Cyclobutane-Type Pyrimidine Dimers in Polynucleotides

Ultraviolet radiation forms dimers that have distinctive properties and affect biological systems.

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In the past few years there have been rapid advances in our knowledge about the molecular mechanisms involved in the effects of ultraviolet radiation on nucleic acids and polynucleotides (*1*). These advances have influenced and been influenced by three related areas in biology: (i) the effects of ultraviolet radiation on viruses and cells; (ii) the genetic control of radiation sensitivity; and (iii) the repair of radiation and chemical damages to DNA in vivo and in vitro. The fundamental research in nucleic acid photochemistry has stimulated new experiments and approaches in such diverse fields as template coding and genetic recombination. In this article I shall summarize some aspects of nucleic acid photochemistry, placing particular emphasis on pyrimidine dimers because such photoproducts have been characterized and because they seem to play an important part in the biological effects of ultraviolet light on nucleic-acid-containing systems. This approach is not meant to imply that pyrimidine dimers are the only important photoproducts, or that they can account for all effects of ultraviolet radiation on biological systems. For example, ultraviolet radiation does affect proteins (*2*), is able to crosslink, in some unknown way, proteins and nucleic acids (*3*), and can form large numbers of photohydrates in irradiated RNA and denatured DNA (but no experimental evidence indicates that such hydrates are formed in native DNA) (*2, 4*). The pyrimidine dimers I shall be concerned with here are presumed to represent adjacent pyrimidine residues, in the same nucleic acid strand, that are linked together by a cyclobutane ring (Fig. 1) (*5*). Formation of a dimer results in a decreased spacing between the pyrimidine rings and a change in their orientation as compared to those found in a Watson-Crick structure, and would be expected to result in a localized distortion of the helix. Before I consider the photochemistry of dimers, I shall briefly review the arguments that implicate them in the biological effects of ultraviolet irradiation of nucleic-acid-containing systems.

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Pyrimidine Dimers As Lesions

It is important to state explicitly the chain of inferences that implicates pyrimidine dimers in DNA as biological lesions, in order to bring out the weak points in the argument, emphasize that dimers cannot explain all the effects of ultraviolet radiation on nucleic acids, and point out that the arguments depend strongly on the photochemical properties of pyrimidine dimers and the interaction of such dimers with various enzymes. The arguments implicating pyrimidine dimers as biological lesions (*1, 6*) may be summarized as follows:

1) The mean lethal doses (the doses that yield 37 percent survival) to bacteria, viruses, and transforming DNA produce one or more than one dimer in the DNA of the irradiated system (*7, 8*). Such doses make a negligible number of chain breaks or crosslinks in double-stranded DNA (*6*). The numbers of other types of photoproducts are not known.

2) Ultraviolet radiation inactivates transforming DNA, destroys the ability of DNA to act as a primer in the calf thymus polymerase system, and results in the formation of nuclease-resistant sequences in DNA. These three effects show the particular photochemical properties (see below) associated with pyrimidine dimers (*9, 10*), properties not associated with chain breaks, crosslinks, or formation of hydrates.

3) Many biological systems show the phenomenon of photoreactivation; that is, when they have been inactivated by ultraviolet radiation they may be reactivated by subsequent exposure to visible or near-ultraviolet light (approximately 350 to 450 millimicrons) (*11*). Photoreactivation in vitro, with purified enzymes, has been shown to destroy pyrimidine dimers in DNA, probably by monomerizing them (*12*). Photoreactivation has not been demonstrated for other types of lesions, and for ultraviolet-irradiated transforming DNA it has been shown that photoreactivation does nothing more than monomerize dimers. (Because the definition of dimers in such systems depends on the photochemical behavior of irradiated DNA, this argument also hinges on the photochemistry of nucleic acids.) Photoreactivation also destroys dimers in vivo in cells that contain photoreactivating enzymes (*8*).

4) Irradiation of sensitive bacterial cells—cells that are killed by small doses of ultraviolet radiation—results in the permanent inhibition of DNA synthesis by doses that give rise to about five pyrimidine dimers per single strand (about 1000 microns) of the bacterial DNA. It is reasonable to suppose that such cells die because they cannot make DNA. Illumination of such irradiated cells with photoreactivating light decreases the inhibition of DNA synthesis and restores the ability to form colonies to the same extent that it monomerizes dimers (*8*).

5) Radiation-resistant bacteria are inactivated appreciably only after doses that produce, say, over 500 dimers per DNA strand. For such bacteria one could assume either that dimers have nothing to do with inactivation or that the cells have some very efficient mechanism for coping, in the dark, with what seems to be a biologically important lesion. The former possibility cannot be disproved, but it has been shown that radiation-resistant cells have enzymatic mechanisms that excise the dimers from their DNA (*13*) (sensitive cells do not), and there are experimental indications that the holes that would be left in the DNA as a

result of excision are repaired by synthesis in which newly incorporated material is distributed randomly along the DNA, rather than along one strand of a double helix as in normal replication (*14*).

6) The amount of reactivation ascribed to dark-repair mechanisms, such as liquid-holding restoration, host-cell reactivation, and *v*-gene reactivation, does not add to that produced by photoreactivation (*15*). Thus the two types of mechanisms nullify the effects of very similar, if not identical, photoproducts—although they may do so by very different molecular mechanisms.

There are several weak but confirming lines of evidence pointing to pyrimidine dimers. (i) The presence of proflavine during irradiation protects some viruses against inactivation by ultraviolet radiation, and Beukers has shown that the binding of this dye inhibits the formation of dimers in DNA (*16*). (ii) Since thymine dimers are formed somewhat more efficiently than cytosine-containing ones in DNA (*17*), it is understandable that the ultraviolet sensitivity of microorganisms should, on the average, increase with the thymine content of the DNA (*18*), but should not necessarily increase in proportion to the frequency of adjacent thymines. (iii) The substitution of thymine by bromouracil results in biological systems which are weakly or no longer reactivable by photoreactivation or by dark-repair mechanisms (*19*). Bromouracil in DNA does not participate in the formation of cyclobutane types of dimers (*1, 20*). (Bromouracil-substituted DNA's are more sensitive to ultraviolet than normal DNA's, but the detailed reasons for this higher sensitivity are not really known.)

Photochemistry of Pyrimidine Dimers

The chain of inferences relating pyrimidine dimers to biological lesions depends not only on the identification of such dimers in DNA, but on the demonstration that the dimers, and not some other photochemical event, are responsible for the observed biological effects. Thymine dimers have been identified as the principal product resulting from the irradiation of thymine in aqueous frozen solutions by molecular-weight determinations, x-ray diffraction, infrared absorption, and nuclear-magnetic-resonance techniques (*5*). Such dimers may be prepared in relatively large amounts and serve as authentic compounds for comparison with the dimers isolated by acid hydrolysis of irradiated DNA. It is probable, but not proven, that most of the dimers formed in ice have the configuration shown in Fig. 1 (*5*). Cyclobutane-type dimers may be characterized by their low ultraviolet absorbance resulting from the saturation of the 5,6 double bond, by their chromatographic mobilities in various solvents, and by the fact that they are monomerized when irradiated in solution (*21*). This last reaction may be written in the general form

$$TT \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \overline{TT} \qquad (1)$$

If, in any irradiated system, the constant k_2 is not zero, then a steady-state concentration of thymine dimers will be attained at high enough fluxes of incident light. The photochemistry of pyrimidine dimers that is of direct application to biological systems concerns the various ways of changing the constants k_1 and k_2 and of varying the steady-state value for dimers.

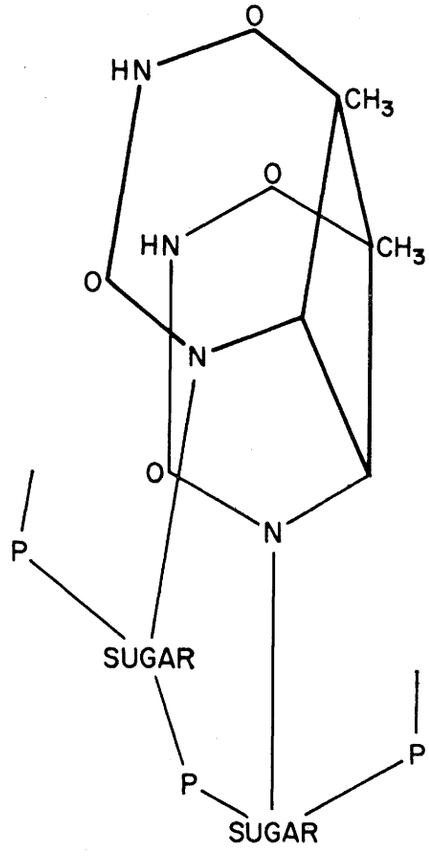


Fig. 1. A schematic diagram of the presumed structure of a thymine dimer in a DNA chain.

Other photoproducts obtained from the irradiation of frozen solution have been identified by analogy as uracil dimers, mixed uracil and thymine dimers, and cytosine-thymine dimers (*22*). (In the last product cytosine can presumably be deaminated to yield a uracil-thymine dimer.) Such photoproducts have characteristic chromatographic mobilities and are monomerized when irradiated in solution. The argument identifying these dimers is really circular because the property of monomerization in solution is taken to indicate the presence of a cyclobutane type of structure. In general, for any two pyrimidines,

$$\text{— PyPy' —} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{— } \overline{\text{PyPy'}} \text{ —} \qquad (2)$$

Photoproducts have been isolated by chromatographic techniques from acid hydrolysates of irradiated DNA (*1, 17, 23*). These photoproducts have chromatographic mobilities similar to those of the known pyrimidine dimers, and they are monomerized in solution with the same kinetics as are thymine dimers. The majority of these photoproducts do not seem to be artifacts arising from the acid-hydrolysis technique, because the observed changes in ultraviolet absorbance of irradiated DNA's can be correlated with the numbers of isolated photoproducts observed, and enzymatic hydrolysis of irradiated DNA's yields sequences that contain pyrimidine dimers to the extent expected from the observed photoproducts in acid hydrolysates (*4, 10*). It is worth emphasizing again that there are photoproducts other than cyclobutane-type dimers in irradiated dinucleotides and DNA (*1, 20, 24*). By definition these photoproducts, unlike pyrimidine dimers, are not monomerized.

Ways of Changing Reaction Rates and Steady-State Values

The rate constants k_1 and k_2 in Eq. 2 are proportional to the intensity of the incident light and to the product of two factors—the absorption coefficient of the molecule (the probability that an incident quantum will be absorbed) and the quantum yield (the

probability that an absorbed quantum will affect the molecule). Some ways in which the ratio of the constants k_1 and k_2 (and therefore the steady-state value for dimers) may be altered are shown in Table 1 and discussed below.

Thymine in frozen aqueous solutions is not uniformly dispersed, but is in the form of small solid aggregates (*25*). As a result, thymine molecules are close together, and an absorbed photon has a good chance of forming a dimer. The quantum yield for formation is between 1 and 2 (*26*), whereas in dilute liquid solution it is zero. On the other hand, the quantum yield for splitting dimers in liquid solution is between 0.5 and 1 (*27*). Thus irradiation of frozen solutions of thymine results in the formation of appreciable numbers of dimers, and irradiation of liquid solutions monomerizes the dimers (*21, 28*). Polynucleotides in liquid solutions contain adjacent pyrimidines that are closer to one another than they would be if not in a polymer. The quantum yield for formation is not zero but about 0.01, and the yield for monomerization is similar to that for free dimers (*23, 29, 30*). Despite the low yield for dimer formation in polynucleotides, appreciable numbers of dimers are formed as a result of irradiation with long wavelengths because of the high absorbance of thymine compared to dimers. The effect of freezing is to inhibit dimer formation in polynucleotides, although freezing increases the ultraviolet-sensitivity of phage and bacteria (*31*).

Table 1. Several ways of changing the constants k_1 and k_2 in Eq. 2, for the dimerization and monomerization of pyrimidines.

Procedure	Change in k_1/k_2	As a result of change in
Freezing solutions of bases	Increases	Quantum yield
Increasing wavelength	Increases	Absorption coefficient
Adding the dye proflavine	Decreases	Quantum yield
Increasing *p*H	Decreases	Absorption coefficient

Two-Wavelength Effects

Saturation of the 5,6 double bond of pyrimidines results in characteristic changes in absorption spectra (Fig. 2). It makes little difference whether the saturation arises from the formation of a cyclobutane ring or from the photochemical addition of water to the pyrimidines. However, the water adducts are not stable and revert to the parent compound upon heating (*2*). Moreover, the water adducts do not exhibit the photochemically reversible reaction indicated in Eq. 1.

The identification of the photochemical reactions of RNA and DNA has been aided by investigations of nucleic acid polymers of known composition. Such polymers are the single-stranded polyuridylic acid (polyU), polycytidylic acid (polyC), and polythymidylic acid (polyT), and the double-stranded ones with complementary chains of deoxyadenylic acid and thymidylic acid (dA:dT), deoxyinosinic acid and deoxycytidylic acid (dI:dC), and alternating deoxyadenylic-thymidylic acid units (dAT).

It is obvious from Fig. 2 that long wavelengths are absorbed strongly by the pyrimidines and very little by the dimers, and that short wavelengths are much more efficiently absorbed by dimers than are long wavelengths. Thus the steady-state concentration of dimers in polynucleotides is wavelength-dependent, and if a polynucleotide containing adjacent pyrimidines is irradiated with long wavelengths, dimers will be formed and the absorbance of the polymer in solution will decrease. Subsequent short-wavelength radiation, strongly absorbed by the dimer, will split some of the dimers and result in an increase in absorption. Typical ex-

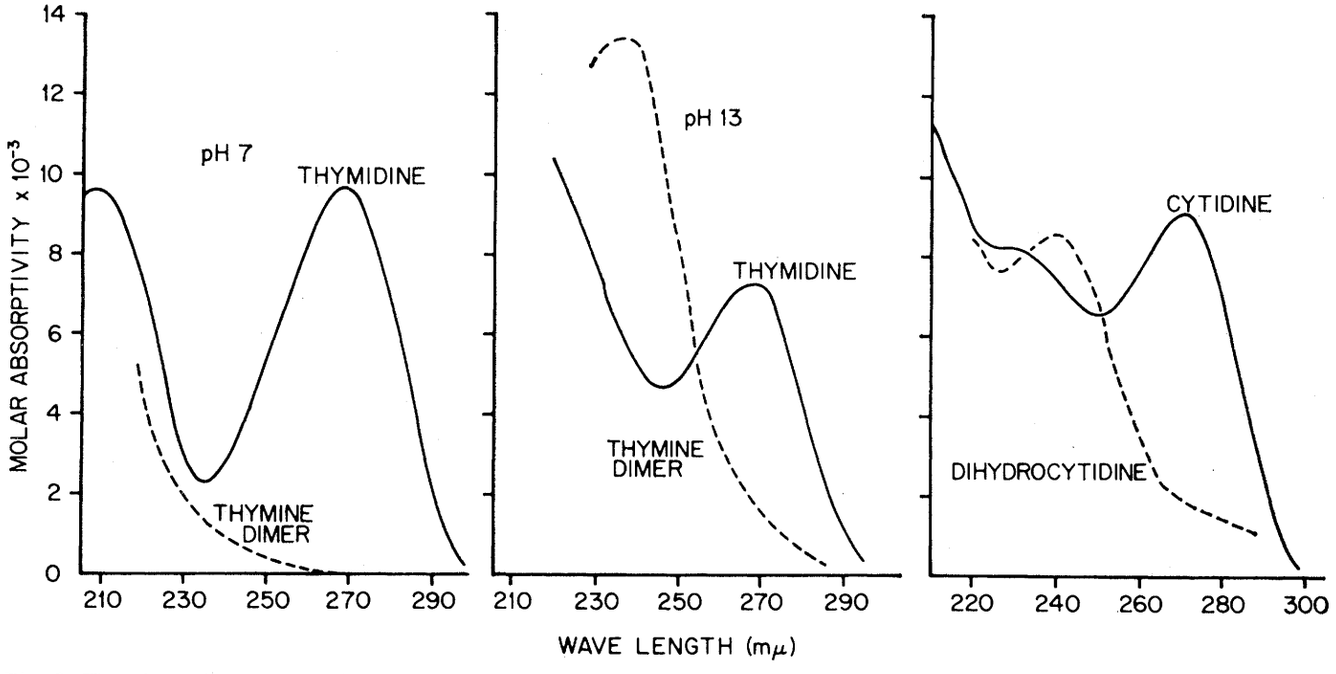


Fig. 2. The absorption spectra of thymidine, thymine dimer, cytidine, and dihydrocytidine (*2, 27, 29*). The deoxycytidine derivatives presumably have spectra similar to those of cytidine, and a cytosine dimer in a polynucleotide probably has a molecular absorptivity twice that of dihydrocytidine.

amples of this two-wavelength effect on the absorbance of irradiated polynucleotides are shown in Fig. 3. The data may be summarized by the scheme

$$\text{— PyPy'} \underset{\text{short } \lambda}{\overset{\text{long } \lambda}{\rightleftharpoons}} \text{— PyPy —} \rightleftharpoons \text{— PyPy —} \qquad (3)$$

The changes in absorbance observed in irradiated DNA are less than those in polyT because there are fewer pyrimidine sequences in DNA. The absorbance changes in dI:dC are also less than those in polyT because the cytosine dimers at long wavelengths have a higher absorption coefficient than thymine dimers. It is also apparent that the splitting of cytosine dimers (as measured by the increase in absorbance during short-wavelength irradiation) is more rapid than the increase for thymine dimers, again because of the higher absorption coefficient of cytosine dimers. The absorbance changes exhibited by irradiated polyU are more complicated than for the other polymers shown in Fig. 3, because ultraviolet irradiation results in the formation of both dimers and hydrates. Short-wavelength irradiation results in the splitting of dimers and in the formation of hydrates, and as irradiation continues, more hydrates are formed and fewer uracils are available for dimerization. The net result for polyU is that a steady-state of dimers is not reached but that with high doses almost the entire polymer may be converted to hydrates. The polymers to which the type of analysis exhibited by the curves of Fig. 3 has been applied are shown in Fig. 4—hydrates being identified by heat-labile changes in ultraviolet absorbance and dimers by changes exhibiting the reversibility shown by Eq. 1.

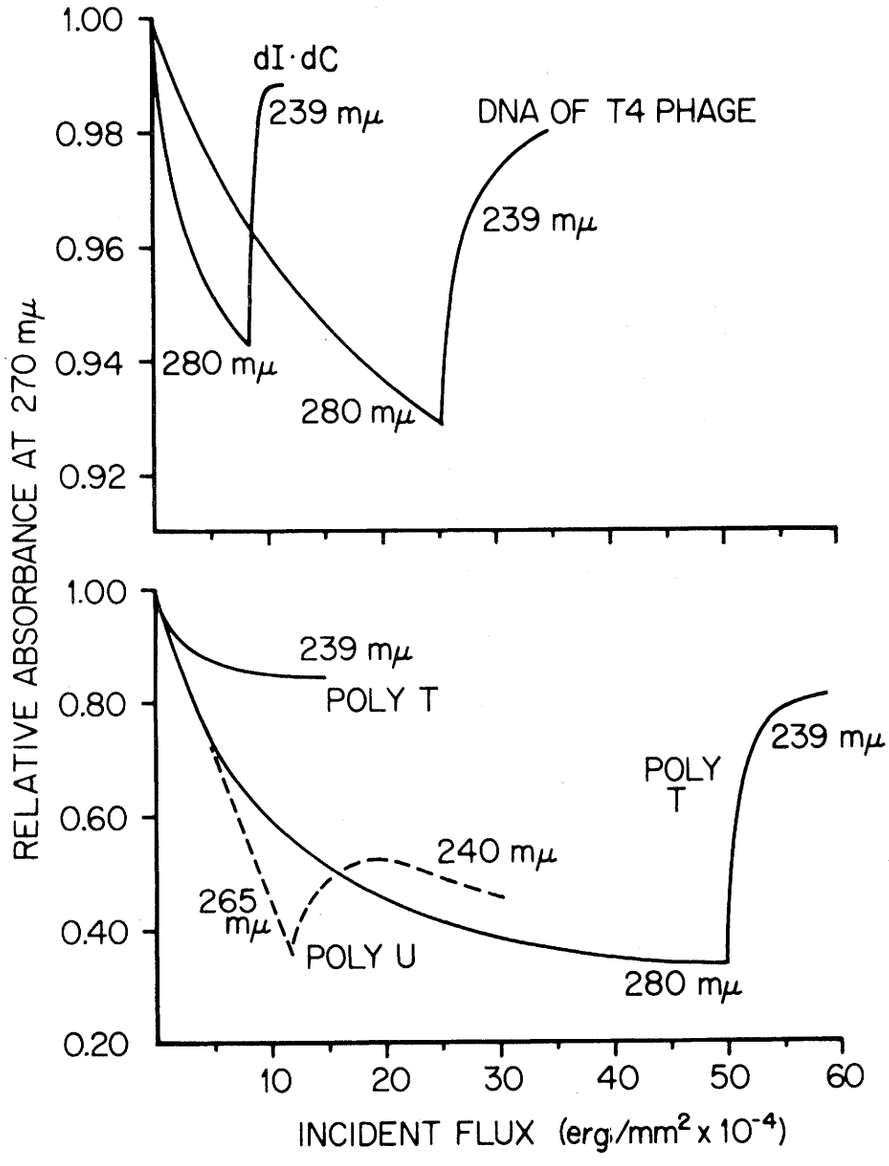


Fig. 3. The relative absorbances of polynucleotides irradiated with a combination of long (265 or 280 mμ), and short (239 or 240 mμ) radiation. The absorbance increases produced by short wavelengths after long-wavelengths irradiation are characteristic of dimer monomerization (*4, 12, 29, 32*).

It is crucial to recognize that, even at short wavelengths, some dimers are formed, and it is only when doses are large in comparison to those usually used for the inactivation of biological systems that the steady-state value is approached. Thus use of the two-wavelength type of experiment on a biologically active system requires doses so large that most biological systems retain no measurable activity (*9, 33*). One of the few biological systems that exhibit activity after large doses of radiation is transforming DNA. Transforming DNA is inactivated by ultraviolet radiation, and one would predict that, if pyrimidine dimers were important in its inactivation, the effects of long-wavelength ultraviolet radiation would be reversed in part by short wavelengths. This, indeed, has been shown to be the case (Fig. 5). The increase in biological activity is quantitatively related to the monomerization of dimers, as indicated by increases in absorption of the irradiated DNA. If dimers alone were responsible for all the inactivation, then short-wavelength radiation should cause only an increase in activity. However, with continued irradiation at short wavelengths a subsequent inactivation is observed (*9*). This decrease is evidence for the inactivation of transforming DNA by other types of photoproducts. At the large doses used in these experiments, it is estimated that between 50 and 70 percent of the inactivation arises from pyrimidine dimers.

Enzymatic photoreactivation removes about 90 percent of the biological damage from transforming DNA irradiated with low doses and thus indicates that about 90 percent of the inactivation at low doses arise from pyrimidine dimers (*11*).

The dye proflavine, when bound to DNA, inhibits the formation of dimers, but does not seem to inhibit their breakage (*16*); that is, it lowers the quantum yield for formation but does

Fig. 4 (right). A schematic representation showing the principal types of pyrimidine photoproducts in irradiated polynucleotides as detected by absorbance changes that are reversible by heat (hydrates) and by short-wavelength radiation (dimers). The sugar phosphate groups that link the bases together are not shown.

not affect the quantum yield for monomerization. Schematically (when h is Planck's constant and ν is the frequency of the emitted radiation), at any *one* wavelength,

$$\begin{array}{l} -\mathrm{TT}- \underset{\text{proflavine}}{\overset{h\nu}{\rightleftharpoons}} -\overline{\mathrm{TT}}- \\ -\mathrm{TT}- \underset{\text{proflavine}}{\overset{h\nu}{\rightleftharpoons}} -\overline{\mathrm{TT}}- \end{array} \qquad (4)$$

Thus one can change the steady-state value of dimers found at high doses by an experiment of the type

$$-\mathrm{TT}- \overset{h\nu}{\rightleftharpoons} -\mathrm{TT}- \overset{h\nu}{\underset{\text{proflavine}}{\rightleftharpoons}} -\overline{\mathrm{TT}}- \qquad (5)$$

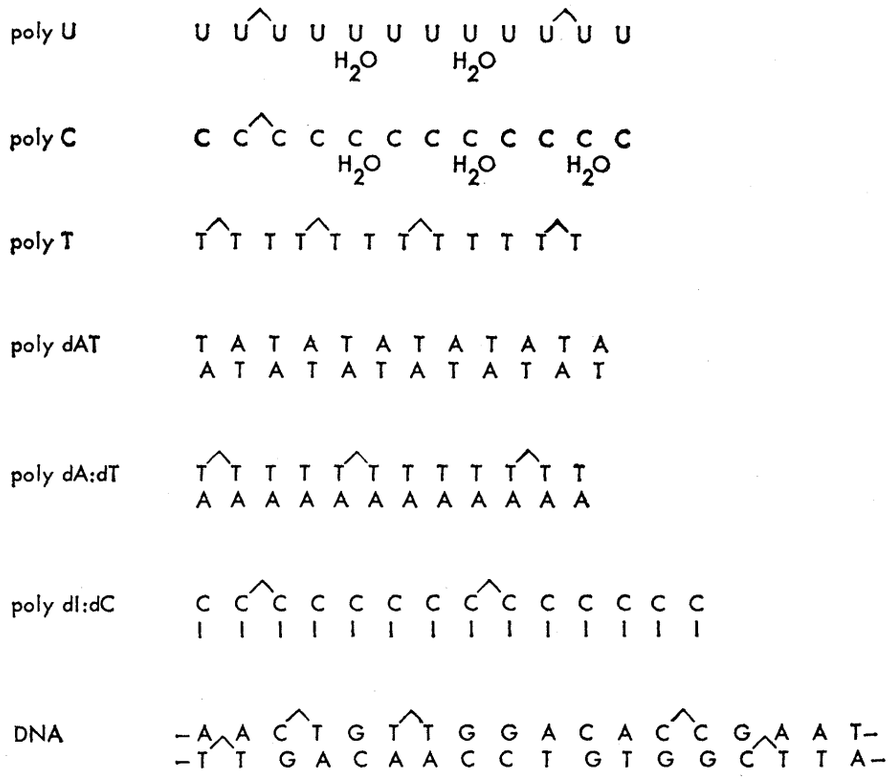
Such changes have biological consequences. Transforming DNA that has been inactivated by 254-millimicron radiation is reactivated by further irradiation, at the same wavelength, in the presence of proflavine.

The absorption spectra of dimers are dependent on *p*H (Fig. 2), but the quantum yields are not (*30*). As a result the steady-state value for dimers is much lower at *p*H 13 than at lower *p*H's, and the steady-state value can be changed by irradiation at, say, 254 millimicrons in the following way:

$$-\mathrm{TT}- \underset{p\mathrm{H}\ 7}{\overset{h\nu}{\rightleftharpoons}} -\mathrm{TT}- \underset{p\mathrm{H}\ 13}{\overset{h\nu}{\rightleftharpoons}} -\mathrm{TT}- \qquad (6)$$

Chromatographic Detection

Chromatographic techniques are much more sensitive than absorbance changes for detecting dimer-containing photoproducts. [Such photoproducts are defined by their composition, chromatographic mobilities, and their quantitative photochemical properties (summarized in Eq. 3).] They have been applied in a very elegant series of experiments to simple model compounds composed of two thymines or two cytosines linked by sugar phosphate groups (thymidylyl thymidine, TpT, and cytidylyl cytidine, CpC) (*34*). Two intramolecular cyclobutane dimers of thymine have been observed. One is formed in higher yield and probably has the form shown in Fig. 1. The other may have a *trans* configuration. Likewise, two cytosine-containing dimers have been observed. Such structures, whose 5,6 double bonds are saturated, tend to deaminate, with the net result that the cytosine dimer is converted into a uracil dimer. Two other photoproducts of irradiated



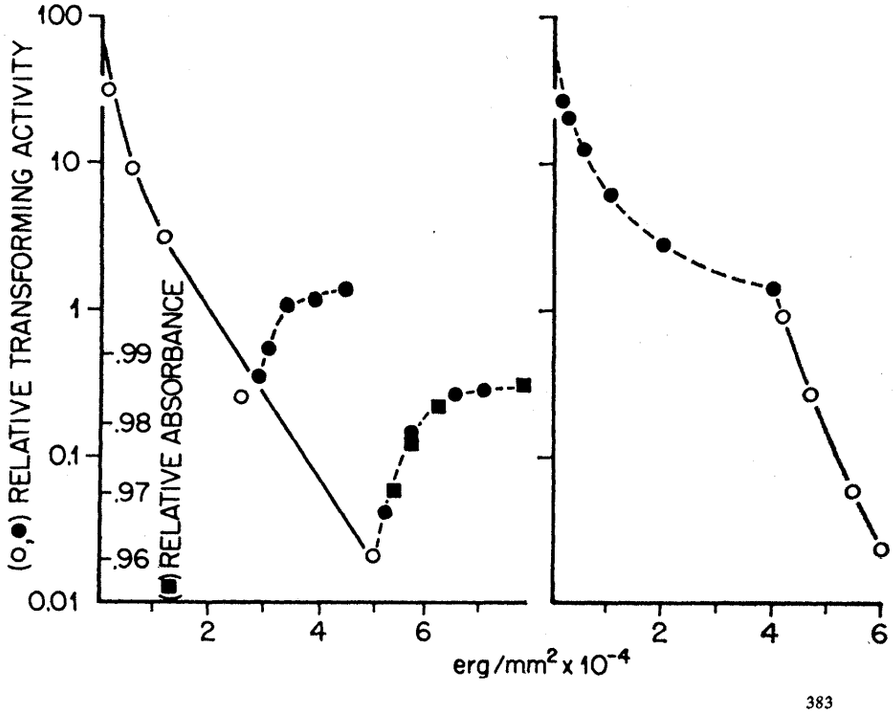


Fig. 5 (right). The effects of sequential irradiation with two wavelengths on the transforming activity of *Hemophilus influenzae* DNA (cathomycin marker); ○, 280 mμ; ●, 239 mμ. The symbol ■ represents the relative absorbance of the DNA and indicates the extent of dimer monomerization as in Fig. 3 (*9*).

TpT have been discovered. These are interconvertible, but they are not monomerized by ultraviolet radiation. It is not clear whether or not all four types of TpT products are formed in DNA.

Chromatographic analysis of acid hydrolysates of irradiated DNA's labeled with either thymine or cytosine may be used to determine the numbers of pyrimidine dimers of the various types that are formed. A typical chromatogram is shown in Fig. 6. The process of acid hydrolysis has converted the cytosine-containing dimers into uracil-containing ones. The relative radioactivity under the several peaks may be measured and, if the photoproduct has been identified, used to determine the numbers of photoproducts. For example, a dose of 2×10^3 ergs per square millimeter at 265 millimicrons to *Escherichia coli* DNA results in the formation of the following numbers of dimers per nucleotide: CC, 1.4×10^{-4}; CT + TC, 6.6×10^{-4}; TT, 11.7×10^{-4}. One may legitimately extrapolate from these doses to lower ones and so calculate that 1 erg per square millimeter (the mean lethal dose for the sensitive bacterium *E. coli* B_{s-1}) results, on the average, in between two and three pyrimidine dimers per DNA strand of the bacterial chromosome of *E. coli*.

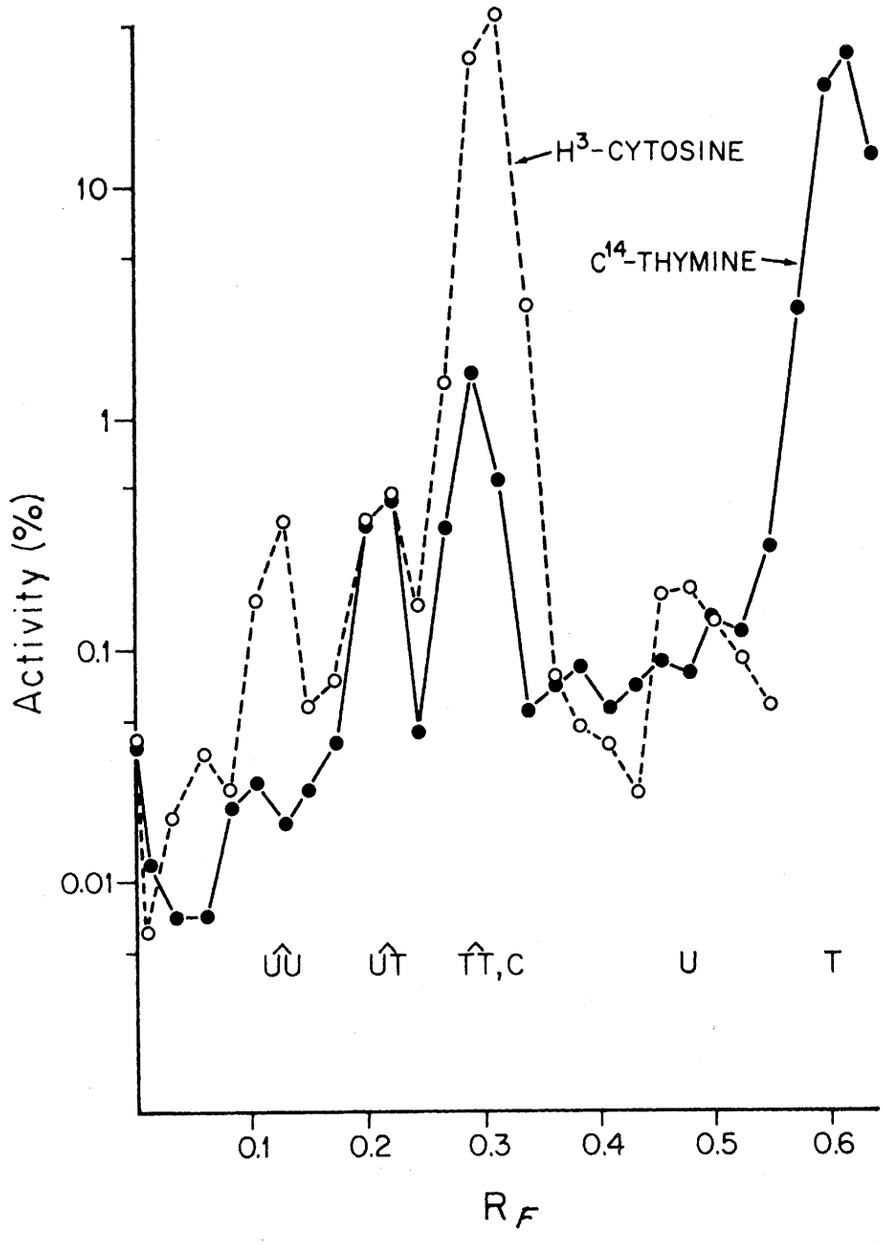


Fig. 6. The distribution of radioactivity along a chromatogram of a hydrolysate of DNA's, labeled with the indicated base, irradiated with 1×10^4 erg/mm^2 at 280 mμ. The positions of known compounds are indicated. The amounts of radioactivity in the dimer peaks depend on wavelength as shown in Fig. 3. The uracil-containing dimers come from deamination of cytosine dimers during acid hydrolysis. The small amount of uracil is not the result of irradiation but represents a slight degree of deamination of cytosine during hydrolysis (*7*). R_F, ratio of the movement of the substance to that of the front.

The relative amounts of the various pyrimidine dimers are shown in Table 2. Obviously, thymine dimers are the most numerous ones for high-AT DNA's, but they represent only a small part of the total for a DNA such as that from *Micrococcus lysodeikticus*. At high ultraviolet doses the relative number of thymine dimers increases because the cytosine-containing ones quickly reach a steady-state value as a result of the high absorption coefficient of the cytosine-containing dimers.

Photoreactivation and Dark Repair

If a DNA that contains pyrimidine dimers is treated with photoreactivating enzyme in the presence of visible light, the dimers disappear from acid hydrolysates of the reaction mixture (*35*). The inference that the dimers are really split or monomerized is on shaky grounds because the photoreactivating treatment may have converted the dimers to some other photoproduct that is not stable to acid hydrolysis and so would not be detected. (The radioactivity associated with such dimers in labeled DNA is usually so small that their monomerization cannot be detected by an increase in thymine or cytosine as a result of photoreactivation.) However, the use of a polymer such as dI:dC allows one to make a definitive decision in this matter. Irradiation of this polymer forms cytosine dimers which, upon heating, are converted to uracil dimers (*12*). An acid hydrolysate of such a polymer contains uracil dimers and cytosine. If the polymer is treated with photoreactivating enzyme plus light, however, the uracil dimers disappear, and in their place appears uracil, a substance very easy to detect in small amounts against the major label of cytosine. Such experiments indicate a quantitative conversion of uracil dimers to uracil, thereby demonstrating, at least for this polymer, that photoreactivating treatment results in monomerization of dimers. The reactions are quantitatively consistent with the scheme shown in Fig. 7. It is reasonable to suppose that

Table 2. The numbers of dimers in DNA's from three species of bacteria after irradiation with 265-millimicron ultraviolet light (2×10^3 erg/mm^2).

Percentage A+T	Total dimers per nucleotide	Percentage		
		$\overline{CC}$	$\overline{CT}+\overline{TC}$	$\overline{TT}$
Hemophilus influenzae				
62	2.7×10^{-2}	5	24	71
Escherichia coli				
50	2.0×10^{-2}	7	34	59
Micrococcus lysodeikiticus				
30	1.4×10^{-2}	26	55	19

photoreactivation represents monomerization of all types of pyrimidine dimers in native DNA.

Dimers may be destroyed by photoreactivation in vivo (*8*), but the inference that all photoreactivation in vivo arises from the monomerization of dimers is not correct. Illumination such as is used in photoreactivation may affect dark-repair systems and result in increased survival. This indirect type of photoreactivation is similar in many respects to what is called photoprotection (*36*). It does not involve dimer destruction. The conceptual difficulties in relating photoreactivation or dark repair (such as excision) to photochemical changes in DNA are great because the effects of repair on colony formation in bacteria or virus infectivity are usually observed long after the irradiation and reactivation have taken place. On the other hand, the inhibition of DNA synthesis can be measured shortly after irradiation, and its measurement permits one to relate dimers to the inhibition because the photoreactivation of this inhibition is associated with dimer monomerization in sensitive and resistant cells (*8*). Moreover, in radiation-resistant cells, DNA synthesis resumes after excision of dimers from DNA (*13*). Obviously, if the repair processes are very efficient, resistant cells may die as a result of photoproducts other than pyrimidine dimers.

Summary

The formation of cyclobutane-type dimers between adjacent pyrimidine residues in model polynucleotides or DNA may be represented by the general scheme

$$-\,\mathrm{PyPy}'\,- \underset{}{\overset{h\nu}{\rightleftharpoons}} -\,\overline{\mathrm{PyPy}}'\,-$$

whereas the formation of all other known photoproducts follows the irreversible path

$$-\,\mathrm{Nucleotide} \xrightarrow{h\nu} \mathrm{product}$$

Thus dimers are distinguished from other photoproducts by the fact that they can be monomerized, as well as formed, by ultraviolet irradiation. At large incident fluxes of photons the steady-state value of dimers depends on wavelength and *p*H, as well as on other characteristics of the surrounding medium. The number of dimers in an irradiated polynucleotide may be decreased by purely photochemical means, whereas this is not true for most other photoproducts, for which continued irradiation, irrespective of wavelength, always results in the formation of more photoproduct (*37*). The wavelength dependence of the steady-state for dimers is also reflected in the biological activity of irradiated transforming DNA. This experiment and the fact that photoreactivating enzyme plus visible light monomerizes dimers (and has not been demonstrated to have any effect on other photoproducts) are the strongest lines of experimental evidence that pyrimidine dimers of the cyclobutane type are biologically important lesions and can account for a large fraction of the effects of ultraviolet light on DNA in solution. Insofar as DNA is one of the more important biological structures, such dimers, when formed, account for a large part of the effects of ultraviolet radiation on biological systems.

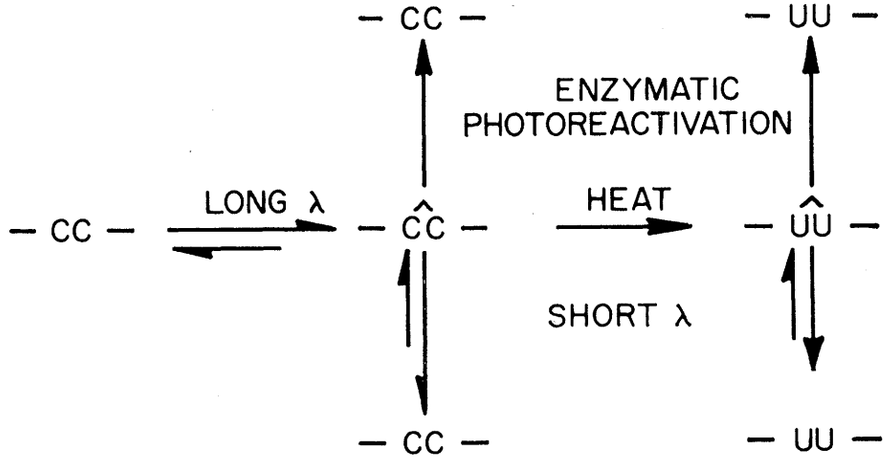


Fig. 7. A schematic diagram showing the reactions of cytosine dimers in a double-stranded polymer, such as dI : dC, in which one strand is deoxycytidylic acid (*12*).

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Research in the Prehistory of Central Western Iran

Recent preliminary excavations promise to document 40,000 years of prehistory in the Zagros Mountains.

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A noticeable recent trend in Near Eastern archeology is toward the study of a particular problem, such as the development of village farming communities or of urbanism, across a number of local time boundaries, accompanied by research in depth in a given geographical region. The activities of Braidwood in Iraq and adjoining countries, Perrot in Israel, Solecki in the Shanidar Valley of Iraq, and Mellaart in southwestern Anatolia, to mention only the best-known instances, have placed in proper perspective the development of culture and cultures across long spans of the food-gathering and food-producing eras in this vital area of the Old World, and amply demonstrate the advantages inherent in interdisciplinary field research (*1–3*). Dyson, working in the Solduz region of northwestern Iran, has demonstrated that this approach can be applied profitably in time ranges as late as the 1st millennium B.C. (*4*).

This article is a preliminary notice of further research of this type recently carried out (1965) in western Iran. One of us (Smith) is a prehistorian concerned mainly with the cultures of the Stone Ages (Paleolithic to Neolithic), while the other is principally interested in the Neolithic to Iron Age Near East. By combining our interests and methods of research in a program of field work over a number of digging seasons we hope to develop, in a relatively restricted region, a nearly continuous archeological sequence from at least Middle Paleolithic to Iron Age times. Studies of the natural setting of this sequence should enable us then to present the human history and the fluctuating Quaternary ecology of the area over an unusually long period of time.

After a broad survey of central western Iran during 1964–65, from northern Luristan to Azerbaijan, we have selected the upper reaches of the Gamas Ab River basin, in the core of the Zagros mountain chain of southern Kurdistan, for long-range study. Here a valley system about 60 kilometers long and 15 kilometers wide, stretching from just east of the town of Kangovar to the villages of Bisitun and Harsin, defines the modern ethnic border between Kurdistan and Luristan and straddles the ancient high road into Iran—the principal route linking the alluvial plains of Mesopotamia with the high Iranian plateau (Fig. 1).

Central western Iran is by no means virgin territory archeologically. A considerable amount of work has been done in areas adjacent to the Kangovar-Bisitun Valley. In 1931–33 G. Conteneau and R. Ghirshman excavated a series of Bronze and Iron Age tombs at Tepe Giyan, near modern Nehavand, in the valley immediately south of Kangovar; and in 1933 Ghirshman sounded Tepe Bad Hora, a Bronze Age site, in the Assadabad Valley just to the northeast of Kangovar (*5*). Within the area of our concern C. S. Coon, in 1949, excavated a Paleolithic rock shelter at Bisitun (*6*). In 1959–60 Braidwood and his associates examined a series of sites ranging from Middle Paleolithic to Uruk times in the Kermanshah Valley to the west (*7*). Recently, a Danish expedition under the general direction of J. Meldgaard has excavated the site of Tepe Guran in the Hulailan Valley to the southwest (*8*). Finally a study of the historical remains of Bisitun is in progress under H. Luschey and the German Archeological Institute of Tehran (*9*).

To date we have examined some 84 mounds and 10 caves and rock shelters

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