Table 1. Protection afforded by parabiosis against the lethal effects of exposure to large doses of x-rays; percent survival during the acute intestinal death phase (*A*) and the 30-day survival rate (*B*). Fifteen animal pairs per treatment group at each exposure dose.

Treatment group*	Exposure dose (r)			
	1200	1500	1800	2400
A. Percent survival at 120 hours (5 days)				
X/C	87	0	0	0
X-X	53		0	
X-C	100	100	100	60
B. Percent survival at 30 days				
X/C	0	0	0	0
X-X	0		0	
X-C	100	94	13	0

* Treatment: X, irradiated; C, shielded; X/C, pairs that are sutured together without vascular anastomosis; X-X, parabiont pairs with both animals exposed; X-C, parabiont pairs with one partner exposed and the other shielded.

indicate that not all of these animals died from acute intestinal injury. However, at 1500, 1800, and 2400 r, all of the irradiated animals that did not have a shielded, anastomosed partner died before 5 days had elapsed. In contrast, 1200, 1500, and 1800 r produced no acute intestinal deaths (3 to 5 days) in any irradiated rats that had a shielded, parabiotic partner. At the 2400-r exposure level it may be observed that, although some deaths did occur among the irradiated members that had a shielded, parabiotic partner, a remarkable survival rate was still apparent even at this extremely high radiation exposure dose.

In addition to the protection afforded by vascular anastomosis against intestinal death, attention is directed to the 30-day survival rates among these groups of parabionts. Table 1 shows that, with the exception of those animals irradiated with 2400 r, there were survivors in all the other groups of irradiated animals that were anastomosed to a shielded partner.

Over a span of about 20 years, numerous studies have been reported on the subject of acute intestinal radiation death. Although there is still no agreement on the mechanism of death, it does appear that there is general agreement among investigators that the small intestine is the target organ which initiates the syndrome. The various hypotheses which have been frequently proposed for the mechanism or mechanisms leading to death include the following: (i) denudation of the intestinal lining as a result of crypt cell destruction, (ii) excessive fluid and electrolyte loss, and (iii) bacteremia. These hypotheses are supported by the work of Quastler and others (*2, 7*) who demonstrated an almost immediate destruction of crypt cells and intestinal villi. Direct measurements of electrolyte and fluid loss have been made by several investigators (*8*) and survival time was shown to be increased markedly by massive infusion of electrolyte solutions and plasma (*4*). Others (*9*) have demonstrated that treatment with antibiotics also leads to increased survival time.

In the present study, we have no direct evidence to support any specific hypothesis that has been discussed. It would seem reasonable to speculate, however, that there is undoubtedly a substantial loss of cells with denudation in the small intestine of the irradiated parabiont at these extremely high doses of irradiation, yet these animals were observed to survive not only the period for acute intestinal radiation death but for 30 days beyond, depending on the dose. It would appear to us that the supportive action of the shielded partner on the irradiated parabiont is probably through maintenance of fluid and electrolyte balance during the critical period. If bacteremia is a factor in the syndrome, the shielded partner may also lend support to the irradiated partner. With regard to the 30-day survival rate, it is conceivable that the shielded partner is capable of providing stem cells in peripheral blood that have the potential for division. These cells then provide hematopoietic support to the irradiated rat and make possible its ultimate survival.

HAROLD W. CARROLL
DONALD J. KIMELDORF
U.S. Naval Radiological Defense Laboratory, San Francisco, California 94135

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Ultraviolet Irradiation of DNA in vitro and in vivo Produces a Third Thymine-Derived Product

Abstract. *A new thymine-derived product was separated from DNA irradiated with utlraviolet light in vitro and in vivo. This compound was mistaken to be thymine homodiner (T=T) by other workers because it is chromatographically indistinguishable from T=T in most eluents. It has absorbancy maximums at 312, 312, and 300 millimicrons in neutral,* p*H 2, and* p*H 11 aqueous solutions, respectively. When it is irradiated in aqueous solution with wavelengths of 360 and 313 millimicrons its spectrum reverts to one similar to that of thymine. Therefore, at least three thymine-derived products can be detected in ultraviolet irradiated DNA, namely the homodimer, a material with absorbancy maximum at 312 millimicrons, and a "minor" product suggested by others to be a dimer of cytosine and thymine. In cells, the latter two are formed in about equal amounts. While these three products were shown to exist in the acid hydrolyzates of ultraviolet irradiated DNA, a material with absorbancy maximum at about 310 millimicrons was demonstrated to form in ultraviolet irradiated DNA without further treatment. The magnitude of this spectral increase varied directly with the increase in the adenine-thymine contents in the DNA as shown by differential transmittance spectra of the irradiated* Micrococcus lysodeikticus, *calf thymus,* Bacillus cereus, *and* Hemophilus influenzae *DNA.*

Two chromatographically separable thymine-derived products have been obtained from acid hydrolyzates of DNA irradiated with ultraviolet light (*1*). The minor product (P_1) (*2*) was taken to be a cyclobutyl type heterodimer (*3*) of cytosine and thymine (*4*) (C=T), while the major one (P_2) (*2*) was considered a homodimer of thymine (T=T) (*5*). Both dimers, C=T and T=T, are con-

Table 1. Average percentage of formation of thymine homodimer (P_2A) and of material with maximum absorbancy at 312 mμ (P_2B) in P_2 ($P_2A + P_2B$). The dose of irradiation was 1200 erg/mm^2 per minute.

Irradiation time (min)	Radioactivity (count/min)		P_2A/P_2	P_2B/P_2
	P_2A	P_2B		
4	17224	2296	88.2	11.8
8	32696	3692	89.9	10.1
12	47882	5761	89.1	10.9
16	61872	6481	90.5	9.5
20	65411	8170	88.9	11.1
24	74147	9678	88.5	11.5

sidered to be responsible for most of the adverse effects produced by ultraviolet irradiation of various biological systems (*6, 7*). Recently, T=T has been isolated from DNA irradiated with ultraviolet light (UV) and has been shown to have the *cis-syn* configuration (*8, 9*). During the process of T=T isolation, it was observed that P_2 is not homogeneous, but contains another product chromatographically indistinguishable in previously reported solvent systems (*10*). We have isolated this product and have studied some of its properties.

Acid hydrolyzates of *Escherichia coli* 15 T$^-$ cells irradiated with ultraviolet light and labeled specifically with radioactive thymine showed peaks of radioactive material, namely, P_1, P_2, and thymine (T), when developed in solvent system I [*n*-butanol, acetic acid, and water (80:12:30)] (Fig. 1). Each of these materials was rechromatographed in the same solvent to remove any contaminants. When the purified T, P_2, and P_1 were chromatographed in solvent system II [*t*-butanol, methyl ethyl ketone, water, and ammonium hydroxide (40:30:20:10)], thymine produced only one peak with a relative flow (R_F) value of 0.65, P_2 produced one major and one minor peak, and P_1 yielded only one peak with R_F value of 0.41 (Fig. 1).

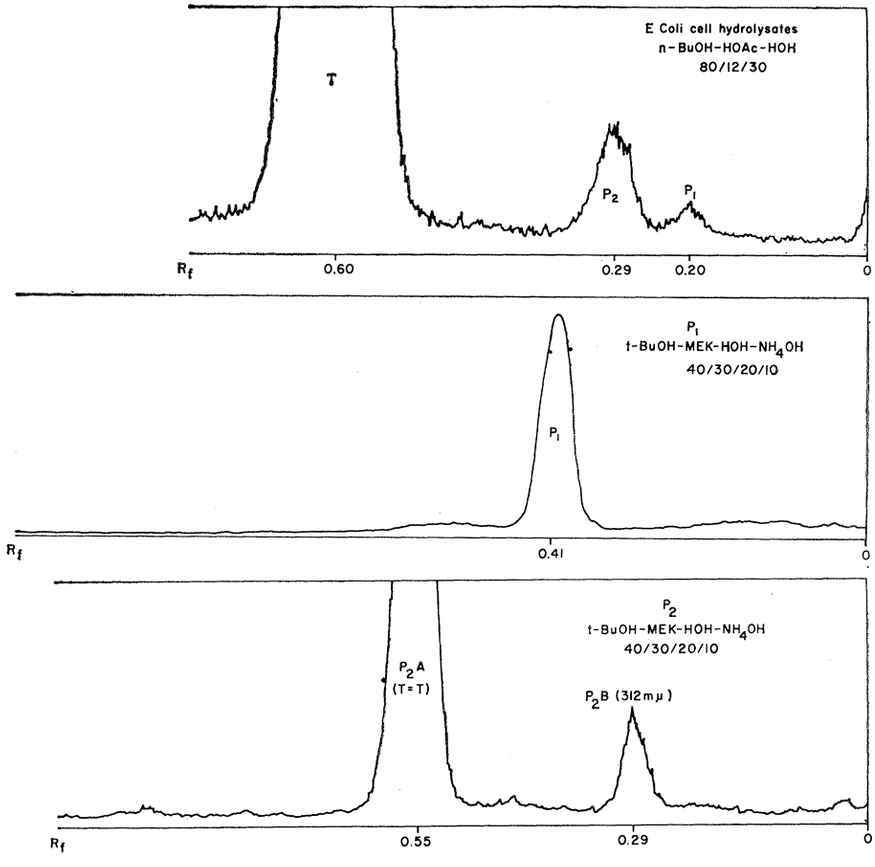

Fig. 1. Radiochromatograms showing distribution of radioactivity. Top: the acid hydrolyzates of *Escherichia coli* 15 T$^-$ DNA irradiated in vivo. Cells were labeled with thymine-2-C^{14} and irradiated with a dose of 1.9×10^4 erg/mm^2. Middle: chromatography of P_1. Bottom: chromatography of P_2. Solvent systems and R_F values are indicated.

The result suggests that P_2 is not a single entity but consists of at least two components, which we shall now designate as P_2A, the major one with R_F value of 0.55, and P_2B, the minor one with R_F value of 0.29. The apparent difference between solvent II and that of the previously reported systems [solvent system I; isopropanol, concentrated HCl, and water (68:15.5:16.5); *n*-butanol and water (86:14); *t*-butanol, methyl ethyl ketone, formic acid, and water (40:30:15:15)] (*10*) is that solvent II is alkaline, while the others are neutral or acidic. To eliminate the argument that P_2B is formed from P_2A (or vice versa) by solvent II during elution, we rechromatographed both P_2A and P_2B, individually and combined, with solvent I. A single peak area with R_F value of 0.29 was observed, an indication that P_2B is a product found in acid hydrolyzates of ultraviolet irradiated cells. Hitherto, P_2B has not been described.

To establish that P_2B is formed in the cells simultaneously with T=T (namely, P_2A) (*8*), we determined the rate of formation of P_2B and the ratios P_2B/P_2 and P_2A/P_2. Equal volumes of the cell suspensions were irradiated for different periods of time (*8, 10*). The irradiated cells were hydrolyzed, and the hydrolyzate was chromatographed in solvent I. From the chromatograms, the P_2 was eluted, and the eluate was rechromatographed in solvent II. The material in two peaks P_2A and P_2B were eluted, and portions of the eluates were counted for radioactivity. The ratios P_2B/P_2 and P_2A/P_2 were calculated for the different samples (Table 1). That these ratios are constant for all samples indicates that P_2B is formed at all doses of irradiation and that it is not a product formed as a result of prolonged ultraviolet irradiation.

This observation prompted us to investigate the possibility of characterizing this product from UV irradiated calf thymus DNA (5 g per 72 liters in each experiment) (*8*). Hydrolyzates of DNA were chromatographed in solvent I. From the paper chromatograms the area corresponding to P_2 was cut out; the product was eluted with distilled water and rechromatographed in solvent II. From the second chromatogram, the area corresponding to P_2B was cut out and the material was eluted, and a UV spectrum of the solution

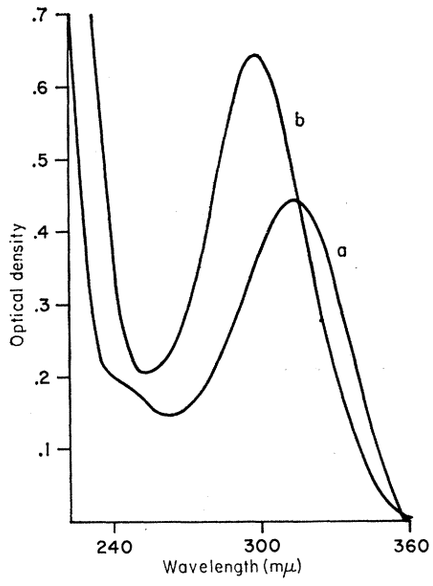

Fig. 2. The ultraviolet absorption spectra of P_2B (a) in neutral and *p*H 2 solutions and (b) in *p*H 12 aqueous solution.

was taken. The UV spectrum revealed the presence of a material which had an absorption maximum at 312 mμ and which was absent in hydrolyzates of unirradiated DNA. The product P_2B from irradiated calf thymus DNA was further purified by repeated chromatography in different solvent systems.

Final purification of the product P_2B was achieved by ion-exchange chromatography on a Dowex 50W-X12(H^+) column. The pure 312 mμ absorbing material was eluted from the column with water. The maximum absorbancy of the pure P_2B obtained was at 312 mμ in aqueous and acidic (*p*H 2) solutions and at 300 mμ in alkaline solution (*p*H 11) (Fig. 2). Irradiation of P_2B in water with wavelengths of 360 mμ resulted in the gradual loss of maximum absorbancy at 312 mμ, with a simultaneous appearance of a spectrum similar to that of thymine. A similar result was obtained with a wavelength of 313 mμ. However, T=T was unaffected under both conditions.

Earlier, we reported the formation of the 312 mμ absorbing material on irradiation of DNA at a wavelength of 254 mμ (*11*). We used transmittance differential spectra with expansion scales to record the spectral changes that would otherwise be unrecognized with the ordinary scale. When 3 ml of DNA solution (40 μg/ml of 0.15*M* NaCl) was irradiated at a distance of 10 cm (6800 erg/mm^2 per minute, about 1 einstein absorbed per mole of P per minute), there was a decrease in absorbancy at 260 mμ with a simultaneous increase in the absorbancy at a wavelength longer than 300 mμ. The magnitude of this increase in absorbancy at about 310 mμ seemed to vary directly with the increase in the adenine-thymine content in the DNA, as indicated by the irradiation of *Micrococcus lysodeikticus*, calf thymus, *Bacillus cereus* and *Hemophilus influenzae* (Fig. 3) DNA (*11*). This observation certainly is consistent with the notion that UV irradiation of DNA produces material that absorbs light at a wavelength of 310 mμ and strengthens the possible biological importance of P_2B, which has an absorbancy maximum at 312 mμ. Pearson *et al.* (see *12*) recently reported the isolation of a new photoproduct (TpT^4) having an absorbancy maximum at 325 mμ from ultraviolet irradiated thymidylyl-thymidine (TpT). However, according to these authors photoproducts of the TpT^4 type cannot be isolated from irradiated DNA by acid hydrolysis because TpT^4 is unstable in acid and alkali. In addition, Smith (and Setlow) (*13*) observed a peak at 320 mμ in the thawed solution of irradiated thymine. We have isolated this compound, and its structure is shown to differ from that of P_2B.

In conclusion, our data indicate that the so-called "thymine-dimer" consists of at least two components: the *cis-syn* thymine homodimer (P_2A) (*7*) and a material with an absorption maximum at 312 mμ, which is found in cells in about the same amount as is P_1 (*9*).

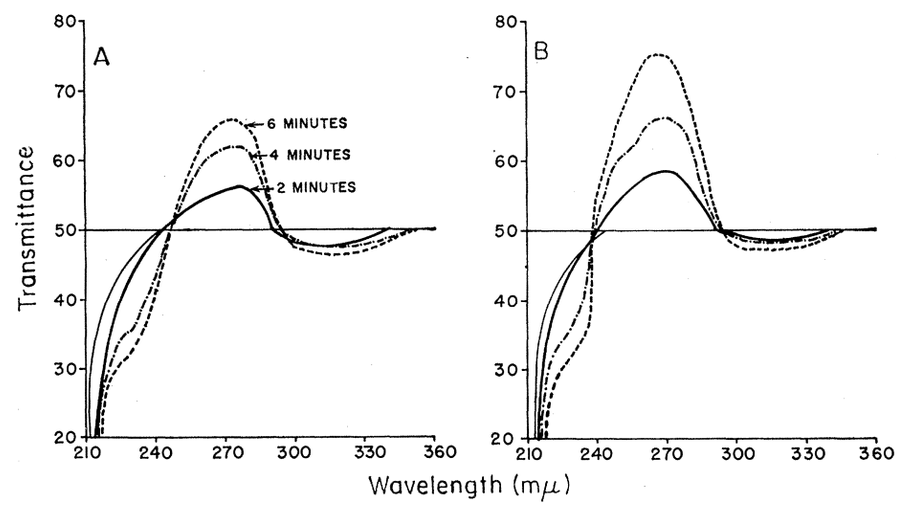

Fig. 3. The differential transmittance spectra of *Hemophilus influenzae* DNA irradiated at 10 cm with 254 mμ light (from a GE germicidal lamp). The times of irradiation are indicated. (A) Native DNA; (B) heat denatured DNA.

A. J. VARGHESE
SHIH YI WANG
Department of Biochemistry,
School of Hygiene and Public Health,
Johns Hopkins University,
Baltimore, Maryland 21205

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3. Homodimer and heterodimer are used to denote those dimers consisting of like or unlike molecules, respectively. The notations T=T, C=T, and so forth, are used to designate the cyclobutyl-type dimers that are linked by two bonds in order to avoid confusion with the single-bonded coupled product, T—T.
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