Pheomelanin as well as Eumelanin Is Present in Human Epidermis

Anthony J. Thody, Elisabeth M. Higgins, Kazumasa Wakamatsu, Shosuke Ito, Susan A. Burchill, and Janet M. Marks

Department of Dermatology (AJT, EMH, SAB, JMM), University of Newcastle upon Tyne, U.K.; and School of Hygiene (KW, SI), Fujita Health University, Toyoake, Aichi, Japan

There are two types of melanin in mammals, the brownish black eumelanin and the reddish yellow pheomelanin. Eumelanin and pheomelanin are present in human hair and this study was carried out to see whether both pigments are also present in human epidermis. Samples of epidermis were obtained from suction blisters raised in the upper arm of 13 Caucasian subjects of skin types I, II, and III and analyzed for both eumelanin and pheomelanin using a procedure involving high-performance liquid chromatography. Eumelanin and pheomelanin were found in all epidermal samples and their relative proportions correlated well with those found in samples of hair taken from the same subjects. The lowest concentrations of eumelanin were found in subjects of skin type I, with higher levels in skin types II and III. The concentrations of pheomelanin were more variable and showed no relationship to skin type. Increases in the concentrations of both pigments occurred following PUVA therapy, but whereas the largest increases in eumelanin were seen in skin types II and III, the increases in pheomelanin showed little relationship to skin type. Unlike eumelanin, epidermal pheomelanin also showed little relationship to PUVA-induced tanning. The present findings could be particularly significant in view of recent suggestions that pheomelanin, rather than protecting the skin against UV radiation, may actually contribute to UV-induced skin damage. *J Invest Dermatol 97:340–344, 1991*

Sunlight, as well as tanning the skin, has many harmful effects and is now recognized as being a major risk factor in the etiology of skin cancers, including malignant melanoma [1–3]. Persons with pale skin that tans poorly and burns in response to ultraviolet radiation (UVR) are particularly at risk and this is thought to be related to the low concentrations of melanin in their skin [4].

Mammalian melanocytes produce two types of melanin, the brownish black eumelanin and the reddish yellow pheomelanin [5]. The initial steps in the synthesis of these two pigments are similar and under the control of the enzyme tyrosinase, which converts tyrosine to dopaquinone. Dopaquinone then undergoes a series of oxidations to give rise to eumelanin or alternatively, to pheomelanin, if cysteine or other related sulphydryl compounds are available.

Eumelanin and pheomelanin differ not only chemically but also in their physical properties. When UV irradiated, pheomelanin produces free radicals [6,7] and in greater quantities than those produced by eumelanin [8]. UV irradiation of pheomelanin also produces extensive cell lysis in Ehrlich ascites carcinoma cells and the release of histamine from mast cells, whereas neither effect is seen following irradiation of eumelanin [9].

In view of the above findings it has been suggested that pheomelanin, rather than protecting the skin against UV, may actually contribute to UV-induced skin damage [6,8]. This could explain why subjects with red hair and "Celtic type" skin are particularly susceptible to the damaging effects of sunlight. However, although pheomelanin is known to be a major pigment in red hair [10,11], much less is known of the relative proportions of pheomelanin and eumelanin in human epidermis. This study was therefore carried out to see whether pheomelanin, as well as eumelanin, is present in human epidermis, whether the relative proportions relate to those in the hair, and whether there is any relationship to skin type.

MATERIALS AND METHODS

Subjects Thirteen Caucasian patients of skin types I, II, and III were studied. All attended for PUVA, as treatment for either psoriasis or mycosis fungoides. One of the subjects with skin type I had red hair, while the remainder had hair ranging in color from fair to dark brown. All patients gave their informed consent.

Procedure Suction blisters were taken from the upper arm immediately before their first PUVA treatment and again after several weeks of treatment. On this second occasion the degree of tan was visually assessed and graded on a scale of 1–5. The blister tops were weighed and freeze-dried prior to assay. All patients received 8-methoxypsoralen (0.6 mg/kg body weight) and PUVA therapy as follows: skin type I, initial dose UVA 1.5 J/cm^2; II, initial dose UVA 2.5 J/cm^2; III, initial dose UVA 3.5 J/cm^2. The treatments were given 3 times weekly and the doses of UVA were increased by 0.5 J/cm^2 after every three treatments if the patients were not progressing and showed no signs of burning.

Hair samples were also obtained from the patients.

Measurement of Eumelanin and Pheomelanin The concentrations of eumelanin and pheomelanin in the blister tops and hair samples were measured by the method of Ito and Fujita [11]. The

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Reprint requests to: Dr. Anthony J. Thody, Department of Dermatology, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 4LP-UK.

Abbreviations:
AHP: aminohydroxyphenylalanine
HPLC: high-performance liquid chromatography
PTCA: pyrrole 2,3,5-tricarboxylic acid
PUVA: psoralen ultraviolet A therapy
UVR: ultraviolet radiation

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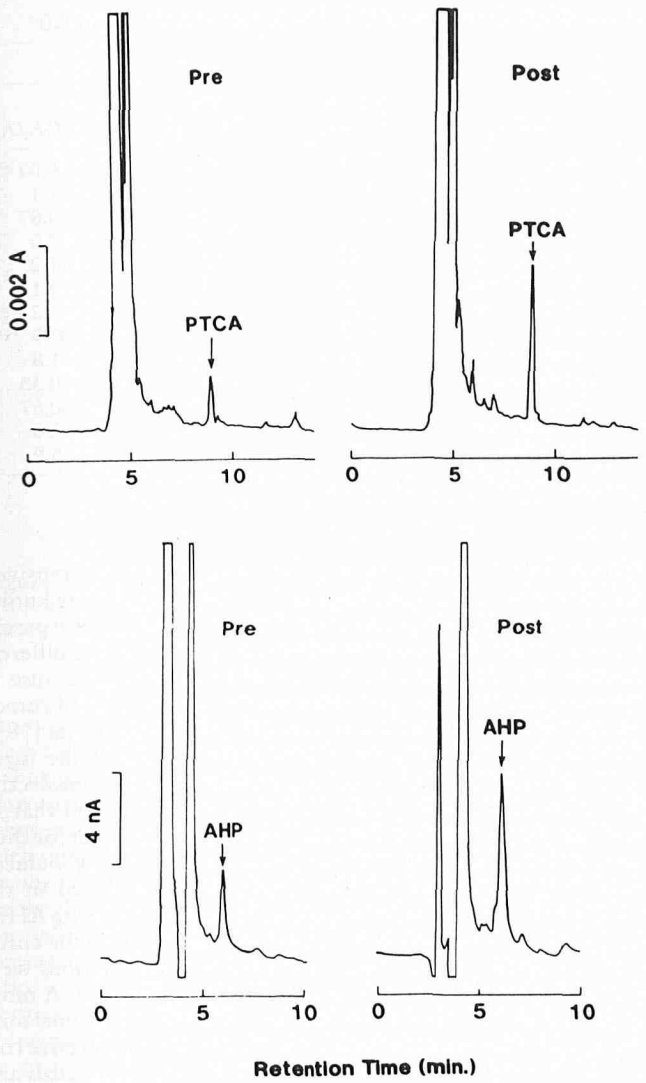

Figure 1. HPLC chromatograms after permanganate oxidation and hydriodic acid hydrolysis of epidermis samples from subject 6. *Upper left panel:* before PUVA therapy, PTCA value 0.52 ng/mg (samples 6.34 mg); *upper right panel:* after PUVA therapy, PTCA value 1.49 ng/mg (sample 6.60 mg); *lower left panel:* before PUVA therapy, AHP value 3.1 ng/mg (sample 4.58 mg); *lower right panel:* 5.1 ng/mg (sample 6.10 mg).

method involves the permanganate oxidation of eumelanin to pyrrole-2,3-5-tricarboxylic acid (PTCA) and the hydriodic acid hydrolysis of pheomelanin to aminohydroxyphenylalanine (AHP). For the measurement of eumelanin in the blister top, a sample weighing usually 2–5 mg was homogenized in 1 ml of 1 M H_2SO_4 and oxidized with 3% $KMnO_4$. The product PTCA was analyzed by high-performance liquid chromatography (HPLC) with ultraviolet detection. For the measurement of pheomelanin, a blister top was transferred to a screw-capped test tube and heated with 500 μl of 57% HI in the presence of H_3PO_2 at 130° for 20 h. The product AHP was analyzed by HPLC with electrochemical detection [11]. For the measurements of eumelanin and pheomelanin in the hair samples, approximately 30 mg of hair were homogenized in water and processed as described by Sponenberg et al [12]. A JASCO Model 880-PU HPLC pump was employed with a JASCO Model 875 UV spectrometric detector for the determination of PTCA. The absorbance for PTCA was monitored at the absorption maximum of 269 nm instead of 254 nm, which had previously been used [11], and this increased the sensitivity several fold. For the determination of AHP, a YANACO Model L-2000 liquid chromatograph was employed with a YANACO Model VMD-101 electrochemical detector coupled with EICOM EC-100 cell (graphite) electrode. The detector was set at +400 mV versus an Ag/AgCl reference electrode. The columns used were a Yanapak ODS-A (YANACO, particle size 7 μm, 25 cm × 4.6 mm) for PTCA and a Catecholpak (JASCO, 15 cm × 4.6 mm) for APH, both being maintained at 45°C. The mobile phases were (a) particle size 5 μm, 0.1 M potassium phosphate buffer, pH 2.1 : methanol, 92 : 8 (v/v) for PTCA and (b) 0.1 M sodium citrate buffer, pH 4.0, containing 1 mM sodium octanesulfonate and 0.1 mM Na_2EDTA : methanol, 98 : 2 (v/v) for AHP. The flow rate was 0.7 ml/min. The sample volume was 50 μl of the 200 μl solution for PTCA and 50 μl of 100 μl solution for AHP. These modifications increased the sensitivity of the method and when signal-to-noise ratio and background peaks were taken into account it was possible to detect approximately 0.1 and 0.2 ng/mg epidermis of PTCA and APH, respectively (Fig 1). Four replicate estimations of PTCA and APH in a sample of epidermis gave mean (± SEM) values of 1.24 ± 0.09 and 7.45 ± 0.58 ng/mg, respectively.

As previously reported, background levels were found to differ for different tissues [11]. Tissues such as liver, kidney, and brain contained less than 0.1 ng PTCA/mg and around 1.0 ng or less AHP/mg and these compare with the values of 0.2 and 0.4 ng/mg of PTCA and AHP, respectively, found previously in albino mouse skin [13]. Background levels in hair tend to be higher and in samples obtained from a subject with tyrosinase-negative albinism were 0.4 ng/mg PTCA and 13 ng/mg AHP. These are similar to those reported previously for other mammalian species [12].

Contents of PTCA and AHP of 1 ng roughly correspond to a eumelanin content of 50 ng and a pheomelanin content of 5 ng, respectively.

RESULTS

Epidermal Eumelanin and Pheomelanin Eumelanin and pheomelanin were found in all epidermal samples (Fig 1). The overall mean concentrations (± SEM) prior to PUVA were 0.79 ± 0.18 ng PTCA and 4.85 ± 0.73 ng AHP/mg wet weight epidermis, respectively (Table I). Following PUVA both eumelanin and pheomelanin increased in concentration to mean levels of 2.22 ± 0.33 ng PTCA and 7.58 ± 0.99 ng AHP/mg epidermis, respectively, which were significantly greater ($p < 0.0001$ and $p < 0.002$, paired t test) than the corresponding pre-PUVA values.

Relationship Between Hair and Epidermal Eumelanin and Pheomelanin Hair samples were obtained from the same patients and analyzed for eumelanin and pheomelanin. Eumelanin and pheomelanin were found in all samples of hair and the concentrations, which ranged from 10–102 ng PTCA/mg hair and 13–713 ng AHP/mg hair, respectively, correlated well with hair color, as previously reported [11] (Table I). Thus, dark brown hair contained relatively more PTCA and light brown/red hair higher proportions of AHP. These differences were reflected in the PTCA/AHP ratios, which ranged from 0.014 for red hair to 5.9 for dark brown hair (Table I).

The highest concentrations of epidermal eumelanin were found in those subjects with dark hair. Epidermal pheomelanin concentrations were more variable and although a high level was found in the subject with red hair they generally related less well to hair color. Nevertheless, there was good correlation between epidermal and hair PTCA/AHP ratios (Fig 2).

Relationship to Skin Type Although there was much overlap between skin types, type I skin contained the lowest mean epidermal eumelanin concentration and higher levels were found in skin types II and III (Fig 3). This was true both before and after PUVA. PUVA increased epidermal eumelanin concentrations in the three skin types, but it was only in skin types II and III that the increases were significant (Fig 3).

In contrast to eumelanin, epidermal pheomelanin concentrations showed little relationship to skin type both before and after PUVA

Table I. Eumelanin (PTCA) and Pheomelanin (AHP) Concentrations (ng/mg wet weight) in Epidermis and Human Hair[a]

		Epidermis				Hair		
Subject	Skin Type	PTCA (ng/mg)	AHP (ng/mg)	PTCA/AHP	Color	PTCA (ng/mg)	AHP (ng/mg)	PTCA/AHP
1	I	0.68	9.0	0.08	Red	10	713	0.014
2		0.22	2.0	0.10	Brown	33	29	1.1
3		0.47	3.4	0.15	Fair	40	46	0.87
4	II	0.70	5.4	0.13	Brown	48	24	2.0
5		1.49	9.4	0.16	Dark brown	48	39	1.2
6		0.52	3.1	0.16	Light brown	40	13	3.1
7		0.49	1.9	0.26	Dark brown	77	24	3.2
8		0.61	1.7	0.35	Brown	77	20	3.9
9	III	0.42	6.3	0.06	Dark brown	25	14	1.8
10		0.26	3.3	0.09	Dark brown	35	105	0.33
11		0.48	5.1	0.10	Brown	40	60	0.67
12		1.41	4.9	0.29	Dark brown	102	36	2.8
13		2.49	7.6	0.33	Dark brown	88	15	5.9

[a] Contents of PTCA and AHP of 1 ng correspond roughly to eumelanin and pheomelanin contents of 50 ng and 5 ng, respectively.

(Fig 4). Moreover, the increases in epidermal pheomelanin concentrations following PUVA also showed no apparent relationship to skin type (Fig 4).

Relationship to Tanning Response PUVA produced a tanning response in all subjects, even in those that were classified as being skin type I. The degree of tan correlated well with the increases in epidermal eumelanin concentration that occurred in response to PUVA (Fig 5). However, no such relationship was found with epidermal pheomelanin (Fig 6).

DISCUSSION

It has long been recognized that pheomelanin is a major pigment in red hair [5,10,11] and this was confirmed in the present study. However, the major finding of this study is that pheomelanin, as well as eumelanin, is also present in human epidermis. Although pheomelanin has been reported to be present in the skin of patients with dysplastic nevi [14], this is, as far as we are aware, the first report of pheomelanin in normal epidermis of subjects unaffected by a pigmentary disorder.

The present results confirm previous reports that hair concentrations of eumelanin and pheomelanin exceed those in the skin [11,12,14]. The reason for this is not clear but can in part be explained by the lower water content of the hair. There was nevertheless a good correlation between the eumelanin-to-pheomelanin ratios in the epidermis and hair and this suggests that common factors may determine the relative proportions of the two pigments in the hair follicle and epidermis. Hair color is dependent on the action and interaction of many genes. This has been extensively studied in mice, where over 50 gene loci and 130 alleles are known to be involved [15–17]. However, little is known of the precise mechanisms that determine the relative proportions of the different melanins. The enzyme tyrosinase could be important because its expression has been shown to differ during the synthesis of eumelanin and pheomelanin in mouse hair follicular melanocytes [18].

Our findings are particularly significant in view of the many suggestions that pheomelanin, rather than having a photoprotective role, is actually phototoxic [6–9]. It has been suspected that its abundance in the skin of red-haired subjects could account for their increased susceptibility to sunburn reactions and other UV-induced skin damage. Only one red-haired subject was included in the present study and his epidermal pheomelanin content of 9 ng AHP/mg epidermis was higher than all but one other value in the entire study. However, on the whole pheomelanin concentrations were quite variable and showed little relationship to skin type. A more obvious relationship was found between skin type and eumelanin, the pigment that is thought to have the greater photoprotective role in the skin. Although there was variation between individuals and considerable overlap between skin types, eumelanin was at its lowest levels in skin type I with increasing amounts in skin types II and III. The synthesis of tyrosinase, the enzyme that catalyses the initial steps in the melanin pathway, has a similar distribution within the different skin types [19,20]. These findings are consistent with the

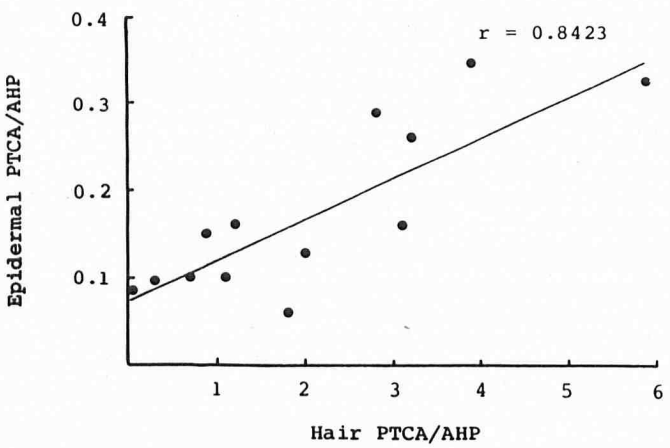

Figure 2. Relationship between epidermal and hair PTCA/AHP.

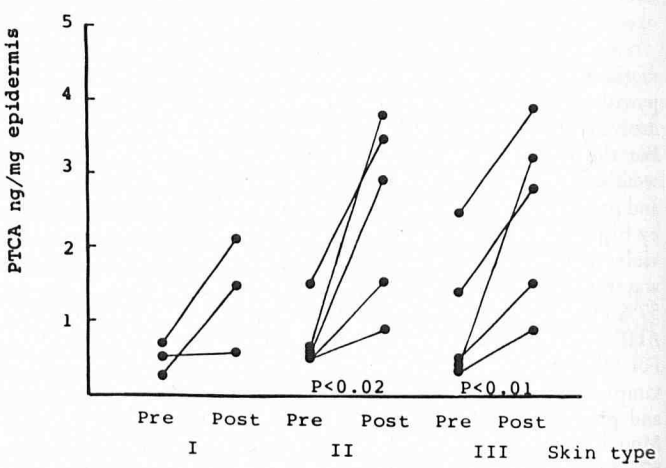

Figure 3. PTCA concentrations in the epidermis of different skin types before and after PUVA therapy. The paired t test was used to test for significance of differences between pre- and post-PUVA values.

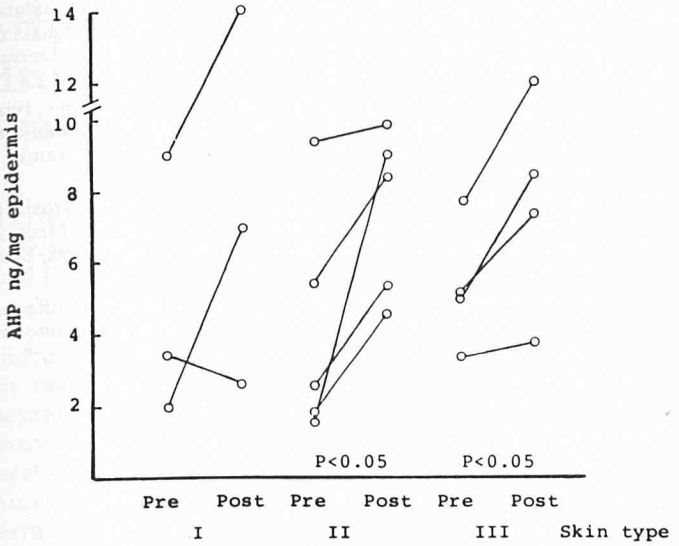

Figure 4. AHP concentrations in the epidermis of different skin types before and after PUVA therapy. The paired t test was used to test for significance of differences between pre- and post-PUVA values.

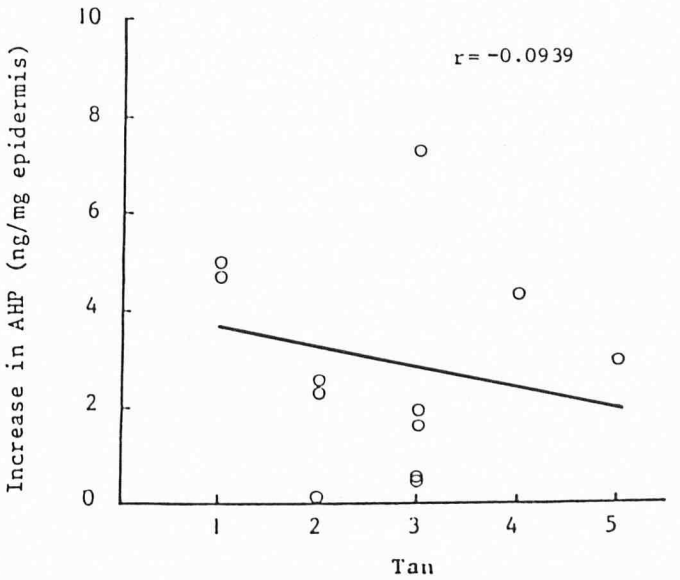

Figure 6. Increases in epidermal AHP concentrations and their relationship to the degree of tanning following PUVA therapy.

view that whereas tyrosinase regulates the synthesis of eumelanin, other factors are of importance in the synthesis of pheomelanin [18].

The present results demonstrate that the concentrations of both pigments in the epidermis increase in response to PUVA. However, whereas the largest increases in eumelanin were seen in subjects with skin type II and III, the increases in pheomelanin appeared to be unrelated to skin type. Epidermal pheomelanin concentrations, unlike those of eumelanin, showed no relationship to the degree of tanning and this would suggest that increases in eumelanin are more important in determining this tanning response following PUVA. This is consistent with the view that eumelanin has the greater photoprotective role, but we now need to examine whether UVA alone and UVB are able to bring about similar changes. It would also be of interest to know whether the increases in eumelanin and pheomelanin concentrations following PUVA are the result of changes in the synthesis or breakdown or a combination of both processes. The same mechanisms may not necessarily operate for both pigments and, as the present findings suggest, the concentrations of epidermal eumelanin and pheomelanin may be differentially controlled.

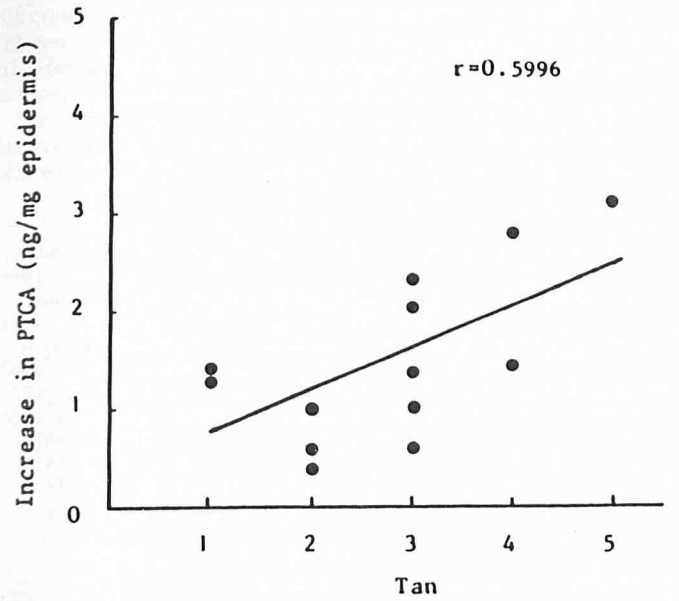

Figure 5. Increases in epidermal PTCA concentrations and their relationship to the degree of tanning following PUVA therapy.

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