Antioxidant Defense Mechanisms in Murine Epidermis and Dermis and Their Responses to Ultraviolet Light

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A comprehensive comparison of antioxidant defenses in the dermis and epidermis and their response to exposure to ultraviolet (UV) irradiation has not previously been attempted. In this study, enzymic and non-enzymic antioxidants in epidermis and dermis of hairless mice were compared. Enzyme activities are presented both as units/gram of skin and units/milligram of protein; arguments are presented for the superiority of skin wet weight as a reference base. Catalase, glutathione peroxidase, and glutathione reductase (units/gram of skin) were higher in epidermis than dermis by 49%, 86%, and 74%, respectively. Superoxide dismutase did not follow this pattern. Lipophilic antioxidants (α-tocopherol, ubiquinol 9, and ubiquinone 9) and hydrophilic antioxidants (ascorbic acid, dehydroascorbic acid, and glutathione) were 24–95% higher in epidermis than in dermis. In contrast, oxidized glutathione was 60% lower in epidermis than in dermis. Mice were irradiated with solar light to examine the response of these cutaneous layers to UV irradiation. After irradiation with 25 J/cm^2 (UVA + UVB, from a solar simulator), 10 times the minimum erythemal dose, epidermal and dermal catalase and superoxide dismutase activities were greatly decreased. α-Tocopherol, ubiquinol 9, ubiquinone 9, ascorbic acid, dehydroascorbic acid, and reduced glutathione decreased in both epidermis and dermis by 26–93%. Oxidized glutathione showed a slight, non-significant increase. Because the reduction in total ascorbate and catalase was much more severe in epidermis than dermis, it can be concluded that UV light is more damaging to the antioxidant defenses in the epidermis than in the dermis. *J Invest Dermatol 100:260–265, 1993*

Reactive oxygen species (ROS) can damage lipids, proteins, and nucleic acids in cells [1]. Skin is easily accessible and is constantly directly exposed to air, solar radiation, and ozone and other air pollutants containing free radicals; thus, it serves as a useful model for free-radical–induced pathology. It is well known that ROS are associated with skin cancers, cutaneous photoaging, and many cutaneous inflammatory disorders, although it is not known whether their appearance is cause, effect, or both. Clarifying the mechanisms of these disorders requires a comprehensive understanding of the antioxidant systems of the epidermis and dermis and their *in vivo* response to environmental stress.

A comprehensive approach is necessary to understand mechanisms of skin damage mediated by oxidative processes and to possibly prevent free-radical–induced skin damage for three reasons. First, antioxidant functions overlap, for example, both catalase and glutathione peroxidase, destroy hydrogen peroxide [2], and both ubiquinol [3] and α-tocopherol [4] may serve as chain-breaking antioxidants in membranes. Second, it is becoming increasingly clear that antioxidants interact in a complex fashion, so that changes in the redox status or concentration of one component may affect many other components of the system. For example, it is well known that ascorbate can regenerate tocopherol from the tocopheroxyl radical [5,6], that such regeneration may occur in skin [7], and that the resulting ascorbyl radical can itself be converted to ascorbate by reduced glutathione (GSH) [8]. Thus, a perturbation in one part of the system affects the entire system. Third, with new techniques it is now possible to measure not only the concentration but the redox status in skin of antioxidants, such as ascorbate, glutathione, and ubiquinol, allowing exquisitely sensitive detection of oxidant stress in skin.

It is also necessary to study both epidermal and dermal antioxidant status because each probably plays a different role in different forms of cutaneous damage (e.g., carcinogenesis mainly involves epidermal events [9]), whereas photoaging involves both epidermal and dermal events [10,11].

Although there have been numerous reports concerning one or a few cutaneous antioxidants and their reactions to oxidative stress, at present a comprehensive understanding is lacking. Previous studies have compared the glutathione system [12,13] and superoxide dismutase (SOD) [14] in epidermis and dermis; others have compared antioxidant enzymes in cultured cell types that represent the major cell types in epidermis and dermis [15]. Other studies have examined the response to ultraviolet (UV) light of epidermal or whole-skin SOD [16,17], ascorbate,* and glutathione [12,13], and others have examined UV-induced changes in antioxidant enzymes in epidermis [18] or in cultured keratinocytes [19,20]. We previously

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Abbreviations:
BHT: butylated hydroxytoluene
DTNB: 5,5′-dithobis-2-nitrobenzoic acid
EDTA: ethylenediaminetetraacetic acid
GR: glutathione reductase
GSH: reduced glutathione
GSSG: oxidized glutathione
HPLC: high-performance liquid chromatography
ROS: reactive oxygen species
SOD: superoxide dismutase
UV: ultraviolet

* Darr D, Combs S, Dunston S, Manning T, Pinnell S: Topical vitamin C treatment inhibits ultraviolet radiation induced damage to porcine skin (abstr). J Invest Dermatol 96:590A, 1991.

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examined the effects of UVA and UVB irradiation on several antioxidants in whole, excised skin [21,22]. The present study, however, is the first to compare all major antioxidant compounds and enzymes (tocopherol, ubiquinol, ascorbate, glutathione, catalase, glutathione peroxidase, glutathione reductase, and SOD) in epidermis and dermis. It is also the first comprehensive investigation of the *in vivo* response of these antioxidants in the epidermis and dermis to a physiologic dose of UV light that closely mimicked natural sunlight. To obtain a more precise understanding of the antioxidant status of the two skin layers, we not only separated epidermis and dermis, but also analyzed both reduced and oxidized forms of glutathione, ascorbic acid, and ubiquinone.

MATERIALS AND METHODS

Preparation of Epidermis and Dermis Female hairless mice (Simonsen, strain Sim HRS/hr hr BR) 14–16 weeks old were used. After cervical dislocation, the skin was cut from the side and back. After adherent subcutis was removed, the whole skin (except in irradiation experiments; see below) was placed dermis-side down on a petri dish and heated at 55°C for 30 sec. It was then separated gently into epidermis and dermis with a scalpel. The separated epidermis and dermis were frozen and stored in liquid nitrogen for up to 2 weeks before analysis.

For glutathione, ascorbate, α-tocopherol, and ubiquinol, we verified that these conditions did not change the concentration, the oxidation status of the antioxidants, or the enzyme activities [SOD, catalase, glutathione peroxidase, and glutathione reductase (GR)] by comparing concentrations and redox status in skin heated to 55°C for 30 sec versus skin held at 0°C for 30 sec; there was no difference.

Irradiation of Mice Mice were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight), and a plate with a 2.3-cm–diameter hole was fixed on the right side of the mouse and irradiated. Light passed through the hole to the skin. Light was provided by a solar simulator (Solar Light Co., Philadelphia, PA; model 14S), which provides light of 290–400 nm with a spectral distribution similar to sunlight. Total dose was 25 J/cm^2 (UVA and UVB), as measured by three meters with overlapping sensitivities (UVX-15,-31,-36, UVX radiometers; UVP, Inc., San Gabriel, CA); because of the overlap, this is probably an overestimate. Using these meters it was found that this dose was the equivalent of exposure to 4–5 h of natural autumn sunlight at our latitude (38°N). This dose was approximately 10 minimum erythemal dose for these mice. Thus, this was a large but not uncommon dose of UV light. After irradiation, mice were killed by cervical dislocation, and irradiated skin on the right side and control skin from the contralateral area on the left side were removed immediately and separated into epidermis and dermis, 4–6 mg of epidermis and 50–70 mg of dermis (wet weight) were obtained in each sample.

Chemicals 5,5′-Dithiobis-2-nitrobenzoic acid (DTNB), reduced nicotinamide adenine dinucleotide phosphate, oxidized glutathione (GSSG), GSH, ferricytochrome *c*, hydrogen peroxide, xanthine, butylated hydroxytoluene (BHT), deferoxamine mesylate, DL-α-tocopherol, ubiquinone 9, ascorbic acid, buttermilk xanthine oxidase, and yeast GR were purchased from Sigma Chemical Co. (St. Louis, MO). 2-Vinylpyridine and 2,3-dimercapto-1-propanol were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Antioxidant Enzyme Assays Buffer A [sodium chloride 130 mM, glucose 5 mM, disodium ethylenediaminetetraacetic acid (EDTA) 1 mM, sodium phosphate 10 mM; pH 7.0], 0.75–1.5 ml, was used for homogenization. Each sample of epidermis and chopped dermis was homogenized with a Teflon homogenizer rotated by an electric drill at maximum speed for 2 min and centrifuged with a bench-top Eppendorf centrifuge model 5415 (10,000 × g for 10 min). The supernatant was kept on ice and used for enzyme assays and protein determination. We verified that this technique produced a supernatant that contained all the enzyme activities by treatment with Triton-X100 or sonication, which were found to not release more activity. The activities of catalase [23], SOD [24], glutathione peroxidase [25], and GR [26] were assayed spectrophotometrically on a Shimazu UV 160 U spectrophotometer according to procedures described in the cited references. One enzyme unit is equivalent to 1 μmol of product formation or 1 μmol of substrate disappearance/minute under the defined conditions, except for SOD. In the case of SOD, the amount of SOD inhibiting the cytochrome *c* reduction rate by 50% under the given assay conditions is defined as 1 unit. All enzyme activities were measured at 30°C. Protein concentration was determined by Bio-Rad DC protein assay. For SOD and catalase, spiking experiments were performed by adding the purified enzymes to skin samples prior to homogenization. In both cases, recovery was 95–100%, and inactivation did not occur.

Antioxidant Assays α-Tocopherol, ubiquinol 9, and ubiquinone 9 contents were analyzed simultaneously by high-performance liquid chromatography (HPLC), as described previously by Lang *et al* [27] using in-line electrochemical detection of tocopherol and ubiquinol 9 and UV detection of ubiquinone 9. For this assay, at least 15 mg wet weight of tissue was necessary. In the experiments comparing untreated epidermis and dermis, the skin of one mouse provided sufficient epidermis for each determination. In the irradiation experiments, a combined mixture epidermal samples from three mice was used for each determination in both control and irradiated skin. Dermis from the same three mice as used for comparison. We report means of three values obtained in this manner (thus, nine irradiated mice).

Glutathione was measured by the DTNB–GR recycling assay [28]. The homogenization solution was 3.3% sulfosalycylic acid, 5 mM EDTA, and 1.5 mM BHT, ice-cold, bubbled with argon gas. Samples were homogenized with the Teflon homogenizer at maximum speed for 1 min, immediately centrifuged at 3000 × *g* for 10 min; then 1 ml of the supernatant was added to 0.6 ml of 2 M sodium citrate (pH 5.5), and the mixture was used for total glutathione (GSH + GSSG) assay. By adjusting the pH in this manner, local areas of high pH, in which GSH oxidation might occur, were avoided. For the GSSG assay, 10 μl of 2-vinylpyridine was added to 500 μl of the above solution, and the mixture was incubated for 1–2 h to derivatize the reduced GSH, rendering it inactive in the assay [29]. As for α-tocopherol, in the case of irradiation, we used a mixture of three irradiated epidermis samples because at least 15 mg wet weight of tissue was necessary for GSSG assay.

Ascorbic acid and dehydroascorbic acid were measured by HPLC using electrochemical detection [30]. In brief, samples were homogenized in ice-cold 90% methanol, 1 mM EDTA, 50 μM deferoxamine mesylate, and 1.5 mM BHT solution bubbled with argon gas, with the Teflon homogenizer at maximum speed for 2 min. After centrifugation (3000 × *g* for 3 min), a 20-μl sample of supernatant was immediately analyzed by HPLC for ascorbic acid. For dehydroascorbic acid, a sample of supernatant was incubated in the dark at room temperature for 10 min with an equal volume of 10 mM 2,3-dimercapto-1-propanol. After incubation, the solution was extracted three times with three volumes of water-saturated ethyl ether. After extraction, samples were purged with nitrogen for 2 min and immediately analyzed by HPLC. Dehydroascorbic acid was calculated as total ascorbic acid minus reduced ascorbic acid. Ascorbic acid standard (2 μM) was freshly prepared for each day's assay. The concentration was determined spectrophotometrically using an extinction coefficient at 265 nm of 14,500 m^{-1} cm^{-1}.

For glutathione, ascorbate, and ubiquinol, recovery experiments were performed by adding the reduced or oxidized form to skin samples prior to homogenization. In all cases, recovery was 95–100%, and oxidation of reduced forms did not occur.

RESULTS

Enzymic Antioxidants We express enzyme activities as both units/gram of skin and units/milligram of protein (Table I). When the data are expressed as units/milligram of protein, all enzyme activities are the same in dermis and epidermis, except for SOD,

Table I. Antioxidant Enzyme Activities in Epidermis and Dermis of the Normal Mouse Skin[a]

Enzyme	Epidermis[b]	Dermis[b]
SOD	708 ± 35	900 ± 78
	11.7 ± 1.4[c]	27.5 ± 2.5
Catalase	1662 ± 204[d]	1109 ± 112
	30.4 ± 4.3	33.3 ± 1.6
Glutathione peroxidase	2.46 ± 0.32[c]	1.32 ± 0.08
	0.049 ± 0.003	0.040 ± 0.004
Glutathione reductase	2.42 ± 0.30[e]	1.39 ± 0.11
	0.042 ± 0.001	0.041 ± 0.001

[a] n = 5; results are mean ± SE.
[b] Upper value; U/g skin; lower value; U/mg protein.
[c] Epidermis different from dermis, $p < 0.001$.
[d] Epidermis different from dermis, $p < 0.05$.
[e] Epidermis different from dermis, $p < 0.01$.

which was higher in dermis than in epidermis (by 135%). When the values are expressed as units/gram of skin, however, catalase, glutathione peroxidase, and GR were higher in epidermis than in dermis by 49%, 86%, and 74%, respectively. SOD activities in epidermis and dermis were not different when expressed as units/gram of skin.

Non-Enzymic Antioxidants All values are expressed as nanomoles/gram of skin (Table II). Concentrations of both lipophilic and hydrophilic antioxidants were higher in epidermis than in dermis. In the case of lipophilic antioxidants, the concentrations of α-tocopherol, ubiquinone 9, and total Q (ubiquinol 9 + ubiquinone 9) were higher in epidermis than in dermis by 44%, 51%, and 50%, respectively. The percent of ubiquinone in the oxidized form was the same for epidermis and dermis. For hydrophilic antioxidants, concentrations of ascorbic acid, dehydroascorbic acid, and total ascorbic acid (ascorbic acid + dehydroascorbic acid) were higher in epidermis than in dermis by 24%, 47%, and 35%, respectively. GSH and total glutathione (GSH + GSSG) were also higher in epidermis than in dermis by 95% and 61%, respectively. In contrast, the concentration of GSSG and percent of total glutathione as GSSG were lower in epidermis than in dermis by 60% and 75%, respectively.

Table II. Concentrations of Lipophilic and Hydrophilic Antioxidants in Epidermis and Dermis of Hairless Mouse Skin[a]

Antioxidant	Concentration in Epidermis (nmol/g tissue)	Concentration in Dermis (nmol/g tissue)
α-Tocopherol	4.81 ± 0.47[b]	3.32 ± 0.33
Ubiquinol 9	1.87 ± 0.20	1.21 ± 0.24
Ubiquinone 9	15.21 ± 1.13[c]	10.03 ± 0.65
Total (ubiquinol + ubiquinone)	17.08 ± 1.08[d]	11.35 ± 0.45
% as ubiquinone 9	88.7 ± 1.5	89.1 ± 2.3
Ascorbic acid	1321 ± 77[b]	1064 ± 54
Dehydroascorbic acid	1324 ± 176[b]	895 ± 109
Total (ascorbic acid + dehydroascorbic acid)	2663 ± 197[c]	1959 ± 139
% as dehydroascorbic acid	48.3 ± 3.9	44.6 ± 2.7
Reduced glutathione	1160 ± 84[d]	594 ± 69
Oxidized glutathione	66 ± 19[b]	163 ± 30
Total (reduced glutathione + oxidized glutathione)	1226 ± 82[c]	757 ± 75
Percent as oxidized glutathione	5.5 ± 1.6[c]	21.7 ± 4.2

[a] n = 5 (ascorbate, n = 10); results are mean ± SE.
[b] Epidermis different from dermis, $p < 0.05$.
[c] Epidermis different from dermis, $p < 0.01$.
[d] Epidermis different from dermis, $p < 0.001$.

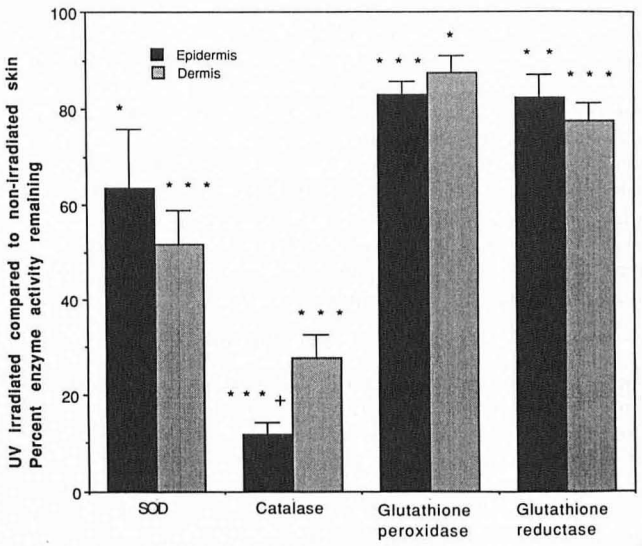

Figure 1. Changes in epidermal and dermal enzyme activities in response to UV irradiation; n = 5; results are mean ± SEM; $^{+}p < 0.05$ compared with dermis; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with non-irradiated skin.

Enzymic Antioxidants After Irradiation The change in activities of antioxidant enzymes in the irradiated side compared with the non-irradiated side is shown as the ratio of activity in skin from the irradiated side to that in control (non-irradiated) skin (Fig 1). Activities of glutathione peroxidase and GR in both epidermis and dermis decreased slightly but significantly (at least $p < 0.05$ for all; see Fig 1 for complete values). SOD and catalase activities exhibited large and significant decreases with irradiation (36% and 48% for SOD; 88% and 72% for catalase; at least $p < 0.05$ for all; see Fig 1 for complete values). The decrease in catalase activity due to irradiation was greater in epidermis than in dermis ($p < 0.05$).

Non-Enzymic Lipophilic Antioxidants After Irradiation α-Tocopherol, ubiquinol 9, ubiquinone 9, and total Q all decreased with irradiation in both epidermis and dermis to approximately the same degree in both (Fig 2). Ubiquinol 9 almost completely disappeared, decreasing in concentration by approximately 90%; ubiquinone 9 decreased by 55–70%; and α-tocopherol decreased by 30%. All changes were significant at $p < 0.001$, except the decrease of α-tocopherol in epidermis, which, owing to a large degree of variability, was not statistically significant.

Non-Enzymic Hydrophilic Antioxidants The concentrations of ascorbate, dehydroascorbate, and total ascorbate decreased by 62%, 44%, and 53%, respectively, in epidermis (at least $p < 0.01$ in all cases) and 68% in dermis (only significant for ascorbate; $p < 0.05$) (Figs 3 and 4). In all cases, the decrease in concentration due to irradiation was greater in epidermis than in dermis, although the difference was not statistically significant.

The concentration of GSH and total glutathione decreased with irradiation in both epidermis ($p < 0.05$ for both) and dermis ($p < 0.01$ for GSH; $p < 0.05$ for total), whereas the concentration of GSSG increased. Owing to a large degree of variation, the changes in GSSG were not statistically significant.

DISCUSSION

ROS are generated both by physiologic oxidative metabolism and by external causes, of which UV light may be the most important in our daily life. ROS have been linked to skin cancers, cutaneous aging, and many inflammatory disorders [1]. Studying the defense mechanisms of the part of skin that is directly exposed to UV light is of considerable interest because UV-induced damage to such defense provides indirect evidence for free-radical processes because

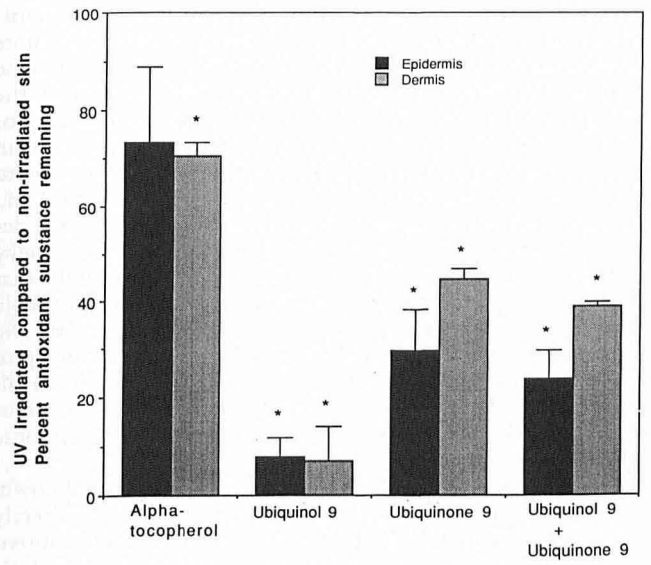

Figure 2. Changes in epidermal and dermal concentrations of lipophilic antioxidants due to UV irradiation; n = 5 (one point is mixture of three mice samples); results are mean ± SEM; *p < 0.001 compared with non-irradiated skin.

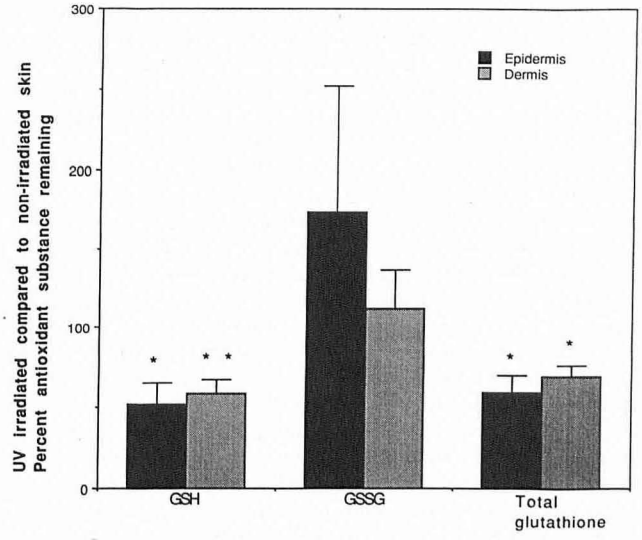

Figure 4. Changes in epidermal and dermal concentrations of reduced and oxidized glutathione due to UV irradiation; n = 5 (one point is mixture of three mice samples); results are mean ± SEM; *p < 0.05, **p < 0.01 compared with non-irradiated skin.

the exact nature of such damage may help elucidate mechanisms of free-radical damage and because rational preventive strategies can only be designed if it is known which antioxidants are most depleted by UV radiation. In this study, we present the first comprehensive analysis of antioxidants, redox status, and effects of *in vivo* UV irradiation in epidermis and dermis.

Choice of Reference Base We expressed enzyme activities as both units/gram of skin and units/milligram of protein and non-enzymic antioxidants as nanomoles/gram of skin. Traditionally, enzyme activities have been expressed as units/milligram of protein to determine degree of purity during separation and purification, but there is no *a priori* reason for referencing activity to protein. We think that values expressed as units or nanomoles/gram of skin are more reasonable than those expressed per milligram of protein because 1) much collagen in dermis is not extracted by the usual homogenization method, so that values expressed per milligram of protein are relatively higher, compared with epidermis, than those expressed per gram of skin, however, because collagen is involved in photodamage to dermis (e.g., photoaging) [10,31], concentrations of antioxidants, which may help prevent such damage, should be expressed in a form that accounts for collagen; that is, per gram of skin. 2) All intracellular and extracellular materials are potentially subject to free-radical attack. For some pathologies (e.g., cancer), the final target may be DNA, but other targets, such as lipids, may serve as important intermediaries in DNA damage. For other pathologies (e.g., cutaneous photoaging), multiple targets, including but not limited to cell proteins, may be involved. Therefore, the reference base selected should reflect all cellular components. Wet weight reflects all cellular components, whereas protein is only one component.

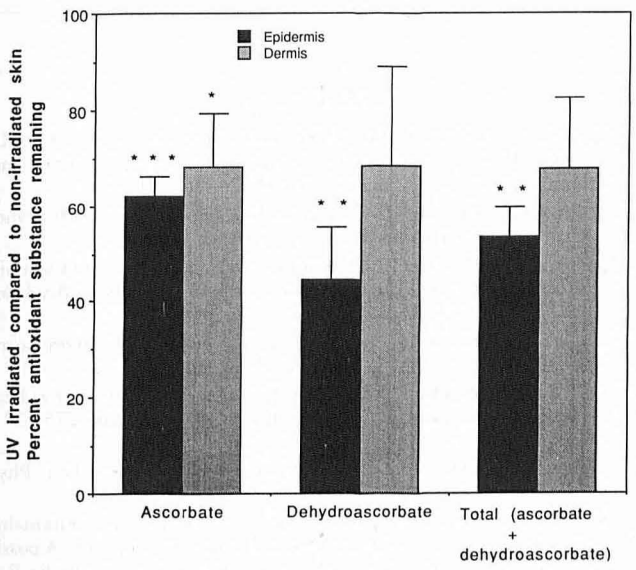

Figure 3. Changes in epidermal and dermal concentrations of ascorbic acid and dehydroascorbic acid due to UV irradiation; n = 5; results are mean ± SEM; *p < 0.05, **p < 0.01, ***p < 0.001 compared with non-irradiated skin.

Antioxidants as Indicators of Free-Radical Formation It is widely hypothesized that UV irradiation induces free-radical formation in skin. This has been shown to occur in isolated whole skin [32,33] and skin homogenates [34,35], but technical difficulties make demonstration of UV-induced free-radical formation *in vivo* extremely difficult. The status of antioxidant defenses that counteract free radicals can, however, serve as indirect evidence of environmentally caused free-radical formation in skin.

If UV light or other environmental factors cause cutaneous free-radical formation *in vivo*, the epidermis, being the outermost layer of the skin, would be expected to have the greatest antioxidant defenses. In this experiment, we found that concentrations of lipophilic (α-tocopherol, ubiquinol 9, and ubiquinone 9) and hydrophilic (ascorbic acid, dehydroascorbic acid, and glutathione) antioxidants were approximately 24–95% higher in epidermis than in dermis and that activities of antioxidant enzymes, except SOD, were 50–85% higher in epidermis when expressed as units/gram of skin. These results are in agreement with the results of Connor and Wheeler [13] for glutathione and related enzymes in skin from mice of the same strain; they reported glutathione concentrations of 750 nmol/g skin in epidermis (3% oxidized) and 320 nmol/g skin in dermis (22% oxidized); we found similar percentages for the oxi-

dized form in the epidermis and dermis (5.5% and 21.7%, respectively), although our absolute values were approximately 50% higher. This may be due to differences in epidermal separation methods because these investigators immersed their samples in water, thus increasing the weight due to water absorption, whereas we were careful not to expose our samples directly to water. Our results for relative activities of GR and glutathione peroxidase in epidermis and dermis, which were 74% and 86% greater in epidermis when expressed per gram of tissue, also agree with those of Connor and Wheeler, who reported approximately twice the activity for these enzymes in epidermis than in dermis, when expressed per gram of tissue [13]. Our results for SOD do not agree with those of Kim and Lee [14] for human skin — our values, when expressed as units/milligram of protein (as these investigators did) show dermis to have over twice the activity of epidermis, whereas these investigators report 20% greater activity in epidermis. This may represent a difference between human skin and mouse skin. These investigators did not report their findings as units/gram of skin. To our knowledge, this is the first time that epidermal and dermal catalase activity and tocopherol, ubiquinol, and ascorbate concentrations have been compared; the fact that these values, as well as those for the other enzymes and for glutathione, were all higher in the epidermis supports the idea of greater epidermal free-radical formation, necessitating greater antioxidant capacity. It should be noted that catalase is contained in erythrocytes at activity levels approximately 30 times those seen in this study for dermis and that dermis is a vascular tissue. No attempt was made to purge blood from the dermis and, although no blood was visible, it is possible that some portion of the catalase activity in the dermis was due to blood and not the tissue itself. In this case, the disparty between catalase activities in dermis and epidermis would be even greater.

More conclusive evidence comes from examining antioxidant status after irradiation. If there is a UV-light–induced free-radical load, then with UV exposure anitoxidant substances would be expected to be oxidized, decrease in concentration, or both. Whereas previous separate studies on UV irradiation of skin have reported *in vivo* decreases in ascorbate (see footnote 1) and glutathione [12,13], and we previously reported simultaneous losses of tocopherol, ascorbate, and glutathione in excised skin [21,22], none has used an *in vivo* system, irradiated with a physiologic dose of UV light closely mimicking sunlight, and examined all the major antioxidants. All of these conditions must be met for the evidence for solar UV-light–induced radical formation to be compelling. Studies of excised skin involve possible artifacts due to hypoxia and skin removal, whereas studies of cell systems using keratinocytes involve complications, such as the state of differentiation of the cells; cell culture can only approximate *in vivo* conditions. All of the major antioxidants must be examined because the decrease of a single antioxidant may be due to effects other than free radicals. For example, α-tocopherol [36] and ubiquinol [37] both absorb strongly at 295 nm and can be directly destroyed by UV irradiation [7]. The destruction and/or oxidation of all of the major antioxidants with a single dose of UV light observed in these experiments presents compelling evidence that solar UV irradiation induces free-radical production *in vivo*.

UV-light–induced decreases in the antioxidant enzymes catalase, SOD, glutathione peroxidase, and GR were seen in this study. Destruction of catalase and SOD has been observed in irradiated keratinocytes* [19,20], and losses in catalase and SOD activity were reported by Pence and Naylor [18] in epidermis of hairless mice irradiated *in vivo;* there was also a non-significant decrease in glutathione peroxidase activity in this study. The results of the latter study differ from ours in that activities immediately after irradiation were not measured and at the first time point (6 h post-irradiation) measured by these investigators, none of the enzyme activities differed significantly from control, however, it is interesting to note that at this first time point, loss of SOD and glutathione peroxidase activity in epidermis was approximately equal to that seen in the present study; the lack of statistical significance in the study of Pence and Naylor may be attributed to greater variation than seen in the present study. In the present study, the loss of catalase due to irradiation was enormous (88% in epidermis, 72% in dermis) and immediate, unlike the report of Pence and Naylor, in which decreases did not occur until 12 h post irradiation. The discrepancy between our work and theirs may be due to our using light that extended into the visible spectrum because it is known that visible light directly destroys catalase [38]. This would also explain the greater destruction in epidermis compared with dermis, owing to the dermis being more shielded from visible light. The small amount of GR destruction that we saw was similar to previous studies, in which little [22] or no [13] destruction of GR in response to UV light was observed.

The exact origin of the damage to these enzymes is unknown, although, as mentioned above, it is known that catalase is directly destroyed by visible light [38]. Damage to skin enzymic antioxidants is not in itself support for the free-radical hypothesis of UV-induced skin damage because the damage is not necessarily mediated by free radicals, however, the destruction of these antioxidant enzymes indicates the possibility of more widespread protein damage, as well as leaving the skin open to further oxidative stress from any source. Inactivation of these enzymes may cause a vicious cycle, accelerating the damage due to free radicals.

Experiments conducted in the hairless mouse system, although offering clues as to the effects of UV light on various antioxidant systems, are not the equivalent of human experiments. Human skin, especially dermis, is thicker than mouse skin and is pigmented to varying degrees. Therefore, these results may not correlate with the human *in vivo* situation directly. In the near future, we plan to repeat these experiments with human skin samples.

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ANNOUNCEMENT

The Dermatology Section of the North Carolina Medical Society will hold its annual meeting on November 6, 1993 at the Adams Mark Hotel in Charlotte, NC. Three hours CME Category 1 credit has been approved. For further information, contact J. Blake Goslen, M.D., Secretary-Treasurer, 1718 E. 4th Street, Suite 304, Charlotte, NC 28204.