NASCENT DNA SYNTHESIS IN ULTRAVIOLET LIGHT-IRRADIATED MOUSE, HUMAN, AND CHINESE HAMSTER CELLS

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ABSTRACT The technique of alkaline sucrose gradient centrifugation was used to study newly synthesized DNA in control and ultraviolet light-irradiated mouse L, human HeLa, and Chinese hamster ovary cells. Nascent DNA molecular weight distributions did not appear to differ among the three cell lines for unirradiated cells. However, at short times after ultraviolet light irradiation, human HeLa cells appeared to synthesize more low molecular weight DNA than either mouse L or Chinese hamster ovary cells. Since this difference was not related to differences in either the rate of DNA synthesis or amount of ultraviolet damage in the irradiated cells it appeared to be a phenotypic characteristic of the cell lines tested. A parallel was noted for these three cell lines between an increase in the synthesis of low molecular weight DNA, detected on alkaline sucrose gradients, and cell killing as measured by the ability of irradiated cells to form colonies.

INTRODUCTION

It has been shown, in bacteria which lack an excision repair system but are competent in recombination, that DNA newly synthesized after ultraviolet (UV) light irradiation is found in short pieces. The molecular weight of these pieces is approximately that expected if each pyrimidine dimer on the template DNA caused an interruption in the continuity of the newly synthesized DNA (Rupp and Howard-Flanders, 1968). Mouse L cells appear to have an undetectable level of dimer excision (Klimek, 1966) though low levels of unscheduled DNA synthesis and repair replication have been detected after UV irradiation (Painter and Cleaver, 1969). Thus, these cells might be expected to resemble excision-deficient, recombination-proficient bacteria. The nascent DNA seen in UV-irradiated mouse L cells using short labeling times (10–30 min) is of a lower molecular weight than that seen in unirradiated cells (Chiu and Rauth, 1972). However this DNA is of a greater molecular weight than would be expected if every dimer in the template DNA caused a detectable gap in the newly synthesized DNA. This discrepancy is especially apparent at low UV exposures (100–200 ergs/mm^2).

Recent work by Buhl et al. (1972) has also indicated that, at short times after UV irradiation of human cells, the newly synthesized DNA is made in segments of lower molecular weight than in unirradiated cells. However in contrast to the results in mouse L cells the size of this DNA is approximately equal to the intradimer distance in the template DNA even at low exposures of UV. Within several hours after irradiation these smaller units are elongated or joined together to yield DNA of the same molecular weight as in unirradiated cells.

These differences between mouse L cells (Chiu and Rauth, 1972) and human cells (Buhl et al., 1972) might be due to differences in the biochemical properties of the cells or differences in the gradient analysis techniques used to measure the effects of UV on new DNA synthesis. In the present work a comparison has been made between mouse L cells and human HeLa cells under identical conditions of gradient analysis using the same gradient system as was used previously for mouse L cells (Chiu and Rauth, 1972). In addition Chinese hamster ovary (CHO) cells were also investigated. The results of this investigation indicate that the differences in nascent DNA synthesis between these UV irradiated cell lines is a function of the cell line used and not the gradient technique. That is, human HeLa cells synthesize more low molecular weight DNA than do mouse L cells after the same UV exposure. In addition, there appears to be a correlation for L, HeLa, and CHO cells between size of the segments of DNA synthesized after UV irradiation and the survival of the cells as measured by their colony forming ability.

MATERIALS AND METHODS

Cells

The cell lines used were (*a*) a subline of mouse L cells (Till et al., 1963), (*b*) human HeLa cells (courtesy of Dr. T. P. Brent), and (*c*) Chinese hamster ovary (CHO) cells (courtesy of Dr. L. H. Thompson). All cell lines were grown in suspension culture in spinner flasks in α-medium (Stanners, 1971) plus 10% undialyzed fetal calf serum at 37°C. All the cell lines had doubling times in the range of 16–20 h under these conditions. For all experiments, asynchronous populations of cells in exponential growth were used.

Irradiation

The source of UV was two low pressure mercury lamps. The predominant wavelength from such lamps is the mercury resonance line at 254 nm. The lamps were mounted side by side at a distance of 40 cm above the surface of the shaker. Cells were irradiated in 1066 medium lacking thymidine and coenzymes (1066 T^- medium) (Parker, 1961) plus 10% undialyzed normal horse serum or in phosphate-buffered saline (Dulbecco and Vogt, 1954) in 5-ml aliquots in 60-mm plastic petri dishes at 37°C in the presence of 5% CO_2 in air with gentle shaking. The average incident exposure was 4 ergs/mm^2 per s in the former case and 8 ergs/mm^2 per s in the latter case. The incident exposure to the cells was measured by malachite green leucocyanide dosimetry, and corrections were made for the absorbancy of the solutions (Johns, 1969).

The cells were labeled with [^{3}H]thymidine (27.8–49.9 Ci/mmol, 1 mCi/ml), at a final concentration of 10–20 μCi/ml at 37°C in suspension cultures in a roller wheel, usually for 30

min. The labeling period was terminated by adding nonradioactive thymidine to give a final concentration of 100 μg/ml. This lowered the specific activity of the [^{3}H]thymidine to the point that appreciable incorporation of radioactivity was stopped. The 1066 medium used contains 10 μg/ml deoxycytidine and no inhibition of DNA synthesis is seen even at concentrations of thymidine up to 1 mg/ml. In some cases incubation was terminated immediately by centrifuging the cells at 200 *g* at 4°C for 7 min to remove the radioactive medium, the cells were resuspended in ice-cold phosphate-buffered saline, centrifuged again and resuspended again in phosphate-buffered saline. In other cases cells were incubated for a time at 37°C after the nonradioactive thymidine addition. The medium was then removed, the cells were washed and resuspended in ice-cold phosphate-buffered saline as before.

In some experiments cells were continuously labeled for 40 h with [^{14}C]thymidine (60 mCi/mmol, 50 μCi/ml) at a final concentration of 0.20 μCi/ml in suspension culture. These conditions do not affect the viability of the cells as measured by their colony forming ability.

Gradient Analysis

DNA was analyzed by sedimentation in an alkaline sucrose gradient prepared as described previously (Chiu and Rauth, 1972). In the present work all gradients were centrifuged at 40,000 rpm for 1.5 or 3.0 h at 20°C using a SW 50.1 rotor in an L-2 model ultracentrifuge (Beckman Instruments, Palo Alto, Calif.). The gradients were collected as described previously yielding 21 fractions (Chiu and Rauth, 1972). Each fraction was prepared for liquid scintillation counting on glass fiber filters. As found previously, a small percentage of the radioactivity went to the bottom of the tube and was not recovered by the fraction collection procedure. The amount of this material was determined by rinsing the bottom of the tube with Nuclear-Chicago Solubilizer (Nuclear-Chicago Corp., Des Plaines, Ill.) and counting as described before (Chiu and Rauth, 1972). The radioactivity of this washing appears as the 22nd fraction in all of the figures. The direction of sedimentation in all figures is from left to right. Controls were always prepared to determine the total amount of radioactivity recovered. The percentage of input radioactivity recovered from the gradient was 70–100%. The gradient was calibrated using [^{14}C]thymidine-labeled λ-DNA (courtesy of Dr. A. Becker). The molecular weight corresponding to each fraction was calculated by using the Burgi-Hershey equation (Burgi and Hershey, 1963) and Studier's formula (Studier, 1965).

Measurement of DNA Synthesis

The relative amount of DNA synthesis, as measured by [^{3}H]thymidine incorporation, occurring after UV irradiation of L and HeLa cells was determined directly from recovery controls of the appropriate gradients. That is, cells in exponential growth were irradiated with 0, 100, 200, or 400 ergs/mm^2 in growth medium at 37°C. The cells were incubated 40–60 min at 37°C before pulse labeling for 30 min with high specific activity [^{3}H]thymidine as described earlier. The same volume and number of cells that were put on the gradient were also placed in 1 ml of 10% alkaline sucrose and left for the duration of the experiment. The radioactivity in this recovery control was determined in the same way as for fractions collected from the gradients. Thus for each UV exposure, the number of counts per minute per cell of [^{3}H]thymidine incorporated into irradiated cells relative to unirradiated cells was determined by taking the ratio of the unirradiated cell counts to the irradiated cell counts and multiplying by 100.

Measurement of Thymine Dimers

[^{14}C]thymidine labeled cells were exposed for various times to UV irradiation in phosphate-buffered saline. The cells were spun down, resuspended in trifluoroacetic acid, and hydrolyzed

at 170°C for 1 h. After the hydrolysates had cooled down the tubes were broken open and the acid was evaporated by a stream of air. The hydrolysate was redissolved in distilled water, streaked on 3 × 57 cm strips of Whatman no. 1 paper and run in the descending mode in *n*-butanol:water:glacial acetic acid, 80:30:12 for 18 h at room temperature. This solvent separates thymine-cytosine dimers (measured as thymine-uracil dimers) from thymine-thymine dimers (Setlow and Carrier, 1966). The distribution of radioactivity on the chromatogram was determined by cutting the strip at 1 cm intervals and counting each 1 cm section of the strip in a liquid scintillation counter. The ratio of total activity on the strip to that at the position of the thymine-thymine dimer was determined for each exposure value and each cell type.

Measurement of Ultraviolet Light Survival Curves

The ability of each cell type to survive UV irradiation as measured by its ability to form a colony of cells was measured as follows. Cells growing in α-medium plus 10% fetal calf serum were centrifuged at 200 *g* for 7 min at room temperature and resuspended in phosphate-buffered saline. 5-ml aliquots of cells were exposed to UV light for different exposure times and the cells were then diluted so a known number of cells was plated for each exposure and dilution point in triplicate. Cells were plated in 5 ml of α-medium plus 10% fetal calf serum in 60 mm Falcon Plastic petri dishes (Falcon Plastics, Los Angeles, Calif.) and incubated for 10–14 days at 37°C in incubators with a 5%-CO_2, 95%-air atmosphere at high humidity. At the end of the incubation period the media was poured off and the cells stained with methylene blue. A cell was considered to have survived if it gave rise to a colony containing 50 or more cells. The plating efficiency of control cells for all three lines in the present experiment was in the range of 0.5–1.0.

RESULTS

A comparison was made of the ability of three different established cell lines to synthesize DNA after various exposures of UV irradiation. The newly synthesized DNA was analyzed on alkaline sucrose density gradients in order to compare the size of the pieces of the DNA made in these cells.

Mouse (L), human (HeLa), and Chinese hamster (CHO) cells were all grown in the same growth medium in separate suspension cultures and maintained in the exponential phase of growth. Aliquots of cells were removed from spinner culture and resuspended in medium deficient in thymidine. The cells were exposed to 0, 100, 200, or 400 ergs/mm^2 of UV light in this medium at 37°C and immediately transferred to a roller wheel where they were maintained in suspension culture at 37°C for 40–60 min. At this time all cultures were labeled with high specific activity [^{3}H]thymidine. 30 min later, 100 μg/ml of thymidine was added to reduce the specific activity of the [^{3}H]thymidine and the cells were transferred to 0°C to stop DNA synthesis. The cells were then washed free of medium, resuspended in lysis buffer, and placed on the top of alkaline sucrose gradients. After 1.5 h lysis time the cells were spun at 40,000 rpm for 1.5 h; fractions were then collected, and radioactivity per fraction was determined. The results are shown in Fig. 1. The DNA profiles for all three cell lines at 0 exposure were found to be similar with peaks occurring at fractions 14–16. The bimodal distribution found for HeLa cells in this particular

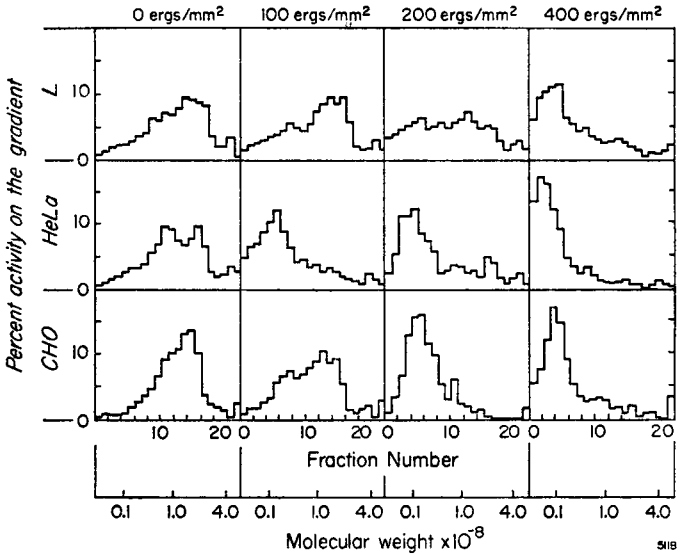

FIGURE 1 Sedimentation profiles of DNA synthesized after exposure of L, HeLa, or CHO cells to 0, 100, 200, or 400 ergs/mm² of UV irradiation. Cells were incubated at 37°C for 45 min after irradiation, then labeled for 30 min at 37°C and immediately layered onto gradients. Conditions of centrifugation: 40,000 rpm for 1.5 h at 20°C.

experiment was the exception and in all other experiments a distribution similar to that in Fig. 3 *a* was seen. The decrease in the apparent molecular weight distribution for L cells as a function of UV exposure is obviously quite different from that observed from HeLa cells. After 100 ergs/mm² the molecular weight distribution for HeLa cells is shifted much more strongly to the low molecular weight region than for L cells. As the irradiation exposure increases the differences become less and at 400 ergs/mm² both L and HeLa cell nascent DNA is shifted to low molecular weight. Chinese hamster ovary cells appear intermediate between L and HeLa cells in the effect of UV irradiation on the molecular weight distribution of newly synthesized DNA.

At the highest UV exposure used, 400 ergs/mm², the DNA is of small molecular weight and, under the conditions of the experiment in Fig. 1, it is at the top of the gradient where it is not possible to accurately measure its molecular weight distribution. Thus the protocol of the experiment shown in Fig. 1 was repeated for the 400 ergs/mm² exposure for L cells and HeLa cells with the following differences. The gradients were spun for 3 h instead of 1.5 h and [^{14}C]thymidine-labeled λ-bacteriophage DNA was added to the L cell gradient as a marker. The results are shown in Fig. 2. Again HeLa cell DNA sediments as a lower molecular weight distribution than L cell DNA but the difference between the two is relatively small. The λ-DNA marker (mol wt 1.5 × 10^7 daltons) sediments at fraction 11, very similar to its location in our previous gradient work (Chiu and Rauth, 1972). In addition, this

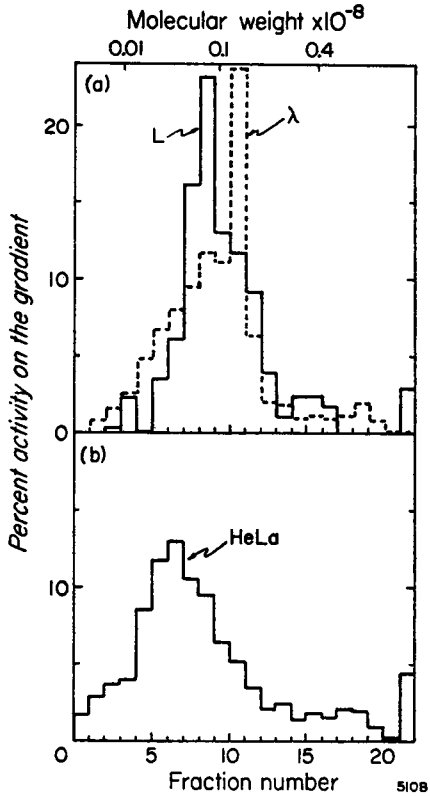

FIGURE 2 Sedimentation profiles of DNA synthesized after exposure of L or HeLa cells to 400 ergs/mm^2 of UV irradiation. Cells were incubated at 37°C for 60 min after irradiation before labeling for 30 min at 37°C. Immediately after labeling, cells were layered on gradients. A [^{14}C]thymidine-labeled λ-DNA marker was run with the L cell gradient (broken line). Conditions of centrifugation: 40,000 rpm for 3.0 h at 20°C.

result indicates that the radioactivity appearing in the low fraction numbers, after high UV exposures (Fig. 1), is due to relatively large pieces of DNA and does not represent small DNA oligonucleotides which cannot be resolved in the present system.

It had been shown previously for mouse L cells in the present gradient system (Chiu and Rauth, 1972) and for human cells in a different gradient system (Buhl et al., 1972) that pulse labeled DNA of lower molecular weight observed in irradiated cells could be chased to higher molecular weight DNA by subsequent incubation at 37°C. To make sure that the human cell DNA behaved the same way on the present gradient system the following experiment was done. Aliquots of HeLa cells in exponential phase growth were resuspended in thymidine-deficient medium, irradiated with 0 or 100 ergs/mm^2 of UV light and incubated for 50 min at 37°C. At this time they were pulse labeled with high specific activity [^{3}H]thymidine. After

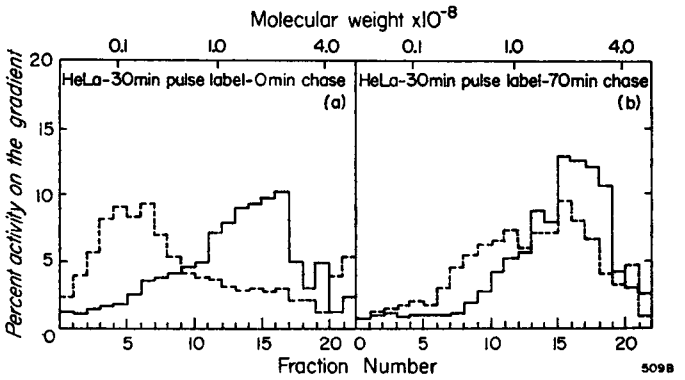

FIGURE 3 Sedimentation profiles of DNA from HeLa cells exposed to 0 (solid line) or 100 ergs/mm² (broken line) of UV irradiation. The cells were incubated for 50 min at 37°C after irradiation before labeling for 30 min at 37°C. The label was chased in the presence of 100 μg/ml of nonradioactive thymidine for (*a*) 0 min or (*b*) 70 min for both unirradiated and irradiated cells. Conditions of centrifugation: 40,000 rpm for 1.5 h at 20°C.

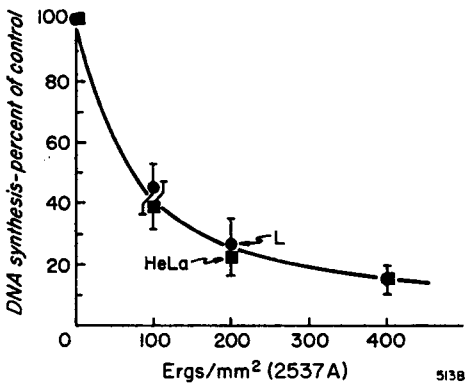

FIGURE 4 Percent incorporation of [³H]thymidine into L (circles) or HeLa (squares) cells as a function of UV exposure. Cells were incubated at 37°C for 45–60 min after irradiation before labeling for 30 min at 37°C. Points are the average values for four to six separate determinations and error bars indicate the standard deviations for these points.

30 min, the pulse was terminated by the addition of 100 μg/ml of thymidine. Samples of both irradiated and control cells were removed and stored at 0°C, while other samples of control and irradiated cells were incubated a further 70 min at 37°C before being placed at 0°C. All samples of cells were then washed and put on gradients; their DNA was sedimented, fractions were collected, and the distribution of radioactivity as a function of fraction number was determined. The results are shown in Fig. 3 *a* and *b*. The distributions observed for the 0 min chase are very similar to those shown in Fig. 1. After the 70 min chase the molecular weight distribution of the irradiated cell DNA had shifted and was now very similar to that of the control. This result shows that the nascent human cell DNA has similar

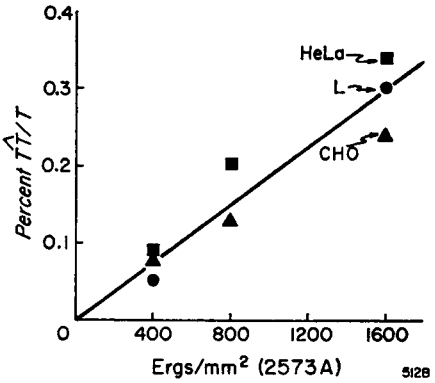

FIGURE 5 Percent of total thymine (T) present as thymine-thymine dimer ($\widehat{TT}$) as a function of UV irradiation for HeLa (squares), L (circles), or CHO (triangles) cells.

pulse-chase properties on the present gradient as has been observed by Buhl et al. (1972), using different techniques.

One explanation for the differences in Fig. 1 is that the amount of DNA synthesis after the same exposure to radiation differs among the different cell lines tested. It has been shown previously that at short labeling times (0–1 h), the apparent low molecular weight to high molecular weight transition is a function of the length of the pulse and the time of the subsequent chase for both mouse (Chiu and Rauth, 1972) and human (Buhl et al., 1972) cells. In Fig. 4 a plot has been made of the amount of trichloroacetic acid insoluble radioactive material incorporated into cells under the conditions of Fig. 1. As can be seen, there is no significant difference in the amount of new DNA synthesis, as measured by [^{3}H]thymidine incorporation, as a function of UV irradiation between L cells and HeLa cells. The data for CHO cells, not shown in Fig. 4, is not signiffcantly different from these two established cell lines. Thus, relative to unirradiated cells, no difference is observed among the cell lines in the amount of DNA synthesized after various exposures of UV irradiation.

A second possible reason for the difference in the molecular weight distributions between these cell lines may be that there was a difference in the actual amount of UV damage produced in the cell. To test this possibility, all three cell lines were prelabeled for one generation time with [^{14}C]thymidine and then exposed to 0, 400, 800, or 1,600 ergs/mm^2 of UV. The percent of the total radioactive thymidine in the cells DNA which was converted to thymine-thymine dimer as a function of exposure was then measured by acid hydrolysis and paper chromatography. The results are shown in Fig. 5. Though the data points scatter, the yield of dimer as a function of a dose is similar, within experimental uncertainty, for the three cell lines tested. Thus, to the degree that the production of thymine dimers is a measure of UV damage, no difference in the amount of initial photoproducts in the three established cell lines was seen in this experiment.

One of the differences observed among the three cell lines was in their ability to

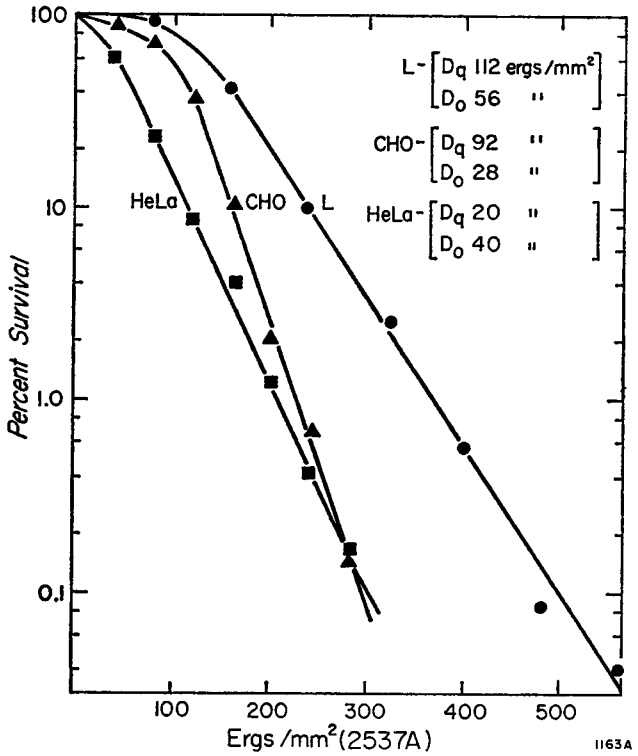

FIGURE 6 Percent survival of colony forming ability as a function of UV exposure for L (circles), CHO (triangles), or HeLa (squares) cells. The D_o (exposure to reduce survival by 37% along the exponential part of the survival curve) and D_q (intercept of the back extrapolation of the exponential portion of the survival curve to the 100% survival level) for each cell line are also listed.

survive UV irradiation at the cellular level. To measure cell survival, samples of the three cell lines were taken from suspension culture in exponential growth phase, resuspended in phosphate-buffered saline, irradiated with different exposures of UV, and assayed for their colony forming ability. Fig. 6 shows the percent survival of colony forming ability as a function of UV exposure. The cell lines do differ in their response to UV. Though these dififerences are no greater than a factor of two in D_o (the dose to reduce survival by 37% along the exponential portion of the survival curve), the difference in D_q (the value of the back extrapolation of the exponential portion of the survival curve to the 100% level) are as large as a factor of 5. There does appear to be a correlation between the resistance of the cells to UV and their ability to produce nascent DNA of a molecular weight similar to unirradiated cells.

DISCUSSION

A comparison of previous work on new DNA synthesis in ultraviolet light irradiated mouse L cells (Chiu and Rauth, 1972) to similar experiments with various strains

and lines of human cells (Buhl et al., 1972) indicates some similarities and some differences. Both UV irradiated mouse and human cells show a decrease in the molecular weight of newly synthesized DNA when compared with unirradiated cells. Part of this decrease is due to the fact that the rate of DNA synthesis is slowed in UV-irradiated cells so that less total DNA is made than in control cells. Since, for unirradiated cells, the molecular weight distribution of newly synthesized DNA is shifted to lower molecular weights for short labeling times (5–30 min) compared with long labeling times, this fact by itself accounts for part of this decrease (Chiu and Rauth, 1972, Buhl et al., 1972).

In mouse L cells, at low UV exposures (0–200 ergs/mm^2), when a correction is made for the effects of UV on the rate of DNA synthesis, little difference remains between unirradiated and irradiated cells in the molecular weight distribution of their newly synthesized DNA. At higher UV exposures, even when conditions are adjusted so unirradiated and irradiated cells make the same total amount of DNA, the molecular weight distribution of the irradiated cells is shifted to lower molecular weights than for unirradiated cells. One possible explanation of this result is that pyrimidine dimers produced in the template DNA cause interruptions in new DNA synthesis. Quantitatively, however, it would appear only a fraction of the dimers produced cause a measurable interruption in the newly synthesized DNA of mouse L cells (Chiu and Rauth, 1972). However in human cells (Buhl et al., 1972) and mouse L5189Y cells (Lehmann, 1972) even when conditions are adjusted so that the total amount of DNA synthesized is the same, newly synthesized DNA in the irradiated cells is still shifted to a lower molecular weight than for unirradiated cells at low UV exposures. Quantitatively each pyrimidine dimer appears to cause an interruption in new DNA synthesis. When such irradiated, pulse labeled cells are incubated for several hours before they are placed on gradients, the molecular weight distribution of their DNA is shifted so it is similar to that of unirradiated cells (Buhl et al., 1972). At the higher UV exposures L cells behave similarly in a pulse-chase experiment (Chiu and Rauth, 1972).

However, the relation between the molecular weight of newly synthesized DNA and dimer frequency seen in human cells at short times after irradiation may be fortuitous. Buhl et al. (1973) have shown that at longer times after irradiation both human cells, which excise dimers, and cells of human mutants xeroderma pigmentosum, which do not, recover their ability to synthesize DNA in segments of normal size similarly as a function of time after irradiation. They interpret this as indicating that pyrimidine dimers may not be the lesions that cause DNA to be synthesized in smaller than normal segments at short times after irradiation.

The alkaline sucrose density gradient techniques used to measure the molecular weight distributions in the mouse L cell (Chiu and Rauth, 1972) differed from that used in the human cell work (Buhl et al., 1972) in a number of ways. These differences could explain the difference in the results obtained using human and mouse cells. That this is not the explaination is indicated by the results in Fig. 1. Here it

can be seen that even when the mouse (L) and human (HeLa) cells are grown, irradiated, and analyzed under as nearly identical conditions as possible a difference remains in the molecular weight distribution of newly synthesized DNA as a function of UV exposure. The difference between L and HeLa cells persists even after 400 ergs/mm^2 of UV, but this difference is much less than at the lower exposures (Fig. 2). The data with CHO cells resemble L cells at 100 ergs/mm^2, but are more similar to HeLa cells at 200 ergs/mm^2. It should be noted that the unirradiated cells have similar molecular weight distributions of newly synthesized DNA after a 30 min pulse of radioactive thymidine. These results indicate that the differences in nascent DNA synthesis between irradiated mouse and human cells is a function of cell type and not the experimental conditions used.

Experiments were done, which are not shown, using the gradient technique of Buhl et al. (1972) and qualitatively similar differences were seen between mouse and HeLa cells. Quantitatively, the DNA molecular weight distribution of unirradiated cells was shifted to a lower molecular weight region than in our gradient procedure. This appeared to be mainly due to the fact that in the technique of Buhl et al. (1972) the cells are preirradiated with 2,000 rads of X-rays before layering them on the gradient. Preirradiation of unirradiated cells prior to layering them on our gradients resulted in a small lowering in their molecular weight distribution but did not change the results of Fig. 1 appreciably.

The ability of UV irradiated HeLa, L, or CHO cells to synthesize new DNA as measured by [^{3}H]thymidine incorporation did not appear to differ (Fig. 4). In addition, the amount of ultraviolet light damage produced, as measured by thymine-thymine dimer production, was similar in all three cell lines (Fig. 5). Thus, these two factors could not be used to explain the above differences. For the three cell lines tested, there did appear to be a correlation between the frequency of small segments of newly synthesized DNA and the amount of cell killing as measured by the ability of an irradiated cell to form a colony (Fig. 6). In fact, if one compares the gradient distributions in Fig. 1 not on the basis of incident UV exposure but at equal survival levels, the sedimentation patterns of the newly synthesized DNA in these three cell lines are rather similar. For example, the nascent DNA distribution for L cells after 400 ergs/mm^2 (survival 0.6%) is similar to that for HeLa and CHO cells at 200 ergs/mm^2 (survival 1.0 and 1.8%, respectively). One explanation for this is that the cell lines have differences in the fine structure of their DNA replication "complexes" which cause the size of newly synthesized DNA to differ after UV irradiation and this results in differences in cell killing. This correlation may be fortuitous however, since it has been shown that the nascent DNA synthesis in unirradiated normal human cells and xeroderma pigmentosum cells is similar (Buhl et al., 1972) though their colony forming abilities after UV irradiation differ greatly (Cleaver, 1970).

The degree to which these results indicate a difference in the mechanisms of L and HeLa cells in coping with UV damage is not clear. As has been pointed out

earlier the same number of gaps may be found in newly synthesized L and HeLa cell DNA after irradiation, but in L cell DNA these may be closed faster at the lower exposures (Chiu and Rauth, 1972). Alternatively a different method of by-passing UV damage may exist in L cells than in HeLa cells (Klimek and Vanicek, 1970). For all cell lines investigated, however, these results once again focus attention on the DNA synthetic phase of the cell cycle and indicate that normal DNA synthesis may be closely involved with the repair or modification of UV damage in mammalian cells.

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