

THE PROTECTIVE ROLE OF EPIDERMAL MELANIN IN A PATIENT WITH PORPHYRIA VARIEGATA AND VITILIGO

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Abstract. To assess the role of epidermal melanin in a patient with porphyria variegata and vitiligo, the MED was determined in pigmented and vitiliginous skin for wavelengths of 310, 405 and 500 nm. The energy required to elicit erythema by irradiating vitiliginous skin at 310 nm was half that for pigmented skin. For 405 nm the differences was 4-fold and at 500 nm 2-fold. A possible explanation for the different protection by melanin against light of 310, 405 and 500 nm is given. UV-B irradiation, as a potent stimulus for melanization of the skin, is proposed as an additive measure in the protection against photosensitivity reactions in porphyria patients.

Key words: Melanin; Light absorption; Porphyria variegata; Vitiligo

It is often assumed that UV light shorter than 320 nm does not penetrate the epidermis beyond the basal lamina (1). However, Everett showed in spectrophotometrical studies on epidermal split skin preparations that a small percentage of shortwave UV is transmitted to the upper dermis, depending on the thickness of the stratum corneum and the stratum malpighii and on the skin colour (4). Two different explanations have been proposed to account for the dermal vascular changes that follow UV-B exposure. According to the indirect diffusion theory (6), light energy is initially absorbed within the epidermis. Then small molecular weight substances released by damaged keratinocytes diffuse into the dermis and dilate the blood vessels. The second theory proposes a 'direct hit' of radiation on dermal blood vessels (9).

In porphyria variegata, excessive quantities of porphyrins may be produced in the liver and skin. These porphyrins are photosensitizers, whereby the photodynamic action takes place in the visible region mainly between 400 and 410 nm and additionally—though to a smaller extent—between 500 and 600 nm. The phototoxicity is thought to be due

to absorption of the visible light by the porphyrin molecules.

The unstable porphyrin takes on a higher energy state and reacts with molecular oxygen present in the tissue. This excited oxygen reacts with lipid bilayers and lysosomal damage may be the result (7).

In a patient of caucasian origin who had variegate porphyria (VP) and vitiligo, we noticed the effects of photodynamic skin damage, which were restricted entirely to vitiliginous lesions on light-exposed parts of the body. In this patient, we determined the minimal erythema dose (MED) for different wavelengths of light in normally pigmented skin and in vitiliginous skin. The aim was to assess the role of epidermal melanin in the absorption of light at different wavelengths.

PATIENT, MATERIALS AND METHODS

Case report

The patient was a 41-year-old female of caucasoid extraction who had suffered from vitiligo for 10 years (Fig. 1). Four years ago she had been treated for hypermenorrhoea with ergot preparations and lynaestrolum (Orgametril®). Subsequently she developed blisters exclusively in the vitiliginous lesions on the dorsa of the hands, especially after solar exposure (Fig. 2).

Her mother and her sister and brother had no complaints, but a nephew from her mother's family had severe porphyria, although he did not have vitiligo.

Pathologic examination of a blister revealed a subepidermal cleavage, minor sclerodermoid changes of the papillary dermis and deposits of PAS-positive material in

Abbreviations used: VP = Porphyria variegata, MED = Minimum erythema dose, ALA = δ -amino l evulinic acid, PBG = Porphobilinogen, UP = Uroporphyrin, CP = Coproporphyrin, PP = Protoporphyrin.

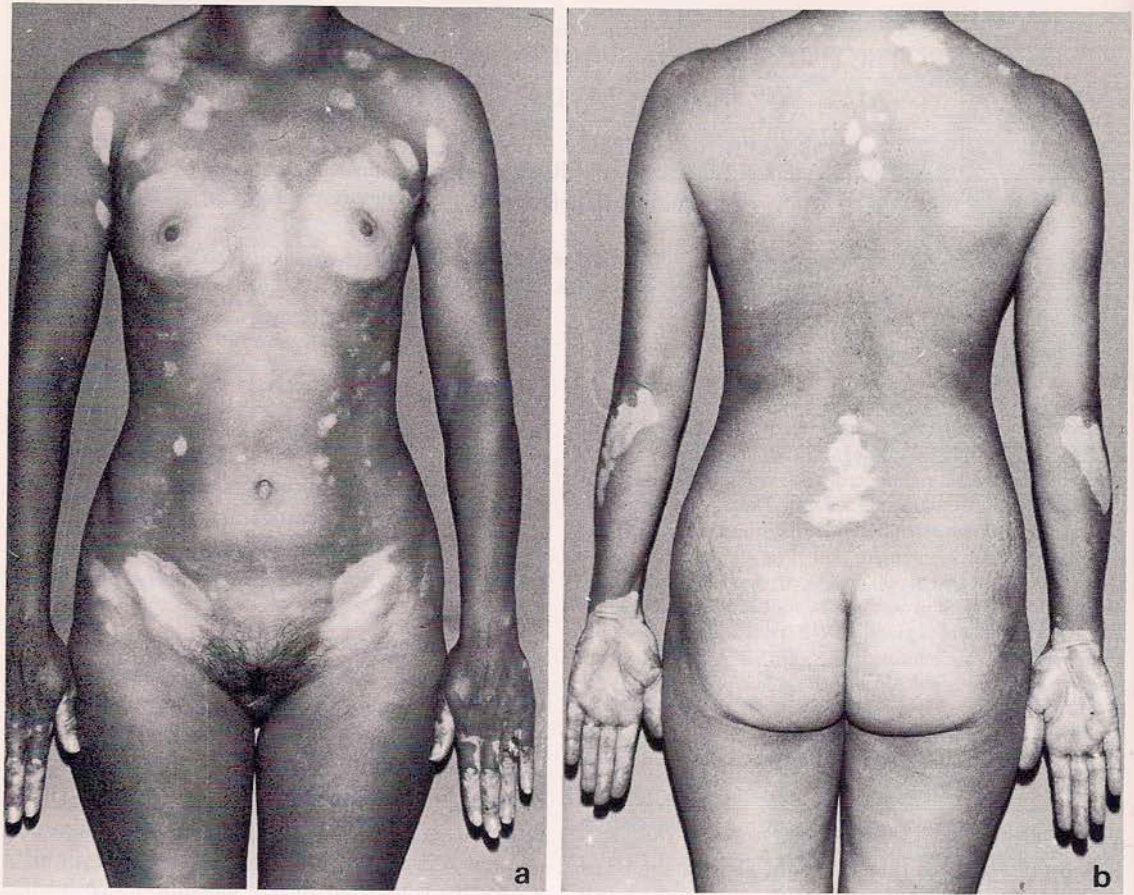


Fig. 1. More or less symmetrically distributed amelanotic macules, typical of vitiligo; (a) patient's front; (b) patient's back.

the walls of the papillary arterioles. The patient had circulating antibodies against thyroid cell cytoplasm and colloid as well as antibodies against parietal cells. Thyroid function tests were normal and no gastric abnormalities were found.

Porphyrin analysis of urine was as follows (normal val-

ues in parentheses): ALA 915 $\mu\text{g/l}$ (1 900–2 600 $\mu\text{g/l}$); PBG 3 645 $\mu\text{g/l}$ (1 400–1 700 $\mu\text{g/l}$); UP 31 $\mu\text{g/l}$ (5–20); porphyrin analysis in faeces: CP 132 $\mu\text{g/g}$ (0–27 g/g wet weight); PP 92 $\mu\text{g/g}$ (0–30 $\mu\text{g/g}$ wet weight) 48-hour sampling. The methods of the porphyrin analysis of faeces and urine are described in detail in a case report on the family (17).

Table I. Results of photosensitivity testing

MED = minimum erythema dose, t_1 = interval after irradiation before erythema develops, t_e = duration of erythema, IP = immediate pigmentation, DP = delayed pigmentation, $\pm \Delta\lambda$ = F.W.H.M. = full-width half-maximum, PF = protection factor

(nm)	$\pm \Delta\lambda$ (nm)	Vitiliginous skin					Pigmented skin					
		MED (J/cm^2)	t_1 (hrs)	t_e (hrs)	IP	DP	MED (J/cm^2)	t_1 (hrs)	t_e (hrs)	IP	DP	PF
310	18	4×10^{-2}	5	72	—	—	8×10^{-2}	5	72	±	+	2
350	28	—	—	—	—	—	—	—	—	+	—	—
405	40	3.5	2	10	—	—	14	2	10	+	—	4
450	55	5.7	2	10	—	—	17	2	10	+	—	3
500	80	8.5	2	10	—	—	17	2	10	+	—	2



Fig. 2. Small blister in a depigmented lesion of the thumb.

Irradiation studies

The patient was subjected to irradiation of the skin at wavelengths of 310 nm (to provoke normal sunburn erythema); 350 nm (no erythema expected); 405 nm (the Soret-band of the porphyrin absorption); 450 nm (no erythema expected); and 500 nm (weaker absorption band by porphyrins).

Vitiliginous and normally pigmented skin of the extensor aspects of the forearms was chosen for the phototesting. The forearms showed no clinical signs of photodynamic damage.

We also recorded for each wavelength: (a) the time lapse after which erythema occurred; (b) the time of maximal erythema; (c) the degree of pigmentation present during and directly after irradiation (immediate pigmentation) as well as the delayed pigmentation.

Two healthy subjects and one patient with vitiligo served as controls. As a light source for irradiation we used a 1600 W Xenon arc lamp operated at maximum power. A monochromator (Zeiss M4Q111) with slits of 2 mm was placed in front of the lamp-house. The beam of 'monochromatic' light was focused with a quartz lens. The output of the Xenon arc lamp behind the lens measured with an EG & G radiometer model 580-25A was at: 310 nm: 2.2 mW; 350 nm: 6 mW; 405 nm: 14 mW; 450 nm: 25 mW; 500 nm: 35 mW.

Before and after the experiment the output of the light source was checked and found to be constant during the

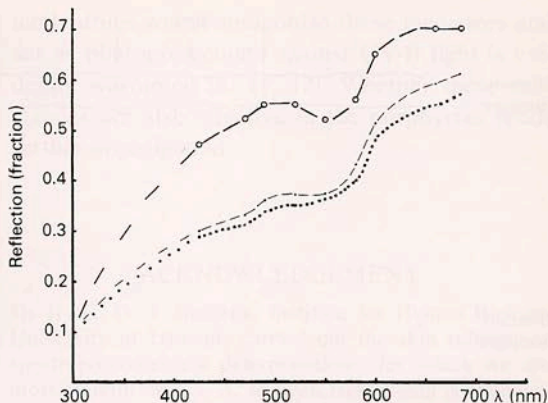


Fig. 3. Reflectance measurements on skin of inner upper arm (· · ·); forearm (normal skin) (---); forearm (vitiliginous skin) (O---O). The reflection is measured at $\lambda=425, 470, 490, 520, 550, 580, 660$ and 685 nm.

experiments. Changes in total radiant flux energy doses at the operational wavelengths were obtained by keeping the intensity of the light source constant and varying the exposure times. A surface of 0.5×1.0 cm² of skin was irradiated and the distance between the lens and the test site was kept scrupulously constant.

Separate areas of skin were marked in advance and each area received a different dose of energy by altering the exposure time. The areas were examined for minimal erythema at 0, 1, 2, 4, 6, 8, 12, 16, 24, 48 and 72 hours.

To obtain an objective parameter for the skin colour of the patient and the two healthy individuals we measured the reflectances of the inner upper arm skin at the different wavelengths (Fig. 3) using an 'EEL' Reflectance Spectrophotometer (16). Additionally we determined in the PV patient the reflectances of the vitiliginous lesion and the neighbouring normal skin of the forearm on which the photosensitivity testing had been carried out.

RESULTS

From the spectrophotometric reflectance studies it appeared that our VP patient was slightly more pigmented than the two healthy controls. From Fig. 3 the reflectances of the inner aspect of the upper arm and of the vitiliginous lesion and neighbouring normal skin of the forearm of the VP patient can be read.

At $\lambda=310$ nm the two healthy subjects had a MED which was equal to that found in the pigmented skin of the VP patient. The MED determined in the patient with vitiligo was comparable to the MED of the vitiliginous lesion of the VP patient. In the healthy individuals and in the control patient with vitiligo no photosensitivity could be demon-

MED's of other authors

Ref.10 (J/cm ²)	Ref.15 (J/cm ²)
4×10^{-2}	—
—	—
4.7	0.5–50
—	—
3.7	—

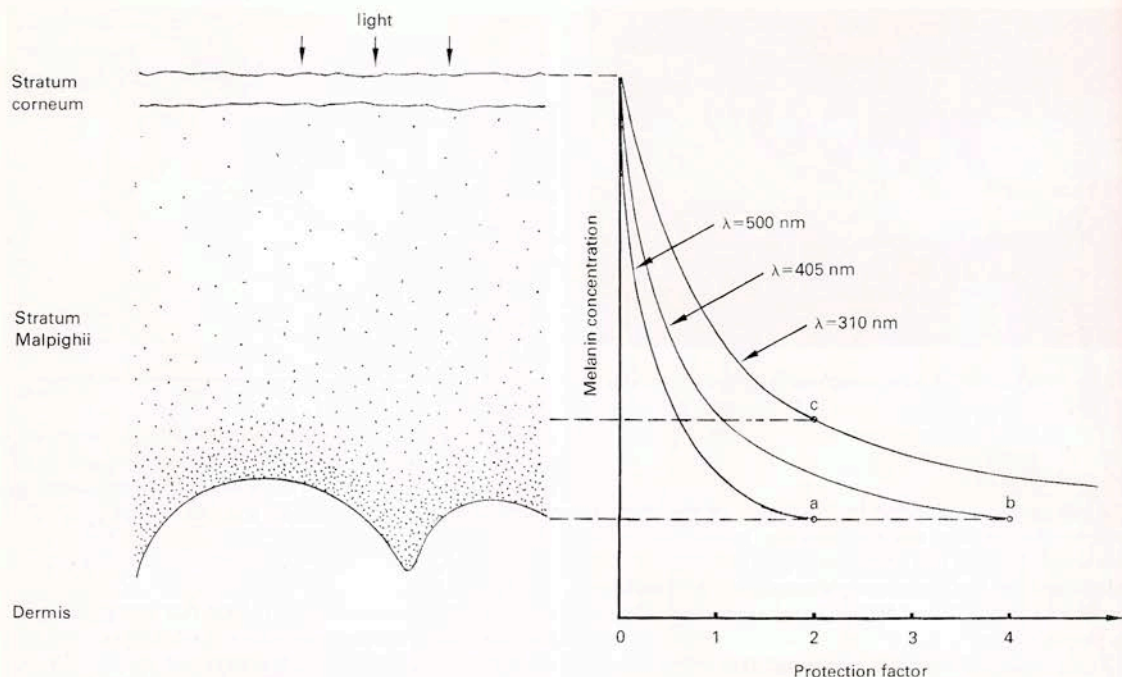


Fig. 4. Schematic drawing of the skin, with the distribution of melanin in the different layers of the epidermis. On the right the absorption of 310, 405 and 500 nm light due to

strated for wavelengths of 350, 405 and 500 nm. In Table I the results of the photosensitivity testing with our VP patient are summarized.

The erythema provoked by irradiation at $\lambda = 310$ nm appeared later but persisted longer than erythema due to longer wavelengths. Immediate pigmentation (IP) was present during and directly after the irradiation, fading after about half an hour. Delayed pigmentation (DP) occurred only when irradiated at $\lambda = 310$ nm and developed after 3–5 days during which the erythema disappeared (Table I). At the wavelengths studied our energies delivered to the test areas never resulted in clinically detectable edema and blistering.

DISCUSSION

In pigmented skin, the energy required at 310 nm to elicit threshold erythema was twice the amount needed for a comparable reaction in vitiliginous skin (Table I). Therefore the protection factor given by the epidermal melanin is 2 at this wavelength. At 405 nm the protection factor given by the pigment barrier is 4, decreasing again at longer wavelengths. When normal and vitiliginous skin measurements

melanin is plotted against the protection factor on the horizontal axis.

are compared the data show that light of $\lambda = 405$ nm is relatively better filtered by the pigment barrier than at $\lambda = 310$ nm. This is unexpected, as it is known that the absorption by the melanized epidermis increases with shorter wavelengths. The higher protection factor in the visible region cannot be explained by the difference in reflection of the skin, either. At wavelengths shorter than 320 nm there is no important difference in the reflectance of non-pigmented compared with pigmented skin. At 425 nm the difference in reflectance between vitiliginous and pigmented skin of our patient was 17% (Fig. 3) which means that more light is reflected by the vitiliginous skin and consequently less light is transmitted through the vitiliginous skin as compared with normally pigmented skin. Therefore this explanation favours the opposite effect.

The results can be better understood if we assume that the photosensitivity for light of 405 and 500 nm (porphyrin absorption bands) depends on a photodynamic mechanism different from that due to UV-B irradiation at 310 nm. The photodynamic processes are not necessarily taking place at the same level in the skin.

Without going into the details of the supposed

photodynamic actions, which basically explain porphyrin photosensitivity and sunburn erythema we have indicated in a schematic drawing of how light of different wavelengths is absorbed by the skin. In order to avoid confusion the aspect of reflection is not indicated. If included it would not alter the explanation. In Fig. 4 the skin with the stratum corneum, the stratum Malpighii and the dermis is shown. Furthermore the distribution of the melanin pigment in the epidermis with a higher density in the basal layer is indicated. On the right we see that the protection factor plotted on the horizontal axis is a function of the melanin concentration. The curve for 500 nm light is steep at first, because there is little pigment, giving only weak absorption and thus a small protection factor. Lower in the epidermis, where more pigment is present, the protection factor increases, giving the curve a flat slope. At the moment the light reaches the dermis (point *a*, Fig. 4) the absorption is a factor 2 higher than in vitiliginous skin. At 405 nm the curve is as indicated, while at 310 nm, bearing in mind the 'direct hit' theory, one expects a curve as indicated, giving a protection factor of 8.

In our experiment a factor 2 only is found, which corresponds to a level in the skin above the basal layer (point *c*, Fig. 4). And thus erythema due to light at 310 nm must be generated at the level of stratum spinosum. Most likely the light absorption here liberates substances which diffuse to the dermis. In agreement with this model is our experimental finding that erythema develops much more slowly after 310 nm irradiation as compared with 405 nm irradiation (see Table I). Substances have to diffuse from the stratum spinosum to the dermis to cause vasodilatation as well as other changes. The photodynamic processes due to porphyrins take place in the dermis next to the blood vessels. Vasoactive substances only have to diffuse over short distances, thus resulting in the rapid development of erythema.

The practical consequences of this study are:

1. Treatment of porphyria patients with UV-B light, which is known to be melanogenic, might be beneficial because the increase in pigmentation gives relatively better protection against 405 nm light (5), as was shown in our study.

2. It supports studies which postulate that certain mediators are liberated in epidermis due to UV-B light, giving rise to erythema and other actinic changes (2, 3, 6, 13, 14). The development of sys-

temic drugs which antagonize these mediators and act as photoprotectants against UV-B light is evidently warranted (8, 11, 12). Whether these substances are also effective in the porphyrias needs further investigation.

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REFERENCES

1. Blum, H. F.: *Carcinogenesis by Ultraviolet Light*. Princeton Univ. Press, Princeton, N.Y., 1959.
2. Crunkhorn, P. & Willis, A. L.: Interaction between prostaglandins E and F given intradermally in the rat. *Br J Pharmacol* 41: 507-512, 1971.
3. Epstein, J. H. & Winkelmann, R. K.: Ultraviolet light induced kinin formation in human skin. *Arch Dermatol* 95: 532-536, 1967.
4. Everett, M. A., Yaergers, E., Sayre, R. M. & Olsen, R. L.: Penetration of epidermis by ultraviolet rays. *Photochem Photobiol* 5: 533, 1966.
5. Van der Leun, J. C., van Weelden, H.: Light induced tolerance to light in photodermatoses. *Br Photobiol Soc Meet* 23rd October 1974.
6. Van der Leun, J. C.: Ultraviolet erythema. A study of diffusion processes in human skin. PhD thesis, Utrecht, 1966.
7. Magnus, I. A.: The Parkes Weber lecture. Porphyria as a model of abnormal skin sensitivity. Tenth Symposium on Advanced Medicine. Proceedings of a conference held at the Royal College of Physicians of London, February 1974, ed. J. G. G. Ledingham, Pitman Medical, 1974.
8. Morison, W. L., Paul, B. S. & Parrish, J. A.: The effects of indomethacin on longwave ultraviolet induced delayed erythema. *J Invest Dermatol* 68: 130-133, 1977.
9. Parrish, J. A., White, H. A. D. & Pathak, M. A.: *Photomedicine. In Dermatology in General Medicine*, 2nd ed. pp 954-956 McGraw-Hill, New York, 1979.
10. Ramsay, C. A., Magnus, I. A., Turnbull, A. L.: Treatment of porphyria cutanea tarda by venesection. *Q J Med* 43: 1, 1974.
11. Snyder, D. S. & Eaglstein, W. H.: Topical indomethacin and sunburn. *Br J Dermatol* 90: 91-93, 1974.
12. — Intradermal anti-prostaglandin agents and sunburn. *J Invest Dermatol* 62: 47-50, 1974.
13. Valtonen, E. J., Jänne, J. & Siimes, M.: The effect of the erythema reaction caused by ultraviolet irradiation on mast cell degranulation in the skin. *Acta Dermatovener (Stockholm)* 44: 269-272, 1964.

14. Veninga, T. S. & de Boer, J. E.: Urinary excretion pattern of serotonin and 5-hydroxy indole acetic acid in ultraviolet induced erythema. *J Invest Dermatol* 50: 1-8, 1968.
15. Wasserman, C. A. & Habermann, H. F.: Photosensitivity: results of investigation in 250 patients. *Can Med Ass J* 113: 1055-1060, 1975.
16. Weiner, J. S.: A spectrophotometer for measurement of skin color. *Man* 51: 253, 1951.
17. Westerhof, W. & Smit, E.: Porphyria variegata, een

familie onderzoek. *Ned T Geneesk*, submitted for publication.

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