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Photochemical alterations in human hair. Part II: Analysis of melanin

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Synopsis

A brief investigation was carried out of changes in the melanin pigment that occur during the irradiation of hair with simulated UV-B, UV-A, visible light, infrared light, and total sunlight. After six weeks of irradiation, the pigments were isolated from hair samples and the changes in their physicocharacteristics were evaluated.

The melanin appears to be affected most by visible light, although the pigment of black and dark brown hair (predominantly eumelanin) is less changed than that of light-brown hair (a mixture of eu- and pheomelanins).

The weight losses of melanin correlate with photochemically induced alterations of the melanin polymer. IR spectroscopic investigations characterize the eumelanin by five peaks and the pheomelanin by six peaks in the range of 1800 to 500 cm^{-1}. The intensity of some of these peaks decreases, depending on the irradiation wavelength and on the origin of melanin pigments.

INTRODUCTION

Melanin constitutes the primary pigment present in the skin and hair of vertebrates. The particular color of the tissue is determined by the quantity and location of the pigment granules as well as by the chemical nature of the pigment (eu- or pheomelanin) (1–3). The black eumelanin is insoluble in acids and alkali and contains no sulfur (2). The reddish-brown pheomelanin is alkali-soluble and contains 8–12 % sulfur (2). The highly complex structures of these forms of melanin remain unresolved; enzymatic conversion of tyrosine or 5,6-dihydroxyindole (DHI) led to formation of pigments of random and unpredictable structure. According to Crippa (3) and Boudier (4), the eumelanin is a product of copolymerization of DHI and DHI-caboxylic acid. Cysteine is involved in the formation of pheomelanin by reaction with dopaquinone to form benzothiazine derivatives; the process intermediates polymerize to form the reddish-brown pigment (3).

While it is generally accepted that melanin has a photoprotective effect, the mechanism of its action has not been elucidated. It is likely that in this respect several modes

of action are operative, and it was shown that melanin has the ability to scavenge molecular oxygen as well as photochemically formed reactive oxygen species such as O_2- and H_2O_2 (5–8). The photooxidation of melanin is accompanied by bleaching (6,9,10), a process well known and practiced in the hair care field.

Eumelanin and pheomelanin respond in a different manner to irradiation. Chedekel *et al.* (11) found that under alkaline conditions the photolysis of pheomelanin leads to a hundredfold higher production on biologically active OH· radicals and O_2-anions than eumelanin. The highly damaging potential of these compounds led Menon *et al.* (12) to suggest that pheomelanin might be a poor protectant against sun-induced skin cancer. A somewhat different perspective on the reactivity of these pigments was provided by Wolfram and Albrecht (13), who examined the photo and chemical oxidation of pheo- and eumelanins in hair. They found pheomelanin to be more resistant to such oxidative attacks than eumelanin.

The purpose of this investigation was to obtain a better understanding of the effect of sunlight on the melanin and hair. Our goal was to provide some explanations on the molecular level. We were fully aware of the difficulties associated with the lack of precise knowledge regarding the complex structure of melanin as well as with the uncertainties of the effect that different segments of the sunlight spectrum may have on the photo-reactions of the pigment. We have thus limited our experiments to black and light-brown hair and carried out the irradiation study with UV-B, UV-A, visible light, and IR segments of the sunlight spectrum. The work was performed in a specially designed irradiation unit, which was described in detail in the first part of this study (14).

An enzymatic technique was used for the isolation of the melanin pigments from hair (15). The isolated pigment was further purified by removal of proteins and lipids. The melanin granules were weighed and characterized by means of IR spectroscopy. The results are discussed in relation to the nature of the pigments and irradiation parameters.

MATERIALS AND METHODS

HAIR SAMPLES

Untreated black and light-brown hair was obtained from Herzig Co. as 25-cm-long tresses of European origin.

PURIFICATION OF HAIR

The hair was extracted for 5 min with dichloromethane and for 30 min with diethylether to remove the sebum and traces of naphthalene, a conservation agent applied by the hair trader. Finally, the hair was washed with a non-ionic detergent, rinsed, and stored at ambient humidity.

IRRADIATION OF THE HAIR

The hair tresses were irradiated for a period of 6 weeks (1008 h) in individual compartments with UV-B, UV-A, visible light, IR, or global radiation at RH $>$ 70 %

(14). In each case approximately 10 g of hair were used, and the hair was spread parallel and rearranged daily to assure uniform exposure to irradiation.

COLOR DETERMINATION

The color of the nonirradiated hair was determined by means of Datacolor® 3890 equipment using the CIELAB-system. The color of the irradiated hair was determined visually by comparing the color quality with nonirradiated light-brown and black tresses.

ISOLATION OF MELANIN

An enzymatic technique to solubilize the keratin was used (15). One gram of hair was stirred into 30 ml of buffer of pH 6.7 containing 83.36 mg papain and 290 mg dithioerythritol (DTE). The reaction was carried out for 72 h at 50° C. The residue was separated by centrifugation (18,000 min^{-1} at 4° C), with twofold washing with water. The residue contains cell membrane complex and melanin granules.

SEPARATION OF THE CELL MEMBRANE COMPLEX AND PROTEINS FROM THE MELANIN

The isolated mixture containing the cell membrane complex and melanin granules was hydrolyzed for 120 h *in vacuo* at 110° C with 6 N HCl and 3% thioglycolic acid. The melanin residue was filtered using a 0.2-μm pore membrane.

DELIPIDIZATION OF MELANIN

The deproteinated melanin was delipidized by three 10-min washings with hexane/isopropanol/water (6:6:1 v/v/v). The residue was freeze-dried and kept at P_2O_5.

SEM EXAMINATIONS

The purity of melanin was evaluated by means of a scanning electron microscope (SEM).

IR SPECTROSCOPY

The spectroscopic examination was carried out using an FTIR 60SXR spectrometer (Nicolet). The melanin samples were examined either as KBr pellets or in a diamond cell. The spectral resolution was 4 cm^{-1} in the range from 400 to 4000 cm^{-1}.

RESULTS

IRRADIATION OF HAIR SAMPLES

The black and light-brown hair samples were irradiated for a period of 6 weeks (1008 h) with UV-B, UV-A, visible light, IR, or global radiation as previously described (14).

COLOR MEASUREMENTS

Before irradiation, color measurements were carried out using the CIELAB-system with notation of L^* for lightness and a^* and b^* for the red and yellow values, respectively (Table I).

As expected, the black hair shows a low L^* value ($L^* = 17.3$) and minimal a^* and b^*. The light brown hair is distinctly lighter ($L^* = 32.6$), more red ($a^* = 6.9$), and more yellow ($b^* = 14.4$). Therefore, we suggest a contribution of the reddish-brown pheomelanin pigments to its original color.

The photochemically induced color changes of black and light-brown hair were subjectively assessed and discussed in the first part of this publication (14). They are referred to in the present paper in Table II to complete the discussion about pigment alterations.

The data in Table II indicate that the light-brown hair photobleaches more extensively than the black hair, a fact already observed in real life by others (16). However, it has become possible for the first time to assign the extent of these color changes to specific segments of the sunlight. The results clearly show that the photobleaching of hair is dependent on the initial color of the hair as well as on the segment of the sunlight spectrum.

ISOLATION OF THE MELANIN PIGMENT

After enzymatic dissolution of the keratin, the insoluble residue consists of the cell membrane complex (CMC) and melanin granules. Separation of the proteins was accomplished by an acid hydrolysis. Thioglycolic acid was added to the hydrolysis mixture to prevent oxidation of the melanin. After 120 h of hydrolysis, followed by a delipidization and a micropore filtration, no fibrous contaminants derived from cells or the cell membranes were present in the granular samples. In that way SEM examinations attested to the satisfactory purity of the melanin obtained (Figure 1).

The pigment granules isolated from black hair show a typical rice-like appearance, with a length of 0.7–1.0 μm and a width of 0.4–0.5 μm (Figure 1a). On the other hand, the melanin granules from the light-brown hair (Figure 1b) are generally smaller and of irregular shape. Some are similar to those of black hair (0.65 μm × 0.3 μm). Most of them appear more circular and smaller (0.35 μm to 0.46 μm in length and 0.17 μm to 0.23 μm in width), which is characteristic of granules consisting of pheomelanin (1). This would suggest that the color of the light-brown hair is primarily due to the pheomelanin pigment and to a much smaller extent to eumelanin.

EFFECT OF IRRADIATION OF HAIR ON THE CONTENT OF MELANIN

It was found that 5–9 % of the melanin, including the CMC, could be isolated upon

Table I
Color Measurements of Nonirradiated Black and Light-Brown Human Hair

	L*	a*	b*
Black	17.3	1.8	1.6
Light-brown	32.6	6.9	14.4

Table II
Alterations in the Color of Light-Brown and Black Human Hair After 6 Weeks of Irradiation With Simulated Segments of the Sunlight Spectrum

Spectral range	Color alteration	
	Light-brown	Black
UV-B	Tips lighter	Unaltered
UV-A	Somewhat lighter	Unaltered
Visible light	Significantly lighter	Somewhat lighter
IR	Unaltered	Unaltered
Global	Lighter	Somewhat lighter

a,b

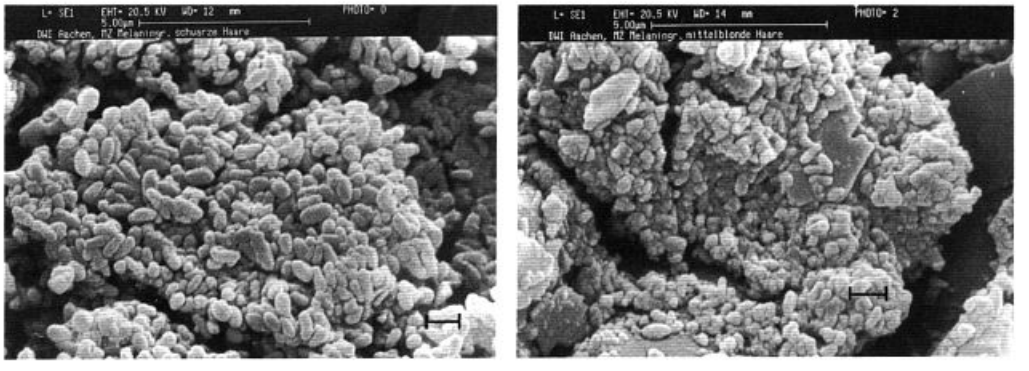

Figure 1. Scanning electron micrographs of isolated melanin granula from untreated human hair. The bars correspond to length of 1 μm. a: Black hair granula. b: Light-brown hair granula.

enzymatic removal of the keratin. After a 24-h hydrolysis of the melanin-CMC mixture, the yield of the remaining granules was determined to assess the effect of the irradiation spectrum on the melanin content (Figure 2). A subsequent long-term hydrolysis and delipidization led to a higher purity of the melanin sample for the IR investigations, but purity could not be determined quantitatively because of the small quantity of the available material and the inherent experimental error.

The yield of melanin granules derived from untreated black hair was 4.8 % (+/− 0.25 %) and from untreated light-brown hair 3.95 % (+/− 0.3 %) of the total hair weight (Figure 2).

While it has been found that the melanin granules of the black hair were only slightly affected by irradiation with visible and global light, the yields of the granules from the light-brown hair were significantly reduced by such irradiation and mostly by UV-A light (44 % less than the unirradiated sample). UV-B showed only limited effect, and the IR segment had no effect on the melanin yield. These results correlate with the results of the color measurements given in Table II.

IR SPECTROSCOPY OF THE MELANIN FROM INTACT AND IRRADIATED HAIR

It is assumed that the IR spectroscopic investigation of the melanin might provide some insight into changes occurring in the melanin polymers as the result of irradiation.

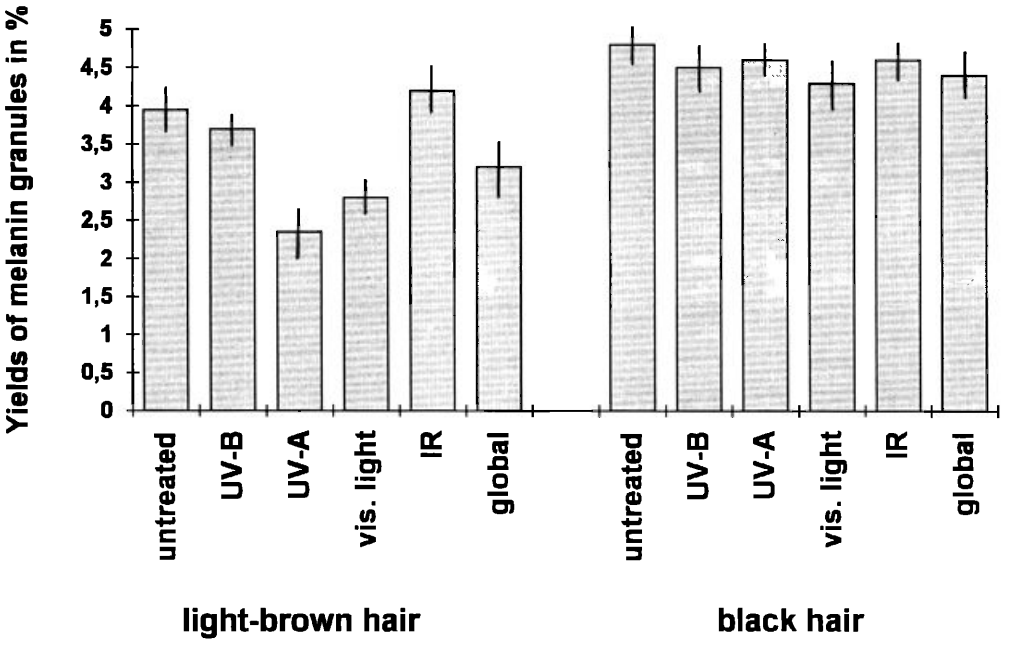

Figure 2. Melanin yields of intact and irradiated black and light-brown hair in relation to the different segments of the sunlight spectrum.

The transmission spectrum (diamond cell) of the melanin samples led to a more satisfactory result than the KBr pellets (better band resolution).

The IR transmission spectra of the melanin isolated from the black and light-brown hair are given in Figure 3. They conform well to the spectra of both synthetic (4) and natural melanin (12,17).

Spectral characteristics of eumelanin. The broad absorptions between 3600 and 2500 cm^{-1}

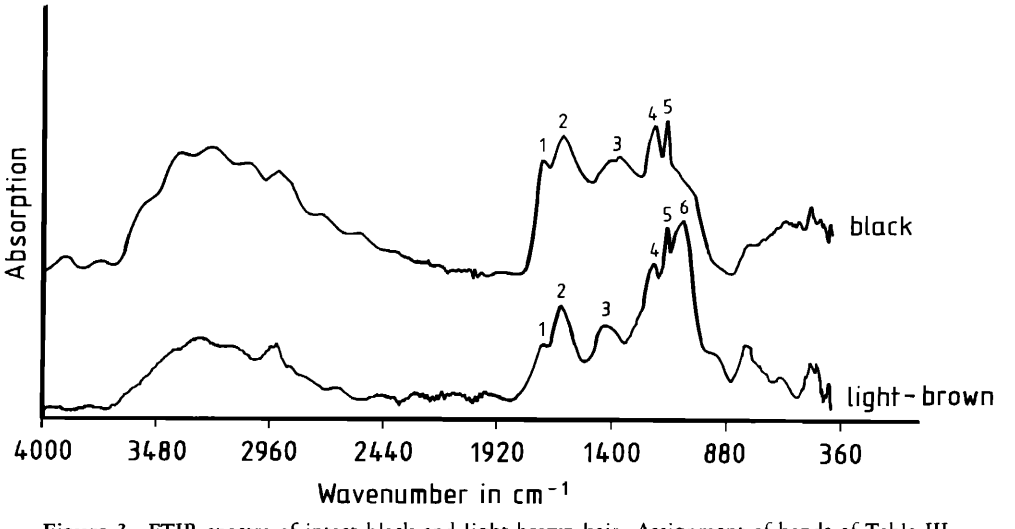

Figure 3. FTIR spectra of intact black and light-brown hair. Assignment of bands of Table III.

are caused by OH- and NH-vibrations. The peak at 1710 cm^{-1} is assigned to a carbonyl group (peak 1, Table III). At 1620–1630 cm^{-1}, ring vibration appears, which might be overlapped by an NH-vibration. Ring structures typical for melanin involve pyrrone, γ-thiopyrrone, indole, and quinones (peak 2) (12). The small peak 3 indicates the presence of heterocyclic moieties. At 1220 and 1157 cm^{-1} two peaks (peak 4 and 5) of similar height are observed; they were identified by Boudier (4) as characteristic vibrations of the dihydroxyindole structure in melanin.

Spectral characteristics of pheomelanin. In the IR spectrum of light-brown hair an additional vibration at 1082 cm^{-1} is found. It is possible that it arises from the presence of sulfur moieties in the pheomelanin that produce vibrations in the region 1070–1110 cm^{-1} (S-aromatic compounds, peak 6 in Table III) (18). This absorption is, apart from the SEM photographs of the melanin granules and color values of the nonirradiated hair (Table I), a third point of evidence indicating the difference in the overall structures of the pigments in the investigated hair samples.

IR spectra of melanin from irradiated hair. The comparison of spectra is made only for the region 1900–550 cm^{-1}, i.e., the absorption bands of melanin: C=O (peak 1), quinone (peak 2), heterocyclics (peak 3), dihydroxyindole (peaks 4, 5), and S-aromatic compounds (peak 6). The corresponding wave numbers are given in Table III.

Figures 4a and 4b provide the spectra of the melanin pigments derived from irradiated and nonirradiated black hair, respectively. The changes in absorption of the black hair melanin are evident only for hair that was irradiated with visible light (Figure 4b); peaks 4 and 5 are slightly visible. A similar but much smaller effect is seen for samples irradiated with UV-A light. It appears thus that visible light, and to much lesser extent UV-A, degrades the DHI structure of the melanin in black hair.

Figures 5a and 5b provide the spectra of melanin pigments derived from irradiated and nonirradiated light-brown hair, respectively. Comparison of the spectra of the irradiated light-brown hair pigments shows clearly the different effects of the segments of sunlight. UV-B, UV-A, visible light, IR, and global irradiation lead to a destruction of the absorption bands of C=O (peak 1) and quinone (peak 2) (Figures 5a and 5b). Visible light, UV-A, and global irradiation show a particularly strong effect. In addition,

Table III
IR Vibrations of the Melanin Polymer and the Corresponding Functional Groups

Peak number	Region (cm^{-1})	Description of peak	Functional groups
	3600–2500	Broad absorption	OH, NH
1[1]	1710	Shoulder	C=O, COOH (9)
2[1]	1628	Significant peak	quinone, pyrrole, thiopyrrole (9)
3[1]	1420	Small peak	heterocycles
4[1]	1220	Two peaks with similar height	dihydroxi-5,6-indol polymer (4)
5[1]	1157		
6[2]	1082	Significant peak	ring vibration with C-S-interaction (18)

[1] In black hair.
[2] Additionally in light-brown hair.

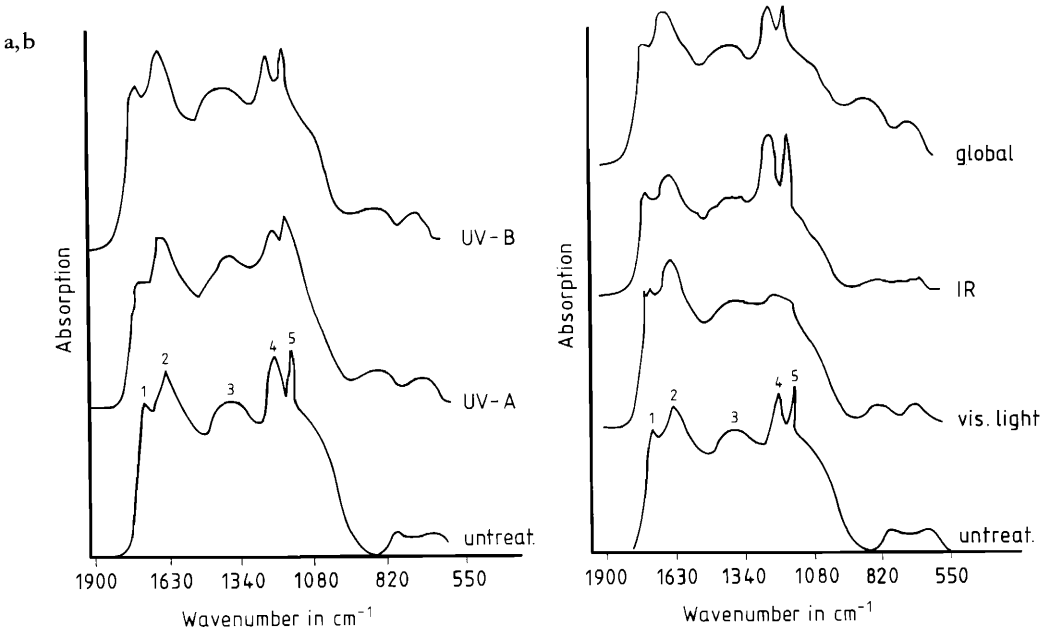

Figure 4. FTIR spectra of melanin from black hair after 6 weeks of irradiation with simulated sunlight (global), UV-B, UB-A, visible light, and IR irradiation in relation to nonirradiated hair. Assignment of bands of Table III. a: Irradiation with UV-A or UV-B. b: Irradiation with visible light, IR or global light.

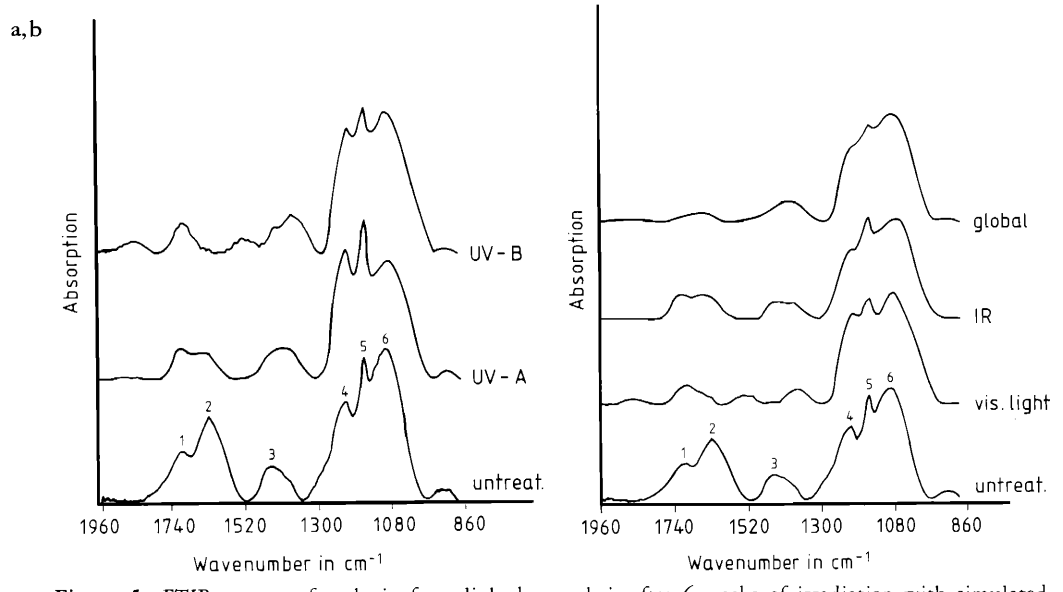

Figure 5. FTIR spectra of melanin from light-brown hair after 6 weeks of irradiation with simulated sunlight (global), UV-B, UB-A, visible light, and IR irradiation in relation to nonirradiated hair. Assignment of bands of Table III. a: Irradiation with UV-A or UV-B. b: Irradiation with visible light, IR, or global light.

visible light, IR, and global irradiation (Figure 5b) bring about a decrease of DHI-assigned bands (peaks 4 and 5). A gradual elimination of the absorption assigned to the sulfur moiety (peak 6) is evident upon exposure to UV-B and UV-A radiation (Figure 5a).

SUMMARY

This investigation provides qualitative and quantitative evidence regarding the different response to sunlight exposure of melanin from black and light-brown hair.

The photochemical degradation by visible light of eumelanin, the predominant pigment of black and light-brown hair, is, under the conditions of these experiments, relatively small and can be shown quantitatively (gravimetry) and qualitatively (color measurements, IR-spectroscopy).

On the other hand, a mixture of pheo- and eumelanins, the pigment of light-brown hair, appears to be affected by all segments of sunlight, particularly by UV-A and visible light. The irradiation brings about drastic degradation of the granules (gravimetry) and substantial changes in the melanin polymer (IR-spectroscopy, see below).

IR investigation of the melanin of irradiated light-brown hair suggests extensive destruction of the quinone system, which according to Crippa (3) is essential for the photoprotective effect of the melanin. In the case of eumelanin the dihydroxyindole moieties are prone to irradiation.

The higher photostability of the eumelanin, particularly the limited degradation of the quinone structure, suggests that eumelanin has a better photoprotective effect on hair than pheomelanin.

REFERENCES

(1) J. P. Cesarini, "Melanin and Color of Hair" (in German), in *Haar und Haarkrankheiten,* C. E. Orfanos, Ed., pp. 137–166 (1979).
(2) S. Ito and K. Fujita, Microanalysis of eumelanin and pheomelanin in hair and melanomas by chemical degradation and liquid chromatography, *Anal Biochem.,* 144, 527–536 (1985).
(3) R. Crippa, V. Horak, G. Prota, P. Svoronos, and L. Wolfram, "Chemistry of Melanins," in *The Alkaloids,* Vol. 36, A. Brossi, Ed. (Academic Press, 1989).
(4) V. Boudier, Etude du comportement photochimique d'eumelanines et du complexe melanine-keratine du cheveu par spectroscopies raman et infrarouge, Thesis, UBP Clermont-Ferrand II (1989).
(5) T. Sarna and R. C. Sealy, Photoinduced oxygen consumption in melanin systems. Action spectra and quantum yields for eumelanin and synthetic melanin, *Photochem. Photobiol.,* 39 (1), 69–74 (1984).
(6) T. Sarna, B. Pilas, E. J. Land, and T. G. Truscott, Interaction of radicals from water radiolysis with melanin, *Biochim. Biophys. Acta,* 883 (1), 162–168 (1986).
(7) W. Korytowski and T. Sarna, Bleaching of melanin pigments, *J. Biol. Chem.,* 265 (21), 12410–12416 (1990).
(8) N. S. Ranadive and I. A. Menon, Role of reactive oxygen species and free radicals from melanins in photoinduced cutaneous inflammations. *Pathol. Immunopathol. Res.,* 5 (2), 118–139 (1986).
(9) M. Tatsuda, M. Uemura, K. Torii, and M. Matsuoka, Studies on hair damage and demelanization by ultra violet light, *J. Soc. Cosmet. Chem. Japan.,* 21, 43–49 (1987).
(10) K. Jimbow, Y. Miyake, K. Homma, K. Yasuda, Y. Izumi, A. Tsutsumi, and S. Ito, Characterization of melanogenesis and morphogenesis of melanosomes by physicochemical properties of melanin and melanosomes in malignant melanoma, *Cancer Res.,* 44 (3), 1128–1134 (1984).

(11) M. R. Chedekal, P. R. Agin, and R. M. Sayre, Photochemistry of pheomelanin: Action spectrum for superoxide production, *Photochem. Photobiol.*, **31** (6), 553–555 (1980).
(12) A. Menon, S. Persad, H. F. Haberman, and C. J. Kurian, A comparative study of the physical and chemical properties of melanins isolated from human black and red hair. *J. Invest. Dermatol.*, **80** (3), 202–206 (1983).
(13) L. J. Wolfram and L. Albrecht, Chemical and photo-bleaching of brown and red hair, *J. Soc. Cosmet. Chem.*, **82,** 179 (1987).
(14) E. Hoting, M. Zimmermann, and S. Hilterhaus-Bong, Photochemical alterations in human hair. Part I: Artificial irradiation and investigations of hair proteins, *J. Soc. Cosmet. Chem.*, in press (1995).
(15) M. Giesen, Isolation and determination of melanin in human hair (in German), Thesis, RWTH Aachen (1981).
(16) G. Reese and N. Maak, Die Beständigkeit von Haarfarben unter dem Einfluß vom Sonne, Wasser, Salz, *Ärztl. Kosmetol.*, **12** (5), 373–379 (1982).
(17) K. Jimbow, O. Ishida, S. Ito, Y. Hori, C. J. Witkop, and R. A. King, Combined chemical and electron microscopic studies of pheomelanosomes in human red hair, *J. Invest. Dermatol.*, **81,** 506–511 (1983).
(18) G. Sokrates, *Infrared Characteristic Group Frequences* (1980).