DOUBLE-STRAND BREAKS FROM SINGLE PHOTOCHEMICAL EVENTS IN DNA CONTAINING 5-BROMOURACIL

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ABSTRACT Ultraviolet irradiation of *Escherichia coli* cells with a low level of 5-bromouracil incorporated produces DNA double-strand breaks by single photochemical events, one such break per 100 single-strand breaks, the latter assayed in alkali-denatured DNA. About 2.5–4 double-strand breaks are produced per "lethal hit," compared with about 6 double-strand breaks per lethal hit induced by gamma rays. These results are consistent with the hypothesis that an unrepaired DNA double-strand break is a major lethal event in both cases. The increase in sensitivity to ultraviolet (measured by colony-forming ability) seems linear in the number of bromouracils incorporated (0–20% of the thymines), and the linear relationship is much the same for incorporation in one or in both strands of the DNA double helix.

INTRODUCTION

The incorporation of the base analogue 5-bromouracil sensitizes DNA to ultraviolet radiation in a wide variety of assays (review: Hutchinson, 1973). The most numerous lesions from ultraviolet irradiation seem to be the conversion of bromouracil to uracil (Wacker, 1961) and the production of a comparable number of single-strand breaks as assayed in alkaline gradients (Lion, 1968). Dodson et al. (1972) showed that uracil was the predominant base to be found at the 5′-termini of the breaks.

Double-strand breaks are clearly formed (Hotz et al., 1971; Smets and Cornelis, 1971; Mönkehaus and Köhnlein, 1973). In none of these experiments was it possible to determine unequivocally if any double-strand breaks result from a single photochemical event, as opposed to coincidences between random single-strand breaks in the complementary strands, which undoubtedly do occur.

This paper presents evidence that double-strand breaks in vivo are produced by single photochemical events and that quantitatively these breaks are as lethal as double-strand breaks caused by gamma rays.

MATERIALS AND METHODS

Bacteria and Growth Media

For all experiments involving the measurement of double-strand breaks, *Escherichia coli* K-12 AB2497 *thyA*$^-$ were grown for at least four generations at 37°C with aeration in a shaking

water bath, in K medium supplemented with [^{14}C]thymine at 46 Ci/mol and 5 μg/ml and 5-bromouracil from 0.08–0.8 μg/ml. K medium consisted of 19 parts M9 buffer (modified as described), 2 parts 10% (wt/vol) Casamino acids (decolorized, vitamin-free), and 1 part 20% (wt/vol) glucose. M9 buffer had 19.7 mM NH_4Cl, 43.7 mM Na_2HPO_4, 23.2 mM KH_2PO_4, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 0.1 μg/ml thiamine. For those experiments in which only the survival of colony-forming ability was measured in cells having various fractions of bromouracil, the growth medium was the same, except [^{14}C]thymine was present at 0.04 μCi/ml and 12 μg/ml (0.1 mM), and 5-[6-^{3}H]bromouracil at 1 μCi/ml, with enough nonradioactive bromouracil to bring the molar ratio of bromouracil to thymine to whatever was wanted. Uracil at 50 μg/ml was also added to minimize uptake of [6-^{3}H]uracil, the major radiolysis product of 5-[6-^{3}H]bromouracil.

Aspartate medium contained M9 buffer, required amino acids (arginine, histidine, leucine, proline, and threonine) each at 50 μg/ml, 0.4% (wt/vol) L-aspartic acid neutralized with NH_4OH, and [^{14}C]thymine as in K medium.

Measurement of Bromouracil Uptake

Bromouracil uptake in cells was determined by the method described by Rydberg (1977). The ratio of ^{3}H (bromouracil) to ^{14}C (thymine) in the acid-insoluble fraction of the cells was compared with the same ratio measured in the medium with a known ratio of bromouracil to thymine. Appropriate controls were counted when necessary to correct for possible different counting efficiencies for radioisotopes in large molecules (DNA) and in small molecules in K medium, as described by Rydberg.

Irradiation Sources and Methods

For 313 nm radiation, a medium pressure mercury arc lamp (Philips SP-50, Philips Electronic Instruments, Inc., Mahwah, N.J.) was used. The radiation was filtered by the lamp cooling water, a Corning 0–53 ultraviolet transmitting clear filter (<0.5% transmission at 254 nm), a Corning 7-54 visible absorbing filter (<5% transmission at 410 nm, Corning Glass Works, Corning, N.Y.), and an interference filter (Ealing Corp., S. Natick, Mass.) with a peak transmission of 16% at 313 nm and a half-peak band width (total) of 12 nm. Two 39-mm diameter (63-mm focal length) planoconvex quartz lenses (A. Jaegers, Lynbrook, N.Y.) first brought the diverging beam to a parallel one for passage through the interference filter at normal incidence, then focused the beam to a spot about 2 cm in diameter. Flux was measured with ferrioxalate dosimetry (Calvert and Pitts, 1966), about 2×10^3 J/m^2 per min.

For 254 nm irradiation, an unfiltered 15 W germicidal lamp at 60 J/m^2 per min was used. Gamma rays were from a ^{60}Co source at 490 rads/min. All irradiations were at ice temperature in M9 buffer. Cell suspensions were stirred during irradiation.

Neutral and Alkaline Sucrose Sedimentation

The procedures used to determine the yields of double-strand breaks have been described in detail (Krasin and Hutchinson, 1977). Briefly, bacteria were converted to spheroplasts and gently layered on top of neutral sucrose gradients, and the DNA was sedimented. The speeds used were low enough so that the sedimentation artifact for very large DNA (Zimm, 1974; Zimm and Schumaker, 1976; Hutchinson and Krasin, 1977) would not invalidate any of the conclusions drawn. (This artifact, a distortion of the random coil form by viscous forces, causes the DNA sedimentation coefficient to decrease with increasing centrifuge speed.)

Alkaline sucrose gradients were capped with 0.1 ml of a detergent mixture (Sarkosyl), followed by 0.05 ml cells, and allowed to stand 30–45 min for lysis before centrifugation in an SW50.1 rotor at 20°C.

Sedimentation profiles were fitted with curves calculated from the equation for the fraction $f(M)\mathrm{d}M$ of linear molecules whose weights are between M and $M + \mathrm{d}M$ after r random cuts per unit mass

$$f(M)\mathrm{d}M = rM(2 + rM_0 - rM)\exp(-rM)\mathrm{d}M \quad (1)$$

for a molecule of initial size M_0, taken here as the genome size, 2.7×10^9 daltons. The molecular weight of a DNA molecule M was related to the distance sedimented D by the relation

$$D/D_{T2} = (M/M_{T2})^{0.38} \quad (2)$$

where D_{T2} is the distance sedimented by marker $T2$ phage DNA of mass $M_{T2} = 1.1 \times 10^8$ daltons. The value of r breaks per unit mass that gave the best fit of Eq. 1 to the observed sedimentation profile defined the number of breaks in that DNA.

Col E1 DNA was assayed in the neutral and alkaline sucrose gradients (5–20% [wt/vol]) described by Kupersztoch-Portnoy and Helinski (1974). Centrifugation was at 48,000 rpm for 100 min in an SW50.1 rotor at 15°C.

Survival of Colony-Forming Ability

Suitable dilutions of the cell suspensions were spread on Luria agar, incubated at 37°C for 24 h and the colonies counted. Luria agar contained (per liter) 10 g Bactotryptone, 5.0 g yeast extract, 0.5 g NaCl, 0.071 g NaOH, and 18.6 g agar.

Isolation and Purification of Col E1 Plasmid DNA

E. coli JC411 (Col E1) was kindly provided by Dr. W. D. Rupp. The procedure for isolating the plasmid DNA was similar to that of Blair et al. (1972). Chloramphenicol (180 μg/ml) was added to a growing culture of JC411 (Col E1) at 3×10^8/ml in K medium at 37°C with thymine (2 μg/ml); 0.6 μCi/ml [^{14}C]thymine was added 90 min later, and the cells incubated overnight. After lysis, cellular debris was centrifuged out and Col E1 DNA was treated with RNase A at 100 μg/ml at 37°C for 5 h to convert supercoiled DNA containing ribonucleotides to open circular form. The remaining supercoiled DNA, free of ribose linkages, was purified by the cesium chloride-ethidium bromide method (Radloff et al., 1967). Ethidium bromide was extracted with isopropanol and the DNA solution dialyzed against buffer (0.01 M Tris – 0.001 M EDTA, pH 8).

Treatment of Col E1 Plasmid DNA with Bromine Free Radical

The bromine free radical was produced by a chain reaction from ultraviolet irradiation of *N*-bromosuccinimide (see Incremona and Martin, 1970). A solution of Col E1 DNA (20 μg/ml) with 0.5 mM *N*-bromosuccinimide, 40 mM Tris, 1 mM EDTA, pH 7.8, was bubbled with highly purified N_2 (saturated with H_2O) to remove oxygen. After 40 min bubbling, the solution was irradiated with a black light (Macalaster Bicknell Company of Connecticut, Inc., New Haven, Conn., emitting mainly 365 nm) for 20 min at 5 cm distance. Immediately after irradiation, 0.1-ml samples were sedimented in neutral and alkaline sucrose gradients.

RESULTS

The Formation of Double-Strand Breaks

E. coli cells, with 1–6% of the thymine content replaced by bromouracil, were irradiated with 313 nm light and the DNA was sedimented on neutral sucrose gradients.

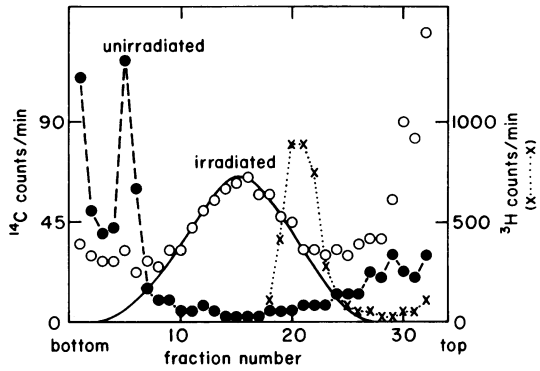

FIGURE 1 Sedimentation profiles of DNA containing bromouracil from *E. coli* cells irradiated with 313-nm light. AB2497 *thy*A$^-$ cells containing bromouracil/thymine at a molar ratio of 0.06 and at 2×10^7 cells/ml in M9 buffer were irradiated with 313-nm light at about 2,000 J/m^2 per min, then immediately converted to spheroplasts. Samples of 0.1 ml containing 10^6 spheroplasts were lysed on top of neutral sucrose gradients, and sedimented 37.5 h at 5,900 rpm in an SW50.1 rotor at 20°C. The two profiles shown were those from cells receiving 0 and 4×10^4 J/m^2 of 313 nm light. The counts per minute (counts/min) in the unirradiated fraction have been divided by two; in this profile the sharp peak at fraction 5 is produced by the sedimentation limit for large DNA as calculated from Zimm's theory (see Materials and Methods). The curve through the experimental points for the irradiated samples is calculated from Eqs. 1 and 2 for r (break/M_{T_2}) = 0.60, M_0 (original size of the DNA) = 25 M_{T_2}. The crosses mark the sedimentation profile for [^{3}H]$T2$ DNA, sedimented in the same gradient.

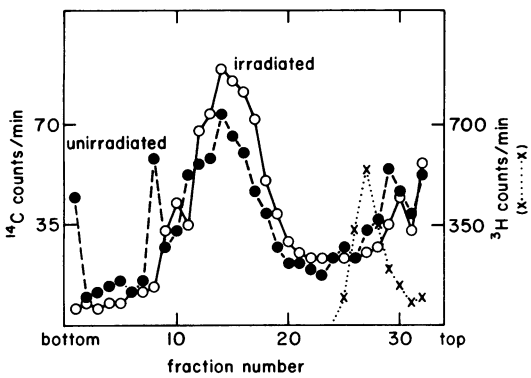

FIGURE 2

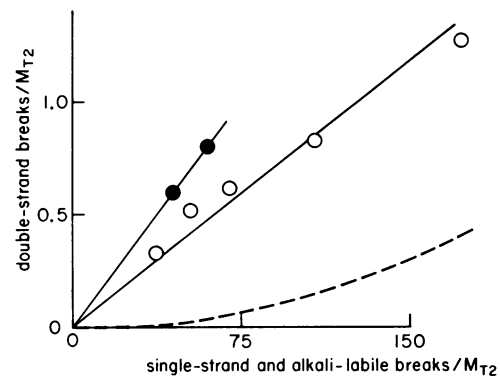

FIGURE 3

FIGURE 2 Sedimentation profiles of DNA from irradiated *E. coli* cells that did *not* contain bromouracil. AB2497 *thy*A$^-$ cells were grown as were those in Fig. 1, but without bromouracil. The DNA was sedimented on neutral sucrose gradients for 51.5 h at 3,360 rpm in an SW50.1 rotor. The profiles are for DNA from cells receiving either 0 or 9.4×10^4 J/m^2 of 313-nm light. Because of the low centrifuge speed, sedimentation anomalies from the Zimm effect are much less pronounced. The crosses show the profile for [^{3}H]$T2$ DNA.

FIGURE 3 The number of double-strand breaks induced by 313-nm radiation in DNA containing bromouracil, plotted against the number of single-strand breaks in the same DNA as observed in alkaline gradients. AB2497 *thy*A$^-$ *E. coli* cells were grown with bromouracil/thymine ratios of 0.01–0.03 and irradiated, and the DNA was prepared for sedimentation as described

A decreased sedimentation rate showed that double-strand breaks were formed (Fig. 1). No breaks were formed in the DNA of irradiated cells that did not contain bromouracil (Fig. 2).

The number of double-strand breaks formed was approximately linear in 313 nm fluence to a given batch of cells, and increased as the bromouracil incorporation increased. The most useful plot of the data was that of the number of double-strand breaks against the number of single-strand breaks (measured in alkali). This tests most directly the prediction from theory that double-strand breaks created by coincidences between randomly produced single-strand breaks will be given by

$$N_D = \frac{2h + 1}{L} N_S^2 \qquad (1)$$

(see Freifelder and Trumbo, 1969, for a summary). Here double-strand DNA L base pairs long contains N_D double-strand breaks as the result of N_S randomly produced breaks in each single-strand L bases long. It is assumed that single-strand breaks in each complementary strand within h base pairs of each other will lead to a double-strand break.

Plotting the data as in Fig. 3 also minimizes experimental errors. The two lines in Fig. 3 give 0.012 and 0.008 double-strand breaks per single-strand break (measured in alkali); it is unclear whether the difference in the two measurements is due to experimental error or suggests that the efficiency of double-strand breakage can depend on conditions with the cells.

It has been reported that double-strand breaks may be produced at sites of preexisting single-strand nicks by enzymes (Bonura and Smith, 1975; Bonura et al., 1975) and shear forces (Hayward, 1974). To investigate the possibility that the observed double-strand breaks were artifacts of such action, *E. coli* AB2497 cells with about 6% thymine replaced by bromouracil were grown in aspartate medium to reduce the DNA content to <1.3 genomes/cell. There is no repair of double-strand breaks in this strain with <2 genomes/cell (Krasin and Hutchinson, 1977). The cells were irradiated with 313 nm light, then incubated 60 min at 37°C in supplemented K medium. During incubation, >90% of the single-strand breaks were repaired, as shown by alkaline sucrose sedimentation (data not shown). Neutral sucrose sedimentation showed the

in Materials and Methods. The number of double-strand breaks was determined by fitting calculated curves (Eqs. 1 and 2) to sedimentation profiles in neutral sucrose gradients. The sum of 'true' single-strand breaks and alkali-labile bonds was measured by fitting curves to profiles from alkaline sucrose gradients. (●) Data for the substrain of *E. coli* AB2497 *thy*A$^-$ cells used in most of the experiments in this paper. The bromouracil/thymine content of the DNA was 2%, as measured by radioactive labeling (see Materials and Methods). (○) Data from a substrain of AB2497 *thy*A$^-$ cells which had been subcultured in another laboratory for a year. The cells were grown in medium containing bromouracil/thymine in the molar ratio 0.03, and the estimated level of bromouracil/thymine in the DNA was 0.01. (— —) A quadratic relationship showing the number of double-strand breaks expected from independently produced single-strand breaks on opposite strands separated by five base pairs and less (Freifelder and Trumbo, 1969). The calculated curve is based on "true" single-strand breaks, about 90% of the total measured in alkaline sucrose gradients (see Results on Alkali-Labile Bonds).

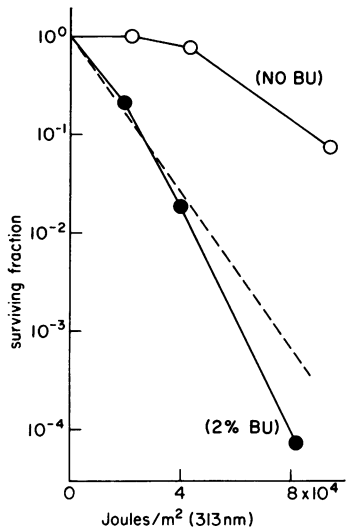

FIGURE 4

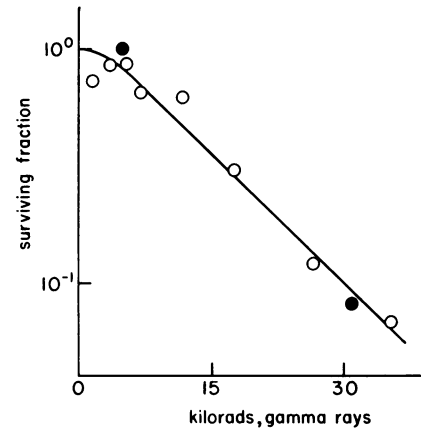

FIGURE 5

FIGURE 4 The fraction of *E. coli* cells, with and without bromouracil (BU), that can still form colonies after irradiation with 313-nm light. AB2497 cells in exponential growth in K medium were resuspended at 2×10^7/ml in M9 buffer and irradiated with 313-nm light. (o) Cells grown in medium with thymine. (•) Cells grown in medium with a bromouracil/thymine ratio of 0.05 and with a measured bromouracil/thymine ratio of 0.02 in the DNA. This is the same batch of cells for which data are shown as filled circles in Fig. 3. (- - -) The effect ascribable to the presence of bromouracil on survival of the ability to form colonies, calculated according to Eq. 4.

FIGURE 5 The fraction of *E. coli* cells, with and without bromouracil, that can form colonies after irradiation with gamma rays. AB2497 cells were prepared for irradiation exactly as described in the legend to Fig. 4, and irradiated with ^{60}Co gamma rays at 490 rads/min at ice temperature. (o) Cells containing no bromouracil. (•) Cells containing 2% bromouracil, the same batch for which data are given in Figs. 3 and 4.

same number of double-strand breaks before and after the single-strand breaks were repaired. If double-strand breaks are produced from the single-strand interruptions, they must be from a special class of single-strand lesions that are not readily repaired.

E. coli *Colony-Forming Ability and Double-Strand Breaks*

Fig. 4 shows colony-forming ability after 313 nm irradiation for cells with 2% bromouracil, and for cells with no bromouracil. With low (2%) bromouracil incorporation, it seems reasonable to assume that survival $S(D)$ after fluence D can be expressed by the product of $g(D)$, the survival for no bromouracil, times a term $b(D)$ depending only on damage caused by the presence of bromouracil.

$$S(D) = g(D) \times b(D). \tag{4}$$

A calculation of $b(D)$ from Eq. 4 gives the dashed line in Fig. 4, a straight line within experimental error. Measurements of DNA double-strand breaks in this batch of cells with bromouracil show $(2.9 \pm 0.7) \times 10^{-4}$ breaks/genome per J/m^2. This corre-

sponds to 2.4–4 breaks for 1.1×10^3 J/m^2, the fluence which reduced $b(D)$ to $e^{-1} = 0.37$, one lethal hit ascribable to the bromouracil.

The effects of gamma rays on colony-forming ability for the same batches of cells are shown in Fig. 5. The presence of 2% bromouracil did not measurably sensitize the cells to gamma rays. The exponential part of the gamma ray survival curve corresponds to 12 krads for a decrease in survival by $0.37 = e^{-1}$. Gamma rays produce 0.50 double-strand breaks/krads per genome in these cells (Krasin and Hutchinson, 1977), or 6 breaks/genome per lethal hit.

For the same level of cell survival, the number of double-strand breaks produced by 313 nm light in DNA incorporating bromouracil is about half the number produced by gamma rays. In assessing the significance of this result, it must not be forgotten that double-strand breaks form a class of lesions, with a number of configurations possible at the broken ends. Thus, one cannot assume that the breaks produced by 313 nm radiation in DNA containing bromouracil will necessarily have the same probability of being repaired as those formed by gamma rays.

Colony-Forming Ability and Bromouracil Content in the Genome

The sensitivity of *E. coli* cells to either 254 or 313 nm radiation increases with fraction f_B of bromouracil incorporated in the genome. Describing the sensitivity by a cross section σ, the lines in Fig. 6 are, for 254 nm radiation, $\sigma = (230 + 3{,}400\ f_B) \times 10^{-4}$m^2/J, and for 313 nm radiation, $\sigma = (0.17 + 16\ f_B) \times 10^{-4}$m^2/J. For those batches of cells for which data were taken with both radiations, cross sections at 254 nm are plotted against the cross sections for 313 nm radiation (Fig. 7). The slope of this line shows that 254-nm radiation is about 200-fold more effective on bromouracil in DNA than 313-nm, compared on the basis of incident energy per unit area, in reasonable agreement with previous results (Hutchinson, 1973). This ratio will be sensitive to the manner in which the "313-nm" radiation is prepared, and hence its true effective wavelength, because of the steep dependence of bromouracil adsorption on wavelength in this region.

Cells in which bromouracil is incorporated in only one strand of the DNA double helix (open circles in Figs. 6 and 7) are sensitized to ultraviolet light to an extent comparable to that for cells in which bromouracil is distributed uniformly in both strands (closed circles). A detailed comparison of the lethal effects of bromouracil in only one strand, and for bromouracil uniformly distributed in both DNA strands, is complicated. In different experiments, bromouracil was incorporated in a single strand in lengths ranging from about $\frac{1}{8}$ of the genome to essentially the full length. There may well be different consequences from a certain number of lesions concentrated in one part of the chromosome, and from the same number distributed throughout the genome.

The overall conclusion is that the effect of ultraviolet light is comparable for cells having bromouracil in one and in both strands of the DNA. This is compatible with the concept that a principal lethal lesion is a DNA double-strand break created by a photochemical event in a single bromouracil residue.

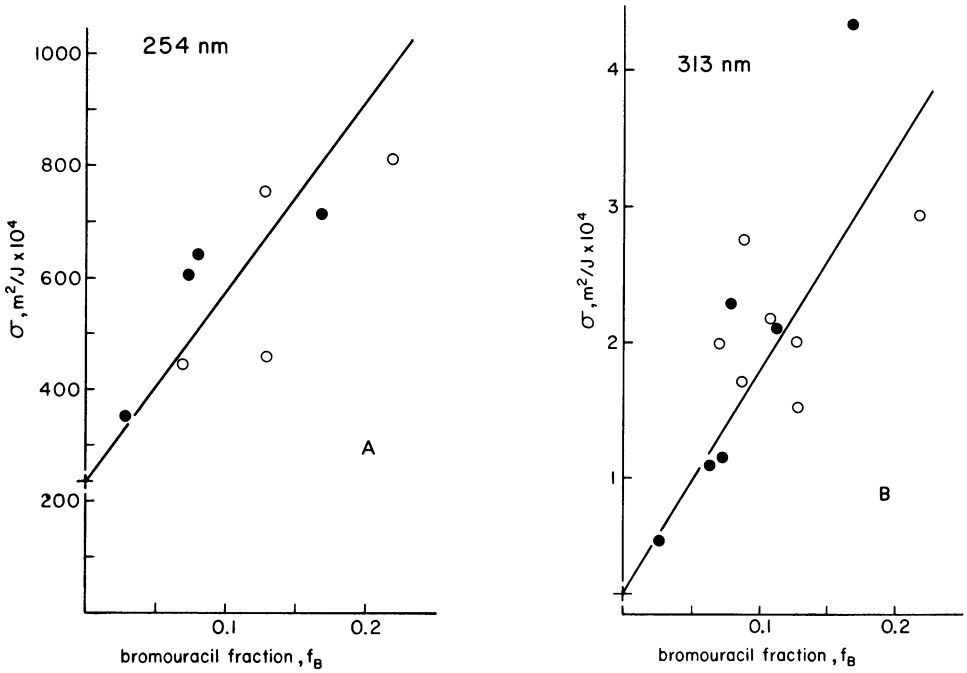

FIGURE 6 Cross sections for loss of colony-forming ability after ultraviolet irradiation as a function of bromouracil incorporation. *E. coli* AB2497 *thy*$^-$ cells were grown in K medium containing 0.1 mM [^{14}C]thymine and varying proportions of [^{3}H]bromouracil. Bromouracil incorporation was measured as described in Materials and Methods; f_B in DNA = bromouracil/(bromouracil + thymine). The cells were resuspended in lambda buffer at 6×10^7/ml and irradiated with either 254 nm (A) or 313 nm (B) light (5–6 different fluences per experiment) with minimum colony-forming ability typically 1–10% that of controls. Plots of ln (survival) against fluence were straight lines, except as described below, defining a cross section $\sigma = -(\ln \text{survival})/\text{fluence}$. The diagrams show σ plotted against f_B. (●) Cells were grown in bromouracil for at least three generations. (○) Cells were grown in bromouracil for 5–35 min, under conditions for which the doubling time was 40 ± 5 min. The bromouracil should all be in one strand; banding in equilibrium neutral CsCl density gradients the DNA from two samples verified this point. The two sets of data for cells grown the shortest time in bromouracil showed plots of ln (survival) with a pronounced "tail" at higher fluences, suggesting nonuniform incorporation of bromouracil in individual cells. The cross sections plotted are those appropriate for the initial slopes of such curves. (+) Approximate cross sections for loss of colony-forming ability of cells with no bromouracil. Plots of ln (survival) against fluence for these cells are not linear (see Fig. 4). However, about 25 measurements of colony-forming ability for fluences of 0–40 J/m^2 (254 nm), or 0–3 x 10^4 J/m^2 (313 nm), could be fitted without serious error by cross sections of the values shown. These cross sections describe the loss of colony-forming ability by damage to the DNA other than that caused by bromouracil over the range of fluences used in the experiments with cells containing bromouracil.

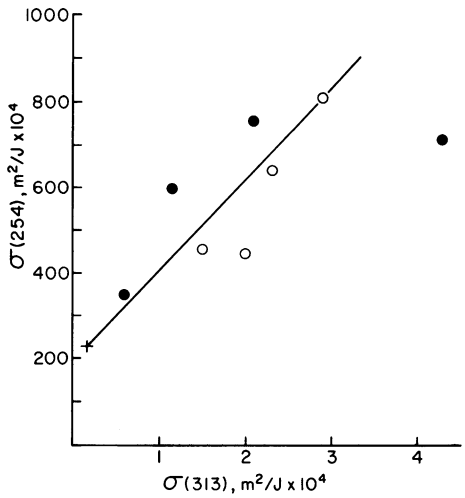

FIGURE 7 The cross sections from Fig. 6 replotted with the cross section for 254 nm radiation as a function of the cross section for 313 nm radiation measured on the same batch of cells.

Single-Strand Breaks and Alkali-Labile Bonds Produced by Bromine Free Radicals

It is reasonable to hypothesize that the free radical Br·, released in the irradiation of DNA containing bromouracil (Hutchinson, 1973), could diffuse to the complementary strand and produce a break there. This would form a double-strand break if the initial event caused a single-strand break.

Col E1 plasmid DNA in supercircular form, free of any ribonucleotides, was exposed to Br· formed by irradiating *N*-bromosuccinimide with a black light. Sedimentation profiles from neutral sucrose gradients showed a 10–15% reduction in the amount of fast sedimenting supercircular DNA, and profiles from corresponding alkaline gradients showed a 60–65% reduction in the amount of fast sedimenting collapsed supercircular DNA. The results indicate that at least five alkali-labile lesions were formed per "true" single-strand break.

The breaks in the DNA could be caused by Br·, or by the succinimide free radical also produced in the photolysis. Incremona and Martin (1970) showed that under conditions like ours, the chain reactions involved lead to Br· as the chief species reacting with other solutes. We have assumed that the same would be true in our experiments.

DISCUSSION

The major conclusion of this paper is that ultraviolet light produces double-strand breaks in DNA containing bromouracil by a one photon process.

Our results support the suggestion that in irradiated *E. coli* cells with low to moderate bromouracil levels in the DNA, double-strand breaks are the most important lesions that block cell replication. About 2.5–4 double-strand breaks are produced by

fluences that reduce colony-forming ability to e^{-1}, compared with about 6 double-strand breaks at the same survival level for the cells irradiated with gamma rays. The loss of colony-forming ability by *E. coli* cells irradiated with ultraviolet light does not depend much on whether 1–20% bromouracil is in one strand or in both strands of the DNA double helix. Roufa (1976) and others[1] have found that the response of mammalian cells to near-ultraviolet light depends strongly on whether high levels (>60%) of bromouracil are in only one strand or in both strands. The difference may reflect a difference in lesions at 1–20% bromouracil substitution and at >60%, or a difference in the response of bacterial and mammalian cells.

It is reasonable to suggest possible mechanisms for the formation of double-strand breaks from the only primary photochemical reaction now known for bromouracil, photodissociation to the 5-uracilyl free radical and the bromine free radical Br·, The single-strand break is in the strand that contains the uracil produced (Dodson et al., 1972) and is presumably caused by extraction of an H atom from a sugar by the uracilyl radical (Hutchinson, 1973). The double-strand break could result from the Br· causing a break in the complementary strand. Our data suggest that Br· can cause such a break.

Several investigators have measured single-strand breaks (in alkali) in irradiated DNA with one strand containing bromouracil and one without (Table I). For such "hybrid" DNA irradiated in cells, there are about 0.01 breaks in the thymine strand per break in the bromouracil strand; the break in the thymine strand occurs because of the bromouracil strand. We have found 0.01 double-strand breaks per single-strand

TABLE I

THE PRODUCTION BY 313 nm LIGHT OF SINGLE-STRAND BREAKS (MEASURED IN ALKALI) IN A DNA SINGLE STRAND CONTAINING ONLY THYMINE, WHICH IS IN DOUBLE-HELICAL FORM WITH A COMPLEMENTARY STRAND CONTAINING BROMOURACIL. THE BREAKS MEASURED ARE THE SUM OF "TRUE" SINGLE-STRAND BREAKS PLUS ALKALI-LABILE BONDS

DNA	Breaks in thymine strand per break in bromouracil strand	Reference
Bacillus subtilis DNA in 0.015 M NaCl, 0.0015 M sodium citrate	0.05	Köhnlein and Mönkehaus, 1972
Mouse DNA, irradiated in the cell	~0.01	Lehmann, 1972
Haemophilus influenzae DNA, irradiated in cells	0.006	Beattie, 1972
E. coli DNA, irradiated in cells	0.01	Ley, 1973

[1] M. Hagan and M. Elkind, Argonne National Laboratory. Personal communication.

break measured in alkali (Fig. 3). The approximate equality of these numbers suggests that a high proportion of strand breaks in the thymine strand of hybrid DNA leads to double-strand breaks.

This model is straightforward if it is assumed that most single-strand interruptions as assayed in alkali can lead to double-strand breaks. Hewitt and Marburger (1975) showed that 80% of the single-strand breaks measured in alkali are alkali-labile bonds for bromouracil-DNA irradiated in Tris-NaCl buffer with ultraviolet light. It is not known if such labile bonds lead to double-strand breaks. However, in the following paper (Krasin and Hutchinson, 1978), we show that for irradiation of bromouracil-DNA in *E. coli* cells (same conditions as for these measurements of double-strand breaks), nearly all the breaks measured after treatment with alkali are "true" breaks.

One remaining question concerns the proportion of alkali-labile bonds to true single-strand breaks produced by the free radical Br·.

A high percentage of such lesions produced in vitro are alkali-labile (see Results, this paper). It is possible that in the cell these will appear as actual single-strand interruptions, for the same (unknown) reasons that few alkali-labile lesions are found in bromouracil-DNA within cells. A direct experimental test of this point is difficult.

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REFERENCES

BEATTIE, K. L. 1972. Breakage of parental DNA strands in *Haemophilus influenzae* by 313 nm radiation after replication in the presence of 5-bromodeoxyuridine. *Biophys. J.* **12**:1573.

BLAIR, D. G., D. J. SHERRATT, D. B. CLEWELL, and D. R. HELINSKI. 1972. Isolation of supercoiled colicinogenic factor E1 DNA sensitive to ribonuclease and alkali. *Proc. Natl. Acad. Sci. U. S. A.* **69**:2518.

BONURA, T., and K. C. SMITH. 1975. Enzymatic production of deoxyribonucleic acid double-strand breaks after ultraviolet irradiation of *Escherichia coli* K-12. J. Bacteriol. **121**:511.

BONURA, T., K. C. SMITH, and H. S. KAPLAN. 1975. Enzymatic induction of DNA double-strand breaks in γ-irradiated *Escherichia coli* K-12. *Proc. Natl. Acad. Sci. U. S. A.* **72**:4265.

CALVERT, J. G., and J. N. PITTS, JR. 1966. Photochemistry. John Wiley & Sons, New York. Chap. 7.

DODSON, M. L., R. HEWITT, and M. MANDEL. 1972. The nature of ultraviolet light-induced strand breakage in DNA containing bromouracil. *Photochem. Photobiol.* **16**:15.

FREIFELDER, D., and B. TRUMBO. 1969. Matching of single-strand breaks to form double-strand breaks in DNA. *Biopolymers.* **7**:681.

HAYWARD, G. S. 1974. Unique double-stranded fragments of bacteriophage T5 DNA resulting from preferential shear-induced breakage at nicks. *Proc. Natl. Acad. Sci. U. S. A.* **71**:2108.

HEWITT, R. R., and K. MARBURGER. 1975. The photolability of DNA containing 5-bromouracil. *Photochem. Photobiol.* **21**:413.

HOTZ, G., R. MAUSER and R. WALSER. 1971. Infectious DNA from coliphage T1. III. The occurrence of single-strand breaks in stored thermally treated and UV-irradiated molecules. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **19**:519.

HUTCHINSON, F. 1973. The lesions produced by ultraviolet light in DNA containing bromouracil. *Q. Rev. Biophys.* **6**:201.

HUTCHINSON, F., and F. KRASIN. 1977. Dependence of the sedimentation of high molecular weight DNA on centrifuge speed. *Biophys. Chem.* **6**:23.

INCREMONA, J. H., and J. C. MARTIN. 1970. N-bromosuccinimide, mechanisms of allylic bromination and related reactions. *J. Am. Chem. Soc.* **92**:627.

KÖHNLEIN, W., and F. MÖNKEHAUS. 1972. Experimental evidence for intramolecular energy transfer in hybrid DNA of *B. subtilis* after irradiation with ultraviolet light. *Z. Naturforsch. Teil. B. Anorg. Chem. Org. Chem.* **B27**:708.

KRASIN, F., and F. HUTCHINSON. 1977. Repair of DNA double-strand breaks in *E. coli,* which requires *recA* function and the presence of a duplicate genome. *J. Mol. Biol.* **116**:81.

KRASIN, F., and F. HUTCHINSON. 1978. Strand breaks and alkali-labile bonds induced by ultraviolet light In DNA with 5-bromouracil in vivo. *Biophys. J.* **24**:657.

KUPERSZTOCH-PORTNOY, Y. M., and D. R. HELINSKI. 1974. Breaks induced in supercoiled factor E1 DNA as a result of the decay of incorporated tritium. *Biochim. Biophys. Acta.* **374**:316.

LEHMANN, A. R. 1972. Postreplication repair of DNA in ultraviolet-irradiated mammalian cells. *J. Mol. Biol.* **66**:319.

LEY, R. D. 1973. Postreplication repair in an excision-defective mutant of *E. coli:* Ultraviolet light-induced incorporation of bromodeoxyuridine into parental DNA. *Photochem. Photobiol.* **18**:87.

LION, M. 1968. Search for a mechanism for the increased sensitivity of bromouracil-substituted DNA to ultraviolet radiation. *Biochem. Biophys. Acta.* **155**:505.

MÖNKEHAUS, F., and W. KÖHNLEIN. 1973. Single- and double-strand breaks in 5-bromouracil-substituted DNA of *B. subtilis* and phage PBSH after irradiation with long-wave length UV and their correlation to intramolecular energy transfer. *Biopolymers.* **12**:329.

RADLOFF, R., W. BAUER. and J. VINOGRAD. 1967. A dye-buoyant-density method for the detection and isolation of closed circular duplex DNA. *Proc. Natl. Acad. Sci. U. S. A.* **57**:1514.

ROUFA, D. J. 1976. 5-bromodeoxyuridine-DNA strand symmetry and the repair of photolytic breaks in Chinese hamster cell chromosomes. *Proc. Natl. Acad. Sci. U. S. A.* **73**:3905.

RYDBERG, B. 1977. Discrimination between bromouracil and thymine for uptake into DNA in *drm*$^-$ and *dra*$^-$ mutants of *Escherichia coli* K12. *Biochim. Biophys. Acta.* **476**:32.

SMETS, L. A., and J. J. CORNELIS. 1971. Reparable and irreparable damage in 5-bromouracil-substituted DNA exposed to ultraviolet radiation. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **19**:445.

WACKER, A. 1961. Strahlenchemische Veränderungen von Pyrimidinen *in vivo* and *in vitro. J. Chim. Phys. Physicochim. Biol.* **58**:1041.

ZIMM, B. H. 1974. Anomalies in sedimentation. IV. Decrease in sedimentation coefficients of chains at high fields. *Biophys. Chem.* **1**:279.

ZIMM, B. H., and V. N. SCHUMAKER. 1976. Anomalies in sedimentation. V. Chains at high fields, practical consequences. *Biophys. Chem.* **5**:265.