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Photohydration of Uridine in the Ribonucleic Acid of Coliphage R17. Lethality of Uridine Photohydrates and Nonlethality of Cyclobutane-Type Photodimers*

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ABSTRACT: Uridine photohydrates (6-hydroxy-5,6-dihydrouridine) have recently been implicated as the major lethal lesions caused by ultraviolet light in the small, single-stranded RNA phage R17. Results on the photochemistry and photobiology of R17-RNA are presented in this paper which strongly support this conclusion and tend to exclude cyclobutane-type photodimers (and other photoproducts which may be formed) as major lethal lesions in ultraviolet-irradiated R17 and R17-RNA. R17-RNA preparations containing comparable amounts of uridine photohydrates but varying amounts of cyclobutane-type pyrimidine dimers were obtained (1) by irradiating free R17-RNA at 280 mμ, (2) at 240 mμ, and (3) by extracting RNA from phage which had been irradiated at 280 mμ. The uridine photohydration cross-section determined at small exposures ranging from 0.1 to 1×10^{-2} μeinstein cm^{-2} was 0.45 cm^2/photon $\times 10^{16}$ at 280 mμ, 0.46 cm^2/photon $\times 10^{16}$ at 240 mμ, and 1.5 cm^2/photon $\times 10^{16}$ for the intact phage irradiated at 280 mμ. Cyclobutane-type pyrimidine dimerization was only observed upon irradiation of free R17-RNA at 280 mμ. Single-hit kinetics were observed for the inactivation of the infectivity of R17-RNA determined on *Escherichia coli* K12 Hfr RNase$^-$ spheroplasts under all three irradiation conditions. Close values ranging from 0.75 to 0.80 were found for the number of uridine photohydrates per RNA chain per biological hit for all three types of RNA preparation independent of the irradiation conditions and, therefore, of the presence or absence of cyclobutane-type photodimers.

The photobiology of double-stranded DNA has been investigated in detail during the last decade and the lethal effect of cyclobutane-type pyrimidine dimers has been clearly demonstrated. The photobiology of single-stranded nucleic acids, on the other hand, is not well understood, but may be equally important. Transfer, messenger, ribosomal, and possibly chromosomal RNA are in part noncomplementary and contain single-stranded loops and tails. More important, the most active portions of a genome, *i.e.*, portions involved in transcription, replication, and recombination, may tempor-

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arily assume a denatured single-stranded conformation. Studies with natural (Cerutti *et al.*, 1969; Small *et al.*, 1968) and synthetic polynucleotides (DeBoer *et al.*, 1967) indicate that single-stranded nucleic acids are more susceptible to photochemical attack than double-stranded nucleic acids. In particular, photohydration of pyrimidine nucleotides and less pronounced cyclobutane-type photodimerization occur more rapidly in single-stranded than in double-stranded polynucleotides. Because of their high photochemical reactivity, single-stranded regions in cellular nucleic acids would therefore be expected to represent targets of extraordinary ultraviolet sensitivity.

As a first step in our efforts to reach a better understanding of the photochemistry and photobiology of single-stranded nucleic acids, we have chosen the small, single-stranded RNA phage R17 as the object for our investigations. Strong indications were obtained that uridine photohydrates are the major lethal products formed by ultraviolet light in the intact phage, while cyclobutane-type photodimerization was found to be suppressed. Photodimerization, however, was observed when free R17-RNA was irradiated (Remsen *et al.*, 1970). In the studies reported in this paper, we have corroborated our previous conclusion that uridine photohydrates represent the major lethal lesions caused by ultraviolet light in R17 phage and R17-RNA, and we have shown that cyclobutane-type photodimers are not lethal in R17-RNA. It is interesting to note the close analogy between the photobiology of R17 (and R17-RNA) and the single-stranded plant virus TMV (and TMV-RNA) (Small *et al.*, 1968; Tao *et al.*, 1966; Tao *et al.*, 1969; Evans and McLaren, 1969).

Materials and Methods

Pancreatic ribonuclease, spleen phosphodiesterase, snake venom phosphodiesterase, alkaline phosphatase, and micrococcal nuclease were purchased from Worthington Biochemical Corp. [$^3H_6$]Uridine was obtained from New England Nuclear Corp., the dinucleotide uridylyl-(3′→5′)-uridine from P-L Biochem., Inc., and alternating poly (A-U) from the Biopolymers Laboratory of General Biochemicals.

1. Preparation of R17 and R17-RNA Labeled in Uridine and Cytidine. Escherichia coli K12 Hfr RNase$^-$ D_{10} was grown in defined medium (Gesteland and Boedtker, 1964) to a cell density of 4×10^8/ml, then infected with R17 at a multiplicity of 10. Five minutes after infection, [$^3H_6$]uridine was added to the culture, 0.044 mCi/ml. The phage was purified by the method of Yamamoto *et al.* (1970). Specific activity of UMP was 353 mCi/mmole and of CMP, 403 mCi/mmole. RNA was extracted with phenol–sodium dodecyl sulfate at 4° according to Gesteland and Boedtker (1964) followed by precipitation with cold ethanol and storage in liquid nitrogen in small aliquots.

2. Determination of Infectivity. All manipulations of irradiated samples were carried out in dim light to exclude possible photoreactivation.

The infectivity of suspensions of R17 phage was determined as described previously (Remsen *et al.*, 1970). The spheroplast assay for infective centers was used to measure infectivity of R17-RNA. *E. coli* K12 Hfr RNase$^-$ was grown to a cell density of 4×10^8 cells/ml in modified 3xD medium (Spiegelman *et al.*, 1964), then converted into spheroplasts with lysozyme and EDTA (Guthrie and Sinsheimer, 1963), as modified by Strauss (Strauss, 1964). Spheroplast preparations were used within 24 hr. R17-RNA was diluted in 0.01 M Tris·HCl–0.005 M $MgCl_2$, pH 7.6 (Argetsinger and Gussin, 1966), then mixed with spheroplasts and incubated at 37° for 10–20 min before plating by agar overlay. Several dilutions of each irradiated sample were plated in triplicate. RNA concentrations between 0.07 and 0.006 μg per ml (based on 23 A_{260} units per mg of RNA) were used. The plating efficiency for unirradiated RNA was $1–2 \times 10^{-7}$.

3. Irradiation Conditions and Dosimetry. The Schoeffel high-intensity ultraviolet monochromator used in this work has been described previously (Cerutti *et al.*, 1969). The high quantum flux of this instrument (up to 5×10^{15} quants/sec at 280 mμ at 4-mμ half-bandwidth) made it possible to use short irradiation times and monochromatic light of high purity. The fluence in microeinsteins per square centimeters was determined according to Johns *et al.* (1964). No correction was made for scattering of light by the phage suspensions. Thorough mixing of the sample in the 1-mm path-length quartz cell used for the irradiations was achieved with a mechanical device moving a microstirring bar vertically through the photolysis solution. R17 phage was irradiated in 10^{-3} M EDTA, pH 8, R17-RNA in 0.15 M NaCl–2.5×10^{-4} M EDTA, pH 8. The absorbance of phage suspensions and of RNA solutions at 260 mμ measured in the 1-mm path-length cell was usually about 0.25 and did not change during irradiation.

4. Reductive Assay of Uridine Photohydration. The assay was essentially as described by Remsen *et al.* (1970). At the small exposures used in these experiments, which led to the conversion of only 0.1–2 uridine residues into photohydrates per R17-RNA chain, approximately 10 μg of RNA containing $1.0–1.2 \times 10^6$ cpm in [$^3H_6$]uridine were used per sample subjected to the reductive assay. A yield of 84% for the production of 1,3-propanediol from uridine photohydrates by the reduction with sodium borohydride under the conditions of the reductive assay had been determined using UMP photolysis as a model (Cerutti *et al.*, 1969). This value, obtained from the monomer, was used in our earlier experiments to compute the photohydrate content of the irradiated polymer, R17-RNA. This may not be completely justified. A considerable amount of work using repeating poly (A-U) and R17-RNA has now been done to study the question whether the polymeric state significantly influences the reduction yield. Some of these experiments are summarized below. It is concluded from these experiments that the polymeric state has only a minor influence on the reduction yield. The best estimate for the yield of 1,3-propanediol produced by the reduction of uridine photohydrates contained in a heteropolynucleotide is 81%, a value which is close to that determined from the UMP photolysis.

(A). KINETICS OF THE REVERSION OF URIDINE-PHOTOHYDRATES IN ALTERNATING POLY (A-U). A difference in the stability of uridine photohydrates contained in a polymer relative to monomeric photohydrates would be expected to lead to a difference in the amount of 1,3-propanediol formed in the reductive assay per uridine residue originally photohydrated for polymer and monomer. The influence of the polymeric state on the stability of uridine photohydrates was therefore investigated using alternating poly (A-U) as a synthetic model system.

Alternating poly (A-U) and an equimolar mixture of UMP

[1] Abbreviations used are: UpU, uridylyl-(3′→5′)-uridine; $\widehat{UpU}$, major isomers of the cyclobutane-type photodimers formed from UpU. $\widehat{PyPy}$, total cyclobutane-type photodimers (major isomers) formed in R17-RNA from UpU, CpU, and UpC; UMP(H_2O), photohydration product of uridylic acid (6-hydroxy-5,6-dihydrouridylic acid).

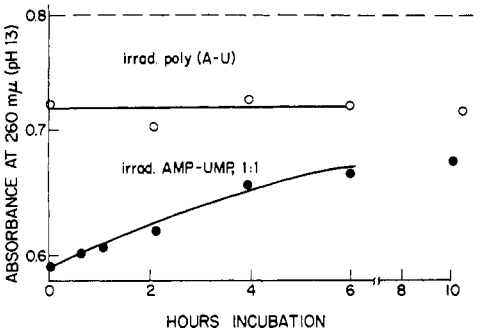

FIGURE 1: The kinetics of the reversion of the photohydrate of monomeric uridylic acid and of uridylic acid contained in alternating poly (A-U). Reversion conditions: 0.5 M NaCl–0.01 M Na_2CO_3 buffer, pH 10.3 at 10° (see section 4A of Materials and Methods).

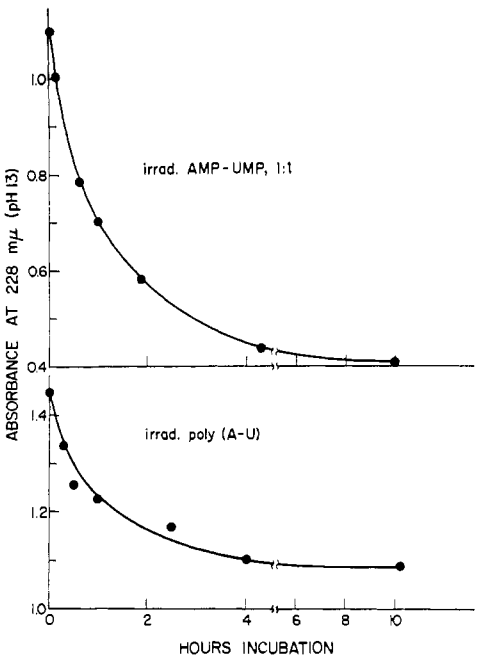

FIGURE 2: The kinetics of the sodium borohydride reduction of the photohydrate of monomeric uridylic acid and of uridylic acid contained in alternating poly (A-U). Reduction conditions: 0.17 M NaCl–0.014 M Na_2CO_3 buffer, pH 10.3–0.34 M $NaBH_4$ (see section 4B of Materials and Methods).

and AMP were irradiated to 10^{-4} M Tris buffer, pH 8, with a mercury low-pressure lamp. The reversion of the photohydrates was studied under the conditions of the reductive assay, namely at 10°, pH 10.3 at a total sodium ion concentration of approximately 0.5 M. Since pyrimidine photodimerization does not occur in poly (A-U) nor in the nucleotide mixture, the formation and reversion of uridine photohydrates could be followed directly by measuring the changes in the absorbance at 260 mμ. All readings were taken at pH 13 to avoid hypochromicity effects immediately after addition of base. The results given in Figure 1 show that photohydrates in poly (A-U) are of higher thermal stability than the photohydrate of UMP. A similar result had been obtained earlier by Logan and Whitmore (1966) in a comparison of the stability of photohydrates contained in oligo- and polyuridylic acid with the stability of the UMP photohydrate. Based solely on these results, a higher yield of 1,3-propanediol per photohydrated residue would be expected for the sodium borohydride reduction of an uridine photohydrate contained in a polymer relative to the monomer.

(B). KINETICS OF THE REDUCTION OF URIDINE PHOTOHYDRATES IN ALTERNATING POLY (A-U). A significant difference in the kinetics of the reduction of monomeric photohydrates and photohydrates contained in a polymer could result in a difference in the yield for the production of 1,3-propanediol in the reductive assay applied to monomer or polymer. It is conceivable that the access of the bulky, negatively charged borohydride moiety to the photohydrated residues in a polymer may be hindered by polymer conformation and in particular by the high negative charge density of the phosphodiester backbone. The kinetics of the sodium borohydride reduction of uridine photohydrates in alternating poly (A-U) and of UMP photohydrates were compared to study this question.

Alternating poly (A-U) and an equimolar mixture of AMP and UMP were irradiated as described in the preceding section. The samples were then treated with sodium borohydride under standard conditions (0.17 M NaCl–0.014 M Na_2Co_3 buffer, pH 10.3, 0.34 M $NaBH_4$) (Remsen *et al.*, 1970). Aliquots were removed as a function of time and the uridine photohydrates remaining determined by measuring the absorbance at 228 mμ immediately after adjusting the pH to 13. Uridine photohydrates, as other 2,4-diketo-5,6-dihydropyrimidines, strongly absorb in the 230-mμ region at alkaline pH but lose their absorption as the heterocyclic ring is reductively opened (Cerutti and Miller, 1967). As mentioned earlier, hypochromicity effects in the experiments with poly (A-U) are avoided by taking the measurements at high pH. The results are given in Figure 2. Comparable reduction kinetics are found for monomer and polymer.

(C). EFFECT OF PREDIGESTION OF IRRADIATED R17-RNA ON THE REDUCTION YIELD. An effect of the conformational state of the RNA on the $NaBH_4$ reduction of uridine photohydrates should essentially be eliminated by digesting the irradiated polymer to mono- and oligonucleotides prior to reduction. Pancreatic RNase has been shown to cleave phosphodiester bonds following uridine photohydrates (Small *et al.*, 1968; Pearson and Johns, 1966) and has been used for this purpose. Irradiated RNA samples were treated with pancreatic RNase (1 μg of RNase per 3 μg of R17-RNA) for 10 min at room temperature and then reduced under standard conditions. The RNase treatment was omitted in control samples which had received the same ultraviolet exposures. A 10% increase in radioactivity released as 1,3-propanediol in the reductive assay was observed for the RNase treated samples relative to the controls. A corresponding correction is included in the value of 81% given above for the production of 1,3-propanediol per uridine photohydrate in R17-RNA.

5. Determination of Cyclobutane-Type Pyrimidine Photodimers in R17-RNA. Cyclobutane-type photodimers were determined as $\widehat{UpU}$ by the procedures of Small *et al.* (1968) following total enzymatic hydrolysis of the irradiated RNA. In a typical experiment, 10 μg of R17-RNA containing 2.4×10^6 cpm in [$^3H_6$]uridine (specific act. 353 mCi/mmole) and [$^3H_6$]cytidine (specific act. 403 mCi/mmole) were exposed to $2–80 \times 10^{-3}$ μeinstein/cm^{-2} at 280 and 240 mμ and subjected to enzyme hydrolysis. The Dowex 50W-X8 (H^+) column and the second paper chromatographic step (washed Whatman No. 1, pretreated with ammonium sulfate; eluent 76% aqueous ethanol) recommended by Small *et al.* (1968) for the reduction of background radioactivity were included

in all our experiments. Nevertheless, relatively high, but reproducible, backgrounds were obtained at small ultraviolet exposures. The overall deviation of the data at exposures leading to the formation of approximately one dimer per RNA chain of 3300 nucleotides is estimated to be ±20%. Despite the large error, the assay has considerable advantages over earlier procedures using rather drastic acidic conditions to achieve hydrolysis of the irradiated polymer. The results are given in Table I. In another series of experiments, intact R17 phage was irradiated, the coat protein removed as described by Gesteland and Boedtker (Gesteland and Boedtker, 1964), and the free RNA analyzed for its content in cyclobutane-type photodimers as described above.

In a separate experiment on R17-RNA, the radioactive material assigned to $\widehat{\text{UpU}}$ was eluted from the second paper chromatogram and further characterized by demonstrating photoreversibility to UpU. The eluate was irradiated at 240 mμ after addition of cold $\widehat{\text{UpU}}$ as carrier. (The carrier had been prepared from the dinucleotide UpU by irradiation at 280 mμ to 70% loss of the absorption at 260 mμ.) The irradiated sample was then reapplied to paper and submitted to chromatography under the conditions of the second chromatogram of Small *et al.* (1968) described above. Over 90% of the radioactivity had a chromatographic mobility identical with or slightly lower than UpU while only background activity remained in the region assigned.

TABLE I: Formation of Cyclobutane-Type Photodimers and of Uridine Photohydrates in R17-RNA upon Irradiation at 280 and 240 mμ.[a]

Wavelength (mμ)	Exposure in μeinsteins cm^{-2}	cpm × 10^{-3}[b]	$\widehat{\text{PyPy}}$/RNA	cpm in 1,3-Propanediol[c]	UMP-(H_2O)/RNA
280	0.62			221	0.18
	0.91			291	0.24
	1.48	2.9	1.0	605	0.50
	7.87	6.6	2.2	2696	2.23
240	0.20	0.5		148	0.12
	0.72	0.1		175	0.14
	1.13	0.1		433	0.36
	6.12	0.6		1852	1.53

[a] Irradiation in 0.15 M NaCl–2.5 × 10^{-4} EDTA buffer, pH 8, at room temperature. [b] Determination according to the procedures of Small *et al.* (1968); corrected for background radioactivity obtained from a nonirradiated sample and for the loss of material during the chromatographic procedures; total input radioactivity per RNA sample, 2.4 × 10^6 cpm (see under Methods and Materials). At the lower exposures, 0.62 and 0.91 × 10^{-2} μeinstein cm^{-2}, the sensitivity of the procedure for the determination of photodimers of Small *et al.* (1968) was not sufficient to yield reliable values. [c] Reductive assay according to Remsen *et al.* (1970); corrected for background radioactivity obtained from a nonirradiated sample and on the basis of an 81% yield for the formation of 1,3-propanediol from uridine photohydrates; total input radioactivity in uridine per RNA sample, 1.04 × 10^6 cpm (see under Methods and Materials).

Results and Discussion

A classical approach in photobiology to implicate a certain type of lesion as the basis for the loss of biological activity is the demonstration of a linear relationship between the logarithm of the fraction of the biological activity remaining after a given dose and the number of specific photoproducts formed. It should be kept in mind, however, that a linear relationship *per se* is not sufficient to rigorously prove the participation of a given product in the deactivation process. The possibility remains that a photoproduct which has escaped detection is instead responsible. We have recently presented evidence suggesting the involvement of uridine photohydrates in the lethal effect of ultraviolet light on coliphage R17 (Remsen *et al.*, 1970). Cyclobutane-type pyrimidine photodimerization was found to be suppressed in the phage below levels detectable by the assay of Small *et al.* (1968) but the presence of other photoproducts, *e.g.*, other types of photodimers (Tao *et al.*, 1969), could not be excluded.

In the experiments reported here we have sought further evidence to identify uridine photohydrates and to exclude other photoproducts as the major lethal lesions caused by ultraviolet light in R17 phage and R17-RNA. The most direct approach, namely to demonstrate recovery of infectivity upon thermal reversion of uridine photohydrates in R17-RNA, was not successful. No reliable data could be obtained probably due to unspecific degradation of the RNA occurring at the high incubation temperatures necessary for the reversion of a substantial portion of uridine photohydrates (*e.g.*, 20 min at 80° for 50% reversion at an initial level of 3 uridine photohydrates per RNA chain). The following alternative approach was chosen. RNA samples were prepared under three different conditions: (1) irradiation of free R17-RNA at 240 mμ, (2) at 280 mμ, and (3) extraction of RNA from phage which had been irradiated at 280 mμ. The preparations were analyzed for their content in uridine photohydrates and cyclobutane-type photodimers, and their infectivity was determined using *E. coli* K12 Hfr RNase$^-$ spheroplasts. From these data the number of uridine photohydrates (and cyclobutane-type photodimers) per RNA chain per biological hit was computed for each preparation and compared. These experiments are based on the following concept. It is unlikely that the rates (cross-sections) for uridine photohydration, pyrimidine cyclodimerization, and for the formation of other photoproducts, would all change in a parallel fashion under the three conditions used in these experiments. RNA samples containing the same amounts of uridine photohydrates, but prepared under the different conditions, would therefore be expected to vary in their content of other photoproducts. As discussed below, comparable values close to unity were obtained for the number of uridine photohydrates per RNA chain per biological hit for all RNA samples regardless of the condition of their preparation and therefore of their content of other photoproducts. This result strongly supports the conclusion that uridine photohydrates are the major lethal lesions introduced by ultraviolet light in R17 and R17-RNA.

The result of the experiments in which free R17-RNA was irradiated with monochromatic light at 240 or 280 mμ are discussed first. The analytical data, *i.e.*, the uridine photohydrate content and cyclobutane-type photodimer content of the RNA as a function of the ultraviolet exposure and the

TABLE II: Photochemical and Photobiological Characteristics of R17 Phage and Its RNA.

Irradiation of	Wavelength (mμ)	Inactivation Cross-Section (cm²/photon × 10^{16})	Uridine Photohydration Cross-Section[c] (cm²/photon × 10^{16})	Uridine Photohydrates per RNA per Biological Hit	Cyclobutane-Type Photodimers per RNA per Biological Hit
R17-RNA[a]	280	0.56	0.45	0.80	1.2
	240	0.57	0.46	0.80	
Intact R17 phage[b]	280	2.0 (1.5)	1.5 (1.4)	0.75 (0.94)	

[a] Irradiation in 0.15 M Na^+–2.5 × 10^{-4} M EDTA, pH 8; infectivity determined on *E. coli* K12 Hfr RNase⁻ spheroplasts. [b] Irradiation in 10^{-3} M EDTA, pH 8 at room temperature; infectivity determined on *E. coli* K12 Hfr RNase⁻ spheroplasts after removal of phage protein by the method of Gesteland and Boedtker (Gesteland and Boedtker, 1964). The values in parentheses are taken from an experiment in which the infectivity of the intact, irradiated phage was determined on *E. coli* K12 Hfr RNase. [c] Since only approximately 0.2 to 2 uridine residues per RNA chain were converted into photohydrates at the small exposures used in these experiments, the values in this column may be considered as true initial cross-sections (*cf.* Cerutti *et al.*, 1969).

wavelength of the incident light are given in Table I. As is evident from these data, photohydration of uridine and cyclobutane-type pyrimidine dimerization proceed at similar rates at 280 mμ. At this wavelength the ratio of uridine photohydrates over cyclobutane-type photodimers at D_{37} is 0.67 (see Table II; D_{37} for the loss of infectivity is 0.030 μeinstein/cm⁻²). While identical photohydration rates are found for irradiation of free R17-RNA at 280 and 240 mμ, no photodimerization could be detected within the sensitivity of the assay of Small *et al.* (1968) at 240 mμ. A value of 2.1 for the ratio of uridine photohydrates over cyclobutane-type dimers at D_{37} is calculated from the data of Small *et al.* (1968) for TMV-RNA irradiated at 253.7 mμ and is about what might be expected from our data for this intermediate wavelength. The absence of cyclobutane-type dimerization in R17-RNA at 240 mμ is unexpected, on the other hand, on the basis of the work of Johns and his collaborators (see, *e.g.*, DeBoer *et al.*, 1967) on poly (U) and the poly (U)–poly A complexes. Depending on the conformational state of poly (U) during irradiation, similar, or even higher dimerization cross-sections were obtained at 240 mμ relative to 280 mμ. The following should, however, be kept in mind when comparing the data obtained with R17-RNA and poly (U) and may in part explain the observed differences: (1) the photochemistry of a heteropolynucleotide (R17-RNA) and a homopolynucleotide (poly (U)) could be significantly different; (2) approximately ten times lower ultraviolet exposures were used in the experiments with R17-RNA than with poly (U). A direct comparison of the data is therefore only possible on the assumption that the dose–response curves are linear in the dose range under consideration.

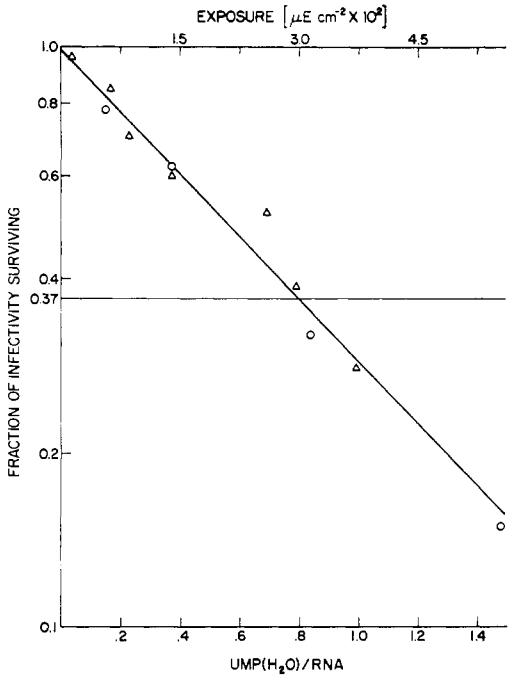

FIGURE 3: Relation between ultraviolet inactivation of free R17-RNA at 240 and 280 mμ and uridine photohydration. The uridine photohydrate content of the irradiated RNA was determined by the reductive assay (see section 4 of Materials and Methods and Remsen *et al.* (1970)) and the infectivity using *E. coli* K12 Hfr RNase⁻ D_{10} spheroplasts (see section 2 of Materials and Methods): △—△, irradiation at 280 mμ; ○—○, irradiation at 240 mμ. (For irradiation conditions, see section 3 of Material and Methods.)

Single-hit kinetics are observed for the inactivation of the infectivity of R17-RNA by ultraviolet light of 240 and 280 mμ. As is evident from Figure 3 and summarized in Table II, both the photohydration cross-sections as well as the inactivation cross-sections are close within experimental error at 240 and 280 mμ. Correspondingly, the same value of 0.80 is computed for the number of uridine photohydrates per RNA chain per biological hit, regardless of the presence or absence of cyclobutane-type photodimers. Irradiation at 280 mμ produces 1.2 photodimers per RNA chain per biological hit, while at 240 mμ no formation of photodimers could be detected. It follows from these results that cyclobutane-type photodimers are nonlethal lesions in R17-RNA. An analogous conclusion has been reached for TMV-RNA most clearly from a comparison of the loss of infectivity upon irradiation in D_2O and H_2O (Tao *et al.*, 1966; Small *et al.*, 1968).

As reported previously (Remsen *et al.*, 1970), cyclobutane-type photodimerization is suppressed below detectability in the RNA of the intact bacteriophage R17 irradiated at 280 mμ. A third set of RNA samples containing increasing amounts of uridine photohydrates but no cyclobutane-type photodimers was therefore prepared from irradiated phage

by removal of the phage protein by phenol–sodium dodecyl sulfate extraction according to Gesteland and Boedtker (1964) and analyzed as described above. The results are given in Figure 4 and Table II. At the small exposures used both the photohydration cross-section as well as the inactivation cross-section are by a factor of about 3.5 larger than for the irradiation of free RNA at 280 mμ. The values for the number of uridine photohydrates per RNA chain per biological hit, however, are comparable for preparations obtained under either condition, 0.80 for free RNA irradiated in solution and 0.75 for RNA extracted from irradiated phage.

In summary, the results reported here identify uridine photohydrates as the major lethal lesions formed by ultraviolet light in free R17-RNA. An analogous conclusion had previously been reached for the intact bacteriophage (Remsen *et al.*, 1970). Important contributions from other detectable photoproducts, *e.g.*, cyclobutane-type photodimers, to the ultraviolet inactivation of R17-RNA can be excluded. The major evidence for these conclusions is the nearly constant value close to unity for the number of uridine photohydrates per RNA chain per biological hit which was obtained for all three RNA preparations investigated despite the differences in their preparation. Attempts are presently being made to identify the step(s) in the viral development where photohydrates exert their inactivating effect. Preliminary experimental support has been obtained for the notion that deactivation of the messenger activity of R17-RNA by photohydrates should be of only minor importance (Remsen *et al.*, 1970). The presence on the average of 14 uridine photohydrates per RNA chain was found to be necessary to decrease the *in vitro* messenger activity of R17-RNA to 37% (J. F. Remsen and P. Cerutti, 1970, unpublished results), a very large value compared with the number of photohydrates per RNA chain per biological hit (0.75–0.80, see Table II) determined for the loss of infectivity. The most likely hypothesis remains therefore that uridine photohydrates interfere with the formation and/or template activity of the replicative intermediate. No explanation can be given at the present time for the difference in the biological effect of photohydrates and photodimers.

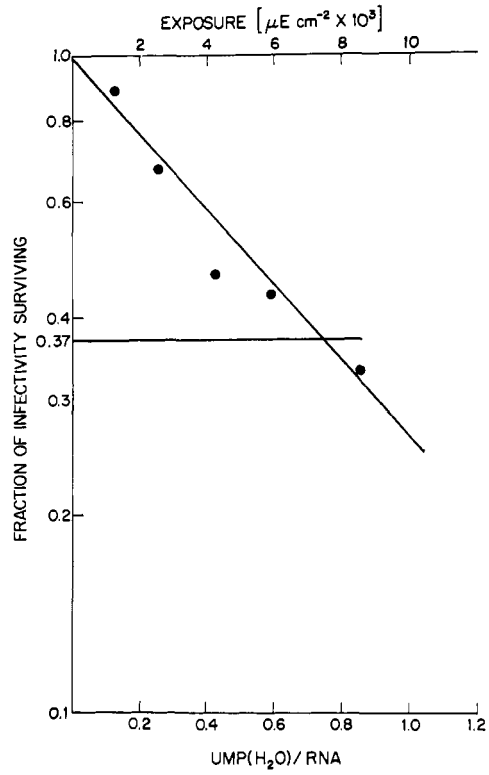

FIGURE 4: The relation between ultraviolet inactivation of R17-RNA obtained from phage irradiated at 280 mμ and uridine photohydration. Intact R17 phage was irradiated at 280 mμ, the phage proteins removed according to Boedtker and Gesteland (1964) and the uridine photohydrate content and the infectivity determined as described in the legend to Figure 3 (for irradiation conditions, see section 3 of Materials and Methods).

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