As the outermost layer of skin, the SC is most directly and frequently exposed to environmental oxidative stressors. Although it does not contain nucleated cells, oxidation products of the lipid- and protein-rich SC may reveal signaling effects on adjacent keratinocytes. Previously, we have demonstrated that SC α-tocopherol depletion is a very early and sensitive marker of ozone- and UVA/UVB-induced photoxidation in skin, and that it can be noninvasively measured in human skin *in vivo*.[10,11] In addition to being the outermost skin layer, the SC is the result of a differentiation process initiated in lower epidermal layers. Therefore, measurement of α-tocopherol gradients in the SC may be of interest for studying not only oxidative stress initiated in the SC, but also oxidative insults that were set during the differentiation period of keratinocytes. Furthermore, differences in the regulation of α-tocopherol in different anatomical sites may be studied less invasively by the tape stripping technique than by regular skin biopsies.

In conclusion, the presented method may be a useful tool for *in vivo* studies of oxidative stress in humans, which are needed to validate the relevance of *in vitro* and animal experiments.

Acknowledgment

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[42] Ultraweak Photon Emission of Human Skin *in Vivo:* Influence of Topically Applied Antioxidants on Human Skin

By GERHARD SAUERMANN, WEI PING MEI, UDO HOPPE, and FRANZ STÄB

Introduction

Since ancient times humans have observed the emissions from fireflies, fungi, and marine organisms. In Bologna in the year 1603, Carcariolo described a stone phosphorescent after exposure to sunlight. In Hamburg in 1669, Brandt observed the light emission of newly discovered white phosphorus, caused by autoxidation and subsequent chemiluminescence. After the development of the theory of light, the quantum theory and

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quantum mechanics are the basis for the explanation of luminescence processes.[1]

Luminescence is the emission of electromagnetic radiation in the UV/VIS/IR spectral range from atoms or molecules that parallel relaxation of electronically exited states to a lower state of energy,[2,3] preferably the ground state. If the excitation of the emitting molecule is caused by chemical reactions, the observed emission is classified as chemiluminescence. If absorbed radiation is triggering the luminescence, the process may be called photoluminescence, which is related to fluorescence and phosphorescence. If the origin of excitation is obscure one may use the term ultraweak photon emission (UPE) or low level chemiluminescence.[4]

Low intensity photon emission can be detected with a photon counting device such as a photomultiplier.[4,5] The monitoring of UPE directly on the skin has the advantages of being noninvasive and providing continuous and convenient monitoring. Especially for the detection of peroxidative processes and the effectiveness of antioxidants for human skin *in vivo,* this method provides a unique technique for routine applications in the laboratory.[6–9] Three generations of UPE detectors have been used in our laboratory. The first investigation was carried out in 1985 using Showa Denko's Shonic Chemiluminescence Counter (Tokyo). After removal of the drawer for measuring samples *in vitro,* the emission from the back of the hands was determined *in vivo,* allowing comparison of two different treatments. The Shonic allowed insertion of cutoff filters to record stepwise emission spectra. An advanced system developed by SI Spectral Instrument (Munich) had comparable sensitivity but allowed recording UPE from other skin areas as well as hands. This was extended to whole body measurements using a newly developed system in cooperation between International Institute of Biophysics (Kaiserslautern) and the Skin Research Centre (Hamburg).

[1] A. K. Campbell, "Chemiluminescence, Principles and Applications in Biology and Medicine." Ellis Horwood, Chichester, UK, 1988.

[2] G. Cilento, *in* "Chemical and Biological Generation of Excited States" (W. Adam and G. Cliento, Eds.), p. 221. Academic Press, New York, 1982.

[3] I. V. Baskakov and V. L. Voeikov, *Biochemistry (Moscow)* **61,** 1169 (1996).

[4] W. P. Mei, Ph.D. Thesis, Hanover University, Germany (1991).

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[6] G. Sauermann, U. Hoppe, and F. Stäb, *Photochem. Photobiol.* **59,** 41s (1993).

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[8] F. Stäb, U. Hoppe, R. Keyhani, and G. Sauermann, *Allergologie* **16,** 163 (1993).

[9] I. Hadshiew, F. Stäb, S. Untiedt, K. Bohnsack, F. Rippke, and E. Hölzle, *Dermatology,* in press (1997).

Most exergonic oxidation reactions produce heat; only a small percentage (<1%) of the enthalpy of the reaction is liberated as light. Some spectacular biological luminescence phenomena optimized by evolution produce "cold" light (i.e., at ambient temperatures) with a very high quantum efficiency (up to 95%). Triplet states of carbonyl are mainly considered a light emitting species in UPE.[1,4,5,10,11] A classical scheme was proposed by Sies[12] illustrating the peroxidative cleavage of olefinic bonds into aldehydes and ketones, producing triplet carbonyl which normally relaxes with low quantum yield (10^{-14}–10^{-10}) preferably in the visible spectral range. This low emission rate of 10–10^3 photons sec^{-1} cm^{-2} is much greater than the blackbody radiation at 37°C of approximately 10^{-9} photons sec^{-1} cm^{-2}.

Method to Detect UPE of Human Skin *in Vivo*

The detector head (with a photomultiplier) is located in a dark room and is shielded from any surrounding light and phosphorescent substances. In order to localize the detector head on human skin, it can be controlled through three stepper motors by computer or manually moved. The dark room should be free from any phosphorescent and synthetic color substance, and during the investigation the same temperature and humidity conditions should be maintained. For light, especially UV light-induced UPE, it is important to avoid any excitation of the control area and other body sites and clothing from stimulation. The volunteer should be kept in a dark environment for at least 5 min before starting the experiment.

The main part of the system is the detector head. A single-photon counting device, the photomultiplier (PM), which has a background noise of about 15 cps (counts/sec), is cooled using both Peltier elements and water cooling. The working temperature of the PM is about −30°. To avoid precipitation of moisture on the quartz window which has direct contact with the surface of the skin, double-vacuum quartz windows are used. To protect against the strong scattering of light during light stimulation (especially when using UVA light), two magnetic shutters are used. Two filter wheels are also employed for the purpose of spectral investigations. A short description of the technical setup of the unit is given in Fig. 1, which contain the following units:

1. Control unit. *Computer:* In this computer (486 DX) there are two special cards, one for I/O of experimental arrangement control (prototype

[10] E. Cadenas, *NATO ASI Ser. Ser. A (React. Oxygen Species Chem. Bio. Med.)* **146,** 117 (1988).
[11] N. Suzuki and H. Inaba, *Photomed. Photobiol.* **11,** 15 (1989).
[12] H. Sies, Ed., "Oxidative Stress: Oxidants and Antioxidants." Academic Press, New York, 1991.

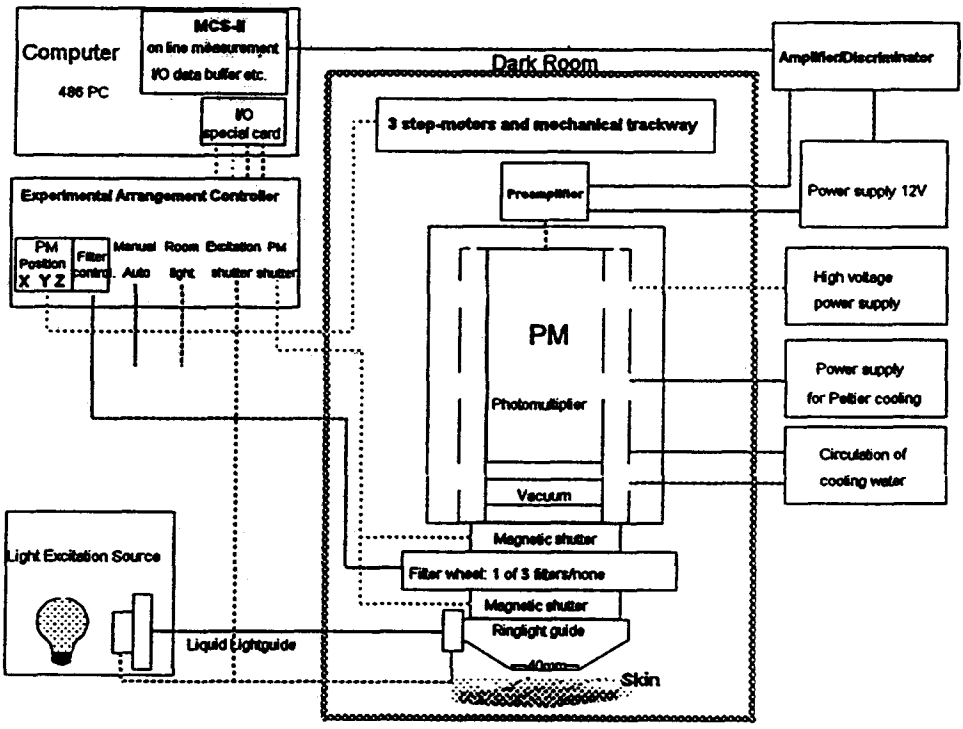

FIG. 1. The technical setup of detecting ultraweak photon emission (UPE) of human skin *in vivo*.

construction) and another for *I/O* of data acquisition (MCS, Oxford Instrument Inc.) *Experimental arrangement control unit:* In this unit (prototype construction) all the mechanical movement (via three stepper motors for movement of the detector head, photomultiplier and light excitation shutters, the stepper motor for the filter wheel, the room light, and the light sensor) can be controlled both manually and automatically.

2. Detector head. *Photomultiplier (PM):* A Thorn-EMI photomultiplier type 9558QA with a 48 mm diameter cathode is used. In order to reduce the noise, the PM should be cooled down to $-30°$ by setting it in a cooling chamber which is cooled by both Peltier elements and cooling water. *Filter wheel:* This is controlled through the computer operating a stepper motor and has four positions with three filters (Schott, Mainz). *Two magnetic shutters:* These parallel shutters (Pronto, E/64) are used to keep the PM from strong light, especially during the light excitation. They can only be opened when the door from the dark chamber is closed; there is no light in the measuring room and the two shutters of the excitation pathway are

closed. *Photosensor:* This is used to protect the PM from being damaged by strong light. This sensor is weakly light sensitive. The computer will only be allowed to open the PM when this photosensor does not sense any light.

3. Light excitation unit: *UV light source:* A 200 W mercury lamp (Lumatec, Superlite, S-UV 201AV-F) is used. *Two magnetic shutters:* These are used for light excitation. They exclude the background light from the surroundings, light source and light guide, and also ensure that there is no phosphorescence going into the PM. *Liquid light guide:* This connects the light source and detector head. Because we work in the UV region, a special liquid light guide (Lumatec, Series 2000) for the UV is used. *Ring light guide:* To meet the various geometric constraints, a ring light guide is used to illuminate the skin homogeneously within the area of interest. This ring light guide is specially constructed to suit UV light stimulation. *Filter wheel:* This is controlled manually and has six positions with four filters (Lumatec).

4. Data processing unit: *Preamplifier* (Otec 9301): This is directly and closely connected to the PM. *Amplifier and discriminator* (Phillips 6930): The signal from the preamplifier is amplified and converted into an electrical pulse chain through discriminating windows. The pulse chain is input to the MCS card. *Multichannel scaler (MCS):* This has two 24-bit 200 MHz counters, on-board data memory, a 48-bit internal dwell timer, and a 48-bit pass counter. This card with standard software transforms the personal computer into a versatile multichannel scaler. A negative TTL input is the only external signal necessary for MCS operation.

Treatment-Induced Photon Emission

As already mentioned, cell[4,13–16] and skin[6,7] show a spontaneous photon emission of 10–100 photons sec^{-1} cm^{-2}. Treatment with UV (especially UVA)[6,7,13,15,17] enhances induced photon emission, which indicates increased oxidative processes in the skin. A typical measurement on forearm *in vivo* (Fig. 2) using UVA-induced emission (12.5 J/25 cm^2) shows the running setup of the experiment (10 volunteers).

[13] W. Mei, *J. Biol. Syst.* **2,** 25 (1994).

[14] R. Van Wijk, J. M. van Aken, W. P. Mei, and F. A. Popp, *J. Photochem. Photobiol. B: Biol.* **18,** 489 (1993).

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[17] W. Mei and F. Popp, "Proceedings of European Symposium on Biomedical Optics. Lille, France," **2331** (Medical Sensors II and Fiber Optics Sensors), 203 (1995).

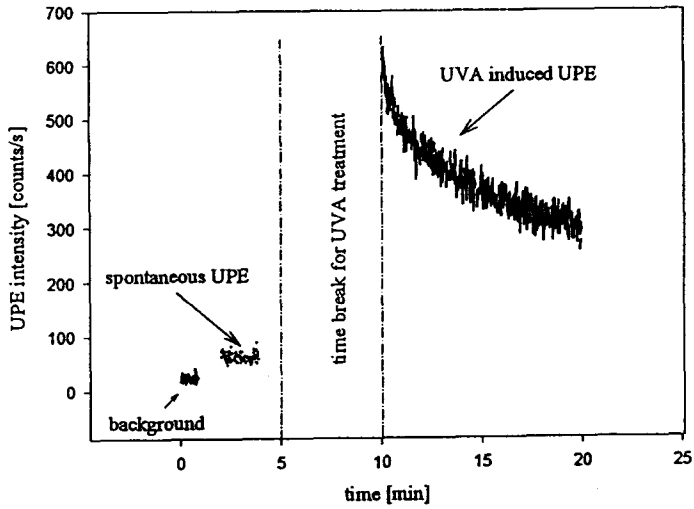

FIG. 2. A typical measurement on forearm of volunteer *in vivo* before and after UVA illumination (UVA doses: 12.5 J/25 cm^2).

Other possible treatments inducing UPE include ultrasound, topical application of peroxides (benzoyl peroxide), iron salts especially in combination with ascorbate, and rubbing contact with other surfaces (textiles).

Emission Spectrum of UVA-Induced UPE. The emission spectrum of spontaneous UPE from human skin tends to be strongest at the red end of the spectrum and weakest at the blue.[17] UVA-induced UPE, however, shows that the maximum is spectrally located between 400 and 580 nm, supporting the assumption that triplet states of newly synthesized carbonyl groups are a main source of UPE and that peroxidative processes induced by mechanisms listed above are basic sources for excited triplets.

Influence of Photosensitizers. Topical application of Rose Bengal or hypericine (both singlet-oxygen producers) and subsequent UVA irradiation cause a dramatic rise in UPE, i.e., induces prooxidative events in skin producing triplet states. Aminolevulinic acid, a precursor of photosensitizing porphyrins, reduces UPE significantly 1 hr after application of a 1% solution in ethanol.

Influence of UV Filters. Topical application of UVA filter Parsol 1789 increases protective power against UVA, with linear dose response, and reduces UVA-induced UPE, but not in a steady dose response. It soon approaches a saturation level with rising concentrations. TiO_2 shows qualitative differences with UVA-induced UPE: uncoated material very strongly triggers the UPE, while coated material reduces this. Uncoated TiO_2 liber-

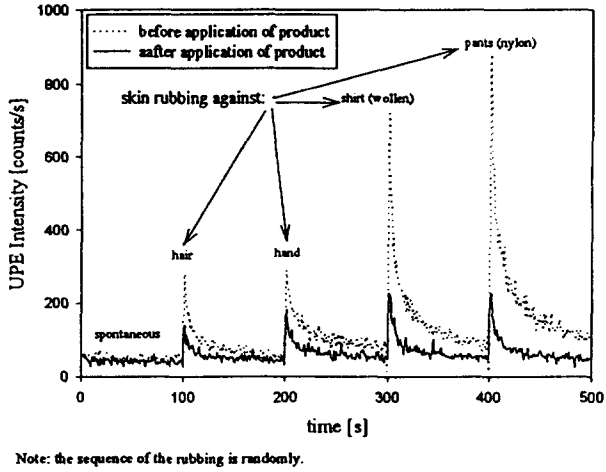

FIG. 3. Rubbing-induced UPE (triboluminescence) of human skin *in vivo* and its dependence on the surface material of rubbing substance and influence of product application.

ates electrons in the surrounding medium under bombardment of UVA and produces O_2^-, another inducer of UPE-stimulating processes.

Influence of Rubbing. Depending on the intensity of contact and movement, of the rubbing material and its surface, more or less UPE will be induced. Materials in contact will have different potentials; separation may cause electron transfer resulting in electric charges (see classical experiments in electrostatics) and subsequent molecular rearrangements, superficial chemical reactions. Therefore, UPE (triboluminescence) seems to represent the sum of oxidative reactions caused by contact, friction, and separation. There are two main triboluminescence influencing factors in skin: surface moisture content and superficial sebum content. Figure 3 shows a typical development of such investigation.

Influence of Horny Layer Moisture Content. Increasing corneal moisture content decreased UVA-induced UPE. Therefore, measurements of UPE should be performed under standardized environmental conditions (relative humidity and temperature). Horny layer proteins are more prone to changes by water than lipids and these changes subsequently affect the solubilities of oxygen species. The oxidation of horny layer proteins is accompanied by UPE and horny layer proteins are oxidized by UVA, peroxides, and ferrous/ferric ions.[18]

Oxidative Status of Young and Elderly Human Skin. The degree of oxidation of stratum corneum proteins increases gradually with increasing

[18] S. Richert, Ph.D. Thesis, Hamburg University, Germany, 1996.

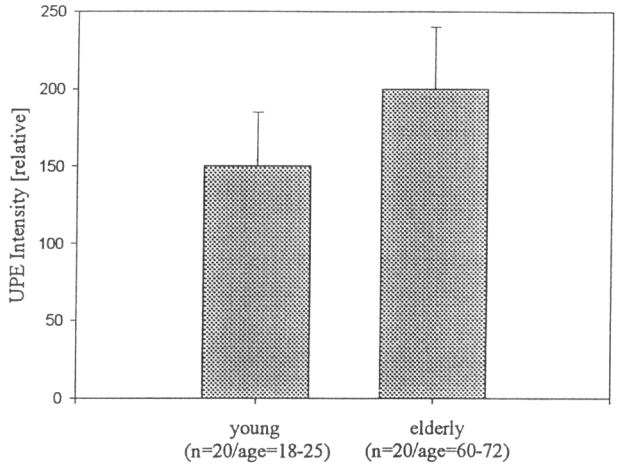

FIG. 4. Oxidative status of young and elderly human skin determined by detecting UPE *in vivo*.

age of the donor and parallels the rising spontaneous and UV-induced UPE. Humidification of the horny layer reduces the tendency of those proteins to oxidize. The investigation of two age groups (Fig. 4) shows a clear correlation of UPE intensity and age. The same results have been reported in several other publications.

Efficacy of Topically Applied Antioxidants on Human Skin: The usefulness of UPE method (Fig. 5) is shown by the efficacy of topically applied antioxidants to reduce the UVA-induced UPE. Figure 5 shows the difference between effects of the test product and placebo after topical application of α-glucosylrutin[9] for 7 days.

Concluding Remarks

Very little work has been done so far on UPE from human subjects. In one report[19] a mouse model was used to evaluate the occurrence of oxidative stress in skin exposed to UVA radiation. The effect of the topical application of α-tocopherol and β-carotene was also determined in this study. It is well known that radiation with UVA decreases the levels of antioxidants, inactivates the antioxidant enzymes, and increases lipid perox-

[19] P. Evelson, C. P. Ordonez, S. Llesuy and A. Boveris, *J. Photochem. Photobiol. B: Biol.* **38,** 215 (1997).

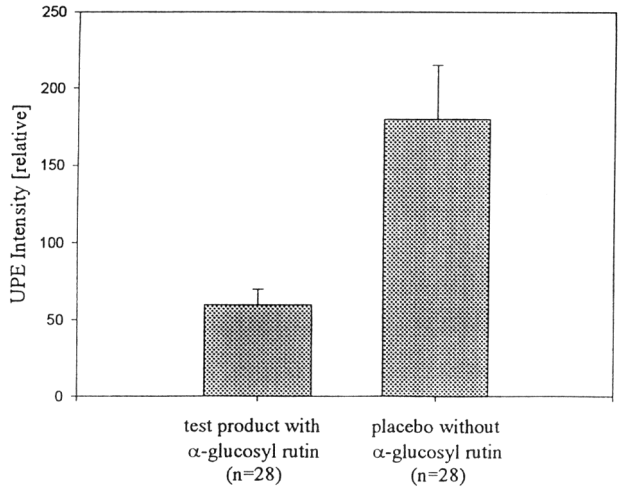

FIG. 5. Efficacy of topically applied antioxidants (the flavonoid α-glucosylrutin) on human skin *in vivo*.

idation in skin.[20–23] The UPE detection method provides a useful technique *in vivo* to determine peroxidative events and efficacy of topically applied antioxidants on human skin.[6–9] In order to record the emissions from the skin of human volunteers *in vivo,* the instruments have to be adapted to special applications. It is necessary to replace small sample compartments and to keep the distance between photocathode and skin surface as short as possible. Avoidance of light from external sources is also necessary. The entire detector head has to be installed in rooms without phosphorescent walls, surfaces, and lamps, and it should be freely movable.

Irrespective of theoretical considerations as to whether some kind of physical or biochemical phenomena may be occurring, skin as an organ designed for protection against noxious materials in the environment (among which numerous UPE triggering influences can be identified) may be useful as an indicator of free-radical metabolism. Because damage in-

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[21] L. Packer and J. Fuchs, Eds., "Vitamin E in Health and Disease." Marcel Dekker, New York, 1993.

[22] Y. Shindo, E. Witt, D. Han, W. Epstein, and L. Packer, *J. Invest. Dermatol.* **102,** 122 (1994).

[23] E. Cadenas and L. Packer, Eds., "Handbook of Antioxidants." Marcel Dekker, New York, 1996.

duced by these radicals is thought to be the cause of many of the phenomena of aging, light could serve as an indicator of the rate of aging and of the success of measures taken to slow this process, for example, by the use of the flavonoid α-glucosylrutin.[9]

[43] Noninvasive in Vivo Evaluation of Skin Antioxidant Activity and Oxidation Status

By RON KOHEN, DAVID FANBERSTEIN, ABRAHAM ZELKOWICZ, OREN TIROSH, and SHARON FARFOURI

Introduction

Most of the existing methods for evaluation of skin antioxidant capacity and its oxidation status are invasive and require removal of the skin, its separation into its various layers, homogenization, and analysis of its antioxidants or its oxidation products. It is obvious that such measurements cannot be done on a large scale in humans. Therefore, only a few comprehensive reviews can be found in the scientific literature which describe the oxidant/antioxidant status of skin. Shindo *et al.*[1] demonstrated that skin possesses antioxidant activity from both antioxidant enzymes and low molecular weight antioxidants. Of the various layers of the skin, the epidermis contains the highest levels of antioxidant molecules.[1] Evaluation of the antioxidant status by invasive means revealed that the total antioxidant activity of young skin is much higher than that of old skin.[2] Since a major contribution to the antioxidant activity is derived from low molecular weight antioxidants (LMWA) which can easily penetrate via cell membranes and provide local protection, we developed a procedure for evaluating antioxidant activity contributed by LMWA which are present on the surface of the skin (stratum corneum) and can be secreted from deeper layers to the outer environment. The method described here is suitable for both indirect and direct evaluation of skin antioxidant activity. It is also suitable for evaluating lipid hydroperoxide levels on the surface of the skin as an indicator of oxidation status of the skin.[3]

[1] Y. Shindo, E. Witt, D. Han, W. Epstein, and L. Packer, *J. Invest. Dermatol.* **102,** 122 (1994).
[2] R. Kohen, D. Fanberstein, and O. Tirosh, *Arch. Gerontol. Geriatr.* **24,** 103 (1997).
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