by Wierzchowski and Shugar (1959). Irradiation of poly U in H_2O and in D_2O showed a gradual increase in kinetic isotope effect as photolysis progresses.

Ultraviolet Irradiation of B. subtilis Transforming DNA. The effect of the solvent on the ultraviolet inactivation of transforming DNA was investigated. It is known that in H_2O, exposure of DNA to 253.7 mμ destroys both transforming ability and its capacity to be taken up by the recipient cell. The loss of the former activity is much more sensitive to ultraviolet than the latter (Lerman and Tolmach, 1959). DNA isolated from a prototrophic strain was irradiated in both H_2O and D_2O. In all irradiations, DNA dissolved in 0.14 M NaCl and 0.01 M sodium citrate was pipetted into H_2O and D_2O to give a solution of 5.5 μg/ml. The final D_2O concentration in the D_2O sample was about 98 mole %. When this DNA was used to transform an indole-requiring strain to prototrophy (168 try_2^-), the results in Figure 3 were obtained. Inactivation of *B. subtilis* transforming DNA has been shown to follow a multicomponent kinetics reaction (Marmur *et al.*, 1961). Only the initial stage of the inactivation was studied. The inactivation kinetic was pseudo first order at this stage. Within our experimental error, the rates of inactivation of transforming DNA in either H_2O or D_2O were identical.

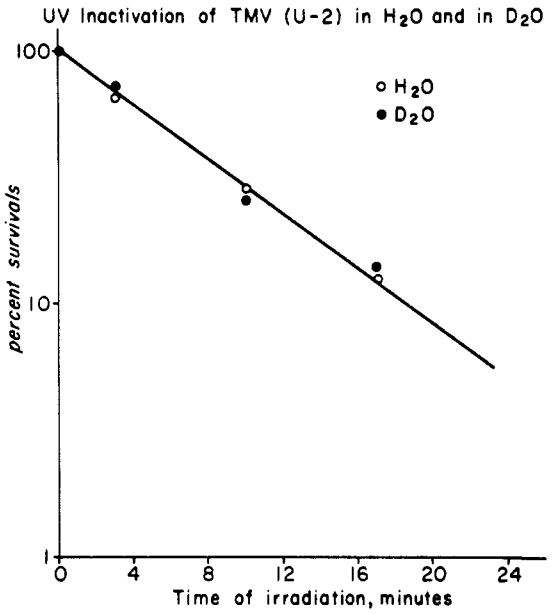


FIGURE 7: Inactivation of U-2 TMV in H_2O and in D_2O by ultraviolet irradiation at 253.7-mμ wavelength. The final D_2O concentration was about 99 mole %. (O——O), in H_2O; (●——●), in D_2O.

Isotope Effects on the Ultraviolet Inactivation of U-1 TMV-RNA. Only U-1 TMV-RNA was investigated. Stock TMV-RNA, dissolved in water, was pipetted into D_2O to give a solution of 20–50 μg/ml in a final D_2O concentration of about 98 mole % or greater immediately before irradiation. Typical survival curves of TMV-RNA irradiated in D_2O or H_2O are shown in Figure 4. The plot of the curves are based on pseudo-first-order kinetics. The rate of inactivation of TMV-RNA in H_2O was faster than that in D_2O. The ratio of the rate constants, K_{H_2O}/K_{D_2O}, as calculated from the ratio of the times required to inactivate to the same percentage survival (t_{D_2O}/t_{H_2O}), was about 1.87 ± 0.09, averaged from several experiments. A similar kinetic isotope effect was observed when TMV-RNA was irradiated in the same salt concentration used in the irradiation of transforming DNA.

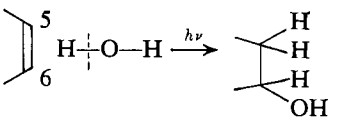
Photoreactivation of TMV-RNA Irradiated in H_2O and in D_2O. It has been shown (Bawden and Kleczkowski, 1959; Rushizky *et al.*, 1960) that ultraviolet-inactivated TMV-RNA may be reactivated when plants are exposed to light immediately after inoculation. Results shown in Figure 5 indicate that the extent of photoreactivation was the same regardless of whether TMV-RNA was irradiated in H_2O or in D_2O. The photoreactivable sector, *fp*, which is defined as 1 − (ultraviolet dose for a given survival in dark/ultraviolet dose to give same survival in light) (Dulbecco, 1950), was about 0.23.

Ultraviolet Inactivation of U-1 and U-2 TMV in H_2O and in D_2O. It has been shown (Siegel and Wildman, 1954; Siegel *et al.*, 1956) that U-2 is more sensitive toward inactivation by ultraviolet light than U-1. The results in Figures 6 and 7 confirm their observations. The ratio of the rates of inactivation of U-2/U-1 as calculated from the ratio of the times required to inactivate to the same extent was about 7.2. However, this value is only an approximation since U-1 and U-2 TMV were not irradiated simultaneously under the same conditions. Figures 6 and 7 also show that irradiations of U-1 and U-2 TMV in either H_2O or D_2O did not affect the inactivation rate. The same result was observed whether the viruses were irradiated with or without preequilibration in D_2O. Equilibration in D_2O was carried out for a period of 8 days at room temperature.

Discussion

Studies on the nature and properties of ultraviolet damage to DNA are being pursued actively by many workers (for a review of this field see Symposium on Recovery of Cells from Injury, 1961; McLaren and Shugar, 1964; Setlow, 1966b). The dimerization of adjacent thymine moieties in DNA either *in vitro* or *in vivo* may contribute significantly to the lethal and perhaps mutagenic effects (Setlow, 1964). In support of this hypothesis, it was shown that thymine dimers can be isolated from hot acid hydrolysates of irradiated DNA (Beukers *et al.*, 1960), DNA of irradiated bacteria (Wacker *et al.*, 1960), and DNA of irradiated bacteriophage (Wulff, 1963). Reactivation of transforming DNA either by short-wavelength ultraviolet irradiation (Setlow and Setlow, 1962) or by photoreactivating enzyme (Wulff and Rupert, 1962) also indicated that thymine dimer formation may be the cause of inactivation. However, Sauerbier (1964) and Setlow (1966a) indicated that aside from the dimerization of thymine, photolysis of cytosine may also play an important role in the inactivation of DNA.

Studies with model compounds have shown that the two main photochemical effects resulting from ultraviolet irradiation of pyrimidines are: (1) hydration at the 5,6 positions of the pyrimidine ring, and (2) dimerization of pyrimidine residues. In an attempt to interpret the effects of ultraviolet irradiation on some pyrimidine derivatives, Shugar and Wierzchowski (see a review by McLaren and Shugar, 1964) carried out the irradiation of these derivatives in H_2O and in D_2O. They showed that the ratio of the rates of photolysis of 1-methyluracil, 2-ethoxyuracil, cytosine, cytidine, and 2-methoxycytosine in H_2O and in D_2O was about 2 ($K_{H_2O}/K_{D_2O} \sim 2$). This would be expected if proton transfer occurred in the addition of water to the 5,6 double bond of the pyrimidine ring at the rate-determining step.



An isotope effect may be observed since the breakage of an O–D bond requires higher energy of activation

than the corresponding O–H bond (Melander, 1960). Wierzchowski and Shugar (1959) also investigated the photolysis of poly U in H_2O and in D_2O. The kinetic isotope effect was found to be as low as 1.2 initially and gradually increased to the value of 2.8, which is comparable to that of 3.0 found for Up. The increase in kinetic isotope effect was well correlated with an increase in hydrate formation. We have extended the kinetic isotope studies to several other compounds in order to obtain further information concerning the effect of ultraviolet light on biologically active nucleic acids. We have found that, in irradiation of CMP, poly C, and poly U, changes in absorbance occurred faster in H_2O than in D_2O. CMP showed a kinetic isotope effect of 3.1. Poly C and poly U showed an initial kinetic isotope effect of about 1.2, and in the case of poly U this value gradually increased as photolysis progresses; this is in agreement with the observations of Wierzchowski and Shugar (1959). Wierzchowski and Shugar (1957) found that irradiation of thymine in H_2O and in D_2O showed a reverse isotope effect where the rate of decrease in optical density was faster in D_2O than in H_2O, $K_{H_2O}/K_{D_2O} \sim 0.44$. However, the isotope effect that they observed was not necessarily due to dimerization since thymine does not readily dimerize in aqueous medium at 10^{-3} to 10^{-4} M. Our investigation with oligothymidylic acid showed that irradiation of $(pT)_4$ in either H_2O or D_2O did not affect the rates of change in absorbance. Irradiation of oligo T has been shown to result predominantly in the formation of dimers. Hydrate formation has been shown to be negligible. Our observations with oligo T indicate that the solvent molecules are not directly involved in the formation of dimers.

The rates of inactivation of *B. subtilis* transforming DNA are the same in H_2O and in D_2O. This suggests that solvent molecules are probably not directly involved in the inactivation of DNA and that dimer formation between adjacent pyrimidines may possibly be the inactivating reaction. Cytosine hydrate either was not formed or its formation was obscured by the predominant dimer effect. Furthermore, it should be noted that cytosine hydrate is relatively unstable and the possibility exists that it may have been reverted to cytosine before we could detect its lethal effects.

In contrast to the observations with transforming DNA, the rate of inactivation of TMV-RNA (U-1) was faster in H_2O as compared to that in D_2O. The ratio of the rates of inactivation of TMV-RNA in H_2O to that in D_2O was about 1.9. This suggests that the addition of a water molecule to the 5,6 double bond of the pyrimidine residues may be a highly significant chemical event leading to damage in TMV-RNA. The observed kinetic isotope effect is of the order of magnitude that would be expected if the inactivation of TMV-RNA were caused predominantly by hydrate formation.

Merriam and Gordon (1966) have isolated pyrimidine dimer from ultraviolet-irradiated TMV-RNA. They suggested that pyrimidine dimers are not biologically important ultraviolet lesions. A similar conclusion may be reached from our observations. If the dimerization of adjacent pyrimidine residues in TMV-RNA were lethal, then the observed kinetic isotope effect would not be approximately 2, but would be about 1, as is seen in the case of the dimerization of adjacent thymine residues in $(pT)_4$. By the same reasoning, if dimer formation were to be reversed in the photoreactivation of TMV-RNA, one would observe a greater degree of photoreactivation in D_2O than in H_2O. Figure 5 shows that the fp's of TMV-RNA irradiated in either H_2O or D_2O were identical. This finding indicates that the rate of formation of photoreparable lesions in TMV-RNA was effected by D_2O to the same extent as the rate of formation of lethal lesions. This behavior gives further support to the hypothesis that, unlike the situation prevailing in DNA, hydrate formation may be important in both lethal and photoreparable lesions in TMV-RNA.

Contrary to the results with TMV-RNA, irradiation of U-1 and U-2 TMV in either H_2O or D_2O did not affect the rate of inactivation. Preequilibration of TMV in D_2O up to 8 days at room temperature gave similar results. The absence of any effect is probably not due to any difficulty of penetration of D_2O into the virus particle. It has been shown that when intact TMV was treated with formaldehyde (Fraenkel-Conrat, 1954) or with nitrous acid (Schuster and Wilhem, 1963) reaction occurred both with the coat protein and the nucleic acid. It is possible that the inactivation of the intact virus by ultraviolet light may follow a mechanism different from that of the RNA and that solvent molecules are not directly involved. The action spectrum of intact TMV was shown to be different from the absorption spectrum of its RNA component, and the quantum yield of inactivation of the intact virus was shown to depend on the wavelength of irradiation (Hollaender and Duggar, 1936). These data suggest that the protein component may be involved in the inactivation of the virus. Further implication of the role of protein subunits in the inactivation of TMV was indicated by the works of Goddard and co-workers (Goddard *et al.*, 1966) and Streeter and Gordon (1966).

The above conclusions are drawn mainly from kinetic isotope studies. More direct experimental verifications are necessary for their support. Attempts are underway in this laboratory to isolate the photoproduct(s) formed in ultraviolet-irradiated TMV-RNA.

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