TRIPLET PHOTOSENSITIZATION AND THE PHOTOBIOLOGY OF THYMINE DIMERS IN DNA

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ABSTRACT

The knowledge of the energy levels of the lowest triplet states of the DNA constituents obtained from photophysical experiments has allowed the design of a photochemical technique which introduces a specific change in the DNA structure, the dimerization of adjacent thymine residues, which, in turn, has led to important conclusions about the role of thymine dimers in the u.v. photobiology of T4 phage and insight into the mechanism of u.v. induced mutations.

INTRODUCTION

During the recent years of great activity in photochemistry several mechanisms for photosensitization have been discovered. These include both mechanisms which require some chemical modification of the sensitizer molecule[1] and mechanisms which involve only weak interactions between sensitizer and substrate, for example triplet-state sensitization involving triplet–triplet transfer from sensitizer to substrate[2,3,4]. Of all the mechanisms the latter is easiest to control. This is because the efficiency of the triplet–triplet excitation process in solution depends primarily on the spectroscopic properties of the donor and acceptor and not on details of structure. Thus these structural details can be modified to be compatible with other important features of the system.

This paper concerns the use of triplet–triplet transfer in the control of DNA photochemistry. Sensitization using acetophenone leads to the production of thymine dimers in *E. coli* DNA to the virtual exclusion of the other products of direct excitation[5,6]. Simple manipulations have allowed extension of the technique to viable T4 bacteriophage[7] and have led to a better understanding of the role of thymine dimers in the photobiology of the phage[8–11].

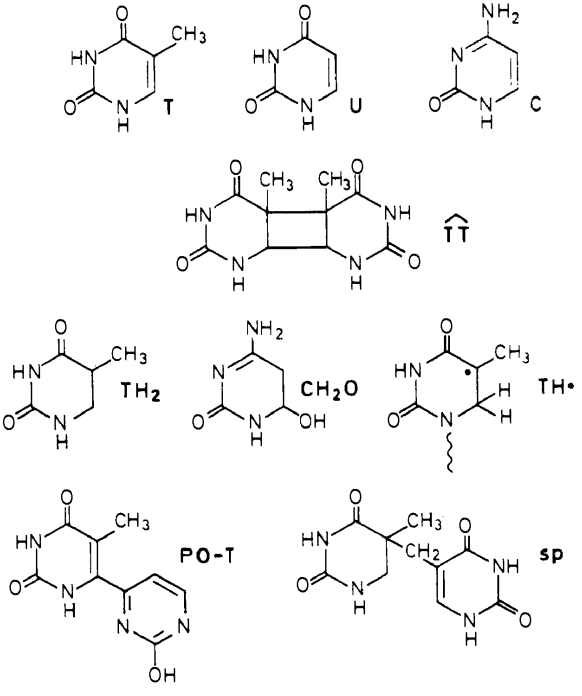
STAHL'S COMPLAINT

In 1959 Stahl[12] complained '... the primary aim in employing radiation in the study of phage is to elucidate the normal state of affairs. However, almost all experiments involving the irradiation of phage have raised far more questions than they have answered.' He was referring to the fact that while radiation (including u.v. light) effects on viruses had been known for

more than three decades[13] and promised to lead to an understanding of the nature of bacteriophage[14] very little had been contributed at the time he wrote his review chapter. But it was just about the time that Stahl made his complaint that the first reports on the structures of the u.v. photoproducts of nucleic acid constituents appeared. These concerned the photohydration of cytosine, uracil and their derivatives[15–18], and the discovery of the photodimerization of thymine[19] which set the stage for important advances. The time around 1959 also saw the first reports on studies of triplet–triplet transfer in fluid solution[20,21], events particularly important for our purposes.

DNA PHOTOPRODUCTS[22]

Sinsheimer and Hastings reported in 1949 that uracil (U) and cytosine (C) derivatives undergo a photochemical reaction in water solution which is reversed by heat or acid[23]. The reaction was postulated by Sinsheimer to be a photohydration of the 5,6 double bond[15]. Proof of this was reported by Moore in 1958 who showed the uracil photoproduct to be 6-hydroxy-5-hydrouracil[16]. The photohydrates of cytosine and its derivatives are very unstable towards dehydration and only recently[24] has a quantitative assay for their formation in nucleic acids been provided. Using this assay Grossman and Rodgers showed that cytosine hydrate (CH_2O) is a major photoproduct in *E. coli* DNA[24]. Thymine and its derivatives do not appear to undergo photohydration.



Results of triplet sensitization experiments as well as quenching experiments indicate that the triplet states of uracil, cytosine and their derivatives are inactive as precursors in the hydration reaction[25-27]. Burr has provided evidence that the primary process in the photohydration of uracil is protonation of the excited singlet state[28]. However, mechanisms involving severely distorted ground state species formed upon internal conversion from the excited singlet state remain as plausible alternatives[29].

It was very shortly after the discovery that irradiation of frozen water solutions of thymine with u.v.-light leads to the formation of dimers[19] ($\widehat{TT}$) that the presence of dimers in irradiated bacterial DNA was demonstrated[30,31]. This photodimerization involves the cycloaddition of the 5,6 double bonds of the monomers to give a cyclobutane ring. In native DNA the dimerization occurs between adjacent thymines on the same strand and leads to the *cis-syn*-stereoisomer[32], the same isomer which is formed in frozen water solutions. The dimerization is photoreversible and a wavelength-dependent photostationary state can be achieved[19]. The photostationary ratio lies on the dimer side for wavelengths below 260 nm where the dimer begins to absorb light strongly (*Figure 1*).

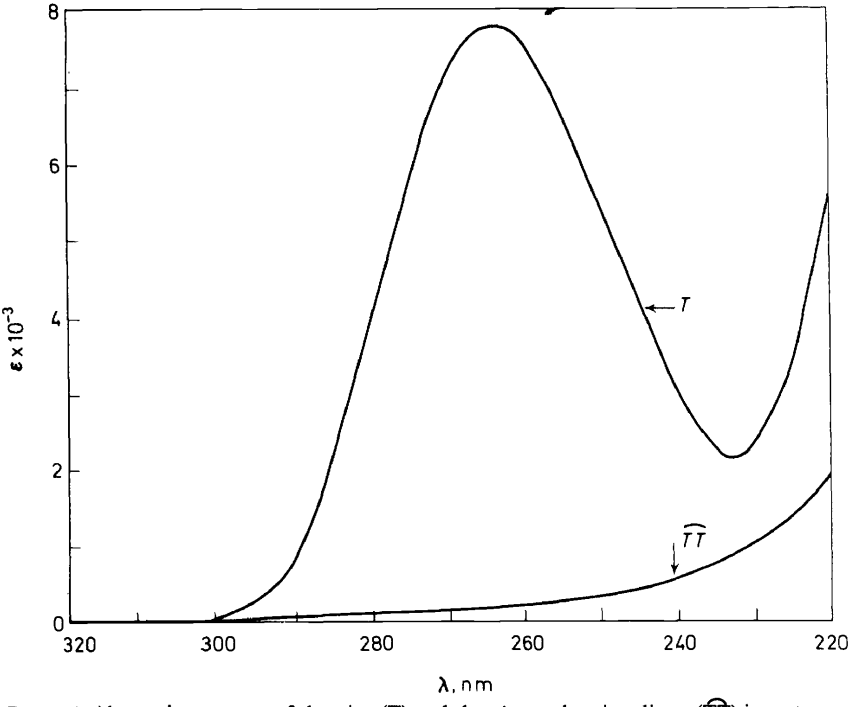


Figure 1. Absorption spectra of thymine (T) and the *cis,syn*-thymine dimer ($\widehat{TT}$) in water.

This photoreversibility was important in implicating thymine dimers in the adverse biological effects of u.v.-light. Then Setlows[33] found that transforming DNA which had been inactivated at 280 nm could be partially

reactivated by subsequent irradiation at 239 nm. Later it was shown that enzymic processes which reverse u.v.-inactivation act to repair thymine dimer damage in DNA[34,35] (see below).

The analogous dimer of cytosine ($\widehat{CC}$) as well as the heterodimer $\widehat{CT}$ are also formed in DNA[36].

Photodimerization of thymine, uracil and cytosine in solution can be sensitized by triplet state sensitizers[25,37,38,39]. The dimerizations can also be effected by direct irradiation. In dilute water solutions they proceed entirely by way of the triplet states as shown by quenching experiments[40,41]. This is expected since the excited singlet states are too shortlived[42] ($\leqslant 10^{-11}$ s) to undergo reaction with ground state monomers present at low concentration. However, under conditions where molecular diffusion is not rate-limiting, for example in aggregates[43,44,45] or in crystals[46], the photodimerizations involve the excited singlet states and are very efficient. No definitive conclusions can be drawn about the relative importance of excited singlet and triplet states as precursors of the pyrimidine photodimers in DNA.

5,6-Dihydrothymine ($\mathbf{TH_2}$) is another major product resulting from the u.v. irradiation of DNA in solution[47,48]. The 6-hydrothyminyl radical ($\mathbf{TH}\cdot$) which is observed in irradiated DNA kept at low temperatures[49] may be the precursor of $\mathbf{TH_2}$. There is some evidence that $\mathbf{TH}\cdot$ is formed by protonation of the thymine radical anion[50]. At this time, however, there is insufficient information to be certain about the relation between these interesting light-induced species.

Another product which appears to arise via a free radical mechanism involves two thymine moieties and is known as the spore product (**sp**) because it is found in greatest abundance in irradiated spores and in irradiated dry DNA[51,52].

Wang and Varghese isolated 6-4′(pyrimidine-2′-one)-thymine (**PO–T**) from irradiated DNA[53]. It is thought that this is the hydrolysis product of the azetane arising from cycloaddition of the 5,6 double bond in thymine and the carbon–nitrogen double bond of the imino tautomer of cytosine. The latter may be the more stable tautomer in the singlet excited state of cytosine in DNA where it is hydrogen-bonded to guanine.

Ultra-violet photoproducts of the purines, adenine and guanine, have been detected in hydrolysates of irradiated DNA but in yields very much smaller than the pyrimidine products mentioned above[48,54]. On the other hand, the purines appear to be major sites of attack in the radiolysis of solutions of DNA[55] and in the dye sensitized photoxidation of DNA[56].

It is important to point out that except for $\mathbf{CH_2O}$ all the products mentioned above were detected in hydrolysed samples of DNA. There may be important products which do not survive acid hydrolysis. In addition it is known that u.v. light can produce crosslinks between the DNA strands, breaks in the sugar phosphate backbone, and covalent bonds between DNA and proteins[57,58,59].

The relative initial yields at which the various known photoproducts are formed in *E. coli* DNA irradiated *in vitro* with 254 nm light are given in *Table 1*. The yields of the several products are comparable within an order of magnitude. Thus it is not immediately evident that one can relate the various biological effects of u.v. light to one or another of these products. Further-

Table 1. The relative initial (low dose) yields of the various photoproducts of *E. coli* DNA irradiated *in vitro* with u.v. light (direct) or with 313 nm light in the presence of acetophenone or acetone.

	Direct	*Sensitized*	
		Acetophenone[e]	*Acetone*[f]
$\widehat{TT}$	(1.0)[a]	(1.0)	(1.0)
$\widehat{CT}$	0.8[a]	0.03	0.14
$\widehat{CC}$	0.2[a]	<0.0025	~0.01
TH_2	0.1[b]	0.02	
CH_2O	0.3[c]	<0.003	
PO–T	0.1[d]	<0.0025	<0.002
Sensitizer addition	—	<0.02	+[g]

(a) Ref. 36; (b) Refs 47, 48; (c) Ref. 24; (d) A. Varghese, private communication; (e) Refs 6, 38; (f) Ref. 64; (g) No yield measured. Reference 65.

more, the number of products alone complicates matters since the specific effectiveness of the different products in leading to a particular biological effect could vary tremendously. For example, the problem is serious in deciding which products are responsible for u.v. induced mutations since the ratio of viable mutations to kills is usually very small so that even minor products must be considered as premutational lesions. The conclusion that pyrimidine dimers are inactivating lesions is based on correlations between dimer repair and u.v. sensitivity in microorganisms. Blockage of the repair processes leads to greater sensitivity towards u.v. light. Since other photoproducts might also be reversed by the repair processes this is not direct proof.

Ideally one would like to be able to produce each photoproduct exclusive of the others. Then any concomitant biological effect can be directly attributed to that product. Through triplet state sensitization we have been able to introduce $\widehat{TT}$ into *E. coli* DNA along with only small yields of some of the other acid-stable products of u.v. irradiation. Extension of the technique to viable T4 bacteriophage followed.

ACETOPHENONE-SENSITIZED DNA PHOTOCHEMISTRY

The energies above the ground states of the lowest excited singlet and triplet states of the five common nucleotides are shown in *Figure 2.* The values were obtained from emission and absorption spectra recorded at 77°K[60,61] but should be very close to the energies required to excite these states in DNA at room temperature[39,62]. The lowest excited singlet and triplet levels of acetone and acetophenone (Acφ) obtained under similar conditions are also shown in the figure. It is readily seen that on the basis of the energetics these carbonyl compounds are suitable classical triplet sensitizers. The triplet energy of acetone is sufficient to excite the lowest triplet levels of all the DNA bases while that of acetophenone is energetic enough to excite only the triplet of thymine efficiently.

We planned sensitization experiments with acetophenone with the view of reducing the number of photoproducts in DNA compared to the number

P.A.C.—24/3—G

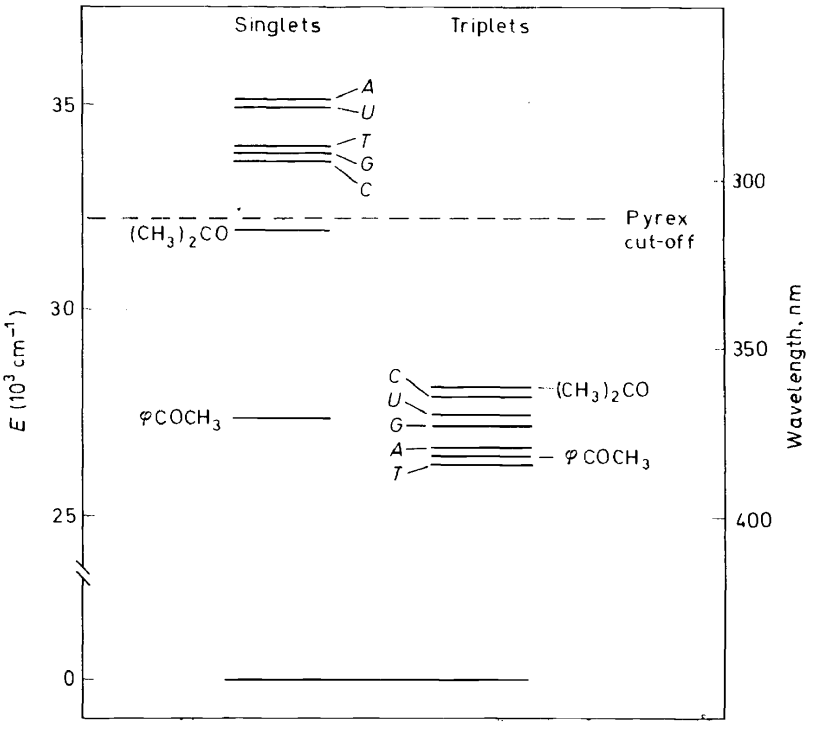


Figure 2. The energies of the lowest excited singlet states and lowest triplet states of the common constituents of nucleic acids, thymine (T), cytosine (C), adenine (A), guanine (G), and uracil (U) as the nucleoside monophosphates, along with the corresponding data for acetone and acetophenone (from refs 60 and 61).

obtained by direct u.v.-excitation. We rationalized that only thymine-containing products would be obtained as a consequence of triplet energy transfer from acetophenone since only the thymine triplet-state would be efficiently excited. Even in the event that activated transfer to the cytosine chromophores was important we rationalized that in the least cytosine photohydrate production would be precluded since the cytosine triplet state does not lead to the hydrate.

Deoxygenated buffered solutions of *E. coli* DNA containing 0.01 M acetophenone were irradiated with 313 nm light† and then analysed for all the known acid-stable photoproducts of u.v. irradiation[5,6,64]. Relative initial yields obtained from dose curves are given in *Table 1*[6,38]. As expected no $\widehat{CC}$ nor CH_2O are detected. Thymine photoproducts, $\widehat{TT}$, $\widehat{CT}$ and TH_2 are obtained but in a ratio much different from the ratio in which they are produced by u.v. irradiation. The acetophenone-sensitized photolysis yields $\widehat{TT}$ in overwhelming proportions compared to $\widehat{CT}$ and TH_2. No other acid-stable products are detected and it was determined by experiments using labelled acetophenone that covalent linkage of the sensitizer to the DNA

† DNA does not absorb appreciably above 300 nm.

is unimportant. Crosslinks and chain breaks also appear to be unimportant relative to $\widehat{TT}$[63]. Thus it appears that the acetophenone-sensitized photolysis of DNA offers a way of introducing $\widehat{TT}$ to the virtual exclusion of other lesions.

The acetone-sensitized photolysis gives $\widehat{CC}$ in addition to $\widehat{TT}$ and $\widehat{CT}$ (*Table 1*) and as expected no cytosine photohydrate[64]. It appears, however, that unlike acetophenone covalent binding of acetone is an important result of photolysis[65].

BIOLOGICAL APPLICATIONS

Motivated by the results with *E. coli* DNA in solution we looked for the possibility of using the triplet sensitization technique with biologically active DNA with the view of determining the specific role of thymine dimers in the biological effects of u.v. irradiation.

The bacteriophage T4† was selected for the first studies because of the wealth of genetic information available for this system, and because the effects of u.v. irradiation on it have been extensively studied. In addition and of great importance, the phage can be irradiated extracellularly in a defined medium avoiding complications on the action of the sensitizer due to components of the host cell. One disadvantage of the phage is that its DNA contains glycosylated 5-hydroxymethylcytosine rather than cytosine. However, the triplet excitation energy of hydroxymethylcytosine (27700 cm^{-1}) is very similar to that of cytosine (27900 cm^{-1})[7].

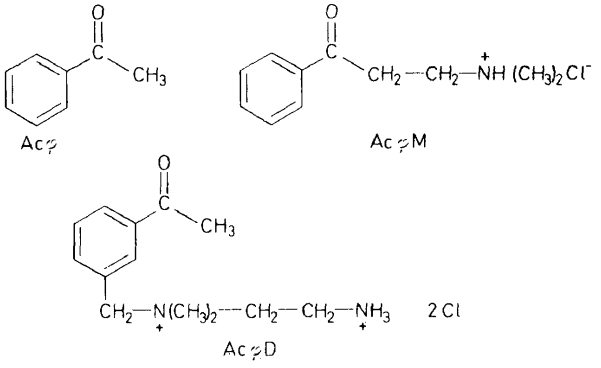
The wild type phage was found to be relatively insensitive to 313 nm light in the presence of acetophenone[7]. This might have been expected on the basis of notions about triplet–triplet energy transfer. The interaction responsible for triplet excitation transfer is the exchange interaction which requires close approach (nearly collisional) of the donor and acceptor chromophores. Thus the sensitizer must be present inside the phage head in order to be sufficiently close to the DNA. Fortunately there exists a mutant of T4 which possesses more porous protein coats capable of passing quite large cationic dye molecules‡. The use of these mutants together with the positively charged acetophenone (E_T = 26500 cm^{-1}) derivatives, AcφM (E_T = 26500 cm^{-1}) and AcφD (E_T = 26500 cm^{-1}), shown below, allowed efficient sensitization of the inactivation of the phage with 313 nm light[7]. The sensitizers have no effect on the phage in the absence of light.

Curves for the survival of plaque-forming ability as a function of irradiation time are given[8] in *Figure 3*. The efficiency of sensitization increases in the order Acφ < AcφM < AcφD, the order of increasing strength of binding of the sensitizers to the DNA polyanion. This together with the much greater sensitivity of the osmotic shock resistant mutant over that of the wild type strongly indicates that the sensitization is due to interaction of sensitizer with the DNA inside the phage head. Several other lines of indirect

† The phage consists essentially of a ball of DNA with associated polyamines contained within a protein coat. A proteinaceous appendage or tail and associated tail fibers have the function of attachment and penetration of the *E. coli* host cell into which the DNA is injected.

‡ Unlike the wild type these mutants can withstand severe osmotic shock due to their ability to pass ions and molecules quickly through the protein coat.

evidence for this conclusion have been obtained such as the insensitivity of the sensitization to the presence of oxygen[7]. At this point one could argue on the basis of analogy with the results for *E. coli* DNA in solution that the sensitization by the acetophenone derivatives is due to the production of thymine dimers in the phage DNA after triplet excitation transfer from the sensitizer. However, it was desirable to demonstrate this in a direct way and two methods were employed to do so.



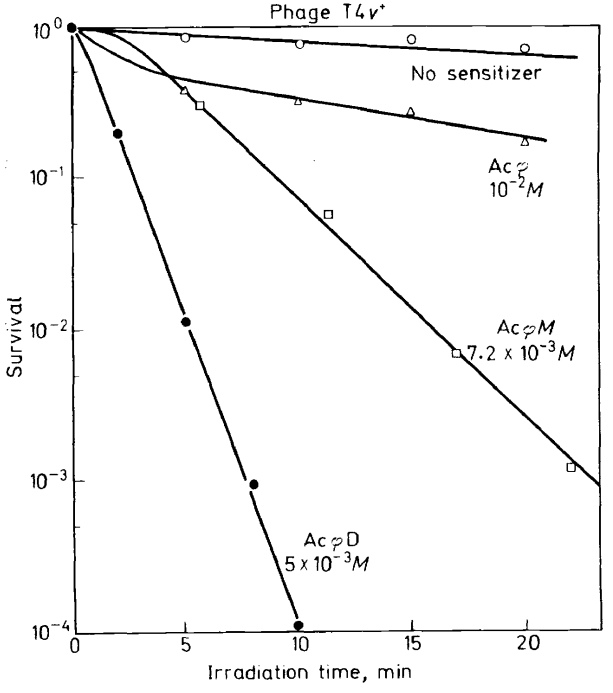


Figure 3. The survival of plaque-forming ability of $T_4BO_1V^+$ as a function of irradiation time (313 nm) in the presence of three different sensitizers and in the absence of sensitizer.

The first was simply to assay for the presence of thymine dimers in the sensitized phage at biological doses (5–10 phage lethal hits†, plh). Phage containing ^{14}C-labelled thymine were grown up and irradiated and the thymine dimer content was determined by radiochromatographic techniques after hydrolysis of the phage[8]. Thirty $\widehat{TT}$ per plh were found in the phage irradiated in the presence of AcφD (313 nm) compared to 10 $\widehat{TT}$/plh for phage irradiated at 254 nm. Thus sensitization with AcφD does lead to $\widehat{TT}$ production in the DNA and it appears that more dimers relative to other photoproducts are introduced by sensitization compared to u.v. irradiation. Radiochromatograms of hydrolysates of phage irradiated at several hundred plh are shown in *Figure 4*. At these doses $\widehat{TT}$ production becomes slow and other photoproducts are built up. Three features of the results are important: (1) the sensitized photolysis appears to give $\widehat{TT}$ as the predominant product while there is at least one additional major product of the direct irradiation; (2) several minor products result from either excitation method, and (3) the products other than $\widehat{TT}$ appear to be different for the sensitized photolysis compared to direct irradiation.

Host cell photoreactivation provided another means of indicating the presence of $\widehat{TT}$ in the phage irradiated in the presence of sensitizers. The *E. coli* host cells contain photoreactivating enzyme which is known to reverse pyrimidine dimer damage in the T4 DNA when the infected cells are exposed to light of wavelengths 300–400 nm. Indeed the phage inactivated by sensitized irradiation were found to be photoreactivated. Furthermore, photoreactivation was observed to reverse up to 80 per cent of the lethal damage caused by sensitized irradiation as opposed to 60 per cent of the u.v. damage[8,9]. This is expected if sensitization introduces more $\widehat{TT}$ relative to other products compared to u.v. light, photoreactivation is connected specifically with the repair of pyrimidine dimers, and thymine dimers are lethal lesions.

Another enzymic repair system–excision repair involves a series of nucleases and polymerases coded for by genes in the phage. This system repairs pyrimidine dimer damage as well as chemically induced damage such as that introduced by alkylating agents although less efficiently. Comparison of the results for phage mutants which are deficient in excision repair with those for phage normal in this respect also indicated that sensitization introduces relatively more $\widehat{TT}$ than does u.v. irradiation[8].

Meistrich measured the relative inactivation rates of several T4 mutants with operable and inoperable repair functions for both sensitized and u.v. irradiation together with their photoreactivable sectors[9]. The data could be analysed[9] on the basis of a simple model which is consistent with the known facts about the various repair modes and has resulted in a quantitative assessment of the relative contributions of $\widehat{TT}$, other pyrimidine dimers, and non-dimer damage products to the u.v.-induced inactivation of the phage and the relative efficiencies with which these three classes of lesions are repaired.

The sensitization technique was also used to gain insight into the mechanism of u.v.-induced mutations[10,11]. A comparison of the mutation frequency

† One plh is the dose required to reduce the survival to $1/e$ of the original.

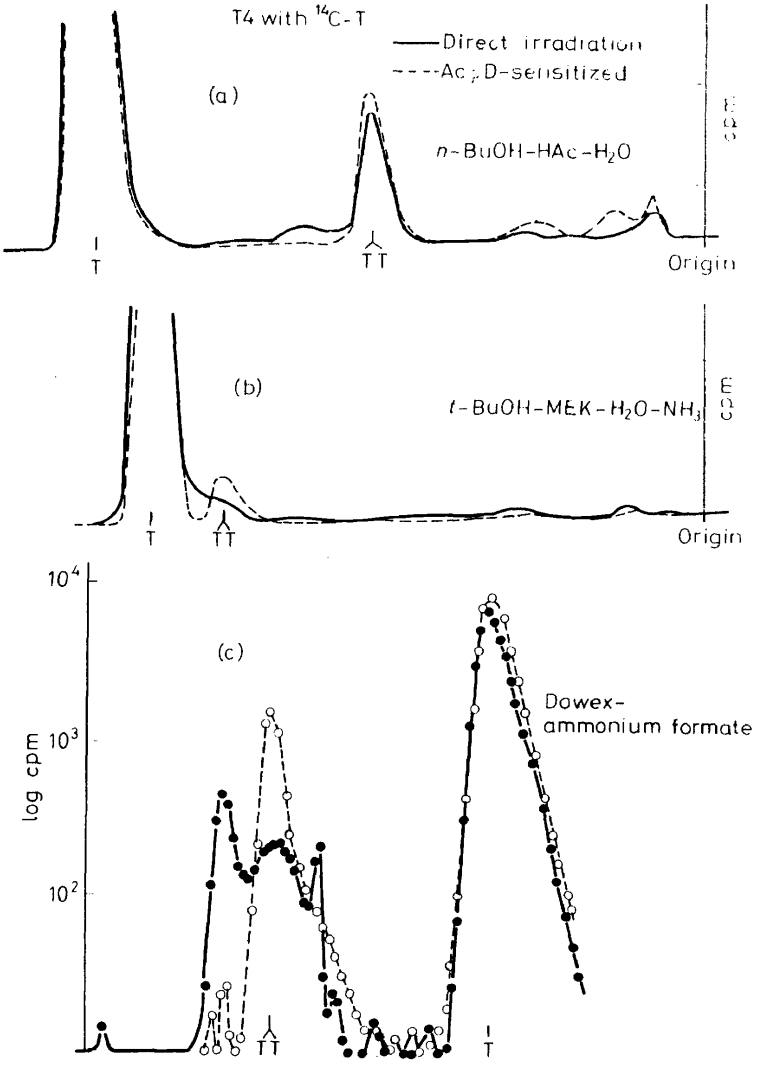


Figure 4. Radiochromatograms of hydrolysates of ^{14}C-thymine labelled $T_4BO_1V^+$ phage irradiated (~1000 plh) at 254 nm (direct) or at 313 nm in the presence of AcφD (AcφD sensitized): (a) *n*-butanol:acetic acid:water 80:12:30 v/v on Whatman No. 1 paper; (b) *t*-butanol:methylethylketone:water:ammonia 40:30:20:10 v/v on Whatman No. 1 paper; (c) Dowex 1-X2 column, ammonium formate gradient.

and reversion analysis for sensitized and u.v.-irradiated phage together with the inactivation results has led to the following conclusions[10,11]: (1) Thymine dimers can act as premutational lesions. (2) Dimers as well as other photoproducts lead to very similar types of mutants with nearly the same frequency, so that (3) an indirect mechanism for mutation is strongly indicated. That is, the mechanism of specific mispairing of bases with the lesion during

replication, which may be responsible for spontaneous and some chemically induced mutations, is not the mechanism responsible for the bulk of u.v.-induced mutants. These mutations may be due to errors introduced in the course of some repair sequence but this has yet to be proved.

REFERENCES

[1] For example the many free radical chain reactions induced by the action of light on photo-initiators.
[2] A. A. Lamola in *Technique in Organic Chemistry*, Vol. XIV, *Energy Transfer and Organic Photochemistry*, p 17. P. A. Leermakers and A. Weissberger, Eds. Interscience: New York (1969).
[3] N. J. Turro, J. C. Dalton and D. S. Weiss in *Organic Photochemistry*, Vol. II, p 1. D. L. Chapman, Ed. Marcel Dekker: New York (1969).
[4] P. J. Wagner and G. S. Hammond, *Advanc. Photochem.* **5**, 21 (1968).
[5] A. A. Lamola and T. Yamane, *Proc. Nat. Acad. Sci. (U.S.)*, **58**, 443 (1967).
[6] A. A. Lamola, *Photochem. Photobiol.* **9**, 291 (1969).
[7] M. L. Meistrich, A. A. Lamola and E. Gabbay, *Photochem. Photobiol.* **11**, 169 (1970).
[8] M. L. Meistrich and A. A. Lamola, to be published.
[9] M. L. Meistrich, to be published.
[10] M. L. Meistrich and R. G. Shulman, *J. Molec. Biol.* **46**, 157 (1969).
[11] M. L. Meistrich and J. Drake, unpublished results.
[12] F. W. Stahl, 'Radiobiology of bacteriophage' in *The Viruses*, Edited by F. M. Burnet and W. M. Stanley, Vol. II, p 353. Academic Press: New York (1959).
[13] F. L. Gates, *Science*, **68**, 479 (1928).
[14] M. Delbrück, *1969 Nobel Lectures*, Elsevier: New York (1970); *Science*, **168**, 1312 (1970).
[15] R. L. Sinsheimer, *Radiation Research*, **1**, 505 (1954).
[16] A. M. Moore, *Canad. J. Chem.* **36**, 281 (1958).
[17] D. Shugar and K. Wierzchowski, *Biochim. Biophys. Acta*, **23**, 657 (1957).
[18] K. L. Wierzchowski and D. Shugar, *Acta Biochim. Polon.* **8**, 219 (1961).
[19] R. Beukers and W. Berends, *Biochim. Biophys. Acta*, **41**, 550 (1960).
[20] H. L. J. Bäckström and K. Sandros, *Acta Chem. Scand.* **12**, 823 (1958); **14**, 48 (1960).
[21] G. Porter and F. Wilkinson, *Proc. Roy. Soc. A*, **264**, 1 (1961).
[22] For a current review see S. Y. Wang in *The Basic Principles in Nucleic Acid Chemistry*. Edited by P. O. P. Ts'O. Academic Press: New York, in press.
[23] R. L. Sinsheimer and R. Hastings, *Science*, **110**, 525 (1949).
[24] L. Grossman and E. Rodgers, *Biochem. Biophys. Res. Commun.* **33**, 975 (1968).
[25] C. H. Krauch, D. M. Krämer, P. Chandra, P. Mildner, H. Feller and A. Wacker, *Angew. Chem. (Internat. Ed.)* **6**, 956 (1957); A. A. Lamola, unpublished.
[26] A. A. Lamola and J. P. Mittal, *Science*, **154**, 1560 (1966).
[27] C. L. Greenstock, I. H. Brown, J. W. Hunt and H. E. Johns, *Biochem. Biophys. Res. Commun.* **27**, 431 (1967).
[28] J. G. Burr, B. R. Gordon and E. H. Park, *Photochem. Photobiol.* **8**, 73 (1968).
[29] J. G. Burr, unpublished results.
[30] A. Wacker, H. Dellweg and D. Weinblum, *Naturwissenschaften*, **47**, 477 (1960).
[31] R. B. Setlow, *Biochim. Biophys. Acta*, **49**, 237 (1961).
[32] D. Weinblum, *Biochem. Biophys. Res. Commun.* **29**, 384 (1967).
[33] R. B. Setlow and J. K. Setlow, *Proc. Nat. Acad. Sci. (U.S.)*, **48**, 1250 (1962).
[34] D. L. Wulff and C. S. Rupert, *Biochem. Biophys. Res. Commun.* **7**, 237 (1962).
[35] See P. Howard-Flanders, *Ann. Rev. Biochem.* **37**, 175 (1968).
[36] R. B. Setlow and W. L. Carrier, *J. Molec. Biol.* **17**, 237 (1966).
[37] I. vonWelucki, H. Matthaus and C. H. Krauch, *Photochem. Photobiol.* **6**, 497 (1967).
[38] A. A. Lamola, unpublished results.
[39] C. L. Greenstock and H. E. Johns, *Biochem. Biophys. Res. Commun.* **30**, 21 (1968).
[40] I. Brown and H. E. Johns, *Photochem. Photobiol.* **8**, 273 (1968).
[41] D. W. Whellans and H. E. Johns, *Photochem. Photobiol.* **9**, 232 (1968).
[42] A. A. Lamola and J. Eisinger, to be published.
[43] G. J. Fischer and H. E. Johns, *Photochem. Photobiol.*, in press.

[44] R. Lesewski and K. L. Wierzchowski, *Chem. Commun.* 348 (1969).
[45] J. Eisinger and A. A. Lamola, *Molec. Photochem.* **1**, 209 (1969).
[46] J. Eisinger and R. G. Shulman, *Proc. Nat. Acad. Sci. (U.S.)*, **58**, 895 (1967).
[47] T. Yamane, B. J. Wyluda and R. G. Shulman, *Proc. Nat. Acad. Sci. (U.S.)*, **58**, 439 (1967).
[48] A. Wacker, *Progress Nucleic Acid Res.* **1**, 369 (1963).
[49] P. S. Pershan, R. G. Shulman, B. J. Wyluda and J. Eisinger, *Science*, **148**, 378 (1965).
[50] A. D. Lenherr and M. G. Ormerod, *Nature, London*, **225**, 546 (1970).
[51] J. E. Donnellan and R. B. Setlow, *Science*, **149**, 308 (1965);
R. S. Stafford and J. E. Donnellan, *Proc. Nat. Acad. Sci. (U.S.)*, **59**, 822 (1968).
[52] A. J. Varghese, *Biochem. Biophys. Res. Commun.* **38**, 484 (1970).
[53] S. Y. Wang and A. J. Varghese, *Biochem. Biophys. Res. Commun.* **29**, 543 (1967); *Science*, **156**, 955 (1967).
[54] A. Wacker *et al.*, *Photochem. Photobiol.* **3**, 369 (1964).
[55] G. Scholes, P. Shaw and R. L. Welson in *Pulse Radiolysis*, p 151. Edited by M. Ebert. Academic Press: New York (1965).
[56] J. P. Spikes and R. Livingston, *Advanc. Radiobiology*, **3**, 29. Academic Press: New York (1969).
[57] K. C. Smith in *Photophysiology*, Vol. II, p 329. Edited by A. C. Giese, Academic Press: New York (1964).
[58] J. K. Setlow in *Comprehensive Biochemistry*, Edited by M. Florkin and E. H. Stotz, Vol. XXVII, p 157. Elsevier: Amsterdam (1967).
[59] R. B. Setlow, *Photochem. Photobiol.* **7**, 643 (1968).
[60] M. Gueron, J. Eisinger and R. G. Shulman, *J. Chem. Phys.* **47**, 4077 (1967).
[61] A. A. Lamola, M. Gueron, T. Yamane, J. Eisinger and R. G. Shulman, *J. Chem. Phys.* **47**, 2210 (1967).
[62] J. Eisinger and A. A. Lamola. unpublished.
[63] B. E. Zierenberg, D. E. Krämer, M. G. Geisert and R. G. Kirste, *Photochem. Photobiol.*, to be published.
[64] R. Ben-Eshai, E. Ben-Hur, and Y. Hornfield, *Israel J. Chem.* **6**, 769 (1968).
[65] R. Ben-Eshai, unpublished results; D. Kramer, unpublished results.