EXCISION REPAIR OF ULTRAVIOLET DAMAGE IN MAMMALIAN CELLS

EVIDENCE FOR TWO STEPS IN THE EXCISION OF PYRIMIDINE DIMERS

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ABSTRACT The incidence of pyrimidine dimer formation and the kinetics of DNA repair in African green monkey kidney CV-1 cells after ultraviolet (UV) irradiation were studied by measuring survival, T4 endonuclease V-sensitive sites, the fraction of pyrimidine dimers in acid-insoluble DNA as determined by thin layer chromatography (TLC), and repair replication. CV-1 cells exhibit a survival curve with extrapolation number $n = 7.8$ and $D_0 = 2.5$ J/m^2. Pyrimidine dimers were lost from acid-insoluble DNA more slowly than endonuclease-sensitive sites were lost from or new bases were incorporated into high molecular weight DNA during the course of repair. Growth of CV-1 cultures in [^{3}H]thymidine or X-irradiation (2 or 10 krads) 24 h before UV irradiation had no effect on repair replication induced by 25 J/m^2 of UV. These results suggest that pyrimidine dimer excision measurements by TLC are probably unaffected by radiation from high levels of incorporated radionuclides. The endonuclease-sensitive site and TLC measurements can be reconciled by the assumption that pyrimidine dimers are excised from high molecular weight DNA in acid-insoluble oligonucleotides that are slowly degraded to acid-soluble fragments.

INTRODUCTION

Excision repair after ultraviolet (UV) light exposure is the best-characterized DNA repair process in cultured mammalian cells (Cleaver, 1974; Painter, 1974; Friedberg et al., 1977). There is a discrepancy in the literature concerning the kinetics of excision repair measured by unscheduled DNA synthesis (Rasmussen and Painter, 1966; Painter and Cleaver, 1969), repair replication (Rasmussen and Painter, 1964; Cleaver and Painter, 1968; Painter and Cleaver, 1969), removal of sites sensitive to prokaryote endonucleases specific for pyrimidine dimers (Paterson et al., 1973; Wilkins and Hart, 1974), and the loss of pyrimidine dimers from acid-insoluble DNA (Horikawa et al., 1968; Setlow et al., 1969; Cleaver, 1974). In general, the removal of pyrimidine dimers measured by acid precipitation of oligonucleotides longer than about 17 residues (Cleaver and Boyer, 1972) and chromatography appears slower than steps measured by

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other methods (Ehmann et al., 1978) and lacks a rapid early response characteristic of repair measured by other methods (Painter and Cleaver, 1969; Paterson et al., 1973).

Excision repair kinetics in previous reports are difficult to compare in detail because of the varieties of cell lines, UV doses, labeling conditions, and experimental procedures employed. We therefore measured excision of UV-induced photoproducts by enzymatic (Friedberg and King, 1971) and chromatographic (Cook and Friedberg, 1976) procedures and by the insertion of new bases as detected in isopycnic gradients (Cleaver, 1975) in the same cell line, African green monkey kidney CV-1 cells. Since this cell line is often used as the host cell for SV-40 and herpes virus studies (Lai and Nathans, 1974; Abrahams and Van der Eb, 1976; Coppey, 1977), our results provide biochemical information with which to interpret host cell reactivation (Abrahams and Van der Eb, 1976; Coppey and Nocentini, 1976) and viral mutagenesis experiments (Cleaver and Weil, 1975; Cleaver 1977*b*).

METHODS

Materials

African green monkey kidney CV-1 cells were obtained from Dr. R. Dulbecco (Imperial Cancer Research Fund, London) and from Dr. Joanne Leong (Department of Biochemistry, University of California, San Francisco, Calif.). No morphological, growth, or radiobiological differences were observed between these two sublines. CV-1 cells were grown in modified Eagle's medium (MEM) (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 15% (vol/vol) fetal calf serum, 2 mM glutamine, and penicillin and streptomycin (each 80 U/ml) (Gibco). T4 endonuclease V, fraction 2 (Friedberg and King, 1971) was a gift of Dr. Errol Friedberg (Department of Pathology, Stanford University, Stanford, Calif.).

Irradiation with UV Light

Cells grown in plastic Petri dishes were drained and 1.0 ml of phosphate-buffered saline (PBS) was added. Cells were irradiated with 254 nm UV light at an incident dose rate of 1.25 $J/m^2/s$, determined with a YSI-Kettering No. 65 radiometer (Yellow Springs Instrument Co., Yellow Springs, Ohio).

Determination of UV Survival Curve

CV-1 cultures set up at low density ($10–10^5$ cells/dish) were incubated overnight, rinsed twice in PBS, and then irradiated under 2.0 ml of PBS. One dish was fixed to determine cell multiplicity at the time of UV-irradiation, and the remainder were incubated in MEM for 10–12 days before the number of colonies with 50 or more cells was scored. The surviving fraction was calculated after correction for multiplicity (Sinclair and Morton, 1965).

T4 Endonuclease V Assay for Endonuclease-Sensitive Sites in CV-1 DNA

CV-1 cells were labeled with [^{3}H]thymidine (1.0 μCi/ml, 11 Ci/mmol) for 24 h. The culture medium was then replaced with unlabeled MEM and cultures incubated overnight to insure that radioactivity was only in high molecular weight DNA. Cultures were washed twice in PBS, irradiated with UV light, and incubated in MEM for various times. The medium was removed and DNA was isolated as described previously (Goth and Cleaver, 1976). The isolated DNA was dialyzed for 24 h against 20 vol of saline citrate and a further 24 h against 20 vol of 0.02 M Tris-HCl, 0.01 M Na_2EDTA, pH 7.8 (T4 buffer). 5–200 μl samples containing

about 20 μg/ml DNA and 4×10^5 cpm/ml were mixed with 75 μl T4 buffer and either 25 μl of a polyethylene glycol isolate of T4 endonuclease V (Friedberg and King, 1971) or 25 μl of T4 buffer and incubated for 1 h at 37°C. The reaction was stopped by layering the samples on 12.4 ml 5–20% alkaline sucrose gradients with 100-μl alkaline lysis layers. Samples were stored on alkaline sucrose gradients for 1 h before the gradients were loaded in a Beckman SW40 rotor and centrifuged in a Beckman L5-75 ultracentrifuge at 39,000 rpm for 3–5 h (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). The centrifugation time was chosen to obtain radioactivity profiles that peaked near the center of the gradient. The number average DNA molecular weight (M_n) was calculated from each radioactivity profile and the number of T4 endonuclease V-induced breaks per 10^8 daltons of DNA calculated from M_n values (Ehmann and Lett, 1973). The number of endonuclease-sensitive sites induced by UV light was calculated with correction for the amount of nonspecific nicking that occurred in unirradiated DNA. The number of breaks introduced into control DNA samples corresponded to about 14 nicks/10^8 daltons of DNA.

DNA Repair Replication

Monkey CV-1 cells were grown in the presence of 10^{-5} M BrdUrd and 2×10^{-6} M FdUrd for 1 h, irradiated with UV light and incubated for an additional 0–19 h. The medium was then replaced with MEM plus 10^{-5} M BrdUrd, 2×10^{-6} M FdUrd, 2×10^{-3} M hydroxyurea, [^{14}C]thymidine (0.1 μCi/ml, 56 mCi/mmol), and [^{3}H]hypoxanthine (2.0 μCi/ml, 0.57 Ci/mmol) and incubation continued for 3 h. The cells were rinsed once in PBS, and the DNA was isolated (Cleaver, 1975) and sheared by five passages through a 25-gauge needle with a 3.0-ml disposable plastic syringe. The sheared DNA was analyzed by alkaline CsCl-Cs_2SO_4 isopycnic centrifugation (Cleaver, 1975). Fractions in the light peak (density = 1.668) were pooled and the absorbance at 260 nm (A_{260}) and the radioactivity in 50-μl aliquots were measured. The specific activities (in counts per minute in 50 μl $\times$ A_{260}^{-1}) were measures of the amount of repair replication using purine and pyrimidine precursors.

Interference between ^{3}H-Decays or X-Rays and UV Light-Induced Repair Replication

To determine the effect of various doses of X-rays or a high level of [^{3}H]thymidine (5.0 μCi/ml, 11 Ci/mmol) before UV irradiation, two protocols were used:

(*a*) CV-1 cultures were given 0, 2, or 10 krads of X-rays (300 kV peak, GE Maxitron with 2 mm Cu nominal filtration, General Electric Co., Medical Systems Div., Milwaukee, Wis.) through the top of Petri dishes containing a 1.2-mm layer of MEM. The cultures were incubated for 24 h before the medium was removed and the cultures irradiated with 0 or 25 J/m^2 UV light. Fresh MEM was added to all cultures and they were incubated for 0, 2, or 4 h before being grown for 0.8–2 h in the presence of 10^{-5} M BrdUrd and 2×10^{-6} M FdUrd, followed by 2×10^{-3} M hydroxyurea, 2×10^{-6} M FdUrd and [^{14}C]BrdUrd (0.1 μCi/ml, 56 mCi/mmol) for 2 h. The cells were then rinsed once in saline citrate, the DNA was isolated, and repair replication was determined by isopycnic gradient centrifugation.

(*b*) Cultures were labeled with [^{3}H]thymidine (5.0 μCi/ml, 11 Ci/mmol) in MEM for 29 h and then grown in unlabeled MEM for an additional 16 h. The medium was then removed and the cultures irradiated with 0–25 J/m^2 of UV light. The amount of repair replication was subsequently determined in the same way as after X-ray and UV irradiation.

Pyrimidine Dimer Removal from CV-1 Cells

CV-1 cells were labeled with [^{3}H]thymidine (0.5 μCi/ml, 11 Ci/mmol) for 24 h before the culture medium on all plates was replaced with unlabeled medium. Incubation was continued overnight to deplete intracellular nucleotide precursor pools of label. The plates were

washed twice in PBS and exposed to UV light under a thin (0.5–0.7 mm) layer of PBS. The PBS was removed and some cultures were analyzed immediately for pyrimidine dimer content by thin layer chromatography (TLC) (Cook and Friedberg, 1976). The remaining cultures were covered with MEM and incubated for 6 or 24 h before being fixed in 5% trichloracetic acid (TCA) at 4°C for 10 min, rinsed once in 5% TCA, and then digested in sealed tubes with 97% formic acid before TLC. A tritium-labeled contaminant peak was consistently identified near the solvent front (R_f = 0.85–0.95) but the amount of this contaminant was not proportional to UV dose and never exceeded 0.1% of the total counts on a chromatogram. Counts in the contaminant peak were therefore routinely included with the monomer peak counts for pyrimidine dimer content calculations.

RESULTS

Survival Curve

Cells of the established monkey cell line CV-1 have a survival curve similar to most normal mammalian cells (Cleaver, 1974) with a D_0 of 2.5 J/m^2 (Fig. 1), but the extrapolation number of 7.8 for CV-1 cells is greater than that usually found in normal mammalian cells (Giese, 1964; Cleaver, 1970; Cleaver, 1972; Cleaver, 1974; Maher and McCormick, 1976). The flattening of the survival curve at high UV doses may be a technical artifact or may be indicative of a resistant subpopulation of CV-1 cells comprising about 5% of all cells.

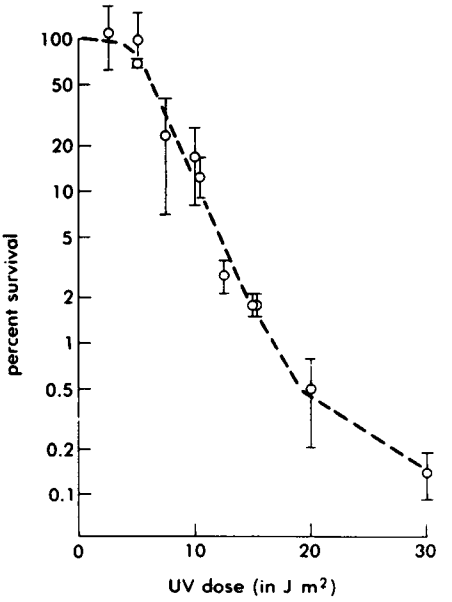


FIGURE 1 CV-1 cell survival curve after treatment with UV light. All data points were corrected for cell multiplicity. Error bars indicate standard errors of the mean among five plates used to determine each point.

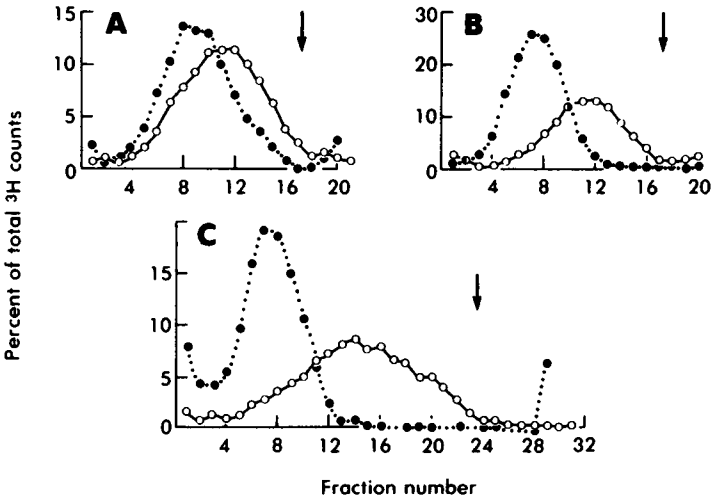


FIGURE 2 Alkaline sucrose isokinetic sedimentation profiles for CV-1 DNA exposed to UV fluences of (A) 0 J/m^2, (B) 12.5 J/m^2, or (C) 37.5 J/m^2 and either treated with T4 endonuclease V for 1 h (●···●···●) or T4 buffer for 1 h (○—○—○) before analysis. Sedimentation is from left to right. Arrows indicate the position of either ^{14}C-labeled SV-40 Form I DNA markers mixed with the CV-1 DNA sample before analysis (A and B) or the sedimentation position of 53S molecules (C) determined by calibration curves of SV-40 Form I DNA molecules sedimenting for various lengths of time. All data are corrected for background counts and ^{14}C spillover with external standards.

TABLE I

NUMBER OF UV-INDUCED SITES IN MAMMALIAN CELL DNA SENSITIVE TO DIMER-SPECIFIC ENDONUCLEASES

Organism	Endonuclease-sensitive sites	Origin of dimer-specific endonuclease (T, T4 coliphage; M, *M. luteus*)	References
	per 10^8 daltons per J/m^2		
Human (WI-38, HeLa)	0.33–0.93	T	Menighini and Hanawalt, 1976
Mitochondrial DNA	0.70–1.14	T	Clayton et al., 1974
SV-40	1.4	T	Williams and Cleaver, 1976
Human (normal primary fibroblasts)	1.0	M	Wilkins, 1973
Chinese hamster ovary	0.74–0.82	M	Clarkson and Hewitt, 1976
Human (normal, xeroderma pigmentosum)	2.5	M	Paterson et al., 1973
Human (WI-38)	0.4–0.7	M	Wilkins and Hart, 1974
Monkey (CV-1)	1.4	T	This report

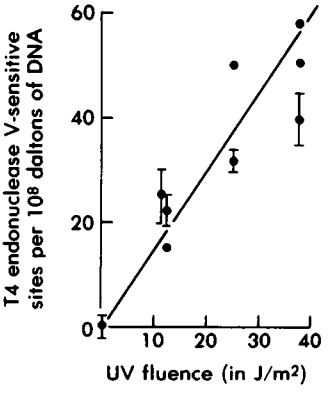


FIGURE 3

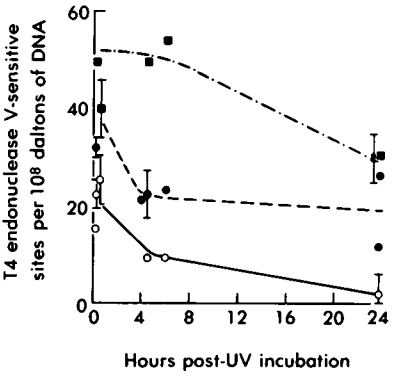


FIGURE 4

FIGURE 3 Endonuclease-sensitive sites in CV-1 DNA as a function of UV fluence. All values are corrected for adventitious nicking. A dose-response line with slope of 1.4 sites per 10^8 daltons per J/m^2 was fitted to the data by eye. Bars indicating standard error of the mean were attached to data points for all samples analyzed more than once.

FIGURE 4 Decrease in number of endonuclease-sensitive sites in CV-1 DNA with post-UV incubation. Curves were fitted by eye to data obtained for cultures exposed to 12.5 J/m^2 (o—o—o), 25 J/m^2 (●--●--●), or 37.5 J/m^2 (■—■—■).

Induction and Loss of T4 Endonuclease V-Sensitive Sites

Irradiation of CV-1 cells with 0–37.5 J/m^2 induced about 1.4 T4 endonuclease V-sensitive sites per 10^8 daltons per J/m^2 (Figs. 2 and 3). This value is similar to published values obtained with both the *Micrococcus luteus* UV endonuclease and T4 endonuclease V (Table I). The number of endonuclease-sensitive sites decreased rapidly in the first 4–6 h after UV irradiation up to 25 J/m^2 of UV light, and slowly thereafter (Fig. 4). These two-component kinetics for endonuclease-sensitive site loss in monkey CV-1 cells resemble those seen in human cells (Paterson et al., 1973). The initial rate of decrease depended on UV dose and was slower at 37.5 J/m^2 than at lower doses. Essentially all sites (approximately 90–95%) are removed in 24 h after 12.5 J/m^2. The total number of endonuclease-sensitive sites removed in 24 h saturated at a fluence of about 25 J/m^2. If the diploid DNA complement of monkey cells is about 6 pg (Abrahamson et al., 1973), about 2,000 endonuclease-sensitive sites/min/cell were excised during the first 6 h after 25 J/m^2.

Induction and Loss of Pyrimidine Dimers in Acid-Insoluble DNA Measured by TLC

The percent of total counts in the pyrimidine dimer region chromatograms increased with increasing UV dose (Fig. 5) and corresponded to a yield of about 1.6 pyrimidine dimers/10^8 daltons/J/m^2 (see Appendix A) or 0.0010% dimers/monomer per J/m^2. The latter value is similar to the published range of 0.0003–0.0014% for other mammalian cells (Swinton and Hanawalt, 1973).

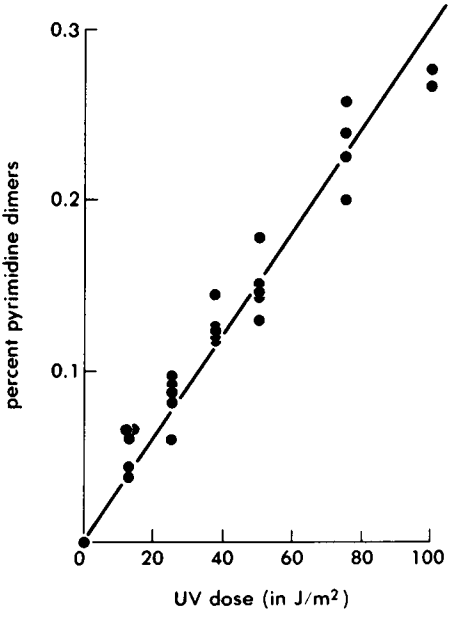


FIGURE 5

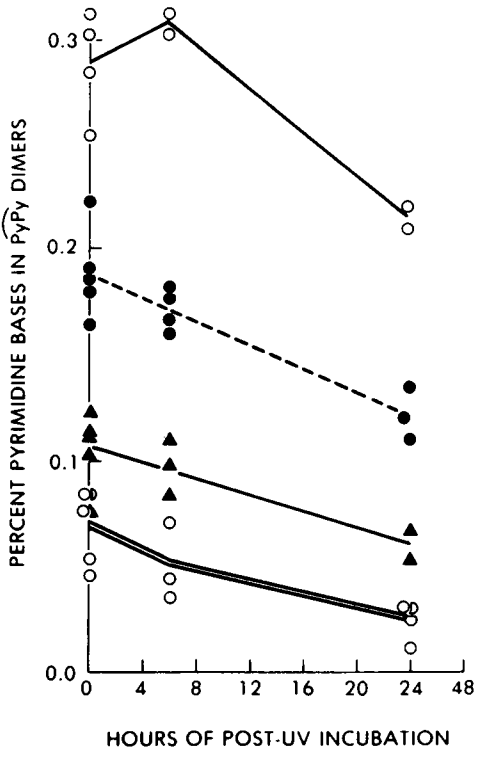


FIGURE 6

FIGURE 5 Percentages of CV-1 DNA tritium counts found in the pyrimidine dimer region of thin layer chromatograms after UV fluences of 0–100 J/m^3. Linear regression analysis was used to fit a smooth line to the data with a slope of 0.0027% of tritium label in dimers per J/m^2. Each point is a single observation and consequently carries no error bar.

FIGURE 6 Removal of pyrimidine dimers from acid-insoluble CV-1 DNA with post-UV incubation. The percent of tritium label in the dimer region of TLC radioactivity profiles is plotted as a function of hours of post-UV incubation at 37°C after UV fluences of 12.5 (o = o), 25 (▲—▲), 50 (●---●), or 75 (o—o) J/m^2.

The fraction of pyrimidine dimers in the acid-insoluble fraction of cultures incubated 0, 6, or 24 h after 0–100 J/m^2 of UV light decreased with increasing times of incubation (Fig. 6; Table II) up to a fluence of 75 J/m^2. The absolute number of dimers removed in 24 h increased with UV dose up to 75 J/m^2 (Table II), suggesting that the capacity of the excision step that removes dimers from damaged DNA does not increase linearly with the amount of damage, but does saturate slowly.

The values for dimer removal at 6 and 24 h of incubation were tested against a null hypothesis by *t*-test (Zar, 1974) to determine whether or not the rate of dimer removal was greater over the first 6 h after UV irradiation than during the subsequent 18 h (Table II, columns G–H). The results show three of the four pairs of dimer removal rates up to 50 J/m^2 are not significantly different ($0.05 \leq P$), and the fourth value is not significant at the 1% level of confidence ($0.01 \leq P \leq 0.05$). The analysis suggests

TABLE II

PYRIMIDINE DIMER CONTENTS OF CV-1 CULTURES INCUBATED 0, 6, OR 24 H AFTER EXPOSURE TO VARIOUS DOSES OF UV LIGHT

A UV dose	B Pyrimidine bases present as dimers at Y hours of incubation after UV light exposure (based on calculations using Appendix A) Y = (0)	C Y = (6)	D (Y = 24)	E Fraction of initial dimers removed in 24 h (1 − D/B)	F Bases removed as dimers in 24 h (B − D)	G Pyrimidine bases present as dimers removed per hour of incubation (F/6) 6-h Incubation	H (F/24) 24-h Incubation
J/m^2					%	%/h	
12.5	0.041 ±0.005	0.030 ±0.007	0.015 ±0.003	0.63	0.026	(1.9 ±0.8) $\times 10^{-3}$	(1.1 ±0.1) $\times 10^{-3}$
25.0	0.063 ±0.005	0.057 ±0.005	0.036 ±0.005	0.43	0.027	(1.0 ±0.6) $\times 10^{-3}$	(1.2 ±0.2) $\times 10^{-3}$
37.5	0.095 ±0.004	0.077 ±0.006	0.065 ±0.008	0.32	0.030	(3.0 ±0.8) $\times 10^{-3}$	(1.3 ±0.2) $\times 10^{-3}$
50.0	0.112 ±0.006	0.102 ±0.003	0.072 ±0.004	0.36	0.041	(1.8 ±0.6) $\times 10^{-3}$	(1.8 ±0.2) $\times 10^{-3}$
75.0	0.172 ±0.008	0.183 ±0.003	0.128 ±0.004	0.26	0.045	—	(1.9 ±0.2) $\times 10^{-3}$
100.0	0.206 ±0.004	0.212 ±0.007	0.212 ±0.002	0	0.0%*	—	—

All errors in columns B–D are standard errors of the mean (Zar, 1974) while errors in columns G and H are standard errors of the difference between the means.

*Failure to observe dimer excision after 100 J/m^2 of UV light is probably due to the rapid cell killing observed at this dose (Fig. 1).

that dimers are removed from TCA-insoluble DNA at an approximately constant rate throughout the 24 h of incubation after UV light exposure.

Repair Replication

The insertion of new bases by repair replication increased with increasing UV dose (Fig. 7) most rapidly at low doses, below 10 J/m^2, for both ^3H and ^{14}C, suggesting that purines (^3H) and pyrimidines (^{14}C) are inserted by similar repair mechanisms. The incorporation of [^{14}C]thymidine into hybrid density DNA was suppressed with increasing UV dose, since the amount of hybrid density DNA is a measure of semiconservative DNA replication.

The rate of repair replication at various times after 25 J/m^2 of UV light (Fig. 8) showed a rapid decrease over the first 7 h followed by a slower rate of decrease up to 19 h after UV irradiation. This type of rate change agrees with that predicted on the basis of previous work (Edenberg and Hanawalt, 1973) and experiments with T4 endonuclease (Fig. 4): there is an initial rapid repair phase when the major part of UV damage is removed followed by a slower repair phase.

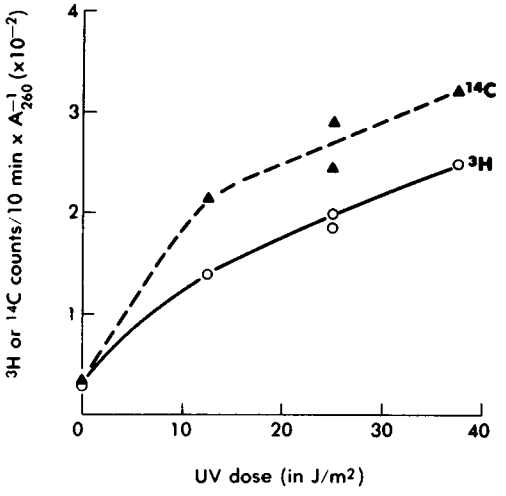


FIGURE 7

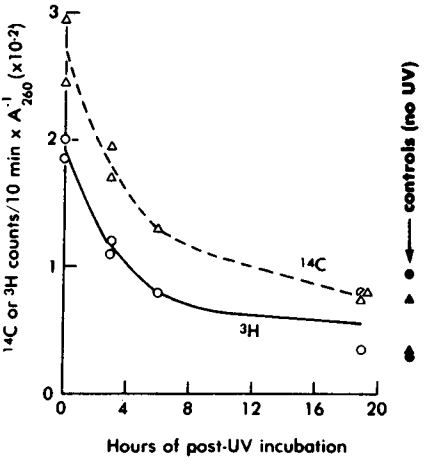


FIGURE 8

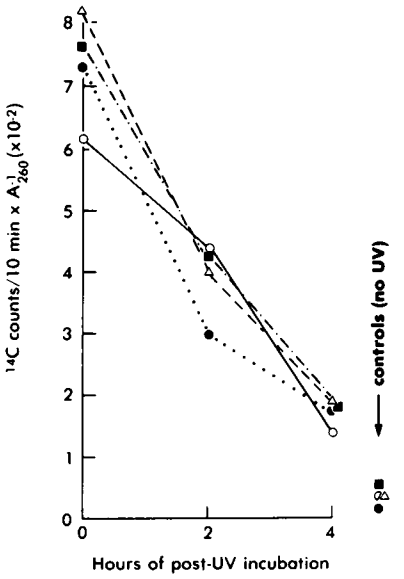
FIGURE 7 Repair replication in CV-1 DNA after exposure to UV light. The specific activity of ^{14}C (▲---▲---▲) or ^{3}H (o—o—o) counts incorporated into light-light DNA after exposure to various UV fluences was determined by counting 50-μl aliquots of pooled fractions of light-light DNA on Whatman 3-cm paper filters (Whatman, Inc., Clifton, N. J.) and dividing the measured radioactivity by the optical density at 260 nm (A_{260}) for the appropriate fractions.

FIGURE 8 The rate of repair replication in CV-1 DNA with various lengths of post-UV incubation time. Specific activities of ^{3}H (o—o—o) or ^{14}C (△---△---△)-labeled light-light CV-1 DNA were plotted for cultures exposed to 0 J/m^2 (closed symbols) or 25 J/m^2 (open symbols) of UV light and incubated for various lengths of time before labeling with [^{14}C]thymidine (0.1 μCi/ml, 56 mCi/mmol) or [^{3}H]hypoxanthine (2.0 μCi/ml, 0.57 Ci/mmol) in the presence of 10^{-5} M BrdUrd for 3 h. Specific activities were computed as described in the legend for Fig. 7.

Repair Replication with [^{3}H] Thymidine or X-Ray Pretreatment of CV-1 Cells

A possible source of artifact in TLC measurements may be that TLC experiments, in contrast to ones measuring endonuclease-sensitive sites and repair replication, require high levels of [^{3}H]thymidine to be incorporated into cellular DNA. To test for the possibility that high doses of ionizing radiation from ^{3}H decays inhibit excision repair, cells were labeled with [^{3}H]thymidine (24 h growth in 5.0 μCi/ml, 11 Ci/mmol) or irradiated with 2 or 10 krads of 300 kV peak X-rays at 24 h before UV irradiation and measurement of repair replication. About 80% of the [^{3}H]thymidine in the medium was incorporated into the cells during this time period (Cleaver, 1977*a*). From the specific activity of the ^{3}H-labeled DNA, it was estimated that the cells were receiving a chronic intracellular X-ray dose of about 1.3–1.8 krads per day (Cleaver et al., 1972*a*).

The rate of UV-induced repair replication decreases during the first 6 h after UV to about 20% of the initial rate (Fig. 9), and there was no significant difference between the control cultures and those exposed to [^{3}H]thymidine or X-rays.



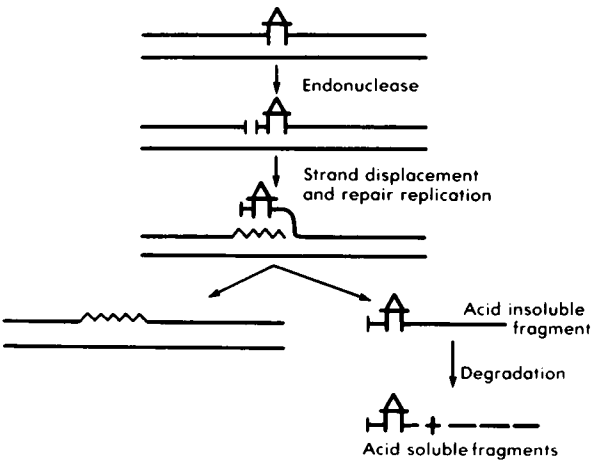


FIGURE 9

FIGURE 10

FIGURE 9 Rates of repair replication at various times after UV irradiation with a fluence of 25 J/m^2 in CV-1 cultures treated 1 day before UV irradiation with nothing (o—o—o), 2 krads X-rays (300 kV peak, 2 mm Cu nominal filtration (●···●···●), 10 krads X-rays (■—■—■), or 24 h incubation in MEM supplemented with [^{3}H]thymidine (5 μCi/ml, 11 Ci/mmol) (Δ---Δ---Δ). Cultures were labeled with MEM plus [^{14}C]BrdUrd (0.1 μCi/ml, 56 mCi/mmol) in the presence of 2 × 10^{-3} M hydroxyurea for 4 h at various times after UV irradiation and ^{14}C specific activities of light-light DNA determined as described in the legend of Fig. 7 (also see Methods).

FIGURE 10 A model for two-stage excision repair in mammalian cell DNA. Pyrimidine dimers (T$\hat{\text{T}}$) are excised in acid-insoluble oligonucleotides after the insertion of new bases (—^^^—) at the damaged site by repair replication. These acid-insoluble oligonucleotides are subsequently degraded to acid-soluble fragments by a slow process.

DISCUSSION

Monkey CV-1 cells efficiently remove and repair UV-induced lesions in their DNA. They lose endonuclease-sensitive sites rapidly after UV fluences of 0–25 J/m^2 (Fig. 4), remove dimers from acid-insoluble DNA for at least 24 h after UV fluences up to 75 J/m^2 (Table II), and do more than 65% of their total repair replication in the first 6 h after exposure to 25 J/m^2 (Fig. 8). The shoulder region of the CV-1 survival curve (Fig. 1) shows CV-1 cells maintain complete viability after induction of up to 2.7 × 10^5 pyrimidine dimers per cell (5 J/m^2 × 1.6 PyPy dimers/10^8 daltons/J/m^2 × 3.6 × 10^{12} daltons/cell). These properties of DNA repair in CV-1 cells are similar to those seen in other mammalian cells (Table I; also Cleaver, 1974), but previous studies have not compared DNA repair kinetics in one cell type by several methods as we have done. The extent of dimer removal in 24 h after UV incubation as detected by TLC is similar to that of endonuclease-sensitive site removal, but the kinetics of dimer release are significantly slower than endonuclease-sensitive site re-

TABLE III

KINETICS AND ABSOLUTE NUMBER OF UV-INDUCED MODIFICATIONS REMOVED IN 24 H AS DETERMINED BY T4 ENDONUCLEASE-SENSITIVE SITES REMOVAL, REPAIR REPLICATION, AND DIMER EXCISION FROM ACID-INSOLUBLE DNA

Type of UV-induced DNA modification	Number removed in 24 h post-UV incubation per 10^8 daltons of DNA after 25 J/m^2 of UV light	Data source	Type of kinetics observed	Data source
Endonuclease-sensitive sites	23	Fig. 4	Biphasic	Fig. 4
Patch sites for repair replication	—	—	Biphasic	Fig. 8
Pyrimidine dimers	21	Table II	Linear	Fig. 6; Table II

moval or repair replication (Table III). Variability in measurements of T4 endonuclease V-sensitive sites (Fig. 3) and pyrimidine dimer content (Figs. 5, 6) necessitates caution in drawing quantitative conclusions, but the similarity in numbers of endonuclease-sensitive sites removed and dimers released into an acid-soluble state (Table III) suggests dimer removal may be complete after 24 h of post-UV incubation.

One explanation of our results is that dimer excision requires two stages, the first detectable by endonuclease-sensitive site removal from high molecular weight DNA and repair replication, and the second by TLC of dimers excised into acid-insoluble fragments. An alternative possibility, that endonuclease-sensitive sites are a class of UV photoproducts other than dimers, is unlikely since T4 endonuclease V has a highly characterized specificity for pyrimidine dimers (Friedberg and Clayton, 1972) and the number of endonuclease-sensitive sites induced at a given fluence did not exceed the pyrimidine dimer content found at the same fluence. It is also possible that the repair capacity of CV-1 cells is damaged by the chronic radiation dose cells receive from incorporated radioactivity during TLC experiments. We eliminated this possibility by showing the rate and extent of repair replication after 25 J/m^2 is not impaired by prior exposure to X-rays or a high level of [^{3}H]thymidine (5.0 μCi/ml) in the medium.

We propose the following model (Fig. 10) for two-stage excision repair, based on the "patch-and-cut" model for *E. coli* (Haynes, 1966): (*a*) A dimer-specific endonuclease cleaves the damaged strand at or near the dimer and a large single-stranded piece of DNA containing the dimer is displaced by insertion of new bases at the damage site. The displaced strand is then cleaved as an acid-insoluble fragment and the high molecular weight DNA is sealed by a DNA ligase. This process occurs rapidly, since the number of single-strand breaks detectable after UV irradiation is much fewer than the number of endonuclease-sensitive sites at any one time (Cleaver et al., 1972b). (*b*) The acid-insoluble oligonucleotide containing the dimer is slowly degraded to acid-soluble fragments undetectable by TLC or is released into the medium surrounding the cells. Oligonucleotides become acid-soluble at sizes less than approximately 17 bases (Cleaver and Boyer, 1972).

Our model and conclusions are similar to those obtained by careful comparison in human cells of dimer excision kinetics and unscheduled DNA synthesis as reported in the preceding paper (Ehmann et al., 1978).

The acid-insoluble oligonucleotides predicted by this model should be found free of high molecular weight DNA either in the cell nucleus or cytoplasm. Trosko and Kasschau (1967) failed to find small acid-insoluble oligonucleotides by column chromatography, but Lucas (1972) identified UV photoproducts in the cytoplasm 6 h after UV irradiation, using immunofluorescent antibodies to pyrimidine dimers and other photoproducts. Ehmann et al. (1978) observed similar kinetics of dimer removal on thin layer chromatograms from both total acid-insoluble DNA and the high molecular weight component (mol wt $> 5 \times 10^6$ daltons) of acid-insoluble DNA, and concluded that dimers are unlikely to be found in small acid-insoluble fragments. However, the resolution of one-dimensional TLC is low and this observation does not eliminate the possibility that dimers at or near sites of base insertion during repair remain for several hours in high molecular weight DNA and subsequently persist in small acid-insoluble oligonucleotides. The chromatographic data of Ehmann et al. (1978) also seem to conflict with the small ratio of DNA single-strand breaks to dimers detected after UV irradiation (Fornace et al., 1976; Cleaver et al., 1972*b*; Hiss and Preston, 1977), since unexcised dimers in high molecular weight DNA should lead to unrejoined single-strand breaks after base insertion has taken place. Poor technical resolution has probably prevented and continues to prevent biochemical detection of the small number of acid-insoluble DNA fragments containing excised dimers.

An indirect benefit of our work is the establishment of the repair competence of the CV-1 cell line, since several recent reports link DNA repair in mammalian cells to reactivation of UV-damaged animal viruses (Rabson et al., 1969; Aaronson and Lytle, 1970; Day, 1974; Lytle et al., 1974; Kaplan et al., 1975*a*, *b*; Wagner et al., 1975; Coppey and Nocentini, 1976). Our results show CV-1 cells could effectively remove pyrimidine dimers from viral DNA. We have investigated this possibility by directly observing the removal of endonuclease-sensitive sites from SV-40 DNA after UV irradiation of SV-40-infected CV-1 cells and made a preliminary report of our findings (Williams and Cleaver, 1976). We have been unable to demonstrate repair replication or pyrimidine dimer removal from SV-40 DNA reproducibly because of the small size of the SV-40 genome (3×10^6 daltons. The larger genome size of herpes virus (10^8 daltons) may make it a better candidate for showing later phases of excision repair of viral DNA in CV-1 cells (Tooze, 1973).

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APPENDIX A

Calculation of Absolute Number of Pyrimidine Dimers from Thin Layer Chromatogram (TLC) Data

The number of pyrimidine dimers per unit mass of cell DNA can be derived from TLC radioactivity profiles if the G-C content of the cell line and the relative yield of TT (thymine-thymine), CT (cytosine-thymine), and CC (cytosine-cytosine) dimers for this G-C content are known. CT dimers are converted to UT dimers by acid hydrolysis and migrate close to TT dimers by the TLC technique we have used (Cook and Friedberg, 1976). The fraction of [^{3}H]thymidine counts in the dimer region of TLC profiles per J/m^2 (P_1) therefore represents both TT and CT dimers and does not detect CC dimers. Setlow and Carrier (1966) have shown that about four times as many TT dimers are formed as CT dimers by moderate fluences of 265 nm UV light in DNA containing 19–25% of all base residues as cytosine (CV-1 cells have a G-C content of 42–44%) (Tooze, 1973). The proportion of [^{3}H]thymidine label from the TLC dimer region that is in TT dimers is then

$$\frac{2\,(4)}{2(4) + 1(1)}\,P_1 = \frac{8}{9}\,P_1,$$

since the average amount of radiolabel in TT dimers is twice that in CT dimers. The fraction of pyrimidine bases per unit mass of DNA per J/m^2 to be found in pyrimidine dimers is then about

$$P_2 = (0.3)\,\frac{(1\ )}{(0.7)}\,\frac{8}{9}\,P_1 = 0.38\,P_1,$$

where 0.7 is the approximate experimental ratio of TT dimers to pyrimidine dimers (Setlow and Carrier, 1966) and the factor (0.3) corrects for the thymine content of DNA. Assuming the average molecular weight of a mononucleotide to be 330 daltons, the absolute number N_{PyPy} of pyrimidine dimers per y daltons of DNA per J/m^2 is

$$N_{PyPy} = (y/330)\,(0.5)\,(P_2 = 0.38\,P_1) = 5.8 \times 10^{-4}\,P_1\,y.$$

If $y = 10^8$ and $P_1 = 2.7 \times 10^{-5}$ (this report), then $N_{PyPy} = 5.8 \times 10^{-4}\,(10^8)\,(2.7 \times 10^{-5}) =$ 1.6 pyrimidine dimers per 10^8 daltons per J/m^2.

REFERENCES

AARONSON, S. A., and C. D. LYTLE. 1970. Decreased host-cell reactivation of irradiated SV-40 virus in xeroderma pigmentosum. *Nature (Lond.).* **228**:359–361.

ABRAHAMS, P. J., and A. J. VAN DER EB. 1976. Host-cell reactivation of ultraviolet-irradiated SV-40 DNA in five complementation groups of xeroderma pigmentosum. *Mutat. Res.* **35**:13–22.

ABRAHAMSON, S., M. A. BENDER, A. D. CONGER, and S. WOLFF. 1973. Uniformity of radiation-induced mutation rates among different species. *Nature (Lond.).* **245**:460–462.

CLARKSON, J. M., and R. R. HEWITT. 1976. Significance of dimers to the size of newly synthesized DNA in UV-irradiated Chinese hamster ovary cells. *Biophys. J.* **16**:1155–1164.

CLAYTON, D. A., J. N. DODA, and E. C. FRIEDBERG. 1974. The absence of a pyrimidine dimer repair mechanism in mammalian mitochondria. *Proc. Natl. Acad. Sci. U.S.A.* **71**:2777–2781.

CLEAVER, J. E., and R. B. PAINTER. 1968. Evidence for repair replication of HeLa cell DNA damaged by ultraviolet light. *Biochim. Biophys. Acta.* **161**:552–554.

CLEAVER, J. E. 1970. DNA repair and radiation sensivity in human (xeroderma pigmentosum) cells. *Int. J. Radiat. Biol.* **18**:557–565.

CLEAVER, J. E. 1972. Excision repair: our current knowledge based on human (xeroderma pigmentosum) and cattle cells. *In* Molecular and Cellular Repair Processes. R. F. Beers, R. M. Herriott, and R. C. Tilghman, editors. *Johns Hopkins Med. J.* **1**:195–211.

CLEAVER, J. E., and H. W. BOYER. 1972. Solubility and dialysis limits of DNA oligonucleotides. *Biochim. Biophys. Acta.* **262**:116–124.

CLEAVER, J. E., G. H. THOMAS, and H. J. BURKI. 1972*a*. Biological damage from intranuclear tritium: DNA strand breaks and their repair. *Science (Wash. D.C.).* **177**:996–998.

CLEAVER, J. E., G. H. THOMAS, J. E. TROSKO, and J. T. LETT. 1972*b*. Excision repair (dimer excision, strand breakage, and repair replication) in primary cultures of eukaryotic (bovine) cells. *Exp. Cell Res.* **74**:67–80.

CLEAVER, J. E. 1974. Repair processes for photochemical damage in mammalian cells. *Adv. Radiat. Biol.* **4**:1–75.

CLEAVER, J. E. 1975. Methods for studying repair of DNA damaged by physical and chemical carcinogenesis. *Methods Cancer Res.* **11**:123–165.

CLEAVER, J. E., and S. WEIL. 1975. UV-induced reversion of a temperature-sensitive late mutant of simian virus 40 to a wild-type phenotype. *J. Virol.* **16**:214–216.

CLEAVER, J. E. 1977*a*. Induction of thioguanine- and ouabain-resistant mutants and single-strand breaks in the DNA of Chinese hamster ovary cells by ^{3}H-thymidine. *Genetics.* **87**:129–138.

CLEAVER, J. E. 1977*b*. Decline in mutation frequency in temperature-sensitive SV-40 viruses before viral DNA synthesis. *Mutat. Res.* **44**:291–298.

COOK, K. H., and E. C. FRIEDBERG. 1976. Measurement of thymine dimers in DNA by thin-layer chromatography. II. The use of one-dimensional systems. *Anal. Biochem.* **73**:411–418.

COPPEY, J., and S. NOCENTINI. 1976. Herpes virus and viral DNA synthesis in ultraviolet light-irradiated cells. *J. Gen. Virol.* **32**:1–15.

COPPEY, J. 1977. Common precursor pathways of herpes DNA and of repair synthesis in ultraviolet irradiated cells. *Nature (Lond.).* **265**:260–262.

DAY, R. S., III. 1974. Cellular reactivation of ultraviolet-irradiated human adenovirus 2 in normal and xeroderma pigmentosum fibroblasts. *Photochem. Photobiol.* **19**:9–13.

EDENBERG, H. J., and P. C. HANAWALT. 1973. The time course of DNA repair replication in ultraviolet irradiated HeLa cells. *Biochim. Biophys. Acta.* **324**:206–217.

EHMANN, U. K., and J. T. LETT. 1973. Review and evaluation of molecular weight calculations from the sedimentation profiles of irradiated DNA. *Radiat. Res.* **54**:152–162.

EHMANN, U. K., K. H. COOK, and E. C. FRIEDBERG. 1978. The kinetics of thymine dimer excision in ultraviolet irradiated human cells. *Biophys. J.* **22**:249–264.

FORNACE, A. J. JR., K. W. KOHN, and H. E. KANN, JR. 1976. DNA single strand breaks during repair of UV damage in human fibroblasts and abnormalities of repair in xeroderma pigmentosum. *Proc. Natl. Acad. Sci. U.S.A.* **73**:39–43.

FRIEDBERG, E. C., and J. J. KING. 1971. Dark repair of ultraviolet-irradiated deoxyribonucleic acid by bacteriophage T4: purification and characterization of a dimer-specific phage-induced endonuclease. *J. Bacteriol.* **106**:500–507.

FRIEDBERG, E. C., and D. A. CLAYTON. 1972. Electron microscope studies on substrate specificity of T4 excision repair endonuclease. *Nature (Lond.).* **237**:99–100.

FRIEDBERG, E. C., J. M. RUDÉ, K. H. COOK, U. K. EHMANN, K. MORTELMANS, J. E. CLEAVER, and H. SLOR. 1977. Excision repair in mammalian cells and the current status of xeroderma pigmentosum. *In* DNA Repair Processes. W. W. Nichols and D. G. Murphy, editors. Symposia Specialists, Miami, Fla. 21–36.

GIESE, A. C. 1964. Studies on ultraviolet radiation action upon animal cells. *Photophysiology.* **2**:203–245.

GOTH, R., and J. E. CLEAVER. 1976. Metabolism of caffeine to nucleic acid precursors in mammalian cells. *Mutat. Res.* **36**:105–114.

GIESE, A. C. 1964. Studies on ultraviolet radiation action upon animal cells. *Photophysiology.* **2**:203–245.

HAYNES, R. H. 1966. General discussion. *Radiat. Res. Suppl.* **6**:231–232.

HISS, E. A., and R. J. PRESTON. 1977. The effect of cytosine arabinoside on the frequency of single-strand breaks in DNA of mammalian cells following irradiation or chemical treatment. *Biochim. Biophys. Acta.* **478**:1–8.

HORIKAWA, M., O. NAKAIDO, and T. SUGIHARA. 1968. Dark reactivation of damage induced by ultraviolet light in mammalian cells *in vitro*. *Nature (Lond.)*. **218**:489–491.

KAPLAN, J. C., S. M. WILBERT, J. J. COLLINS, T. RAKUSANOVA, G. B. ZAMANSKY, and P. H. BLACK. 1975*a*. Isolation of simian virus 40-transformed inbred hamster cell lines heterogeneous for virus induction by chemicals or radiation. *Virology*. **68**:200–214.

KAPLAN, J. C., L. F. KLEINMAN, and P. H. BLACK. 1975*b*. Cell cycle dependence of simian virus 40 induction from transformed hamster cells by ultraviolet irradiation. *Virology*. **68**:215–220.

LAI, C.-J., and D. NATHANS. 1974. Mapping the genes of simian virus 40. *Cold Spring Harbor Symp. Quant. Biol.* **39**:53–60.

LYTLE, C. D., S. G. BENANE, and L. E. BOCKSTAHLER. 1974. Ultraviolet-enhanced reactivation of Herpes virus in human tumor cells. *Photochem. Photobiol.* **20**:91–94.

LUCAS, C. J. 1972. Immunological demonstration of the disappearance of pyrimidine dimers from nuclei of cultured human cells. *Exp. Cell Res.* **74**:480–486.

MAHER, V. M., and J. J. MCCORMICK. 1976. Effect of DNA repair on the cytotoxicity and mutagenicity of UV irradiation and of chemical carcinogens in normal and xeroderma pigmentosum cells. *In* Biology of Radiation Carcinogenesis. J. M. Yuhas, R. W. Tennant, and J. D. Regan, editors. Raven Press, New York. 129–145.

MENIGHINI, R., and P. HANAWALT. 1976. T4 endonuclease V-sensitive sites in DNA from ultraviolet-irradiated human cells. *Biochim. Biophys. Acta*. **425**:428–437.

PAINTER, R. B., and J. E. CLEAVER. 1969. Repair replication, unscheduled DNA synthesis, and the repair of mammalian DNA. *Radiat. Res.* **37**:451–466.

PAINTER, R. B. 1974. DNA damage and repair in eukaryotic cells. *Genetics*. **78**:139–148.

PATERSON, M. C., P. H. M. LOHMAN, and M. L. SLUYTER. 1973. Use of a UV endonuclease from *Micrococcus luteus* to monitor the progress of DNA repair in UV-irradiated human cells. *Mutat. Res.* **19**:245–256.

RABSON, A. S., S. A. TYRELL, and F. Y. LEGELLAIS. 1969. Growth of ultraviolet-damaged herpes virus in xeroderma pigmentosum cells. *Proc. Soc. Exp. Biol. Med.* **132**:802–806.

RASMUSSEN, R. E., and R. B. PAINTER. 1964. Evidence for repair of ultraviolet-damaged deoxyribonucleic acid in cultured mammalian cells. *Nature (Lond.)*. **203**:1360–1362.

RASMUSSEN, R. E., and R. B. PAINTER. 1966. Radiation-stimulated DNA synthesis in cultured mammalian cells. *J. Cell Biol.* **29**:11–19.

SETLOW, R. B., and W. L. CARRIER. 1966. Pyrimidine dimers in ultraviolet-irradiated DNA's. *J. Mol. Biol.* **17**:237–254.

SETLOW, R. B., J. D. REGAN, J. GERMAN, and W. L. CARRIER. 1969. Evidence that xeroderma pigmentosum cells do not perform the first step in the repair of ultraviolet damage to their DNA. *Proc. Natl. Acad. Sci. U.S.A.* **64**:1035–1041.

SINCLAIR, W. K., and R. A. MORTON. 1965. X-ray and ultraviolet sensitivity of synchronized Chinese hamster cells at various stages of the cell cycle. *Biophys. J.* **5**:1–25.

SWINTON, D. C., and P. C. HANAWALT. 1973. The fate of pyrimidine dimers in ultraviolet-irradiated *Chlamydomonas*. *Photochem. Photobiol.* **17**:361–375.

TOOZE, J. 1973. The Molecular Biology of Tumor Viruses. Cold Spring Harbor Laboratory, New York.

TROSKO, J. E., and M. R. KASSCHAU. 1967. Study of pyrimidine dimers in mammalian cells surviving low doses of ultraviolet irradiation. *Photochem. Photobiol.* **6**:215–219.

WAGNER, E. K., M. RICE, and B. SUTHERLAND. 1975. Photoreactivation of herpes simplex virus in human fibroblasts. *Nature (Lond.)*. **254**:627–628.

WILKINS, R. J. 1973. DNA repair: a simple enzymatic assay for human cells. *Int. J. Radiat. Biol.* **24**:609–613.

WILKINS, R. J., and R. W. HART. 1974. Preferential DNA repair in human cells. *Nature (Lond.)*. **247**:35–36.

WILLIAMS, J. I., and J. E. CLEAVER. 1976. Removal of UV damage from simian virus 40 (SV40) DNA. Abstracts of 24th Annual Meeting. *Radiat. Res.* **67**:619. (Abstr.).

ZAR, J. H. 1974. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, N. J.