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EFFECT OF ULTRAVIOLET IRRADIATION ON BIOSYNTHESIS OF DNA IN GUINEA-PIG SKIN IN VIVO*

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ABSTRACT

The molecular and metabolic alterations preceding the clinical manifestation of a photobiologic process, the erythematous or sunburn reaction, were investigated in mammalian skin in vivo. The effect of a moderate (2.5–3 times the minimal erythema dose [MED]) and a large (6–8 times MED) dose of ultraviolet radiation (290–320 nm) on the incorporation of [*Me*-^{3}H]-thymidine into epidermal cell DNA of guinea pigs was studied. The epilated half of the back of each animal was irradiated with various doses of ultraviolet light, and the other half served as the nonirradiated control. The amount of intraperitoneally injected [*Me*-^{3}H]-thymidine incorporated into the DNA was determined by the isolation of DNA at various time intervals and the measurement of its radioactivity. Significant inhibition of the biosynthesis of DNA (64% of the control) immediately after ultraviolet irradiation was observed. The duration of this inhibition is related to the total dose of ultraviolet light delivered and to the degree of the erythema reaction (i.e., the degree of cellular damage) and can persist from 2–24 hr. With a moderate dose, the biosynthesis of new DNA occurs 2 hr after irradiation.

Deoxyribonucleic acid (DNA), by virtue of high extinction coefficients of its constituent bases in the ultraviolet (UV) spectrum, its ultimate importance to the replication of the cells, and its sensitivity to alteration upon exposure to UV radiation, appears to be the principal target for the damaging effect of UV photons on cells. The distinctive occurrence of basal cell epitheliomas, squamous cell carcinomas, and lesions of xeroderma pigmentosum on the exposed areas of the body implicate solar radiation of wavelengths 290–320 nm as a major factor in the induction of these human skin cancers [1–3]. Under both in vitro and in vivo conditions, irradiation of mammalian DNA by UV of wavelengths shorter than 300 nm produces cyclobutyl pyrimidine dimers [3–7]. In our recent studies of guinea-pig skin in vivo, we also observed that one of the early effects of UV irradiation (at 290–320 nm) is the formation of thymine dimers in epidermal DNA [8, 9].

In addition, UV irradiation has a profound effect on a number of cellular functions in living mammalian epidermis [3, 10–12]. The results of these studies indicate that a marked depression of the synthesis of DNA, RNA, and protein and of cell mitosis occurs within 1–3 hr after irradiation of the skin at wavelengths shorter than 320 nm. These early effects of ultraviolet light in human and mouse skin have been well illustrated recently by Epstein et al [13]. After the administration of [*Me*-^{3}H]-thymidine, these investigators employed light microscope and autoradiographic techniques to determine the labeling pattern of 1,000 basal cells before and after UV irradiation. Cell nuclei that contained more than 5 silver grains were considered to be in the DNA-synthesizing phase. The results showed a significant depression in the labeling pattern of the basal cell nuclei that was detectable shortly after UV irradiation and became most noticeable within 2–6 hr after exposure. Acceleration of DNA synthesis was observed 24–48 hr after irradiation.

Although such autoradiographic studies have revealed that synthesis of DNA is inhibited by UV irradiation, we decided to obtain additional proof of this phenomenon by studying the incorporation of thymidine into isolated DNA at various time intervals after UV irradiation. The structural changes in the DNA strand at thymine sites appear to interfere with normal DNA replication and account for the inhibition of scheduled DNA synthesis. In this study, we present our findings on the early effects of UV irradiation on the biosynthesis of epidermal DNA. Results pertaining to single-strand breaks, damaged bases, and other physical and chemical alterations in the epidermal DNA will be published elsewhere.

MATERIALS AND METHODS

In Vivo Effect of UV Irradiation on the Yield of Extractable DNA

Fourteen adult albino guinea pigs (1000–12000 gm) were epilated [14]. Two days after epilation, 4 of these animals were treated as unirradiated controls. The remaining 10

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were divided into two groups. Group A included 6 animals which were exposed to UV radiation of 290–320 nm. Group B included 4 animals which received topical application of 4,5′,8-trimethylpsoralen (TMP 50 μg/2.5 cm^2) and UV radiation of 320–400 nm. Animals were immobilized on wooden boards so that in vivo the skin of the entire back (7″ x 3″ area) could be irradiated. Animals of group A were irradiated with sunburn-producing spectrum ($\lambda > 290$ nm) emitted by a 410-watt, high-pressure quartz mercury-arc tube equipped with a replaceable filter (see below) that transmitted wavelengths between 285–350 nm. Each animal received an ultraviolet dose of 8.0×10^5 ergs/cm^2 which was approximately 2–2.5 times the minimal erythema dose (MED) of the animal. Animals of group B, 1 hr following the application of TMP, were exposed to long-wave UV radiation emitted by a 250-watt, high-pressure mercury-arc lamp equipped with a Wood's filter that transmitted wavelengths between 320 and 400 nm. Each animal received an ultraviolet exposure of 1.94×10^7 ergs/cm^2, over 80 percent of this dose being 365 nm radiation. Immediately after irradiation, the entire skin of the back was removed under anesthesia. Irradiated and nonirradiated skin samples (135 cm^2) were processed immediately for the isolation of DNA as described below [14, 15].

Determination of the Effect of Moderate Dose of UV Light (2.5–3 Times MED) on DNA Biosynthesis

The backs of 5 albino guinea pigs (800–1000 gm) were epilated using a mixture of beeswax and rosin [14, 16]. The animals were housed in their individual cages for 6 days to allow the epidermis to return to a steady state of proliferation. We have observed that epilation with a mixture of beeswax and rosin causes inhibition of DNA synthesis in the first 12 hr and subsequently hyperplasia of the epidermis, manifested by increased population of the epidermal cells and increased synthesis of DNA, RNA, and protein during the first 72 hr after epilation. We therefore decided to irradiate the animals on the sixth day after epilation so that the primary effect of UV irradiation could be examined when the biosynthesis of the essential macromolecules was proceeding in vivo under almost steady-state conditions.

Six days after epilation, the animals were immobilized on a wooden board with strings. The back of each animal was divided into two halves; in the right central half of the back, a rectangular area measuring 3.5 × 10 cm was demarcated for UV irradiation. Likewise, on the left central half, a similar area was demarcated and covered with black paper to serve as nonirradiated control skin. During irradiation the entire animal, except the right half, was covered with black paper. Irradiation of the animals with wavelengths of 285–350 nm was carried out at a distance of 55 cm from the light source. The source of ultraviolet irradiation was a Hanovia high-pressure 410-watt, quartz mercury-vapor lamp. This lamp was equipped with a replaceable cellophane amber-yellow filter,¶ which cuts off wavelengths below 285 nm and allows transmission of only 285–350 nm. Cellophane filters were replaced after three irradiations. Radiant energy flux of UV radiation impinging on the skin surface was measured by a calibrated thermocouple (Eppley Laboratory, Newport, R. I.). The dose of UV irradiation which reached the skin surface of each animal was 4.5×10^6 ergs/cm^2 and was delivered to the back of the animal in 20 min. This ultraviolet energy corresponds to 2.5–3 times the MED. The MED is defined as the minimal dose of UV radiation (290–320 nm) that produces definite, but minimally perceptible redness, at 24 hr after exposure. In a separate experiment, the MED was determined by exposure of the backs of 2 normal epilated animals to the increasing dose of UV irradiation (5, 7, 10, 12.5, and 15 min) in two separate rows, each of which contained 5 skin windows, 2 × 2 cm each. The erythemal response at 24 hr was then recorded. The MED was found to be in the range of 7–8 min, which, in terms of total energy at the skin surface, was equal to 1.5×10^6 ergs/cm^2. Approximately 90 percent of this energy is absorbed in the nonviable cells of the stratum corneum and only about 5–10 percent is transmitted into the viable cells of the epidermis where the major effects of UV radiation are produced (unpublished observations).

The effect of UV light on the biosynthesis of DNA was examined at 0, 2, 5, 11, and 22 hr after irradiation. Each of the 5 animals received an intraperitoneal injection of 1 mC of [*Me*-^{3}H]-thymidine (New England Nuclear Corp., Spec Act 20 C/mmole) in 1 ml normal saline at 0, 2, 5, 11, and 22 hr after irradiation. Two hr after injection of [*Me*-^{3}H]-thymidine, the animals were anesthetized with ether and sacrificed. Irradiated and nonirradiated skin samples were obtained and processed immediately for the isolation of DNA. DNA from the control and the irradiated sites was isolated separately [14, 15]. The results concerning biosynthesis of DNA at these various time intervals were expressed as the effects observed at 2, 4, 7, 13, and 24 hr after the injection of [*Me*-^{3}H]-thymidine.

The epidermis, including the horny layer of irradiated and nonirradiated skin of the back was isolated by the stretch method [16] and transferred into precooled mortars containing 4 ml of Kirby-I solution [14, 15]. The tissue was homogenized at about 0°C, and the homogenate was transferred into polyethylene flasks, using 1.5 ml of Kirby-I solution twice for rinsing purposes. The two phenol steps followed by one butoxyethanol precipitation step, as recommended by Kirby and Cook [15], were carried out. After centrifugation, the pellet was washed first with 5 ml of sodium acetate:ethanol:water (2:75:25, w/v/v), followed by two washings with 5 ml of absolute ethanol and dried in vacuo over $CaCl_2$.

The DNA was dissolved in 1 ml Tris buffer (0.015 M Tris, pH 7.6 + 0.05 M CsCl); 0.5 ml of this solution was added to 13.5 ml of a CsCl solution (142.8 gm CsCl + 107.2 gm Tris buffer). By refractive-index measurement, the average density at 20° C of the final solution was found to be 1.7175. Centrifugation was carried out in a Beckman Spinco ultracentrifuge, using a rotor No. 40, at 69,500 × *g* and 20° C for 120 hr. A linear gradient is established within this time over most of the range. The samples were fractionated into 15 fractions (each containing about 0.8 ml) by using a tube-piercer device obtained from Measuring and Scientific Equipment Co., Ltd., London, England. Optical density of each fraction, after appropriate dilution, was measured at 260 nm; the DNA-containing fractions were pooled and dialyzed at 5° C against standard saline citrate buffer (pH 7) with five separate changes of the buffer. The dialyzed samples were added to 4 ml standard saline citrate buffer, and aliquots of the diluted solutions were taken for the recording of the ultraviolet spectra. Another aliquot was diluted 1:10 with water and used for determination of the radioactivity. The radioactivity was measured in a Packard Model 3320 liquid scintillation spectrometer, using a scintillation fluid containing 8 gm Omnifluor (New England Nuclear Corp.), 120 gm naphthalene in 1 L dioxane. The counting efficiency with respect to T_2O was 40 percent. The radioactivity of the isolated DNA is

¶ Manufactured by E. I. DuPont de Nemours and Co., Wilmington, Delaware. Transmission spectrum was checked on a recording spectrophotometer (Bausch & Lomb, Spectronic 505).

expressed as counts/min/100 μg DNA, or as percentage of the control (nonirradiated skin DNA).

Determination of the Effect of High Dose of UV Light (6–8 Times MED) on DNA Biosynthesis

Although the Kirby and Cook method [15] is ideal for the isolation of purified DNA in the native form, it is time consuming. Two separate methods were, therefore, employed in this study: one was the Schmidt–Thannhauser procedure [17] which enables determination of radioactivity in DNA without isolation of the macromolecule; and the other was the isolation of DNA [15] and its subsequent centrifugation in a CsCl density gradient. At 72 hr after epilation, 5 albino guinea pigs (each 400–500 gm) were irradiated under a 250-watt, high-pressure mercury lamp (Kromayer Lamp, Model Q 250) equipped with a W.G.-6 filter that allowed transmission of wavelengths greater than 290 nm. The irradiation dose was equal to 6–8 times the MED for each animal and was delivered to 2–4 skin areas, each measuring 1.5 × 3 cm. Twenty-three hr after irradiation, 500 μc of [*Me*-^{3}H]-thymidine (2 C/mmole) in 0.5 ml of sterile water were injected intraperitoneally; 2 hr later the animals were sacrificed. The skin was separated and the epidermis was obtained by the stretch method. Without further homogenization, the samples were washed twice with 5% ice-cold trichoroacetic acid, followed by two washings with 1% potassium acetate in absolute ethanol (to remove any unincorporated, free [*Me*-^{3}H]-thymidine). The samples were dehydrated by immersion in absolute ethanol at 60° C for 20 min, two washes with ethanol and ether, and drying in vacuo over $CaCl_2$ [17]. About 8 mg of dry epidermis were obtained from the control and irradiated areas, from which 1–3 samples, each weighing 0.5–2 mg, were taken for determination of radioactivity. Two samples were taken for estimation of DNA content by the method of Kissane and Robins [18].

For determination of radioactivity, samples were transferred into 20-ml Packard glass flasks, and 0.2 ml of 70% perchloric acid was added. After 20 min, 0.4 ml H_2O_2 (30%) was added, and the flasks were firmly closed and kept at 75° C for 3 hr. After cooling, 10 ml toluene scintillator fluid (6 gm PPO/1) and 5 ml ethylene glycol–monoethyl ether were added. The radioactivity of the samples was recorded in a liquid scintillation spectrometer (Packard Tri-Carb, Model 544). A highly reproducible estimation of tritium in biologic materials is achieved by this procedure.** To establish the observations thus obtained, we also isolated epidermal DNA and subsequently centrifuged it in a CsCl density gradient. An area of 3 × 8 cm of dorsal skin on the left side of one animal was irradiated with a dose of 6–8 times MED. A similar area on the right side served as a nonirradiated control. The epidermis from the two areas was separately processed for isolation of DNA [14, 15]. The radioactivity per optical unit of DNA was determined, and the aliquots of the isolated DNA samples were then subjected to centrifugation in CsCl density gradient, as described in the section on the determination of the effect of moderate UV light (2.5–3.0 times MED) on DNA biosynthesis.

RESULTS

In Vivo Effect of UV Irradiation on the Yield of Extractable Epidermal DNA

The yield of extractable DNA from the control, nonirradiated animals averaged about 2159 μg/135 cm² skin surface (Table I). From the animals exposed to UV light, when DNA was extracted immediately after irradiation, the average yield of the extractable DNA was only 984 μg/135 cm² skin surface, indicating a decrease of nearly 55 percent in extractable DNA. Likewise, in 4 animals which were photosensitized by topical application of trimethylpsoralen and UV light irradiation (320–400 nm), the average yield was 861 μg/135 cm² skin surface. This low yield of extractable DNA appears to be due to physicochemical changes in the macromolecules such as those involving single- and double-strand breakage (to be published elsewhere).

Effect of Moderate Dose of UV Light on DNA Biosynthesis

Epidermal DNA was obtained from the nonirradiated and irradiated skin of albino guinea pigs. A typical profile of the DNA isolated from a sample of irradiated skin after CsCl density-gradient centrifugation is shown in Figure 1. The same type of profile was obtained from both the irradiated and nonirradiated skin samples.

Data concerning the yields of DNA per 35 cm² of skin surface are shown in Table II. The average yield of DNA obtained from the control samples was 245 μg/35 cm². The yield of DNA isolated at 2 hr after UV irradiation was only 58 μg/35 cm²

TABLE I

Effect of ultraviolet irradiation on DNA in guinea pig skin in vivo

Yield of extractable epidermal DNA (μg/135 cm² skin)		
Unirradiated (control)	Irradiated (290–320 nm) Group A	Irradiated (320–400 nm) Group B
2170	1070	880
2260	993	760
1750	750	615
2455	930	1200
	1155	
	1105	
Mean 2159	984	861

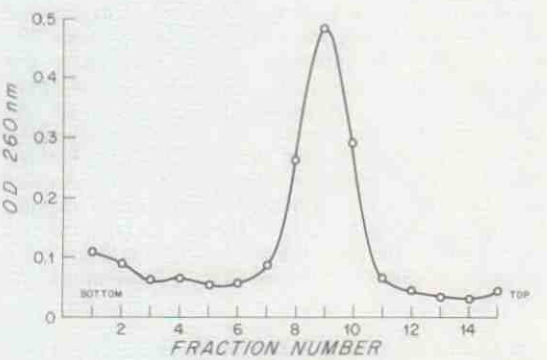

FIG. 1: CsCl equilibrium density-gradient profile of DNA isolated from guinea-pig epidermis.

** D Kasangh, personal communication

TABLE II

Yields of epidermal DNA and incorporation of [Me-^{3}H]-thymidine into epidermal DNA after moderate dose of ultraviolet light

Animal No.	Hours after irradiation	μg DNA isolated		Counts/min/100 μg DNA		Incorporation (% of control)
		Non-irradiated control	Irradiated	Non-irradiated control	Irradiated	
1	2	310	58	20 200	7 250	36
2	4	268	268	18 800	13 500	72
3	7	132	128	19 000	16 500	87
4	13	236	218	17 900	28 000	157
5	24	268	192	26 000	27 500	106

(about 24% of the yield from the control). The amount of DNA isolated at 4, 7, and 13 hr, respectively, from the irradiated sites appeared to reach the value obtained from the corresponding control nonirradiated sites. For the interpretation of the effect of UV irradiation on DNA biosynthesis in vivo, we believe that the total DNA values, although desirable, are not so critical as are the incorporation values of [*Me*-^{3}H]-thymidine. The incorporation of [*Me*-^{3}H]-thymidine into DNA 2 hr after irradiation was only 36 percent of the control values. At 4 and 7 hr after irradiation, the rates of incorporation of [*Me*-^{3}H]-thymidine into DNA were 72 and 87 percent, respectively, compared to control (Fig. 2).

Effect of High Dose of UV Light on DNA Biosynthesis

Using the Schmidt–Thannhauser method [17], we found that the rate of *Me*-^{3}H -thymidine incorporation into DNA at 24 hr after irradiation was depressed by a factor of 3 (Table III). On the other hand, no difference in DNA content, as determined by the method of Kissane and Robins [18], between irradiated and nonirradiated areas was observed. In 40 irradiated and in 40 nonirradiated specimens, a content of 1.94 ± 0.5 mg DNA per 100 mg epidermal tissue was established.

Using the CsCl density-gradient method, we confirmed that DNA biosynthesis was inhibited for a prolonged period (24 hr) by a high dose of UV irradiation. A significant inhibition, by a factor of 6, was found in the radioactivity of DNA when the values from the irradiated specimens were compared with the corresponding control values.

As can be seen from the standard deviations of counts per min per mg of epidermis, the Schmidt–Thannhauser procedure has much greater error than the CsCl density-gradient centrifugation method. Both methods, however, revealed that irradiation of skin with heavy doses of UV light can result in a significant depression of DNA biosynthesis, lasting at least up to 24 hr.

DISCUSSION

Until recently, experiments on the effect of UV irradiation on mammalian cells at both cellular and molecular levels were restricted primarily to established cell lines, such as Chinese hamster cells, human HeLa cells, and mouse L-cells grown as suspensions on monolayers [5]. All these studies, however, showed that the rate of DNA synthesis can be markedly inhibited for several hours after UV irradiation. The same conclusions were reached by Epstein and his associates [11, 13] who used autoradiographic techniques and silver-grain counts to study the early effects of UV light on DNA synthesis in human skin and in the skin of hairless mice.

Our results agree with the previous findings and indicate that UV irradiation produces a direct effect on DNA biosynthesis. We observed a significant inhibition of DNA synthesis immediately after UV irradiation. This depression of DNA synthesis, however, appears to be related to the degree of cellular damage produced by UV irradia-

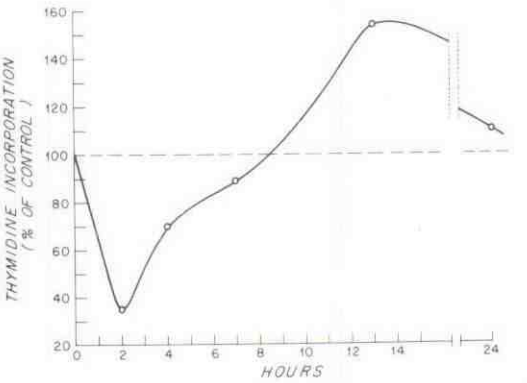

FIG. 2: [*Me*-^{3}H]-thymidine incorporation into epidermal DNA at various time intervals after a moderate dose of ultraviolet light irradiation. DNA was isolated from the control (nonirradiated) and from the irradiated skin sites. The radioactivity of the isolated DNA from the irradiated sites (solid line) is expressed as percentage of the control (dotted line).

TABLE III

Incorporation of [Me-^{3}H] Thymidine into DNA and the effect of high dose of ultraviolet light on DNA biosynthesis 24 hr after irradiation

Method	Animal No.	Nonirradiated control sites		Irradiated sites	
		Counts/min/mg dry epidermis	Standard deviation ±	Counts/min/mg dry epidermis	Standard deviation ±
Schmidt and Thann-Hauser [10]	1	859 (5)*	199	148 (8)	115
	2	696 (6)	96	292 (8)	241
	3	936 (8)	265	264 (8)	314
	4	718 (11)	205	212 (16)	144
	5	592 (11)	112	330 (11)	234
Average:		741		248	
CsCl	6	371†		61†	

* The numbers in ( ) indicate the number of samples from which radioactivity was determined.

† Disintegration/min/μg DNA; determined using T_2O as an internal standard; counting efficiency 20%.

tion. A significant depression of DNA biosynthesis occurred after moderate doses of UV irradiation that produced a mild erythematous reaction, but, within 4–7 hr after irradiation, a rapid recovery in the rate of DNA biosynthesis was observed. When a heavy dose of UV irradiation was used, however, DNA biosynthesis remained markedly depressed for as long as 24 hr. This finding is in agreement with the findings of several other workers [19–21] who showed that the rate of DNA synthesis after moderate dose of UV irradiation decreased rapidly up to 1–2 hr after irradiation and then recovered in a dose-dependent fashion.

Using autoradiography, one can obtain a qualitative measure of the degree of inhibition of DNA synthesis and the amount of DNA synthesized in cells that have been irradiated with UV light. To support these findings and to overcome some of the limitations of autoradiography, we isolated DNA from the cells and determined its radioactivity at various time intervals after exposure to UV light. In addition, each animal served as its own control. Although our observations are in general agreement with those of Epstein et al [11, 13], they differ to some extent. From their reports, one gets the general impression that irrespective of the total dose of irradiation, a single dose of UV exposure will cause a depression of DNA synthesis which persists for at least 6 hr. We believe that the duration of this inhibition is not for a fixed time interval, but is related to the total dose of UV irradiation that is absorbed by the skin and to the degree of cellular damage evoked by the absorbed energy. This inhibition of DNA synthesis can last for 2 hr or less, if the UV irradiation dose is low (2–3 MED). However, if the dose of UV irradiation is high, DNA synthesis can remain inhibited for 24 hr or longer.

Studies in vitro concerning UV irradiation (λ 254 nm) of DNA have revealed disruption of hydrogen bonds causing the DNA molecule to become more flexible and more coiled [22–24]. In addition, an increase in buoyant density, hyperchromicity, double-strand breakage, and cross-linking of complementary strands have also been reported [22, 24–30]. Recently the effects of acetophenone-sensitized and unsensitized longwave UV irradiation ($\lambda > 295$ nm) on the conformational behavior of DNA have also been examined [31]. Thymine dimerization and single- and double-strand breakage of the sugar phosphate backbone in DNA were observed. In addition, a decrease in molecular weight and melting temperature were detected, indicating a gross conformational alteration in the microstructure of the DNA.

It appears that UV radiation can produce two types of damage in the nucleic acids. The first involves damage to the pyrimidine base, and the second chain breaks. The structural changes in DNA interfere with the normal replication of the macromolecule and account for the inhibition of DNA synthesis after UV irradiation observed in this study. The second type of damage indicates a gross conformational alteration in the microstructure of the DNA and is reflected by the low yield of extractable DNA following irradiation as well as the inhibition of DNA synthesis.

Those of us who have known Dr. Irvin H. Blank are uniquely privileged. I wrote this paper to honor a man who has dedicated his entire scientific life to strengthening the bonds between basic research and clinical dermatology. I share his philosophy that when scientists and clinicians of diverse diciplines meet together or work together and share information, the progress of science is undoubtedly helped and accelerated by an interplay of dialogue between observation and interpretation, fact and theory, reality and concept, comment and criticism.—M. A. Pathak

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