Excision of Pyrimidine Dimers from Epidermal DNA and Nonsemiconservative Epidermal DNA Synthesis following Ultraviolet Irradiation of Mouse Skin

G. T. Bowden, James E. Trosko, B. G. Shapas, and R. K. Boutwell

McArdle Laboratory for Cancer Research, University of Wisconsin Medical School, Madison, Wisconsin 53706

SUMMARY

Pyrimidine dimer production and excision in epidermal DNA were studied at five different dose levels of ultraviolet light in the skin of intact mice. Dimer production increased with dose up to 50,400 ergs/sq mm. Approximately 30% of the thymine-containing dimers were excised by 24 hr after irradiation at three lower dose levels of ultraviolet light. Nonsemiconservative DNA replication in ultraviolet-irradiated mouse skin was shown to continue for at least 18 hr. The rate of nonsemiconservative replication decreased with time, but did so slowly. The initial rates of nonsemiconservative replication increased with ultraviolet light dose level up to about 4,200 ergs/sq mm, after which the initial rates were decreased. Semiconservative epidermal DNA synthesis was shown to be inhibited by hydroxyurea, but hydroxyurea had no effect on ultraviolet light-induced nonsemiconservative DNA replication. The observed pyrimidine dimer excision and nonsemiconservative DNA replication suggest that in the intact mouse the cells of the epidermis are capable of DNA excision repair after ultraviolet irradiation of mouse skin.

INTRODUCTION

One type of repair of damaged DNA has been shown to involve the replacement of damaged regions in DNA, using the complementary intact strand for base-pairing information. This repair process (excision repair) was proposed as a result of the demonstration that certain UV-resistant strains of bacteria selectively remove UV-induced pyrimidine dimers from the DNA (5, 46). Direct evidence for the postulated replacement of the excised regions was shown in bacteria by the demonstration of nonsemiconservative DNA synthesis after UV irradiation (38, 39).

Similar studies have been carried out with mammalian cells grown in culture. In general, human cells grown in culture are capable of excision of UV-induced pyrimidine dimers (43). Within 12 to 24 hr after irradiation of human amnion cells, HeLa cells, or diploid cell strain WI-38, approximately 50% of the UV-induced pyrimidine dimers were lost from their DNA. HeLa cells have also been shown to perform nonsemiconservative DNA synthesis following UV irradiation (12, 41), and this repair replication after irradiation (20) is measurable for 18 hr or longer. Although no direct evidence has been provided that this repair replication is involved in the recovery of cells from damage induced by UV irradiation, it has been shown in HeLa cells that DNA that has undergone repair replication can later participate in normal semiconservative synthesis (37).

In contrast to human cells in culture, rodent cells in culture in general have not been found to excise pyrimidine dimers. Pyrimidine dimers have been induced in Chinese hamster (51, 54, 55) and mouse L-cells (33); however, in these studies there was no evidence for the loss or excision of dimers from the DNA subsequent to UV irradiation. Contrary to this finding, it has been shown that both of these rodent cell lines are capable of carrying out nonsemiconservative DNA synthesis after UV irradiation but to a lesser extent than human cells (11). Recently, Setlow et al. (47) and Lehmann (34) have shown that there was a small amount of excision of UV-induced pyrimidine dimers at low doses in rodent cells. Also, Ben-Ishael (1) has reported that cells of mouse embryos in earlier passages excise pyrimidine dimers, but those cells of later passages, as well as those cells of established lines, do not. It is not known why these rodent cells do not excise pyrimidine dimers but do perform nonsemiconservative DNA synthesis in response to UV irradiation.

All the studies of the pyrimidine dimer excision and nonsemiconservative DNA synthesis have been carried out in cells grown in culture. The question remained as to whether the results obtained in cell culture could be extrapolated to the intact animal. Because of the observation that many rodent cells grown in culture cannot excise thymine dimers (54, 55), it was of special interest to study in the intact mouse whether skin epidermal cells were capable of excising UV-induced thymine-containing dimers and were capable of performing nonsemiconservative DNA synthesis. Moreover, UV irradiation has been shown to cause skin tumors in mice when used both as a complete carcinogen (2) and also as an initiator in a 2 stage system of carcinogenesis (40).
MATERIALS AND METHODS

Animals. Female Charles River CD-1 mice were purchased from Charles River Farms, North Wilmington, Mass. Mice 7 to 9 weeks old were carefully shaved with surgical clippers 4 days before use. Hair not removed by shaving was removed 1 day before use with the depilatory agent Nudil. Only those mice in the resting stage of the hair cycle were used.

Chemicals. Solvents and chemicals used were reagent grade and were used without further purification unless otherwise stated. BrdUrd, 5-FU, and hydroxyurea were purchased from Sigma Chemical Co., St. Louis, Mo. 2-Butoxyethanol (scintillation grade) was obtained from Eastman Kodak Co., Rochester, N. Y. [methyl-3H]Thymidine (40 to 60 Ci/m mole) was obtained from New England Nuclear, Boston, Mass.

UV Light Source and Irradiation of the Mice. A bank of 4 15-W G 15T8 ITT germicidal A lamps (primarily 254 nm) was used to irradiate the backs of the mice. The lamps were held 6.5 cm from the surface of the backs of the mice, which were retained in a wire mesh cage in individual compartments. The cage measured 23 x 25 cm and contained 24 rectangular compartments. The intensity of the UV, measured at 6.5 cm from the lamps with a Blak-Ray short-wave (J-225) UV meter (Ultra-Violet Products, Inc., San Gabriel, Calif.), was 280 ergs/sq mm/sec for each irradiation. The various UV doses were obtained by varying the time of exposure. The mice were kept in semidarkness for the duration of the experiments.

Pyrimidine Dimer Measurements in Mouse Skin

Epidermal DNA. Mouse skin epidermal basal cell DNA was prelabeled by the following procedure. Groups of 10 mice kept in semidarkness were given i.p. injections of 2.5 mg of 5-FU (in 0.2 ml 0.9% NaCl solution) at 0 hr. After 1 hr, the mice received an injection of 200 μCi [methyl-3H]thymidine (40 to 60 Ci/m mole) in a 5-ml air pouch underneath the skin of the back (3). One hr after the air-pouch injection, the mice received another i.p. injection of 2.5 mg 5-FU, and 3 hr after the 1st 5-FU injection, the mice received another air-pouch injection of 200 μCi [methyl-3H]thymidine. Five hr after the 1st 5-FU injection, the mice were irradiated with the proper dose of UV in semidarkness and killed either immediately after irradiation (0 hr) or 24 hr after irradiation. With this method of prelabeling, we obtained epidermal DNA with specific activities from 1000 to 4000 cpm/μg of DNA. It will be shown in “Results” that, 2 hr after the last [methyl-3H]thymidine injection, there is no more incorporation of the labeled DNA precursor. After cervical dislocation, the whole skin was removed from the mouse and the epidermis was separated from the whole skin by a modification (4) of the method of Marrs and Voorhees (35). After isolation, the epidermal tissue was frozen in liquid nitrogen and ground to a powder. DNA was extracted from the powdered epidermis by a modified (4) Kirby-Cook (31) procedure. Typical DNA preparations contained no protein and less than 3% RNA.

Epidermal DNA in standard saline citrate (0.15 M sodium chloride-0.015 M sodium citrate, pH 7.4) was dialyzed against distilled water at 4° for 2 days with 1 change of distilled water. The dialyzed DNA was then lyophilized to dryness and stored in the dark at 4° until the pyrimidine dimer content of the samples was measured. Epidermal DNA (300 μg) was hydrolyzed in formic acid, and the DNA hydrolysate was chromatographed on paper in a 2-dimensional system in order to separate and quantify radioactivity associated with thymine and thymine-containing dimers, by the method described by Carrier et al. (6). The pyrimidine dimer data are expressed as the percentage radioactivity associated with thymine-containing dimers versus the radioactivity associated with thymine.

Nonsemiconservative DNA Synthesis following UV Irradiation. It has been shown that BrdUrd is incorporated into replicating epidermal DNA of mouse skin (4), and this technique was used to distinguish between semiconservative and nonsemiconservative DNA synthesis following UV irradiation of mouse skin. DNA repair synthesis was studied in 3-hr intervals from 0 to 24 hr after UV irradiation. For the 0- to 3-hr interval, groups of 10 mice were given i.p. injections of 0.5 mg BrdUrd and 0.5 mg 5-FU 1 hr and one-half hr before irradiation. It will be shown in “Results” that these injections are necessary to make certain that any DNA replicated after irradiation would have sufficient BrdUrd incorporated to shift its density on a CsCl gradient. At 0 hr, the mice were irradiated and, immediately thereafter, were given an i.p. injection of 0.5 mg BrdUrd, 0.5 mg 5-FU, and 30 μCi [methyl-3H]thymidine (in 0.1 ml 0.9% NaCl solution). This injection was repeated every half-hr for 2.5 hr (a total of 6 injections). The mice thus received, over a 3-hr period, a total of 180 μCi of [methyl-3H]thymidine. The mice were killed at the end of 3 hr, and epidermal DNA was isolated (4). For the other 3-hr intervals, the mice were treated similarly but, in addition, 1 hr and one-half hr before the 3-hr labeling period, the mice received i.p. injections of 0.5 mg BrdUrd and 0.5 mg 5-FU.

Nonsemiconservative DNA synthesis was distinguished from semiconservative synthesis through the use of CsCl gradient isopycnic centrifugation. Neutral CsCl as well as alkaline CsCl, Cs₂SO₄ gradients were made according to a modified (3) method of Gautschi et al. (24).

Specific Activity Measurement of Nonsemiconservative DNA Synthesis. The radioactivity and absorbance profiles from neutral and alkaline gradients showed the relative distribution of radioactive and UV-absorbing material but, in most cases, these profiles were not used to obtain quantitative estimates of nonsemiconservative [methyl-3H]thymidine incorporation. Rather, quantitative estimates were obtained by pooling DNA from appropriate fractions and dialyzing to remove CsCl. The specific activity of the DNA was determined by measuring the radioactivity in 1.0 ml of the dialyzed DNA, using Scintisol counting solution (4). The quantity of DNA in a dialyzed sample was determined by a modification (4) of the fluorometric determination of Kissane and Robins (32).
Modified Schmidt-Thannhauser Procedure for Extraction of Epidermal DNA from Mouse Skin. The mice were killed by cervical dislocation and the whole skin was excised. Epidermis was isolated from whole skin by the Cowdry (15) cold acetic acid procedure. DNA was extracted from the epidermis by a modification (49) of the Schmidt-Thannhauser procedure (44). The specific activity of the DNA was measured by counting aliquots of the hydrolyzed DNA in 10 ml of Scintisol on a Packard Tri-Carb liquid scintillation counter and determining the DNA concentration by the diphenylamine reaction (45).

RESULTS

Pyrimidine Dimer Production and Excision in Mouse Skin. Pyrimidine dimer induction and possible excision were studied in mouse skin at 5 different dose levels of UV irradiation. It was first established by autoradiographic studies that prelabeling with [methyl-3H]thymidine labeled only basal cells of the epidermis. Experiments were also performed to show that, 2 hr after the last [methyl-3H]thymidine air-pouch injection, which was part of the prelabeling procedure, there was no further incorporation of [methyl-3H]thymidine into epidermal DNA, which would lead to erroneous values for pyrimidine dimer contents. In this experiment, mice that had been prelabeled with [methyl-3H]thymidine were given a series of 8 injections of BrdUrd and 5-FU every 0.5 hr for 4 hr starting 2 hr after the last [methyl-3H]thymidine injection. The results of this experiment are shown in Chart 1A. Most of the radioactivity was associated with the non-BrdUrd-substituted DNA present toward the top of the gradient. These results indicate that, 2 hr after the last [methyl-3H]thymidine injection, there is no further incorporation of the labeled precursor into epidermal DNA. For confirmation that the BrdUrd labeling procedure is capable of shifting the density of replicated DNA on a CsCl gradient after an air pouch injection of [methyl-3H]thymidine, mice were given air pouch injections of [methyl-3H]thymidine at the same time that they received the BrdUrdd-FU i.p. injections. The results of this experiment are shown in Chart 1B. Most of the tritium radioactivity banded at the BrdUrd-substituted (replicated DNA) position of the gradient. This experiment demonstrated the validity of the BrdUrd labeling procedure.

The results of the thymine-containing dimer production and excision experiments are given in Table 1 in which the data are expressed as the percentage of radioactivity associated with thymine-containing dimers versus the amount of radioactivity associated with thymine. The 6 dose levels of UV irradiation studied were 0, 4,200, 8,400, 16,800, 50,400, and 84,000 ergs/sq mm. Thymine-containing dimer yields at 0 erg/sq mm of UV irradiation were at a very low background level (average yield, 0.005%). The initial (0 hr) thymine-containing dimer yields increased with dose at the 4 lower dose levels (4,200, 0.076%; 8,400, 0.130%; 16,800, 0.322%; 50,400, 0.566%). At the highest dose level (84,000 ergs/sq mm), the thymine dimer yield increased no further (0.560%). The ratio of thymine-containing dimers to thymine measured 24 hr after UV irradiation had decreased, compared with the ratio immediately after irradiation at all dose levels. The decrease ranged from 29 to 61%, had no relation to irradiation dose, and was statistically significant (39% reduction) at the dose level (4,200 ergs/sq mm) at which sufficient observations were made to allow statistical analysis.

The Effect of Dose Level of UV on the Loss of Prelabeled Skin Epidermal DNA. In order to determine whether there were large cell losses 24 hr after the various dose levels of UV irradiation which could bias the dimer excision results, mouse skin epidermal DNA was prelabeled with [methyl-3H]thymidine. Control (unirradiated) groups of mice were compared with UV-irradiated groups in terms of epidermal DNA-specific activity 24 hr after irradiation. It has been shown previously (29) that, after an i.p. injection of [methyl-3H]thymidine, the level of precursor incorporation into epidermal DNA plateaus after one-half hr and remains at the same level for 24 hr. Thus, for each determination, 2 groups of 5 mice each were given i.p. injections of 30 pCi [methyl-3H]thymidine and, 2 hr later, 1 group of mice was irradiated. Both groups of mice were killed 24 hr after...
irradiation, and epidermal DNA was isolated by a modified Schmidt-Thannhauser procedure. The specific activity in dpm/μg DNA was determined, and the data are presented in Table 2. At the lowest dose level (4,200 ergs/sq mm), there was no significant difference in specific activities between control and irradiated groups (p = 0.2). However, significant differences were found at the 4 remaining dose levels of irradiation. The specific activity data indicated approximately a 20% loss of prelabeled epidermal DNA 24 hr after irradiation of mouse skin with 8,400, 16,800, 50,400, and 84,000 ergs/sq mm of UV.

Nonsemiconservative DNA Synthesis following UV Irradiation. The procedures previously described for distinguishing semiconservative DNA synthesis (replicative) from nonsemiconservative DNA synthesis (repair) were used to study normal and repair DNA synthesis after all 6 dose levels of UV irradiation. The results from 3 of the 6 dose levels are depicted in Chart 2. For each dose level of UV irradiation, a neutral CsCl gradient and an alkaline CsCl, Cs2SO4 reband of the non-BrdUrd-substituted, light-light DNA from a group of mice that had received a UV dose of 8400 ergs/sq mm. Nonsemiconservative DNA synthesis was evident at this dose of UV irradiation. When hydroxyurea was used during the 3-hr labeling period after irradiation, the alkaline reband depicted in Chart 3D was obtained. Again nonsemiconservative DNA synthesis was evident, and the incorporation of [methyl-3H]thymidine was not reduced by hydroxyurea treatment.

In order to quantify the amount of repair replication as a function of dose of UV irradiation, repaired DNA was isolated in a double-stranded form on a CsCl gradient, and its specific activity was measured. Hydroxyurea was given along with [methyl-3H]thymidine, BrdUrd, and 5-FU during labeling. Thus, groups of mice were given i.p. injections of 0.5 mg BrdUrd and 0.5 mg 5-FU at −1 hr and −0.5 hr and were irradiated with the specified dose of UV at 0 hr. Immediately after irradiation, the mice received 30 μCi [methyl-3H]thymidine, 0.5 mg BrdUrd, 0.5 mg 5-FU, and 2.5 mg hydroxyurea in a single i.p. injection. A similar injection was administered every one-half hr for 2.5 hr, and the mice were killed 3 hr after irradiation. Skin epidermal DNA was banded on neutral CsCl gradients and the light-light double-stranded DNA was pooled from 7 fractions, dialyzed to remove CsCl, and the specific activity was determined.

Quantitative measurements of the effect of increasing levels of irradiation with UV on the amount of repair replication are shown in Table 3. The specific activity of the

### Table 1
Thymine-containing dimer production and excision in mouse skin epidermis after exposure of mice to UV

<table>
<thead>
<tr>
<th>UV irradiation (ergs/sq mm)</th>
<th>Time after irradiation (hr)</th>
<th>Dimers/thymine (%)</th>
<th>Dimers* excised (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.005 ± 0.005</td>
<td>43</td>
</tr>
<tr>
<td>4200</td>
<td>0</td>
<td>0.076 ± 0.023</td>
<td>39</td>
</tr>
<tr>
<td>8400</td>
<td>0</td>
<td>0.046 ± 0.016</td>
<td>0.017</td>
</tr>
<tr>
<td>16,800</td>
<td>0</td>
<td>0.130 ± 0.010</td>
<td>29</td>
</tr>
<tr>
<td>32,000</td>
<td>0</td>
<td>0.134 ± 0.02</td>
<td>16</td>
</tr>
<tr>
<td>50,400</td>
<td>0</td>
<td>0.586 ± 0.059</td>
<td>61</td>
</tr>
<tr>
<td>84,000</td>
<td>0</td>
<td>0.560 ± 0.24</td>
<td>43</td>
</tr>
<tr>
<td>84,000</td>
<td>24</td>
<td>0.320 ± 0.036</td>
<td>43</td>
</tr>
</tbody>
</table>

* The thymine-containing dimer production is expressed as the ratio (in terms of percentage) of radioactivity associated with thymine-containing dimers to the radioactivity associated with thymine.

Table 2
The effect of various doses of UV on the loss of prelabeled epidermal DNA

<table>
<thead>
<tr>
<th>UV irradiation (ergs/sq mm)</th>
<th>Control DNA (dpm/μg DNA)</th>
<th>Irradiated DNA (dpm/μg DNA)</th>
<th>Irradiated DNA/Control DNA (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>4200</td>
<td>149 ± 34*</td>
<td>172 ± 17*</td>
<td>108</td>
<td>0.2</td>
</tr>
<tr>
<td>8400</td>
<td>117 ± 14</td>
<td>93 ± 12</td>
<td>79</td>
<td>0.04*</td>
</tr>
<tr>
<td>16,800</td>
<td>100 ± 2</td>
<td>80 ± 5</td>
<td>80</td>
<td>0.0005*</td>
</tr>
<tr>
<td>50,400</td>
<td>121 ± 12</td>
<td>92 ± 22</td>
<td>76</td>
<td>0.11*</td>
</tr>
<tr>
<td>84,000</td>
<td>124 ± 4</td>
<td>114 ± 11</td>
<td>81</td>
<td>0.01*</td>
</tr>
</tbody>
</table>

* Mean ± S.D. of from 3 to 5 values, each determined with a different group of mice.

At these dose levels, the p values suggest that irradiation with UV caused a significant loss of prelabeled epidermal DNA.
DNA Excision Repair in Epidermis of Intact Mice

Chart 2. Repair replication in mouse skin epidermis following in vivo treatment with various dose levels of UV. Groups of 10 mice were given i.p. injections of 0.5 mg BrdUrd and 0.5 mg 5-FU 1 hr and 0.5 hr before irradiation with UV lamps. Immediately after irradiation, the mice received an i.p. injection of 30 μCi [methyl-3H]thymidine, 0.5 mg BrdUrd, and 0.5 mg 5-FU. The mice received this injection every 0.5 hr for 2.5 hr. At 3 hr after irradiation, the mice were killed and DNA was isolated from the epidermis of the excised skin of the back. The DNA was subjected to neutral CsCl gradient centrifugation, and light-light double-stranded DNA from neutral CsCl gradients was then subjected to alkaline CsCl, Cs2SO4 gradient centrifugation. Neutral gradients: A, no UV control; B, 4,200 ergs/sq mm; C, 50,400 ergs/sq mm; D to F are the corresponding alkaline CsCl, Cs2SO4 rebands of light-light double-stranded DNA from gradients A to C. ○, absorbance at 260 nm; ◦, tritium disintegrations.

normal density DNA of the nonirradiated mice was significantly above background. This might indicate that some radioactivity from the BrdUrd-substituted density region contaminated the normal density region. Consequently, normal density DNA from the 1st neutral CsCl gradient, rebanded on a 2nd neutral CsCl gradient, still contained radioactivity, thereby establishing the validity of the method. The level of nonsemiconservative replication increased with increasing UV dose levels up to 4200 ergs/sq mm, beyond which the level of nonsemiconservative incorporation decreased (Table 3).

Quantitative measurements of repair replication at various time intervals after irradiation are shown in Table 4. The experimental procedure was the same as that used to study the effect of dose of irradiation, except that repair replication was studied in 3-hr intervals from 0 to 24 hr following irradiation of mouse skin with 8400 ergs/sq mm of UV. Repair replication was maximal from 0 to 3 hr, and was lower at successive time intervals.

DISCUSSION

Our data corroborate existing evidence that treatment with carcinogens causes DNA repair in the intact organism. Earlier studies on the disappearance of bound carcinogens or their metabolites from DNA (14, 26, 30, 42, 53, 56, 57) showed that the rate of loss soon after exposure sometimes exceeded that which could be attributed to DNA turnover and to depurination (14); thus the data are compatible with the possibility that a part of the loss was the result of repair processes. Furthermore, the early transient inhibition of mouse epidermal DNA synthesis following carcinogen treatment may be attributed in part to interference with semiconservative DNA synthesis until repair is accomplished and/or to temporary inactivation of enzymes involved directly or indirectly in DNA synthesis (49, 50).

Although unscheduled DNA synthesis has been demonstrated after UV irradiation of normal skin of mice (22) and humans (23) by autoradiography, the 1st studies in the intact organism to provide direct evidence that carcinogens caused damage to repaired DNA were based on alkaline sucrose gradient analyses of changes in the size of single-stranded DNA of rat liver (16, 17, 26). DNA repair in rat liver may also be monitored by determining the extent of nonsemiconservative DNA synthesis, as has been done by Goodman (25), to confirm that treatment with liver carcinogens provokes DNA repair. We have utilized the latter technique to show that DNA repair replication occurs in mouse skin following exposure to UV, the major cause of skin cancer in man. We also investigated pyrimidine dimer excision.

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UV irradiation (ergs/sq mm) Specific activities of nonsemiconservative replicated DNA (dpm/µg DNA)

0 8.9, 9.6
560 9.7, 11.4
1,400 16.5, 18.3
2,800 16.9, 19.7
4,200 28.6, 26.4
8,400 18.7, 22.9
16,800 18.6, 22.6
50,400 15.1, 18.6
84,000 15.9, 23.2

* The data are from 2 separate experiments. In each experiment, 2 CsCl gradients were run at each level of irradiation, and the variation of the values for specific activity from the mean did not exceed 15%.

The data presented in Table 1 show that thymine-containing dimer production in mouse skin basal cells was approximately linear, with UV dose levels up to about 50,400 ergs/sq mm. However, the dimer yield at 84,000 ergs/sq mm was approximately equal to that obtained at 50,400 ergs/sq mm, possibly indicating that higher pyrimidine dimer yields in mouse skin cannot be obtained. In Chinese hamster cells grown in culture, the thymine dimer yield was found to be linear with UV doses up to 400 ergs/sq mm, and there were 0.05% thymine dimers produced at 100 ergs/sq mm (54). In a different study utilizing mouse L-cells grown in culture, UV doses up to 10,000 ergs/sq mm yielded 3% thymine dimers (33). It can be seen that irradiation of mouse skin basal cells through 1 or 2 cell layers of differentiating cells plus keratin is a less efficient process than irradiation of a monolayer of rodent cells grown in culture. At approximately equal thymine-containing dimer yields (0.13 and 0.122% for mouse skin basal cells and Chinese hamster cells, respectively) a UV dose of 8,400 ergs/sq mm was required for mouse skin basal cells and 200 ergs/sq mm for Chinese hamster cells. In contrast to mouse L-cells in which dimer yields as high as 3% were reached.
When specific excision of pyrimidine dimers from mouse skin basal cell DNA was investigated by measuring dimer yields 24 hr after irradiation, from 29 to 61% of the thymine-containing dimers were found to be excised. In contrast to this finding, most rodent cells grown in culture have been found to poorly excise pyrimidine dimers from their DNA (33, 51, 54, 55). However, human cells grown in culture will excise up to 50 to 60% of the UV-induced dimers within the 1st 12 to 24 hr after irradiation (43). These dimer excision studies in hamster and human cells were carried out on sublethal doses of UV irradiation. Presently, we have no direct measure of cell survival of mouse skin basal cells. Thus we do not know what effect the dose levels of UV had on basal cell survival.

We estimated basal cell loss by studying the loss of [methyl-3H]thymidine-prelabeled DNA after the various dose levels of UV irradiation and found no apparent cell (DNA) loss at the lowest dose of UV irradiation (within 24 hr after irradiation). There was approximately a 20% loss at the 4 higher dose levels of UV irradiation. However, we do not feel that this 20% loss of prelabeled DNA affected the dimer excision data because dimer yields were expressed as percentage of radioactivity associated with dimers versus radioactivity associated with thymine. Losses of cellular DNA, and thus radioactivity associated with thymine, were corrected for by expressing the dimer data as a percentage. There is, however, the possibility that there were basal cells of the mouse's back with very different dimer yields and that we are observing the loss of cells with a very large number of thymine-containing dimers. This specific cell loss probably would not be corrected for by determining the dimer yields as percentage of labeled thymine. This specific cell loss could not be taking place at the lowest UV dose (4200 ergs/sq mm), in which case no prelabeled DNA was lost, but it must be considered when analyzing the dimer excision data at the 4 higher dose levels. Thus, no meaningful statement can be made regarding the relationship of thymine dimer excision from the DNA of mouse skin basal cells to basal cell survival.

We have also demonstrated nonsemiconservative DNA synthesis in mouse skin epidermal cells, a phenomenon that has been associated with DNA repair after UV irradiation of mammalian cells. This molecular phenomenon is very similar to repair replication which *Escherichia coli* cells perform after UV irradiation during the repair of pyrimidine dimers (39). However, we have no evidence that this molecular phenomenon observed in mouse skin has any relationship to enhancement of survival as it does in bacteria, nor do we have evidence to show that repair replication in mouse skin is involved in the excision of thymine-containing dimers. It must be emphasized that UV irradiation causes damage to DNA other than pyrimidine-dimer formation, and therefore the repair replication we observed may represent the repair of these other types of damage to DNA. There is some evidence that repair replication in human fibroblasts and Chinese hamster cells influences the response of these cells to UV damage. The absence of repair replication in fibroblasts from people with the disease xeroderma pigmentosum has been correlated with the clinical symptoms of sensitivity of these people to UV (7, 8, 13, 48), and on the basis of reduced unscheduled DNA synthesis, a UV-sensitive mutant has been derived from the Chinese hamster cell line V-79 (9).

The conflicting observations concerning the excision of UV-induced pyrimidine dimers in rodent cells might be resolved by the hypothesis that development and differentiation influence the level of excision repair. This hypothesis is based on a number of reported observations which show that, as cells in culture differentiate or are passaged, there is progressive loss of unscheduled DNA synthesis or excision repair induced by chemical or physical damage to the DNA. Hahn et al. (27) have demonstrated decreasing amounts of unscheduled DNA synthesis induced by methyl methanesulfonate in rat embryo muscle cells as they progress from myoblasts to fully developed muscle fibers. Similarly, Stockdale (52) has shown a progressive decrease in unscheduled DNA synthesis induced by UV irradiation in chick embryo muscle cells as they differentiate. Darzynkiewicz (18) and Darzynkiewicz and Chelmicka-Szorc (19) have shown that either the stimulation of lymphocytes with phytohemoagglutinin or cell fusion of dormant and active avian erythrocytes causes transformation of cells showing little or no semiconservative and unscheduled DNA synthesis to cells with both increased DNA synthesis and DNA repair. Hart and Setlow (28) have reported a direct correlation between the amount of excision repair and the life-span of an organism. Similarly, Ben-Ishael (1) reported that excision repair of DNA in the cells of mouse embryos decreased with successive passages. These observations are not necessarily inconsistent with our observation that excision repair takes place in the epidermal skin cells of the adult mouse, because the basal cells of the epidermis are constantly turning over. Thus, the skin basal cells are probably in a quasiembryonic state, and genetic repression of those genes necessary for excision repair is less effective in the skin basal cells in contrast to the more differentiated cells of the mouse.

Contrary to many previous investigations of pyrimidine dimer excision in rodent cells, this work demonstrated that, in the intact mouse, epidermal cells excised 30 to 60% of the UV-induced thymine-containing dimers from their DNA within 24 hr. This work also established that UV irradiation of the intact mouse initiated repair replication (nonsemiconservative DNA synthesis) in epidermal cells. A cause-effect relationship between the excision of thymine dimers from mouse skin basal cells and repair replication after UV irradiation of mouse skin remains to be established, as well as any potential role of UV-induced pyrimidine dimers in skin carcinogenesis.

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