Photochemistry and Photobiology, 1996, 64(6): 918–922

EPR Detection of Free Radicals in UV–Irradiated Skin: Mouse *Versus* Human

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Received 28 June 1996; accepted 28 August 1996

ABSTRACT

Ultraviolet radiation produces free radicals in Skh-1 mouse skin, contributing to photoaging and carcinogenesis. If a mouse model is a general indicator of free radical processes in human skin photobiology, then radical production observed in mouse and human skin should be directly comparative. In this work we show that UV radiation ($\lambda > 300$ nm, 14 μW/cm^2 UVB; 3.5 mW/cm^2 UVA) increases the ascorbate free radical ($Asc^{\bullet-}$) electron paramagnetic resonance (EPR) signal in both Skh-1 mouse skin (45%) and human facial skin biopsies (340%). Visible light ($\lambda > 400$ nm; 0.23 mW/cm^2 UVA) also increased the $Asc^{\bullet-}$ signal in human skin samples (45%) but did not increase baseline mouse $Asc^{\bullet-}$, indicating that human skin is more susceptible to free radical formation and that a chromophore for visible light may be present. Using EPR spin-trapping techniques, UV radiation produced spin adducts consistent with trapping lipid alkyl radicals in mouse skin (α-[4-pyridyl 1-oxide]-*N-tert*-butyl nitrone/alkyl radical adduct; $a^N = 15.56$ G and $a^H = 2.70$ G) and lipid alkoxyl radicals in human skin (5,5-dimethylpyrroline-1-oxide/alkoxyl radical adduct; $a^N = 14.54$ G and $a^H = 16.0$ G). Topical application of the iron chelator Desferal® to human skin significantly decreases these radicals (≈50%), indicating a role for iron in lipid peroxidation; Desferal has previously been shown to decrease radical production in mouse skin. This work supports the use of the Skh-1 mouse as a predictive tool for free radical formation in human skin. These results provide the first direct evidence for UV radiation-induced free radical formation at near physiological temperatures in human skin and suggest that iron chelators may be useful as photoprotective agents.

INTRODUCTION

The ultimate test animal for therapeutic experiments is humans, but there are ethical limitations. The hairless mouse is a widely used model for studying photoaging of skin. Ultraviolet radiation produces free radicals in Skh-1 mouse skin, contributing to photoaging and carcinogenesis (1). The ascorbate free radical ($Asc^{\bullet-}$)‡ is detectable by electron paramagnetic resonance (EPR) spectroscopy at low steady-state levels in a manifold of biological samples (2–6), including skin (7–10). It has been demonstrated that $Asc^{\bullet-}$, which is naturally present in biological systems, can be used as a noninvasive indicator of oxidative stress (11). As such, $Asc^{\bullet-}$ has been useful in the study of free radical oxidations in numerous biological systems (12–15) including mouse skin (7–10). Because $Asc^{\bullet-}$ is a resonance-stabilized free radical, it is detectable directly by EPR. However, the nonresonance-stabilized free radicals initially produced in the skin by UV radiation have very short lifetimes at room temperature. Thus in this work $Asc^{\bullet-}$ serves as a marker of UV radiation-induced free radical formation in human skin, while EPR spin-trapping techniques have been applied to detect the nonresonance-stabilized radicals produced by UV radiation.

A common approach for studying free radicals in tissues has been to use homogenized samples (16,17). But, homogenizing the sample may produce artifacts; thus, in this study sections of mouse or human skin were examined *ex vivo*, epidermis and dermis intact, to study real-time free radical formation by UV radiation.§

There are significant biochemical and structural differences between mouse and human skin that could affect their responses to UV radiation and other environmental insults. If this mouse model is to be used as a general indicator of free radical processes in human skin photobiology, then radical production observed in mouse and human skin should be directly comparative. Thus, in this research we compare standard Skh-1 hairless mouse skin to human facial skin bi-

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‡*Abbreviations:* $Asc^{\bullet-}$, ascorbate free radical; DMPO, 5,5-dimethylpyrroline-1-oxide; EPR, electron paramagnetic resonance spectroscopy; POBN, α-(4-pyridyl 1-oxide)-*N-tert*-butyl nitrone.

§Homogenization of the sample will disrupt and damage membranes resulting in decompartmentalization of cell contents. In addition, high levels of oxygenation are achieved that will amplify the oxidation process. Thus, many peroxides may be introduced into the sample. By comparison, an intact biopsy will have only minor decompartmentalization of cellular contents, but the lack of blood flow will reduce nutrient levels; oxygenation will be lower than when *in vivo*. This low oxygenation may actually blunt the oxidative processes we are attempting to monitor.

opsies as models for UV radiation-induced free radical formation.

MATERIALS AND METHODS

The Skh-1 hairless mice were obtained from Charles River Laboratory, Portage, MI and were given a standard diet and water *ad libitum*. Animal care strictly followed University of Iowa and NIH animal welfare policies. Mice were sacrificed, and whole dorsal skin was harvested immediately and cut into EPR-usable pieces (≈1 cm^2).

Intact human facial skin sections (≈1 cm^2) were obtained from pre- or postauricular regions of caucasian patients undergoing facial surgery and were immediately frozen at 77 K. In our previous work with murine skin we have demonstrated that this storage technique does not significantly influence the UV light-mediated free radical response (8,9). (All appropriate guidelines of the Helsinki Declaration of 1975 as revised in 1983 were followed.) Because EPR samples of skin can be no more than approximately 500 μm thick, some of the dermis was cut from the human skin samples prior to EPR examination. In all experiments the same radiation source setup was used for irradiating the epidermal surface of the skin sample in the EPR cavity.

The radiation source was an Oriel 150 W Photomax® xenon arc lamp operating at 30 W. The filtered light-fluence rate at the sample, assuming the cavity grid transmits 75% of the incident radiation, was estimated using a model IL 1400A International Light Inc. radiometer with UVA detector, model SEL 033; UVB detector model SEL 240. In those experiments examining the effects of solar radiation, wavelengths below ≈300 nm were filtered out using a Schott WG305 filter. (This 305 nm cutoff filter provides 4% at 290 nm, 46% transmission at 305 nm and 79% at 320 nm. The resulting fluence rates were 14 μW/cm^2 UVB; 3.5 mW/cm^2 UVA.) For visible light experiments, wavelengths below 400 nm were filtered out using an Oriel 59472 filter (0.23 mW/cm^2 UVA). Infrared radiation was removed by a 5.0 cm quartz water filter. Typical ambient levels of light in the laboratory produced no change in EPR-detectable free radical levels.

For EPR examination, the skin samples ≈1 cm^2 (≈500 μm thick) were placed in the well of a Wilmad Glass Co. (Buena, NJ) tissue cell (WG-806-B) that had the lower positioning rod removed for easier tuning of tissue samples. The epidermal surface was facing "out." A coverslide over the sample was held in place with Parafilm ties. In the ascorbate experiments the Bruker ESP-300 EPR spectrometer (Karlsruhe, Germany) settings were: microwave power, 40 mW; modulation amplitude, 0.66 G; time constant, 0.3 s; scan rate, 8 G/41.9 s; receiver gain, 2×10^6. For the spin-trapping experiments 50 μL of the stock spin trap in H_2O (250 m*M* α-[4-pyridyl 1-oxide]-*N-tert*-butyl nitrone [POBN]; 1.0 *M* 5,5-dimethylpyrroline-1-oxide [DMPO]) was topically applied to the epidermis for 10 min, then blotted and immediately examined by EPR. No increase in background EPR signal occurred when the aqueous solutions of either POBN or DMPO was exposed to UV radiation with identical parameters to that in the skin experiments. The EPR instrument settings for the POBN spin-trapping experiments were: microwave power, 40 mW; modulation amplitude, 0.76 G; time constant, 0.3 s; scan rate, 60 G/41.9 s; receiver gain, 1×10^6. The EPR spectrometer settings for the DMPO experiments were: microwave power, 40 mW; modulation amplitude, 1.06 G; time constant, 0.3 s; scan rate, 80 G/84 s; receiver gain, 1×10^6.

RESULTS

Previous work, using room temperature EPR, has demonstrated the presence of the endogenous, resonance-stabilized ascorbate free radical at a low steady-state level in mouse (Skh-1) skin (7,8). In the present work we have for the first time demonstrated the presence of $Asc^{\cdot-}$ (g = 2.0053, $a^{H4} \cong$ 1.8 G‖) in human skin biopsies.

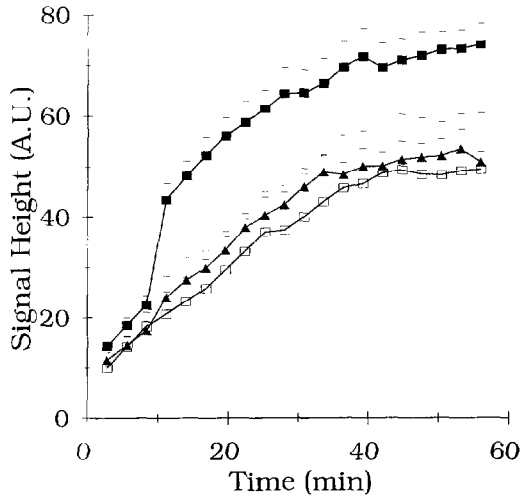

Figure 1. The $Asc^{\cdot-}$ increases in mouse skin when exposed to UV radiation but not visible light. ■, $Asc^{\cdot-}$ signal intensity from skin that was exposed to UV and visible light (305 nm cutoff filter); ▲, $Asc^{\cdot-}$ signal intensity from skin exposed to visible light only (400 nm cutoff filter); □, $Asc^{\cdot-}$ signal intensity from skin that was exposed to room light only. The data represent the mean of four separate experiments. The light was turned on after the third data point. After UV radiation exposure, a paired comparison of each curve showed the visible light-exposed samples not to be statistically different from those samples exposed to room light.

Exposure of mouse skin, while in the EPR cavity, to a combined UVA and UVB radiation source results in an approximately twofold increase in the $Asc^{\cdot-}$ signal height relative to unirradiated mouse skin, indicating that during UV radiation exposure the skin is undergoing free radical oxidative stress (Fig. 1). However, when human skin is exposed to the same UV radiation source there is an approximate fourfold increase in the $Asc^{\cdot-}$ EPR signal intensity (Fig. 2). The kinetics of the observed changes in $Asc^{\cdot-}$ EPR signal height between human and mouse skin also differed, as did the patterns of spontaneous $Asc^{\cdot-}$ radical accumulation in nonirradiated skin.

Visible light photons have generally been considered innocuous because of their low energies; however, in skin naturally occurring photosensitizers could produce free radicals in the presence of visible light. Thus, the potential involvement of visible light in free radical production was examined. Using only wavelengths above ≈400 nm, we observed that exposure of mouse skin to visible light does not increase the $Asc^{\cdot-}$ EPR signal above ambient light levels (Fig. 1). This indicates that visible light has no detectable effect on free radical formation in mouse skin.

However, in human skin, visible light was found to increase $Asc^{\cdot}$; an approximate twofold increase was observed (Fig. 2). In these Caucasian facial skin biopsies there may be a visible light chromophore that is not significantly pres-

‖Each line of the ascorbate radical doublet is actually a triplet of doublets: a^{H4} = 1.76 G, a^{H6} (2) = 0.19 G and a^{H5} = 0.07 G (18). However, these EPR instrument settings were selected for maximum sensitivity (19); thus, only a doublet with $a^{H4} \cong$ 1.8 G is observed.

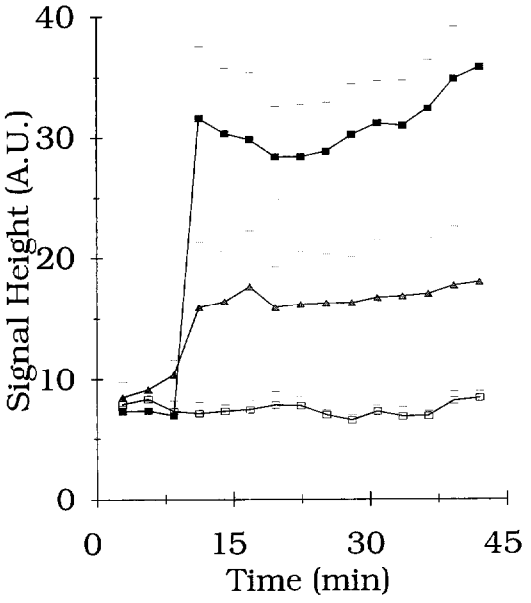

Figure 2. The $Asc^{\cdot-}$ in human skin increases on exposure to UV radiation and visible light. ■, $Asc^{\cdot-}$ signal in biopsies exposed to UV radiation (305 nm cutoff and IR filters); ▲, $Asc^{\cdot-}$ signal in biopsies exposed to visible light (400 nm and IR filters); □, $Asc^{\cdot-}$ signal in biopsies exposed to room light only. The samples were exposed to light after collection of the third data point. Each data point represents the mean of four samples ± SEM. All $Asc^{\cdot-}$ data represent two signal-averaged scans.

ent in Skh-1 mouse skin, such as flavins and melanin, which when excited may produce an oxidizing species that can react directly with $AscH^-$ producing $Asc^{\cdot-}$. This would be another indication of the oxidation processes that can be initiated in skin by light.

Spin-trapping techniques were applied to further identify the short-lived initial free radicals produced by UV radiation in skin. Using the spin-trap POBN, a carbon-centered POBN spin adduct as well as $Asc^{\cdot-}$ is observable in UV radiation-exposed mouse skin Fig. 3. The spectra exhibit hyperfine splittings characteristic of POBN/alkyl radicals, a^N = 15.56 G and a^H = 2.70 G, possibly generated from membrane lipids as a result of β-scission of lipid alkoxyl radicals causing generation of alkyl radicals, such as ethyl and pentyl radicals (20,21).

The spin-trap DMPO was used in our mouse skin model to further identify the radicals involved in UV radiation-induced free radical formation. The DMPO was topically applied to the skin in the same manner as the POBN; however, no spin-trapped adduct was observable in the absence or presence of UV radiation. Only the ascorbate radical was observed (data not shown).

These spin traps were also applied to the human skin biopsies. When POBN was topically applied to the human skin biopsies no EPR signal was observable in the absence of UV radiation. Exposure to UV radiation resulted in a triplet of doublets as well as the ascorbate free radical signal. The hyperfine splittings of the POBN adduct were a^N = 15.62 G and a^H = 3.1 G, possibly indicating a carbon dioxide radical anion (21,22). This signal was very weak, thus further spin-trapping experiments were done using DMPO.

When human skin biopsies were treated with DMPO, no

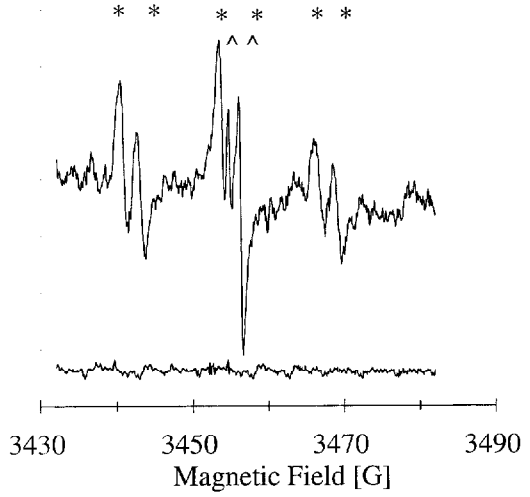

Figure 3. The POBN spin traps a carbon-centered radical upon UV radiation exposure of mouse skin. The lower spectrum is from skin exposed to room light. The upper spectrum is from skin exposed to the combined UVA and UVB radiation source (305 nm cutoff filter). When skin was exposed to UV radiation a carbon-centered POBN spin adduct (*) (a^N = 15.56 G, a^H = 2.70 G) as well as the ascorbate radical ( ) ($a^{H4} \cong$ 1.8 G) were observed.

EPR signal was detected in room light alone. Upon UV radiation exposure, $Asc^{\cdot-}$ as well as a six-line DMPO adduct signal were observed, (Fig. 4). The hyperfine splittings (a^N = 14.54 G and a^H = 16.0 G) are characteristic of DMPO/$SO_3^{\cdot-}$ and DMPO/alkoxyl radical adducts (10,21,23); however, a lipid-derived alkoxyl radical is more likely in this system. The presence of lipid-derived radicals is consistent with our previous observation of UV radiation-induced lipid alkyl radicals in mouse skin (8,9).

Because lipid peroxidation products were indicated in both our mouse and human skin spin-trapping experiments, iron may be involved in these free radical processes. Skin is a significant site of iron excretion. In addition chronic exposure of skin to UV radiation has been found to cause a significant increase in the basal levels of non-hemoglobin iron (24). This excess iron could lead to increased free radical damage (25). Indeed, using the Skh-1 mouse model top-

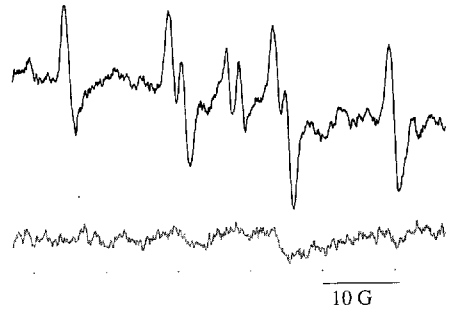

Figure 4. The EPR spectra of UV radiation-induced DMPO radical adducts in human skin biopsies. The top spectrum is of human skin exposed to UV radiation; the lower spectrum is of human skin exposed to room light. The upper spectrum results from two radical species: a central $Asc^{\cdot-}$ doublet (a^{H4} = 1.8 G) and a six-line DMPO adduct (a^N = 14.54 G, a^H = 16.0 G). Both spectra are the result of 10 signal-averaged scans.

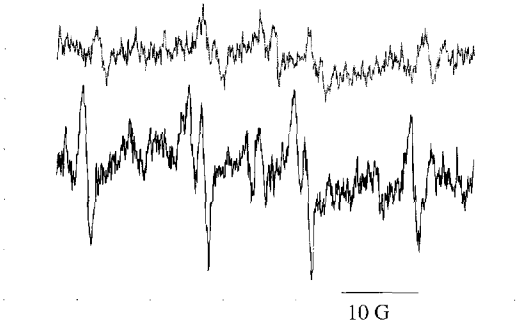

Figure 5. Desferal® decreases the UV radiation-induced DMPO adduct signal in human skin biopsies. The lower spectrum is of the DMPO signal adduct from human skin exposed to UV radiation. The upper spectrum is of human skin treated with Desferal and exposed to UV radiation. The spectral species and parameters are the same as observed in Fig. 4. Both spectra are the result of five signal-averaged scans.

ical application of iron chelators to skin has been found to be photoprotective by delaying the onset of UV light-induced skin tumor formation (24). In previous work we demonstrated that topical application of Desferal®, an iron-chelating agent, to mouse skin significantly reduced UV-induced free radical formation (8). Desferal has no significant UV absorption at wavelengths greater than ≈280 nm. In our experiments a 305 nm UV cutoff filter was used, thus Desferal would not act as a simple UV blocking agent. Therefore, to determine if the action of Desferal is similar in human skin to that observed in mouse skin we examined the effect of topically applied Desferal on protection against UV radiation-induced free radical production in human skin. Using DMPO spin-trapping techniques, Desferal or vehicle was topically applied to the human skin prior to EPR examination. After exposure to UV radiation the same six-line DMPO adduct, as well as $Asc^{\cdot-}$, was observable in both treatment groups. Desferal reduced the DMPO radical signal by ≈50% (Fig. 5), which is parallel to our earlier observations in mouse skin (8).

DISCUSSION

The data presented in this work demonstrate that in both mouse and human skin biopsies the ascorbate free radical signal intensity increases with UV radiation exposure, indicating oxidative stress. Visible light also caused a modest increase in free radical formation in the human biopsies, which is in contrast with our findings in the mouse model. Direct detection of $Asc^{\cdot-}$ by EPR is consistent with ascorbate's role as the terminal small-molecule antioxidant. Due to ascorbate radical's low reduction potential, +282 $E^{o\prime}$ mV (26), nearly every oxidizing radical that can arise in a biological system will react with ascorbate forming the semidehydroascorbate radical ($Asc^{\cdot-}$), a resonance-stabilized, tricarbonyl free radical species. It has been demonstrated that the ascorbate radical EPR signal can serve as an indicator of oxidative events (5,8,11). Thus, UV light increases the flux of radicals in both mouse and human skin.

Using EPR spin-trapping techniques we have for the first time detected, *in situ,* a free radical spin adduct during UV radiation exposure of human skin. The trapped radical in human biopsies appears to be different from the radicals trapped from mouse skin, *i.e.* an alkoxyl *versus* an alkyl radical. Timmins *et al.* used DMPO to trap organic peroxide-induced radicals in pieces of mouse skin (10). They observed spin adducts characteristic of trapping peroxyl, alkoxyl and alkyl radicals, consistent with our findings here.

Photooxidation of lipids may be one of the initial steps involved in UV radiation-induced skin damage (27). The spin-trapping of alkyl and alkoxyl radicals indicates that iron may be involved in the lipid peroxidation processes occurring in skin.

$$Fe^{2+} + LOOH \rightarrow Fe^{3+} + LO^{\cdot} + OH^{-}$$

$$LO^{\cdot} \xrightarrow{\beta\text{-scission}} \text{ethyl or pentyl radicals} + \text{aldehydic products}$$

$$LO^{\cdot} + DMPO \rightarrow DMPO/LO^{\cdot}$$

$$^{\cdot}CH_2R + POBN \rightarrow POBN/^{\cdot}CH_2R$$

Both human and mouse skin naturally have a basal level of non-heme iron; sun-exposed sites have significantly increased levels of this iron (24,28). Iron can serve as a catalyst to enhance lipid peroxidation. Thus, sequestration of this iron by chelators should lead to a reduction in free radical formation. This scenerio is consistent with our observation of reduced spin adducts in skin treated with Desferal. Desferal, not chelated to metals, can be a donor antioxidant. However, we see no hint of the aminoxyl radical that would be expected, but this of course does not completely rule out this possibility. However, we favor the iron sequestration hypothesis because other iron chelators have been shown to be effective in skin protection (24). Thus, the inclusion of appropriate metal chelators in topical skin formulations may be beneficial.

Although our results indicate definite differences between the free radical response in mouse and human skin, the increase in $Asc^{\cdot-}$ and the presence of lipid-derived radical products with UV light exposure in both models indicate that the mouse model can be a predictive tool for the study of human skin photochemistry. In addition this work demonstrates that human skin biopsies can directly be used as an *ex vivo* indication of UV-induced free radical processes occurring in skin and may be a useful tool in studying other dermato-oxidation events.

Acknowledgements—This work was supported in part by The Procter & Gamble Co. B.A.J. was partially supported by a University of Iowa Center on Aging NIH Training Grant (AG-00214). G.R.B. was supported in part by NIH grant P01 CA-66081.

REFERENCES

1. Black, H. S. (1987) Potential involvement of free radical reactions in ultraviolet light mediated cutaneous damage. *Photochem. Photobiol.* **46**, 213–221.
2. Stegmann, H. B., P. Schuler, S. Westphal and E. Wagner (1993) Oxidative stress of crops monitored by EPR. *Z. Naturforsch. C* **48**, 766–772.
3. Rose, R. C. and A. M. Bode (1993) Biology of free radical scavengers: an evaluation of ascorbate. *FASEB J.* **7**, 1135–1142.
4. Minetti, M., T. Forte, M. Soriani, V. Quaresima, A. Menditoo and M. Ferrari (1992) Iron-induced ascorbate oxidation in plasma as monitored by ascorbate free radical formation. *Biochem. J.* **282**, 459–465.

5. Sharma, M. K. and G. R. Buettner (1993) Interaction of vitamin C and vitamin E during free radical stress in plasma: an ESR study. *Free Radicals Biol. Med.* **14**, 649–653.
6. Buettner, G. R. and W. Chamulitrat (1990) The catalytic activity of iron in synovial fluid as monitored by the ascorbate free radical. *Free Radicals Biol. Med.* **8**, 55–56.
7. Buettner, G. R., A. G. Motten, R. D. Hall and C. F. Chignell (1987) ESR detection of endogenous ascorbate free radical in mouse skin: enhancement of radical production during UV irradiation following topical application of chlorpromazine. *Photochem. Photobiol.* **46**, 161–164.
8. Jurkiewicz, B. A. and G. R. Buettner (1994) Ultraviolet light-induced free radical formation in skin: an electron paramagnetic resonance study. *Photochem. Photobiol.* **59**, 1–4.
9. Jurkiewicz, B. A., D. L. Bissett and G. R. Buettner (1995) The effect of topically applied tocopherol on ultraviolet-radiation mediated free radical damage in skin. *J. Invest. Dermatol.* **104**, 484–488.
10. Timmins, G. S. and M. J. Davies (1993) Free radical formation in murine skin treated with tumour promoting organic peroxides. *Carcinogensis* **14**, 1499–1503.
11. Buettner, G. R. and B. A. Jurkiewicz (1993) Ascorbate free radical as a marker of oxidative stress: an EPR study. *Free Radicals Biol. Med.* **14**, 49–55.
12. Tomasi, A., E. Albano, A. Bini, A. C. Iannone and V. Vannini (1989) Ascorbyl radical is detected in rat isolated hepatocytes suspensions undergoing oxidative stress: and early index of oxidative damage in cells. *Adv. Biosci.* **76**, 325–334.
13. Arroyo, C. M., J. H. Kramer, B. F. Dickens and W. B. Weglicki (1987) Identification of free radicals in myocardial ischemia/reperfusion by spin trapping with nitrone DMPO. *FEBS Lett.* **221**, 101–104.
14. Nohl, H., K. Stolze, S. Napetschnig and T. Ishikawa (1991) Is oxidative stress primarily involved in reperfusion injury of the ischemic heart? *Free Radicals Biol. Med.* **11**, 581–588.
15. Sharma, M. K., G. R. Buettner, K. T. Spencer and R. E. Kerber (1994) Ascorbyl free radical as a real-time marker of free radical generation in briefly ischemic and reperfused hearts. *Circ. Res.* **74**, 650–658.
16. Ogura, R., M. Sugiyama, J. Nishi and N. Haramaki (1991) Mechanism of lipid radical formation following exposure of epidermal homogenate to ultraviolet light. *J. Invest. Dermatol.* **97**, 1044–1047.
17. Ogura, R., J. Nishi, M. Sugiyama, N. Haramaki and M. Kotegawa (1990) Lipid radical formation of the epidermis exposed to ultraviolet light (ESR study). *Photomed. Photobiol.* **12**, 179–184.
18. Laroff, G. P., R. W. Fessenden and R. H. Schuler (1972) The electron spin resonance spectra of radical intermediates in the oxidation of ascorbic acid and related substances. *J. Am. Chem. Soc.* **94**, 9062–9073.
19. Buettner, G. R. and K. P. Kiminyo (1992) Optimal EPR detection of weak nitroxide spin adduct and ascorbyl free radical signals. *J. Biochem. Biophys. Methods* **24**, 147–151.
20. North, J. A., A. A. Spector and G. R. Buettner (1992) Detection of lipid radicals by electron paramagnetic resonance spin trapping using intact cells enriched with poyunsaturated fatty acid. *J. Biol. Chem.* **267**, 5743–5746.
21. Buettner, G. R. (1987) Spin trapping: ESR parameters of spin adducts. *Free Radicals Biol. Med.* **3**, 259–303.
22. Li, A. S. W., K. B. Cummings, H. P. Roethling, G. R. Buettner and C. F. Chignell (1988) A spin trapping database implemented on the IBM PC/AT. *J. Magnet. Res.* **19**, 140–142.
23. Smith, F. L., R. A. Floyd and M. P. Carpenter (1981) Prostaglandin synthase-dependent spin trapped free radicals. In *Oxygen and Oxy-Radicals in Chemistry and Biology* (Edited by M.A.J. Rodgers and E. L. Powers), pp. 734–745. Academic Press, New York.
24. Bissett, D. L., R. Chatterjee and D. P. Hannon (1991) Chronic ultraviolet radiation-induced increase in skin iron and the photoprotective effect of topically applied iron chelators. *Photochem. Photobiol.* **54**, 215–223.
25. Miller, D. M., G. R. Buettner and S. D. Aust (1990) Transition metals or catalysts of "autoxidation" reactions. *Free Radicals Biol. Med.* **8**, 95–108.
26. Williams, N. H. and J. K. Yandell (1985) Reduction of oxidized cytochrome c by ascorbate ion. *Biochim. Biophys. Acta* **810**, 274–277.
27. Shibamoto, T. (1994) The role of lipid peroxidation caused by ultraviolet light in skin diseases. *J. Toxicol.* **13**, 193–202.
28. Bissett, D. L. and J. F. McBride (1992) Iron content of human epidermis from sun-exposed and non-exposed body sites. *J. Soc. Cosmet. Chem.* **43**, 215–217.