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Discontinuities in the DNA synthesized in an Excision-defective Strain of *Escherichia coli* following Ultraviolet Irradiation

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Although *Escherichia coli* K12 *uvrA6* is defective in the excision of pyrimidine dimers from its DNA, 37% of cells survive a dose of ultraviolet light which is equivalent to about 50 pyrimidine dimers per 10^7 nucleotides. The amount of tritiated thymidine incorporated into the DNA of irradiated cells indicates that pyrimidine dimers in the DNA inhibit DNA synthesis but are not permanent blocks. Zone sedimentation of single-strand DNA was performed in alkaline sucrose gradients. To minimize degradation by shearing, the DNA was released from spheroplasts layered on top of the gradients. Newly synthesized, denatured DNA from unirradiated cells sediments with a molecular weight of greater than 100×10^6, whereas the newly synthesized, denatured DNA from cells irradiated with 60 ergs/mm^2 has a molecular weight of about 14×10^6. During subsequent incubation of the irradiated cells, the sedimentation rate of the DNA synthesized immediately after irradiation increases and approaches that of normal DNA. However, at any time during this incubation period, the incorporation of tritiated thymidine into fast-sedimenting DNA is minimal, suggesting that the daughter-strand DNA synthesized after ultraviolet-irradiation contains gaps, or alkali-labile bonds. These dicontinuities disappear during further incubation, as a higher rate of sedimentation is found. The number of these daughter-strand defects is similar to the number of pyrimidine dimers in an equivalent length of parental DNA.

1. Introduction

Strain *Escherichia coli* K12 *uvrA6* is defective in the excision of dimers from its DNA (Boyce & Howard-Flanders, 1964), and it has a 1/e survival dose of ultraviolet light that induces about 50 dimers per chromosome. In contrast, a strain carrying *uvrA6* plus *recA13* has a 1/e survival dose that corresponds to between one and two dimers per chromosome (Howard-Flanders & Boyce, 1966). One plausible interpretation of these data is that normal cells contain a mechanism that allows survival with unexcised dimers, but cells carrying *recA13* do not.

The effect of ultraviolet light on the incorporation of [^{3}H]thymidine into the DNA of several strains of *E. coli B* was recently examined (Swenson & Setlow, 1966). After a dose of 20 ergs/mm^2, strains B_{s-3}, B_{s-8} and B_{s-12}, which are defective in dimer excision, incorporate about one-third as much [^{3}H]thymidine as the wild type. Recent studies (Mattern, Zwenk & Rorsch, 1966; Greenberg, 1967) show that B_{s-1}

is a double mutant, and the very low level of incorporation observed in this strain is therefore not related in a simple manner to the number of unexcised dimers in DNA.

In this paper, we show that *E. coli* K12 *uvrA6*, irradiated with ultraviolet doses of 25 to 100 ergs/mm^2, also exhibits an intermediate level of incorporation of [^{3}H]thymidine into acid-insoluble material. The DNA of this strain was analyzed in alkaline sucrose gradients by the method of McGrath & Williams (1966). Newly synthesized DNA from ultraviolet-irradiated cells sediments more slowly than DNA from unirradiated cells, but with further incubation, its sedimentation rate approaches that of the control DNA. These results are considered in terms of a possible genetic mechanism in which, by sister exchanges, information lost in one DNA duplex is recovered from the corresponding intact region of a sister DNA duplex.

2. Materials and Methods

(a) *Bacterial strain*

E. coli AB2500 *uvrA6* is a thymine-requiring strain derived by the method of Stacey & Simson (1965) from the parent strain AB1886 *uvrA6* (Howard-Flanders, Boyce, Simson & Theriot, 1962). The nutritional requirements in addition to thymine are: threonine, leucine, proline, histidine, arginine and thiamine.

(b) *Media*

K medium (Weigle, Meselson & Paigen, 1959) is a salts–glucose medium supplemented with Casamino acids and thiamine. K + 2 is K medium supplemented with 2 μg thymidine/ml. K + 10 is K medium supplemented with 10 μg thymidine/ml. and is used for routine growth of AB2500. M9 buffer is M9 medium (Adams, 1959) with glucose omitted.

(c) *Materials*

[^{3}H-*methyl*]Thymidine (8·7 c/m-mole) was obtained from the New England Nuclear Corporation, and [2-^{14}C]5-bromouracil (31·0 mc/m-mole) was obtained from Calbiochem.

(d) *Ultraviolet irradiation and radioisotope incorporation*

Log-phase cells grown to about 2×10^8 cells/ml. in K + 10 medium were harvested on Millipore membrane filters and resuspended in 15 vol. of K + 2 medium for irradiation with a low-pressure mercury germicidal lamp giving predominantly 2537 Å radiation. The dose rates were measured with the same General Electric germicidal u.v. meter used by Boyce & Howard-Flanders (1964) when measuring dimer yield. A meter constructed and calibrated by Dr R. Latarjet registers a dose rate 13% lower than the General Electric meter. Samples were irradiated in Petri plates on a rotating platform and the doses were corrected (Morowitz, 1950). Immediately after irradiation, 10 μc of [^{3}H]thymidine were added to 1·0 ml. of the cell suspension in K + 2 medium. The cells were incubated at 37°C and at the desired times, 0·1-ml. samples were pipetted onto Whatman 3 MM paper disks (2·4 cm diameter) to which 0·1 ml. of 1 N-NaOH had previously been added. After drying in the air, the disks were washed 3 times with ice-cold trichloroacetic acid (5% w/v), once with cold 95% ethanol and then with acetone. When dry, the disks were counted in vials with 10 ml. scintillator fluid in a scintillation counter. The liquid scintillator contained per litre: 670 ml. toluene, 330 ml. ethanol, 2·7 g 2,5-diphenyloxazole and 33 mg 1,4-bis 2-(5-phenyloxazolyl)benzene.

(e) *Sedimentation in alkaline sucrose gradients*

Log-phase cultures were grown to approximately 2×10^8 cells/ml. in K + 10 medium and 1-ml. portions were used for each part of an experiment. Medium transfers were done by membrane filtration at room temperature and the cells were washed with the appropriate medium. Labeling was accomplished by suspending the cells in 1 ml. K medium at 37°C and adding 100 μc [^{3}H]thymidine. For ultraviolet irradiation, cells were resuspended in 13 vol. of K medium. The method of McGrath & Williams (1966) was used

for sedimenting DNA in alkaline sucrose gradients. Spheroplasts were produced at ice temperature by suspending about 3×10^8 cells in 0·3 ml. M9 buffer plus 0·06 ml. of a 30% w/v sucrose in 0·6 M-Tris (pH 8·1). To this suspension were added 0·04 ml. lysozyme (10% solution) and 0·1 ml. of 32 mM-EDTA. After 5 min in ice, 10 μl. of the spheroplast suspension were placed on top of a 5·0-ml. gradient of 5 to 20% w/v sucrose, adjusted to pH 12 with NaOH (final concentration about 0·04 to 0·15 N). The spheroplasts were mixed into a 0·1-ml. top layer of 0·5 N-NaOH by stirring several times with a pin to release the DNA. In this procedure, the gradients are ready for centrifugation within 10 min after the cells are harvested. The tubes were centrifuged in the SW50 rotor for 120 min at 30,000 rev./min at 20°C in a Spinco L2 centrifuge. After centrifugation, drops were collected on filter disks which were washed and counted as described above. The recovery of input radioactivity was greater than 75% and was not affected by irradiation.

(f) *Use of bacteriophage DNA's as molecular weight markers*

Bacteriophages T2 and λc26 were lightly labeled with [^{14}C]thymidine, using the precautions suggested by Thomas (1966) to minimize radiochemical damage to the phage DNA. The phage particles were layered directly onto the alkaline sucrose gradients for release and denaturation of the DNA. After centrifugation, the acid-precipitable radioactivity was measured as described. The presence of divalent cations in the phage suspension caused rapid sedimentation in the centrifuge tube, presumably because of aggregation, or co-precipitation of the DNA with the insoluble hydroxides. This was avoided by the presence of 10 mM-EDTA in the phage stocks. In the calculation of molecular weight from the sedimentation data, we have used the values obtained by electron microscopy (Abelson & Thomas, 1966). These values for the single-strand DNA's of T2 and λ are 59×10^6 and 17×10^6, respectively.

(g) *Cesium chloride gradients*

Cells were lysed in 0·5 ml. vol. of 0·1 M-Tris, pH 8·1, by the lysozyme–pronase procedure of Billen, Hewitt & Jorgensen (1965). Each lysate was diluted to a final vol. of 1·8 ml. and forced through a no. 27 syringe needle 3 times to fragment the DNA (Richards & Boyer, 1965). For centrifugation, 2·94 g of CsCl were dissolved in 1·9 ml. of water and 0·3 ml. of lysate mixed in. The tubes were filled up with 2 ml. of paraffin oil and centrifuged for 40 to 60 hr at 30,000 rev./min at 20°C in the SW50 rotor of the Spinco L2. Drops were collected and counted as described above.

3. Results

(a) *Incorporation of [^{3}H]thymidine into ultraviolet-irradiated cells*

Figure 1 shows that the incorporation of [^{3}H]thymidine into acid-insoluble material in the *uvrA6* strain is reduced more and more as the ultraviolet dose is increased. The yield of dimers is about six per erg/mm^2 per 10^7 nucleotides†. The strain carrying

† The yield of pyrimidine dimers in the DNA of *E. coli* exposed to 1 erg/mm^2 of 2537 Å light is about $2{\cdot}6 \times 10^{-6}$ pyrimidine dimer per thymine residue, or about 6 pyrimidine dimers/genome of 10^7 nucleotides. This estimate is based on the following evaluation of published data. Setlow, Swenson & Carrier (1963) reported that 5×10^{-6} of [^{3}H]thymidine radioactivity was converted to dimer per erg/mm^2 at 2650 Å. Wulff (1963) and Boyce & Howard-Flanders (1964) reported this yield to be $3{\cdot}4 \times 10^{-6}$ and $3{\cdot}6 \times 10^{-6}$ respectively at 2537 Å. Assuming the yield of biologically significant photoproducts to be 25% higher at 2650 Å than at 2537 Å (Setlow & Setlow, 1960), the mean of these yields is $3{\cdot}7 \times 10^{-6}$ per erg/mm^2 radioactivity in photoproducts per erg/mm^2 at 2537 Å.

The types of products formed must be considered when converting from the observed radioactivity in photoproducts to the yield of pyrimidine dimers. It was found by Setlow & Carrier (1966) that the relative yields of the pyrimidine dimers are T–T, 50%; T–C, 40%; and C–C, 10%. The ratio between the radioactivity in one [^{3}H]thymidine molecule to the average radioactivity per pyrimidine dimer is therefore 0·71. For a u.v. dose of 1 erg/mm^2 at 2537 Å, the yield of pyrimidine dimers (T–T + T–C + C–C) is $2{\cdot}6 \times 10^{-6}$ dimers/thymine, or 6·5 dimers per 10^7 nucleotides, the latter number being close to the number of nucleotides in the genome of *E. coli* (Cairns, 1963).

uvrA6 was unable to excise dimers either after a relatively high u.v. dose, 960 ergs/mm^2 (Boyce & Howard-Flanders, 1964), or after 30 ergs/mm^2 at 2650 Å (Setlow, 1967). Moreover, as a greatly increased sensitivity to u.v. light in strains carrying *uvrA6* is evident at doses down to 10 ergs/mm^2 (and down to 0·2 erg/mm^2 in the strain carrying *uvrA6* and *recA13*), it is likely that a large fraction of the dimers formed by these lower doses remain in the DNA during the post-irradiation incorporation of [^{3}H]thymidine. Thus, it is apparent that the incorporation of [^{3}H]thymidine is not blocked permanently by unexcised dimers. One explanation of these results is that each dimer delays the polymerase for a few seconds during which time the dimer is passed and synthesis resumes on the other side.

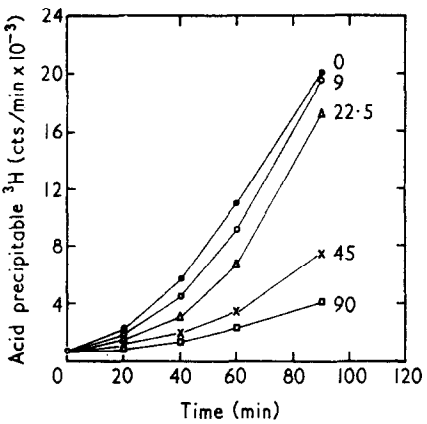

FIG. 1. Incorporation of [^{3}H]thymidine into acid-insoluble material after exposure to u.v. light. *E. coli* K12 AB2500 *uvrA6* was examined after doses of 0, 9, 22·5, 45 and 90 ergs/mm^2 as labeled on the curves.

(b) *Sedimentation in alkaline sucrose of DNA synthesized after ultraviolet irradiation*

These experiments were carried out to determine the rate of sedimentation of the DNA synthesized in cells containing dimers. Figure 2 shows that the DNA labeled before u.v. irradiation sedimented at the same rate as DNA from control cells. In contrast, it can be seen from Figs 2 and 3 that the average sedimentation rate of the DNA labeled after u.v. irradiation decreased with increasing dose.

Figure 4 shows the sedimentation pattern of the DNA synthesized during the first ten minutes after u.v. irradiation. The DNA labeled for ten minutes after u.v. irradiation has a reduced rate of sedimentation. In a number of similar experiments, the amount of tritiated thymidine incorporated in the first ten minutes after irradiation with 60 ergs/mm^2 is about 25% of that incorporated into the unirradiated cells. In other comparable experiments, these manipulations had little effect upon the time taken to double the DNA content. Thus, with a generation time of about 45 minutes for unirradiated cells, an average of 5% of each chromosome, or about 150×10^6 molecular weight of DNA, has been replicated in the irradiated cells. The average number of pyrimidine dimers in this length of DNA is about 16. If after ten minutes in [^{3}H]thymidine the cells are transferred to [^{1}H]thymidine medium for further incubation, the sedimentation rate of the [^{3}H]DNA approaches that of

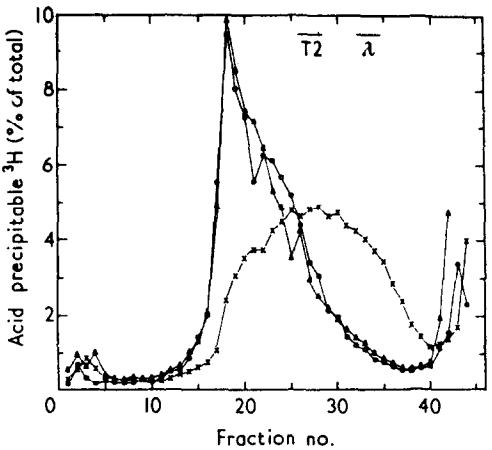

FIG. 2. Sedimentation in alkaline sucrose of radioactive DNA labeled with [^{3}H]thymidine in K medium. One sample (▲) was labeled for 40 min, exposed to a u.v. dose of 60 ergs/mm^2, and then incubated 40 min in K + 2 medium. The second sample (×) was incubated in K + 2 medium for 40 min, irradiated with 60 ergs/mm^2 and then transferred to K medium + [^{3}H]thymidine for 40 min. The control sample (●) was labeled for 40 min and then transferred to K + 2 medium for 40 additional min. Finally, the cells were converted to spheroplasts and lysed on top of the gradients. Conditions of centrifugation were 30,000 rev./min for 120 min at 20°C. The positions of intact strands from T2 and λ centrifuged under identical conditions are indicated. Cells used were *E. coli* K12 AB2500 *uvrA6*.

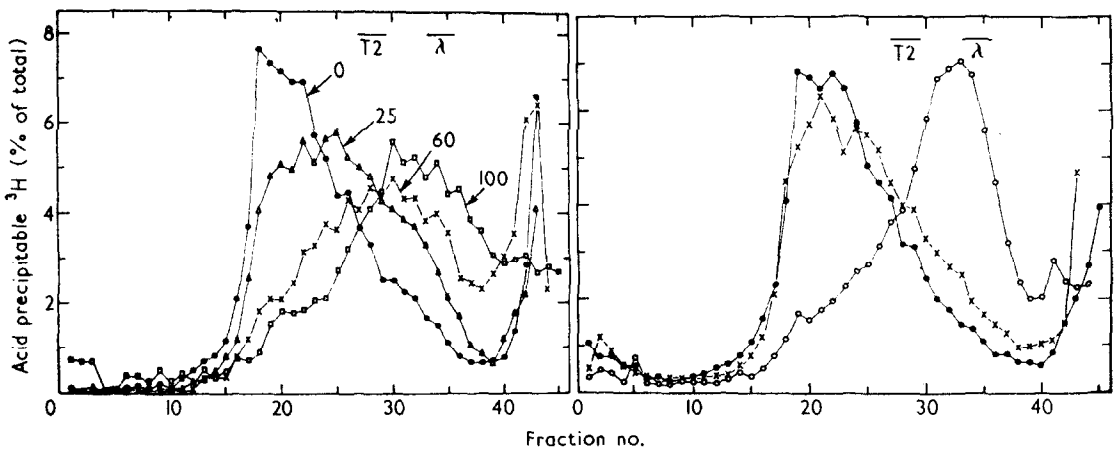

FIG. 3

FIG. 4

FIG. 3. Effect of several u.v. doses on sedimentation rate in alkaline sucrose of DNA from *E. coli* K12 AB2500 *uvrA6* labeled with [^{3}H]thymidine after exposure to u.v. After exposure to 0, 25, 60 or 100 ergs/mm^2 (see labels on curves), cells were incubated in K + [^{3}H]thymidine for 40 min. The procedures for lysis and sedimentation were as in Fig. 1. Positions of T2 and λ indicated as in Fig. 2.

FIG. 4. Effect of further incubation on sedimentation rate in alkali of DNA pulse-labeled after u.v. irradiation. *E. coli* K12 AB2500 *uvrA6* cells were irradiated with 60 ergs/mm^2 and incubated in K medium + [^{3}H]thymidine for 10 min. Cells were analyzed immediately (○) or shifted to non-radioactive K + 2 and incubated for 70 min at 37°C (×). No acid-precipitable radioactivity was lost during the incubation in non-radioactive medium. The third sample was an unirradiated control that was incubated in K + [^{3}H]thymidine for 10 min (●). The procedures for lysis and sedimentation were as in Fig. 1. Positions of T2 and λ indicated as in Fig. 2.

[^{3}H]DNA from unirradiated cells. If in these strains DNA synthesis after u.v. irradiation continues only from existing replicating forks, the newly synthesized daughter strands contain gaps or alkali-labile bonds. These regions become stable to alkali during subsequent incubation.

A second possibility is that ultraviolet light causes initiation of replication at new points (Hewitt & Billen, 1965). The results in Fig. 4 could be interpreted as evidence for the initiation of new daughter strands after u.v. irradiation. Thus, during continued incubation in [^{1}H]thymidine medium, the extension of these newly initiated chains would be expected to lead to faster sedimenting material resembling DNA from unirradiated cells.

The following experiment was designed to distinguish between these alternatives. Cells were incubated in [^{1}H]thymidine medium. If the increase in sedimentation rate observed in Fig. 4 were due to the extension of newly initiated DNA strands, the [^{3}H]thymidine incorporated after a period of incubation in [^{1}H]thymidine would be expected to be at the growing end of substantial chains and should appear in DNA sedimenting faster than [^{3}H]DNA labeled in the first ten minutes after irradiation. In contrast, if the newly synthesized DNA contains single-strand defects, incubation in [^{1}H]thymidine before addition of [^{3}H]thymidine should have little effect on the sedimentation profile of the [^{3}H]DNA. Figure 5 shows that the sedimentation profile of [^{3}H]DNA labeled after incubation in [^{1}H]thymidine is quite similar to [^{3}H]DNA labeled immediately after u.v. irradiation, and thus favors the daughter-strand gap or labile-bond interpretation.

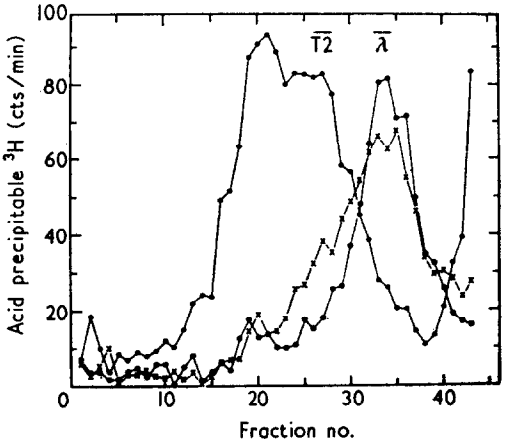

FIG. 5. Sedimentation in alkaline sucrose of DNA pulse-labeled for 10 min beginning at 0 or 50 min after u.v. irradiation. *E. coli* K12 AB2500 *uvrA6* cells were irradiated with 60 ergs/mm^2 and were labeled with [^{3}H]thymidine for 10 min immediately (○) or after 50 min incubation in K + 2 medium (×). An unirradiated control sample was labeled with [^{3}H]thymidine for 3 min (●). The procedures for lysis and sedimentation were as in Fig. 1. Unlike the other Figures which show the cts/min as percentage of total, this Figure shows the actual cts/min, to facilitate a direct comparison of the yields of radioactivity in various fractions. Positions of T2 and λ indicated as in Fig. 2.

(c) *Effect of extent of synthesis on sedimentation rate of newly synthesized DNA*

In control experiments, where unirradiated cells were labeled with [^{3}H]thymidine for short periods of time, such as in Fig. 5, the sedimentation profile was broader

than for radioactive DNA from cells labeled for longer periods of time. Figure 6 shows an example of this difference in unirradiated cells. It was expected that newly synthesized DNA would be covalently linked to pre-existing DNA and would sediment at the same rate as the bulk of the cellular DNA. However, this effect is insufficient to explain the changes observed after u.v. irradiation. Experiments in which the extent of synthesis in the unirradiated control cells is reduced to compare directly with that in the irradiated cells, such as in Fig. 5, show that the low sedimentation rate of DNA synthesized after u.v. irradiation cannot be attributed to the reduced synthesis in irradiated cells.

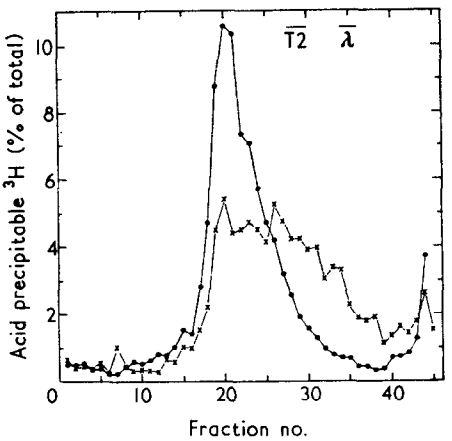

FIG. 6. Effect of time of labeling on sedimentation of newly synthesized DNA in unirradiated cells. *E. coli* K12 AB2500 *uvrA6* cells were labeled with [^{3}H]thymidine for 1 min (×), or 30 min (●) before analysis as described in Fig. 1. The procedures for lysis and sedimentation were as in Fig. 1. Positions of T2 and λ indicated as in Fig. 2.

(d) *Estimation of molecular weight*

The molecular weight of the DNA strands was estimated by comparing their sedimentation rates with that of phage DNA's. With the standard conditions (30,000 rev./min, two hours, 20°C), DNA from T2 concentrated in a narrow zone with a peak 16 fractions from the meniscus, and when centrifuged at twice the usual centrifugal force (42,500 rev./min, two hours, 20°C), the peak has moved to fraction 31 or 32. This indicates that the distance sedimented is proportional to the sedimentation coefficient in these gradients. The molecular weights of two DNA preparations (M_1 and M_2) are related to the distances sedimented in the sucrose gradient (D_1 and D_2) as described by Abelson & Thomas (1966):

$$\frac{D_1}{D_2} = \left(\frac{M_1}{M_2}\right)^{0.38}.$$

The calculated results for several of the preparations are given in Table 1. The value for λ DNA agrees well with the estimate of 17×10^6 daltons derived by electron microscopy (Abelson & Thomas, 1966).

It is pertinent to determine the numerical relation between gaps in the daughter strands and dimers in parental strands. The approximate number of dimers per 10^7 nucleotides after a dose of 60 ergs/mm^2 is 360, or 1 dimer per $9{\cdot}2 \times 10^6$ daltons. At

TABLE 1

Estimation of molecular weight

Source of DNA	$\frac{D}{D_{T2}}$	$M \times 10^{-6}$
Fig. 5 (labeled 3 min)	1·24	104
Fig. 5 (labeled 10 min after 60 ergs/mm^2)	0·66	20
Fig. 5 (labeled from 50 to 60 min after 60 ergs/mm^2)	0·72	25
Fig. 6 (labeled 30 min)	1·43	151
Fig. 6 (labeled 1 min)	1·21	97
λ Bacteriophage	0·625	17·1
λ Bacteriophage	0·612	16·3

DNA from ^{14}C-labeled T2 was used as a standard with an intact single-strand molecular weight of 59×10^6 daltons (Abelson & Thomas, 1966). For phage DNA, D is the number of fractions from the meniscus to the peak of the radioactivity. For bacterial DNA, D is the number of fractions from the meniscus to the median of the radioactivity sedimenting in fractions 10 through 40.

this dose, the average size of daughter strands, ten minutes after u.v. irradiation, is equivalent to a length of parental DNA with an average of 2·2 dimers (see Table 1). The molecular weights in Table 1 are based on the median sedimentation rate of radioactivity from a polydisperse population, and a better estimate of the number of gaps in the daughter strands is obtained from the number average molecular weight calculated from the following formula:

$$M_n = \frac{\sum f_i}{\sum (f_i/M_i)}$$

where f_i is the fraction of the total radioactivity in the ith fraction and M_i is the molecular weight for DNA in this fraction calculated from the relation of Abelson & Thomas (1966). Since slowly sedimenting radioactivity contributes strongly to M_n, centrifugation was also carried out at twice the usual centrifugal force in order to move the radioactivity further from the meniscus, and molecular weights calculated by the two methods. The M_n values listed in Table 2 show that the number average size of daughter strands corresponds to a region of parental DNA containing less than two dimers. Also included in Fig. 7 is a theoretical curve (derived and normalized to the sedimentation pattern as described in the Appendix) for the distribution of lengths of DNA between dimers. The maximum value of the theoretical curve corresponds to a length 1·62 times greater than the average length between defects. Since the maximum in Fig. 7 occurs at a molecular weight of 14×10^6, the apparent average molecular weight between daughter-strand defects is $8{\cdot}6 \times 10^6$. This figure is not very different from $9{\cdot}2 \times 10^6$, the average molecular weight between dimers. If replication is delayed at a dimer, regions of DNA with few dimers would presumably be replicated faster than regions of DNA with many dimers. Thus, the labeled daughter strands may represent predominantly parental DNA with a lower than average density of dimers. Also, some of the original daughter-strand defects may

have become alkali-stable before centrifugation. It is striking therefore that the number of daughter-strand gaps differs by less than a factor of two from the number of dimers in an equivalent length of parental DNA.

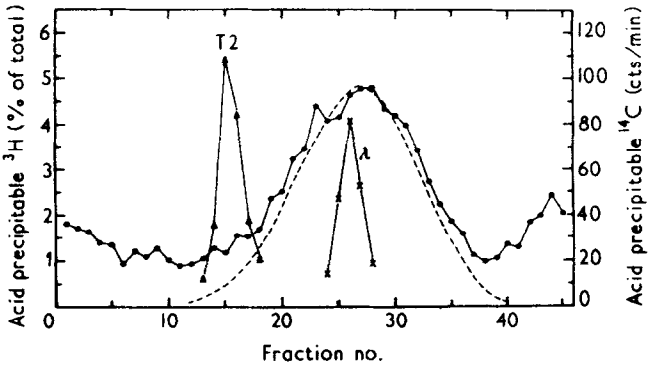

FIG. 7. Sedimentation in alkaline sucrose of DNA labeled for 10 min after u.v. irradiation. *E. coli* K12 AB2500 *uvrA6* cells were irradiated with 60 ergs/mm^2 and were then labeled with [^{3}H]thymidine for 10 min. Cells were converted to spheroplasts and were lysed on the gradient. In the other tubes of the same run, ^{14}C-labeled DNA's from the bacteriophages T2 (46 fractions collected) and λ (45 fractions collected) were sedimented as molecular weight references. The conditions of centrifugation were 42,500 rev./min, 120 min and 20°C. The broken line is the theoretical sedimentation pattern for DNA with random breaks occurring at a frequency of 360 breaks per 10^7 nucleotides (see Appendix).

TABLE 2

Molecular weight of DNA daughter strands synthesized after exposure to 60 ergs/mm^2 u.v. light.

Portion of Fig. 7 used for calculation	M_{median} ($\times 10^{-6}$)	M_n($\times 10^{-6}$)
Fractions 1–38	20·7	10·9
Fractions 1–40	19·9	7·4
Fractions 8–38	17·9	9·6
Fractions 8–40	17·5	6·8

Molecular weights are calculated from the relations of Abelson & Thomas (1966) using λ DNA as a reference with a molecular weight of 17×10^6. M_{median} is calculated from the median of radioactivity sedimenting in the indicated fractions. M_n is determined as indicated in the text.

(e) *Centrifugation in cesium chloride gradients*

Several experiments were performed with a density label to investigate how strands were distributed among the duplexes formed after u.v. irradiation. Certain recombination-like events evoked by u.v. photoproducts could lead to the exchange of single-strand partners between the daughter helices, and so to the

formation of regions of DNA that resemble conservatively replicated DNA. Normal control cells and cells irradiated with 10 ergs/mm^2 were shifted to [2-^{14}C]bromouracil medium after irradiation. No differences were observed between the irradatedi and unirradiated samples: in particular, there was no indication of premature appearance of heavy DNA (Fig. 8(a)), and the ratio of heavy DNA to hybrid DNA was similar for the control and u.v.-irradiated cultures (Fig. 8(b)). Thus, extensive exchange in which two parental strands appear in one light duplex and two heavy daughter strands in the other duplex does not occur. However, such abnormal structures could exist for lengths of DNA of molecular weight less than one million daltons without being detected.

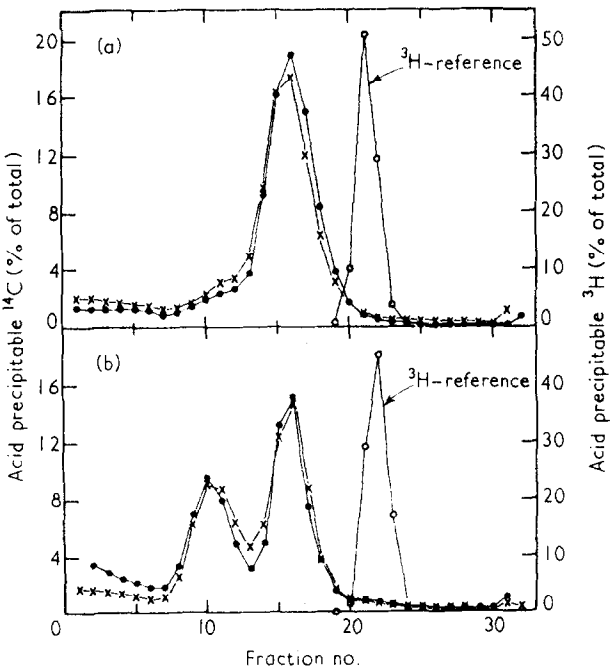

FIG. 8. Equilibrium centrifugation in CsCl of DNA labeled with [^{14}C]bromouracil after exposure to u.v. light. After exposure to 0 (●) or 10 (×) ergs/mm^2, cells were incubated in K supplemented with [^{14}C]bromouracil (5 μg/ml.) for 30 min (a) or 60 min (b). Reference DNA (○) from cells grown in [^{3}H]thymidine is banded in the third tube in each run. In part (b), the unirradiated sample is plotted from fractions 2 to 31 because only 30 fractions were obtained.

4. Discussion

After ultraviolet irradiation, DNA replication presumably continues at a normal rate along the chromosome until a dimer is reached, at which point replication is delayed for several seconds before normal synthesis is resumed on the other side. An estimate of the average delay can be obtained from Fig. 1. After a dose of 45 ergs/mm^2 (about 270 dimers per genome), the irradiated cells required about twice as long as unirradiated cells to incorporate a given amount of [^{3}H]thymidine. The doubling time for the unirradiated cells is about 45 minutes, and it takes 45 additional minutes

to overcome the effects of 270 dimers per chromosome; this corresponds to an average delay of about ten seconds per dimer. On the basis of *in vitro* studies with calf polymerase, Bollum & Setlow (1963) have suggested that dimers in DNA might be bypassed by the insertion of two or more non-complementary bases into the daughter strand. An alternative possibility is that the polymerase skips at a dimer and leaves a gap in the daughter strand.

The low sedimentation rate in alkali of [^{3}H]DNA synthesized after u.v. irradiation can be explained by the presence of defects in the daughter strands. The present experiments do not allow a precise determination of the relation between the number of defects in the daughter strands (synthesized after u.v.) and the number of dimers in the template strands, but the best estimate is close to one defect per dimer.

Our experiments do not show whether the defects present in new daughter strands are formed during replication or are introduced later by an enzyme that recognizes abnormal linkages in DNA opposite the modified bases. The increase in sedimentation rate during subsequent incubation indicates that the defects in the daughter strands are converted to alkali-stable regions.

The relation of these observations to the ability of a cell to survive with unexcised dimers in its DNA is uncertain. However, the mechanism that replaces the daughter-strand defects by intact DNA must be important to the cell, since about 50 dimers per chromosome are required to kill mutants unable to excise dimers. Several possible structures resulting from the replication of DNA with unexcised dimers are illustrated in Fig. 9; (a) represents a region of double-stranded DNA that contains four dimers; (b) would result if, during conventional replication, a dimer in the parental strand were skipped leaving a gap at that position in the daughter strand. If a dimer in the parental

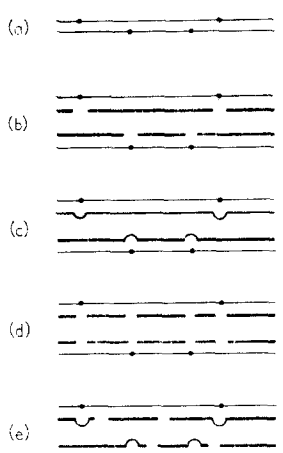

FIG. 9. Schematic model of possible structures resulting from the replication of DNA that contains unexcised dimers.

(a) Parental DNA with dimers.

(b) Gap in daughter strand at position of dimer in complementary parental strand.

(c) Daughter strands contain non-complementary material opposite dimers in parental strand.

(d) Dimer in parental strand results in gaps on both daughter strands at that point.

(e) Daughter strands contain non-complementary material opposite dimers in parental strand and random gaps.

strand caused the insertion of random bases (Bollum & Setlow, 1963), or other non-complementary material into the daughter strand, structure (c) would result. Alternatively, skipping a dimer could result in structure (d) with simultaneous gaps on both daughter strands. Structures (b), (d) and also (e) would be consistent with the low sedimentation rate of the DNA synthesized after u.v. irradiation. If (c) resulted from the insertion of random bases, the daughter strand should remain intact in alkali and sediment normally. However, if the loops in (c) contained alkali-labile bonds, it would also have a reduced rate of sedimentation. Both (c) and (d) could be converted to (b) by repair enzymes, and enzymes resembling those excising dimers from DNA but having a different specificity could remove the loops and convert (c) to (b). In (d), half of the daughter-strand gaps occur opposite normal parental DNA, and thus resemble excision gaps that can be filled in by repair replication (Pettijohn & Hanawalt, 1964).

As excision-defective cells surviving u.v. irradiation usually produce normal rather than mutant daughter cells, it is unlikely that the gaps in the daughter strands are filled by the insertion of bases at random. One possibility is that genetic exchanges occur between the sister duplexes. If only one of the duplexes in structure (b) is considered, the information contained in the region of the dimer would be permanently lost. Filling in the gaps with random bases or other non-complementary material could lead to a chemically more stable molecule, but it might be highly mutagenic and produce a non-viable daughter cell.

If both duplexes in (b) are examined, it is apparent that the region containing a dimer in one duplex is intact in the sister duplex. If a structure similar to (b) is present after u.v. irradiation, the ability of a cell to recover potentially lost information from a sister duplex would seem to be extremely advantageous. Since two DNA duplexes would be involved in such a process, some of the enzymes promoting genetic recombination might be required.

Unfortunately, meaningful experiments of the type described in this paper have not been carried out on double mutants defective in both recombination and excision, because the strains investigated to date incorporate very little exogenous thymidine into their DNA after irradiation.

It is suggested that a duplex in which the daughter strand was completed in this way would contain a complete copy of genetic information in at least one strand (probably the daughter strand), but not necessarily in both strands. This mechanism for post-replication correction would not necessarily be limited to overcoming effects of unexcised pyrimidine dimers, but might also be effective for damaged bases in DNA resulting from a range of other treatments. This correction could occur efficiently only if the gaps are opposite the damaged bases.

A post-replication mechanism for the reconstruction of the correct base sequence could complement the excision mechanism in wild-type cells. While the cut and patch process would be effective before replication, the post-replication genetic repair mechanism would act on abnormalities in the daughter strands. Thus, if the replication apparatus passes an unexcised dimer, defective or missing information in the daughter strand might subsequently be replaced with genetic information derived from the sister duplex.

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APPENDIX

Theoretical Sedimentation Pattern of DNA with Random Breaks

The distribution of distances between randomly spaced defects in a long uniform rod can be calculated from h, the mean number of defects per unit length. The probability $\mathrm{d}P$ of the length between two defects being between l and $l + \mathrm{d}l$ is the product of the chance of no defect in l and of there being a defect in $\mathrm{d}l$. The former is obtained from the Poisson distribution for zero events and is e^{-hl}, while the probability of a defect in $\mathrm{d}l$ is $h\mathrm{d}l$. Thus,

$$\mathrm{d}P = h\mathrm{e}^{-hl}\mathrm{d}l.$$

The contribution to $\mathrm{d}W$, the weight of material between defects, is proportional to the product of $\mathrm{d}P$, the length l, and ρ, the mass per unit length. Thus,

$$\mathrm{d}W = \rho l\mathrm{d}P = \rho lh\mathrm{e}^{-hl}\mathrm{d}l. \quad (1)$$

This can be used as an approximation for the distribution of material between u.v.-induced pyrimidine dimers in a long single-strand molecule of DNA, such as in the bacterial chromosome. It may also be used to calculate the hypothetical distribution which will be obtained if the lengths of the newly synthesized single-strand fragments are limited to the distances between the dimers, provided the

gaps at the dimers are short and the average fragment contains a large number of nucleotides. The length of DNA single strands and the distance D sedimented in an alkaline sucrose gradient are connected by the relation of Abelson & Thomas (1966):

$$\left(\frac{D}{D_1}\right)^n = \frac{l}{l_1} \tag{2}$$

where $1/n$ was found to be 0·38 and the subscript $_1$ denotes the values for reference material such as phage DNA. Differentiating

$$\frac{n D^{n-1}}{D_1^n} \mathrm{d}D = \frac{\mathrm{d}l}{l_1} \tag{3}$$

and from equations (1), (2) and (3)

$$\frac{\mathrm{d}W}{\mathrm{d}D} = \frac{\rho n h l_1^2}{D_1}\left(\frac{D}{D_1}\right)^{2n-1} \mathrm{e}^{-l_1 h (D/D_1)^n}. \tag{4}$$

Equation (4) gives the distribution of the amount of material as a function of the distance sedimented. This was calculated for *E. coli* exposed to 60 ergs/mm^2 of u.v. light at 2537 Å and is plotted in Fig. 7. To facilitate comparison between the theoretical curve and the experimental results, an arbitrary constant was introduced to equalize the height of the calculated distribution to that of the experimental curve. The following parameters were used for the yield of pyrimidine dimers produced by this dose, and for the molecular weight and distance sedimented of reference DNA from λ bacteriophage:

$h = 360$ dimers per 10^7 nucleotides,

$l_1 = 5{\cdot}1 \times 10^4$ nucleotides.

$D_1 = 19$ fractions from the meniscus.

As the average number of nucleotides per fragment is large, the approximations used in the derivation of the distribution function are not important.

By differentiating equation (4), it is found that the maximum value of the distribution function occurs when

$$l = \frac{2n-1}{nh} = \frac{1{\cdot}62}{h}.$$

Since h is defined as the mean number of defects per unit length, $1/h$ is the average length between defects. Thus, if the DNA being sedimented in alkaline sucrose gradients has a large number of random single-strand breaks, the peak value on the sedimentation pattern is expected at a molecular weight that is 1·62 times greater than the average molecular weight of DNA between breaks.