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Photochemistry and Photobiology. 1964. Vol. 3, pp.1 –10. Pergamon Press Ltd. Printed in Great Britain

THE PHOTOCHEMISTRY OF THYMINE AND BROMOURACIL *IN VIVO*

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(*Received* 20 *September* 1963)

Abstract—A study of the relative sensitivity of bromouracil and thymine to photochemical alteration (2537Å) when incorporated into the DNA of bacterial cells has led to the conclusion that bromouracil is about twice as reactive as thymine. This offers a chemical basis for the sensitization of bacteria to ultraviolet light by incorporated bromouracil.

Bromouracil forms at least five photoproducts *in vivo*. The relative proportions of these several photoproducts is significantly different in *E. coli* B/r and *Enterococcus* Stei. Uracil is the chief photoproduct of bromouracil in *Enterococcus* Stei but in *E. coli* B/r the major photoproduct (which is a minor product in *Enterococcus* Stei) is as yet unidentified.

Preliminary data on the five to nine decomposition products of the dimer of bromouracil and the mixed dimer of uracil and bromouracil (produced in irradiated frozen solution) have suggested a tentative explanation for the differences in products in the two organisms. The results for *Enterococcus* Stei appear consistent with the asumption that the major photoproduct of bromouracil is the dimer and this then decomposes to yield mainly uracil (as it does *in vitro*). *E. coli* B/r takes up less bromouracil and the photoproducts formed resemble more closely those found for the *in vitro* decomposition of the bromouracil-uracil dimer. These results suggest that the primary photoproduct of bromouracil in *E. coli* B/r may be either a mixed dimer (intrastrand) with cytosine (with the subsequent deamination of the cytosine) or an interstrand crosslink with either bromouracil or cytosine.

Thymine forms at least four photoproducts other than the dimer. Two of these photoproducts are significantly elevated in that portion of the bacterial DNA that is rendered non-extractable by detergent after ultraviolet irradiation (remains associated with protein) and are also found in irradiated poly-dAT.

From the rate of formation of thymine dimers in irradiated (2537Å) *E. coli* B/r it can be calculated that approximately 1 erg/mm^2 produces one thymine dimer.

INTRODUCTION

THE incorporation of the thymine analog, 5-bromouracil, into the deoxyribonucleic acid (DNA) of bacterial and mammalian cells results in an increased sensitivity of these cells to the lethal effects of ultraviolet light[5, 7, 9, 10]. Beukers and Berends[1] have reported that the ultraviolet (u.v.) irradiation of frozen aqueous solutions of thymine brings about the formation of a dimer. This dimer is also obtained when DNA is irradiated *in vitro*[2] or *in vivo*[27]. This dimer appears to be the major photoproduct within the DNA and accounts for most of the biological inactivation of transforming DNA[15]. It seemed reasonable, therefore, to postulate that bromouracil might exhibit a similar but enhanced photochemical response to ultraviolet light as the basis for its radiation sensitizing properties. Moore and Thomson[13] have reported that bromouracil is somewhat more sensitive than thymine

*This investigation was supported by Grant CA-02896 from the National Cancer Institute of the U.S. Public Health Service.

when irradiated in solution. A previous paper[23] dealt with the photochemistry of the several pyrimidines when irradiated in frozen solution and in dried films.

The present paper is concerned with the photochemical alterations of thymine and bromouracil when incorporated into bacterial DNA and irradiated in the bacteria. The relative sensitivity of these two compounds has been investigated as well as the number and identity of the photoproducts produced *in vivo*. The photochemistry of bromouracil has been shown to differ in two different species of bacteria and a possible explanation is offered. A chemical basis for the sensitization of bacteria to ultraviolet light by incorporated bromouracil has been elucidated. Certain of these data have appeared in a preliminary communication[18] and in a review article[22].

METHODS

E. coli B/r were inoculated into 100 ml of a salts–glucose medium containing 0·2 per cent sulfanilamide to block thymidylate synthesis[10]. To one mg (25μc) of thymine-2-C^{14} or bromouracil-2-C^{14} (Calbiochem, Los Angeles) was added to the cultures and they were incubated at 37°C for 24 hr. The replacement of thymine by bromouracil under these conditions was usually around 70 per cent[10]. The cells were harvested, divided into four portions (approximately 3×10^{10} cells), suspended in 10 ml phosphate buffer and irradiated on a rotating platform under an unfiltered General Electric germicidal lamp (G8T5) whose output was 13·3 ergs/mm^2/sec at 43 cm distance. Cells were irradiated for various lengths of time, harvested, washed twice in 5 per cent trichloroacetic acid and twice in ethanol–ether (3:1), hydrolyzed in trifluoroacetic acid[6], and chromatographed in butanol/acetic acid/water (80/12/30)[23]. Chromatograms were photographed[17] to locate the u.v. absorbing spots and run through a strip scanner for the detection of radioactive areas. The radioactive areas were cut out, eluted and re-counted in a liquid scintillation counter[19] and the amount of parent compound and photoproducts was calculated as percent of the total radioactivity. In the experiments with thymine, more than 99 per cent of the incorporated radioactivity could be recovered as thymine in the unirradiated controls. In the experiments with bromouracil (radiopurity$>$99 per cent), more than 90 per cent of the total incorporated radioactivity was present as bromouracil in the unirradiated controls; less than 10 per cent appeared in products which would have been expected if the bromouracil had been debrominated[26].

Enterococcus Stei, a gift of Dr. A. Wacker, was cultured for 36 hr in the special medium required by this organism[25]. Efficient incorporation of thymine-2-C^{14} or bromouracil-2-C^{14} was accomplished by simply adding them to the medium at about 10 μg/ml[26].

The Br^{82}-bromouracil was a gift of Dr. J. P. Kriss[11].

RESULTS and DISCUSSION

Relative photochemical sensitivity of bromouracil and thymine in vivo

When *E. coli* (labelled either with thymine-2-C^{14} or bromouracil-2-C-^{14}) are irradiated with u.v. light one can determine the sensitivity of the labelled compounds by following their rate of disappearance (and concomitant appearance of photoproducts) with increasing doses of ultraviolet light. The experimental details of such experiments are given in the section on Methods and the results are given in Fig. 1. The data for thymine were obtained from three independent experiments while those for bromouracil came from four experiments. The lines were fitted by regression analysis and the standard error of the slope when expressed as the percent of the slope was 5·4 per cent for thymine and 4·7 per cent

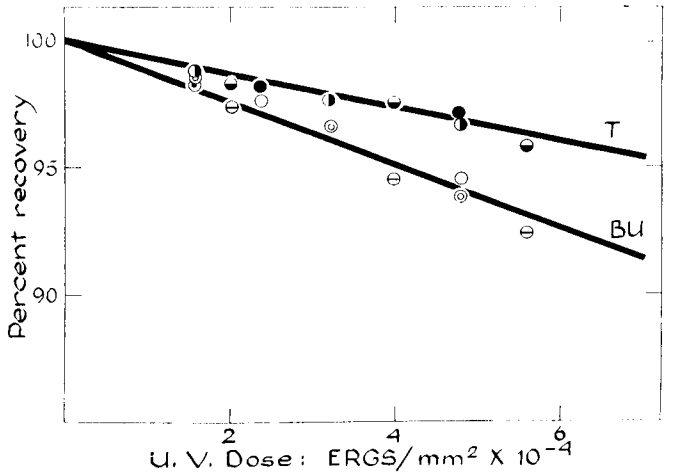

FIG. 1. The relative sensitivity of Thymine (T) and Bromouracil (BU) to alteration by untraviolet light when incorporated into the DNA of *E. coli* B/r and irradiated *in vivo*. Experimental conditions are described in the section on METHODS. The symbols indicate the several independent experiments.

for bromouracil. From the slope ratio of the two lines it is calculated that, *in vivo*, bromouracil is 1·9 times more sensitive to irradiation by u.v. light than is thymine. This, then, offers one explanation for the enhancement of bacterial sensitivity to u.v. when thymine has been replaced by bromouracil.

Photoproducts of bromouracil

The disappearance of parent compound due to the action of u.v. light is accompanied by the appearance of photoproducts. The data for the appearance of photoproducts with

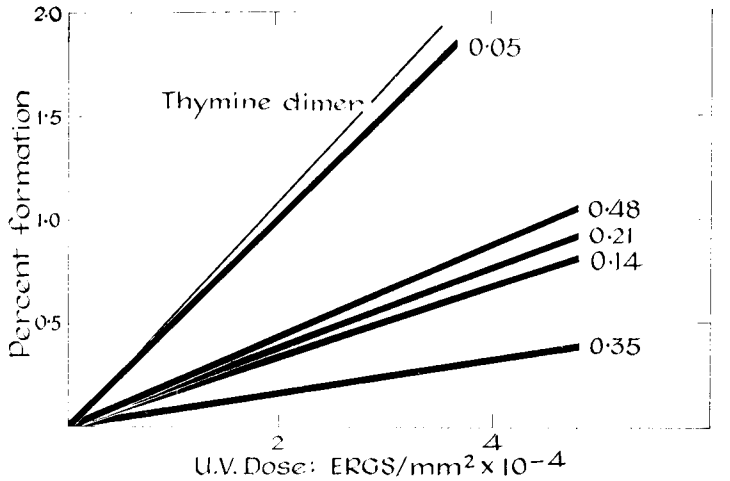

FIG. 2. The number and rate of appearance of photoproducts of Thymine and Bromouracil incorporated into the DNA of *E. coli* B/r and irradiated with u.v. light *in vivo*. The data are taken from the experiments partially described in Fig. 1 and further described in the text. The numbers that identify the lines are the R_f values for the bromouracil photoproducts chromatographed in butano/acetic acid/water (80/12/30). Representative R_f values in this solvent are given in Table 1 of a previous publication[23].

increasing doses of u.v. light are taken from the experiments described above in and Fig. 1 and are plotted in Fig. 2. The photoproducts are identified by their R_f in butanol–acetic acid/water (80/12/30)[23]. The curves are plotted from the slopes calculated by regression analysis. The standard error of the slope expressed as the percent of the slope was 3·2 per cent for the thymine dimer, and for the bromouracil photoproducts, listed in increasing order of R_f value, 5·7 per cent; 11·7 per cent; 10·9 per cent; 18·9 per cent and 13·3 per cent, respectively.

By irradiation *in vitro* it is possible to form a mixed dimer of bromouracil and uracil and also a compound that appears to be the dimer of bromouracil[23], but these are unstable except at acid pH and they probably would not survive the procedures used in the present experiments. In fact, when the above experiments were performed on bacteria grown on Br–82 bromouracil, no bromine-containing photoproducts were isolated although the concentration of unaltered bromouracil remaining after irradiation decreased with increasing dose of u.v. It must be concluded, therefore, that (when assayed as described here) debromination is the chief result of the absorption of ultraviolet radiation by bromouracil when incorporated in DNA. This statement, however, tends to make the photochemistry of bromouracil appear quite simple. On the contrary, it is remarkably complicated. Five debrominated photoproducts of bromouracil are obtained from irradiated *E. coli* B/r (Fig. 2). One of the minor products is uracil (R_f 0·48). The other photoproducts remain to be identified. From R_f considerations it is not improbable that the chief photoproduct formed in *E. coli* (R_f 0·05) may be dimeric in nature, although not necessarily possessing the same skeletal structure as the thymine dimer discovered by Beukers and Berends[1]. Thus far we have little data on the structure and properties of the R_f 0·05 material except that is seems to be rather alkali labile. Pretreatment of the cells with alkali (to remove the RNA) significantly reduces the amount of this photoproduct with the resultant formation of at least one new product in both *E. coli* B/r and *Enterococcus* Stei.

A comparison of the R_f values for the photoproducts of bromouracil produced *in vitro*[23] with those produced *in vivo* (Fig. 2) is of little help in identifying those produced *in vivo*. The R_f values obtained *in vitro* were 0·01; 0·08; 0·14; 0·26 and 0·43[23]. The latter two *in vitro* photoproducts still contain bromine, unlike any of those detected after *in vivo* irradiation. The only agreement is at R_f 0·14. The bromine containing photoproducts [bromouracil dimer (R_f 0·43) and bromouracil–uracil mixed dimer (R_f 0·26)] are unstable in neutral pH and decompose to form products that more closely correspond in R_f to the products isolated from *in vivo* experiments (see below).

We find that the block of thymidylate synthesis in *E. coli* B/r by sulfanilamide is about 5 per cent leaky as judged by the amount of the thymine compartment (thymine plus bromouracil) labelled by formate-C^{14} in the presence of sulfanilamide and bromouracil. Since some thymine is incorporated in the presence of bromouracil we felt that perhaps the bromouracil photoproduct in *E. coli* B/r (at R_f 0·05) might be a thymine-bromouracil mixed dimer (although we have thus far been unable to produce such a compound *in vitro*) or a derivative thereo . We made use of this leak in the block of thymidylate synthesis to label some of the thymine in the presence of the bromouracil and hoped therefore to find the same photoproduct labeled with C^{14} regardless of whether the cells were labelled with formate-C^{14} or bromouracil-C^{14}. Twelve radioactive areas were present on the chromatogram when the formate labelled cells (acid washed) were hydrolyzed and chromatographed. None of these showed a significant alteration in amount following a dose of 6×10^4 ergs/

mm^2 (2537Å). This would seem to preclude the hypothesis that the R_f 0·05 material is a mixed dimer of thymine and bromouracil.

Wacker *et al.*[28] have performed similar experiments using a different species of bacteria, *Enterococcus* Stei. Their results indicated a much larger conversion of bromouracil (per unit u.v. dose) to photoproducts than was found in *E. coli*. This may possibly be due to a difference in dose of u.v. absorbed by the nuclei as a consequence of the difference in size of the two bacteria. Furthermore, these authors found that the major photoproduct formed was chromatographically similar to uracil. This apparent discrepancy between the two laboratories could be caused by the use of different u.v. sources, different acid hydrolysis conditions (we used trifluoroacetic acid while Wacker used perchloric acid) or different species of bacteria. We have repeated their experiment on *Enterococcus* Stei (kindly supplied by Dr. Wacker) using our lamp and hyrdolysis conditions and obtained essentially the same results as did Wacker *et al.*[28]. The major photoproduct was in fact uracil but it should be emphasized that essentially all of the same photoproducts that we had found in *E. coli* B/r were also present here, although the relative amounts were different (Fig. 3).

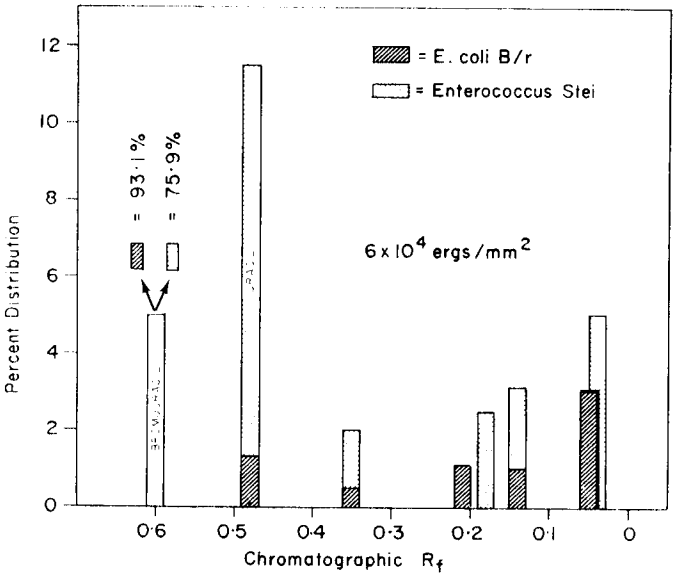

FIG. 3. Percent Distribution of Photoproducts Isolated from Bromouracil-2-C^{14} Labelled *E. coli* B/r and *Enterococcus* Stei.
Cells were irradiated (2537Å), hydrolyzed[6] and chromatographed in butanol/acetic acid/water (80/12/30)[23].

It is also of interest that the sensitivity of bromouracil relative to thymine was approximately the same in *Enterococcus* Stei (1·6 times) as it was in *E. coli* B/r (1·9 times) (Fig. 4).

Therefore, when one speaks of the photoproducts of bromouracil formed by u.v. irradiation one must specify the environment in which the bromouracil is irradiated. Two significantly different results have been obtained for *E. coli* B/r and *Enterococcus* Stei. The base composition of the DNA of *Enterococcus* Stei is (G+C)/(A+T)=0·69 (Wacker *et al.*[28]) while that for *E. coli* is 1·0. This difference in base composition is probably also reflected in the sequence of the bases in the DNA of these species; both factors undoubtedly play an important role in determining these results.

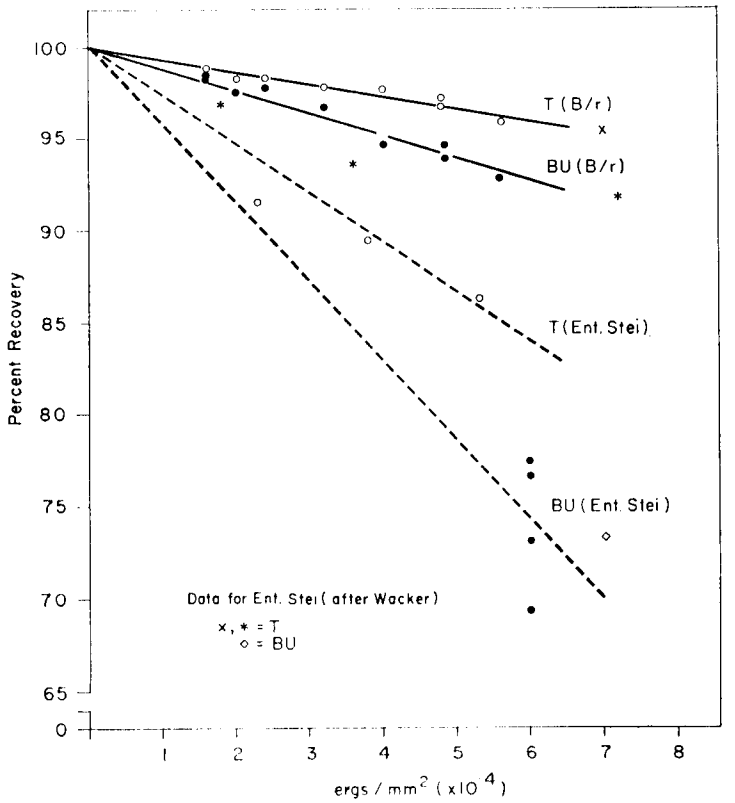

FIG. 4. A comparison of the photochemical response of thymine-2-C^{14} and bromouracil-2-C^{14} when incorporated into either *E. coli* B/r or *Enterococcus* Stei. Data for *E. coli* B/r are reproduced from Fig. 1. Data for *Enterococcus* Stei connected by dotted lines are those of the present author. Other data for *Enterococcus* Stei (as indicated) are taken from Wacker *et al.*[27, 28].

Native DNA (*Enterococcus* Stei) containing bromouracil-2-C^{14} forms 26 per cent uracil after u.v. irradiation ($4{\cdot}8 \times 10^4$ ergs/mm^2) whereas heat denatured DNA forms only 11 per cent and apurinic acid only 2 per cent[29]. No mention, however, was made of the relative appearance of the other photoproducts of bromouracil under these several conditions. This would be very important to know in view of the difference in results obtained for *E. coli* and *Enterococcus* Stei.

Further evidence that the photoproducts formed from bromouracil are extremely dependent upon its environment comes from the results cited in a previous paper[23]. At the doses of u.v. used (2537Å), bromouracil was completely inert when irradiated in frozen solution but on the addition of cytosine, uracil, uridine, or NaOH, bromouracil became nearly as reactive as thymine. Therefore, the fact that a compound is sensitive or insensitive when irradiated by itself in frozen solution may have little or no relation to its radiation sensitivity in a molecule of nucleic acid.

Enterococcus Stei also appears to incorporate a greater amount of bromouracil per optical density unit (260 mμ) of DNA than does *E. coli* B/r. Just from a consideration of the A-T content of the two bacteria one would expect about a 20 per cent increase, however, we observed about a 2·7-fold difference. A 44 per cent replacement in *E. coli* B/r and a 100 per

cent replacement in *Enterococcus* Stei could account for this difference. Clearly the percent replacement of thymine by bromouracil would have an effect on the type of photoproducts produced by bromouracil. One would expect different photoproducts depending upon whether the neighbour of a bromouracil molecule was another bromouracil or not. However, using large inocula of *Enterococcus* Stei and short incubation times so as to achieve a labelling comparable with that obtained with *E. coli* B/r we still obtained uracil as the major photoproduct. We have assumed that all the cells would be partially labelled but if only part of the population was almost completely labelled then it would not be surprising to obtain the same results as when a long incubation was performed and a high degree of labelling was achieved for all the cells. Wacker[29] makes the observation that in *Enterococcus* Stei the amount of uracil produced in irradiated DNA substituted with bromouracil depends upon the irradiation dose "but the percentage of bromouracil that forms uracil seems to be independent of the amount of bromouracil incorporated".

When *E. coli* B/r are grown in mineral medium without added sulfanilamide but in the presence of added bromouracil, incorporation into DNA was only about 1 per cent of the amount of bromouracil incorporated in the presence of the sulfanilamide. When the photoproducts of bromouracil produced in these slightly analog-substituted bacteria were investigated, the one with R_f 0·22 predominated, and that at 0·34 was also observed. There was no difference between the irradiated and unirradiated samples at R_f 0·05 and no counts at all were noted at R_f 0·48 (uracil). The observed photoproducts (R_f 0·22 and 0·34) appear in relatively lower yield in these cells when more fully substituted by bromouracil.

One factor that further complicates the comparison of the photochemistry of these two organisms is that a different proportion of the incident radiation apparently reaches the DNA within these two organisms. It was of interest, therefore, to compare the relative proportion of photoproducts within the two organisms under conditions where exactly the same amount of bromouracil had been photolysed. From a consideration of the data in Fig. 4 it was decided to irradiate bromouracil substituted *Enterococcus* Stei with doses up to $1{\cdot}8 \times 10^4$ ergs/mm^2. At a dose of $0{\cdot}6 \times 10^4$ ergs/mm^2, where only about 5 per cent of the bromouracil had been photolysed, uracil was still the major photoproduct. This preferential formation of uracil in *Enterococcus* Stei (as compared to *E. coli*) therefore does not seem to be due to a dose phenomenon.

The isolation of uracil as the major photoproduct from irradiated, bromouracil substituted, *Enterococcus* Stei may well be the result of the destruction of a primary photoproduct during the hydrolysis procedures. Wacker *et al.*[28] postulated that uracil might arise from the formation of a bromouracil dimer which would be unstable and yield uracil dimer. They erroneously adjudged uracil dimer to be unstable (for a discussion on the stability of uracil dimer see[23]) and concluded that this would split to form uracil during the hydrolysis of the DNA by acid. We have rather good evidence for the formation of a dimer of bromouracil when bromouracil is irradiated *in vitro*[23]. This compound is very labile except in acid media and decomposes in water to form nine separate products, of which the major one (about 40 per cent) has been identified as uracil. The decomposition of the bromouracil dimer *in vitro* also yields a minor product with about the same R_f (0·05) as the chief photoproduct of bromouracil produced in *E. coli* B/r.

We have also isolated the mixer dimer of bromouracil and uracil[23]. As is the case for the bromouracil dimer this compound is unstable except at acid pH. When the bromouracil –uracil dimer (labelled with bromouracil-2-C^{14}) undergoes decomposition in neutral aqueous

solution a high proportion of it is converted to material chromatographing near the origin, and a much smaller amount is converted to uracil.

Our data are not yet sufficient to completely explain the marked difference in the photochemistry in these two species of bacteria but we can offer two tentative hypotheses.

1. If the same primary photoproduct of bromouracil (the bromouracil dimer) is formed in the two species, then the observed difference in the final products isolated must be a reflection of the manner in which the bromouracil dimer decomposes as a consequence of differing environmental conditions occurring within the two types of cells. In *Enterococcus* Stei the bromouracil dimer must decompose by pathways very similar to those observed *in vitro*, at least the end result is quite similar. In *E. coli*, however, in order to explain the *in vivo* results on the basis of our knowledge gained from *in vitro* experiments we must postulate that the bromouracil dimer is first converted to a mixed dimer of bromouracil and uracil. The end result of the decomposition of this dimer *in vitro* yields results comparable to those obtained in *E. coli*.

2. Another hypothesis is that the primary photoproducts of bromouracil are not the same in the two bacteria. *Enterococcus* Stei appears to incorporate bromouracil much more efficiently than does *E. coli* B/r. *Enterococcus* Stei also contains 20 per cent more thymine per molecule of DNA than does *E. coli* B/r and might be expected to contain a higher proportion of adjacent thymine molecules. The results for *Enterococcus* Stei seem to be consistent with the conclusion that bromouracil dimer is the primary photoproduct formed. However, since the formation of uracil is apparently not dependent upon the percent replacement of thymine by bromouracil[29, and above] and since less uracil is formed in denatured as compared to native DNA[29]; this seems to argue against dimer formation.

We know that the block in thymidylate synthesis of *E. coli* B/r by sulfanilamide is about 5 per cent leaky so that every thymine molecule in a new strand of DNA will not be replaced by a bromouracil. This would cut down the chances that two bromouracil molecules would be incorporated side by side and therefore reduce the chances of bromouracil dimer formation. The results for *E. coli* are more consistent with the conclusion that the primary photoproduct is a dimer of bromourcail and uracil. This could arise by a preferential decomposition of the bromouracil dimer (hypothesis 1) but it could also arise by other mechanisms. The dimerization of bromouracil and cytosine with the subsequent elimination of the amino group of the cytosine could yield the bromouracil–uracil dimer. This mechanism has some merit since it has recently been shown[23, 29] that uracil dimer can be formed when cytosine is irradiated in frozen solution,

When DNA is irradiated with u.v. the two strands become cross-linked[12]. This is believed to be caused by the photochemical interaction of two thymine molecules[12] but it cannot be through the formation of a dimer of the type found within a single strand of DNA[22]. Bromouracil substituted DNA has been shown to be much more sensitive to interstrand crosslinking than is normal DNA[14]. In view of the relatively more limited substitution by bromouracil in *E. coli* B/r the possibility exists that the major photoproduct produced in *E. coli* B/r is not an intrastrand dimer, but rather, interstrand crosslinks (with either bromouracil or cytosine). This could account for the differences in the relative amount of the several photoproducts of bromouracil produced in *Enterococcus* Stei and *E. coli* B/r.

The fact that bromouracil is about twice as photochemically reactive as thymine (*in vivo*) would certainly explain in large part the mechanism by which bromouracil incorporated in to the DNA of cells causes these cells to become more radiation sensitive[5, 7, 9, 10]. Other mechanisms must also be considered. The photoproducts of bromouracil do not

appear to respond to repair by the several recovery mechanisms and in fact appear inhibitory to the enzymes[22]. Cells containing bromouracil substituted DNA show a five-fold greater sensitivity to the u.v. crosslinking of DNA and protein than do normal cells[22]. Bromouracil may also play a role in other mechanisms of radiation sensitization but these are reviewed elsewhere[22].

Photoproducts of thymine

When thymine is incorporated into the DNA of bacteria it forms mainly one photoproduct, the dimer. Other photoproducts, however, are also formed. These have R_f's in butanol/acetic acid/water (80/12/30) of 0·0; 0·13; the dimer at 0·24; 0·33 and 0·42 (and thymine at 0·60). These other photoproducts are present at less than one-fourth the amount of dimer present after $2{\cdot}9 \times 10^4$ ergs/mm^2 at 2537Å.

The DNA from unirradiated bacteria is almost quantitatively extracted with sodium lauryl sulfate using the method of Smith[20]. However, after increasing doses of u.v. (2537Å), more and more of the DNA becomes non-extractable[20, 21, 22]. It is this non-extractable fraction of the DNA that contains the greater portion of these new photoproducts (although the amount of dimer is not increased) and chiefly the ones at R_f 0·0 and 0·42 (see below; poly-dAT). It is expected that these may play a role in the u.v. crosslinking of DNA and protein[20, 21, 22]. Dellweg and Wacker[4] have also reported finding *in vivo* photoproducts of thymine other than the dimer. Photoproducts of thymine other than the dimer have also been produced *in vitro* Smith[23], Sztumpf and Shugar[24], Dellweg and Wacker[4], Johns[8]).

The experiments described above were largely performed at one dose of u.v. In the multidose experiments described in Figs. 1 and 2 a thymine photoproduct with an R_f of 0·0 was formed in variable amounts. In one experiment this compound increased linearly with dose but in other experiments it was largely absent or was maximally increased with our first dose of u.v. ($1{\cdot}6 \times 10^4$ ergs/mm^2). Submitting this material to further acid hydrolysis did not effect its R_f nor did pre-hydrolysis of the cells with NaOH remove this photoproduct of thymine. We can conclude that it is not unhydrolyzed thymidylic acid or a component wholly arising from RNA. The absence of thymine photoproducts other than the dimer, and occasionally the material at R_f 0·0, from the experiments described in Fig. 1 and 2 may stem from the fact that less sensitive detecting equipment was used here than in subsequent experiments (see addendum[23]).

When poly-dAT (a gift of Dr. Carl Schildkraut) labelled with thymine-C^{14} was irradiated with doses of u.v. (2537Å) up to $1{\cdot}8 \times 10^4$ ergs/mm^2, hydrolyzed and chromatographed, there was no evidence for the appearance of the thymine dimer of the type originally described by Beukers and Berends[1]. A similar conclusion has been reached by Deering and Setlow[3] from a consideration of the lack of appropriate spectral changes occurring in irradiated poly-dAT We found that several other photoproducts of thymine were formed, however. The two major ones appeared at R_f 0·00 and 0·42. These are the same R_f values for the major photoproducts of thymine (other than the dimer) that are formed in irradiated *E. coli* (see above). These products may have some relation to the phenomenon of interstrand crosslinking described by Marmur and Grossman[12].

From the results in Figs. 1 and 2 on the rate of formation of thymine dimers in irradiated (2537Å) *E. coli* B/r one can calculate the dose required to produce a dimer. If we assume for *E. coli* that there are $8 \times 10{-}^{15}$g of DNA per cell then there should be approximately $3{\cdot}9 \times 10^6$ molecules of thymine per cell. At the 99 per cent killing dose for *E. coli* B/r (1800 ergs/mm^2) 0·085 per cent of the thymine has been dimerized, i.e., 1660 molecules of thy-

mine dimer have been produced. Since thymine dimer production was linear with increasing dose under the conditions used in these experiments, it may be concluded that approximately 1 erg/mm² of u.v. at 2537Å produces one molecule of thymine dimer.

Setlow and Carrier[16] have calculated the rate of dimer formation from spectral changes in irradiated DNA and deduce that approximately 3 ergs/mm² (2800Å) are required per dimer in phage T2. Wulff[30] found that for phage $T4_{v1}$ that 4·8 dimers were formed per lethal hit and 2·5 ergs/mm² (2600Å) caused the formation of one dimer molecule.

Except for the fact that about three times as much dimer is produced by the same incident dose of u.v. (2537Å) in *Enterococcus* Stei as for *E. coli* B/r (Fig. 4) there appear to be no other marked differences in the photochemical response of thymine when incorporated into these two organisms.

Acknowledgement—I gratefully acknowledge the valuable assistance of Mrs. Mary E. O'Leary in these experiments.

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