0022-202X/84/8304-0401$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 83:401–404, 1984
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Vol. 83, No. 6
Printed in U.S.A.

REPORTS

UV Irradiation and Cutaneous Vitamin A: An Experimental Study in Rabbit and Human Skin

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The effect of UV irradiation on the concentration of cutaneous retinoids (retinol and 3-dehydroretinol) in rabbit skin in vivo and in human skin in vitro was investigated. Irradiation with 4 different narrow-wavelength bands produced dose-dependent reductions of retinol in epidermis and dermis. The maximal effect was obtained at 334 nm, a wavelength which coincides with the absorption maximum for retinol in organic solutions. 3-Dehydroretinol was not reduced to the same extent as was retinol. In human skin the photodecomposition of retinol was most extensive in epidermis and progressively less so in dermis, presumably reflecting the extent to which 334 nm radiation penetrates the tissue. The regeneration of cutaneous retinol took over a week in the rabbit. The nutritional and biologic implications of the UV-induced reduction of cutaneous retinol remain to be established.

The photodecomposition of vitamin A in organic solvents has been widely studied. Maximal destruction is produced by radiation around 330 nm [1]. Since vitamin A is present in skin [2] and UV radiation penetrates the integument to certain depths, it is reasonable to assume that photodecomposition of the vitamin could occur in vivo. In fact, we recently showed that the vitamin A concentration of human epidermis is significantly reduced by repeated, whole-body irradiations, using a combination of UVA (320–380 nm) and UVB (280–320 nm) [3]. The mechanisms involved have not, as yet, been elucidated in humans. In this communication, data obtained by irradiating rabbit skin in vivo and human skin in vitro are presented.

MATERIALS AND METHODS

Materials and Equipment

Adult albino rabbits (generously supplied by Pharmacia AB, Uppsala, Sweden) weighing 2.5–3.5 kg were used. The animals were kept in a climated room with water and food available ad lib. The vitamin A content of the pellets was 12,000 IU/kg. Before irradiation, the outer surfaces of the ears were depilated by applying thioglycolate (Opilca, Olivin, Hamburg) followed by washings with a mixture of water and ethanol.

Full-thickness human skin was obtained in connection with reductive mammary surgery. The skin specimens were kept cool until processed.

All solvents were of spectro-grade quality from Merck, Darmstadt, Germany or from Rathburn Chemicals, England. The retinoids were of crystalline purity [2].

Manuscript received December 27, 1983; accepted for publication June 7, 1984.

This work was supported by grants from the Finsen Foundation, the Einar Jarl Foundation, the Welander Foundation, and the Swedish Medical Research Council (Proj. No. 03X-05174).

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Abbreviations:
HPLC: high-performance liquid chromatography

The high-performance liquid chromatography (HPLC) system has been described in detail elsewhere [2].

The UV-irradiation source and UV-measuring equipment were described by Alsins et al [4]. Briefly, the irradiation source consisted of 4 Philips SP 500 W water-cooled high-pressure mercury lamps with water-cooled filters. The band width at half the maximum intensity was for each of the 4 filter combinations less than 10 nm. The intensities at the distance used were: 313 nm, 50 mW/cm^2; 334 nm, 10 mW/cm^2; 365 nm, 200 mW/cm^2; and 405 nm, 100 mW/cm^2. The heat caused by the irradiation was negligible.

Study Design

The irradiations were performed by applying the light aperture (2.2 cm in diameter) directly to the skin. The irradiation times varied between 30 s and 15 min depending on the wavelength and irradiation dose studied.

Rabbit ears were irradiated as follows: (1) Five animals were irradiated with 4 different wavelengths (313, 334, 365, and 405 nm) using the same dose (3 J/cm^2). (2) Four animals were irradiated at 334 nm either with single doses of varying energies (0.5, 1.0, or 3.0 J/cm^2) or with 6 repeated doses of 0.5 J/cm^2. (3) Two animals were irradiated at 334 nm with a single dose of 3 J/cm^2 and skin samples removed after 1, 24, 72, and 168 h.

The irradiated skin was cut out as soon as possible after locally anesthesizing the ears with lidocaine (Xylocaine 1%, Astra, Sweden). Epidermis and dermis were separated using 1% acetic acid at +4°C for 2–4 h [5], a particularly useful method for rabbit skin with its thin epidermis. Acetic acid did not interfere with the vitamin A assay.

Human skin was irradiated in vitro as follows: (1) Fresh skin was irradiated at 334 nm with 3 and 10 J/cm^2 at ambient temperature. (2) Frozen skin was kept on dry ice during irradiation with the same wavelength and doses as in (1). The irradiated skin was cut out and separated into epidermis and dermis by ordinary heat treatment [6,7]. In some samples, the dermis was subdivided by cutting parallel to the upper surface. Sections approximately 200 μm thick were obtained with a cryostat (Leitz Wetzlar, West Germany).

In all experiments untreated control skin was obtained not less than 1 cm from the irradiated area. The samples were stored at −70°C until processed.

Analysis

The samples were hydrolyzed in KOH-ethanol and extracted with hexane as described elsewhere [2]. HPLC was performed on a Nucleosile 5-μm ODS column, isocratically eluted with acetonitrile and water (80:20) [2]. Duplicate protein analyses were performed on the extracted samples [7].

Mean and SEM were used when data were compiled from 3 or more experiments. Alternatively, the mean and range are given. The data were evaluated statistically by applying Student's t-test or variance analysis.

RESULTS

Untreated Rabbit and Human Skin

Table I shows the concentrations of retinol and 3-dehydroretinol in rabbit ear and human mammary skin. The human values are twice those reported earlier for normal back skin [2], possibly reflecting regional variations. The high concentration of retinol in rabbit dermis (24.9 nmol) is probably due to contamination by small amounts of vitamin A-rich subcutis.

TABLE I. *Concentrations of retinol and 3-dehydroretinol (nmol/g protein) in normal rabbit ear skin and human mammary skin (mean and SEM)*

Specimen	n[a]	Retinol	3-Dehydroretinol
Epidermis			
Rabbit	18	6.16 ± 0.35	2.50 ± 0.18
Human	10	12.53 ± 1.08	1.20 ± 0.03
Dermis			
Rabbit	14	24.96 ± 1.95	5.07 ± 0.03
Human	10	6.55 ± 0.74	ND[b]

[a] Number of analyses.
[b] Not detectable.

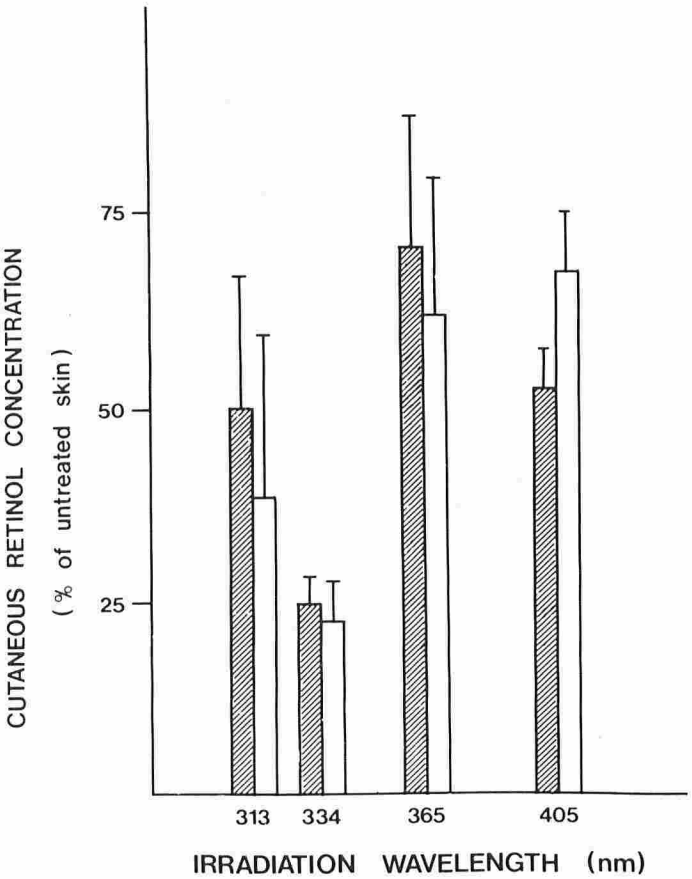

FIG 1. Cutaneous retinol concentrations in rabbit ear skin after irradiation (3 J/cm^2) at 4 different wavelengths. Epidermis, *closed bars*; dermis, *open bars*. Mean (+ SEM) values for 2–5 samples are given. Variance analysis showed that the 334 nm values were significantly ($p < 0.01$) lower than the 365 and 405 nm values.

Irradiated Rabbit Skin

Action spectrum: Fig 1 shows the retinol concentrations in epidermis and dermis after irradiation with identical doses at 4 different wavelengths. The concentrations are expressed as a percentage of those in adjacent untreated skin. The lowest values (24% and 22% for epidermis and dermis, respectively) were noted after irradiation at 334 nm. Both values are significantly lower ($p < 0.01$) than those obtained after irradiation at 365 nm and 405 nm.

The 3-dehydroretinol concentration in epidermis decreased to 71% after 334 nm irradiation and to 79% after 365 nm irradiation. The lowest dehydroretinol value in dermis (51%) was observed after 334 nm irradiation. The differences compared to untreated skin were not, however, statistically significant.

Dose response: Dose-dependent reduction of the retinol concentration was observed after irradiation at 334 nm (Table II). Six repeated irradiations of 0.5 J/cm^2 given over a period of 9 days reduced retinol by the same amount as that resulting after a single dose of 1.0 J/cm^2.

Regeneration: Fig 2 shows the regeneration of retinol following a single irradiation at 334 nm (3.0 J/cm^2). The initially low values (18% and 10% for epidermis and dermis, respectively) increased gradually to about 70% at the end of a week.

Irradiated Human Skin

Dose-response: Pilot experiments showed that, in comparison with rabbit skin, higher doses were needed to affect vitamin A in human skin. Irradiation of frozen human skin at 334 nm induced the following reductions of epidermal retinol: 88% (1 J/cm^2), 74% (5 J/cm^2), and 51% (10 J/cm^2).

In another set of experiments, the importance of the skin temperature during irradiation was studied. At ambient temperature (22°C), 10 J/cm^2 of 334 nm radiation reduced the epidermal retinol concentration to 38 ± 8%, which is similar to the value observed in frozen specimens (see above).

Analyses of different skin layers: Fig 3 compares the retinol content in different layers of untreated and irradiated skin samples. Down to middermis, the values in irradiated skin are significantly below those in untreated skin, indicating that the effect of 334 nm radiation extends to a depth of about 1 mm.

DISCUSSION

The present study shows that UV irradiation reduces the cutaneous vitamin A concentration in vivo as well as in vitro. The reduction is dose-dependent and additive if repeated doses are given (Table II). Also, there is a wavelength dependence as revealed by applying equal doses of 313, 334, 365, and 405 nm

TABLE II. *Dose-response data for UV-induced (334 nm) lowering of the cutaneous retinol concentrations in rabbit ear skin*[a]

	UV dose (J/cm^2)			6 × 0.5
	0.5	1.0	3.0	
	(Mean concentration in % of untreated skin)			
Epidermis	52.0	48.5	23.8	44.5
Dermis	48.5	32.5	17.0	32.5

[a] For experimental details see under *Study Design*.

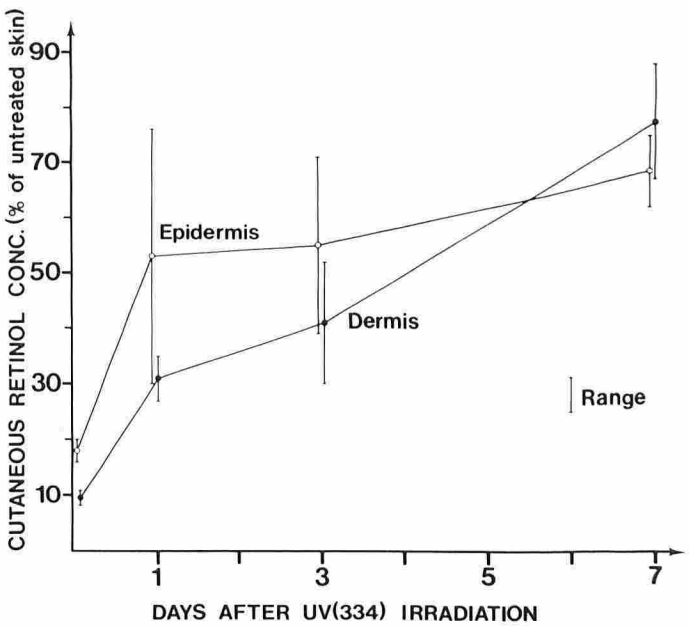

FIG 2. Variations in the cutaneous retinol concentrations in rabbit ear skin after a single exposure to 334 nm UV irradiation (3 J/cm^2). Each point represents the mean value of 2 experiments.

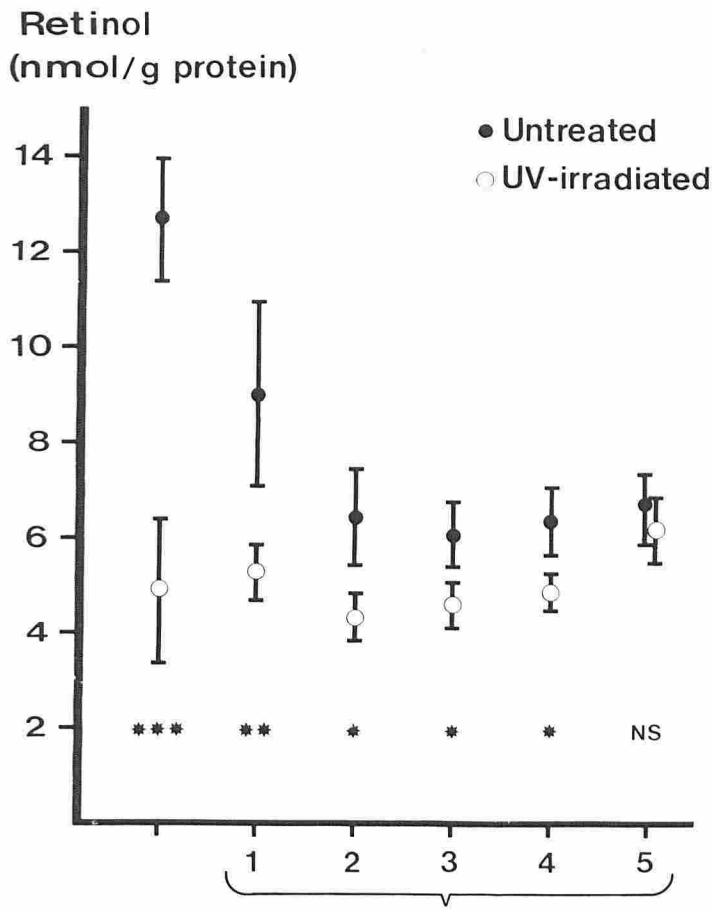

FIG 3. Retinol concentrations in untreated and UV-irradiated (334 nm, 10 J/cm^2) human skin. Epidermis and 5 dermis sections (200 μm) were prepared from paired samples. Each point represents the mean ± SEM of 6 analyses. *Stars* denote level of significance of untreated vs UV-irradiated skin: *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$. *NS* denotes not significant, $p > 0.05$.

(Fig 1). The maximum effect was seen after irradiation with the 334 nm band whereas at the other wavelengths the effect was less pronounced. Since the optical absorption spectrum of retinol in organic solvents has a broad maximum centered at 327 nm [8], it is assumed that the action spectrum closely resembles the absorption spectrum.

In a previous study in humans, a reduction of cutaneous retinol was observed after a series of 12 UV irradiations over a period of 2–3 months [3]. Whether the reduction was a direct consequence of irradiation or secondary to metabolic rearrangements could not be decided. In the present study, we found that the retinol level was lowered directly after a single irradiation, and that the reduction was similar in frozen and in fresh skin, thus suggesting independence of cellular metabolism. A direct photochemical process is more likely to be involved.

Photochemical damage of retinoids in organic solvents is a well-established event and will lead to destruction of the activity of the compounds [1,9]. The polyunsaturated side chain of vitamin A is particularly sensitive to irradiation. Isomerization and disruption of double bonds result in a range of decomposition products with different chemical characteristics [1,10]. Recent investigations using pulsed irradiation of vitamin A have demonstrated several short-lived intermediates [11]. These include triplet states, radical cations and anions, and retinylic carbenium ions. It was suggested that these intermediates might elicit photosensitivity when applied to the skin. Whether the photodecomposition of endogenous retinoids in the skin proceeds in the same manner is unknown, however.

A study of the various skin layers showed that retinol degradation was most extensive in the epidermis and progressively less so in the deeper layers of the skin, presumably reflecting the extent to which 334 nm radiation penetrates the tissue (Fig 3) [12]. In rabbit ear skin, on the other hand, similar reductions of retinol were observed in epidermis and dermis (Fig 1, Table II). A probable explanation is that the thin and nonpigmented rabbit epidermis allows the radiation to penetrate into the underlying dermis. The different morphology of rabbit and human epidermis may also help to explain why the UV radiation dose needed to reduce the retinol concentration by 50% was 10 times less in the former tissue.

3-Dehydroretinol, present in both rabbit and human epidermis, was not reduced to the same extent by irradiation as was retinol. One possible explanation is that 3-dehydroretinol has a different absorption maximum than retinol (352 vs 327 nm). Analogously, we recently reported that, in patients treated with an aromatic retinoid (etretinate; absorption maximum 365 nm), the epidermal concentration of the drug was apparently unaffected by UVB therapy, while at the same time the retinol concentration decreased to 30% of the original value [13]. Another possible explanation for why retinoids are differently affected by UV irradiation, is that some compounds are protected from photochemical damage by compartmentalization within the keratinocytes or by binding to receptor proteins. By analogy, serum retinol-binding protein protects vitamin A on irradiation [14].

The regeneration of the cutaneous vitamin A levels after irradiation seems to be a slow process. In rabbit skin, it takes over a week (Fig 2). Since it is not known whether carotenoids of the skin can be recruited for a local formation of vitamin A, it appears that the regeneration requires a continuous supply of vitamin A from the blood. Preliminary data show that the epidermal uptake of [^{3}H]retinol from serum retinol-binding protein is increased after UV irradiation [15]. Whether or not whole-body UV irradiation causes secondary changes in the blood vitamin A concentration is a matter of debate [3,16–19] and needs further investigations under controlled conditions. Such experiments are now in progress using an animal model.

In conclusion, we have shown that retinol levels in rabbit and human skin decrease significantly upon exposure to UV radiation of the type and intensity present in sunlight at the surface of earth. The nutritional and biological implications of these findings remain to be established. We are currently interested in the possibility that radiation-induced depletion of cutaneous vitamin A and formation of photosensitizing retinoid intermediates may promote UV carcinogenesis.

The technical assistance of Ms. I. Pihl-Lundin and Ms. Eva Hagforsen is gratefully acknowledged.

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