ULTRAVIOLET LIGHT ALTERATION OF CELLULAR DEOXYRIBONUCLEIC ACID IN VIVO*

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Abstract.—DNA after irradiation with ultraviolet light was immunogenic in rabbits and elicited serum antibodies reacting specifically with UV-irradiated DNA. The serological reactions were demonstrated by immunodiffusion, complement fixation, and immunofluorescence. By immunofluorescence, antisera reacted with cell nuclei of irradiated tissue sections but not with unirradiated tissue. This method was employed to show the presence of UV lesions in tissues of mice exposed to UV light. UV lesions in DNA were present in nuclei of epidermal cells, and in heavily irradiated animals they were also detected in the corium immediately below the epidermis. The method is useful not only for directly demonstrating UV lesions of DNA but also for localizing such lesions in tissues.

Native deoxyribonucleic acid appears to be a weak immunogen, since many attempts to produce antibodies to native DNA in animals have been unsuccessful.[1] However, denatured DNA has been shown to be immunogenic, and in these instances antibody specificities were directed mainly against purines or pyrimidines.[2, 3] Recently, Levine et al.[4] reported the production of antibodies against altered moieties in DNA caused by ultraviolet light. For antigens, they used heat-denatured DNA irradiated with ultraviolet light and showed that antibodies to UV photoproducts were obtained in addition to antibodies against purine and pyrimidine bases.

In a preliminary report,[5] it was shown that *native* DNA irradiated with ultraviolet light was immunogenic. This report describes immunological studies characterizing serum antibodies reacting with irradiated DNA and the use of immunofluorescence to demonstrate UV-induced DNA lesions *in vivo*.

Materials and Methods.—Preparations of DNA: Calf-thymus DNA was purchased from Worthington Biochemicals, Freehold, N.J. Solutions of native DNA were prepared by dissolving strands of DNA in phosphate-buffered saline (0.01 M phosphate, 0.15 M NaCl), pH 7.0. Aliquots of 5 ml DNA (500 μg per ml) in 50-mm-diameter Petri dishes were irradiated with a G30T8 germicidal lamp (General Electric, Schenectady, N.Y.). The Petri dishes were placed on a platform that was slowly rotated during irradiation, and the bottoms of the dishes were 5 cm from the light source. Some evaporation occurred after long periods of irradiation, and in these instances the solutions were made up to the original volumes by the addition of saline. For immunodiffusion and complement fixation studies, DNA that had been irradiated for 60 min was employed. Heat-denatured DNA used in serological reactions was prepared by heating 500 μg/ml native DNA in a boiling water bath for 10 min, followed immediately by rapid chilling in ice-cold water.

Preparation of antiserum to UV-irradiated DNA: DNA was irradiated for 120 min at concentrations of 500 μg per ml and complexed with equivalent amounts (by weight) of methylated bovine serum albumin (MBSA) according to the method of Plescia et al.[3]

The antigen, consisting of UV-irradiated DNA-MBSA complex, was homogenized in an equal volume of complete Freund's adjuvant. Rabbits were injected weekly with 2 ml of antigen-adjuvant mixture containing a total of 500 μg UV-irradiated DNA. Injections were given intramuscularly and subcutaneously.

Demonstration of serologic activity in antisera: Precipitin reactions between antiserum and antigen were demonstrated by double diffusion in agarose according to a method described previously.[6] Complement fixation methods used were those of Wasserman and Levine.[7] Immunofluorescent reactions were performed on cryostat sections of frozen mouse kidney.[8] In instances where tissue sections were irradiated, the sections were thawed, air-dried, and irradiated with UV light from the G30T8 germicidal lamp, placed 5 cm away from the sections. Antiserum to rabbit gamma globulin was prepared in sheep, and the gamma globulin fraction of sheep antiserum was conjugated with fluorescein by the method of Wood et al.[9]

Demonstration of UV alteration of cellular DNA: Hairless mice were purchased from Charles River (Wilmington, Mass.), and were exposed to UV light from either a germicidal lamp (G15T8, General Electric) or from a Hanovia Sunlamp (model Luxor Alpine, Hanovia Lamp Division, Englehard, N.J.). The animals were placed in open cages 11 × 7 inches and were allowed to roam freely in the cages during irradiation. The germicidal lamp was 8 inches and the sunlamp 21 inches above the bottom of the cages. After the animals were irradiated for different time periods, they were killed immediately and portions of skin from the back were frozen at $-60°C$ for immunofluorescent studies.

Results.—Antibodies to UV-irradiated DNA: Four rabbits were immunized with UV-irradiated DNA, and all four responded by making antibodies that had specificity for UV-irradiated DNA. The immunological reactions were demonstrated by immunodiffusion, complement fixation, and immunofluorescence.

Immunodiffusion: The immunodiffusion reactions are demonstrated in Figure 1. Serum (Me) from a patient with systemic lupus erythematosus was tested against native DNA, heat-denatured DNA, and UV-irradiated DNA. This serum reacts principally with native DNA and only weakly with the preparation of heat-denatured DNA. It also reacts strongly with UV-irradiated DNA. On the other hand, antiserum from a rabbit (91) immunized with UV-DNA reacts to give two precipitin lines with UV-DNA but does not react with native DNA or heat-denatured DNA. Sera from the other three rabbits immunized with
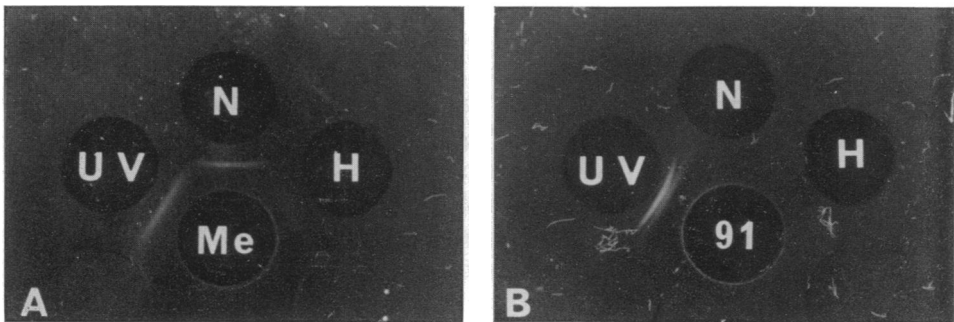

FIG. 1.—Precipitin reactions with native DNA (*N*), heat-denatured DNA (*H*), and UV-irradiated DNA (*UV*). *Me*, a serum from a patient with systemic lupus erythematosus, reacts primarily with native DNA and weakly with heated DNA. It also reacts with UV-DNA, and the precipitin line is identical with that of native DNA. Rabbit 91, immunized with UV-DNA, reacts only with irradiated antigen to give two precipitin lines, but does not react with native or heat-denatured DNA.

UV-DNA also reacted with UV-DNA to give precipitin lines. Two of the four rabbits have at least two antibodies of different specificities to UV-DNA, as illustrated here, while the other two have demonstrated only single-line precipitin reactions. Precipitating antibodies to native DNA or heat-denatured DNA were not demonstrated in any animal sera.

Complement fixation: The specificity of rabbit serum antibodies was further demonstrated by complement fixation. Representative studies for one such serum (91) are depicted in Figure 2. At 1/400 and 1/600 dilutions there was
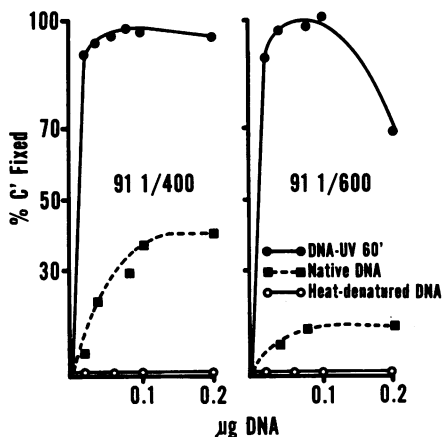

FIG. 2.—Total fixation of complement with UV-DNA by rabbit serum 91 at 1/400 and 1/600 dilutions. There was no complement fixation with heat-denatured DNA and only partial fixation with native DNA at 1/400. At 1/600 dilution, fixation of less than 20% of complement with native DNA is within the range of experimental error of method.

total fixation of complement with UV-DNA. At 1/400 dilution there was partial fixation of complement with native DNA. But at 1/600 dilution, fixation of less than 20 per cent complement with native DNA fell within the range of experimental error of the method. There continued to be significant complement fixation with UV-DNA at 1/1000 and 1/1500 dilutions of this antiserum but no reaction with native DNA. At none of the serum dilutions at which the complement-fixation method could be used was there any reaction with heat-denatured DNA. Serum 91 was the only serum which showed some complement fixation with native DNA. All other sera fixed complement only with UV-DNA.

Immunofluorescence: The substrate for immunofluorescent studies consisted of 4-μ cryostat sections of frozen mouse kidney.[8] With unirradiated tissues, two rabbit antisera were negative for antinuclear antibodies by the indirect immunofluorescent technique, and two others were weakly positive (Table 1). However, after the tissues were irradiated with ultraviolet light, all sera were strongly positive for antinuclear antibodies. Serum 91, for example, reacted with nuclei of UV-irradiated tissue at serum dilution of 1/1024, whereas nuclear staining could not be detected above $^1/_8$ dilution with unirradiated tissue. An example of this is shown in Figure 3 where serum 91 diluted at $^1/_{40}$ did not react with unirradiated tissue (A), but reacted strongly with UV-irradiated tissue (B). Preimmune sera of these animals and sera of animals immunized with unrelated antigens, such as bovine serum albumin, showed no nuclear staining of either

TABLE 1.   *Antibody to UV-irradiated DNA demonstrated by immunofluorescence.*

Rabbit serum	Titer* of Antinuclear Antibody on	
	Unirradiated tissue	Irradiated tissue
Ra 89	0	256
Ra 90	8	512
Ra 91	8	1024
Ra 92	0	128

* Titer is reciprocal of highest dilution of serum showing nuclear staining.

irradiated or unirradiated tissues.   The specificity of this reaction for UV-irradiated DNA alone and not for other nuclear components altered by UV light was shown by the complete absence of nuclear staining when antisera were absorbed at equivalence with UV-irradiated DNA.   It was observed that the duration of exposure to UV light was an important factor in the immunofluorescent method.   Tissue sections exposed for one minute gave optimum reactions with antisera.   Tissues exposed for 5, 10, and 30 minutes showed progressively weaker reactions with antisera.

UV alteration of cellular DNA in vivo: Since rabbit antisera at the appropriate dilutions reacted only with nuclei of irradiated tissues, and since this reaction was specific for UV-irradiated DNA, the immunofluorescent method was used to determine whether ultraviolet light altered cellular DNA *in vivo*.

Hairless mice were exposed to UV light from a single germicidal lamp (G15T8) for periods of 10, 30, and 180 minutes and to a sunlamp (Hanovia) for 70 and 700 seconds under the conditions described in *Materials and Methods*.   Immediately after exposure, the animals were killed and the skin of the back was studied for the presence of UV-altered DNA.   Cryostat sections of frozen skin were thawed and air-dried, made to react with rabbit serum 91 at 1/40 dilution, and stained with fluorescein-conjugated sheep antirabbit gamma globulin.   The skin from unirradiated animals showed no staining of any structures in the skin, but the nuclei of epidermal cells from all the irradiated animals showed altered DNA (Fig. 4).   The extent of DNA lesions produced by UV light paralleled duration of exposure.   In this strain of hairless mice the epidermis was three to four cell layers thick and the UV-induced DNA lesions were seen in the ma-
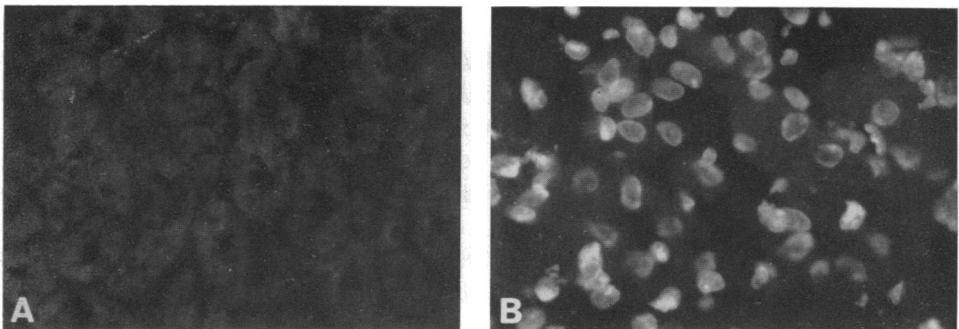

FIG. 3.—Indirect immunofluorescence with serum 91 at 1/40 dilution and mouse kidney section as substrate.   There was no reaction with unirradiated tissue section (*A*), but strong reaction with nuclei in UV-irradiated tissue section (*B*).
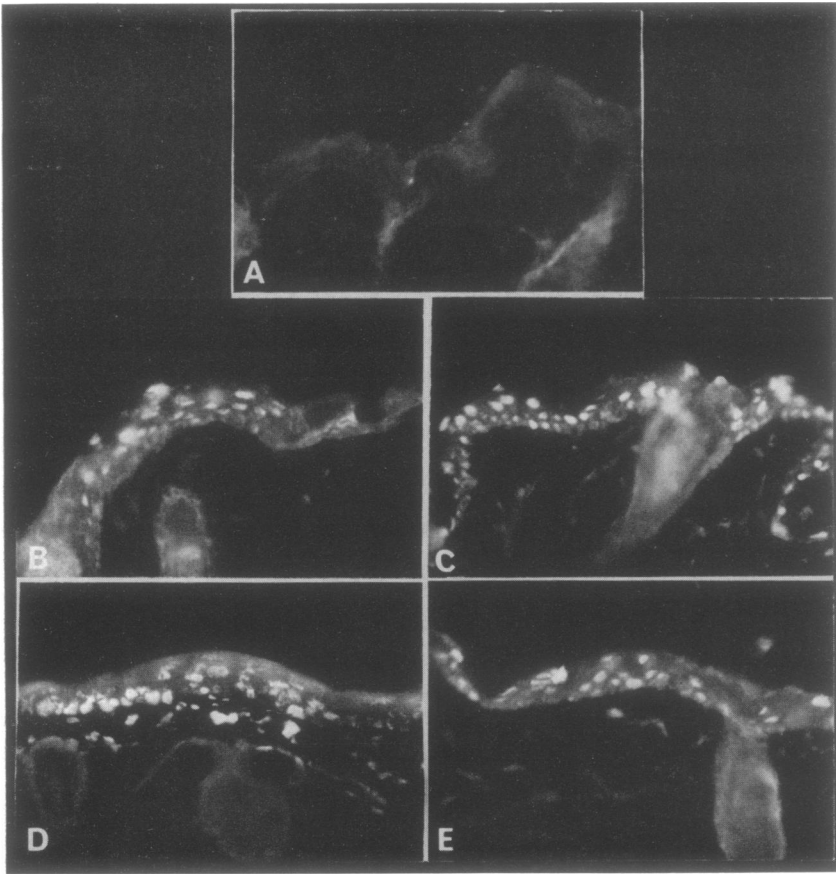
Fig. 4.—DNA lesions in skin of hairless mice exposed to UV light.   Indirect immunofluores-
cence was employed and serum 91, diluted 1/40, was used to detect altered DNA.   (A) Skin
of unirradiated mouse.   (B) Mouse irradiated with germicidal lamp for 10 min.   (C) Germi-
cidal lamp used for 30 min.   (D) Germicidal lamp used for 180 min.   (E) Sunlamp used for
700 sec.   The DNA lesions were located in epidermal cell nuclei.   After 180 min under a
germicidal lamp, altered DNA was also detected in the corium below the epidermis.

jority of cells of the epidermis (Fig. 4B–E).   In animals irradiated for 180 min-
utes with the germicidal lamp, material reacting with serum 91 can be seen
subjacent to the epidermis, at the dermal-epidermal junction (Fig. 4D).   Ani-
mals irradiated for 70 seconds with the sunlamp showed trace staining of nuclei,
but irradiation for 700 seconds showed unquestioned alteration of DNA in
epidermal cells (Fig. 4E).

   Discussion.—In contrast to native DNA, UV-irradiated DNA is highly anti-
genic.   Employing the indirect immunofluorescent technique on irradiated tis-
sues, we detected antibodies to UV-altered DNA in the sera of all four rabbits
after the second injection of antigen.   The titers of antibodies continued to rise
following subsequent injections;   after the fourth injection, precipitating
antibodies to UV-altered DNA were detected in all animals.   It was clear that

the antibodies were preponderantly those against irradiated DNA, but there also appeared to be low concentrations of antibodies with specificity for native DNA determinants since two animals (90 and 91) had low titers of antinuclear antibodies, detected by immunofluorescence using unirradiated tissue, and one animal (91) showed complement fixation with native DNA at low serum dilutions. However, no antibodies against heat-denatured, single-strand DNA were detected by immunodiffusion or complement fixation.

The antigenic determinants in irradiated DNA have not been investigated in this study. By immunodiffusion, it was clearly demonstrated that some rabbit antisera contained antibodies with at least two distinct specificities for irradiated DNA. By inhibition of complement fixation, Levine et al.[4] showed that irradiated tri- and tetrathymine oligonucleotides were the best inhibitors, suggesting that the antigenic determinant may be thymine polymers. It has been established that a number of photoproducts, including pyrimidine dimers and hydrates, are present in irradiated DNA,[10] and it appears that at least two UV-photoproducts are capable of eliciting antibody responses.

It has been shown that the deleterious effects of ultraviolet light on certain viruses and bacteria can in some instances be directly attributed to UV-induced lesions in DNA.[11, 12] Tissue cultures of mammalian cell lines irradiated with ultraviolet light have also been shown to result in DNA lesions.[13-15] We believe that the present study demonstrates for the first time that ultraviolet light produces DNA lesions *in vivo* in the whole animal. Further, the use of specific antiserum and the immunofluorescent method allow the direct demonstration of UV alteration of cellular DNA in contrast to methods that detect the repair or reversion of UV-induced DNA lesions.

The germicidal lamp employed in this study emits its major radiant energy at 2537 Å, but there are also minor radiations in the near ultraviolet at 3200 and 3600 Å. The sunlamp also has major emission energies at these wavelengths.[16] The wavelength or wavelengths of the UV spectrum responsible for the *in vivo* alteration in DNA were not determined, and the precise amount of energy delivered at the skin sites was not measured. With the sunlamp, 70 seconds of exposure would have been equivalent to seven times the minimal erythema dose for human skin. However, the animals in this study were not restrained, and since the targets were constantly moving and changing, the energy actually received was probably much less. It was noteworthy that even with this small exposure to the sunlamp, alterations in DNA were detected in the epidermal cells.

Certain human diseases are either initiated or aggravated by exposure to sunlight. Systemic lupus erythematosus is an example, and this disease is characterized in particular by the presence of serum antibodies to DNA. This report shows that UV-irradiated native DNA is antigenic and, further, that UV irradiation produces DNA lesions *in vivo*. It has yet to be shown that DNA lesions induced *in vivo* may eventually result in induction of antibody to DNA in the irradiated host. Further investigations along these lines may provide information leading to a better understanding of the role of photosensitivity in the inception of certain diseases.

We appreciate the able technical assistance of Ingrid Ekvall and Maria Ashton.

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