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Photodegradation of human hair: An SEM study

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Synopsis

This study uses field emission scanning electron microscopy (FESEM) to monitor the effects of UV irradiation on the physical nature of hair fibers. Long-term UV irradiation/humidification cycling causes thinning and fusion of the surface cuticle cell, as well as fusion of the cuticular sheath into a solid, rigid, and brittle unit. While intercellular cohesion within the cuticular sheath is high, possibly due to crosslinking of the proteins in the intra- and intercellular domains, the cells themselves are brittle. A newly observed fracture pattern of long-term UV-exposed fibers suggests fusion of the regions attacked most severely by UV light into one rigid and brittle mass, incapable of extension due to loss of all original elastic properties.

Unlike chemical oxidation, which results in partial dissolution (1 h H_2O_2) and then complete solubilization (4 h H_2O_2) of the melanin granules, photochemical oxidation produces entirely different results. Even after long-term UV irradiation/humidification (95% RH) cycling, the melanin granules appear physically intact. Loss of color does not occur as long as the melanin granules are intact.

The severity of photodegradation during UV irradiation/humidification cycling becomes apparent upon brief (seconds) contact of these fibers with alkaline hydrogen peroxide. Such contact results in instantaneous disintegration of the components within the cuticle cells. Formation of sac-like structures (Allwörden sacs) occurs due to osmotic pressure within seconds of exposure to alkaline hydrogen peroxide caused by photochemically degraded proteins within the surface cuticle cells. The cells swell until they burst and their contents drain, leaving behind cuticular membranes, which may detach or fuse to the fiber surface. UV irradiation has also severely photodegraded the melanin granules and preconditioned them for accelerated solubilization upon contact of the fibers with alkaline hydrogen peroxide.

The effects of both relative humidity and spectral energy distribution on the photochemical oxidation of the hair fiber are studied. Results obtained at various relative humidities in two different fading units, namely, the QUV Accelerated Weathering Tester and the Atlas Weather-Ometer® ("AW") are compared. Scale thinning and fusion observed during UV/humidification cycling are greatly reduced with exposure at low humidities without humidification cycles. Upon post-treatment with water, fibers irradiated at a constant 10% RH in the QUV show scale thinning and fusion similar to that of fibers exposed to UV/humidification cycling. This indicates that photodegradation occurs at low humidity as well. Fibers exposed at constant 20%, 50%, and 70% humidity in the "AW" show only moderate scale thinning, even after post-treatment with water. The total solar spectrum used in the "AW" apparently causes less severe photodegradation of the proteins than the UV light of the QUV.

INTRODUCTION

When exposed to sunlight, hair is known to undergo changes in morphological, chemical, and mechanical characteristics (1–4). The lower wavelength range of the UV com-

ponent of sunlight is known to be responsible for these changes. In recent years, UV radiation at lower wavelengths has significantly increased due to the deterioration of the ozone layer. While the earth's atmosphere filters out most radiation below 295 nm, depletion of the ozone layer, and therefore the reduction in the screening effect of the atmosphere, permits lower wavelength components to reach the earth's surface. These lower wavelength regions of the UV radiation received by the earth are the most energetic and therefore can cause severe photodegradation. The UV range of the sunlight can be divided into three wavelength regions, namely UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (<280 nm). UV-C radiation is totally filtered out by the atmosphere and is only experienced in space.

We have carried out extensive studies, investigating by various microscopic techniques the effects of photochemical oxidation on specific aspects of hair damage such as the appearance of the surface cuticle cell, intercellular cohesion, scale lifting during longitudinal extension, the integrity of the cuticular sheath, the physical nature of the melanin granules, characteristic fracture patterns, and loss in hair color. In other words, we have examined radiation-induced changes in the physical rather than chemical nature of the hair fiber. Hair fibers used in these studies were exposed to UV radiation/humidification cycling in a QUV Accelerated Weathering Tester. Various follow-up treatments of these long-term UV-irradiated fibers illustrate the extent of photodegradation inflicted upon the hair proteins.

More recently, we have also examined the effects of both the relative humidity and the spectral energy distribution in the radiation on the photochemical oxidation of the hair fiber. Comparisons were made between the results obtained at various relative humidities in two different fading units, namely, the QUV Accelerated Weathering Tester (290–400 nm; UV-A; λ_m = 340 nm) and the Atlas Weather-Ometer®, "AW" (Xenon solar simulator, 250–800 nm). Using FESEM, we characterized by different approaches the extent of photodamage inflicted upon the physical nature of the hair fiber in general and the cuticula in particular.

EXPERIMENTAL

MATERIALS

Hair type. Root sections of 14-in-long, brown European hair fibers from DeMeo Brothers were used. The major axis of these hair fibers ranged from 70 μm to 120 μm. Because of this variation in size, single hair fibers also varied in the depth of shade. Fibers with the larger major axis were more elliptical than fibers with a smaller major axis.

UV EXPOSURE CONDITIONS

QUV Accelerated Weathering Tester. The QUV simulates the sunlight in the range of 290–400 nm, with an irradiance maximum of the fluorescent bulb at 340 nm. The irradiance intensity factor has been chosen to be 1.35 compared to 1.0 for regular sunlight. The energy density at the 340-nm wavelength is kept constant at 0.97 W/m^2. The total energy density of the UV light in the wavelength range of 300–400 nm is 5.06 mW/cm^2.

Hair fibers were exposed to two sets of conditions in the QUV: (a) 0, 100, 300, 500, and

700 hours at alternating three-hour cycles of humidification (at 95% RH; 40°C) and UV irradiation (50°C; RH decreasing from 95% to 10% within 30 min, then remaining at 10% for 2.5 h); and (b) 0, 100, 200, and 300 hours continuous UV irradiation at constant 10% RH and 50°C.

Atlas Weather-Ometer®, ("AW"). The "AW" has a spectral distribution ranging from UV through the visible range, simulating sunlight. These exposure conditions correspond to an "average" 45° Miami summer sunlight. The energy density at the 340-nm wavelength is kept constant at 0.3 W/m^2. This means that at the specific wavelength of 340 nm, the energy density in the QUV (0.97 W/m^2) is approximately three times greater than that of the "AW" (0.3 W/m^2). However, in the wavelength range of 300–400 nm, the energy densities of the QUV (300–400 nm yield 5.06 mW/cm^2) and the "AW" (300–400 nm yield 4.46 mW/cm^2) are similar, although the distribution is quite different. The specific spectral distributions in the "AW" in the range of 250–300 nm yield 0.012 mW/cm^2, and 400–800 nm yield 36.80 mW/cm^2. The total energy density in the "AW" is 41.272 mW/cm^2. Specific exposure conditions of the hair fibers to UV/visible radiation in the "AW" were 0, 100, 200, and 300 hours at constant 20%, 50%, and 70% RH and 50°C.

Post-treatment of hair fibers in alkaline hydrogen peroxide. Hair fibers exposed to UV irradiation/humidification (at 95% RH) cycling were subjected to post-treatment in 6% alkaline hydrogen peroxide from seconds up to two hours. This was done to illustrate and characterize the severe extent of photodegradation inflicted upon the fiber during treatment in the QUV.

Post-treatment of samples irradiated at low and intermediate humidities with water. UV irradiation/humidification (at 95% RH) cycling lead to extensive "thinning" of the surface cuticle cells and "fusion" of the scale edges. Differentiation of the surface cuticle cell, so characteristic of the untreated hair fiber, eventually disappears at longer exposure times. We concluded that the presence of moisture in the highly swollen fiber during the humidification cycle is responsible for transporting the degraded, low-molecular-weight protein fragments out of the cuticle cell and possibly into the cortex, thereby causing the collapse and thinning of the surface cuticle cell.

Hair fibers irradiated at low humidities (from 10% to 70% RH) in the two fading units do not show this feature of thinning and fusion of the cuticle cell. Apparently, the lack of mobile water in the hair fiber and the lack of swelling of the fiber eliminate the transport of photodegraded fragments out of the cuticle cell. To mobilize the photodegraded proteins, hair fibers irradiated under low RH conditions in both the QUV and "AW" were subjected to a water post-treatment. The fibers were immersed for 60 minutes in lukewarm, deionized water, air-dried, and then examined longitudinally in the SEM. This was to establish whether post-treatment in warm water would result in thinning and fusion of the cuticle cell by diffusing photodegraded materials out of the cuticle cell, assuming that photo-oxidation of the hair proteins had occurred at all.

INVESTIGATIVE METHOD

Field emission scanning electron microscopy. Longitudinal and cross-sectional segments of untreated and UV-exposed fibers were mounted on double-sided tape and coated with approximately 90 Å of platinum. The hair fiber topography and interior were examined

for photo-oxidative damage in a Hitachi S-4500 digital field emission scanning electron microscope (FESEM).

RESULTS AND DISCUSSION

BACKGROUND

Photochemical degradation of hair affects both hair proteins and hair pigments and has been shown to occur primarily in the wavelength region of 254 to 400. In recent infrared spectroscopic studies of hair exposed to weathering, Dubief (3) shows a decrease in lysine and histidine, and a drastic increase in cysteic acid content in tip ends of hair compared to root ends. This suggests scission of the disulfide crosslinks during the photochemical oxidation process and formation of cysteic acid. This assumption is supported by Robbins and Bahl (5), who have shown by electron spectroscopy for chemical analysis (ESCA) that both UV-B (280–320 nm) and UV-A (320–400 nm) oxidize sulfur in hair. Oxidation was shown to occur primarily in the fiber periphery, namely, the cuticular sheath, producing a steep gradient to less oxidized hair in the fiber core. These results are not surprising, since disulfide is at its highest concentration in the A-layer and the exocuticle, where the highest level of photochemical oxidation occurs. The ESCA spectra suggest that high levels of cystine S-sulfonate and cysteic acid are formed in hair exposed to photochemical oxidation in the wavelength region of 254–500 nm.

Our research (6) strongly supports the results of Dubief (3) and Robbins and Bahl (5). Using UV microspectrophotometry, we have shown that high levels of photodegradation products are formed throughout the hair fiber cross section during long-term exposure in the 290–400 nm range. UV irradiation-induced photodegradation products of the hair proteins are revealed by an extension of the absorbance plateau and a shift in peaks from 290 nm in untreated hair to 315 nm in UV-exposed hair. There is also the development of an absorbance shoulder in the 330–340 nm range, well isolated from the absorbance of the bulk of the hair fiber (Figure 1). Formation of the photo-oxidized hair proteins can be traced and mapped, even quantified, by scanning across hair fiber cross sections at the wavelengths of the absorbance shoulder, (λ_m = 330 nm). These photodegradation products are especially pronounced in blond (unpigmented) Piedmont hair, with the highest level of photodegradation occurring in the cuticular region (A-layer and exocuticle), where cystine is at its highest concentration (Figure 2).

It is a generally accepted concept that the mechanism for photochemical oxidation of cystine follows the C-S scission pathway, whereby oxidative scission yields S-sulfonic acid that is finally degraded by light to cysteic acid (7,8). In contrast to the chemical oxidation that follows the S-S scission pathway and yields two moles of cysteic acid per mole of reacted disulfide, only one mole of cysteic acid is produced from each mole of reacted disulfide in the C-S scission pathway. The progressive oxidation pathways for the two types of scission are:

S-S Scission (chemical oxidation):

$$\text{R-S-S-R} \rightarrow \text{R-SO-S-R} \rightarrow \text{R-SO}_2\text{-S-R} \rightarrow \text{R-SO}_2\text{-SO-R} \rightarrow \text{R-SO}_2\text{-SO}_2\text{R} \rightarrow 2\text{R-SO}_3\text{H}$$

C-S Scission (photochemical oxidation):

$$\text{R-S-S-R} \rightarrow \text{R-S-S-OH} \rightarrow \text{R-S-SO}_2\text{H} \rightarrow \text{R-S-SO}_3\text{H} \rightarrow \text{R-SO}_3\text{H} + \text{H}_2\text{SO}_4 + \text{R-OH}$$

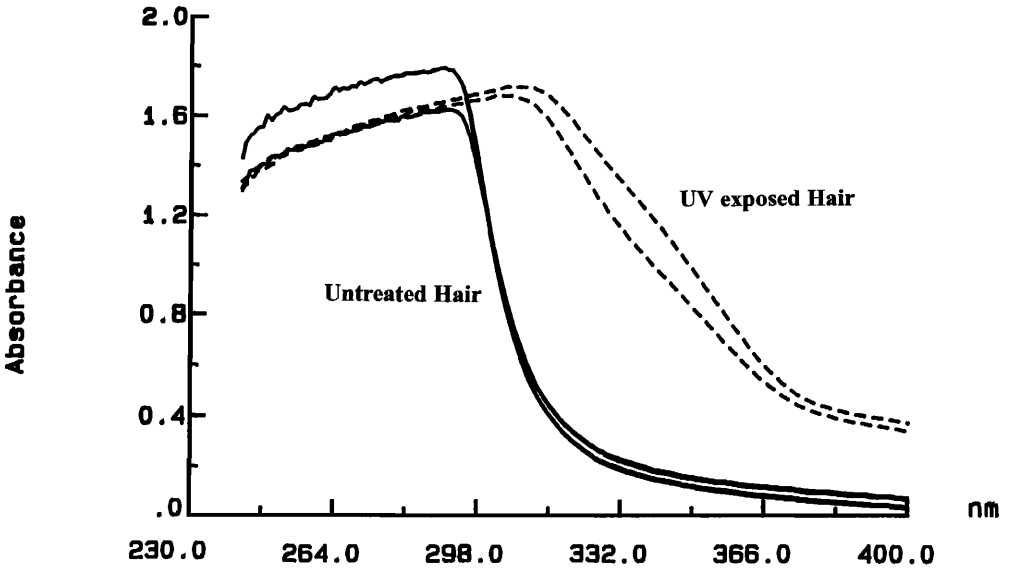

Figure 1. Absorbance spectra of untreated blond Piedmont hair obtained on longitudinally viewed hair fibers before and after UV exposure.

COLLAPSE AND FUSION OF THE SURFACE CUTICLE CELL

The conditions we have chosen in this study may seem somewhat extreme; however, we felt they were necessary to be able to learn about the behavior of the hair fiber and the changes of its physical nature brought about by severe UV irradiation. Observations in the FESEM had shown that nearly all hair fibers exposed to long-term alternating cycles of UV irradiation (290–400 nm; λ_{max} = 340 nm) and humidification (95% RH; 42°C) displayed an unusual topography. The hair fibers have a smooth topography similar to that of a man-made fiber, with little of the characteristic differentiation of the cuticle cell of undamaged hair. Figure 3a shows the typical appearance and thickness of a normal cuticle cell of unaltered, untreated hair fibers. However, after only 100 hours of UV irradiation and humidification in the QUV, a slight thinning of the surface cuticle cell and fusion at the scale edges are apparent (Figure 3b). After 300 hours of UV exposure, a more pronounced collapse of the surface cuticles and fusion of the scale edges to the underlying cuticle cells is seen (Figure 3c). 700 hours of UV exposure has produced hair fibers with a smooth topography, lacking clear differentiation of the cuticle cells (Figure 3d) because of extreme cuticular thinning and fusion to the underlying cuticle cells. The overall decrease in thickness of the surface cuticle cell as a function of exposure time to UV irradiation was obtained in the FESEM using built-in software for measuring distances on a nanometer scale in the axial, radial, and diagonal direction of fibers. Decreases in scale thickness were measured at the same high magnification and converted to the appropriate scale. The results are shown in Figure 4.

Our hypothesis explaining this photochemical damage phenomenon is as follows: this progressive thinning and fusion of the surface cuticle cell, under the conditions we used, is most likely due to photochemical degradation of the proteins in the surface cuticle cell

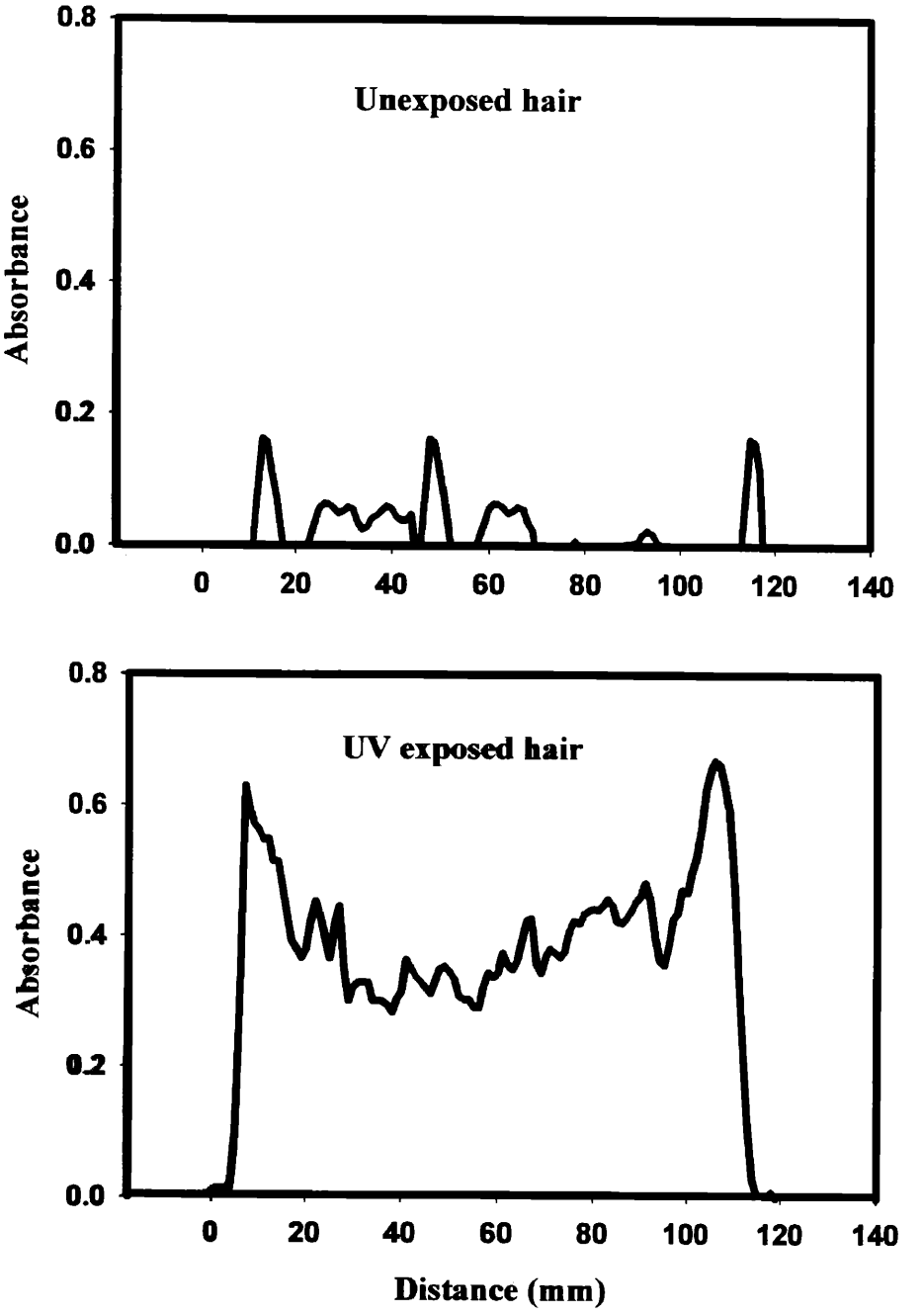

Figure 2. Formation of photodegradation products in the hair fiber cross section. Absorbance scans were made at 330 nm across hair fiber cross sections before (top) and after (bottom) UV exposure.

into soluble, low-molecular-weight peptides, which are capable of diffusing into lower cuticle cell layers (or out of the fiber) while in a highly swollen state during the humidification cycle at 95% RH.

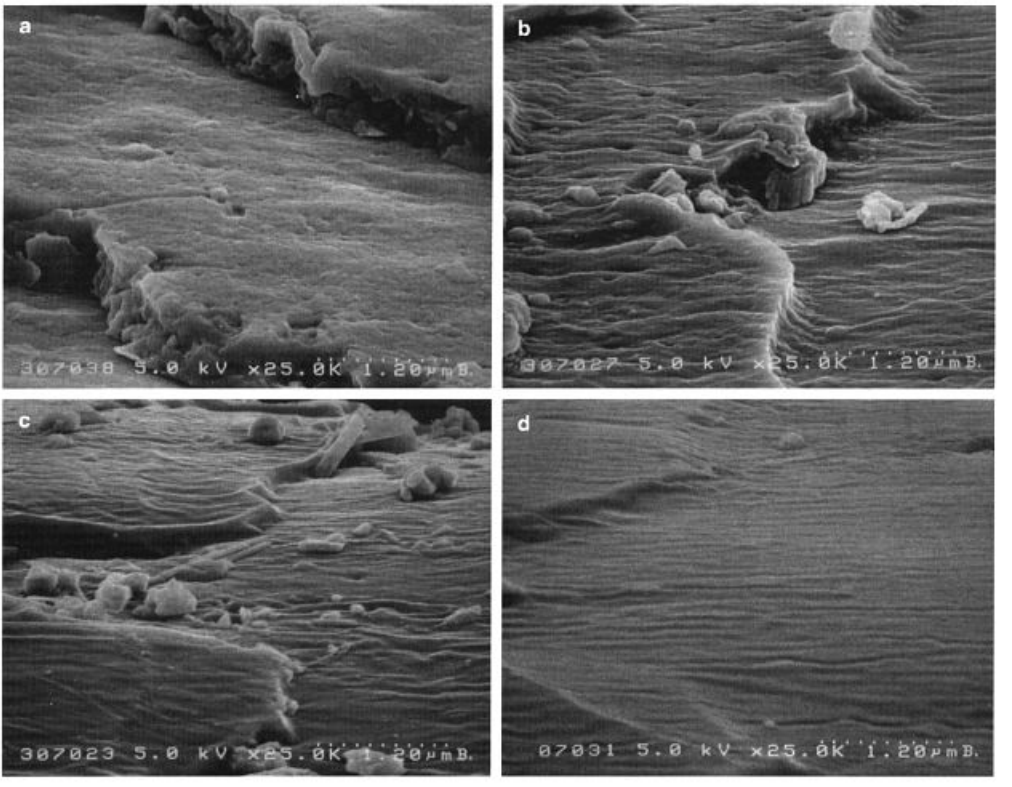

Figure 3. FESEM micrographs of hair fibers after exposure to UV radiation. (a) control, 0 h; (b) 100 h; (c) 300 h; and (d) 700 h UV exposure.

FUSION OF THE CUTICULAR SHEATH

We were also interested in exploring the changes in the physical nature of the cuticular sheath, which had shown such thinning and fusion of the surface cuticle cell during long-term UV exposure. Cross-sectional views of the freeze-fractured untreated hair fiber showed the individual layers of the cuticular sheath to be easily identifiable (Figure 5a1,a2). However, after 300 hours of UV exposure, fusion of all cuticular layers into a monolithic, brittle unit had occurred preferentially on the side of the hair fiber oriented towards the damaging rays of the light source (Figure 5b). In that region of the fiber, individual cuticle cells were no longer identifiable. Also, fibers exposed to 300 hours of UV light exhibit deep radial cracks, which are parallel to each other and absolutely perpendicular to the fiber axis (Figure 6), fracturing easily under minimal amounts of stress during bending or extension.

More drastic results were observed in hair fibers exposed for an even longer time (700 h) to UV light under similar conditions. Upon extension of hair fibers exposed for 700 hours to UV irradiation/humidification cycling, most fibers failed instantaneously at the start of extension. These fibers had become so brittle that they were incapable of extension due to loss of all original elastic properties. Upon failure, these hair fibers displayed an unusual fracture pattern. Figure 7 shows this fracture phenomenon, which occurs as the fibers snap apart. Fusion of the complete cuticula and possibly the outer

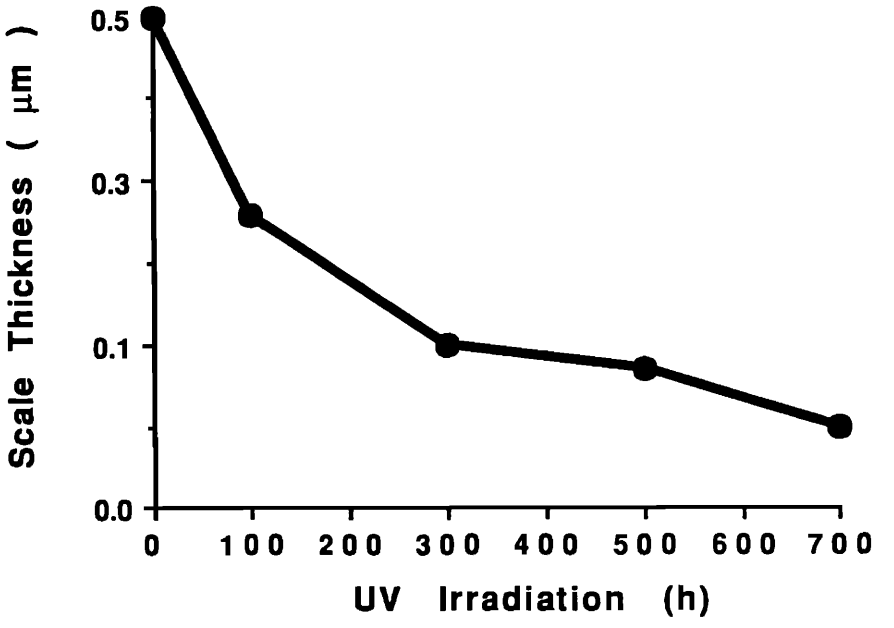

Figure 4. Progressive thinning and fusion of the surface cuticle cells as a function of exposure time to UV irradiation.

layer of the cortex into one rigid unit causes brittle failure circumferentially and displaces the stress of extension to those regions of the hair fiber that are still untouched by this progressive fusion. Multiple, successive fractures develop at individual sites along the cortical cell boundaries, then change direction and travel radially across individual cortical cells, and this pattern continues until it tapers off towards the core of the fiber, thereby creating what we call the "cathedral spire" fracture pattern (Figure 7a). The opposite, corresponding site of the "cathedral spire" fracture shows a hollow opening (Figure 7b), surrounded by a firmly fused wall. Higher magnification shows that this wall consists of a firmly fused cuticular sheath and possibly also the outer layer of cortical cells (Figure 7c,d). Some cuticular regions, preferentially those on the side of the fiber oriented towards the damaging light source, have become indistinguishable, rigid, and very brittle.

The "cathedral spire" fracture pattern clearly shows primary levels of photodegradation in the fiber periphery (cuticular sheath) and a drop-off to lesser levels of degradation in the fiber interior.

PHOTOCHEMICAL VERSUS CHEMICAL OXIDATION

Differences between chemical and photochemical oxidation of hair proteins and melanins have been widely discussed in the literature. Robbins (9) reports that both chemical and photochemical oxidation attack both the hair pigments and proteins, and within the proteins, primarily the amino acid cystine. Up to 25% of the disulfide bonds in human hair are degraded by "normal" bleaching, and 45% of the disulfide bonds may be broken

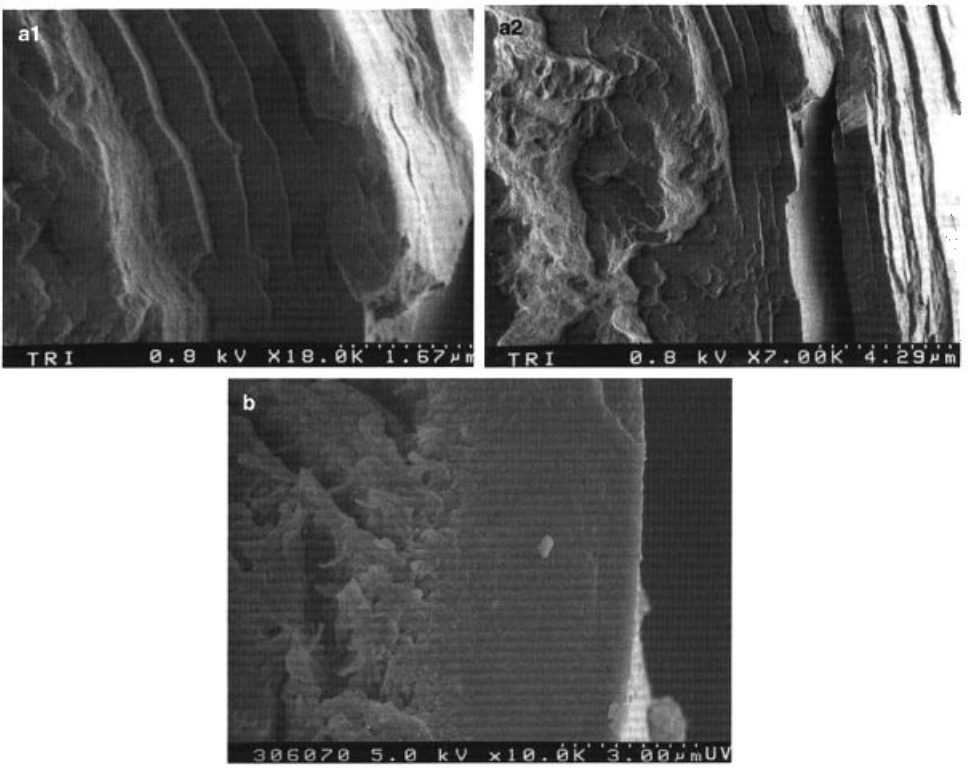

Figure 5. Representative cross-sectional views of the cuticular sheath of an untreated (a1,a2) and a 300-h UV-exposed (b) hair fiber.

during severe bleaching. However, chemical oxidation of the hair pigments occurs faster than the degradation of the proteins.

UV irradiation in the wavelength region between 254 and 400 nm has been shown (9) to degrade hair protein and pigment. Although both hair proteins and pigments absorb light in the UV/visible region, the longer wavelengths have been found to be less effective in causing photodamage. As with chemical oxidation, photodegradation of cystine is the most extensive phenomenon because of the reactivity of the disulfide bond.

Examination of cross sections of hair fibers exposed up to 700 hours UV-irradiation/humidification cycling showed, quite surprisingly, that the physical appearance of the melanin granules had not changed much, if at all. Figure 8a,b shows melanin granules in cross sections of untreated hair fibers. These granules are of various sizes, spherical or elliptical in shape, and appear to consist of smaller granular entities. The granules are housed in small cavities and appear to be connected to the cell walls by some intercellular material.

Even after long-term UV-irradiation/humidification cycling, the physical nature of the melanin granules appears intact and undegraded (Figure 9a,b). In unpublished work carried out at TRI, we have shown that hair fibers retained their dark brown color and that only a few had been faded slightly to a lighter brown color. Since the melanin granules retained their physical bulk and appearance, the melanin pigment was pro-

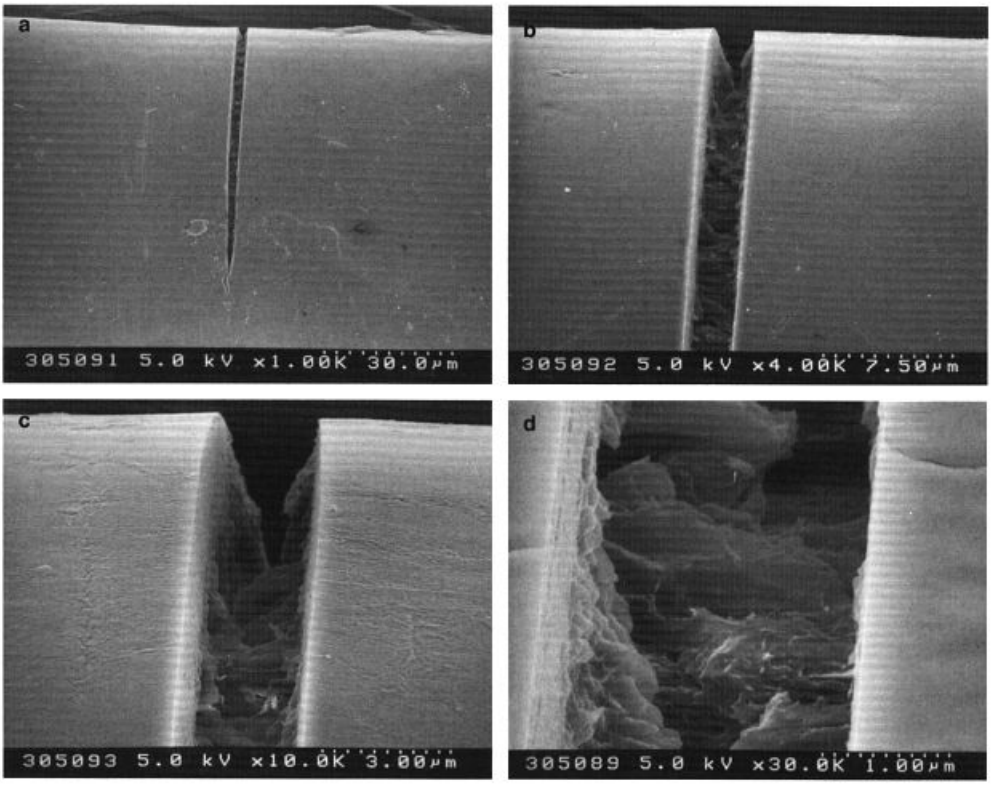

Figure 6. a–d: Typical longitudinal views of a long-term UV-exposed hair fiber, displaying severe radial cracking, a typical radiation-induced damage phenomenon.

tected from degradation, which, in turn, prevents a loss of hair color. The assumption was made that photochemical oxidation of the melanin chromophor would have to occur *in situ* if color loss was to occur at all.

This is quite in contrast to the swelling and dissolution of the melanin granules by chemical oxidation with hydrogen peroxide. After 1 hour of oxidation with 6% alkaline hydrogen peroxide, moderate disintegration of the melanin granules had occurred (Figure 10b,c). The subgranular structures, which make up the melanin granules, appear more pronounced. There are various stages of degradation, from mile to severe, due to partial solubilization of the melanoprotein. Solubilization appears to occur preferentially from the center of the granules (Figure 10c).

After the hair fibers were exposed for four hours to oxidation with 6% alkaline hydrogen peroxide, most of the granular cavities were empty (Figure 10d). The assumption was made that the majority of the cavities were empty because the granules were dissolved away by the peroxide. Progressive solubilization of the melanoproteins is the prerequisite for solubilization and/or dispersion of the melanin pigments. With the melanoprotein dissolved and diffused into the bulk of the hair fiber, the spread-out melanin pigment becomes the unrestricted target of bleaching or loss in color of the human hair. Wolfram and Hall (10) have observed similar behavior with melanin granules isolated from hair and exposed to peroxide bleaching.

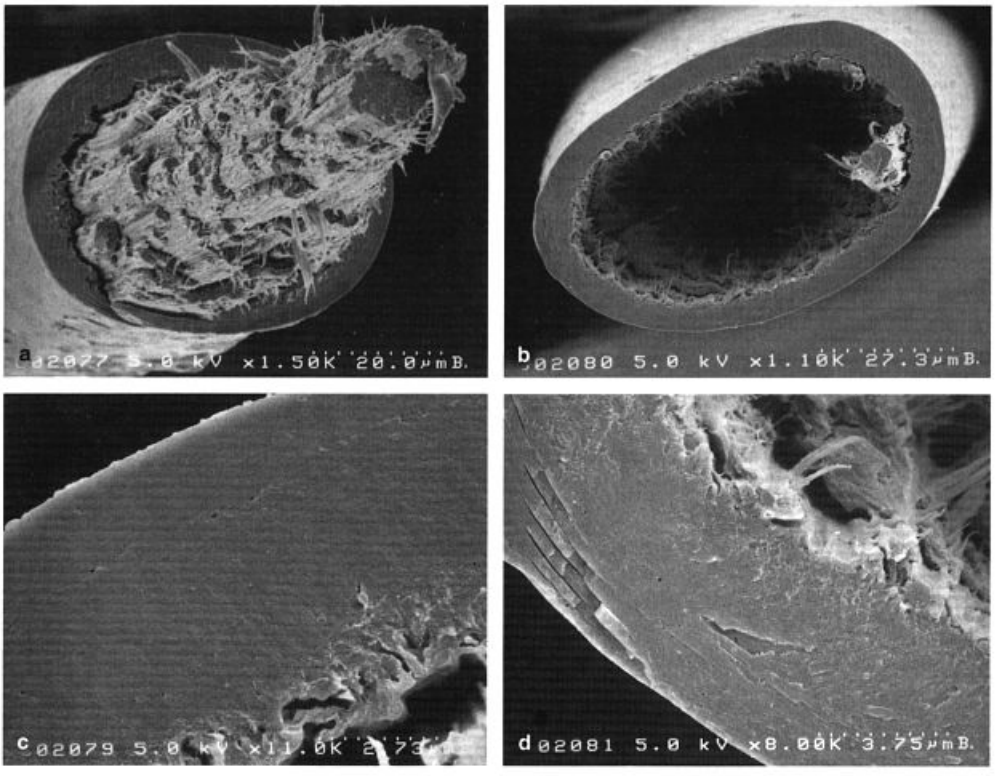

Figure 7. a–d: The "cathedral spire" fracture pattern seen upon extension of hair fibers exposed to 700 h of UV irradiation/humidification cycling.

Using microspectrophotometry, we have shown that under the selected conditions of progressive exposure to UV radiation/humidification cycling in the QUV, no or insignificant loss of color occurs after short exposure times and that only moderate lightening of the color is observed after 700 hours of UV exposure. The melanin granules remain intact as observed in the FESEM. Chemical oxidation, on the other hand, results in increased lightening of the hair color with increased exposure time, while solubilization of the melanin granules occurs.

Since microspectrophotometry is a non-destructive technique, the change in hair color is measured in transmitted light at the very same (previously marked) locations on the very same hair fibers under identical instrumental settings before and after the various exposure times to UV radiation. Increases in transmission intensity are directly proportional to loss in hair color. The same technique is used on hair fibers exposed to chemical oxidation. Figure 11 compares the decrease in hair color caused by chemical oxidation with 6% alkaline hydrogen peroxide versus progressive UV irradiation.

MANIFESTATION OF ADVANCED UV DEGRADATION OF HAIR PROTEINS (CUTICULA AND MELANIN GRANULES) DURING SUBSEQUENT HYDROGEN PEROXIDE TREATMENT

Hair fibers exposed to long-term (700 h) UV irradiation/humidification cycling, and

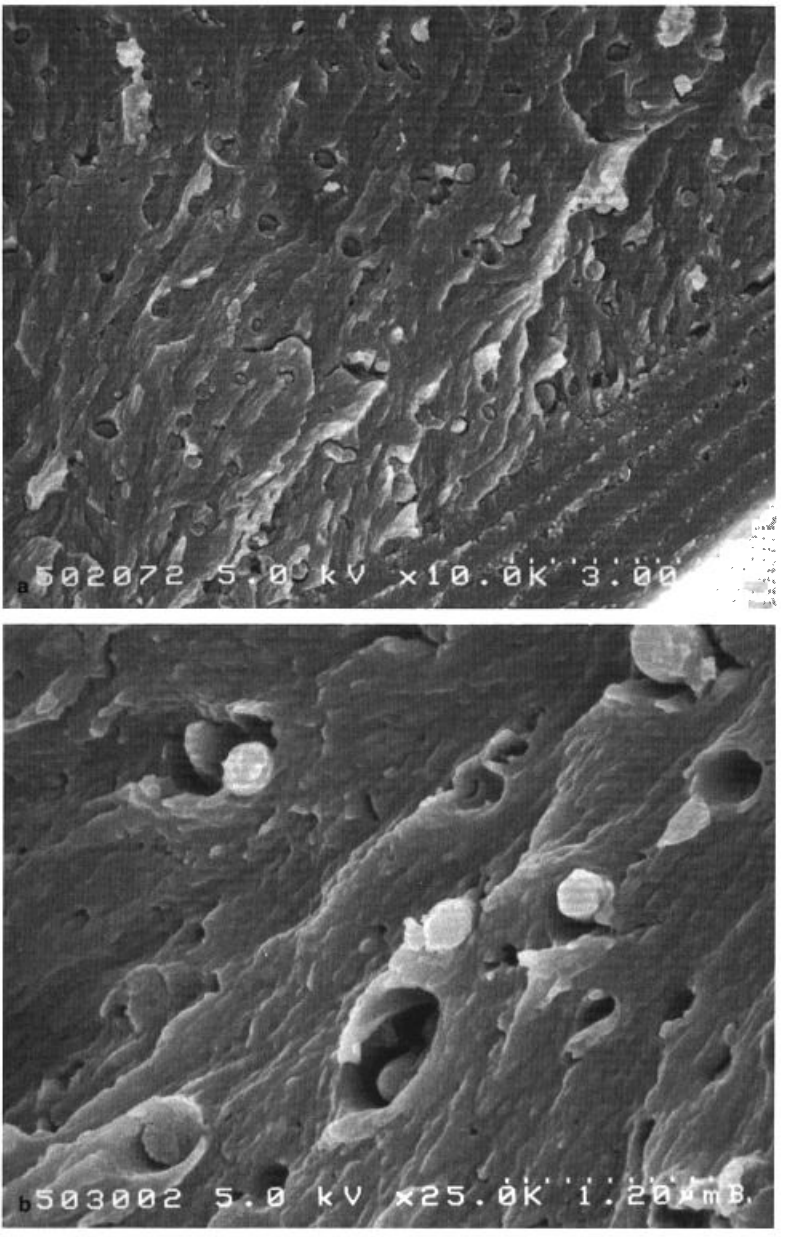

Figure 8. a,b: Melanin granules in cross sections of untreated hair fibers.

subsequently subjected to treatment with alkaline hydrogen peroxide, reveal significantly more damage to the hair fiber than had originally been suspected.

Short-term alkaline peroxide bleaching of long-term UV-irradiated hair fibers results in instantaneous disintegration and dissolution of elements of the cuticular cells, fusing the hair fibers firmly together. After drying, these fibers can no longer be freely separated, and forcefully pulling them apart results in their tearing and fibrillation (Figure 12a,b).

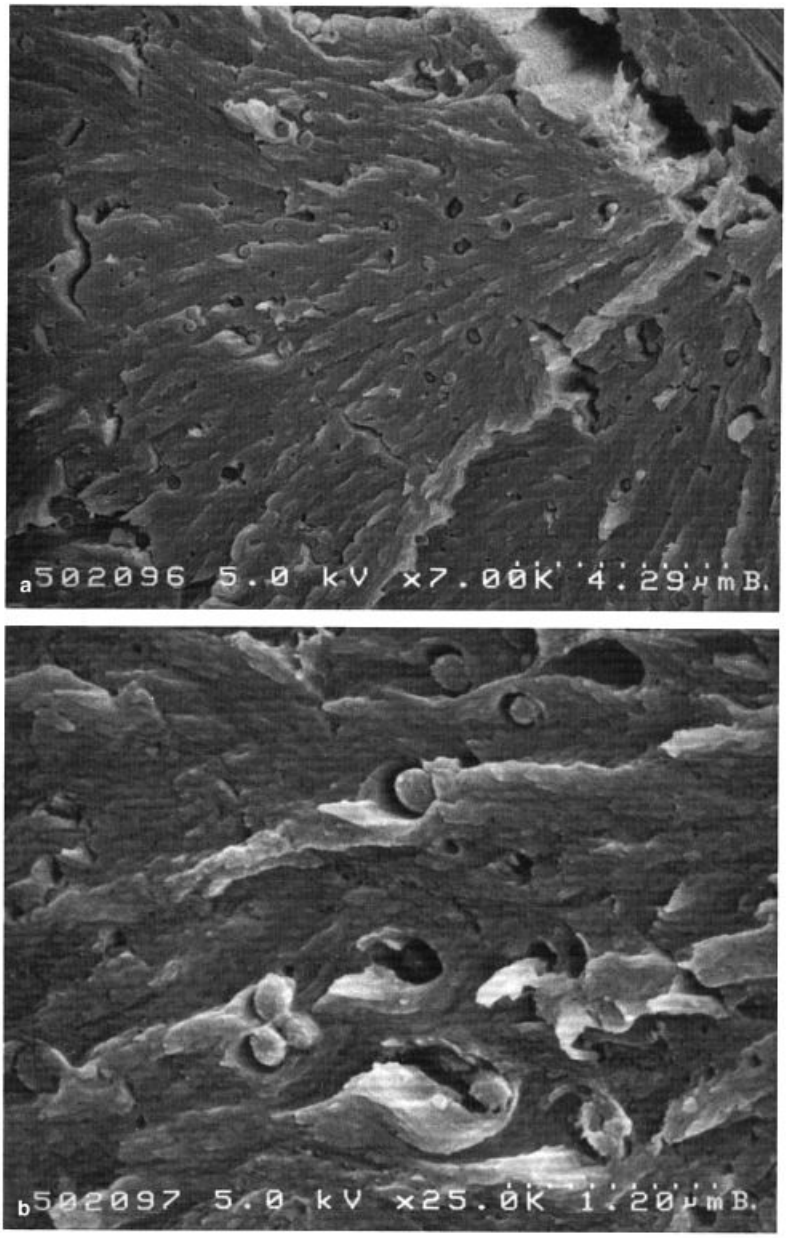

Figure 9. a,b: After long-term UV-irradiation/humidification cycling, the physical nature of the melanin granules appears intact.

The cross-sectional view of these fibers confirms the observations made during the longitudinal study. The cross sections show partially disintegrated, dissolved, and fused surface cuticle cells (Figure 12c,d). We also observed that some of the melanin granules appear to have been solubilized after merely 15 minutes of peroxide treatment. Such solubilization of the melanin granules in untreated hair occurs usually after several hours (4 h) of bleaching. Long-term UV exposure has, without doubt, severely degraded the

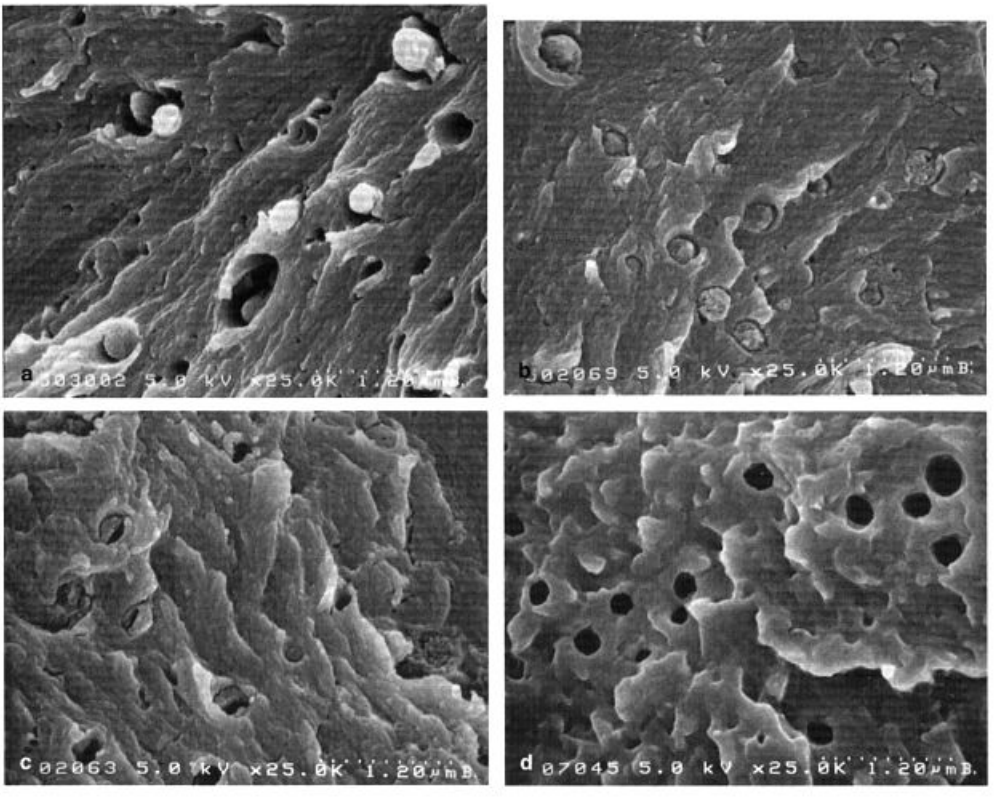

Figure 10. Intact (a) and partially (b,c) and completely (d) solubilized melanin granules before and after 1 h and 4 h of oxidation with 6% alkaline hydrogen peroxide, respectively.

hair fibers (cuticle cells and melanin granules) and preconditioned them further to accelerated disintegration.

After one and two hours of alkaline peroxide bleaching of long-term UV-irradiated hair fibers, the cuticula has completely disintegrated and dissolved into an unrecognizable, thin, film-like layer of cuticular membranes enveloping the outer cells of the cortex (Figure 13a,b). The disintegration, dissolution and fusion of the cuticle cells are so advanced that the individual cuticle cells are no longer identifiable. The original topography of the hair fiber no longer exists. Instead of surface cuticle cells with their characteristic scale-like structure, a bumpy surface covered by a thin film is displayed. This thin film may consist of cuticular remnants, the cell membranes, and the CMC, which exists between cuticula and cortex. The corresponding cross-sectional views of these fibers demonstrate the progressive damage inflicted upon the hair fiber by long-term UV exposure, (Figure 13c,d). The low magnification cross section (Figure 13c) shows the fused cuticula and merely a cortex containing empty cavities (Figure 13d) where once the melanin granules were housed. Besides severe oxidative damage to the cuticula, long-term UV irradiation has also photochemically degraded the melanin granules, and has preconditioned them for accelerated dissolution during subsequent short-term treatment with alkaline hydrogen peroxide.

These observations clearly show that long-term UV exposure causes severe chemical

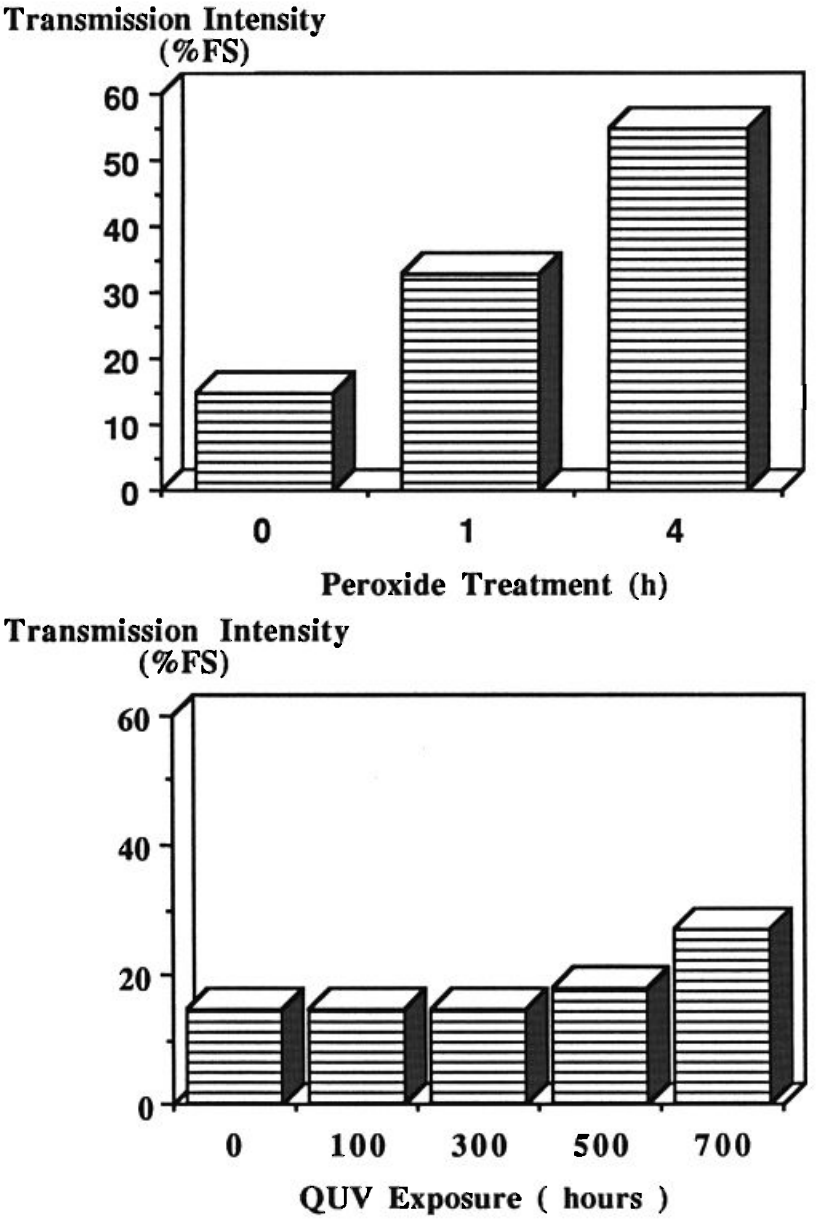

Figure 11. Comparison of loss in hair color due to chemical oxidation with 6% alkaline hydrogen peroxide (top) and UV irradiation (bottom).

degradation not only of the cuticula and melanin granules, but also of the hair fiber in general.

Optical microscopy. The unusual results demonstrated in the SEM study produced several questions about the drastic changes in the physical nature of the fiber's topography. Therefore, long-term UV-irradiated hair fibers were observed in the optical microscope during alkaline peroxide treatments, which provided some answers to questions about

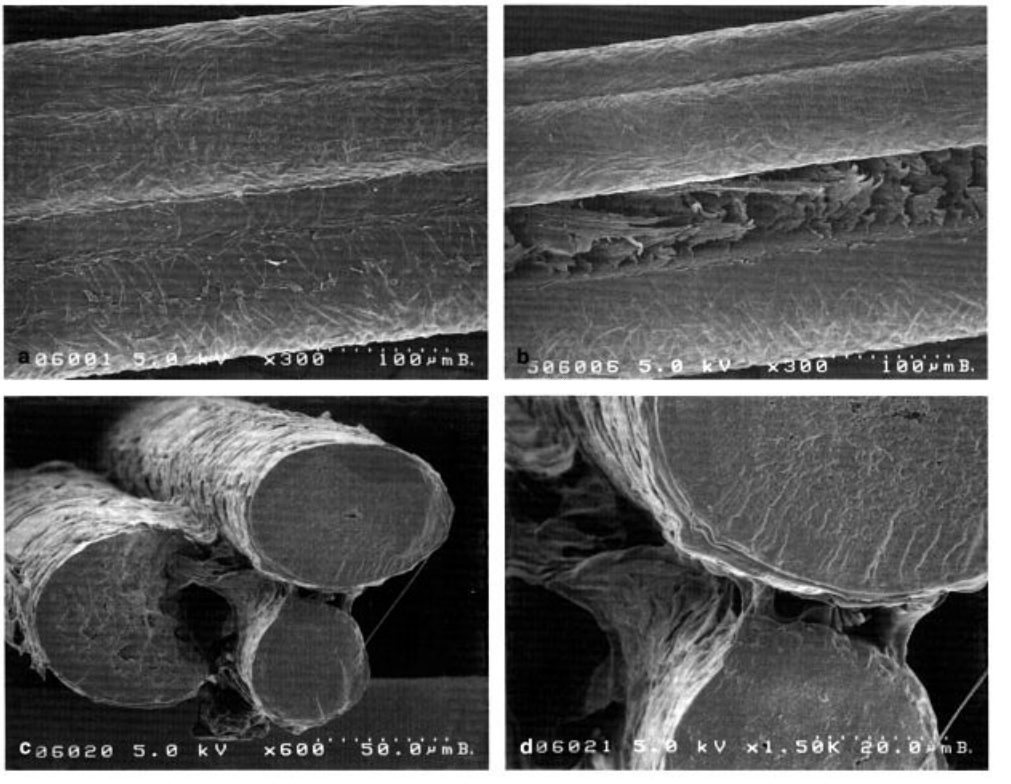

Figure 12. Longitudinal (a,b) and cross-sectional (c,d) views of long-term UV-irradiated hair fibers after subsequent 15-min treatment with 6% alkaline hydrogen peroxide.

this extreme cuticular disintegration during chemical oxidation of UV-exposed fibers. Long-term UV irradiation causes extensive oxidative degradation of the proteins of the cuticula as manifested in an Allwörden-like reaction during subsequent chemical oxidation of UV-exposed hair fibers (Figure 14a–f). After only seconds of alkaline peroxide treatment (Figure 14b,c), small bubbles develop along the hair surface. These small bubbles or half domes resemble the famous Allwörden sacs formed by the Allwörden reaction. Allwörden first detected these sacs at the surface of wool fibers during treatment with chlorine water. He thus also discovered the epicuticle surrounding the cuticle cell. The epicuticle is a semipermeable, partly proteinaceous membrane, ~25 A in thickness, which allows small molecules (water) to diffuse into the cell, rapidly swelling degraded, hydrophilic, low-molecular-weight peptide fractions. The highly hydrophilic molecules are not capable of diffusing out and remain trapped inside the cell. In Allwörden's reaction, the hydrophilic molecules continue to attract water from the outside solution, and thus these sacs are formed by the resulting osmotic pressure.

In our study concerning long-term UV exposure of human hair, the bubbles resembling Allwörden sacs are formed by diffusion of peroxide, ammonium hydroxide, and water through the epicuticle into the cuticle cell, where the already photolytically degraded proteins are now further degraded by the peroxide and swollen by water. The hydrophilic protein fractions are still too bulky to diffuse out through the semipermeable epicuticle. They remain trapped within the cuticle cell, attracting water and forming the sac-like

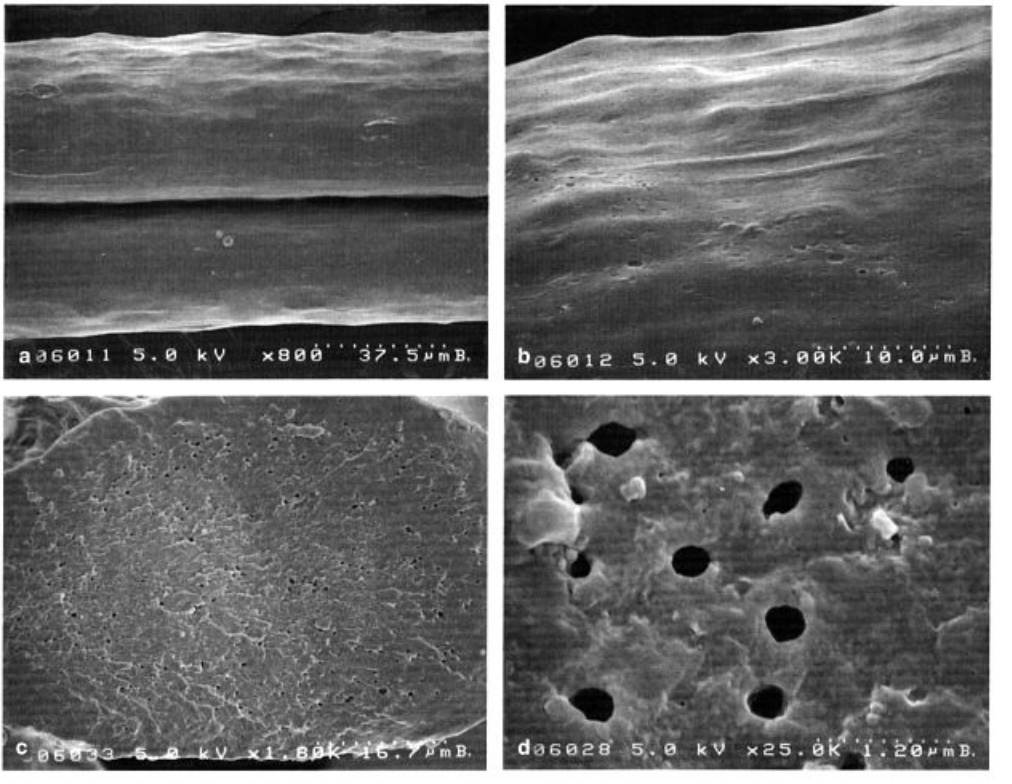

Figure 13. Longitudinal (a,b) and cross-sectional (c,d) views of long-term UV-irradiated hair fibers and subsequent 2-h treatment with 6% alkaline hydrogen peroxide.

structures (Figure 14b,c). Eventually, these sac-like structures (swollen cuticle cells) burst and their contents drain (Figure 14d), leaving behind membranes or shells of cuticle cells (Figure 14e), which may detach from the hair fiber and drift away (Figure 14f), or may remain attached and form a thin film-like layer enveloping the outer cortical cells, as has been shown in the SEM study.

EFFECTS OF RELATIVE HUMIDITY AND SPECTRAL ENERGY DISTRIBUTION ON PHOTOCHEMICAL OXIDATION

Moisture plays an important role in the photobleaching of human hair (3,11,12). Photochemical oxidation (also termed "weathering" or "photo-bleaching") damages hair at any given RH and increases with duration of exposure. The wet mechanical properties of photo-bleached hair fibers decrease with increasing extent of damage (11). Reduction in wet mechanical properties was found to be the most severe when hair is weathered at high or low RH. These fiber properties are least affected when hair is exposed at a RH of 30%.

We have also investigated the effects of both the relative humidity and the spectral energy distribution on photochemical oxidation of hair fibers. We compared the results obtained at various relative humidities in two different fading units, namely, the QUV accelerated weathering tester and the Atlas Weather-Ometer®.

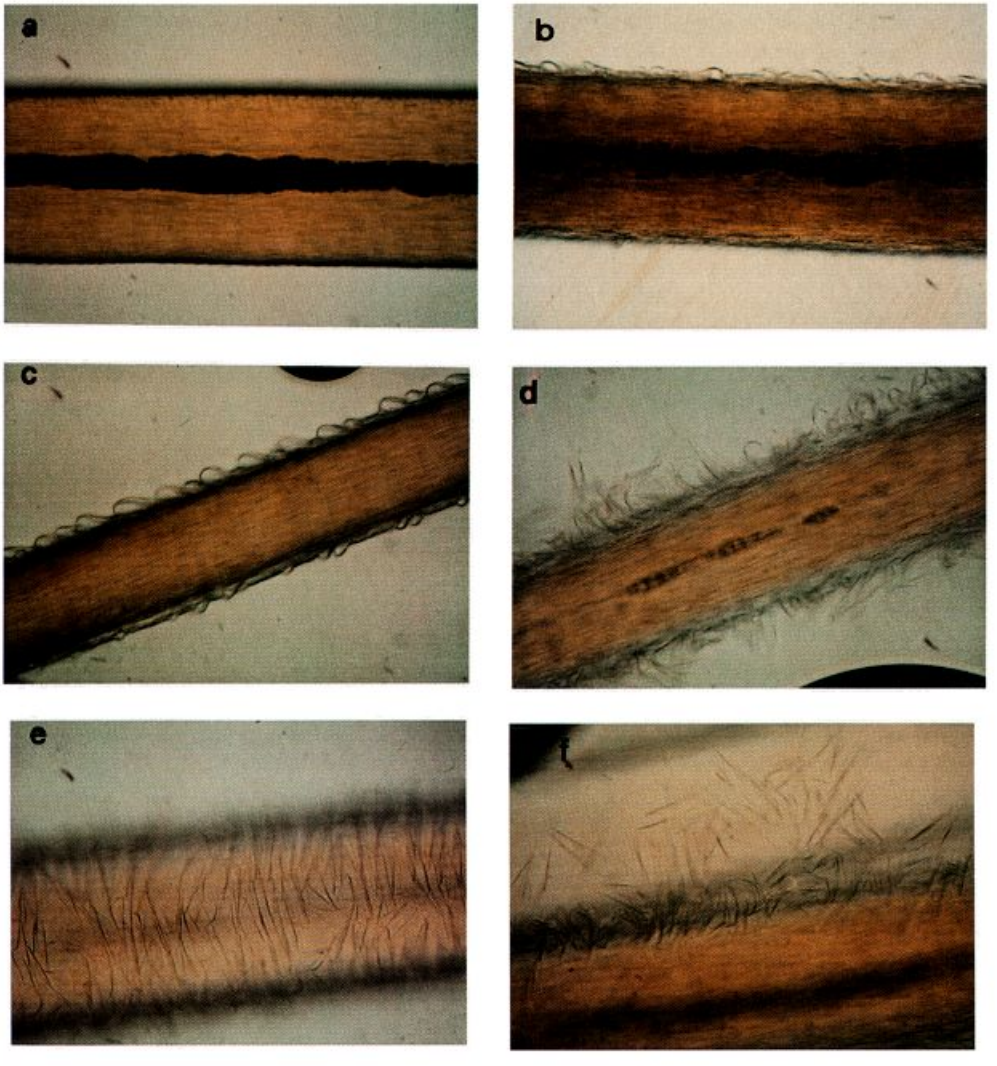

Figure 14. Longitudinal views of long-term UV-irradiated hair fibers and subsequent exposure for various times to 6% alkaline hydrogen peroxide: (a) no peroxide post-treatment, (b) 5 s, (c) 20 s, (d) 15 min, (e) 15 min (focused on dome of fiber), and (f) 60 min of peroxide post-treatment.

UV exposure in the QUV. Fibers exposed to continuous UV light at constant 10% humidity in the QUV do not show thinning and fusion of the cuticle cells (Figure 15a), as was observed in our earlier studies in which hair had been exposed to UV irradiation/humidification (at 95% RH) cycling in the QUV. However, upon subsequent immersion in water, collapse and fusion of the surface cuticle cell occurs (Figure 15b), with a severity similar to that observed in hair fibers exposed to irradiation/humidification cycling in the QUV. This indicates that photodegradation had occurred at a low concentration of water in the fiber structure, but that the degraded protein fractions (unlike in the swollen fiber), were not able to diffuse from the cuticle cell due to lack of adequate amounts of water to promote swelling.

This thinning and fusion phenomenon can be easily explained. At very low relative

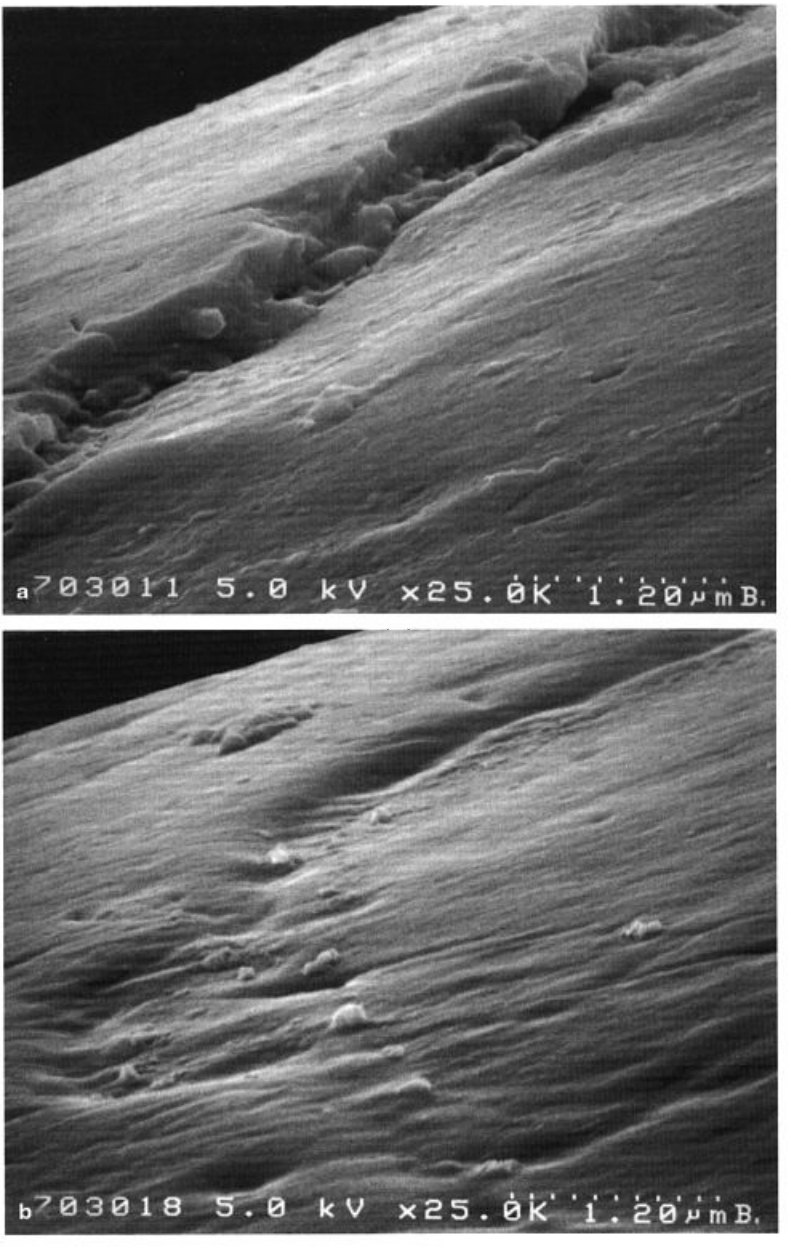

Figure 15. Longitudinal views of hair fibers exposed to (a) 300 h of continuous UV irradiation at constant 10% RH, 50°C, and (b) subsequent 1-h immersion in lukewarm deionized water.

humidities, below 25%, water molecules are principally bound to hydrophilic sites of the keratin fiber by hydrogen bonds, and the water is not mobile (13). As the humidity increases, additional water is sorbed, occupying the remaining adsorption sites associated with the protein. At high RH, when all the adsorption sites of the keratin are occupied, water molecules are no longer bound and are more mobile within the fiber. This facilitates diffusion of degraded, highly soluble, low-molecular-weight peptide fractions

from the cuticle cell. This accelerates degradation because now the radiation can attack new proteins rather than the already degraded proteins. Similar phenomena have been observed by Ratnapandian *et al.* (11).

Exposure to the solar spectrum in the "AW." Fibers exposed to continuous irradiation at constant 20%, 50%, and 70% relative humidity in the "AW" show the extent of photodegradation to be less than in hair fibers exposed in the QUV (to either continuous or cycling conditions), even after subsequent immersion in water. This was somewhat unexpected, especially since hair fibers were exposed to UV irradiation in the 250–400 nm range as well as to visible light at 400–800 nm wavelengths. While fibers exposed to continuous UV light at constant 10% RH in the QUV show extreme collapse and fusion of the cuticula after subsequent immersion in water, fibers exposed in the "AW" display more moderate cuticular collapse and fusion. Micrographs depict large pores in the scale faces (Figure 16b) and small pores or openings at the fused scale edges (Figure 16b,c), most likely areas where moisture and solubilized materials escaped during immersion in water. We concluded that the increased damage to fibers exposed in the QUV must be due to radiation emitted at λ_m of 340 nm in the UV-A range, since it is that wavelength at which the radiation energy in the QUV is approximately three times greater than that of the "AW."

There is no SEM evidence of melanin granule degradation, even in the presence of moisture for either wavelength range, (QUV or "AW"). After long-term exposure to irradiation in both fading units, the melanin granules appear physically intact, even though in some instances the melanin granules may have become slightly "grainier" at moderate levels of degradation. As long as the melanin granules are intact, loss of hair color does not occur.

Loss of hair color. Using microspectrophotometry in transmitted white light, we have measured loss in hair color as a function of exposure time to radiation in both the QUV and "AW." Since the transmission intensity was measured at the same location on the same hair fibers before and after the various exposure times, the true color loss was monitored, for example, for heavily or weakly pigmented hair fibers. Figure 17 shows the increases in transmission intensity (TI_t–TI_o), indicative of loss in color as a function of exposure time to radiation in both the QUV and "AW."

The following summarizes the effects of both the RH and the spectral nature of radiation on the photolysis of hair pigment:

(a) At low RH conditions (10%, 20%, and 50% RH), *independent of the spectral range,* there is a lack of loss in hair color in both the UV range of the QUV and the UV/visible range of the "AW." In other words, at low RH, neither UV nor UV/visible radiation appear to cause significant photodegradation of melanin in brown hair.

(b) Moderate loss of hair color is observed when long-term exposure to radiation in the UV/visible range is combined with a higher relative humidity (70% RH).

(c) Moderate loss of hair color is also observed upon long-term exposure to alternating three-hour cycles of humidification at 95% RH and UV radiation, during which the relative humidity is decreased within 30 minutes from 95% to a low of 10% RH, then remaining at the 10% RH for the duration of the radiation cycle.

From this microspectrophotometric study it can be concluded that the *high relative humidity is the primary contributing factor to accelerated loss in hair color,* since low RH

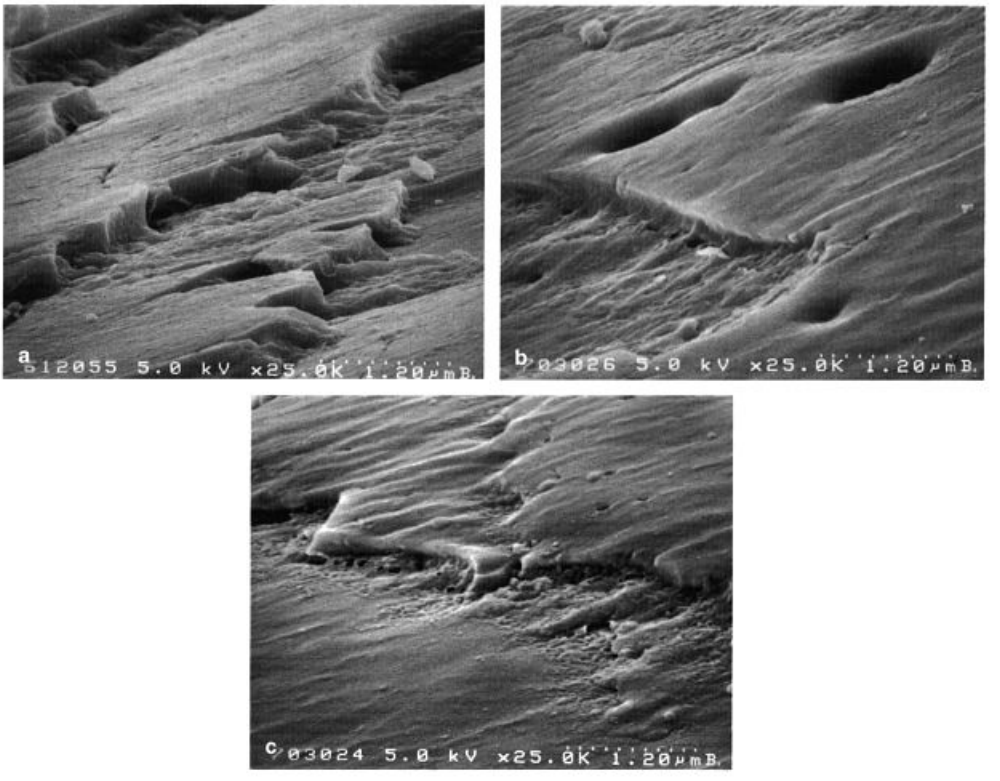

Figure 16. Longitudinal views of hair fibers exposed to (a) 300 h of continuous UV radiation at constant 20% RH and 50°C in the "AW," and (b,c) subsequent 1-h immersion in lukewarm deionized water.

conditions do not result in significant loss in hair color, even after long-term exposure. Increased exposure time and spectral range appear to be secondary factors. Also, there appears to be an initial period of great resistance to fading, since color loss does not occur during short exposure times under any of the conditions investigated. However, it is clearly shown that the combination of both the high RH conditions and the light in the visible range has the most damaging effects on hair color.

According to Wolfram and Albrecht (2), partial solubilization of the melanoproteins is a prerequisite for the decolorization by disintegration and dispersion of the melanin pigments within the hair fiber.

CONCLUSIONS

Long-term exposure to UV irradiation in the QUV results in photo-oxidative degradation of the hair proteins, especially of cystine, which occurs at its highest concentration in the cuticular domains where the degradation is most severe. UV irradiation (QUV) results in amino acid degradation as indicated by shifts in the UV absorbance spectrum to higher wavelengths and development of an absorbance shoulder.

Cycling UV irradiation at 10% RH with humidification at 95% RH (QUV) leads to extensive "thinning" of the surface cuticle cell and "fusion" of its scale edge to the

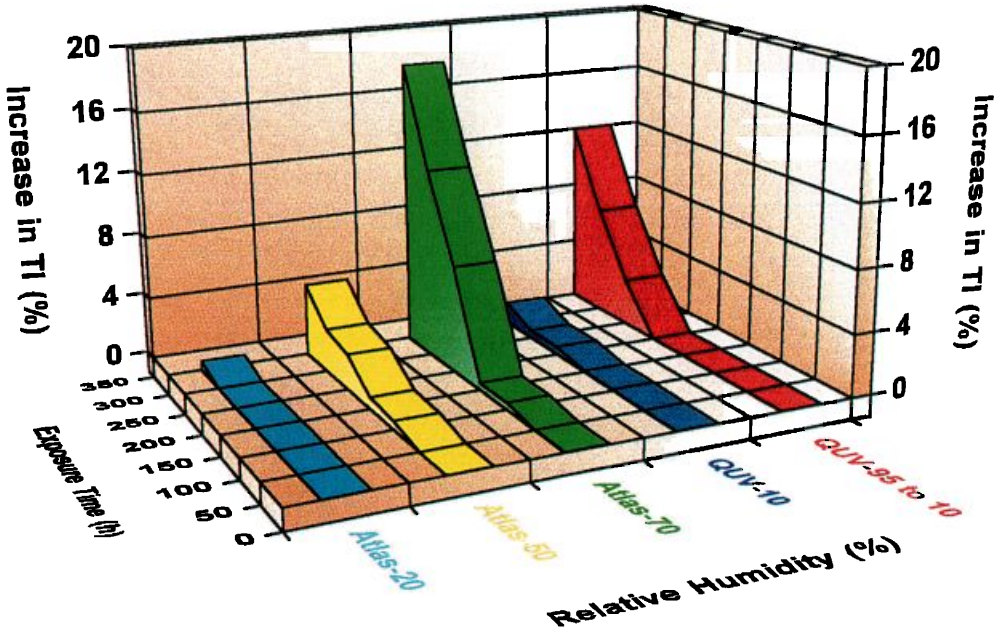

Figure 17. Loss in hair color due to progressive light exposure in the QUV and "AW," shown as increase in transmission intensity (%) of hair fibers due to light exposure.

underlying cuticle layer. The characteristic differentiation of the surface cuticle cell of untreated hair gradually disappears with increasing exposure. Besides thinning and fusion of the surface cuticle cell, there also occurs fusion of the complete cuticular sheath into a rigid, brittle unit. While intercellular cohesion within the cuticular sheath is high, possibly due to free radical-initiated crosslinking of the CMC, the cuticle cells themselves are brittle. A newly observed fracture pattern of long-term UV-exposed fibers suggests fusion of the regions attacked by UV light into one rigid and brittle mass, incapable of extension due to loss of all original elastic properties.

While chemical oxidation results in partial (1 h H_2O_2) and then complete solubilization (4 h H_2O_2) of the melanin granules, photochemical oxidation produces entirely different results. Even after long-term exposure to severe conditions of UV irradiation/humidification cycling, the melanin granules appear physically intact with little change in their physical nature. Loss of color does not occur as long as the melanin granules are intact.

Long-term UV irradiation/humidification cycling has severely damaged the hair proteins and preconditioned them for accelerated disintegration during subsequent treatments with alkaline hydrogen peroxide. Such contact results in rapid disintegration and dissolution of elements of the cuticle cells, resulting in formation of Allwörden sacs via osmosis. UV irradiation has also severely damaged the melanoproteins and preconditioned them for accelerated disintegration and solubilization by the alkaline hydrogen peroxide, as indicated by SEM micrographs of the empty cavities where once the melanin granules were housed.

While photochemical oxidation of hair occurs at all humidity conditions, it appears to be more pronounced and rapid at high relative humidity and/or humidification cycling.

Continuous UV irradiation at constant 10% RH (QUV), without alternating cycles of humidification, does not show the thinning and fusion of the surface cuticle cells. However, photochemical degradation has occurred, as subsequent immersion in water causes cuticle thinning and fusion of a magnitude similar to that experienced by fibers exposed to UV irradiation/humidification cycling (QUV).

Low and moderate RH conditions during exposure to unfiltered solar light in the "AW" also fail to produce cuticle thinning and fusion, and hair fibers still display the characteristic cuticle cell differentiation. Subsequent immersion in water shows only moderate cuticle thinning and fusion. This suggests that the solar spectrum of the "AW" causes less severe levels of photo-oxidative degradation of the proteins than the UV irradiation in the QUV.

High relative humidity is the primary contributing factor to accelerated loss in hair color. The combination of both the high RH conditions and the unfiltered solar light have the most damaging effects on hair color.

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