INHIBITION OF DNA REPLICATION BY ULTRAVIOLET LIGHT

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ABSTRACT DNA replication in ultraviolet-irradiated HeLa cells was studied by two different techniques: measurements of the kinetics of semiconservative DNA synthesis, and DNA fiber autoradiography. In examining the kinetics of semiconservative DNA synthesis, density label was used to avoid measuring the incorporation due to repair replication. The extent of inhibition varied with time. After doses of less than 10 J/m^2 the rate was initially depressed but later showed some recovery. After higher doses, a constant, low rate of synthesis was seen for at least the initial 6 h. An analysis of these data indicated that the inhibition of DNA synthesis could be explained by replication forks halting at pyrimidine dimers. DNA fiber autoradiography was used to further characterize replication after ultraviolet irradiation. The average length of labeled segments in irradiated cells increased in the time immediately after irradiation, and then leveled off. This is the predicted pattern if DNA synthesis in each replicon continued at its previous rate until a lesion is reached, and then halted. The frequency of lesions that block synthesis is approximately the same as the frequency of pyrimidine dimers.

INTRODUCTION

Ultraviolet irradiation (UV) is known to inhibit DNA synthesis in mammalian cells, although the mechanism(s) of this inhibition is not known. This paper presents the results of two approaches to this problem: a kinetic analysis of semiconservative DNA synthesis and an examination by DNA fiber autoradiography of the lengths and arrangement of the DNA segments synthesized.

An early attempt at kinetic analysis (Cleaver, 1967) concluded that replication halted as discrete blocks were reached, but that blocks were only about 1/10 as frequent as pyrimidine dimers (i.e. dimers were probably not the blocks). Cleaver's study, however, was presented prior to the demonstration that in mammalian cells many replicons along each DNA fiber are active at any one time (Huberman and Riggs, 1968). Klímek and Vlašinová (1966) noted that DNA synthesis was not completely blocked after UV, and suggested that continued synthesis was partly due to the presence of multiple replication units. In experiments conducted to measure the time-course of DNA repair replication in HeLa cells (Edenberg and Hanawalt, 1973) we noticed that after UV, repair replication made a significant contribution to the total thymidine incorporation. For this reason, I have re-examined the kinetics of the inhibition of semiconservative DNA synthesis in UV irradiated HeLa cells, using

density labeling to distinguish semiconservative synthesis from incorporation due to repair. An analysis of these results in terms of our present knowledge of the many replicons active in mammalian cells (for review see Edenberg and Huberman, 1975) shows that the inhibition could be explained by replication forks halting (or pausing) at pyrimidine dimers (Edenberg, 1975).

This analysis could not itself rule out the alternate possibilities that the rate of replication fork movement was uniformly slowed, or that the initiation of new replicons was inhibited. To directly approach these problems, I have studied post-UV DNA synthesis by a different technique—DNA fiber autoradiography. The results presented here demonstrate that the inhibition of DNA replication is due to a halt (or long pause) when replication forks reach discrete lesions in the template DNA, and that these lesions are present in approximately the frequency of pyrimidine dimers.

METHODS

Kinetic Studies

The details of cell growth, labeling, irradiation, gradient preparation and collection have all been described (Edenberg and Hanawalt, 1973). In calculating the extent of synthesis after UV, each experiment (i.e. each set of cultures from one bottle of prelabeled cells) was treated individually. The tritium incorporation into the hybrid and intermediate density regions of each gradient, representing semiconservative DNA replication in the presence of ^{3}H-dThd+BrdUrd (Edenberg, 1973), was normalized to the amount of ^{32}P-prelabeled DNA in the gradient. This corrected value for incorporation due to semiconservative DNA synthesis was then compared to the incorporation (measured identically) in the unirradiated control culture of that experiment.

Rate of DNA Synthesis in BrdUrd. Cells were prelabeled with [^{32}P]phosphate, divided into petri dishes and allowed to attach. FdUrd (10^{-6} M) and several different mixtures of BrdUrd and dThd (total concentration 10^{-5} M; 0, 20, 50, 80, and 100% BrdUrd) were added. After 1 hr, ^{3}H-dThd (1 μCi/ml, 8 Ci/mmol) was added to each culture The incubation was terminated after 4 h, the DNA extracted, centrifuged in neutral CsCl, and collected; all as described previously (Edenberg and Hanawalt, 1973).

DNA Fiber Autoradiography

Equal aliquots of exponentially growing HeLa cells (in Joklik-modified Minimum Essential Medium + 10% horse serum) were plated onto 35 mm petri dishes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) and incubated overnight at 37°C in an atmosphere of 95% air + 5% CO_2. The medium was removed immediately before irradiation and the cells rinsed with 37° sterile buffer (0.14 M NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 25 mM Tris, pH 7.4). The cell layers, without covering liquid, were irradiated with light from a germicidal lamp at 0.5 J/m^{2}s (monitored with an ultraviolet meter from UV Products, Inc., San Gabriel, Calif.). Immediately after irradiation, 1.0 ml of fresh 37° medium containing 100 μCi/ml of ^{3}H-dThd (65 Ci/mmol) was added to each dish, and the dishes returned to the incubator. Control cultures were mock-irradiated in the same way. After an appropriate time, the cell layer was rinsed three times with ice-cold buffer containing 10 mM unlabeled dThd, and the cells were resuspended in 1 ml of this buffer by scraping with a rubber policeman. Care was taken to leave a ring approximately 4 mm wide around the edge of the dishes unscraped, so that any cells that might have been shielded from the UV by the rim of the petri dish would not be resuspended.

Preparation of Autoradiograms. The cell suspensions (on ice) were diluted to approximately 10^4 cells/ml, and 20 μl of cell suspension placed on each glass slide (previously "subbed" with gelatin). 20 μl of a solution of 2% SDS in 10 mM EDTA (adjusted to pH 8.1 by addition of Trizma base [Sigma Chemical Co., St. Louis, Mo.]) was added and the drops allowed to mix for about 10 min before being drawn across the slide in the manner of a blood smear. Slides were allowed to dry, rinsed twice with 5% trichloracetic acid, once with 1 N HCl, and once with 95% ethanol, and again dried. Kodak NTB-3 nuclear emulsion was applied to the slides at 44°C, the slides dried overnight and stored at 4°C in light tight plastic boxes containing dessicant.

Development and Examination. Slides were developed after 4 mo, using Kodak D-19 developer for 3 min (20°C), water rinse (0.5 min), Kodak fixer (7 min), and a running water rinse (1 h). The long exposure time was chosen to insure that labeled segments were very dark so the ends could be clearly discerned. Only internal segments in clearly separated tandem arrays were scored to avoid measuring segments that might have been broken at the ends of the fiber. The slides were examined under brightfield illumination in a Nikon microscope with a 40x objective lens and an ocular micrometer mounted in a 10x ocular; one unit of the ocular micrometer represents 2.5 μm.

RESULTS

Kinetic Studies

An initial experiment was performed to determine whether growth in 5-bromodeoxyuridine (instead of thymidine) slowed the rate of DNA synthesis, since such an effect might alter the inhibition seen after UV irradiation. Cultures incubated in various mixtures of bromodeoxyuridine and thymidine all showed approximately the same rate of DNA synthesis (data not shown).

The use of a density label to distinguish semiconservative DNA synthesis from repair replication can be important. In cells irradiated with 18.6 J/m^2 and labeled for 1.5 h after irradiation, for example, about one third of the total incorporation is due to repair replication. This effect is even more pronounced at higher doses, where the semiconservative DNA synthesis is further depressed. It is this fact that makes the present study (in which density label is used to distinguish semiconservative DNA synthesis) a more accurate reflection of DNA replication after UV than previous studies.

The rate of DNA replication after ultraviolet irradiation changes with time. Fig. 1 shows the normalized rates of synthesis in each of four 1.5 h intervals after irradiation. It is clear from this figure that for doses below 10 J/m^2 the rate of semiconservative DNA synthesis is depressed in the first 1.5 h after irradiation, but that it increases at later times. Both the degree of inhibition and the delay before recovery are related to the dose. A surprising aspect of these results is that after the two lowest doses studied the rate of semiconservative synthesis (although at first depressed) later exceeded that in the control. At doses above 10 J/m^2 the rate of synthesis was much lower than the control and was constant for the first 6 h after irradiation. An experiment carried out to 20 h after a dose of 10 J/m^2 showed the same general pattern: synthesis was initially depressed to about 20% of the control rate, and later recovered somewhat. Between 9 and 12 h after irradiation the rate was 75% of the control, and between 15 and 21 h the rate was up to 88% of the control.

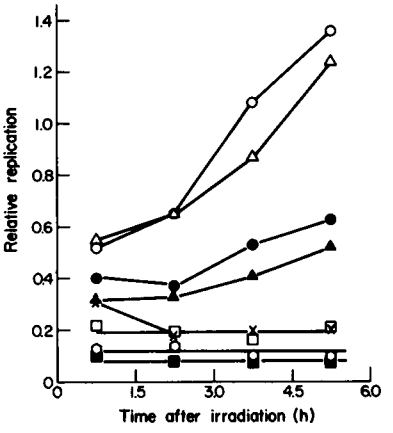

FIGURE 1

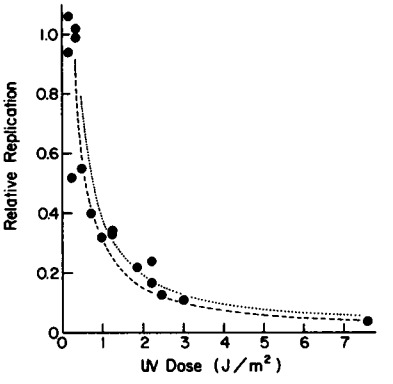

FIGURE 2

FIGURE 1 Rate of semiconservative synthesis at various times after irradiation. Cells were labeled for 1.5 h intervals at different times after irradiation; the DNA was extracted, sedimented to equilibrium in CsCl, and collected, all as described (Edenberg and Hanawalt, 1973). The rate of semiconservative synthesis in each 1.5 h interval, normalized to the rate in the unirradiated culture from each experiment, is shown in the center of that interval. Each set of symbols represents an experiment at a different dose. ○ = 2.4 J/m^2; △ = 4.8 J/m^2; ● = 7.2 J/m^2; ▲ = 9.7 J/m^2; □ = 18.6 J/m^2; ○ = 24.4 J/m^2; ■. 29.9 J/m^2; x = 16.8 J/m^2 (with partially synchronized cells).

FIGURE 2 Relative replication after various doses of UV. The relative rate of replication in the initial 1.5 h interval after irradiation is plotted against the dose. The two curves represent the relative replication predicted from a model in which the replication forks within each replicon continue at their original rate until they encounter the first dimer (in each direction) and then stop. In that case the relative replication equals the ratio of the lengths of the average interval between dimers into which origins fall to the lengths of the average replicon. The curves are for average replicon sizes of 20 μm (upper curve) and 25 μm (lower). The average replicon size which best fits the data (3.3 J/m^2 and above) is 22.3 μm. If dimers are randomly distributed with respect to origins, the average interval into which origins fall can be calculated by Poisson statistics and is equal to twice the average distance between dimers. Although this might seem paradoxical at first, it is explained by the fact that the probability of an origin falling into an interval of length (i) is directly proportional to length (i). The average intervals were calculated from the frequency of thymine dimers in the DNA of cells irradiated in the manner used here (0.045% of thymine as dimers after 10 J/m^2 [Cleaver and Trosko, 1970; Edenberg, 1973] corrected for other pyrimidine dimers according to Setlow et al., 1969).

Fig. 2 shows the relative replication in the first 1.5 h after irradiation as a function of the UV dose. The curves shown represent the predicted values based upon a model in which the inhibition is due to replication forks halting (or pausing for a significant time) when the first pyrimidine dimer is reached within each replicon; they are shown for average replicon sizes of 20 and 25 μm. The assumptions of this model are that bidirectional DNA synthesis within each replicon continues at its original rate until the first dimer on each strand (in each direction) is reached, and then halts; and that there is no inhibition of initiation of replicons. In that case the steady state ratio of DNA synthesis in irradiated cells to that in unirradiated cells (i.e. the relative replication)

should equal the ratio of the lengths synthesized per origin, or:

$$\text{Relative replication} = \frac{\text{length of interval between dimers into which origins fall}}{\text{length of average replicon}}.$$

The inverse relationship between relative replication and dose predicted by this model is obvious in Fig. 2, as is the fact that the theoretical curves (for average replicon sizes of between 20 and 25 μm) fit the data very well. These average replicon sizes are quite reasonable for human cells (other data show ranges of 10–80 μm with means and modes between 20 and 30 μm) (Edenberg and Huberman, 1975).

DNA Fiber Autoradiography

Rate Measurements: Use of a Single Pulse. Autoradiographic measurements of the rate of fork movement are often done using two pulses with tritiated thymidine at different specific activities to distinguish those segments which have been replicating during the entire time of one of the pulses. For this experiment, however, the lengths expected after the higher doses of UV were so short that the presence of label at a second specific activity would have confused the results. For this reason, only one pulse was used.

The use of only one pulse makes it impossible to determine whether a given segment had been synthesised by one or two replication forks, or whether the fork(s) had been operating throughout the pulse. However, an analysis of the expected distribution of segments in an exponentially growing population of cells (H. Edenberg, unpublished; for a similar analysis see the appendix to Blumenthal et al., 1973) reveals that for pulse times below that needed to synthesize the average-sized replicon, the average length of the labeled segments gives an accurate measure of the length of DNA synthesized by a single replication fork.

Unirradiated Cells. The sizes of the labeled segments in autoradiograms of unirradiated HeLa cells increase as the length of the pulse increases, as shown in Fig. 3. The increase in mean segment length is linear (Fig. 5) as expected. In pulses of up to 40 min the histogram shows one strong mode at a size that approximates the mean segment length (Fig. 3, A-D), as expected when the pulse time is shorter than the time required to synthesize an average replicon. The average rate of fork movement in these cells is 0.35 μm/min, comparable to previously reported data for HeLa cells (about 0.5 μm/min; see Edenberg and Huberman, 1975, for review).

UV-Irradiated Cells. The distribution of labeled segment lengths in fiber autoradiograms from cells irradiated with 10 J/m^2 of UV are shown in Fig. 4. There is some increase in the mean length between 20 and 30 min, but not beyond that. The mode of the histogram remains the same, and the distribution remains fairly narrow, even after 90 min of labeling. This is in marked contrast to the unirradiated controls (cf. Fig. 3).

The data on mean segment length vs. time are shown in Fig. 5. The curves for cells irradiated with 10 and 17.5 J/m^2 show a sharp break and level off completely. That for cells irradiated with 5 J/m^2 seems to have leveled off from about 60 to 90 min.

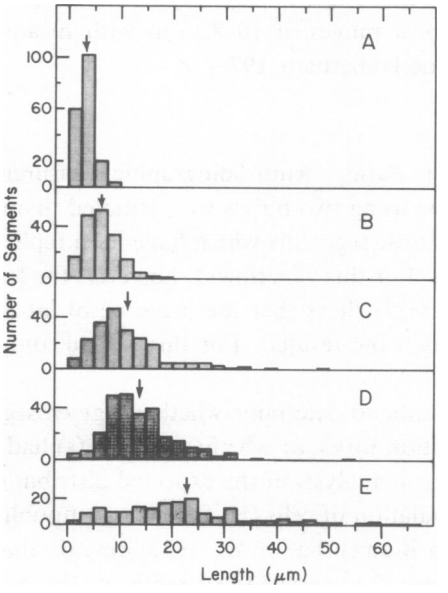

FIGURE 3

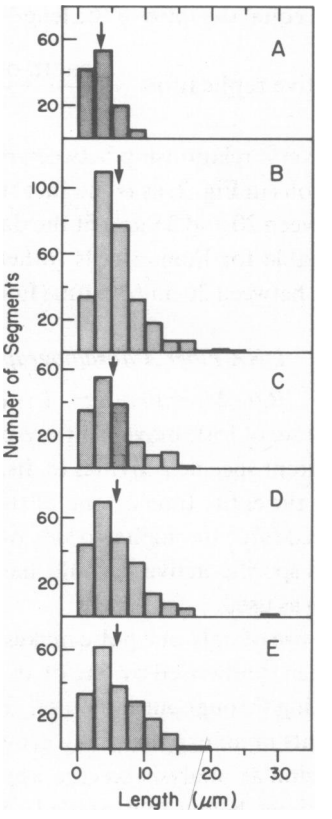

FIGURE 4

FIGURE 3 DNA synthesis in unirradiated cells: segment lengths. The lengths of internal segments in tandem arrays are shown for unirradiated cells labeled with tritiated thymidine for various times, as noted: *A* = 0–10 min; *B* = 0–20 min; *C* = 0–30 min; *D* = 0–40 min; *E* = 0–60 min. Arrows represent the mean segment length.

FIGURE 4 DNA synthesis after ultraviolet irradiation (10 J/m^2): segment lengths. Cells were labeled for the times noted, starting immediately after irradiation: *A* = 0–20 min; *B* = 0–30 min; *C* = 0–40 min; *D* = 0–60 min; *E* = 0–90 min. Arrows represent mean segment length.

The curve for cells irradiated with only 2.5 J/m^2 shows a break after 40 min, but continues to rise at a slower rate.

DISCUSSION

The measurements of semiconservative DNA synthesis in UV irradiated HeLa cells reported here do not include a contribution from repair replication, and are thus more accurate measurements of the inhibition of DNA replication than previous work using total incorporation. In cultures irradiated with less than 10 J/m^2 the rate of DNA synthesis varies markedly with time, as had been reported by others (Cleaver, 1965,

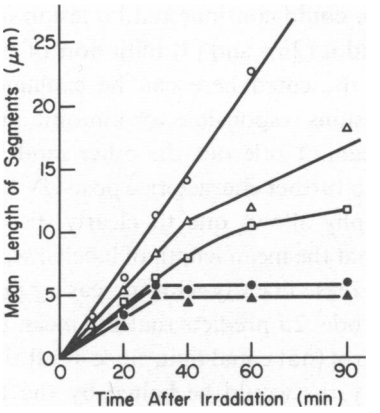

FIGURE 5 Effect of UV on DNA synthesis. The mean length of the segments synthesized during pulses of increasing duration, starting immediately after irradiation (or mock irradiation) is shown for the various cultures. Unirradiated cells (data in Fig. 3) = ○; cells irradiated with 2.5 J/m^2 = △; 5.0 J/m^2 = □; 10 J/m^2 (data in Fig. 4) = ●; 17.5 J/m^2 = ▲.

1966*a*,*b*, 1967; Klímek and Vlašínová, 1966; Klímek and Vaníček, 1970; Domon and Rauth, 1968). For doses over 10 J/m^2, a low but constant rate of DNA synthesis was seen for at least the first 6 h; the choice of 1.5 h labeling periods would tend to obscure transient changes in the interval immediately after irradiation.

The recovery in synthetic rate observed after low doses of UV may have several causes. Repair of UV-induced lesions might have two effects: replicons that initiate after some repair has occurred would synthesize longer before encountering a lesion, and replication forks held up at a lesion might be able to resume synthesis. In addition, Domon and Rauth (1968) have shown some accumulation of cells in S phase after UV. (It should be noted that total synthesis in the first 6 h after UV did not exceed that in unirradiated cells.)

These present data are in qualitative agreement with several earlier measurements of total thymidine incorporation in mouse L cells (Cleaver, 1965; Klímek and Vlašínová, 1966; Klímek and Vaníček, 1970; Domon and Rauth, 1968) which showed that the rate of thymidine incorporation in those cells dropped within the first few hours after UV irradiation, and that some recovery occurred after low doses. Klímek and Vlašínová (1966) interpreted the results of their study as being due to multiple replication units each proceeding up to a dimer. Klímek and Vaníček (1970; Vaníček and Klímek, 1971) analyzed this situation, assuming unidirectional replication and a constant rate of chain elongation during S phase (recent evidence argues against both assumptions [Edenberg and Huberman, 1975]). Their general approach, however, is similar to that taken here and the simplification they propose as a method of estimating replicon size (Vaníček and Klímek, 1971) is analogous to the equation used here.

There are three general models for the inhibition of DNA replication by ultraviolet light: (1) the rate of polymerization (replication fork movement) could be slowed;

(2) synthesis in each replicon could continue until a lesion is reached, and halt (2*a*) or pause, then reinitiate beyond it (2*b*); and (3) initiation of new replicons could be inhibited. The kinetic data presented here can be explained by model 2, in which pyrimidine dimers are the lesions responsible for inhibition (Fig. 2; see also Edenberg, 1975). Those data alone cannot rule out the other models. For that reason, fiber autoradiography was used to further characterize post-UV DNA synthesis.

DNA fiber autoradiography allows one to clearly discriminate among the three models. Model 1 predicts that the mean length of labeled segments in cells exposed at a given dose of UV should increase linearly with increasing pulse time, but at a rate less than that of the control. Model 2*a* predicts that the mean length should increase at a rate close to that of the control (not equal to it, since at all times after irradiation some fraction of the replication forks would be halted by the lesions) until the segment length approximates the average distance between lesions, and then should level off. Model 2*b* would predict the same behavior as 2*a* if the pause before reinitiation is long compared to the duration of the experiment, since then reinitiations during the period of labeling would not be significant. However, if the pause were short, and several lesions could be skipped past during the labeling, a continued steady increase in length would be predicted since a gap of about 800–1,000 nucleotides (as suggested by Lehmann, 1972, 1975, and Buhl et al., 1972*a*) would not be detected in these experiments and the adjacent segments would be scored as one. (Beta particles emitted by the tritiated thymidine decay can travel up to about 1 μm, so that labeled nucleotides in segments on either side of a putative 0.3 μm gap would expose the emulsion over the gap, making such very close adjacent segments appear as one.) Model 3 predicts no change detectable by fiber autoradiography, since only those replicons that do synthesize DNA during the pulse can be examined.

As shown in Fig. 5, the mean segment lengths in UV-irradiated cells increase with time in the initial intervals and then level off. The leveling is most dramatic after 10 and 17.5 J/m^2, but also seen after 5 J/m^2 (and even partially after only 2.5 J/m^2). This is the pattern expected if the replication forks hit discrete blocks and most do not reinitiate during the 90 min experiment (Model 2*a*).

These results clearly rule out the possibility that the inhibition is due to a general slowdown in the rate of fork progression (Model 1). The decreased slope of the curves seen before the leveling might appear to argue that a reduced rate of fork progression is partly responsible for the inhibition, but this decreased slope can be accounted for by the expectation that at *all* times after irradiation some fraction of the forks will reach the blocks and stop. Model 2 thus predicts a decreased average length of DNA synthesized at all times, with the decrease more marked in the more heavily irradiated cultures, as has been found (Fig. 5). The results also rule out the possibility that the inhibition of DNA synthesis in HeLa cells is to any large extent due to an inhibition in replicon initiations (Model 3) since if inhibition of replicon initiations were the major factor, those replicons that did initiate synthesis (the only ones detectable by this technique) would be expected to behave as the control. In addition, the decrease in

average labeled length seen in Fig. 3 is sufficient to account for the decreased incorporation. A small effect on initiations cannot, however, be completey ruled out.

The data presented here demonstrate that the inhibition of DNA replication in UV irradiated HeLa cells is due primarily, if not entirely, to the replication forks stopping when they encounter lesions in the DNA (Model 2). Moreover, the fact that the average labeled length remains level for the last 60 min in cells irradiated with 10 and 17.5 J/m^2 (Fig. 5) strongly suggests that reinitiations beyond the lesion do not occur to a significant extent during this period. This argues that if reinitiations beyond the lesion occur (Model 2*b*), the delay before reinitiation occurs is long.

It should be noted that the leveling in the mean length of labeled segments does not imply that DNA synthesis has stopped in these cells, since new replicons are continually initiating during S phase and synthesis in each newly initiated replicon until a block is reached would result in the decreased but steady rate of incorporation seen here, while the mean length of the segments into which they are incorporated would not increase (i.e. with time there would be an increase in the number of segments labeled after irradiation but not in their average length).

These fiber-autoradiographic data, while showing that the inhibition of DNA replication is due to discrete lesions blocking replication forks, do not directly reveal the nature of the lesions. As shown in Fig. 2, the kinetics of semiconservative DNA synthesis after UV are consistent with pyrimidine dimers action as the lesions. Similarly, an analysis of the average length at which the segments seen in the autoradiograms level off (shown in Table I) is consistent with dimers acting as the lesions that block replication forks. The data in Table I fall between the values predicted if a dimer on one strand blocked synthesis only of the daughter strand copied from it ("independent strands") and those predicted if a dimer on either strand blocked synthesis along both

TABLE I

COMPARISON OF THE LENGTH AT WHICH SYNTHESIS LEVELS OFF WITH THAT PREDICTED IF PYRIMIDINE DIMERS WERE LESIONS RESPONSIBLE FOR STOPPING DNA SYNTHESIS

The length of the average interval between dimers was calculated as described in the legend to Fig. 2. The data in parentheses from 2.5 J/m^2 cannot be used for this comparison, since the predicted length is larger than the replicon size (and thus the model does not apply) and since it is not clear whether the length has indeed leveled off.

UV dose	Predicted length		Observed length
	Independent strands	Either strand	
J/m^2	μm	μm	μm
2.5	(30.2)	15.1	(18.4)
5.0	15.1	7.6	11.8
10.0	7.6	3.8	5.8
17.5	4.3	2.2	4.8

("either strand"). The data thus demonstrate that pyrimidine dimers are present in approximately the frequency of the lesions which block replication, but do not allow a judgment as to whether a dimer in one strand can block synthesis in the opposite (as suggested by Buhl and Regan, 1974). Uncertainties in the measurement of dimers, the extent to which they are repaired, the replicon sizes, and the experimental measurements all make a more precise determination very difficult.

The model (2) presented here, in which the inhibition of DNA replication after UV is due to replication forks halting when they encounter pyrimidine dimers in the DNA, is consistent with other reported data. In a study of mammalian mitochondria, where there is no detectable dimer removal, Clayton et al. (1974) have suggested that replication of mitochondrial DNA ceases when a dimer on the parental strand is reached. Several groups (Lehmann, 1972; Lehmann and Kirk-Bell, 1972; Buhl et al., 1972*a*,*b*, 1973, 1974; Buhl and Regan, 1974; Lehmann et al., 1975; Fujiwara, 1975) but not all (cf. Chiu and Rauth, 1972; Rauth et al., 1974) have reported that the DNA strands synthesized after UV irradiation are of lengths approximating the distance between dimers in the parental DNA (although problems in obtaining reliable estimates of the number average molecular weight of the segments from these gradients make such correlations very rough). This too is consistent with the idea that DNA synthesis is halted at pyrimidine dimers. Further evidence that dimers are the lesions that reduce the size of the DNA strands synthesized after UV was obtained by Buhl et al. (1974) and Lehmann and Stevens (1975) who showed that in marsupial and chick cells photoreactivation led to an increase in the segment size approximately equal to that predicted from the decrease in dimer content. Lehmann and Stevens (1975) report that the inhibition of thymidine incorporation was also partially reversed by the photoreactivation, although not to the extent expected.

In the present autoradiographic study, there was no evidence for reinitiations of DNA replication beyond the dimers within the first 90 min after irradiation. This suggests caution in assessing the interpretations given to the eventual "chase" of the short strands synthesized in UV irradiated cells to higher molecular weight DNA (cf. Cleaver and Thomas, 1969; Meyn and Humphrey, 1971; Lehmann, 1972, 1975; Lehmann and Kirk-Bell, 1972; Buhl et al., 1972*a*,*b*, 1974; Lehmann et al., 1975). Although such chasing is generally interpreted as being due to the filling of 1,000 nucleotide gaps left when synthesis is reinitiated beyond a dimer, it might represent a continuation of normal semiconservative DNA synthesis after a delay during which the dimers are repaired or altered to a form that the replication forks can bypass (cf. Klímek and Vlašínová, 1966; Klímek and Vaníček, 1970). This delay might be as long as 90 min, since there is no indication that reinitiations are occurring during this period (cf. Fig. 5). This alternate suggestion might explain why the putative gap filling is sensitive to inhibitors of normal semiconservative synthesis (such as hydroxyurea, cf. Lehmann, 1972; Buhl et al., 1972*b*; Fujiwara, 1975).

The finding that at long times after irradiation DNA is synthesized in segments as long as in unirradiated cells (Buhl et al., 1973; Lehmann et al., 1975) despite the continued presence of dimers in the DNA is puzzling, since the same reports and the data

discussed here indicate that at short times after UV dimers block replication. It has also been found that in Chinese hamster cells the dimer content of replicated and unreplicated DNA is the same (Meyn et al., 1974). These two reports suggest that an as yet unknown mechanism allows eventual bypassing of the dimers.

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