# AN EFFECT OF ULTRAVIOLET LIGHT ON RNA AND PROTEIN SYNTHESIS IN NONDIVIDING HUMAN DIPLOID FIBROBLASTS

G. J. KANTOR AND D. R. HULL, *Department of Biological Sciences, Wright State University, Dayton, Ohio 45435 U.S.A.*

ABSTRACT Nondividing human diploid fibroblasts maintained in medium containing 0.5% calf serum do not survive when exposed to low doses of UV (254 nm). The extent of killing is dose and strain dependent. DNA excision repair-proficient cells are more resistant than excision repair-deficient cells. Results of measurements of the effect of UV on RNA and protein synthesis in repair-proficient and -deficient (XP12BE) cells are reported. UV causes an immediate and equal depression of the RNA synthesis rate in both kinds of cells. A recovery to control rates was observed only at low (5 $J/m^2$) doses in repair-deficient cells and at higher doses (20 $J/m^2$) in repair-proficient cells. No recovery was observed at doses that cause substantial reductions in survival (>5 $J/m^2$ for XP12BE; >40 $J/m^2$ for repair-proficient populations). No initial effect on rate of protein synthesis was detected at doses <20 $J/m^2$. However, in XP12BE populations, a decreased rate first evident at 15–30 h post-UV and before any cell degeneration and loss was observed for doses as low as 7 $J/m^2$. This delayed effect was not observed in repair-proficient populations. The results are consistent with the hypothesis that the lethal action of UV in nondividing cells is one on DNA that leads to an inhibition of required protein synthesis by preventing RNA transcription.

## INTRODUCTION

Cultured populations of human diploid fibroblasts (HDF) arrested with respect to division by incubation in medium containing 0.5% calf serum can be maintained in a quiescent, nondividing state for long periods (>6 mo) without loss of cells (1). The cells remain viable and can reenter a state of rapid proliferation upon incubation with medium containing 10% serum. The derived population can be subcultured and will divide a number of times equivalent to the number of population doublings achieved for an unarrested, continuously subcultured population (1). Irradiation of arrested populations with reasonably low doses of ultraviolet light (UV, 254 nm) causes cells to detach from the surface of the culture vessel (2). The detached cells are not viable, as indicated by uptake of viable stains and unsuccessful attempts at cultivation. The extent of cell detachment is dose and strain dependent. DNA repair-deficient cells (XP12BE) are more sensitive than DNA repair-proficient cells (WI-38, WS-1)(2). These results suggested to us that DNA repair processes play a role in maintaining irradiated cells in the arrested state. We had suggested previously that the lethal event caused by UV in these populations may be an effect on RNA transcription leading to an inhibition of required protein synthesis (2–4). The purpose of this paper is to present our observations of the effect of biologically significant doses of UV on synthesis rates of RNA and protein in nondividing populations of repair-proficient and -deficient HDF strains. The results are consistent with the above hypothesis for lethality.

## MATERIALS AND METHODS

### *Cell Culture*

Cell strains used were human diploid fibroblasts in the Phase II stage of the proliferative life-span. Strain WS-1 was isolated by Hay (5) from embryonic lung tissue. The xeroderma pigmentosum strains XP12BE (ATCC No. CRL 1223) and XP4BE (XP variant, CRL 1162) were obtained from the American Type Culture Collection, Rockville, Md. Strains WS-1 and XP4BE are DNA excision repair-proficient (4, 6); XP12BE is repair deficient (7). Cell culture techniques and procedures for establishing and maintaining nonproliferating populations have been previously described (2). Briefly, midpassage cells cultured at 37°C in a humidified incubator using Eagle's minimum essential medium supplemented with nonessential amino acids, 30 mM HEPES buffer (pH 7.5), 10% fetal calf serum (Grand Island Biological Co., Grand Island, N.Y.), and penicillin and streptomycin were used. Nondividing populations were established and maintained by using a similar medium supplemented with 0.5% rather than 10% fetal calf serum (1, 2). Medium was routinely changed every 5 d. Populations were maintained in this medium for 10 to 20 d before the initiation of an experiment. Routine tests of cultures for mycoplasma contamination using procedures previously described were negative.

### *UV-Irradiation Technique*

Techniques for UV-irradiation have been described in detail previously (2). Medium was removed from the appropriate cultures, which were then washed with HEPES-buffered saline (HBS) and exposed through air to UV. Fresh, prewarmed (37°C), serum-deficient (0.5%) medium was added immediately after irradiation, and incubation at 37°C was continued. The UV source was a Westinghouse 15 W germicidal lamp (Westinghouse Electric Corp., Pittsburgh, Pa.). Incident intensities were determined with a Yellow Springs Instruments radiometer (Yellow Springs Instrument Co., Yellow Springs, Ohio). All irradiations and subsequent manipulations were done at 24°C under subdued lighting conditions or in the dark.

### *RNA and Protein Synthesis Measurements*

Uptake of $^3$H-uridine into RNA was used to detect RNA synthesis. Cultures (60-mm plates, Falcon Plastics, Oxnard, Calif.) of control and irradiated nondividing populations were incubated in serum-deficient medium containing $^3$H-uridine (uridine, 5-$^3$H, International Chemical & Nuclear Corp., Burbank, Calif., 25 Ci/mmol, 5 $\mu$Ci/ml). At appropriate times, cells from replicate cultures were rinsed twice with 5 ml HBS, removed from the culture vessel surface with either a trypsin solution or a rubber policeman, collected by centrifugation, resuspended in 2 ml HBS, counted with a hemacytometer, and precipitated with an equal volume of ice-cold 10% trichloroacetic acid (TCA). $^3$H activity in RNA was determined as described by Enger et al. (8). The TCA precipitate was washed with ice-cold ethanol, resuspended in HBS containing ribonuclease (50 $\mu$g/ml; ribonuclease A, NBCO 1656 (NBCO Biochemicals, ICN Pharmaceuticals, Inc., Cleveland, Ohio) dissolved in water and heated at 60°C for 10 min to inactivate DNase) and incubated at 37°C for 20 min. The digested solution was precipitated with ice-cold 0.2 M perchloric acid for 30 min. Radioactivity in the supernatant was determined using a toluene-based triton-X containing scintillation fluid and a liquid scintillation counter (Searle Analytic Isocap 300 LSC, Searle Analytic, Inc., Des Plaines, Ill.). In some instances, data were corrected for quench by adding internal standards to each sample. No significant differences in quench correction between various samples were detected.

Protein synthesis was detected by measuring incorporation of $^3$H-leucine (leucine-$^3$H, New England Nuclear, Boston, Mass., 20 Ci/mmol, 0.5 $\mu$Ci/ml) into acid-insoluble material. Cultures incubated with medium containing $^3$H-leucine were drained and washed twice with HBS before releasing cells with a trypsin solution. Cells were quantitated with a hemacytometer and the total suspension was precipitated with an equal volume of ice-cold 10% TCA for 30 min. Precipitates were collected on membrane filters (Gelman Instrument Co., Ann Arbor, Mich., 0.2-$\mu$m pore size) and washed with ice-cold 5% TCA and ice-cold 95% ethanol. After drying, filters were placed in scintillation fluid and counted in a liquid scintillation counter.

## RESULTS

### *Effect of UV on RNA Synthesis*

The initial effect of UV on RNA synthesis in a DNA excision repair-proficient strain (WS-1) and an excision repair-deficient strain (XP12BE), measured by incorporation of $^3$H-uridine into RNA, is shown in Fig. 1. In control populations, incorporation occurred at a linear rate for about the first 2 h of the experiment. An immediate depression in this rate was observed in both strains with doses as low as 5 J/m$^2$. The size of the depression increased with dose, at least up to 40 J/m$^2$. No differences were observed at any given dose in the size of the depression of the initial rate between the two strains in this or in three other similar experiments. In the data presented, the results obtained for WS-1 are superimposable on those obtained for XP12BE. A least-squares regression analysis indicates no dose-dependent decrease in the $y$-intercept value. All lines could have been drawn through zero, given the goodness of fit of the data.

Uptake of $^3$H-uridine into RNA in control populations was not linear at times between 120 min and 8 h after initiation of the experiment. Nonlinear uptake kinetics were also observed for irradiated populations at times beyond 120 min. These results were observed in experiments similar to those of Fig. 1 and in experiments to be discussed later.

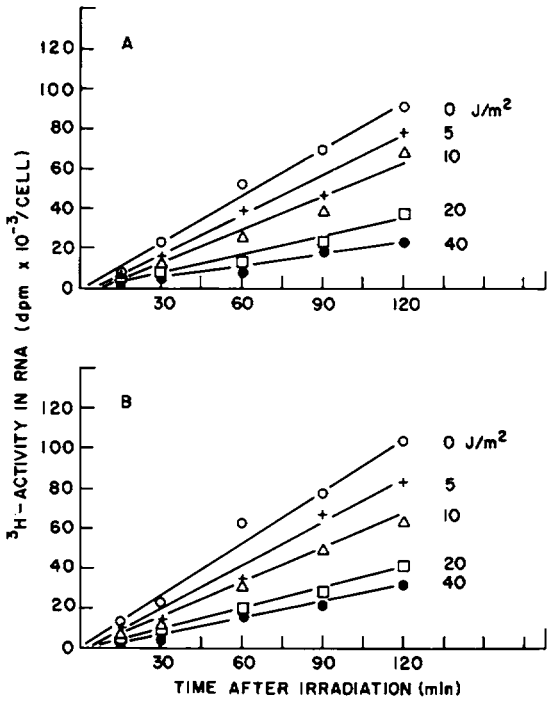


FIGURE 1 Effect of UV on RNA synthesis in nondividing human diploid fibroblast strains. Cell populations maintained in serum-deficient medium for 14 d were irradiated at time zero and incubated in medium containing $^3$H-uridine (5 $\mu$Ci/ml) for the times indicated before a determination of radioactivity in RNA. Each datum point is an average value determined by using two replicate cultures. Lines are best fits of the data as determined by a least-squares regression analysis. (A) WS-1; (B) XP12BE. Symbols: (O), unirradiated control; (+), UV-irradiated, 5 J/m$^2$; (△), 10 J/m$^2$; (□), 20 J/m$^2$; (●), 40 J/m$^2$.

Data obtained for measuring the effect of UV on the initial rate of RNA synthesis by using nondividing populations of three HDF strains, determined from several experiments similar to those of Fig. 1 and from pulse-labeling experiments, are summarized in Fig. 2. Data were normalized to control rates and are plotted as a function of dose. The line drawn is the curve

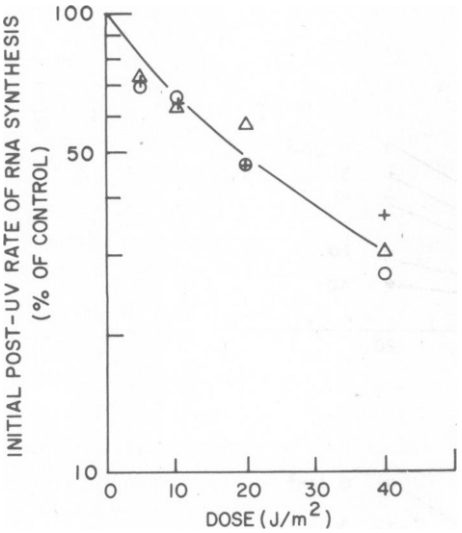


FIGURE 2

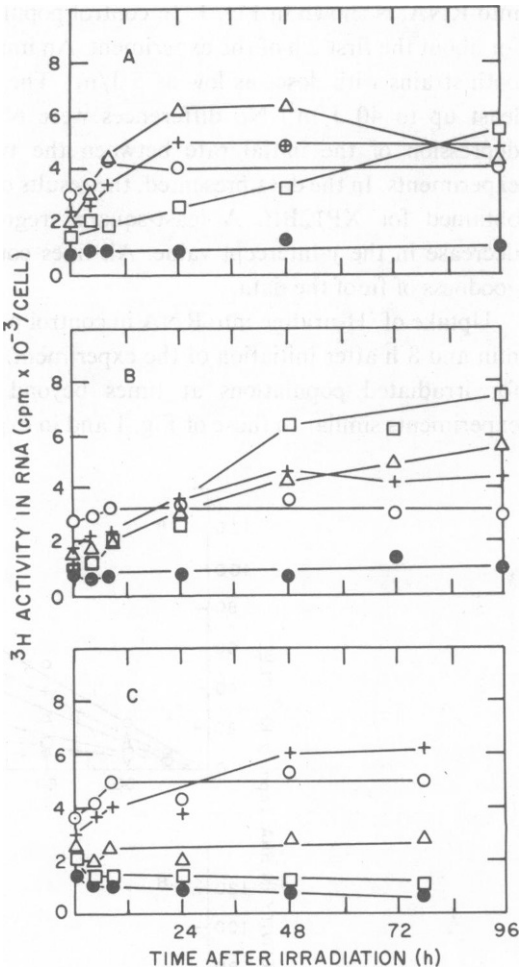


FIGURE 3

FIGURE 2 Effect of UV on the rate of RNA synthesis in nondividing HDF populations. Data obtained as described in Fig. 1 from several experiments, and from pulse-labeling experiments, were combined and plotted as percent of the appropriate unirradiated control. Each datum point is an average determined from at least three experiments. Line drawn is the curve for the equation $Ruv/Ro = (1 - e^{-D})/D$ (see text). Symbols: (○), WS-1; (+), XP12BE; (△), XP4BE.

FIGURE 3 Recovery of the rate of RNA synthesis in UV-irradiated HDF strains. Cell populations maintained in serum-deficient medium for 13 d were irradiated at time zero. $^3$H-uridine (5 μCi/ml) was added for 15 min at the times indicated; cell samples were immediately extracted and assayed for activity in RNA. Each datum point is an average of two replicate cultures treated in identical fashion. Lines are approximations of the data. The average number of cells used per determination in controls was: (A) WS-1, $7.0 \times 10^4$; (B) XP4BE, $8.3 \times 10^4$; (C) XP12BE, $7.1 \times 10^4$. The radioactivity detected in the final control sample was: (A) WS-1, 300 cpm; (B) XP4BE, 310 cpm; (C) XP12BE, 350 cpm. Symbols: (○), unirradiated control populations; (+) UV-irradiated, 5 J/m$^2$; (△), 10 J/m$^2$; (□), 20 J/m$^2$; (●), 40 J/m$^2$.

for the equation $Ruv/Ro = (1 - e^{-D}/D)$, where *Ruv/Ro* is the RNA synthesis rate in irradiated samples (*Ruv*) compared to an unirradiated control (*Ro*), and *D* is the average number of transcription-terminating lesions per average transcription template (chosen here as 1 at a dose of 12 J/m$^2$, since RNA synthesis is reduced to 63% of controls at this dose). This equation has been presented by others (9–11) to describe the nonlinear, nonexponential effect of UV on the rate of RNA synthesis. The data presented in Fig. 2 show that similar results were obtained for three HDF strains examined, and that the overall effect is similar to that previously described by others for UV-irradiated bacteriophage (12) and bacterial (13, 14) and other mammalian cells (10, 15–18) (for a review of the effect of UV on RNA synthesis, see reference 11).

To determine the effect of DNA excision repair on UV-depressed RNA synthesis, rates of RNA synthesis in irradiated populations at considerably longer times post-irradiation (to 96 h) were determined. Results are presented in Fig. 3. Pulse-labeling conditions were used in these experiments to avoid problems associated with RNA processing and to make observations in the linear part of the $^3$H-uridine uptake curve. In all three strains (WS-1, XP4BE, XP12BE), the rate of synthesis in the unirradiated controls was constant, except at times earlier than 12 h post-irradiation. In all irradiated populations, an immediate dose-dependent depression in the synthesis rate compared to controls was observed. An increase in the rate was observed in strains WS-1 and XP4BE at later times for doses as large as 20 J/m$^2$. A similar increase was observed for strain XP12BE only at significantly lower doses (5 J/m$^2$); no increase in the rate of RNA synthesis was observed at higher doses. A recovery to a rate exceeding that of the controls was observed in all populations that exhibited any ability to recover from the UV-depressed synthesis rate. No recovery at doses of 40 J/m$^2$ or higher was observed in any population. Similar observations were made in three other experiments. Although in different experiments the initial effect of a specific UV dose was always the same, rates of recovery were not. This may be due to unknown uncontrolled culture variations, because similar rates were observed when the same batch of cells was tested on the same days.

The increase in the control rate during the first 12 h of the experiment may be due to experimental procedures. Just before initiation of the experiment, medium is removed from cultures, which are then rinsed with HBS. Fresh medium is returned to the cultures immediately after UV-irradiation. We observe a constant rate of incorporation in unirradiated controls during all 15-min pulses when the medium removal and buffer rinse are not employed.

### *Effect of UV on Protein Synthesis*

The effect of UV on protein synthesis in nondividing populations of WS-1 and XP12BE, as measured by incorporation of $^3$H-leucine into acid-insoluble material, is shown in Fig. 4. An immediate decrease in the rate of protein synthesis was detected only at relatively high doses ($\geq$20 J/m$^2$). The immediate effect was about the same in both strains. A delayed decrease, usually evident at 15–30 h post-irradiation, was detected at low doses (7 and 10 J/m$^2$) in XP12BE and at the higher doses in both strains. The lower doses of 7 and 10 J/m$^2$ had no immediate or delayed effect in WS-1. A delayed effect at doses lower than 7 J/m$^2$ (3.5 and 5 J/m$^2$, data not shown) in XP12BE was not detected. The rate of protein synthesis per cell in the low-dose irradiated XP12BE populations appeared to increase at later times and

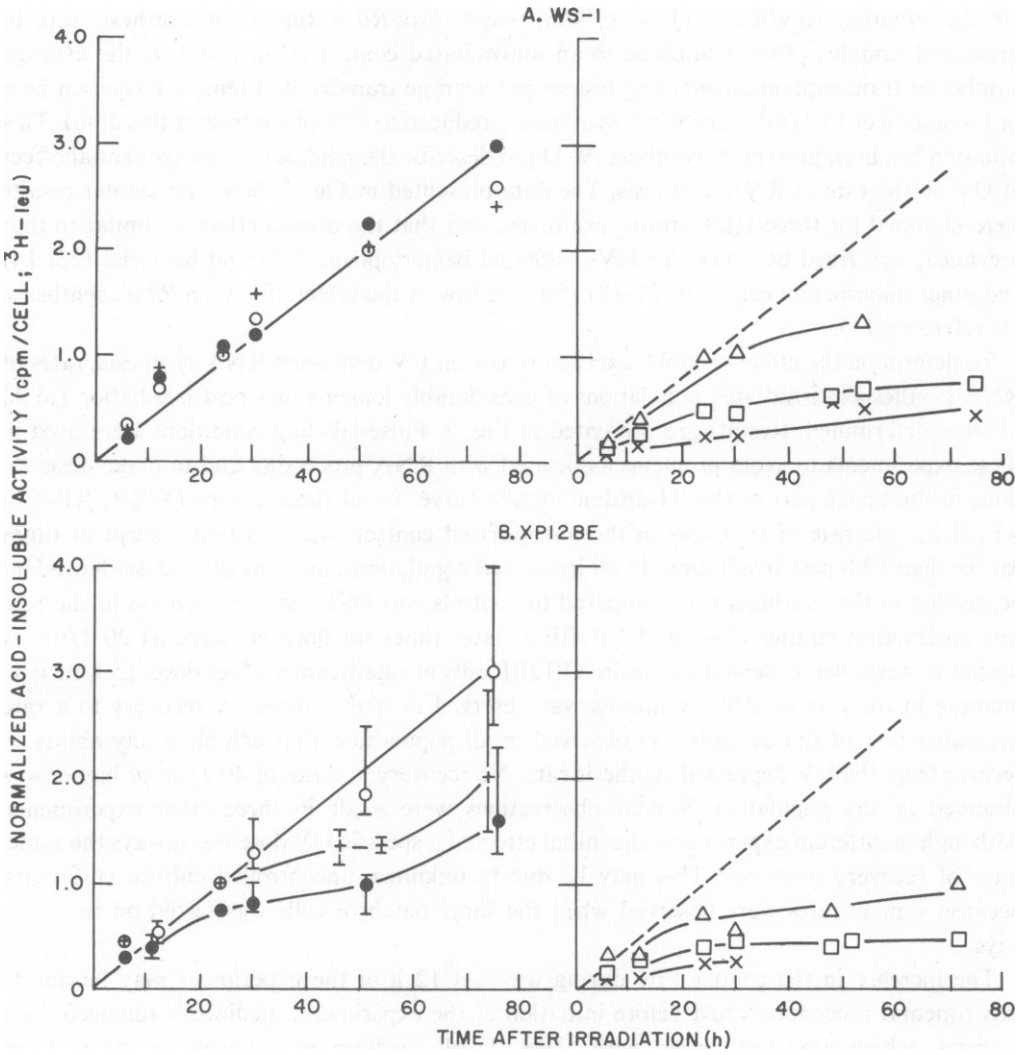


FIGURE 4 Effect of UV on protein synthesis in nondividing HDF populations. HDF populations maintained in a nondividing state for 14 d were irradiated at time zero and immediately incubated in serum-deficient medium containing $^3$H-leucine (0.5 $\mu$Ci/ml). Radioactivity in the acid-insoluble fraction was determined in replicate cultures at the times indicated. The data were obtained from four experiments. All data were normalized to a value of 1 for the control at 24 h. In each experiment duplicate cultures were used for each point; results were averaged. The data points and bars presented for XP12BE at lower doses represent the average of the four experiments and the extreme values. All other data points represent the average from the four experiments. For clarity, results for the two strains at low and high doses are presented in two graphs. The dashed line is the same as that drawn through control values. Symbols: (○), unirradiated control samples; (+), UV-irradiated, 7 J/m$^2$; (●), 10 J/m$^2$; (△) 20 J/m$^2$; (□), 50 J/m$^2$; (x), 100 J/m$^2$.

approached that of controls. UV-induced cell loss starts at ~40 h post-UV; the apparent increase in protein synthesis is probably a reflection of this loss and an indication of the rate of protein synthesis in survivors. A clearer demonstration of this is seen in Fig. 5.

The results presented in Fig. 4 depended on long-term (3–4 d) labeling with radioactive

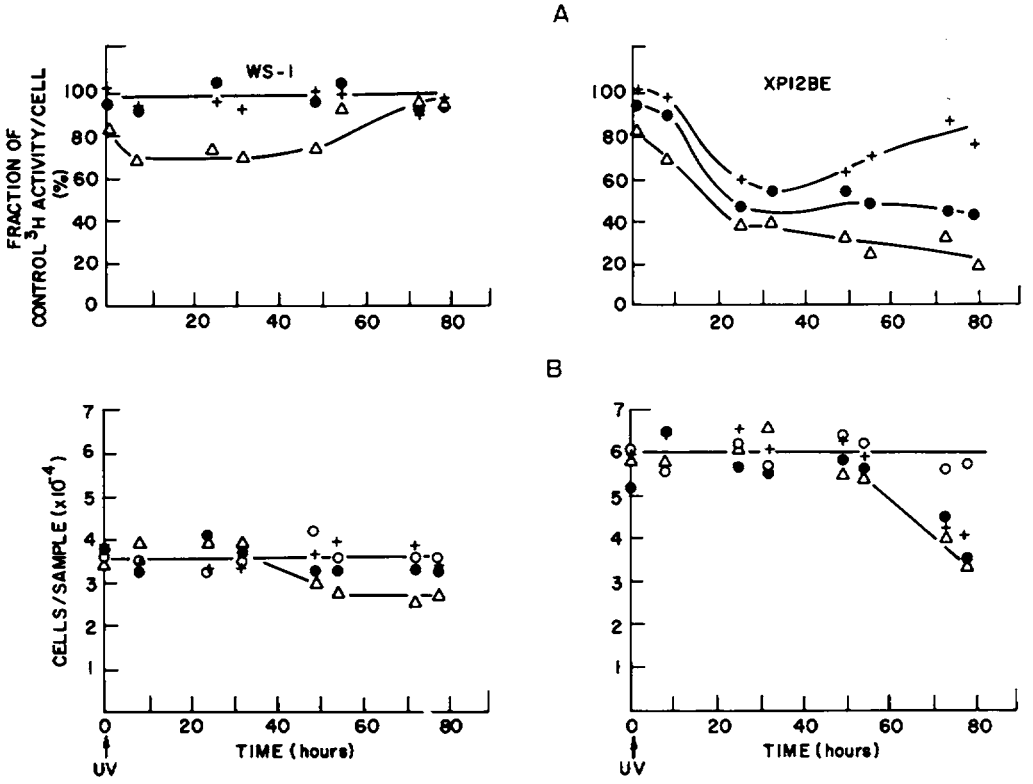


FIGURE 5 Effect of UV on protein synthesis and maintenance of cell attachment in nondividing HDF populations. The experiments are similar to those described in Fig. 4, except that $^3$H-leucine (0.5 $\mu$Ci/ml) was added to the serum-deficient medium at the times indicated and incubation was continued for 1 h before determining cell number and acid-insoluble radioactivity. Results are from a single experiment and are representative of other experiments. Each datum point is an average determined from two replicate cultures. (A) Effect of UV on protein synthesis in WS-1 and XP12BE. (B) Corresponding effect of UV on maintenance of cell attachment in WS-1 and XP12BE. Symbols: (○), unirradiated control cultures; (+), UV-irradiated, 7 J/m$^2$; (●), 10 J/m$^2$; (△), 20 J/m$^2$.

isotope. Although no depletion of $^3$H-leucine was observed, other potential problems, such as loss of labeled proteins into the medium (not observed) and UV-induced cell loss without total loss of cell protein, could complicate the interpretation of the long-term labeling results. Experiments similar to those of Fig. 4 were done using instead pulse–labeling conditions. Results of these experiments are presented in Fig. 5. Data in part A represent the $^3$H-leucine uptake in a l-h incubation period at the times indicated and in part B the corresponding cell numbers in the same cultures. Little or no effect on protein synthesis was observed immediately after irradiation with 10 J/m$^2$ in either strain. The delayed effect in XP12BE was readily observable at later times. No similar delayed effect was observed with WS-1 except at the higher doses (20 J/m$^2$) that also had an immediate effect. The delayed decrease in synthesis occurred before any cell loss. Cell loss was not detectable before 40 h post-irradiation. An apparent increase in protein synthesis rate occurred at about the time as cell loss, and is most likely due to a nearly normal synthesis rate in the survivors.

## DISCUSSION

Several recent studies have reported a dose-dependent effect of UV on RNA synthesis in different biological systems (see references 10, 15, 19; for a recent review, see reference 11). To get a measurable effect, reasonably high doses compared to those that affect DNA synthesis are usually required. Thus the effect on RNA synthesis has not normally been implicated as a primary event in death. Some evidence has been presented by Sauerbier et al. (12), however, to show that the effect of UV on RNA synthesis is responsible for at least some of the killing of T2 phage when placque-forming ability is measured. Here we present data that shows again an effect of UV on the rate of RNA synthesis. The effect is measured in nondividing HDF populations. The effect on RNA synthesis is probably caused by UV-induced DNA lesions, as argued by others (11). We suggest that the effect on RNA synthesis can lead to an eventual decline in protein synthesis and that the decline in protein synthesis is responsible for the detachment and death of nondividing HDF. Thus the effect of UV on RNA synthesis is the primary event leading to cell death. The correlations in our data between the effects of UV in excision repair-proficient and -deficient strains with respect to survival of attachment, rates of RNA synthesis, recovery of these rates, and protein synthesis support these suggestions and are discussed in the following paragraphs.

### *Effect on RNA Synthesis is Caused by UV-Induced DNA Lesions*

The effect of UV on the RNA synthesis rate is similar to that predicted by an equation that successfully describes the effect in other systems. The equation is based on the assumption that lesions in DNA affect RNA synthesis. Evidence has been presented previously to support this assumption (11). The dose response observed here is the same in the three HDF strains used. This is as expected if DNA lesions were responsible. Sauerbier et al. (12) have demonstrated that RNA-chain termination by UV-induced DNA lesions is the primary occurrence responsible for depressed synthesis rates. If so, removal of transcription-terminating lesions should permit a recovery of this rate. Excision repair-proficient strains remove UV-induced pyrimidine dimers from DNA and reconstruct the DNA molecule (for reviews of DNA repair, see references 20–22). These repair processes occur in serum arrested cells (4,25). The excision repair-deficient strain used here, XP12BE, is incapable of removing most of the dimers from DNA. The greater capability of repair-proficient strains compared to repair-deficient strains to recover from the effect of UV on RNA synthesis is consistent with the hypothesis that the observed effect is due to DNA lesions and that excision repair removes transcription-terminating lesions.

The rate of recovery of RNA synthesis rates is also consistent with known rates of excision repair in the cells used here. Measurements of dimer excision in proliferating (23, 24) and arrested cells (4) indicate that $<10\%$ of the dimers are removed in the first 2 h after irradiation. No recovery of RNA synthesis is observed in this time (Fig. 1). However, by 24 h, a time at which a significant fraction of the dimers are removed (50–70%), a recovery in the RNA synthesis rate is observed. The recovery continues during later post-UV times. The reduced capacity for recovery of RNA synthesis rates in XP12BE is consistent with the small amount of excision repair observed in this strain (7).

### *The Effect on RNA Synthesis can Lead to an Eventual Decline in Protein Synthesis*

UV doses of 5 to 10 J/m$^2$ affect the RNA synthesis rate. No recovery from this effect is observed in XP12BE cells. These same doses do not immediately affect the protein synthesis rate. The initial UV effect in repair-proficient cells is the same. However, recovery from the effect on RNA synthesis occurs. A delayed effect on protein synthesis caused by these doses is evident in XP12BE but not in repair-proficient cells. It is observed 15–30 h post UV. This is a time-span during which recovery of the RNA synthesis rate (Fig. 3) occurs in repair-proficient strains. We suggest that the delayed effect on protein synthesis is due to a lack of necessary RNA. A delayed effect on protein synthesis is also evident in repair-proficient cells at larger UV doses ($\geq$20 J/m$^2$). The delayed effect is observed in those cultures that show either a slower recovery of the RNA synthesis rate (20 J/m$^2$) or no recovery (40 J/m$^2$).

### *The Decline in Protein Synthesis is Responsible for Detachment and Death of Nondividing HDF*

Protein synthesis continues in nondividing HDF populations, probably throughout the entire time they are in the arrested state (26, 27). The data of Figs. 3 and 4 demonstrate a constant rate of protein synthesis through a 3-d period. We have observed a nearly constant rate throughout a 50-d arrested period.[1] Incubation with the protein synthesis inhibitors puromycin, cycloheximide, and actinomycin D causes arrested cells to detach from the culture vessel (4). The detached cells are not viable and appear similar to those in UV-irradiated cultures. Exposure to UV causes a delayed effect on protein synthesis at lower doses and an immediate effect at higher doses (Fig. 4). The immediate effect is about the same in both repair-proficient and repair-deficient strains. It is evident at doses that kill large fractions of the populations (>20 J/m$^2$). The delayed effect, evident in both strains, occurs at lower doses in the more sensitive strain. It can be detected only at UV doses that have a measurable effect on survival, regardless of strain. It is not detected at UV doses that have no effect on survival. For example, a dose of 2 J/m$^2$ has no measurable effect on survival or protein synthesis in XP12BE; likewise, doses of 10 J/m$^2$ have no effect in repair-proficient cells. The UV-induced delayed effect on protein synthesis is always observed before any cell detachment.

## CONCLUSIONS

UV-irradiated nondividing human cells in culture detach from the culture vessel surface, are incapable of reattaching during culture conditions that are favorable for either maintenance or growth of unirradiated cells, and take up a viable stain rapidly. The detached cells are considered dead with respect to these criteria. When observed in the microscope, the irradiated cells appear to degenerate gradually. As attachment for the vessel surface is gradually lost, cells lose their fibroblastic morphology, begin to round up and become refractile, and can be observed dangling to some extent in the medium. In some instances,

[1]Hull, D. R., and G. J. Kantor. Unpublished data.

nuclei are lost before total detachment; some cell lysis has been observed. Our conclusions as to how this effect is caused by UV, based on these observations and the data and arguments presented here, are as follows: (*a*) The killing of nondividing HDF cells by UV is caused primarily by DNA damage; (*b*) The damage to DNA leads to a decreased rate of RNA synthesis; (*c*) The decreased rate of RNA synthesis eventually causes a decreased rate of protein synthesis; (*d*) The decreased rate of protein synthesis leads to cell degeneration and death, possibly because proteins lost by turnover are not replaced; (*e*) Killing in DNA excision repair-proficient and -deficient strains is due to the same mechanism—a decrease in protein synthesis; and (*f*)DNA excision repair-proficient strains are more resistant to UV because they can remove to some extent the DNA damage that leads to an effect on RNA synthesis.

With respect to (*e*) and (*f*), killing of repair-proficient cells may involve initial events in addition to DNA damage. The doses that kill a majority of cells ($\geq$40 J/m$^2$) have an effect on RNA synthesis from which the cells do not recover and an immediate effect on protein synthesis. The immediate effect of UV on protein synthesis may be great enough to lead to cell degeneration before the occurrence of a sufficient degree of DNA repair that would permit a recovery of the RNA and protein synthesis rates.

### *General Considerations*

Although an overall effect of UV on the RNA synthesis rate can be demonstrated, no attempt to define the kinds of RNA molecules affected at the doses used has been made. We suspect that because the majority of RNA synthesis is ribosomal, the measured effect is one on these RNA species. However, because of assumed random distribution of UV-induced DNA lesions, one would expect some effect on all RNA species, even though the inactivation cross-sections for different species vary considerably (10, 18).

In those UV-irradiated populations where a recovery in the RNA synthesis rate was detected (Fig. 3), an increase to a rate greater than that of controls was observed. In some instances, the increased rate was observed to return to the control rate. This increased synthesis may reflect an attempt by cells to correct for an imbalance created by the originally depressed rate. No adequate explanation can be given without further study of this effect.

Dell'Orco and colleagues (28) suggested that nondividing HDF in serum-deficient (0.5%) medium may represent an appropriate model system for the study of human cells usually found as nonmitotic in vivo. A comparison of the study presented here with in vivo studies of the effect of UV on human, mouse, and guinea-pig skin (29–32) suggests similarities which further support the use of this sytem. An effect of UV on RNA and protein synthesis in the nondividing Malpighian and granular skin cells was detected. Cellular degeneration was also observed. Although accurate comparisons of dosimetry and kinetics of inhibition are difficult, the observations are consistent with those reported here. Our results suggest that in vivo, the occurrence of DNA repair in nondividing cells may to some extent prevent cell degeneration that is a consequence of RNA and protein synthesis inhibition caused by DNA damaging agents. As an example, a greater loss of nondividing skin cells in xeroderma pigmentosum patients compared to normal individuals upon exposure to sunlight UV may be expected. In addition, some of these patients have neurological abnormalities due to the premature death of the central nervous system neurons (6). The results presented here suggest a possible explanation for the premature death of these DNA repair-deficient nondividing cells.

We gratefully acknowledge the encouragement of Dr. R. B. Setlow during the preparation of this manuscript. This investigation was supported by Public Health Service grant CA-16477 from the National Cancer Institute.

*Received for publication 1 March 1979.*

## REFERENCES

1. DELL'ORCO, R. T., J. G. MERTENS, and P. F. KRUSE, JR. 1973. Doubling potential, calendar time, and senescence of human diploid cells in culture. *Exp. Cell Res.* **77**:356–360.
2. KANTOR, G. J., C. WARNER, and D. R. HULL. 1977. The effect of ultraviolet light on arrested human diploid cell populations. *Photochem. Photobiol.* **25**:483–489.
3. KANTOR, G. J., and D. R. HULL. 1978. A comparison of the response of arrested HDF populations to UV and X-rays. *J. Supramol. Struct.* **8**:81.
4. KANTOR, G. J., and D. R. HULL. 1977. DNA repair in arrested human diploid fibroblast cultures irradiated with ultraviolet light. *Biophys. J.* **17**:144*a* (abstr.).
5. COREFIELD, V. A., and R. J. HAY. 1978. Effects of cystine or glutamine restriction on human diploid fibroblasts in culture. *In Vitro.* **14**:787–794.
6. ROBBINS, J. H., K. H. KRAEMER, M. A. LUTZNER, B. W. FESTOFF, and H. G. COON. 1974. Xeroderma pigmentosum: an inherited disease with sun sensitivity, multiple cutaneous neoplasms, and abnormal DNA repair. *Ann. Intern. Med.* **80**:221–248.
7. CLEAVER, J. E. 1970. DNA repair and radiation sensitivity in human (Xeroderma pigmentosum) cells. *Int. J. Radiat. Biol. Relat. Stud. Phys. Chem. Med.* **18**:557–565.
8. ENGER, M. D., R. A. TOBEY, and A. G. SAPONARA. 1968. RNA synthesis in Chinese hamster cells. I. Differential synthetic rate for ribosomal RNA in early and late interphase. *J. Cell Biol.* **36**:583–593.
9. HAGEN, U., M. ULLRICH, E. E. PETERSON, E. WERNER, and H. KRÖGER. 1970. Enzymatic RNA synthesis on irradiated DNA. *Biochim. Biophys. Acta.* **199**:115–125.
10. HACKETT, P. B., and W. SAUERBIER. 1975. The transcriptional organization of the ribosomal RNA genes in mouse L cells. *J. Mol. Biol.* **91**:235–256.
11. SAUERBIER, W. 1976. UV damage at the transcriptional level. *Adv. Radiat. Biol.* **6**:49–106.
12. SAUERBIER, W., R. L. MILLETTE, and P. B. HACKETT, JR. 1970. The effects of ultraviolet irradiation on the transcription of T4 DNA. *Biochim. Biophys. Acta.* **209**:368–386.
13. MICHALKE, H., and H. BREMER. 1969. RNA synthesis in *Escherichia coli* after irradiation with ultraviolet light. *J. Mol. Biol.* **41**:1–23.
14. HACKETT, P. B., and W. SAUERBIER. 1974. Radiological mapping of the ribosomal RNA transcription unit in *E. coli*. *Nature (Lond.).* **251**:639–641.
15. PAINTER, R. B., M. G. GOODMAN, and B. L. REISNER. 1970. The effect of ultraviolet light on RNA metabolism in bromouracil deoxyriboside-grown HeLa Cells. *Photochem. Photobiol.* **12**:49–55.
16. GIORNO, R., and W. SAUERBIER. 1976. A radiological analysis of the transcription units for heterogeneous nuclear RNA in cultured murine cells. *Cell.* **9**:775–783.
17. GOLDBERG, S., H. SCHWARTZ, and J. E. DARNELL, JR. 1977. Evidence from UV transcription mapping in HeLa cells that heterogeneous nuclear RNA is the messenger RNA precursor. *Proc. Natl. Acad. Sci. U.S.A.* **74**:4520–4523.
18. GIORNO, R., and W. SAUERBIER. 1978. Units of transcription for cytoplasmic RNA in mouse myeloma cells. *Proc. Natl. Acad. Sci. U.S.A.* **75**:4374–4378.
19. SWENSON, P. A., and R. B. SETLOW. 1966. Effects of ultraviolet radiation on macromolecular synthesis in Escherichia coli. *J. Mol. Biol.* **15**:201–219.
20. DNA Repair Mechanisms. 1978. P. C. Hanawalt, E. C. Friedberg, and C. F. Fox, editors. *ICN-UCLA Sympos. Mol. Cell. Biol.* **IX**:1–800.
21. SETLOW, R. B., and J. K. SETLOW. 1972. Effects of radiation on polynucleotides. *Annu. Rev. Biophys. Bioeng.* **1**:293–346.
22. PAINTER, R. B. 1972. Repair of DNA in mammalian cells. *Curr. Top. Radiat. Res. Q.* **7**:47–70.
23. REGAN, J. D., J. E. TROSKO, and W. L. CARRIER. 1968. Evidence for excision of ultraviolet-induced pyrimidine dimers from the DNA of human cells in vitro. *Biophys. J.* **8**:319–325.
24. SETLOW, R. B., J. D. REGAN, J. GERMAN, and W. L. CARRIER. 1969. Evidence that Xeroderma pigmentosum cells do not perform the first steps in the repair of ultraviolet damage of their DNA. *Proc. Natl. Acad. Sci. U.S.A.* **64**:1035–1041.

25. DELL'ORCO, R. T., and W. L. WHITTLE. 1978. Unscheduled DNA synthesis in confluent and mitotically arrested populations of aging human diploid fibroblasts. *Mech. Ageing Dev.* **8**:269–278.
26. DELL'ORCO, R. T., and J. H. NASH. 1973. Effects of ascorbic acid on collagen synthesis in nonmitotic human diploid fibroblasts. *Proc. Soc. Exp. Biol. Med.* **144**:621–622.
27. BIRCKBICHLER, P. J., W. L. WHITTLE, and R. T. DELL'ORCO. 1975. Amino acid uptake in growing and arrested human diploid cell populations. *Proc. Soc. Exp. Biol. Med.* **149**:530–533.
28. DELL'ORCO, R. T., H. A. CRISSMAN, J. A. STEINKAMP, and P. M. KRAEMER. 1975. Population analysis of arrested human diploid fibroblasts by flow microfluorometry. *Exp. Cell Res.* **92**:271–274.
29. BADEN, H. P., and C. PEARLMAN. 1964. The effect of ultraviolet light on protein and nucleic acid synthesis in the epidermis. *J. Invest. Dermatol.* **43**:71–75.
30. FUKUYAMA, K., W. L. EPSTEIN, and J. H. EPSTEIN. 1967. Effect of ultraviolet light on RNA and protein synthesis in differentiated epidermal cells. *Nature (Lond.)*. **216**:1031–1032.
31. EPSTEIN, W. L., K. FUKUYAMA, and J. H. EPSTEIN. 1969. Early effects of ultraviolet light on DNA synthesis in human skin *in vivo*. *Arch. Dermatol.* **100**:84–89.
32. EPSTEIN, J. H., K. FUKUYAMA, and K. FYE. 1970. Effects of ultraviolet radiation on the mitotic cycle and DNA, RNA and protein synthesis in mammalian epidermis *in vivo*. *Photochem. Photobiol.* **12**:57–65.