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Antioxidant Defense Systems in Skin

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INTRODUCTION

As the outermost organ of the body, the skin is frequently and directly exposed to a prooxidative environment, including ultraviolet radiation, drugs, and air pollutants (1). Besides external inducers of oxidative attack, the skin has to cope with endogenous generation of reactive oxygen species (ROS) and other free radicals, which are continuously produced during physiological cellular metabolism. To counteract the harmful effects of ROS, the skin is equipped with antioxidant systems, which maintain an equilibrium between prooxidants and antioxidants.

In the course of skin evolution, a variety of primary (preventive, e.g., vitamin C) and secondary (interceptive, e.g., vitamin E) antioxidant mechanisms have been developed, which form an ‘‘antioxidative network’’ of closely interlinked components (Fig. 2). While some antioxidants can be synthesized by humans (e.g., glutathione or ubiquinol-10), others have to be supplied by intake (e.g., antioxidant vitamins C and E, and trace metals). Antioxidants intervene at different levels of oxidative processes: (1) scavenging free radicals; (2) scavenging lipid peroxyl radicals; (3) binding metal ions; or (4) removing oxidatively damaged biomolecules (2). However, the antioxidant defense in cutaneous tissues can be overwhelmed either by an increased exposure to exogenous (e.g., UV exposure) or endogenous (e.g., inflammatory disorders) sources of ROS, or by

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a primarily depleted antioxidant defense (e.g., malnutrition) facing a normal level of prooxidative challenge. Such a disturbance of the prooxidant/antioxidant balance may result in oxidative damage of biomolecules, such as lipids, proteins, and DNA, and has been termed ''oxidative stress'' (3,4). In skin, the induction of oxidative damage by environmental stimuli such as UVA, UVB, and ozone was demonstrated to occur in lipids (5–7), proteins (8), and DNA (9,10).

The chapter summarizes the currently available knowledge on (1) the presence and physiological distribution of natural antioxidants in skin; (2) their response to oxidative environmental stressors; and (3) the photoprotective potential of topically applied antioxidants.

CONSTITUTIVE SKIN ANTIOXIDANTS

Water-Soluble Antioxidants

Ascorbate

Antioxidant Properties Ascorbate, a ketolactone, is also known as vitamin C (see Fig. 1). While most mammals are able to synthesize ascorbate from glucose-derived glucuronic acid, guinea pigs, monkeys, and humans lack the enzyme gulonolactase and therefore require the dietary intake of this vitamin. Dietary ascorbate is absorbed and distributed throughout the body within a few hours. The biochemical importance of vitamin C is primarily based on its reducing potential, as is required in a number of hydroxylation reactions. Several hydroxylases involved in collagen synthesis require ascorbate as a reductant (11). Due to its high reduction potential, ascorbate is an efficient scavenger of superoxide anion radicals, hydroxyl radicals, hypochlorite, singlet oxygen, thiyl radicals, and water-soluble peroxyl radicals (2,12,13). Oxidation of ascorbate results in the formation of dehydroascorbate via the ascorbyl radical, which can be recycled back to ascorbate in the presence of thiols (Fig. 2), or irreversibly decomposes

Figure 1 Chemical Structures of Selected Antioxidants. *1* L-ascorbic acid (176.1 g mol^{-1}, $pKa_1 = 4.2$, $pKa_2 = 11.6$), *2* uric acid (168.1 g mol^{-1}, pKa_1 (37°C) = 5.2 [197]), *3* D-α-lipoic acid (206.3 g mol^{-1}, pKa = 5.4), *4* tocopherols (α: $R_1 = R_2 = CH_3$, 430.7 g mol^{-1}; β: $R_1 = CH_3$, $R_2 = H$, 416.7 g mol^{-1}; γ: $R_1 = H$, $R_2 = CH_3$, 416.7 g mol^{-1}; δ: $R_1 = R_2 = H$, 402.7 g mol^{-1}), *5* tocotrienols (α: $R_1 = R_2 = CH_3$, 424.7 g mol^{-1}; β: $R_1 = CH_3$, $R_2 = H$, 410.6 g mol^{-1}; γ: $R_1 = H$, $R_2 = CH_3$, 410.6 g mol^{-1}; δ: $R_1 = R_2 = H$, 396.6 g mol^{-1}), *6* ubiquinone ($n = 9$: 795.3 g mol^{-1}; $n = 10$: 863.4 g mol^{-1}), *7* ubiquinol ($n = 9$: 797.3 g mol^{-1}; $n = 10$: 865.4 g mol^{-1}), *8* vitamin A precursors (R = H: β-carotene, 536.9 g mol^{-1}; R = OH: cryptoxanthin, 552.9 g mol^{-1}), *9* vitamin A (all-trans-retinol: R = CH_2-OH, 286.5 g mol^{-1}; all-trans-retinoic acid: R = COOH, 300.4 g mol^{-1}).

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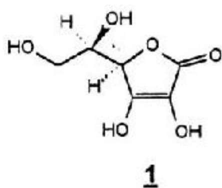

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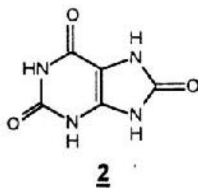

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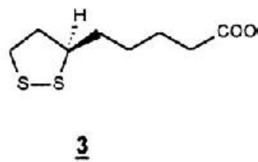

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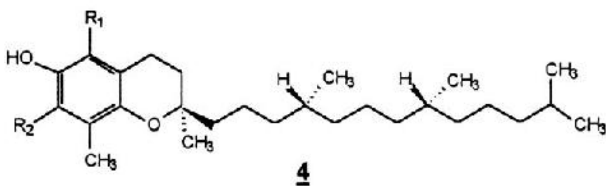

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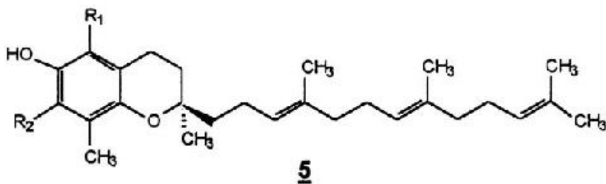

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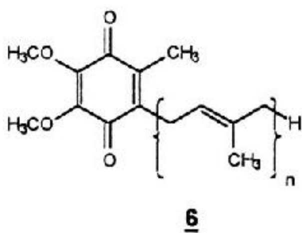

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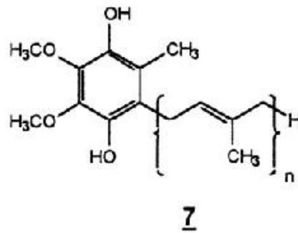

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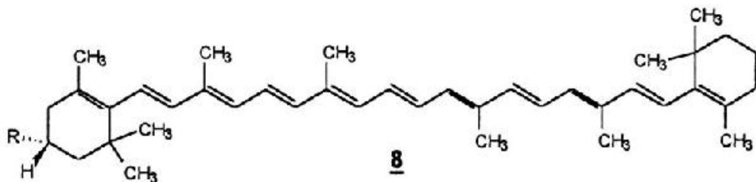

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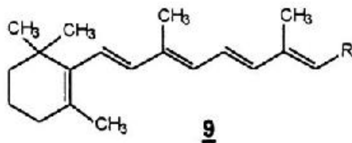

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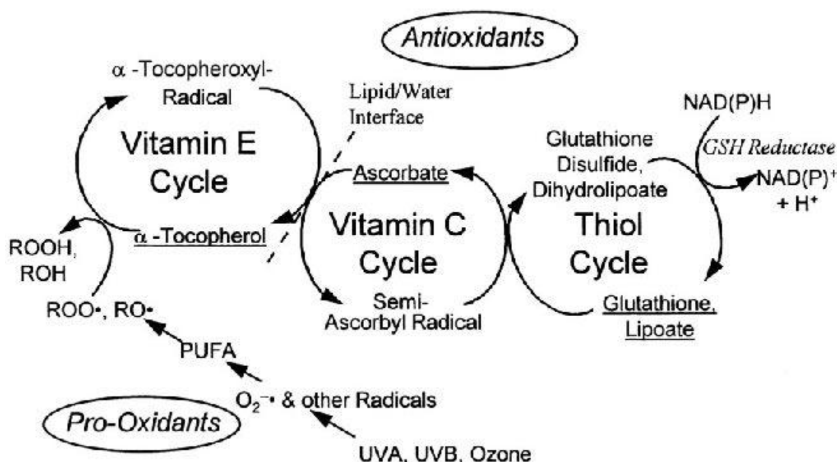

Figure 2 Activation of the Antioxidant Network by Environmental Oxidative Stressors O_2^{-}•: Superoxide anion radical; PUFA: polyunsaturated fatty acids; ROO•, RO•: lipid (per-) oxy radicals; ROOH, ROH: Lipidhydro(per)oxides. Please note that some of the depicted recycling mechanisms have been found in other than cutaneous systems (see ''Antioxidant Properties'').

to the unstable diketogulonic acid. Although ascorbate is not able to scavenge lipophilic radicals directly, in the presence of vitamin E it synergistically reduces lipid peroxyl radicals by reacting with tocopheroxyl radicals. This leads to regeneration of active tocopherol (14) (Fig. 2). Ascorbate has also been reported to show prooxidant properties. Mixtures of copper or iron salts with ascorbate are well known to stimulate lipid peroxidation in vitro (15). With the exception of pathological metal overload disease states, however, the prooxidative potential of ascorbate is not considered to be of relevance in vivo (16).

Prevalence in Skin In skin, the data available on ascorbate concentrations are limited and variable due to differences in species, skin layer analyzed, and method of analysis (Table 1). Importantly, however, vitamin C is present at significant levels in both the dermis and epidermis of animals and humans. In hairless mice, vitamin C levels are only slightly higher in the epidermis than in the dermis (5,17). In human skin, which is dependent on dietary vitamin C, the epidermis apparently contains approximately fivefold higher levels than the dermis (18). This difference in dermal and epidermal vitamin C levels may reflect an increased utilization in the dermis for the regulation of collagen and elastin biosynthesis (19), or facilitated transport mechanisms for vitamin C from the dermal

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Table 1 Physiological Levels of Ascorbate in Cutaneous Tissues

Skin layer	Species	Concentration	References	Year
Total skin	Rat	0.2 g/kg tissue	Salomon and Stubbs (198)	1961
Total skin	Human	41 μg/g dry weight	Stüttgen and Schaefer (199)	1974
Total skin	Mouse	6–7 nmol/mg protein	Fuchs et al. (101)	1989
Epidermis	Mouse	1321 ± 77 nmol/g tissue	Shindo et al. (17)	1993
Dermis	Mouse	1064 ± 54 nmol/g tissue	Shindo et al. (17)	1993
Epidermis	Human	3798 ± 1016 nmol/g tissue	Shindo et al. (18)	1994
Dermis	Human	723 ± 320 nmol/g tissue	Shindo et al. (18)	1994

vasculature to the epidermis. The epidermis is not only more directly exposed to the environment than the underlying dermis and therefore might have a higher demand of antioxidant protection, but also requires the presence of ascorbate for efficient formation of the stratum corneum barrier (20). Isolated human stratum corneum was reported to contain only very low ascorbate levels, as compared with levels in subjacent epidermal layers (6). The latter phenomenon is most likely due to both the hydrophobicity and, due to its location, the high degree of environmental exposure of the stratum corneum.

Glutathione

Antioxidant Properties Glutathione (γ–glutamyl-cysteinyl-glycine; GSH), present intracellularly at millimolar concentrations, is an important water-soluble antioxidant and reducing compound. Oral GSH is poorly absorbed and is not required to be provided by dietary intake (21). In cells, glutathione is synthesized from glutamate, cysteine, and glycine (22). It acts as a substrate for numerous reducing enzymes, among them glutathione peroxidase and the phospholipid hydroperoxide glutathione peroxidase. Therefore, the absence of glutathione may lead to an accumulation of lipid hydroperoxides (2). Importantly, glutathione also protects cells by reacting directly with reactive oxygen species such as singlet oxygen ($^1O_2$), hydroxyl radicals ($HO^{\bullet}$), and superoxide radicals ($O^{\bullet}{}_2{}^-$), resulting in the formation of thiyl radical ($GS^{\bullet}$), and subsequently glutathione disulfide (GSSG). The latter can be recycled to GSH by the NADPH-dependent enzyme glutathione reductase (Fig. 2). The ratio of GSH/GSSG in tissues in normally high (i.e., >100) (23). In many biological systems the GSH/GSSG ratio is lowered upon prooxidative conditions and therefore is frequently used as an indicator of oxidative stress. In mice, ascorbate supplementation increases GSH levels in lung epithelial tissue (24), and glutathione deficiency increases hepatic ascorbic acid synthesis (25), suggesting that the antioxidant actions of

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glutathione and ascorbate are closely linked. In humans, who are dependent on dietary vitamin C intake, this link remains to be clarified.

Prevalence in Skin Although a number of studies are available on glutathione (GSH) and glutathione disulfide (GSSG), absolute values obtained for levels in total skin, epidermis, and dermis are highly variable (Table 2). However, comparing the relative levels, most studies demonstrated higher glutathione levels in the epidermis than in the dermis. Furthermore, the epidermis reveals a higher ratio of GSH/GSSG than the dermis, indicating either a lower oxidative challenge or a better antioxidative protection. Since the epidermis is more directly exposed to the environment, it seems also possible that the pathways leading to the endogenous formation of epidermal glutathione are upregulated by chronic environmental factors, as was shown for glutathione peroxidase in ozone-exposed lung epithelium (26). It must be considered that the cell turnover rate in the epidermis is very high, as well as cellular differentiation processes; since GSH is an important substrate for essential enzymes and GSSG can inactivate enzymes by forming disulfides (27), a high GSH/GSSG ratio could be essential for the stratified and keratinized epidermis.

Urate

Antioxidant Properties Uric acid (deprotonated form: urate) is a small water-soluble molecule (Fig. 1) that accumulates in human tissues as the end-product of purine metabolism. In blood plasma, urate has been shown to be a powerful scavenger of singlet oxygen, peroxyl-, and hydroxyl radicals (28). Further studies have demonstrated that urate scavenges ozone (15) and hypochlorous acid (29). In addition to its radical-scavenging potential, urate was proposed to stabilize reduced vitamin C in serum. This stabilizing effect appears to be due to inhibition of iron-catalyzed oxidation of ascorbate, which largely results from the formation of a stable, noncatalytic urate–iron complex (30). Unlike radical-scavenging reactions, this protective effect provided by iron chelation is not associated with depletion of urate. Direct free-radical attack upon urate generates allantoin, which has therefore been proposed as a marker molecule for free-radical reactions in vivo (31).

Prevalence in Skin Only little data are available on urate levels in cutaneous tissues. Lopez-Torres et al. reported values of 147 $\pm$ 5 nmol g^{-1} tissue in the epidermis, and 75 $\pm$ 9 nmol g^{-1} in the dermis of hairless mice (32). In humans, Shindo et al. reported levels of 1071 $\pm$ 242 nmol g^{-1} tissue in the epidermis, and 182 $\pm$ 24 nmol g^{-1} tissue in the dermis, respectively (18). Thus, as found for other antioxidants, the highest cutaneous urate levels are present in epidermal tissue.

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Table 2 Physiological Levels of Glutathione in Cutaneous Tissues

Skin layer	Species	Concentration	References	Year
Epidermis	Human	1.8 μmol/g tissue (GSH) 0.09 μmol/g tissue (GSSG)	Halprin and Ohkawara (200)	1967
Total skin	Guinea pig	0.7–1.1 μmol/g tissue (GSH) 1.4–1.5 μmol/g tissue (GSSG)	Benedetto et al. (201)	1981
Epidermis	Mouse	0.75 μmol/g tissue (GSH)	Wheeler et al. (65)	1986
Dermis	Mouse	0.32 μmol/g tissue (GSH)	Wheeler et al. (65)	1986
Epidermis	Human	1.2 μmol/g tissue (GSH)	Connor and Wheeler (66)	1987
Total skin	Mouse	3.9–6.3 μmol/g protein (GSH) 1–1.5 μmol/g protein (GSSG)	Fuchs et al. (27,101)	1989
Epidermis	Mouse	1.16 μmol/g tissue (GSH) 0.07 μmol/g tissue (GSSG)	Shindo et al. (17)	1993
Dermis	Mouse	0.59 μmol/g tissue (GSH) 0.16 μmol/g tissue (GSSG)	Shindo et al. (17)	1993
Epidermis	Human	0.46 μmol/g tissue (GSH) 0.02 μmol/g tissue (GSSG)	Shindo et al. (18)	1994
Dermis	Human	0.08 μmol/g tissue (GSH) 0.01 μmol/g tissue (GSSG)	Shindo et al. (18)	1994

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Lipid-Soluble Antioxidants

Vitamin E

Antioxidant Properties Vitamin E is the major lipophilic antioxidant in plasma, membranes, and tissues (33). The term ''vitamin E'' collectively refers to the eight naturally occurring molecules (four tocopherols and four tocotrienols), which exhibit vitamin E activity. Tocotrienols differ from tocopherols in that they have an isoprenoid instead of a phytyl side chain (see Fig. 1); the four forms of tocopherols and tocotrienols differ in the number of methyl groups on the chromanol nucleus (α– has 3, β– and γ– have 2, and δ has 1). In humans, α–tocopherol is the most abundant vitamin E homologue, followed by γ–tocopherol.

Vitamin E is among the early recognized biological antioxidants, and its redox and free-radical chemistry are well documented (33). Vitamin E acts as an antioxidant by scavenging free radicals, which can, either directly or indirectly, initiate ($HO^{\bullet}$, and $O^{\bullet}_2{}^{-}$) or propagate (lipid peroxyl radicals) lipid chain reactions (34). Vitamin E can also react with nitric oxide (35). The major antioxidant role of vitamin E is generally considered to be the arrest of chain propagation by scavenging lipid peroxyl radicals. The initial oxidation product of tocopherol is the metastable tocopheroxyl radical (Fig. 2), which can be either reduced to tocopherol by coantioxidants, or reacts with another lipid peroxyl radical, yielding tocopherol-quinone (36). Thus, one molecule of tocopherol is able to scavenge two peroxyl radical molecules. Since the physiological molar ratio of tocopherols to polyunsaturated phospholipids, first-line targets of oxidative attack, is less than about 1:1000 in most biological membranes, regeneration of tocopherol is essential for its high antioxidant efficacy in vivo. As mentioned above, several hydrophilic coantioxidants, such as ascorbate and glutathione, can regenerate vitamin E from the tocopheroxyl radical and thus enhance the antioxidant capacity of vitamin E (14).

Furthermore, there is some in vitro evidence that ubiquinol-10 protects α–tocopherol from photo-oxidation by recycling mechanisms (37). In vitro, unphysiologically high concentrations of α–tocopherol were reported to induce prooxidative effects leading to acceleration of lipid peroxidation (38,39). In human skin in vivo, however, such adverse health effects have not been reported.

Prevalence in Skin As demonstrated in other body tissues, α-tocopherol is the predominant vitamin E homologue in murine and human skin (Table 3) (5,6,18). In addition, γ–tocopherol is present in murine and human epidermis, dermis, and stratum corneum. The α–tocopherol/γ–tocopherol molar ratio in the human dermis and epidermis is approx. 10:1. Notably, a vitamin E gradient has recently been demonstrated in human upper arm stratum corneum. The highest α–tocopherol levels were found in the lower stratum corneum, whereas the lowest levels were present in the upper layers. The α–tocopherol/γ–tocopherol ratio

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Table 3 Physiological Levels of α- and γ-Tocopherol in Cutaneous Tissues

Skin layer	Species	Concentration	References	Year
Total skin	Mouse	200 pmol α-tocopherol/mg protein	Fuchs et al. (27,101)	1989
Epidermis	Mouse	4.8 ± 0.5 nmol α-tocopherol/g tissue	Shindo et al. (17)	1993
Dermis	Mouse	3.3 ± 0.3 nmol α-tocopherol/g tissue	Shindo et al. (17)	1993
Epidermis	Human	31 ± 3.8 nmol α-tocopherol/g tissue	Shindo et al. (18)	1994
		3.3 ± 1 nmol γ-tocopherol/g tissue		
Dermis	Human	16.2 ± 1.1 nmol α-tocopherol/g tissue	Shindo et al. (18)	1994
		1.8 ± 0.2 nmol γ-tocopherol/g tissue		
Stratum corneum	Mouse	8.4 ± 1.3 nmol α-tocopherol/g tissue	Thiele et al. (5)	1997
		2.9 ± 0.9 nmol γ-tocopherol/g tissue		
Stratum corneum	Human	33 ± 4 nmol α-tocopherol/g tissue	Thiele et al. (6)	1998
		4.8 ± 0.8 nmol γ-tocopherol/g tissue		

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decreased from about 10 : 1 in the lower layers to about 3 : 1 in the upper stratum corneum. The α–tocopherol levels in human dermis and epidermis were severalfold higher than in corresponding layers of hairless mouse skin (17,18). Consistently, human stratum corneum contains almost tenfold higher α–tocopherol levels than measured in murine stratum corneum (5,6). As observed for hydrophilic antioxidants, higher vitamin E levels were found in murine and human epidermis, as compared with dermal levels. It remains to be clarified whether the uptake and transport of α–tocopherol in the epidermis is an unspecific and passive process or, as described for human hepatocytes (33), is regulated by a mechanism involving a specific binding enzyme (α–tocopherol transfer protein).

Ubiquinols/Ubiquinones (''Coenzyme Q'')

Antioxidant Properties The terms ''coenzyme Q,'' as well as ''ubiquinone,'' are commonly used for the redox couple ubiquinol/ubiquinone (see Fig. 1). Ubiquinones are lipid-soluble quinone derivatives with an isoprenoid side chain. In nature, ubiquinone homologues containing 1 to 12 isoprene units occur; the predominant form of ubiquinone in humans is ubiquinone-10 (contains 10 isoprene units), and in mice ubiquinone-9. In liver cells, about 40 to 50% of the total cellular ubiquinone is located in the mitochondria, 25 to 30% in the nucleus, 15 to 20% in the endoplasmic reticulum, and only 5 to 10% in the cytosol (40). In vitro, the reduced forms of ubiquinones, the ubiquinols, are by two to three orders of magnitude more potent antioxidants (41). The role of ubiquinol/ubiquinone as a redox carrier in the respiratory chain is well established, participating in the transfer of protons across the inner mitochondrial membrane (42). Ubiquinols can react with reactive oxygen species and thus prevent direct damage to biomolecules and initiation of lipid peroxidation. Although ubiquinones cannot prevent autocatalytic free-radical reactions by donating a phenolic hydrogen atom (unlike ubiquinols and tocopherols), it scavenges singlet oxygen and inhibits lipid peroxidation in model membranes (43). Furthermore, there is some in vitro evidence that ubiquinol-10 protects α–tocopherol against superoxide-driven oxidation (37). In low-density lipoproteins, its protective potential against lipid peroxidation was shown to exceed that of α–tocopherol (44). However, it must be noted that the antioxidant properties reported for ubiquinones are strongly dependent on the length of the side chain and the model systems used. A growing scientific and commercial interest in ubiquinones has led to its incorporation into skin-care products; however, further research is needed to better understand its protective antioxidant mechanisms in human skin.

Prevalence in Skin In both mouse and human skin, the highest ubiquinol levels were found in the epidermis. In human skin, the majority of ubiquinone is present in its oxidized form (ubiquinone-10) (Table 4). This is in accordance with the ratios determined in brain and lung tissues, but different from those in

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Table 4 Physiological Levels of Ubiquinone/Ubiquinol in Skin

Skin layer	Species	Concentration	References	Year
Total skin	Mouse	20–48 pmol ubiquinol-9/mg protein 98–136 pmol ubiquinone-9/mg protein	Fuchs et al. (27,101)	1989
Epidermis	Mouse	1.9 ± 0.2 nmol ubiquinol-9/g tissue 15.2 ± 1.1 nmol ubiquinone-9/g tissue	Shindo et al. (17)	1993
Dermis	Mouse	1.2 ± 0.2 nmol ubiquinol-9/g tissue 10.0 ± 0.7 nmol ubiquinone-9/g tissue	Shindo et al. (17)	1993
Epidermis	Human	3.5 ± 0.8 nmol ubiquinol-10/g tissue 4.1 ± 0.6 nmol ubiquinone-10/g tissue	Shindo et al. (18)	1994
Dermis	Human	0.4 ± 0.1 nmol ubiquinol-10/g tissue 2.9 ± 0.8 nmol ubiquinone-10/g tissue	Shindo et al. (18)	1994
Stratum corneum	Mouse	ubiquinol-9 and ubiquinone-9: not detectable (<0.1 pmol/mg)	Thiele et al. (5)	1997
Stratum corneum	Human	ubiquinol-10 and ubiquinone-10: not detectable (<0.1 pmol/mg)	Thiele et al. (6)	1998

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heart, kidney, liver, and blood plasma, where the majority of ubiquinone is present in the reduced form (45). Interestingly, all three organs—skin, brain, and lung—are well known to be challenged by a high load of oxidative stress. Despite its high lipid content, the stratum corneum appears to be very low in ubiquinol/ubiquinone levels (5,6). Most likely, this results from the loss of nuclei and organelles, both rich in ubiquinones, during the terminal differentiation process of keratinocytes into the stratum corneum barrier.

Carotenoids and Vitamin A

Antioxidant Properties Dietary vitamin A is available in the form of provitamin A compounds (e.g., α– and β–carotene, and cryptoxanthin) (see Fig. 1), or directly from animal food (liver, milk, egg, and fish) (46). In comparison with α–tocopherol, β–carotene membrane levels are severalfold lower; however, β–carotene accumulates significantly in skin and may achieve levels far exceeding those of α–tocopherol in subjects on a β–carotene-supplemented diet (47). There are at least three known mechanisms by which carotenoids protect cells from oxidative stress: (1) by quenching triplet-state sensitizers; (2) by quenching singlet oxygen; and (3) by scavenging peroxyl radicals (48,49). Triplet sensitizers, such as flavins and porphyrins, may abstract a hydrogen atom or an electron from various molecules; this can lead to further radical-mediated damage (type I) or formation of singlet oxygen (type II) by reaction with ground-state oxygen. The quenching of singlet oxygen by carotenoids is almost entirely an energy transfer process yielding ground-state oxygen and a triplet excited carotenoid (2). The role of carotenoids within the ''antioxidant network'' is not clear. It has been demonstrated in liver homogenates that dietary carotenoids increase the resistance to lipid peroxidation primarily by enhancing α–tocopherol membrane levels, while direct antioxidant effects provided by carotenoids were less protective (50). Several forms of vitamin A (13-cis-retinoic acid, all-trans-retinoic acid, all-trans-retinol) (see Fig. 1), however, were shown to effectively inhibit lipid peroxidation in liver microsomes (2,51). Carotenoids protect biological systems against triplet sensitizers and singlet-oxygen-mediated oxidative damage largely without being sacrificed. Both carotenoids and retinoids act at physiological oxygen tension as peroxyl radical scavengers, thus preventing oxidative damage (49). As opposed to the reducing antioxidants ascorbate and dihydrolipoic acid, β–carotene was not effective in recycling α–tocopherol in mouse skin homogenates irradiated with solar-simulated UV irradiation (52).

Prevalence in Skin Data available on carotenoid and vitamin A levels in skin are very limited. Vahlquist et al. revealed that the levels of β–carotene in human skin (epidermis: 2.2 $\mu g\ g^{-1}$ protein; dermis: 0.7–0.8 $\mu g\ g^{-1}$ protein; subcutis: 18.9 $\mu g\ g^{-1}$ protein) is severalfold higher than that of vitamin A (retinol; epidermis: 0.3 $\mu g\ g^{-1}$ protein; dermis: 0.2–0.4 $\mu g\ g^{-1}$ protein; subcutis: 6.4 $\mu g\ g^{-1}$

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protein) (53). Furthermore, the same investigators detected carotene and retinol in skin surface lipids, but no data are yet available on stratum corneum levels of these compounds (53). Recently, Stahl et al. detected relatively high basal levels of carotenoids in human skin of the forehead (0.40 ± 0.09 nmol g^{-1}), back (0.22 ± 0.13 nmol g^{-1}), and palmar hand (0.32 ± 0.08 nmol g^{-1}), while significantly lower levels were present in the skin of the dorsal hand (0.03 ± 0.10 nmol g^{-1}) and the inside of the forearm (0.07 ± 0.05 nmol g^{-1}). Furthermore, skin carotenoid levels were increased after oral carotenoid supplementation (with daily doses of 20–25 mg carotenoids), and correlated well with increased serum carotenoid levels (54). Higher levels of β–carotene (1.4 ± 0.7 nmol g^{-1} tissue) and lycopene (1.6 ± 0.6 nmol g^{-1} tissue) in human skin samples were found when the subcutaneous fat was included in the whole skin samples (55). Thus, as was reported for other skin antioxidants, β–carotene levels are higher in the epidermis than in the dermis. This difference seems to be less pronounced for vitamin A (53,56).

Enzymatic Antioxidant Systems

The Enzymatic Glutathione System

Antioxidant Properties The major components of the enzymatic glutathione system are glutathione (GSH) peroxidase (GSH-Px), GSSG reductase, phospholipid hydroperoxide GSH-peroxidase, and GSH-S-transferase. GSH-Px is a selenoenzyme consisting of four identical subunits, each of which contains a selenocysteine residue in its active site. In eukaryotes, the majority of its enzymatic activity is localized in the cytosol, and, to a lesser extent, in mitochondria (57,58). GSH-Px reduces H_2O_2 and lipid hydroperoxides at the expense of two molecules of GSH, which are oxidized to GSSG. GSSG-reductase, a dimeric enzyme containing FAD in its active site, catalyzes the reduction of GSSG using reducing equivalents such as NADPH (59) (Fig. 2). Nonselenium-dependent GSH-peroxidases (GSH-S-transferases) and phospholipid hydroperoxide GSH-peroxidase are able to catalyze the reduction of lipid hydroperoxides, but not of hydrogen peroxide (60). A developing body of data indicates that polymorphism at GSH-S-transferases (GSTs) genes influences skin cancer susceptibility. It was proposed that GSTs influence tumorigenesis because these enzymes detoxify the products of UV-induced oxidative stress (61), and that heritable deficiency of specific GSTs may be a genetic determinant of individual skin sensitivity toward UV irradiation (62). Recently, increased tumorigenesis has been demonstrated in mice lacking π-class GSTs (63).

Prevalence in Skin As compared to liver, kidney, and brain, skin GSH-Px and GSH-reductase activities are markedly lower (47). The baseline levels measured in epidermis and dermis vary considerably between different studies

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and therefore do not point to a clear preferential distribution of GSH-Px in skin (see Table 5). GSH-S-transferase is expressed during all stages of differentiation of cultured human keratinocytes, but was reported to lack substrate specificity and catalytic activity for reduction of lipid hydroperoxides (64). GSH-S-transferase and GSH-reductase activities have been detected at similar levels in murine epidermis (19.6–53.3 U/mg protein, and 22.5–31.6 U/mg protein, respectively) and dermis (33.8–64.8 U/mg protein, and 14.3–27.6 U/mg protein, respectively) (65,66). While little is known about absolute levels of GSTs in distinct layers of human skin, π-, μ-, and α-class GSTs have been localized immunohistologically in normal skin, naevi, and melanoma (67): π-GSTs were found in the stratum basalis and, to a lesser extent, in the superficial epidermal layers. Distribution of GST π in the epidermis showed that only the stratum basale, where melanocytes are located, stained well. The α-GSTs were relatively abundant in the upper strata and to a lesser extent, in the basal layers.

Superoxide Dismutases

Antioxidant Properties Superoxide dismutase (SOD) catalyzes in the dismutation reaction of superoxide radicals ($O^{\bullet}_2{}^-$) to H_2O_2. SODs are found in virtually all eukaryotic cells. Three types of human SOD have been purified: Cu/Zn-SOD (a cytosolic enzyme); Mn-SOD (a mitochondrial enzyme); and an extracellular SOD (EC-SOD, a tetrameric glycoprotein which contains Cu^{II} and Zn^{II}) (68,69). Cu/Zn-SOD consists of two protein subunits, each of which has an active site containing one Cu^{II} and one Zn^{II}. The Cu ion serves as an active redox site, while the Zn ion maintains the protein structure (68). The Mn-SOD consists of four subunits, each containing Mn^{II}, and is more labile than Cu/Zn-SOD. The presence of SOD in various compartments of the body may facilitate immediate dismutation of $O^{\bullet}_2{}^-$ at the site where it is generated.

Table 5 Activities of GSH-Px in Cutaneous Tissues

Skin layer	Species	Concentration	References	Year
Epidermis	Mouse	80.2 U/mg protein	Wheeler et al. (65)	1986
Dermis		37.0 U/mg protein		
Epidermis	Mouse	80.2 U/mg protein	Connor and Wheeler (66)	1987
Dermis		36.5 U/mg protein		
Total skin	Mouse	35 U/mg protein	Fuchs et al. (27,101)	1989
Epidermis	Mouse	11.7 ± 1.4 U/mg protein	Shindo et al. (17)	1993
Dermis		27.5 ± 2.5 U/mg protein		
Epidermis	Human	17.8 ± 1.0 U/mg protein	Shindo et al. (18)	1994
Dermis		15.0 + 1.3 U/mg protein		

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Prevalence in Skin Many investigators measured SOD activities in epidermal and dermal tissues, mostly using unspecific spectrophotometric activity assays determining total SOD activity (70). The reported activity levels are highly variable and do not allow clear conclusions about its preferential distribution within layers of skin (Table 6). Both in human and in pig epidermis, the Cu/Zn-SOD activity seems to be five- to tenfold higher than that of Mn-SOD (71,72). As compared with other body tissues, SOD activity is relatively low in skin (47).

Catalase

Antioxidant Properties Catalase is a tetrameric enzyme that is expressed in all major body organs. Each of its four subunits contains a heme-group in its active site and one tightly bound molecule of NADPH (73). Highest catalase activities are found in the peroxisomes, where it constitutes about 50% of the peroxisomal protein. The major role of catalase as an antioxidant is its ability to detoxify H_2O_2 by decomposing two H_2O_2 molecules to two molecules of water and one of oxygen.

Prevalence in Skin Epidermal activities were first measured in mice by Solanki et al., who reported 78–175 U/mg protein (74). Shindo et al. measured activities of 30.4 ± 4.3 U/mg protein in murine epidermis, and 33.3 U/mg protein in murine dermis, respectively. The same authors reported higher catalase activities in human epidermis (62 ± 6 U/mg protein), but lower activities in human dermis (14.6 ± 2.9 U/mg protein).

Effect of Environmental Stressors on Skin Antioxidants

UVB and UVA irradiation induce the formation of ROS in cell cultures (75,76), skin homogenates (52,77,78), and intact murine and human skin (79,80). Evalua-

Table 6 Physiological Activities of Superoxide Dismutase in Cutaneous Tissues

Skin layer	Species	Concentration	References	Year
Epidermis	Human	12.0 U/mg protein	Kim and Lee (202)	1987
Epidermis	Pig	11.4 U/mg protein	Ohkuma et al. (72)	1987
Dermis	Human	10.5 U/mg protein	Kim and Lee (202)	1987
Epidermis	Mouse	0.6 U/mg protein	Carrao and Pathak (203)	1988
Epidermis	Guinea pig	0.5 U/mg protein	Carrao and Pathak (203)	1988
Total skin	Mouse	3.0–4.3 U/mg protein	Fuchs et al. (27,101)	1989
Epidermis	Mouse	11.7 ± 1.4 U/mg protein	Shindo et al. (17)	1993
Dermis	Mouse	27.5 ± 2.5 U/mg protein	Shindo et al. (17)	1993
Epidermis	Human	17.8 ± 1.0 U/mg protein	Shindo et al. (18)	1994
Dermis	Human	15.0 + 1.3 U/mg protein	Shindo et al. (18)	1994

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tion of the protective mechanisms of skin have included measurements of baseline levels of antioxidants in the dermis and epidermis (17,18) and the antioxidant response to UVB and UVA light in these layers (27,81). Terrestrial UVR consists of UVB (280–320 nm) and UVA (UVA-II: 320–340 nm, UVA-I: 340–400 nm). Radiation of less than 280 nm (UVC) does not reach the Earth's surface, since they are absorbed by stratospheric ozone.

While ozone (O_3) in the upper atmosphere (stratosphere) occurs naturally and protects skin by filtering out harmful solar ultraviolet radiation, O_3 at ground level (troposphere) is a noxious, highly reactive oxidant pollutant. The precursors of photochemical oxidants are volatile organic compounds (e.g., vapor-phase hydrocarbons and halogenated organics), oxides of nitrogen (NO_x), NO and other radicals, O_2, and sunlight (82). As a major pollutant in photochemical smog, O_3 occurs at concentrations between 0.1 and 0.8 ppm and represents a severe urban air quality problem (83). In addition to photochemical smog, O_3 is generated during operation of high-voltage devices and dermatological phototherapy equipment (83). There is ample evidence that acute (2–6 h) and chronic in vivo exposure to O_3 causes airway inflammation and affects pulmonary function in humans (84–86). The biological effects of O_3 are attributed to its ability to cause ozonation, oxidation, and peroxidation of biomolecules, both directly and via secondary reactive reactions. Hydrogen peroxide, hydroperoxides, hydroxyl radical, superoxide anion, and singlet oxygen have been proposed as intermediates in these secondary reactions (83,87–90). Analogous to the respiratory tract and the surface tissues of plants, a primary function of the skin is to provide a protective barrier against noxious environmental agents including oxidative air pollutants.

Numerous studies have documented the effects of O_3 on the respiratory tract in animals and humans (83,91,92) and on plants (93–96). In contrast, only little is known about the effect of O_3 on cutaneous tissues. Recently, a series of studies were published investigating the impact of O_3 on skin antioxidants (1,5,7,97). Since O_3 levels are frequently highest in areas where exposure to ultraviolet radiation is also high, the concomitant exposure to O_3 and ultraviolet radiation in photochemical smog could be of relevance for skin pathologies, as has been implicated for plants (93,98).

Hydrophilic Skin Antioxidants

Ascorbate and Urate High acute doses of solar simulated UVA/UVB (SSUV) have been demonstrated to deplete ascorbate and urate in cultured human skin equivalents. The SSUV dose needed to deplete these hydrophilic antioxidants was much higher than those necessary to deplete lipophilic antioxidants ubiquinol-10 and α–tocopherol (99). In hairless mice, however, Shindo et al. (100) observed depletion of ascorbate already at lower SSUV doses than those needed to deplete lipophilic antioxidants or GSH. A single acute ozone exposure

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depletes ascorbate in the upper epidermis of hairless mice, but not in lower skin layers (7).

Glutathione Fuchs et al. (27,101) reported that single exposures to UVB, but not to UVA, deplete GSH and increase GSSG in excised mouse skin, while ascorbate levels remained unchanged. However, UVA irradiation of human fibroblasts depleted intracellular glutathione levels (102). Treatment of hairless mice with 8-methoxypsoralene plus UVA (PUVA) resulted in a significant depletion of cutaneous glutathione after 24 to 48 h (65). Epidermal GSH levels of UVB-treated hairless mice were depleted by 40% within minutes after exposure and returned to regular levels after half an hour (66).

Lipophilic Skin Antioxidants

Vitamin E Recently, it was demonstrated that a single suberythemogenic dose of SSUV light (UVA and UVB; 0.75 MED) depletes human stratum corneum α–tocopherol by almost 50%, and murine stratum corneum α–tocopherol by 85% (6). These findings were in contrast to previous studies investigating the effects of SSUV light on dermal and epidermal antioxidants, in which doses equivalent to 3 MED or more were necessary to detect a significant depletion of α–tocopherol (17,81,103). Hence, it was concluded that α–tocopherol depletion in the stratum corneum is a very early and sensitive event of photooxidative damage in skin (6). The high susceptibility of stratum corneum vitamin E to SSUV may be, at least in part, due to a lack of coantioxidants in the stratum corneum. Ubiquinol-10 was undetectable in human stratum corneum at levels found in epidermis and dermis (6). Additionally, ascorbate, the major hydrophilic coantioxidant that is capable of recycling photooxidized α–tocopherol (52,78) is present only at very low levels in murine and human stratum corneum, as compared to epidermal and dermal tissue (Thiele et al., unpublished observations).

Vitamin E may be depleted (1) directly, by absorption of UVB-radiation; and/or (2) indirectly, by excited-state singlet oxygen or reactive oxygen intermediates that are generated by photosensitizers upon UV absorption also in the UVA range. Since both UVB and UVA alone have been shown to deplete murine α–tocopherol (6), both mechanisms may be relevant. The absorption maxima of α– and γ–tocopherol fall between 290 and 295 nm (104,105) and thus extend well into the solar UV spectrum. Interestingly, a large part of terrestrial UVB (around 290–300 nm) is absorbed in the human stratum corneum (106). Furthermore, depletion of α–tocopherol by UVR is maximal at wavelengths in the range of its absorption maximum in skin homogenates of hairless mice (52). This congruency suggests that α–tocopherol is directly destroyed upon short-wavelength UVB absorption. Indeed, tocopheroxyl radical formation occurs in UVB-irradiated skin homogenates (52). Direct depletion of α–tocopherol and formation of its radical

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may also affect other endogenous antioxidant pools. As mentioned previously, α–tocopherol is readily regenerated from its radical at the expense of reductants like ascorbate (52,107) (Fig. 2), which itself can be regenerated by glutathione (25). In addition to direct depletion by UVB, skin α–tocopherol levels may also be consumed as a consequence of its chain-breaking antioxidant action. The absorption of UVB and UVA photons by endogenous photosensitizers (e.g., porphyrins, riboflavin, quinones, and bilirubin) results in its electronically excited state (108,109). The excited sensitizer subsequently reacts with another substrate (type I reaction) to form radicals or radical ions, or with oxygen (type II reaction) to generate singlet oxygen (110). Photosensitizers, such as melanin, are present in variable amounts in the stratum corneum (111). Hence, their wavelength-dependent potential to generate or to quench free radicals, and to absorb UVR, may modulate α–tocopherol depletion during and after solar exposure.

Recently, Thiele et al. investigated the effects of the air pollutant ozone on skin antioxidants: while no depletion of vitamin E was observed in whole skin (97), α–tocopherol depletion was detected in the outer epidermis when skin layers were analyzed separately (7). It was concluded that ozone itself is too reactive to penetrate deeply into skin and reacts rapidly with skin barrier lipids and proteins (8). Consequently, it was demonstrated that the stratum corneum is the most susceptible skin layer for ozone-induced vitamin E depletion (5).

Ubiquinol/Ubiquinone Ubiquinol-9 has been shown to be the most susceptible nonenzymatic antioxidant in murine skin, with respect to SSUV-induced (280–400 nm) depletion in vivo (17). Similar results were obtained for ubiquinol-10 in SSUV-irradiated human cell culture models (99). Exposure of purified ubiquinol-9 and α–tocopherol to SSUV in vitro resulted in the depletion of both compounds, which have similar absorption maxima around 295 nm (81). Since ubiquinol depletion precedes that of α–tocopherol in UVR-challenged skin in vivo, it is thought that ubiquinol protects vitamin E, as demonstrated in vitro (37).

Vitamin A/Carotenoids A single exposure of human volar forearm skin to SSUV (3 MED) was found to lower the skin lycopene (Ψ,Ψ–carotene) level by 31 to 46%, whereas the same UV dose did not induce significant changes in the skin β–carotene level (55). However, repeated exposures of human volunteers to solar light (total UV dose of about 10 kJ cm^{-2}) depleted also β–carotene levels in skin (112).

Enzymatic Skin Antioxidants

Catalase, SOD, GSH-Px, GSSG Reductase It was demonstrated by Aronoff et al. more than three decades ago that photooxidation of a single porphyrin ring in catalase results in complete inhibition of its activity (113). Superoxide anion radicals (114) and ozone (115) have also been shown to inactivate catalase

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activity. Punnonen et al. demonstrated that UVB (116), as well as UVA or PUVA therapy (117) decreases the activity of both catalase and SOD in cultured human keratinocytes. In cultured human fibroblasts, a single UVA exposure decreased catalase activities immediately, while GSH-Px and GSSG reductase remained unaffected, and SOD activity decreased only 3 days after exposure (118). In vivo exposures of hairless mouse skin using SSUV light demonstrated that dermal and epidermal catalase is more susceptible to photoinactivation than SOD, and far more than GSH-Px and GSSG reductase (17,81). In vitro, purified catalase was demonstrated to be directly inactivated by SSUV light, while SOD activity remained unaffected (81). Hence, while direct photodestruction appears to account for catalase inactivation, other mechanisms, possibly involving free-radical-mediated oxidative protein damage, may account for the observed UV-induced loss of skin SOD activity in vivo. Notably, chronic UVB irradiation was recently shown to upregulate human epidermal SOD activity in vivo, whereas the activities of other antioxidant enzymes remained unchanged (119).

ROLE OF ANTIOXIDANTS IN THE PHOTOPROTECTION OF SKIN

Topical Application of Antioxidants

UVR-induced skin damage includes acute reactions, such as erythema, edema, and pain, followed by exfoliation, tanning, and epidermal thickening. Premature skin aging (photoaging) and carcinogenesis are generally believed to be consequences of chronic UVR exposure (120). ROS and other free radicals, particularly the highly damaging hydroxyl radical, deplete the skin of its antioxidant defense and, when the latter is overwhelmed, can damage biomolecules such as lipids, proteins, and nucleic acids (27,81,101). Therefore, apart from using chemical and/or physical sunscreens to diminish the intensity of UVR reaching the skin, preventing ROS from reacting with these biomolecules by strengthening the skin's antioxidative capacity is an emerging approach in limiting UVR-induced skin damage (121–123). Topical application of antioxidants, such as vitamin E, provides an efficient means of increasing antioxidant tissue levels in epidermis and dermis (103,124). The stratum corneum, which was shown to be the most susceptible skin layer for UVR-induced depletion of vitamin E (6), may particularly benefit from an increased antioxidant capacity.

A selected overview of animal and human studies investigating acute and chronic photoprotection of skin by topical administration of antioxidants is given in Tables 7 to 10.

Vitamin E

The photoprotective effect of vitamin E (α–tocopherol) and its acetyl ester have been studied extensively (see Table 7). Numerous topical studies demonstrated

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Table 7 Photoprotective Effects of Topically Applied Vitamin E (α–Tocopherol) and Derivatives In Vivo

Compound(s)	Species	Endpoint(s)	Efficacy	Remarks	References
Vitamin E Vitamin E acetate	Rabbit	Erythema (MED)	Vitamin E protective; vitamin E acetate not protective	BHT also protective; vitamin E also protective when applied after UVR exposure	Roshchupkin et al., 1979 (125)
Vitamin E	Human	Mechanoelectrical properties of skin	Protection against UVR- and PUVA-induced damage		Potapenko et al., 1983 (139)
Vitamin E Vitamin E derivatives with shorter hydrocarbon chains Vitamin E acetate	Human, rabbit	PUVA-induced erythema and changes in mechanoelectrical properties of skin	Vitamin E and derivatives with shorter hydrocarbon chain protective; vitamin E acetate not protective	No protection of vitamin E and derivatives when applied after UVR exposure	Potapenko et al., 1984 (38)
Vitamin E	Mouse	Lipid peroxidation	Protective	Vitamin A, BHT, and β–carotene also protective	Khettab et al., 1988 (130)
Vitamin E	Mouse	Skin wrinkling, skin tumor incidence, and histology	Protective		Bissett et al., 1989 (136)
Vitamin E	Human	Erythema (MED)	Protective	SPF determination	Möller et al., 1989 (126)
Vitamin E Trolox® Vitamin E acetate Vitamin E succinate Vitamin E linoleate Vitamin E nicotinate	Mouse	Skin wrinkling and sagging, skin tumor incidence, and histology	Vitamin E esters not as protective as vitamin E or vitamin E analog Trolox®; no protection against UVA-induced skin sagging	Glutathione, β–carotene, BHT, and mannitol not protective	Bissett et al., 1990 (137)
Vitamin E	Mouse	Skin tumor incidence and immunosuppression	Protective	Prolonged pretreatment	Gensler et al., 1991 (131)

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Vitamin E Vitamin E acetate	Rat	UVA-induced binding of 8-MOP and CPZ to epidermal biomacromolecules	Vitamin E protective after single application; vitamin E acetate only protective after prolonged application	Limited conversion of vitamin E acetate into vitamin E after single application	Schoonderwoerd et al., 1991 (133)
Vitamin E acetate	Mouse	Lipid peroxidation and DNA synthesis rate	Protective		Record et al., 1991 (140)
Vitamin E	Mouse	Skin wrinkling, skin tumor incidence, and histology	Protective	Additive protection in combination with anti-inflammatory agents	Bissett et al., 1992 (135)
Vitamin E acetate	Mouse	Erythema, edema, and skin sensitivity	Protective	Treatment immediately after UVR-exposure	Trevithick et al., 1992 (141)
Vitamin E acetate	Mouse	Edema and histology	Protective	Delayed treatment after UVR-exposure; increased skin vitamin E concentration	Trevithick et al., 1993 (142)
Vitamin E Vitamin E acetate Vitamin E sorbate	Mouse	Skin wrinkling	Vitamin E and sorbate ester protective; vitamin E acetate ester only modestly protective	Sorbate ester more protective than free vitamin E	Jurkiewicz et al., 1995 (80)
Vitamin E Vitamin E acetate	Human	Erythema (skin color)	Moderate protection of vitamin E and vitamin E acetate when applied occlusively after UVR exposure	No protection when applied occlusively before UVR exposure	Montenegro et al., 1995 (179)
Vitamin E Vitamin E acetate	Rat	UVA-induced binding of 8-MOP to epidermal biomacromolecules	Vitamin E protective; vitamin E acetate only protective after prolonged application	Conversion of vitamin E acetate into vitamin E slow	Beijerbergen van Henegouwen et al., 1995 (132)
Vitamin E acetate Vitamin E succinate	Mouse	Skin tumor incidence and immunosuppression	No protection		Gensler et al., 1996 (143)

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Table 7 Continued

Compound(s)	Species	Endpoint(s)	Efficacy	Remarks	References
Vitamin E	Yorkshire pig	Sunburn cell formation	Protection against UVR-induced damage	Minimal protection in reducing PUVA-induced damage	Darr et al., 1996 (128)
Vitamin E	Mouse	Immunosuppression and lipid peroxidation	Protective	No protection when applied after UVR exposure	Yuen et al., 1997 (105)
Vitamin E	Mouse	Histology (sunburn cell formation and skin thickness)	Protective		Ritter et al., 1997 (129)
Vitamin E Vitamin E acetate Vitamin E methyl ether	Mouse	Formation of DNA photoadducts	Vitamin E derivatives less protective than vitamin E	Sunscreening properties of vitamin E	McVean et al., 1997 (10)
Vitamin E	Mouse	Chemiluminescence after UVA exposure	Protective	β–Carotene also protective	Evelson et al., 1997 (134)
Vitamin E	Mouse	Lipid peroxidation	Protective	Skin's enzymatic and non-enzymatic antioxidant capacity investigated	Lopez-Torres et al., 1998 (124)
Vitamin E	Human	Erythema (skin color and skin blood flow)	Moderate protection	No protection when applied after UVR exposure; SPF (determined in vitro) = 1	Dreher et al., 1998 (127,180)

Abbreviations: BHT, butylated hydroxytoluene; CPZ, chlorpromazine; MED, minimal erythema dose; 8-MOP, 8-methoxypsoralen; PUVA, 8-methoxypsoralen and UVA treatment; SPF, sun protection factor.

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significantly reduced acute skin responses when vitamin E was applied before UVR exposure, such as erythema and edema (125–127), sunburn cell formation (128,129), lipid peroxidation (105,124,130), DNA adduct formation (10), immunosuppression (105,131), as well as UVA-induced binding of photosensitizers (132,133) and chemiluminescence (134). Chronic skin reactions due to prolonged UVR exposure, such as skin wrinkling (80,135–137), and skin tumor incidence (131,135–137) were also diminished by topical vitamin E. However, most studies used animal models, while only few studies exist demonstrating photoprotection by topical application of vitamin E in humans (126,127,138,139).

Vitamin E esters, particularly vitamin E acetate, were also shown to be promising agents in reducing UVR-induced skin damage (80,132,133,137,140–142) (see Table 7). However, their photoprotective effects appeared to be less pronounced as compared to vitamin E; moreover, some studies failed to detect photoprotection provided by vitamin E esters (125,138,143). Since the free aromatic hydroxyl group is responsible for the antioxidant properties of vitamin E, vitamin E esters need to be hydrolyzed during skin absorption to show activity. Vitamin E acetate was shown to be absorbed and penetrate skin easily (144–146). A skin bioavailability study demonstrated that vitamin E and vitamin E acetate behave similarly with regard to penetration of rat epidermis (132). The authors concluded that the aromatic hydroxyl group in vitamin E is not dissociated in the skin penetration limiting layer, the stratum corneum. Consequently, the difference between physicochemical parameters determining skin transport for vitamin E and its esters seem negligible. Notably, the bioconversion of vitamin E acetate to its active antioxidative form, α–tocopherol, was found to be slow and to occur only to a minor extent in vivo (132,147). Hence, the less pronounced or missing photoprotective effects of topically applied vitamin E acetate after a single application might be explained by a limited bioavailability of the ester-cleaved form during oxidative stress at the site of action (e.g., superficial skin layers). As was further shown by the same authors, photoprotection was obtained only after several topical applications of vitamin E acetate. A photocarcinogenesis study by Gensler et al. (143) even demonstrated an increased skin tumor incidence after topical application of vitamin E esters compared to nontreated, but UVR-exposed, hairless mice. Confirming the results obtained by Beijersbergen van Henegouwen et al. (132), Gensler and coworkers found an accumulation of vitamin E acetate in the skin after prolonged topical application, whereas the level of free α–tocopherol remained relatively low. A human study further demonstrated that topically applied α–tocopherol acetate, though substantially absorbed into skin, is not significantly metabolized to the hydrolyzed form, even after long-term administration (147).

In addition to the antioxidative properties of vitamin E, further photoprotective mechanisms have been discussed. Recent studies on vitamin E using a liposome dispersion model to estimate the photooxidation of biomolecules (148), or

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measuring DNA-adduct formation in vivo (10), indicated that vitamin E may also have substantial sunscreening properties. On the other hand, a determination of the sun protection factor (SPF) of a vitamin E lotion (2 w%) in vitro resulted in no significant sunscreening effect when administered at a dose of 2 mg cm^{-2} (127). Additionally, interactions of vitamin E with the metabolism of arachidonic acid have been described. Vitamin E was shown to modulate the activity of cyclooxygenase and to depress the biosynthesis rate of prostaglandin E_2, possibly by inhibiting the release of arachidonic acid by phospholipase A_2 (33,149). Interactions with the eicosanoid system may result in an anti-inflammatory effect and thus complement antioxidative photoprotection in skin.

Vitamin C

Few studies have reported photoprotective effects for vitamin C (see Table 8). Using a porcine skin model, Darr and associates proposed that topically applied vitamin C is only effective when formulated at high concentration in an appropriate vehicle (150). Vitamin C is highly unstable and is only poorly absorbed into the skin, possibly explaining its modest photoprotective effect when applied topically (151). Hence, more lipophilic and more stable vitamin C esters, such as its palmitatyl, succinyl, or phosphoryl ester (151–153), might be promising derivatives providing increased photoprotection, as compared to vitamin C. As described for vitamin E esters, such compounds must be hydrolyzed to vitamin C to be effective as antioxidants.

Other Antioxidants

Besides vitamin E and vitamin C, several other compounds with antioxidative potential have been suggested to lower photodamage when topically applied (see Table 9). Administration of different plant extracts, particularly flavonoids, were reported to diminish acute and chronic skin damage after UVR exposure (154–159). Flavonoids (e.g., apigenin, catechin, epicatechin, α–glycosylrutin, and silymarin) are polyphenolic compounds that occur in plants and, due to their free phenolic groups, exhibit antioxidative capacity. Furthermore, flavonoids may possibly also have anti-inflammatory properties (160). Thiols, such as N-acetylcysteine and derivatives, are another important group of potent radical scavengers (161,162). It was demonstrated in several rat studies that topical administration of thiols diminishes UVA-induced binding of photosensitizers to epidermal lipids and DNA (163,164) and afforded some protection against the damaging effects of UVB on epidermal DNA (165). Treatment with cysteine derivatives, like N-acetyl-cysteine, resulted in increased intracellular glutathione (GSH) levels in human keratinocytes (166). Thus, thiol-induced stimulation of GSH biosynthesis may be a key mechanism accounting for the observed photoprotective effects. Exogenously applied GSH penetrates the cell membrane and the skin only poorly

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Table 8 Photoprotective Effects of Topically Applied Vitamin C (Ascorbic Acid) and Derivatives In Vivo

Compound(s)	Species	Endpoint(s)	Efficacy	Remarks	References
Vitamin C Vitamin C palmitate	Mouse	Skin wrinkling and sagging, skin tumor incidence, and histology	Vitamin C palmitate less protective than vitamin C; no protection against UVA-induced skin sagging		Bissett et al., 1990 (137)
Vitamin C	Yorkshire pig	Erythema (skin blood flow) and sunburn cell formation	Protection against UVR- and PUVA-induced damage	High vitamin C concentration	Darr et al., 1992 (150)
Vitamin C	Mouse	Skin wrinkling, skin tumor incidence, and histology	Protective	Additive protection in combination with anti-inflammatory agents	Bissett et al., 1992 (135)
Vitamin C palmitate	Human	Erythema (skin color)	Poor protection when applied occlusively after UVR exposure	No protection when applied occlusively before UVR exposure	Montenegro et al., 1995 (179)
Vitamin C	Yorkshire pig	Sunburn cell formation	No protection against UVR-induced damage, protective against PUVA-induced damage	Additive protection in combination with sunscreens	Darr et al., 1996 (128)
Vitamin C	Human	Erythema (skin color and skin blood flow)	Poor protection	SPF (determined in vitro) = 1	Dreher et al., 1998 (127)

For abbreviations see Table 7.

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Table 9 Photoprotective Effects of Topically Applied Plant Extracts, Flavonoids, N-Acetyl-Cysteine and Derivatives, and Other Antioxidants In Vivo

Compound(s)	Species	Endpoint(s)	Efficacy	Remarks	References
Green tea extract	Mouse	Skin tumor incidence	Protective	Green tea contains catechin and epicatechin derivatives	Wang et al., 1991 (154)
Polypodium leucotomos (tropical fern) extract	Guinea pigs, human	Erythema (skin color)	Protection against UVR- and PUVA-induced damage	Extract with immunomodulating properties	González et al., 1996 (155)
Polypodium leucotomos (tropical fern) extract	Human	Erythema (MED), immediate pigment darkening, delayed tanning, minimal phototoxic dose, and histology	Protection against UVR- and PUVA-induced damage		González et al., 1997 (156)
Epigallocatechin-3-gallate	Mouse	Skin tumor incidence	Protective	Not immunosuppressive; isolated from green tea	Gensler et al., 1996 (157)
Apigenin	Mouse	Skin tumor incidence	Protective		Birt et al., 1997 (158)
Silymarin	Mouse	Edema, sunburn and apoptotic cell formation, and skin tumor incidence	Protective	Isolated from milk thistle plant	Katiyar et al., 1997 (159)
N-acetyl-cysteine Captopril Other thiols	Rat	UVA-induced binding of 8-MOP and CPZ to epidermal biomacromolecules	N-acetyl-cysteine and captopril most protective thiols	Vitamin E less protective	Van den Broeke et al., 1993 (164)
N-acetyl-cysteine	Rat	DNA synthesis rate	Protective		Van den Broeke et al., 1994 (165)

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N-acetyl-cysteine Several cysteine derivatives	Rat	UVA-induced binding of 8-MOP and CPZ to epidermal biomacromolecules	Protective	High epidermal bioavailability of N-acetyl-cysteine	Van den Broeke et al., 1995 (163)
Melatonin	Human	Erythema (skin color)	Protective	Also protective when applied after UVR exposure	Bangha et al., 1996 (204)
Melatonin	Human	Erythema (skin color)	Protective	No protection when applied after UVR irradiation; melatonin without sunscreening properties	Bangha et al., 1997 (170)
Melatonin	Human	Erythema (skin color and skin blood flow)	Protective	No protection when applied after UVR irradiation; melatonin with sunscreening properties	Dreher et al., 1998 (127,180)
Superoxide dismutase	Guinea pig	PUVA-induced erythema and edema	Protective	β–Carotene also protective; vitamin E, vitamin E acetate, and glutathione not protective	Carraro et al., 1988 (174)
Superoxide dismutase	Guinea pig	Erythema	Not protective		Hamanaka et al., 1990 (177)
Superoxide dismutase	Human	Erythema (skin color)	Protective when applied occlusively after UVR exposure	No protection when applied occlusively before UVR exposure	Montenegro et al., 1995 (179)
Superoxide dismutase	Mice, human	PUVA-induced erythema and edema	Protective	Prolonged pretreatment	Alaoui et al., 1994 (175) Filipe et al., 1997 (176)
2,4-Hexadienol	Mouse	Skin wrinkling and sagging, and skin tumor incidence	Protective, not protective against UVA-induced skin sagging	Also other conjugated dienes tested	Bissett et al., 1990 (205)

For abbreviations, see Table 7.

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and does not prevent photodamage in mice when applied topically (137) or injected intraperitoneally (167).

A photoprotective effect for the redox couple α-lipoate/dihydrolipoate (also referred to as ''α-lipoic acid'') has been proposed for skin (168). Dihydrolipoate, the reduced form of lipoic acid, is a reductant with a more negative redox potential (−0.32 V for the couple lipoate/dihydrolipoate) than ascorbate (0.08 V for the couple dehydroascorbate/ascorbate), which is thus able to regenerate ascorbate from its oxidation products (see Fig. 2). In liposomes irradiated with solar-simulated UV light, dihydrolipoate in combination with ascorbate was shown to strongly enhance the recycling of α–tocopherol (52). It was demonstrated in hairless mice that α-lipoate readily penetrates skin and thereafter is reduced to its more potent antioxidant form, dihydrolipoate (169). Fuchs et al. reported anti-inflammatory properties of dihydrolipoate in dermatitis induced by reactive oxidants in hairless mice (168).

Regarding the pineal hormone melatonin (N-acetyl-5-methoxytryptamine), Bangha and coworkers showed a suppression of UVR-induced erythema by topical melatonin in humans (170). Besides melatonin's antioxidant (171) and dose-dependent sunscreening properties (127,170), it may also act in an immunomodulatory way (172,173). Photoprotective effects were also reported for topical application of several other substances with antioxidant properties. Interestingly, topical administration of *superoxide dismutase* SOD resulted in reduction of PUVA-induced skin reactions after single application in guinea pigs (174), or after prolonged pretreatment of murine (175) and human skin (176), respectively. In contrast, Hamanaka and associates did not observe significantly lowered UVB-induced erythema reaction after topical administration of SOD in guinea pigs (177). However, they demonstrated that, while cutaneous SOD activity was decreased in nontreated control animals after UVB exposure, topical SOD diminished this decrease in activity. Due to its high molecular weight, SOD is unlikely to penetrate into deeper skin layers. Yet, it was shown to be capable of inhibiting PUVA-induced erythema, suggesting that oxidative processes initiated at the skin surface may induce an inflammatory response in lower skin layers (123,175).

Antioxidant Combinations

The cutaneous antioxidant system is complex and far from being completely understood. As pointed out above, the system is interlinked and operates as an antioxidant network (Fig. 2). Thus, an enhanced photoprotective effect may be obtained by applying appropriate combinations of antioxidants (see Table 10). As was shown in a human study, application of vitamin C or vitamin E alone resulted in modestly decreased erythema reaction (127). However, a much more pronounced effect was obtained by combining these two vitamins. Notably, the most dramatic improvement resulted from the coformulation of melatonin to-

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Table 10 Photoprotective Effects of Topically Applied Antioxidant Combinations In Vivo

Compounds	Species	Endpoint(s)	Efficacy	Remarks	Reference
Vitamins E and C, BHT, and glutathione	Mouse	Erythema (MED)	Protective	BHT alone also protective	De Rios et al., 1978, (206)
Vitamins E and C	Yorkshire pig	Sunburn cell formation	Protective	Maximal protection in combination with sunscreens	Darr et al., 1996 (128)
Vitamin E acetate and α–glycosylrutin Vitamin E acetate, α–glycosylrutin and ferulic acid	Human	Chemiluminescence and reflection spectrometry of experimentally provoked polymorphous light eruption	Protective	Polymorphous light eruption induced by UVA radiation	Hadshiew et al., 1997 (178)
Vitamins E and C Vitamins E and C, and melatonin	Human	Erythema (skin color and skin blood flow)	Protective; maximal protection when vitamins E and C are combined with melatonin	No protection when administered after UVR-exposure; melatonin with sunscreening properties	Dreher et al., 1998 (127,180)

For abbreviations see Table 7.

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gether with vitamin E and vitamin C. Studying the effect of distinct mixtures of topically applied antioxidants in photodermatoses, Hadshiew and associates demonstrated that the development and severity of polymorphous light eruption were significantly reduced by administration of a combination consisting of α-glycosylrutin, ferulic acid, and tocopheryl acetate (178). The authors hypothesized that a sunscreening effect of the substances employed was negligible, and that the photoprotection observed was due to reduction of UVA-induced oxidative stress.

Whereas the photoprotective effect of topical antioxidants applied before UVR exposure has been recognized, the effect of these compounds administered after irradiation is less obvious (see Tables 7 to 10). Diminished erythema formation was reported when antioxidants, such as α–tocopherol or α–tocopherol acetate, were topically administered after UVR exposure (125,141,142,179). However, these findings are in contrast to other studies that found no diminished UVR-related skin damage when antioxidants were applied after the irradiation (105,138,170). As was shown in a recent human study by Dreher and coworkers, neither vitamin E, nor vitamin C, nor melatonin, nor combinations thereof, led to a significantly lowered erythema formation when administered after UVB exposure (180). The authors concluded that ROS-induced skin damage is a rapid event, and antioxidants possibly prevent such damage only when present in relevant concentration at the site of action (e.g., superficial skin layers) at the beginning and during occurrence of oxidative stress.

Topical Application of Substances Other Than Conventional Antioxidants

Apart from increasing the skin's antioxidant capacity by topical application of antioxidants, other substances may serve to enhance the antioxidative capacity by preventing the formation of ROS or by increasing the formation, stability, or activity of constitutive skin antioxidants. Skin contains substantial amounts of iron, and chronic exposure to UVR was shown to increase the skin levels of nonheme iron (181). Iron participates as a catalyst in the formation of the highly damaging hydroxyl radical (15). Hence, topical application of certain iron chelators such as 2-furildioxime were demonstrated to be efficient in providing photoprotection alone (182) or in combination with sunscreens (183). Furthermore, a possible role of 1,25-dihydroxy-vitamin D_3-induced formation of metallothionein in cutaneous photoprotection was reported; Hanada and coworkers found a significantly lowered level of sunburn cell formation in mouse skin after UVB exposure by topical application of the active form of vitamin D_3 (184). The authors postulated that the cysteine-rich metallothionein may act as a radical scavenger. Supplementation with selenium is a further interesting approach in reducing UVR-induced skin damage. Selenium is an essential trace element in humans

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and animals and is the required constituent for GSH peroxidase. Applying topical selenium in the form of L-selenomethionine proved to reduce acute and/or chronic skin damage in mice (185) as well as in humans (186). Topical application of L-selenomethionine led to increased skin selenium levels, whereas free selenium was apparently not absorbed (187,188).

SUMMARY AND CONCLUSION

Animal and human studies have convincingly demonstrated significant photoprotective effects of ''natural'' and synthetic antioxidants when applied topically before UVA and UVB exposure. However, particularly with respect to UVB-induced skin damage, the photoprotective effects of most antioxidants were modest as compared to sunscreens. More successful in preventing such damage were appropriate combinations of antioxidants resulting in a sustained antioxidant capacity of the skin, possibly due to antioxidant synergisms. On the other hand, regarding photoprotective effects against UVA-induced skin alterations, which are largely determined by oxidative processes (75,189–192), topical administration of antioxidants might be particularly promising (193–195). In fact, topical application of antioxidants resulted in a remarkable reduction of UVA-induced ROS generation in mice (134), and diminished UVA-induced polymorphous light eruption in humans (178). Furthermore, topical application of antioxidants, particularly of vitamin C, was reported to diminish PUVA-induced erythema and sunburn cell formation (128,138,150,155,156).

Since UVA- and UVB-induced skin damage is not solely dependent on ROS formation and their reaction with numerous skin biomolecules, topical (as well as systemic) antioxidant supplementation cannot be presumed to give complete photoprotection (196). Other ROS-independent processes, such as DNA dimer formation, will persist in causing skin damage, regardless of the effectiveness of the antioxidant(s) administered. Therefore, efficient sunscreens are indispensable in the effective prevention of skin photodamage. However, antioxidants, in combination with sunscreens (128) or anti-inflammatory agents (135), seem to be highly effective adjuncts increasing the safety and the efficacy of photoprotective products.

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12
Protective Creams

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INTRODUCTION

Contact dermatitis is the most frequent manifestation of occupational skin disease. Since the course may be chronic leading to disability, and since treatment is frequently of limited efficacy, prevention should be emphasized to reduce the incidence and prevalence of irritant contact dermatitis (ICD) and allergic contact dermatitis (ACD). Apart from total elimination of cutaneous exposure to hazardous substances and the use of gloves or protective clothing, protective creams (PC) are additional tools in an integrated concept of preventive measurements. Skin protection in the workplace consists of preexposure PCs, mild skin cleansers, and postexposure skin-care products. PCs are designed to prevent skin damage due to irritant contact; skin cleaning should remove aggressive substances from the skin; and skin care is intended to enhance epidermal barrier regeneration (1).

CHEMISTRY AND MODE OF ACTION

Protective Creams with a Physical/Chemical Mode of Action

Even in recent years the prevailing opinion has been that PCs are effective in a purely physical way. Due to their composition, a barrier is built up that cannot

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be penetrated easily. In addition, lipophilic ointments should have benefit against hydrophilic irritants and lipophobic ointments against hydrophobic irritants. Water-in-oil emulsions are recommended against water-soluble irritants such as detergents, acids, alkalics, metal-working fluids, and even plain water. On the other hand, oil-in-water emulsions are offered against lipophilic irritants such as oils, varnishes, and organic solvents.

Special investigations have been undertaken to develop preparations with a dual mode of action, combining the different effects of hydrophilic and hydrophobic ingredients or developing foamy skin protectors containing stearic acid, propylene glycol, glycerol, sorbitol, and dimethylpolysiloxane. However, these so-called ''invisable gloves'' failed in a repetitive irritation test against the anionic detergent sodium lauryl sulfate (SLS) and against the solvent toluene (TOL) (2). Other preparations include a fatty amine amide acetate that binds to negatively charged carboxyl groups of keratin and the positive fatty ammonium ion of these substances binds firmly to the negative charge of the epidermis. This is supposed to build up a firm second layer on the skin, which prevents penetration of various agents in a steric manner (3).

Protective Creams with Special Ingredients

Some ingredients purportedly have special protective properties such as natural or synthetic tannery substances, zinc oxide, talcum, chelating agents, or other substances that can bind metal ions or reduce penetration through the skin. Zinc oxide has a covering effect. Tannin is used as a skin astringent in order to increase the mechanical resistance of the skin surface against microtraumas. Additionally, tannery agents cause a local decrease of perspiration, which seems to be helpful while wearing gloves (4). The decrease of swelling is caused by direct binding of the tanning substance to keratin. Chelating agents are used in order to protect against sensitizing substances. Tartaric acid and glycine chelate chromate and reduce chrome VI to chrome III, which is less allergenic (5).

EFFICACY OF PROTECTIVE CREAMS

Though PCs are one of the most common measures to prevent CD, their actual benefit at the workplace is still regarded with skepticism (6) and debated in recent reviews (7,8). Due to the fact that PCs are not considered to be drugs, but rather cosmetics, valid methods to show their efficacy have not been legally necessary. Because of new European Union (EU) laws for cosmetic standards, producers are now forced to provide better claim support. In addition, European Community (EC) regulations require the employer to provide PCs to workers at exposed workplaces for prevention of ICD. It is in the employers' interest that this invest-

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ment is not based on unfounded claims, but on scientific data. Double-blinded, placebo-controlled clinical tests of PCs are still lacking because of methodological difficulties, ethical doubts, and the enormous expenditure for tests regarding the preventive benefit of PCs in practice. Therefore, in vivo and in vitro tests are used for the evaluation of PC efficacy, even though they are not considered to be close to real workplace situations.

Since Suskind introduced the slide test to evaluate PCs in the 1950s (9) various in vitro techniques and in vivo tests on animals or human skin were developed to investigate the efficacy of PCs as preexposure skin protectors (10–12). In recent years, noninvasive biophysical measurements have achieved great importance especially for clinically weak reactions. Mahmoud and Lachapelle (13,14) showed PCs to have some effect against the acute irritative and locally toxic action of solvents using skin biopsies and Doppler flowmetry. Also using a guinea pig model, Frosch et al. (15,16) carried out cumulative irritation by SLS, sodium hydroxide (NaOH), and TOL. Irritation was measured by a visual score and biophysiological techniques (evaporimetry and Doppler velocimetry).

Considering human models for PC evaluation, Frosch et al. (17) proposed the model of a repetitive irritation test (RIT) to examine efficacy of barrier creams in a human test model. After 30 min of treatment with two different products, SLS was applied daily to the ventral forearm of healthy volunteers for 2 weeks. Cutaneous irritation was evaluated by a visual score, evaporimetry, laser-Doppler velocimetry, and colorimetry. The authors observed a significant suppression of irritancy with one of the tested creams. In a subsequent paper, Frosch and Kurte (3) reported on the RIT with a set of four standard irritants (10% SLS, 1% NaOH, 30% lactic acid, and undiluted TOL) using the midback as a larger area than the forearm. Thus, three products could be compared simultaneously to a nonpretreated control site. The irritant cutaneous reactions were quantified by erythema score, transepidermal water loss, blood flow volume, and stratum corneum hydration. The tested products demonstrated a specific profile of efficacy against the four irritants used. Using the RIT, our group showed that four products tested were very effective against 10% SLS and three products showed a partial protective effect against all ionic irritants (18). However, the necessity of a 2-week period of cumulative irritation is still discussed and a model with repeated irritation of the forearms has been evaluated for further testing (19,20). Grunewald et al. (21) developed a repetitive washing procedure with SLS on the forearms for 7 days, demonstrating protection of skin function for the creams tested. Zhai and Maibach (22) presented an in vivo method using cyanoacrylate strips of protected skin samples to measure the effectiveness of PCs against two dye indicator solutions: methylene blue in water and oil red O in ethanol, representative of model hydrophilic and lipophilic compounds. One formulation was protective against the permeation of methylene blue and oil red O while the other was protective against oil red O only.

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Recently, perfluoropolyethers were shown to have some benefit in the prevention of irritation due to hydrophilic and lipophilic substances (23). As petrolatum is effective against water-soluble and water-insoluble irritants, it was recommended as a standard substance against which PCs may be compared (24).

Although PCs have been shown to reduce ACD in sensitized individuals under experimental conditions (25,26), their use in the prevention of ACD has been disappointing under practical conditions. However, recent publications indicate a benefit for some PCs used as ''active'' creams in the prevention of ACD like nickel dermatitis or poison ivy/oak ACD (5,27–31).

APPLICATION

PCs should be applied before contact with irritants, including an application after every break; repeated application is suggested. It is clear that for PCs to be effective, they must be applied frequently and in adequate amounts to all skin areas that need protection. In particular, application should be made with attention to the interdigital spaces. In a recent study, a simple method of determining and quantifying how exactly self-application of a PC was performed at the workplace is described. Using a fluorescence technique, it was shown that application was often incomplete, especially in the dorsal aspects of the hands and wrists (32). These findings indicate that people miss certain areas. Individuals should apply the cream systematically by anatomical regions, ensuring that each region is adequately covered.

To improve daily application, instructive brochures may be given to workers but they are usually not very successful. It was shown that the fluorescence technique is also a useful tool in demonstrating the most common mistakes in conjunction with an instructive videotape (33).

ADVERSE EFFECTS AND CONTRAINDICATIONS

While some authors reported a satisfactory protective action of PCs, others found no protection from or even aggravation of ICD. A foamy skin protector was not convincing in a guinea pig model and impressed by its aggravating effect of the irritation due to NaOH (2). Also using a guinea pig model, Goh showed that treatment with PC increases skin irritation by cutting oil fluids (34). Bomann and Mellström showed that absorption of butanol through stripped skin treated with PC was higher than absorption through skin not so treated (35). Recently, a PC was shown to cause an amplification of inflammation by TOL (18) and the protective properties against systemic absorption of solvents are less than adequate (35–37).

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Besides less efficacy against irritants or even amplification of barrier damage the creams themselves can induce ICD or ACD (38,39). Preservatives, cream bases such as wool alcohols, emulsifiers, and fragrances are potential allergens. Preparations marketed as invisible glove may feign a seeming protection that causes workers at risk to be careless about contact to irritants. Additionally, it is of utmost importance to apply PCs on intact skin only. They are not intended to be used on diseased skin, due to the irritant properties of some formulations (7,40,41).

CONCLUSION

Current PCs are still not perfect. Much effort is necessary to develop products that will give more protection and less side effects. Efficacy and cosmetic acceptance are both important qualities of PCs to be used for protective success at the workplace but the knowledge how they are used correctly is a basic condition. It goes without saying that their benefit in the prevention of ICD and ACD has to be evaluated in reliable studies. Results of animal experiments may not be valid for humans, particularly when dealing with irritants, in view of their complex action mechanisms and the high interindividual variability in susceptibility of human skin (22). Regarding the various models of investigation, the validation of a sensitive, standardized, and widely accepted model proved by interlaboratory standardization or controlled clinical studies at the workplace seems to be necessary. Clearly, studies both under experimental conditions and in the workplace are needed before a rational recommendation can be made as to whether a product is safe and effective for skin protection. PCs cannot be of benefit in all cases—only against individual irritants. The data of in vitro and in vivo tests underline the importance of careful selection of PCs for specific workplaces. Choosing the wrong preparation may worsen the effect of an irritant. Based on the data presented, PCs should be used more critically due to the noxious substances used at the workplace and complete labeling of the ingredients should be given on the packages.

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