REPAIR REPLICATION IN *ESCHERICHIA COLI* AS MEASURED BY THE PHOTOLYSIS OF BROMODEOXYURIDINE

R. D. LEY *and* R. B. SETLOW

From the Institute of Radiation Biology, University of Tennessee, Knoxville, Tennessee 37916, and the Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830. Dr. Ley's present address is the Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois 60439.

ABSTRACT The ability to selectively photolyze bromouracil-(BrUra-)containing repaired regions in cellular DNA has allowed us to estimate the average size of repaired regions in ultraviolet (UV) light-irradiated *Escherichia coli*. Cells were labeled with thymidine-^{3}H, irradiated at 254 nm, and incubated in nonradioactive bromodeoxyuridine (BrdUrd). After incubation the cells were exposed to 10^6 ergs·mm^{-2} at 313 nm, lysed, and sedimented in alkaline sucrose gradients so as to measure the average molecular weight of single DNA strands. In strains that had excised ~45 cyclobutane pyrimidine dimers/10^8 daltons, the 313 nm treatment resulted in ~6 single-strand breaks/10^8 daltons. In an excisionless strain, the same treatment resulted in only 1.5 breaks/10^8 daltons. From the determination of the sensitivities of fully substituted DNAs to 313 nm light, we calculate that the repaired regions in excising strains of *E. coli* contain an average of 4–6 BrUra residues. Photoreactivation experiments indicate that the excision of pyrimidine dimers in the presence of BrdUrd is the primary source of repaired regions selectively photolyzed by 313 nm radiation.

INTRODUCTION

The mechanism proposed (Hanawalt, 1968; Howard-Flanders, 1968; Setlow, 1968; Strauss, 1968) for the excision of pyrimidine dimers from DNA in UV-irradiated *E. coli* (Setlow and Carrier, 1964; Boyce and Howard-Flanders, 1964) is as follows: (*a*) a single-strand incision is made near the pyrimidine dimer by a UV-specific endonuclease, (*b*) the dimer and adjacent nucleotides are excised by an exonuclease, (*c*) repair replication inserts undamaged nucleotides into the single-strand gaps, and (*d*) the replaced nucleotide sequence is joined to the DNA strand by ligase action. According to this scheme, the incorporation of BrUra into parental DNA after irradiation, as observed by Pettijohn and Hanawalt (1963, 1964), presumably represents the insertion of undamaged nucleotides into single-strand gaps created by the excision of pyrimidine dimers and adjacent nucleotides (i.e., step *c*).

Repair replication has been measured primarily by the incorporation of labeled BrUra into light-density parental DNA as detected by isopycnic CsCl centrifugation. Recently the selective photolysis of BrUra-containing repaired regions has been used as a rapid method for detecting repair replication in mammalian cells (Regan et al., 1971). Exposure of DNA with BrUra-containing repaired regions to 313 nm radiation reduces the sedimentation rate of the DNA in alkaline sucrose. Hence the irradiation has reduced the molecular weight presumably by inducing single-strand breaks (or alkaline-labile bonds) in the BrUra-rich regions. At 313 nm, the ratio in strand-break sensitivities between BrUra-substituted DNA and unsubstituted DNA is greater than at shorter wavelengths (unpublished data). This technique has been adapted here to measure the average size of repaired regions in *E. coli*.

MATERIALS AND METHODS

Bacteria and Growth Media

We used the following thymine-requiring strains of *E. coli*: 15 TAU-bar (from P. C. Hanawalt) and *E. coli* K-12 strains AB2497 (uvr^+) and AB2500 (uvrA-6) (from R. P. Boyce).

The basic medium, a glucose-salts minimal medium, M9 (Roberts et al., 1957), was supplemented for 15 TAU-bar with 2.5 mg/ml Casamino acids (Difco Laboratories, Detroit, Mich.), 2 μg/ml thymidine, 40 μg/ml uridine, and 25 μg/ml tryptophan and for the K-12 strains with 2.5 mg/ml Casamino acids, 2 μg/ml thiamine, and 2 μg/ml thymidine.

Radionuclide Labeling of Cells

Logarithmic phase cells at an initial density of 5×10^7 cells/ml were grown for two to three generations in the presence of 4 μCi of thymidine-methyl-^{3}H (6 Ci/mmole, Schwarz Bio-Research Inc., Orangeburg, N. Y.)/ml of growth medium.

Irradiation with 254 nm Light

Labeled cells were harvested by centrifugation, washed once with unsupplemented M9 medium, and resuspended at 10^8 cells/ml in unsupplemented medium. 5 ml of cell suspension in a small Petri dish (6 cm in diameter) were exposed to 254 nm radiation from a germicidal lamp at an incident exposure rate of 12 ergs $\cdot$ mm^{-2} sec^{-1} as measured by a Jagger (1961) meter.

Incubation in Thymidine or Bromodeoxyuridine

After the 254-nm irradiation, glucose, Casamino acids, and factors required for growth were added to the bacterial suspensions and the suspensions were incubated for 30 or 45 min at 37°C in the presence of 5 μg/ml unlabeled thymidine (dThd) or unlabeled bromodeoxyuridine (BrdUrd). The incubation was terminated by adding KCN to 0.01 M and cooling to ice bath temperature. The number of pyrimidine dimers excised during the incubation period was determined on a portion of the cells by the method of Carrier and Setlow (1971 *a*).

Irradiation with 313 nm Light

Cells incubated in dThd or BrdUrd were harvested by centrifugation, washed once, and resuspended at 10^8 cells/ml in M9 "buffer" (M9 without glucose) for the 15 TAU-bar strain

and in Tris buffer (0.05 M, pH 8.0) for the K-12 strains. 0.2 ml of cell suspension in an ice-cooled, quartz microcuvette (1 cm light path) was exposed to radiation centered at 313 nm. The radiation was obtained from a quartz prism Hilger monochromator (Hilger & Watts, Inc., Morton Grove, Ill.) illuminated by a 1000 w Philips high-pressure, mercury arc lamp (Philips Electronic Instruments, Mount Vernon, N. Y.), and each slit had a width of 5 nm band pass. The radiation was passed through a thin Mylar film (10% transmission at 308 nm, 1% at 306 nm) to eliminate any scattered shorter wavelengths. The average incident exposure rate reaching the sample was 2.3×10^3 ergs·mm^{-2} sec^{-1} as determined by a bolometer (Yellow Springs Instrument Co., Yellow Springs, Ohio) calibrated against a standard lamp (National Bureau of Standards, Washington, D. C.).

Sedimentation in Alkaline Sucrose Gradients

The technique of McGrath and Williams (1966) was used to estimate the sizes of single-stranded DNA. Immediately after irradiation at 313 nm, the cells were prepared for lysis by incubation of 15 TAU-bar with lysozyme for 5 min at 37°C (Fraser et al., 1957) or by the addition of ethylenediaminetetraacetate (EDTA) (to 5×10^{-3} M) to the K-12 suspensions (Kato and Kondo, 1970). 50 μl of the treated cells were layered onto 0.2 ml of lysing solution (0.5 M NaOH, 0.05% Sarkosyl), on top of a 3.6 ml gradient of 5–20% sucrose, containing 0.5 M NaCl, 0.2 M NaOH, and 0.01 M EDTA. The gradients were allowed to stand at room temperature for 30 min and then centrifuged at 20°C in an SW-56 rotor at 42,500 rpm for 65 min (Beckman Model L ultracentrifuge, Beckman Instruments, Inc., Fullerton, Calif.).

The distribution of radioactivity in the gradients was determined by puncturing the bottom of the tube and collecting approximately 30 eight-drop fractions on Whatman No. 17 paper strips (1 × 57 cm). This collection procedure is a modification by Carrier and Setlow (1971 *b*) of the filter paper disk technique. The strips were washed in 5% trichloroacetic acid, ethanol, and acetone, and then dried. They were cut into sections and the acid-insoluble radioactivity determined by counting in a toluene-BBOT[1] scintillator. The data were treated as previously described (Regan et al., 1971). The output from the scintillation counter was punched on paper tape that served as the input to a computer that plotted graphs of percentage of radioactivity vs. distance sedimented. In separate experiments, we determined the relation between distance sedimented and molecular weight by using phage T4 DNA as a standard. The molecular weight was assumed proportional to $(s^0_{20,w})^{2.5}$ according to Studier's (1965) relation between molecular weight and sedimentation constant. This calibration was used to calculate the number average molecular weights (M_n) of the sedimented DNA with the top and bottom three fractions of the gradient omitted from the calculation.

Photolysis of Fully Substituted DNA by 313 nm Radiation

Labeled BrUra-containing DNA was obtained by incubating 15 TAU-bar and K-12 (uvr$^+$) cells for 90 min at 37°C in the usual supplemented M9 medium but containing 7 μCi/ml of 5-bromo-2'-deoxyuridine-^{3}H (12.7 Ci/mmole, Schwarz BioResearch, Inc.) and 10 μg/ml of unlabeled BrdUrd instead of thymidine. The cell suspensions were then treated as in the previous two sections. The 313 nm irradiation was carried out with a 500 w mercury arc illuminating the monochromator, and the exposure rate through a thin Mylar film was 1.6×10^2 ergs·mm^{-2} sec^{-1}. The alkaline sucrose centrifugations were at 40,000 rpm for 100 min.

[1] BBOT = 2,5-bis-2-(5-*tert*-butylbenzoxazolyl)-thiopene.

RESULTS

Sensitivity of Repaired DNAs to 313 nm Radiation

BrUra-substituted DNA is more sensitive than unsubstituted DNA to the induction of single-strand breaks (or alkaline-labile bonds) by UV radiation (Hutchinson and Hales, 1970; Lion, 1970; Hotz and Walser, 1970; Hewitt and Marburger, 1971). Based on this observation, one would predict that DNA which has undergone repair replication in the presence of BrdUrd would exhibit an increased sensitivity to 313 nm radiation compared with cells that carry out repair replication in thymidine. The following data show that this is indeed true. UV-irradiated cells were incubated in the presence of dThd and then exposed to 313 nm radiation. With an exposure of 10^6 ergs·mm^{-2} at 313 nm, the DNA from the excisionless, uvr$^-$, K-12 strain (AB2500) had a number average molecular weight (M_n) of 45 × 10^6 daltons (Fig. 1 *a*); the DNA from the uvr$^+$ strain had a M_n of 35 × 10^6 daltons (Fig. 1 *b*). Those UV-irradiated cells which were incubated in the presence of BrdUrd, however, showed an increased sensitivity to 313 nm radiation, as judged by the lower M_n's. M_n's from uvr$^-$ and uvr$^+$ cells incubated in BrdUrd were 27 × 10^6 daltons (Fig. 1 *a*) and 10 × 10^6 daltons (Fig. 1 *b*), respectively. The number of single-strand breaks resulting from incubation in BrdUrd is calculated by subtracting the breaks observed in cells incubated with dThd from those observed in the BrdUrd-

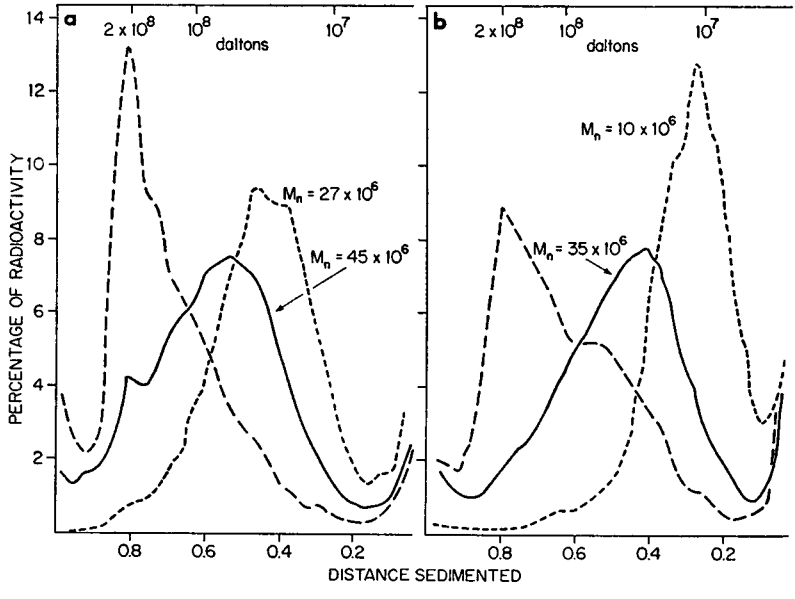

FIGURE 1 Sedimentation profiles of DNA from UV-irradiated (300 ergs·mm^{-2}) *E. coli* K-12 strains incubated in BrdUrd or dThd and subsequently exposed to 10^6 ergs·mm^{-2} of 313 nm radiation. (*a*) uvr$^-$ cells, (*b*) uvr$^+$ cells. There were approximately 3000 cpm in each gradient. dThd, 313 nm—; BrdUrd, 313 nm ----; BrdUrd, no 313 nm — —.

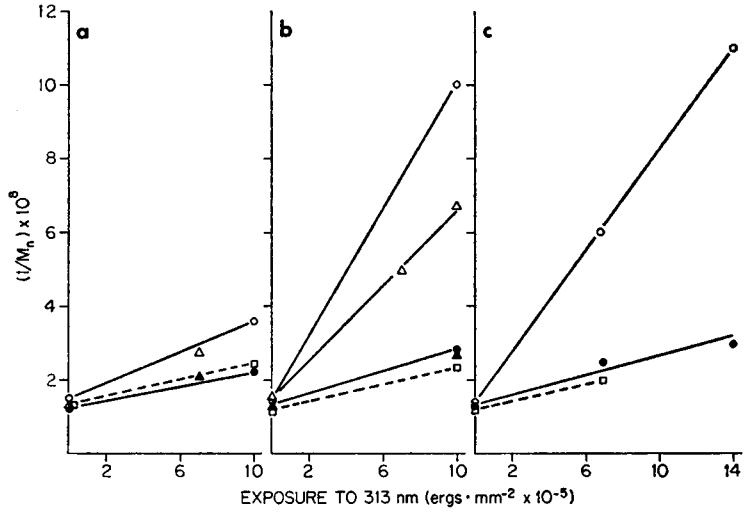

FIGURE 2 The effect of 313 nm radiation on the number average molecular weight (M_n) of uvr$^-$ K-12 (*a*), uvr$^+$ K-12 (*b*), and 15 TAU-bar (*c*) DNA. 300 ergs·mm^{-2} of 254 nm radiation plus BrdUrd (○) or dThd (●); 150 ergs·mm^{-2} of 254 nm radiation plus BrdUrd (△) or dThd (▲); no UV plus BrdUrd (□).

treated cells. For example, for UV-irradiated uvr$^+$ cells in dThd there were, after 313 nm irradiation, $10^8/(35 \times 10^6) = 2.9$ breaks/10^8 daltons, whereas if incubated in BrdUrd there were $10^8/(10 \times 10^6) = 10$ breaks/10^8 daltons. Thus, incubation of UV-irradiated cells in BrdUrd and subsequent exposure to 313 nm radiation resulted in 7 single-strand breaks/10^8 daltons for the uvr$^+$ cells and, by a similar calculation, 1.5 single-strand breaks/10^8 daltons for the uvr$^-$ cells.

We interpret these data as follows: 313 nm treatment of UV-irradiated cells incubated in dThd results in a small number of randomly induced single-strand breaks in the DNA; however, incubation in the presence of BrdUrd followed by exposure to 313 nm radiation results in the selective photolysis of BrUra-containing repaired regions in addition to the random breaks. The source of the slight increase in sensitivity obtained by incubating UV-irradiated, nonexcising cells in the presence of BrdUrd is not known. This increased sensitivity may be due to the occurrence of a low level of repair replication not associated with the excision of pyrimidine dimers (postreplication repair?), whereas the large increase in sensitivity to 313 nm radiation seems associated with the excision of dimers in the presence of BrdUrd.

A comparison of 313 nm sensitivities of DNAs from cells treated under various conditions may be made by plotting $1/M_n$ vs. exposure to 313 nm radiation. It is apparent that for the uvr$^+$ K12 strain (Fig. 2 *b*) both the initial 254 nm dose and incubation in the presence of BrdUrd are necessary to obtain the high sensitivity to 313 nm radiation. In control experiments where either the 254 nm dose or the

TABLE I

FRACTION OF BrUra-CONTAINING REPAIRED REGIONS PHOTOLYZED BY 313 nm RADIATION

E. coli strain	Dose of 254 nm radiation	No. of pyrimidine dimers excised per 10^8 daltons*	No. of single-strand breaks per 10^8 daltons in BrUra repair-replicated DNA after exposure to 10^6 ergs·mm^{-2} at 313 nm‡	Ratio of single-strand breaks to No. of dimers excised§
	ergs·mm^{-2}			
15 TAU-bar	300	40	5.5	0.10
K-12 (uvr$^+$)	300	45	7	0.12
(AB2497)	150	25	4.5	0.14
K-12 (uvrA)	300	0	1.5	
(AB2500)	150	0	1	

* The numbers of pyrimidine dimers excised were calculated from the percentages of labeled thymine occurring in dimers before and after incubation in the presence of BrdUrd. For example, exposure of 15 TAU-bar to 300 ergs·mm^{-2} at 254 nm resulted in the dimerization of 0.10% of the labeled thymine. Based on the assumption that in *E. coli* DNA the ratio of T—T:T—C:C—C = 5:4:1 (Setlow and Carrier, 1966), 0.10% equals 56 dimers/10^8 daltons. After incubation, only 0.028% of the label in DNA occurred in dimers; therefore, 40 dimers/10^8 daltons had been excised during the incubation.

‡ Number of single-strand breaks observed in BrdUrd-incubated cells minus the number of breaks observed in cells incubated in thymidine.

§ The number of breaks measured for the uvr$^-$ strain was subtracted from the number observed in the excising strains for equivalent 254 nm exposures. The net value was then divided by the number of dimers excised per 10^8 daltons.

BrdUrd treatment was withheld, low levels of sensitivity were observed. Reductions in M_n's for 15 TAU-bar DNA exposed to 313 nm radiation after repair replication in the presence of BrdUrd or dThd (Fig. 2 *c*) are comparable to those obtained with the uvr$^+$ K-12 strain.

The cellular DNA of the uvr$^-$ K-12 strain (Fig. 2 *a*) also exhibited low levels of sensitivity to 313 nm radiation when the 254 nm or BrdUrd treatments were withheld, and, as previously mentioned only a small increase in sensitivity when subjected to both the UV and BrdUrd treatments.

The number of repaired regions selectively ruptured by a given dose of 313 nm radiation (Table I, column 4) was calculated from data presented in Fig. 2. Since photoreactivation abolishes the enhanced sensitivity of uvr$^+$ cells incubated in BrdUrd (see below), we assume that the number of repaired regions equals the number of excised cyclobutane pyrimidine dimers. Thus we may calculate the number of BrUra residues per repaired region from the knowledge of the numbers of dimers excised during post-UV incubation and the number of breaks per BrUra residue per given exposure of 313 nm radiation. Breaking efficiencies were determined by 313 nm irradiation of cellular DNA fully substituted with BrUra.

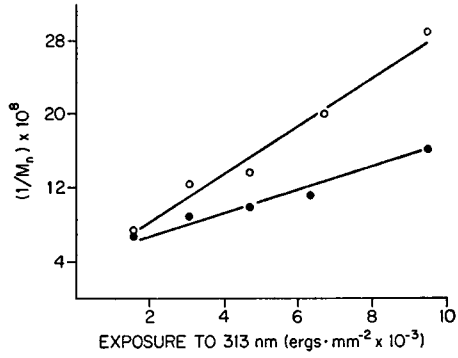

FIGURE 3 The effect of 313 nm radiation on the number average molecular weights (M_n) of *E. coli* DNA fully substituted with BrdUrd. 15 TAU-bar (●); uvr$^+$ K-12 (○).

Sensitivity of Fully Substituted DNAs to 313 nm Radiation

E. coli 15 TAU-bar and the uvr$^+$ K-12 strains were labeled by incubation for 90 min in BrdUrd-^{3}H and subsequently exposed to 313 nm radiation (see Methods).

The labeled DNAs synthesized under these conditions were observed by isopycnic CsCl centrifugations to be fully substituted (data not presented). The data shown in Fig. 3 as $1/M_n$ vs. exposure at 313 nm permit us to calculate the numbers of strand breaks per BrUra residue per erg·mm^{-2}. For example, for the uvr$^+$ K-12 strain, the change in $1/M_n$ per unit exposure is 25×10^{-12} mm^2/dalton-erg. Thus, 1 erg·mm^{-2} makes 25 breaks in 10^{12} daltons or 1 break/4×10^{10} daltons. In such a mass, there are 3.1×10^7 BrUra residues. The breakage sensitivity is the reciprocal of this number or 3.2×10^{-8} breaks/BrUra residue per erg·mm^{-2}.

A similar calculation for 15 TAU-bar gives a value of 1.8×10^{-8} breaks/BrUra residue per erg·mm^{-2}. The difference between the two bacterial strains does not indicate any intrinsic photochemical differences between the two DNAs, but rather a difference in the amount of repair, or the decay of alkaline-labile regions, after 313 nm irradiation. Strain 15 TAU-bar was incubated at pH 7 for 5 min during the treatment with lysozyme before the bacteria were layered on top of the alkaline gradients. In this time there is appreciable loss of single-strand breaks or alkaline-labile regions (unpublished data) as there is after X-irradiation of wild-type *E. coli* strains (Town et al., 1971). Using the above sensitivities and the data presented in Table I, we calculate (see Appendix) that each repaired region in *E. coli* contains an average of 4–6 BrUra residues.

Photoreactivation

Photoreactivation experiments were conducted to determine if the excision of pyrimidine dimers is a major source of BrUra-containing repaired regions in *E.*

TABLE II

PHOTOREACTIVATION OF BrdUrd SENSITIZATION IN UV-IRRADIATED *E. COLI* K-12 (uvr$^+$)

Treatment*	M_w	
	(*a*) −313 nm	(*b*) +313 nm
	daltons ($\times 10^{-6}$)‡	
1. No UV; no PR	96	76
2. No UV; PR	86	53
3. UV; no PR	90	36
4. UV; PR	93	46

* Treatments were before incubation in the presence of BrdUrd and the resulting weight average molecular weights (M_w) were calculated after exposure to 10^6 ergs·mm^{-2} at 313 nm. Cells were labeled with thymidine-^{3}H (see Methods), resuspended in M9 buffer, and exposed to 200 ergs·mm^{-2} of 254 nm radiation. Half of the irradiated cells were held for 20 min at room temperature, and the remainder were exposed for 20 min, also at room temperature, to 405 nm radiation (exposure rate = 8×10^3 ergs·mm^{-2} min^{-1}.) The 405 nm radiation was obtained from a Hilger monochromator illuminated by a 500 w mercury arc. The radiation was passed through a thick Mylar film to eliminate any scattered shorter wavelengths. Photoreactivated and nonphotoreactivated cells were incubated in the presence of BrdUrd for 45 min before exposure to 313 nm radiation (see Methods).

‡ Weight average molecular weights were calculated for these experiments since the photoreactivation treatment resulted in a nonrandom breakdown of the DNA upon exposure to 313 nm radiation and small fluctuations near the tops of the gradients would give large errors in M_n .

coli. Cyclobutane pyrimidine dimers are presumably the only photoproduct altered by photoreactivation (Setlow, 1968).

The data in Table II (column *a*) show that the photoreactivation procedure itself did not result in a significant change in weight average molecular weights (M_w) for either irradiated or unirradiated cells. Photoreactivation treatment of unirradiated cells followed by incubation in the presence of BrdUrd did result in a decrease (reason unknown) in M_w upon exposure to 313 nm radiation (column *b*, line 2). The opposite effect, however, was observed for UV-irradiated cells; photoreactivation, followed by incubation with BrdUrd, resulted in a decreased sensitivity to 313 nm radiation (column *b*, line 4). Correcting for the decrease in molecular weights observed in unirradiated cells, we calculate that the number of single-strand breaks per 10^8 daltons weight average in UV-irradiated cells as

$$10^8 \left(\frac{1}{36 \times 10^6} - \frac{1}{76 \times 10^6} \right) = 1.5$$

without photoreactivation and as

$$10^8\left(\frac{1}{46\times 10^6}-\frac{1}{53\times 10^6}\right)=0.3$$

with photoreactivation. Thus photoreactivation appears to have reduced the sensitivity to 313 nm radiation by 80% for UV-irradiated cells incubated with BrdUrd. The photoreactivating treatment reduced the number of pyrimidine dimers present in DNA by 65% (from 0.05% labeled thymine in dimers to 0.017%). Therefore we assume despite the large errors in this experiment that the excision of pyrimidine dimers is the major source of BrUra-containing repaired regions selectively photolyzed by 313 nm radiation.

DISCUSSION

The data demonstrate that the DNA of excising strains of *E. coli* that are incubated after UV irradiation in the presence of BrdUrd exhibits an increased sensitivity to breakdown by 313 nm radiation. Our interpretation is that the BrdUrd is inserted by repair replication into the single-strand gaps which result from the excision of pyrimidine dimers and that upon exposure to 313 nm radiation these BrUra-substituted regions are selectively photolyzed.

The data (Table I) on the number of repaired regions ruptured by a given exposure to 313 nm radiation and the breaking sensitivities toward 313 nm radiation of BrUra-substituted DNA (Fig. 3) have allowed us to calculate the average size of the repaired regions in *E. coli*. For each pyrimidine dimer excised, 4–6 BrUra residues are incorporated into parental DNA. Since *E. coli* DNA contains 25% thymidine, the presence of 4–6 BrUra residues implies the incorporation of approximately 12–20 nucleotides per repaired region; however, since intracellular pools of thymidine may compete with BrdUrd for incorporation into repaired regions, the value of 12–20 nucleotides incorporated per dimer excised is a minimum number.

The following experiment was performed to determine the effect of intracellular pools of thymidine on the level of BrdUrd incorporation. UV-irradiated cells (150 ergs·mm^{-2}) were incubated at 37°C for 45 min in labeled BrdUrd (total concentration, 5 μg/ml), and the level of substitution in semiconservatively replicated DNA determined by CsCl density centrifugation (Billen et al., 1965). The 15 TAU-bar and uvr$^+$ K-12 strains treated in this manner exhibited BrUra substitution levels of 90 and 75%, respectively (data not shown). It cannot be assumed that these observed levels of substitution also occur in the repair-replicated regions because Kanner and Hanawalt (1968) showed that the repair polymerase is selective for thymine over BrUra by a factor of 2.5–4 as compared with the normal replication processes. Even so, if this factor is applicable to repair replication experiments using BrdUrd, the repaired regions in *E. coli* would be expected to contain ~50% BrUra based on the substitution levels observed in normal replication

after UV irradiation. Therefore, the value of 12–20 nucleotides incorporated for each pyrimidine dimer excised is probably not in error by more than a factor of 2 because of intracellular pools of thymidine.

Other Estimated Values for the Sizes of Repaired Regions

Pettijohn and Hanawalt (1964), using isopycnic CsCl centrifugations, observed 1% BrUra substitution in 15 TAU-bar DNA after a 254 nm incident exposure of 500 ergs·mm^{-2} and incubation with BrUra. Using the data of others for the number of pyrimidine dimers formed, and assuming that all dimers were excised, they calculated that approximately 20 nucleotides were incorporated for each dimer excised.

Billen (1968) reported that 2% of the parental DNA of *E. coli* strain B/r was replaced by repair replication after a 254 nm exposure of 200 ergs·mm^{-2}. Based on our data, 40 dimers/10^8 daltons are formed by this dose and are excised during the period observed. Thus 2% replacement would represent an average of 140 nucleotides incorporated per repaired region.

Both the BrUra photolysis method and the isopycnic centrifugation technique give average sizes for the repair-replicated regions. If, however, the sizes of the repaired regions varied widely as indicated by Cooper and Hanawalt (1971), the averages determined by the BrUra photolysis method and the isopycnic centrifugation technique would be different. For example, if 1% of the repaired regions were made 100-fold as large as the remainder, this change would increase the average size, as determined by isopycnic centrifugation, by ~100% but would only increase the size estimated by BrUra photolysis by ~10% at the 313 nm exposures we have used.

Sources of Error and Uncertainty in the BrUra Photolysis Determination

Three readily apparent sources of error exist in determining the magnitude of repair replication in *E. coli* by the BrUra photolysis method:

(*a*) To determine the number of repaired regions ruptured by 313 nm radiation requires measuring a reduction in single-strand molecular weight of repair-replicated DNA. The molecular weights calculated for DNAs which have not sedimented out of the upper one-third of the alkaline sucrose gradient (M_n less than 15×10^6 daltons) may be in error by as much as twofold. Therefore, the calculated number of repaired regions ruptured may be in error by a factor of 2.

(*b*) The total number of nucleotides incorporated per repaired region may be found from the number of BrUra residues per repaired region only if the level of BrUra substitution in the repaired region is known. This source of uncertainty has been considered above.

(*c*) We have assumed that the number of repaired regions in the DNA was equal

to the observed number of pyrimidine dimers excised. If photoproducts other than pyrimidine dimers are also excised, the number of repaired regions will be higher than the value used for our calculations, and the calculated sizes will be too large. The photoreactivation data in Table II indicate that this assumption does not introduce a large error.

The BrUra photolysis method offers a rapid means of detecting repair replication in *E. coli*, mammalian cells (Regan et al., 1971), and presumably other cellular systems, in spite of errors which do exist in making quantitative measurements.

The authors thank Doctors A. R. Lehmann, M. L. Randolph, and J. K. Setlow for suggestions concerning the manuscript.

The Oak Ridge National Laboratory is operated by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation.
During this research Dr. Ley was supported by training grant 730 from the National Institutes of Health to the Institute of Radiation Biology, University of Tennessee.

Received for publication 29 September 1971.

REFERENCES

BILLEN, D. 1968. *Int. At. Energy Ag. Monogr.* 103.
BILLEN, D., R. HEWITT, and G. JORGENSEN. 1965. *Biochim. Biophys. Acta.* **103**:440.
BOYCE, R. P., and P. HOWARD-FLANDERS. 1964. *Proc. Nat. Acad. Sci. U.S.A.* **51**:293.
CARRIER, W. L., and R. B. SETLOW. 1971 *a. In* Methods in Enzymology. L. Grossman and K. Moldave, editors. Academic Press, Inc., New York. **21**(Pt. D):230.
CARRIER, W. L., and R. B. SETLOW. 1971 *b. Anal. Biochem.* **43**:427.
COOPER, P., and P. HANAWALT. 1971. *Biophys. Soc. Annu. Meet. Abstr.* **11**:192a.
FRASER, D., H. R. MAHLER, A. L. SHUG, and C. A. THOMAS. 1957. *Proc. Nat. Acad. Sci. U.S.A.* **43**:939.
HANAWALT, P. C. 1968. *In* Photophysiology. A. C. Giese, editor. Academic Press, Inc., New York. **4**:204.
HEWITT, R., and K. MARBURGER. 1971. *Biophys. Soc. Annu. Meet. Abstr.* **11**:27a.
HOTZ, G., and R. WALSER. 1970. *Photochem. Photobiol.* **12**:207.
HOWARD-FLANDERS, P. 1968. *Annu. Rev. Biochem.* **37**:175.
HUTCHINSON, F., and H. B. HALES. 1970. *J. Mol. Biol.* **50**:59.
JAGGER, J. 1961. *Radiat. Res.* **14**:394.
KANNER, L., and P. HANAWALT. 1968. *Biochim. Biophys. Acta.* **157**:532.
KATO, T., and S. KONDO. 1970. *J. Bacteriol.* **104**:871.
LION, M. B. 1970. *Biochim. Biophys. Acta.* **209**:24.
MCGRATH, R. A., and R. W. WILLIAMS. 1966. *Nature (London).* **212**:534.
PETTIJOHN, D. E., and P. C. HANAWALT. 1963. *Biochim. Biophys. Acta.* **72**:127.
PETTIJOHN, D. E., and P. C. HANAWALT. 1964. *J. Mol. Biol.* **9**:395.
REGAN, J. D., R. B. SETLOW, and R. D. LEY. 1971. *Proc. Nat. Acad. Sci. U.S.A.* **68**:708.
ROBERTS, R. B., D. B. COWIE, P. H. ABELSON, E. T. BOLTON, and R. J. BRITTEN. 1957. *Carnegie Inst. Wash. Publ.* No. 607.
SETLOW, R. B. 1968. *Progr. Nucl. Acid Res. Mol. Biol.* **8**:257.
SETLOW, R. B., and W. L. CARRIER. 1964. *Proc. Nat. Acad. Sci. U.S.A.* **51**:226.
SETLOW, R. B., AND W. L. CARRIER. 1966. *J. Mol. Biol.* **17**:237.
STRAUSS, B. S. 1968. *In* Current Topics in Microbiology and Immunology. Springer-Verlag KG., Berlin. **44**:1.
STUDIER, F. W. 1965. *J. Mol. Biol.* **11**:373.
TOWN, T. D., K. C. SMITH, and H. S. KAPLAN. 1971. *Science (Washington).* **172**:851.

APPENDIX

Calculation of Numbers of BrUra Residues in Repaired Regions

The number of repaired regions photolyzed in Table I can be used in conjunction with the breaking efficiencies of 313 nm radiation on BrUra-substituted DNA (Fig. 3) to calculate the number of BrUra residues per repaired region.

The probability (P) of a break occurring in a repaired region is equal to $1 - e^{-kND}$, where k = breaking efficiency, N = number of BrUra residues per repaired region, and D = dose of 313 nm radiation. For 15 TAU-bar, the observed probability of 10^6 ergs·mm^{-2} photolyzing a repaired region equals 0.10 (Table I) and therefore $kND = 0.105$. Since $k = 1.8 \times 10^{-6}$ breaks/BrUra residues per erg·mm^{-2} (Fig. 3) $N = 6$ BrUra residues per repaired region. Similarly for the K-12 (uvr$^+$) strain, where $P = 0.12$ (300 ergs·mm^{-2} at 254 nm) and $k = 3.2 \times 10^{-6}$, $N = 4$; and where $P = 0.14$ (150 ergs·mm^{-2} at 254 nm), $N = 4.5$. Thus, 4–6 BrUra residues are incorporated into *E. coli* parental DNA for each pyrimidine dimer excised.