

## THE PIGMENTARY RESPONSE TO PHOTOCHEMOTHERAPY<sup>1</sup>

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**Abstract.** Previous studies on skin topically photosensitized with trimethylpsoralen and subsequently irradiated with long-wave UV light have demonstrated an increase in melanosome size and changes in the distribution patterns of melanosomes, suggesting the possibility of gene derepression or the induction of a somatic mutation of melanocytes. The present investigation was performed to determine whether identical changes are induced by systemic photochemotherapy using 8-methoxypsoralen and UVA (PUVA) under therapeutic conditions. Our results show that PUVA stimulates melanogenesis but does not induce significant changes in the average size of melanosomes nor in their distribution patterns within keratinocytes. Thus they indicate that under therapeutic conditions PUVA does not induce morphologically detectable cytogenetic changes in pigment cells.

**Key words:** Photochemotherapy; 8-Methoxypsoralen; Melanocytes; Melanosomal size; Distribution pattern of melanosomes; Skin pigmentation

Photochemotherapy with psoralens and long-wave ultraviolet light (PUVA) is gaining in importance for the treatment of psoriasis (15, 29, 30). Psoralens, applied topically or administered systemically, plus subsequent exposures to UVA, produce photosensitization reactions in the skin which are followed by hyperpigmentation. The mechanism responsible for the beneficial effect of PUVA on psoriasis is thought (15, 29) to be due to a photo-mediated binding of psoralens to DNA, leading to monofunctional photoadducts and DNA crosslinks (3, 13) and, to a lesser extent, to interactions of psoralens with ribosomal RNA (16, 17). To date, it is not clearly established whether the erythema produced by the photosensitization reaction and the ensuing pigmentation are due to the same

macromolecular events, or represent two different interrelated or even independent phenomena.

Psoralens and UVA have been shown to stimulate melanogenesis in vitro (2) and the clinical experience of the present authors with photochemotherapy as well as that of Kaidbey & Kligman (8) using high oral doses of TMP suggest that pigmentation can be achieved even in the absence of erythema. Thus a direct effect of PUVA on the melanocyte in vivo appears probable and it is in this context that the findings of Toda et al. (21) acquire considerable significance; these authors noted that topical application of trimethylpsoralen to Caucasoid skin and subsequent exposure to UVA resulted in an increase not only in the number of melanosomes but also in their size; in addition there was a change in the distribution pattern of melanosomes in the keratinocytes from the aggregated (complexed) to the non-aggregated (single) state. The persistence of these changes for more than 6 months suggested the possibility of a long-term gene derepression or the induction of a somatic mutation of the melanocytes (12, 21, 23).

In view of the encouraging results of PUVA treatment in psoriasis (5, 6, 15, 19, 29, 30) and the consequent potential widespread use of this technique, the suggestion of cytogenetic changes and thus potential long-term adverse effects must be a matter of concern. It was therefore deemed necessary to determine whether systemic PUVA will induce permanent melanosomal changes, indicating a genetic transformation of the melanocytes in the patients thus treated.

### MATERIALS AND METHODS

Uninvolved skin from 11 Caucasoid adults, 10 males and one female, aged between 30 and 75 years, all suffering from severe generalized psoriasis, was investigated. For

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Table I. Clinical data of patients, indicating their individual sensitivity to UV-light

Pat. no.	Initials	Age	Sex	Pigmentation <sup>a</sup>	Eye colour	Hair colour	Skin type <sup>b</sup>	PPI <sup>c</sup>
1	K. F.	60	♂	c	Blue	Brown	III	0.9
2	H. E.	30	♂	a	Blue	Blond	II	1.8
3	B. J.	44	♀	a	Green	Brown	II	0.8
4	B. F.	66	♂	a	Brown	Brown	III	0.6
5	W. J.	53	♂	c	Blue	Brown	III	1.0
6	H. F.	55	♂	c	Blue	Brown	III	0.9
7	K. L.	75	♂	a	Blue	Brown	IV	0.8
8	S. G.	55	♂	a	Blue	Blond	III	0.9
9	P. J.	47	♂	c	Brown	Brown	IV	0.4
10	G. A.	40	♂	a	Blue	Blond	III	1.3
11	B. R.	66	♂	a	Brown	Brown	IV	0.8

<sup>a</sup> Pigmentation of skin in exposed areas before start of treatment: a=light, b=light with freckles, c=medium or moderate, d=well tanned, e=highly tanned.

<sup>b</sup> The following criteria were used (31):

Skin type: I=Always burn, never tan, II=Always burn, then slight tan, III=Sometimes burn, always tan, IV=Never burn, always tan.

<sup>c</sup> Photosensitivity: pigmentation index (PPI), which indicates the patient's propensity to tan, relative to his propensity to burn (31).

reference purposes the skin type (relative to the pigmentary capacity) and photosensitivity-pigmentation index (PPI) (31) of these patients are summarized in Table I. Except for case 2 all had a moderate to pronounced tanning propensity. None of the patients was or had been undergoing topical or systemic treatment other than oral 8-MOP and there was no history of previous exposure of the "unexposed" sites to ultraviolet light, except during photochemotherapy.

#### Treatment

The treatment regimen comprised the ingestion of 8-MOP capsules in a dosage related to the patient's weight (29) followed 2 hours later by exposure to a high intensity long-wave ultraviolet light (UVA) system (29) with an emission spectrum between 320 and 390 nm (peak at 365 nm). The initial UVA doses ranged from 1 joule/cm<sup>2</sup> to 4.0 J/cm<sup>2</sup> and were gradually increased up to 10 J/cm<sup>2</sup>. During the initial phase, four irradiations/week were given until the psoriatic skin lesions were completely cleared (clearing phase). Maintenance therapy (29) was then started, the frequency of irradiations being initially maintained at once per week. During the clearing phase, all patients developed a significant degree of uniform pigmentation which, during the maintenance therapy period, gradually decreased over the course of several months and finally disappeared several months after the treatment had been discontinued.

#### Biopsies and biopsy schedule

Biopsies were taken under local anaesthesia from the uninvolved skin of the lower back before treatment and at various intervals during and after therapy. The frequency and times of biopsy in each patient and the total dose of UVA energy delivered to the patient up to the respective biopsies are summarized in Fig. 1. The longest treatment periods at the time of biopsy ranged between 11 and 12 months. Biopsies were also done in 2 patients 2 and 9 months after treatment had been discontinued (Fig. 1).

The tissue was cut into small pieces and immersed in half-strength Karnovsky's paraformaldehyde fixative (9) for 5 hours at room temperature. It was rinsed overnight and postfixed in 3% osmic acid in distilled water for 1½ hours in an icebath, stained en bloc in uranylacetate for 2 hours at room temperature, dehydrated in a graded series

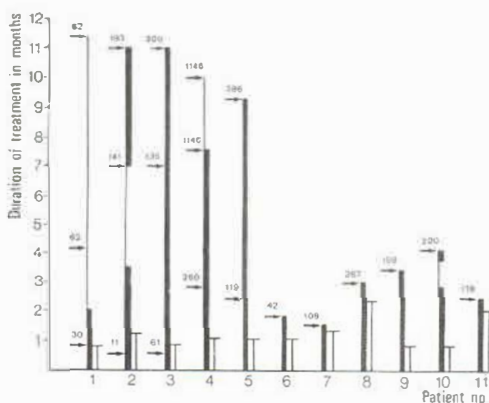
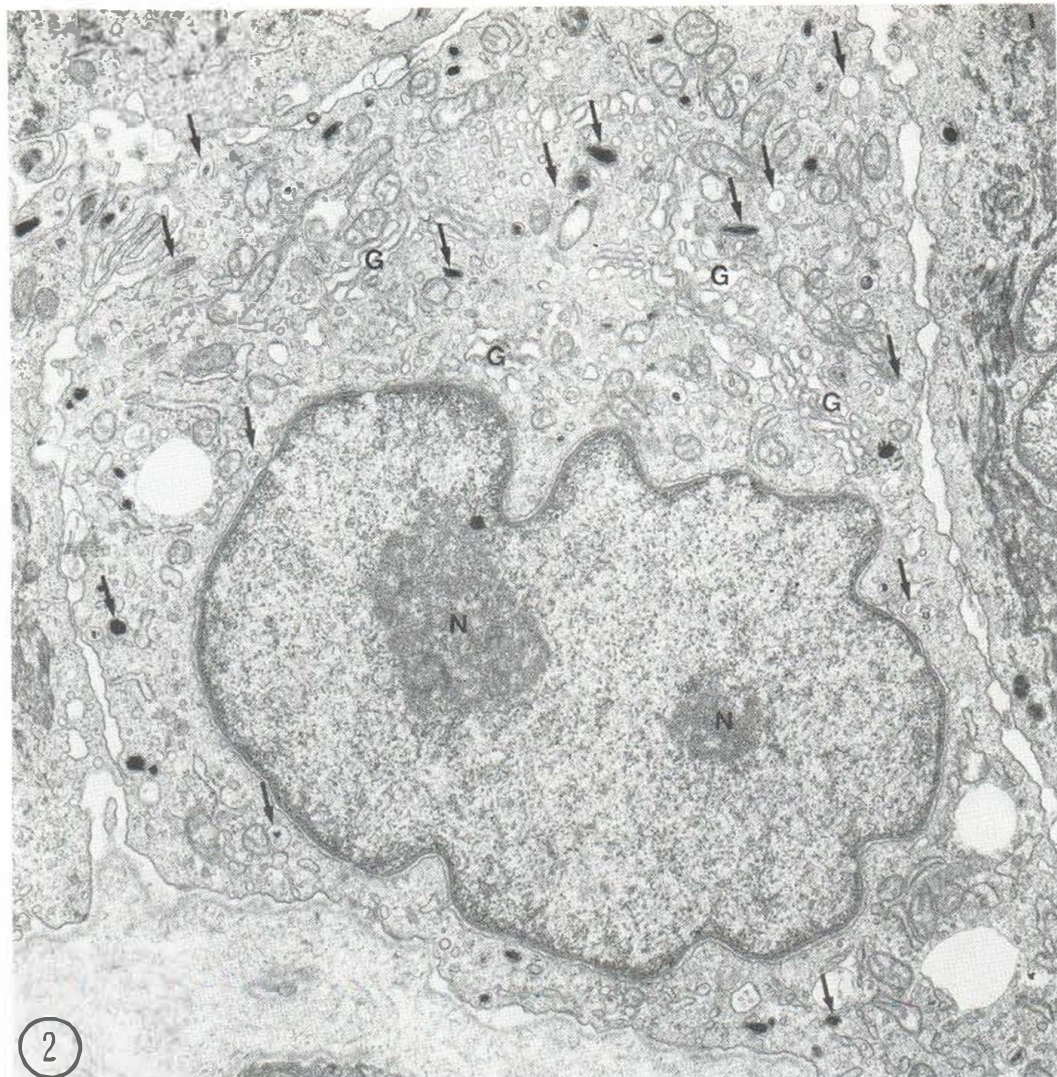


Fig. 1. Irradiation schedule. Arrows indicate biopsies during or after therapy. A control biopsy was taken from each patient before start of the treatment. The numbers at the columns indicate the total amount of UVA energy (joule/cm<sup>2</sup>) delivered up to the time of the biopsy. The thin columns at the base indicate duration of the clearing phase (4 irradiations/week) as opposed to the maintenance treatment phase.



**Fig. 2.** Highly active melanocyte 14 days after start of PUVa treatment. There is an increase in the number of melanosomes in all stages of maturation (arrows). Promi-

nent Golgi apparatus (G). Large nucleus with prominent nucleolar profiles (N)  $\times 22200$ .

of alcohol and embedded in Epon 812. Ultrathin sections were cut with Dehmer diamond knives in a Reichert and LKB ultramicrotome, stained with lead citrate and examined with a Zeiss EM 9 S electron microscope.

#### *Evaluation of melanocytes*

Five blocks of each biopsy specimen were examined and electron micrographs were taken from at least 15 melanocytes exhibiting a nuclear profile. Melanocytes were not quantitated but they were evaluated according to the following criteria: general appearance, arborization, distribution of melanosomes within the cytoplasm, evalua-

tion of melanosome morphology and stage of melanization. All melanocytes were carefully screened for any morphological abnormalities.

#### *Determination of melanosome size and distribution*

At least five blocks of each biopsy specimen were examined. Twenty electron micrographs of keratinocytes of the basal and occasionally of the first suprabasal layer were taken randomly from each biopsy specimen and the prints were enlarged to a final magnification of 24 000. Ten of these 20 prints were randomly selected for analysis. As

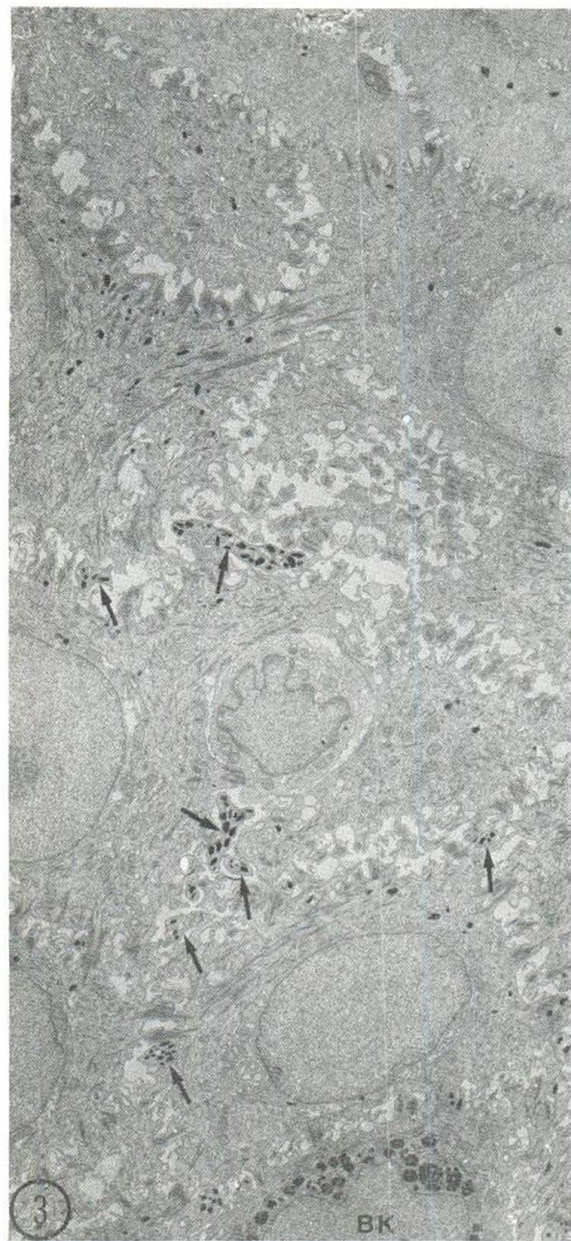
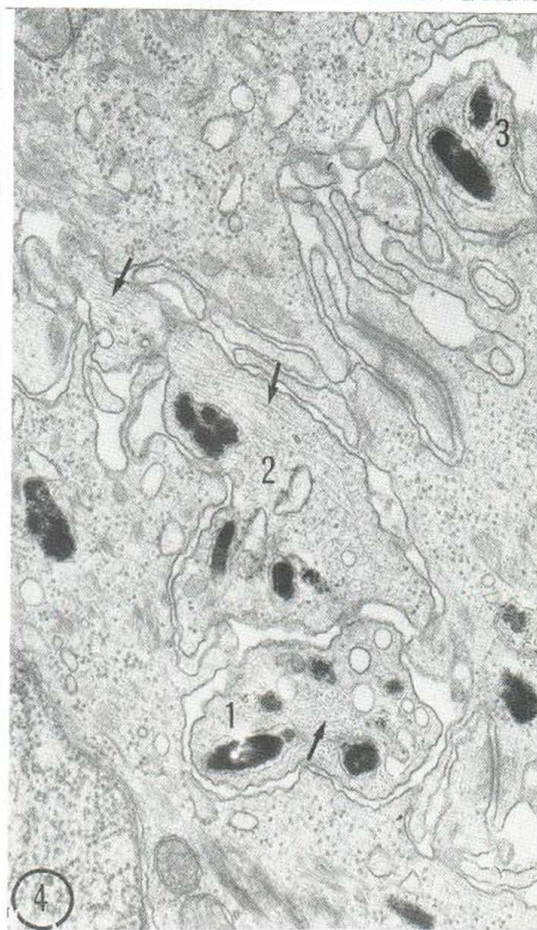
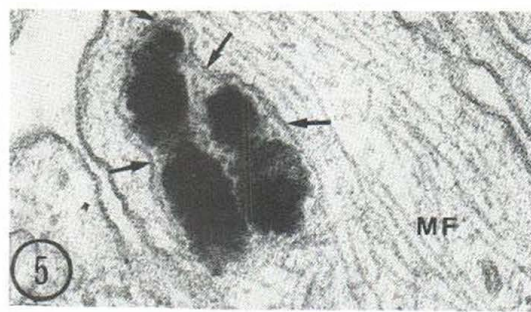


Fig. 3. After 8 weeks of continuous PUVA treatment. Melanocytic dendrites (arrows) filled with mature melanosomes reach up to the third layer of the epidermis. Basal keratinocyte (BK) with numerous melanosome complexes.  $\times 4200$ .

Fig. 4. After 8 weeks of PUVA treatment. Profiles of 3 melanocytic dendrites (1, 2, 3) between keratinocytes of the second and third layer. Fully developed melanosomes



and numerous microfilaments (arrows) within the dendrites.  $\times 27000$ .

Fig. 5. Detail from Fig. 4. 3 melanosomes within the melanocytic dendrite are surrounded by a common membrane (arrows), thus resembling a melanosome complex usually occurring only in keratinocytes. MF: microfilaments.  $\times 62000$ .

Table II. *Melanosomes in melanocytic dendrites*<sup>a</sup>

Total no. of melanosomes	1 107
Total no. of single melanosomes	979
Total no. of melanosomes in complexes	128
Total no. of complexes	60
Average size of single melanosomes <sup>b</sup>	0.13±0.04 μm
Average size of melanosomes in complexes <sup>b</sup>	0.1 ±0.03 μm

<sup>a</sup> Based on counting and measurement on 310 photomicrographs (magnification ×24 000) of the basal area of the epidermis from all biopsy specimens.

<sup>b</sup> As determined by measuring the largest cross diameter of all melanosomes (10).

an increase of the number of melanosomes after PUVA has been documented (17, 24) the absolute numbers of melanosomes were not determined and attention was focused instead on melanosome size. The transverse diameter of all melanosomes within keratinocytes was measured as this provides a more reliable estimate of melanosomal size than the longitudinal diameter, which is subject to greater variability in ultrathin sections (10). Fragmented melanosomes and "melanosome dust" were excluded. For each specimen the average size of all melanosomes was computed. Independently, all the melanosomes with a cross diameter of more than 0.17 μm were also counted and the percentage ratio of these large melanosomes to the total number of melanosomes was calculated for each biopsy. The distribution of all melanosomes was charted and the number of single melanosomes within keratinocytes was determined for each specimen.

## RESULTS

### (1) *Melanocytes*

No attempt was made to quantitate the increase in the number of melanocytes, since this has been shown to occur by other authors (8).

Melanogenesis was greatly stimulated during the initial phase of treatment; this started at 72 hours and reached maximal activity at 2 weeks after the start of PUVA (Fig. 2). From then on, no more activation was noted. The melanocytes increased in size and developed longer and wider dendrites which frequently reached the third and even the fourth suprabasal layer (Fig. 3). The hypertrophy of the melanocytes was accompanied by an increase in melanosome production manifested as an increase in the number of melanosomes at all stages of melanosomal maturation (Fig. 2). The rough endoplasmic reticulum, Golgi apparatus and mitochondria became more prominent, corresponding to the increased activity (Fig. 2). Numerous fully developed melanosomes and microfilaments were

noted in dendrites in suprabasal layers, suggesting increased transfer of melanosomes even at higher levels in the epidermis (Figs. 3 and 4). On several occasions the melanosomes within the dendrites were seen to occur in small groups of 2 or 3, surrounded by a common membrane, thus resembling melanosome complexes in the keratinocytes (Figs. 4 and 5). In an attempt to quantify this phenomenon we found that of 1 107 melanosomes in melanocytic dendrites surrounding basal keratinocytes, 128 were in aggregated form; the total number of these complexes was 60 (Table II). When the largest cross diameter of the melanosomes in the melanocytic dendrites was measured (Table II) it became evident that the melanosomes in these complexes were smaller (0.1 μm±0.03) than of those occurring in single state (0.13 μm±0.04). Thus it seems probable that melanosome size is an important determining factor in the distribution and packaging of melanosomes, not only in the keratinocytes (25, 26) but that this phenomenon holds true also in the melanocytes.

During maintenance therapy the melanocytes were much less active and were frequently noted to approach the normal as evaluated by comparison with the pretreatment biopsies (Fig. 6). The dendrites decreased in both number and size and were more often seen between the basal cells. After discontinuation of the treatment the melanocytes returned completely to normal state.

At no time during or after the treatment period did the melanocytes show any morphological abnormalities. It should be noted that in these patients no signs of overdosage (severe phototoxic reactions) had occurred throughout the entire treatment period.

### (2) *Melanosomes*

(a) *Number.* Starting with the 7th to 10th day after commencement of treatment there was a definite overall increase in the number of melanosomes within keratinocytes, both in basal and suprabasal layers, but there was a considerable variability between the different patients. After changing to maintenance treatment with a frequency ranging from 1 irradiation/week to 1 irradiation/3 weeks the number of melanosomes gradually decreased and only the basal keratinocytes still contained more melanosomes than before treatment. There was a close correlation between the degree of clinical tanning and the number of melanosomes at the

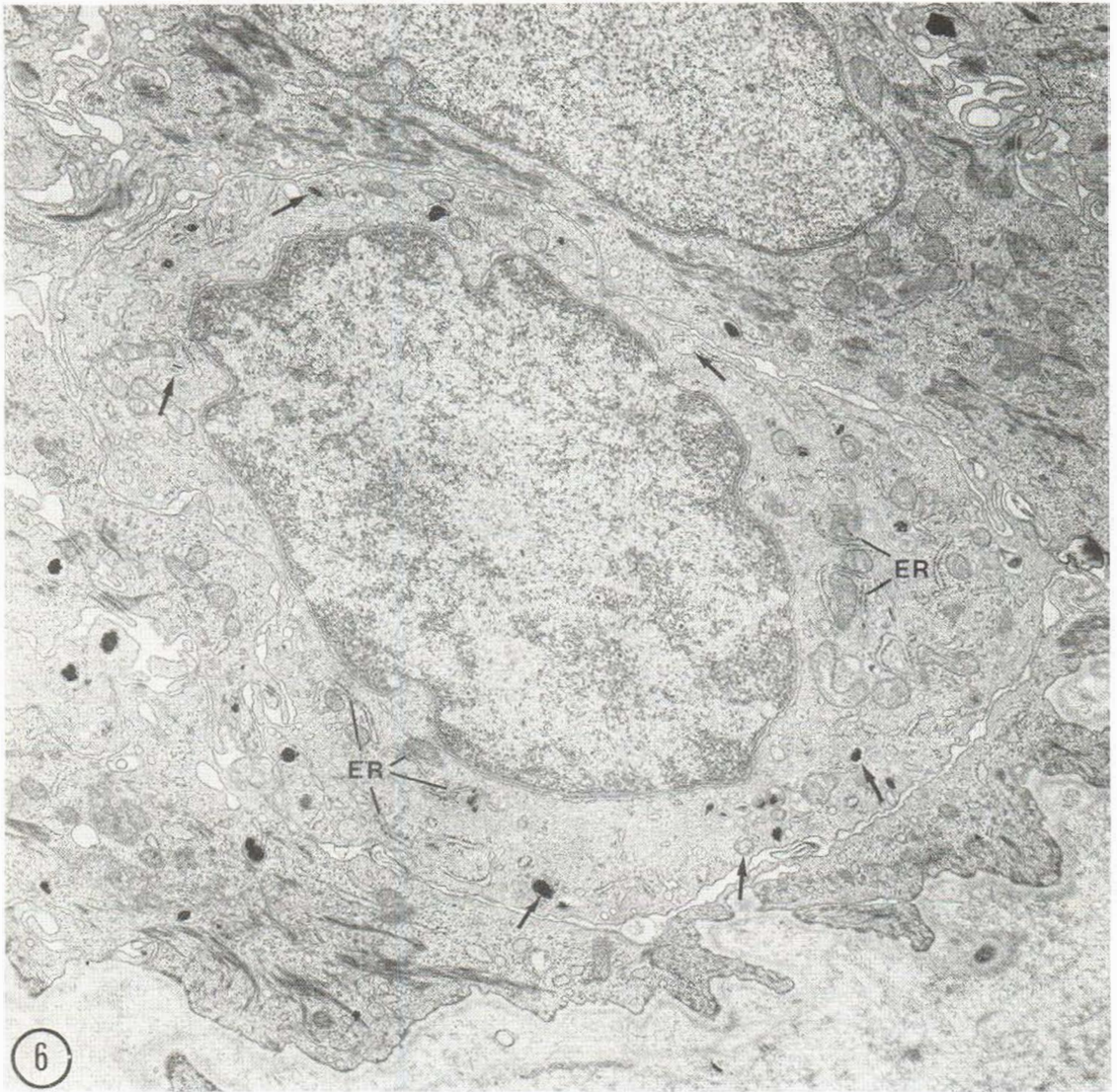


Fig. 6. Maintenance treatment with 1 irradiation/10 days. The melanocyte shows signs of increased metabolic activity (prominent endoplasmic reticulum (ER), melanosomes

in all stages of maturation (arrows)) but is much less active than during the initial clearing phase. Compare with Fig. 2.  $\times 21\ 500$ .

ultrastructural level. In addition, patients receiving a rather large dosage of UVA energy over a period of time exhibited more melanosomes than those irradiated with low doses of UVA. The number of melanosomes returned to the pretreatment level within about 2 months following discontinuation of treatment.

(b) *Size.* 7765 melanosomes within keratinocytes were measured. Fig. 7 shows the average size of melanosomes for all biopsies taken at different time

intervals during treatment. There was no significant difference between the size of melanosomes in the control specimens and in those taken at various intervals during PUVA therapy. Fig. 8 and Table III show the average melanosomal size for each patient separately. Patients 5, 7 and 11 showed no significant change following PUVA, while patient 3 and 6 had some decrease in average melanosomal size. On the other hand, patients 2, 4, 8, 9 and 10 showed a mild to moderate increase. Patient 1 did not show

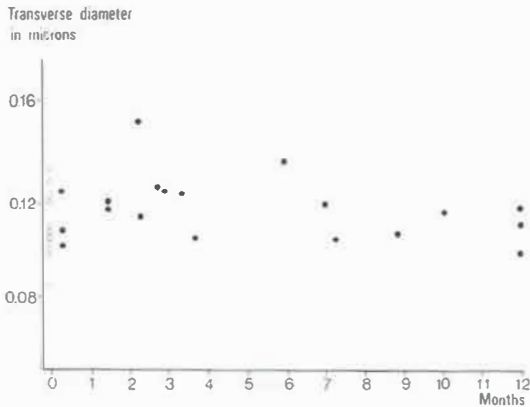


Fig. 7. Average melanosomal size (transverse diameter in  $\mu\text{m}$ ) of pooled patient population. A total of 7765 melanosomes are measured. There is no significant difference between sizes of melanosomes in the control specimens ( $\circ$ ) and in those taken at various intervals during PUVa ( $\bullet$ ).

any significant change after 2 weeks of PUVa but there was a definite increase in melanosome size when the patient was biopsied 4 months after discontinuation of treatment and a return to normal 9 months after PUVa was discontinued.

Fig. 9 shows the percentage of larger melanosomes ( $>0.17 \mu\text{m}$ ) computed for each specimen. There is a close correlation between these results and those obtained when the average size of all melanosomes was determined (compare Figs. 8 and 9).

(c) *Distribution.* Fig. 10 shows the percentage of single melanosomes within the whole melanosome population for each patient and biopsy specimen. Once again no consistent trend is observed and the

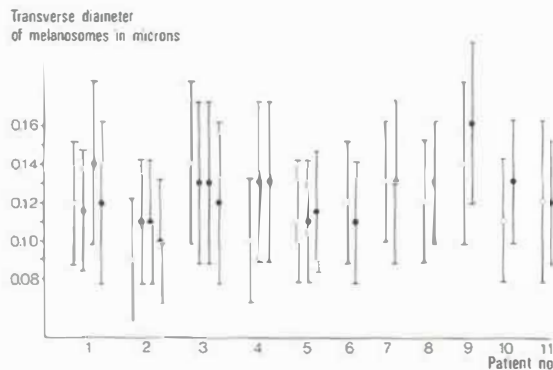


Fig. 8. Average size of all melanosomes shown for each patient.  $\circ$  (controls) and  $\bullet$  (during treatment) denote the mean values; range bars show the standard deviation.

Table III. Average size of all melanosomes, of single melanosomes and of melanosomes in complexes for each patient before, during or after PUVa treatment

Pat. no.	Biopsy no. <sup>a</sup>	Average size of all melanosomes ( $\mu\text{m}$ ) <sup>b</sup>	Average size of single melanosomes ( $\mu\text{m}$ ) <sup>b</sup>	Average size of melanosomes in complexes ( $\mu\text{m}$ ) <sup>b</sup>
1	1 control	$0.12 \pm 0.03$	$0.13 \pm 0.03$	$0.11 \pm 0.03$
	2	$0.11 \pm 0.03$	$0.13 \pm 0.04$	$0.10 \pm 0.03$
	3	$0.14 \pm 0.04$	$0.17 \pm 0.04$	$0.13 \pm 0.04$
	4	$0.12 \pm 0.04$	$0.16 \pm 0.04$	$0.11 \pm 0.03$
2	1 control	$0.09 \pm 0.03$	$0.10 \pm 0.03$	$0.09 \pm 0.03$
	2	$0.11 \pm 0.03$	$0.12 \pm 0.03$	$0.10 \pm 0.03$
	3	$0.11 \pm 0.03$	$0.12 \pm 0.04$	$0.10 \pm 0.03$
	4	$0.10 \pm 0.03$	$0.14 \pm 0.05$	$0.10 \pm 0.03$
3	1 control	$0.14 \pm 0.04$	$0.015 \pm 0.04$	$0.13 \pm 0.03$
	2	$0.13 \pm 0.04$	$0.15 \pm 0.04$	$0.12 \pm 0.03$
	3	$0.13 \pm 0.04$	$0.14 \pm 0.04$	$0.12 \pm 0.03$
	4	$0.12 \pm 0.03$	$0.13 \pm 0.03$	$0.11 \pm 0.03$
4	1 control	$0.10 \pm 0.03$	$0.12 \pm 0.03$	$0.10 \pm 0.03$
	2	$0.13 \pm 0.04$	$0.15 \pm 0.04$	$0.11 \pm 0.03$
	3	$0.13 \pm 0.04$	$0.15 \pm 0.04$	$0.12 \pm 0.03$
5	1 control	$0.11 \pm 0.03$	$0.13 \pm 0.03$	$0.10 \pm 0.03$
	2	$0.11 \pm 0.03$	$0.13 \pm 0.03$	$0.11 \pm 0.03$
	3	$0.12 \pm 0.03$	$0.13 \pm 0.03$	$0.11 \pm 0.03$
6	1 control	$0.12 \pm 0.03$	$0.13 \pm 0.03$	$0.11 \pm 0.03$
	2	$0.11 \pm 0.03$	$0.14 \pm 0.03$	$0.10 \pm 0.03$
7	1 control	$0.13 \pm 0.03$	$0.15 \pm 0.03$	$0.12 \pm 0.03$
	2	$0.13 \pm 0.04$	$0.14 \pm 0.04$	$0.12 \pm 0.04$
8	1 control	$0.12 \pm 0.03$	$0.13 \pm 0.03$	$0.10 \pm 0.03$
	2	$0.13 \pm 0.03$	$0.14 \pm 0.03$	$0.10 \pm 0.03$
9	1 control	$0.14 \pm 0.04$	$0.16 \pm 0.04$	$0.13 \pm 0.04$
	2	$0.16 \pm 0.05$	$0.18 \pm 0.04$	$0.14 \pm 0.04$
10	1 control	$0.11 \pm 0.03$	$0.13 \pm 0.03$	$0.10 \pm 0.03$
	2	$0.13 \pm 0.03$	$0.14 \pm 0.03$	$0.13 \pm 0.03$
11	1 control	$0.12 \pm 0.04$	$0.14 \pm 0.04$	$0.14 \pm 0.03$
	2	$0.12 \pm 0.03$	$0.14 \pm 0.03$	$0.11 \pm 0.03$

<sup>a</sup> See the irradiation schedule shown in Fig. 1.

<sup>b</sup> The average size of the melanosomes was determined by measuring the longest cross diameter (10).

overall impression is that there is no definite increase in the number of single melanosomes during PUVa treatment. Even so, larger melanosomes tended to occur in singles whereas smaller ones tended to be complexed and this was true in both control and PUVa-irradiated skin (Table III).

## DISCUSSION

The size of melanosomes is under genetic control and is probably governed by several loci (18). It has been shown that in Caucasoids, Mongoloids and

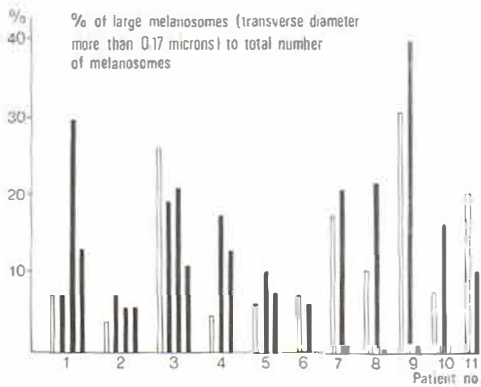


Fig. 9. The percentage ratio of large melanosomes (transverse diameter more than  $0.17 \mu\text{m}$ ) to the total number of melanosomes, computed for each specimen, is indicated by each column (white columns: control before treatment).

American Indians the melanosomes are rather small and that groups of two or more are aggregated within membrane-bound complexes in the epidermal keratinocytes (20). On the other hand, melanosomes in negroid skin (20) and those of Australian aborigines (11) are larger and are individually dispersed in the cytoplasm. Light- and medium-complexioned Blacks have melanosomes of intermediate size and distribution (14, 22), and a similar situation is true of Caucasoid skin, when patients with varying ethnic background, degree of pigmentation, and various pigmentary disorders are examined: large melanosomes are found to be singly distributed, whereas small melanosomes occur in complexes (10).

Our results do not show any significant change in melanosomal size or distribution in the PUVA-treated skin and thus support the preliminary observations made by Jimbow et al. (7). The wide variation in size of normal melanosomes, the regional differences and the possible change in melanosomal size at different time intervals preclude any conclusions as to the slight enlargement of melanosomes following PUVA therapy in some of our patients. Furthermore, the Caucasoid pattern of melanosomal distribution is by no means absolute and patients 3 and 9 are good examples to prove this point. In their non-exposed control skin a significant number of large single melanosomes was found, yet they both denied having had any sun-exposure or exposure to artificial ultraviolet sources prior to treatment. In addition to these two cases we have often come across a similar

melanosomal pattern, both in hyperpigmentation states in Caucasoids (10) and in normal non-exposed Caucasoid skin and this is probably more common than the literature seems to suggest.

The distribution pattern of large melanosomes in this study is in agreement with what Toda et al. (21), Wolff & Konrad (25) and Wolff (27) have reported. In both control as well as irradiated skin, large melanosomes tended to occur more in the discrete non-aggregate form in keratinocytes.

The melanocytes showed marked changes during the initial phase of PUVA treatment (clearing phase) (29) characterized by hypertrophy, increased activity and arborization of dendrites which became laden with fully melanized melanosomes and  $100 \text{ \AA}$  filaments. The fact that these broad and long dendrites frequently reached the third and even the fourth epidermal layer suggests that the melanosomal transfer to the keratinocytes occurs at higher epidermal levels in addition to the normally occurring transfer to the basal keratinocytes. Melanosomal production was enhanced and an overall increase in the number of melanosomes in the keratinocytes was noted. These findings confirm those of Jimbow et al. (7), and Kaidbey & Kligman (8) who, at the light microscope level, reported increased DOPA stainability of the melanocytes. It is worth noting that during maintenance treatment the activity of the melanocyte was markedly depressed, despite continued, albeit less frequent, PUVA exposures; after discontinuation of treatment, melanocytes returned to their normal state. No signs of abnormal mitosis or degenerative changes were noted in the melanocytes and no permanent morphological change was observed.

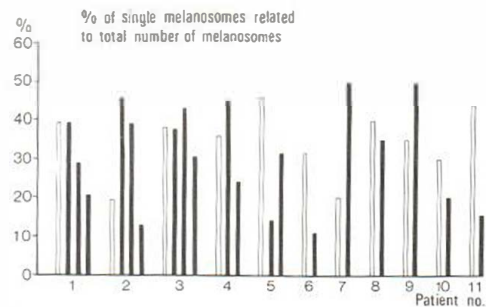


Fig. 10. The distribution pattern of melanosomes within the keratinocytes is shown. The columns indicate the percentage ratios of single melanosomes to the total number of melanosomes. White columns give values before start of treatment.

Although this does not exclude macromolecular alteration, it is reassuring that no recognizable structural defects are induced in the melanocytes by this treatment.

When Toda et al. (21) observed an increase in the size of melanosomes following treatment of Caucasoid skin with topical trimethyl-psoralen and UVA irradiation, they also noted that there was a concomitant shift in the distribution pattern, as these larger melanosomes tended to occur singly.

Similar changes were noted to occur in Mongoloid skin following prolonged and frequent sun exposure (21). It was concluded that the distribution pattern of melanosomes in keratinocytes is a size-dependent phenomenon and that the normal genetic racial expression can be altered by non-genetic factors, which could cause long-term gene derepression. Wolff & Konrad (25, 26), using polystyrene latex beads, showed that small beads were engulfed by keratinocytes in groups, whereas larger beads were taken up singly. This was further supported by experiments on guinea pig skin, using melanosomes from C57 mice and B16 mouse melanoma (28). The results showed conclusively that the formation of melanosome complexes is a size-dependent phenomenon. Conversely, the application of nitrogen mustard to the skin of Caucasoids with mycosis fungoides or psoriasis resulted in non-aggregation of melanosomes within the keratinocytes, yet without any concomitant increase in melanosomal size (4). Similar findings were noted by Allen & Hunter (1) following the prolonged use of topical fluorinated corticosteroids on the faces of Caucasoids suffering from chronic dermatitis. While this suggests that particle size is probably not the only factor determining the distribution, it provides additional evidence that racial characteristics of melanosome packaging can be altered by experimental methods (4).

In this study our main concern was to find out if significant changes in melanosomal size and distribution pattern occur after long-term treatment with PUVA which could be considered to herald somatic mutation and whether these changes are accompanied by abnormalities in the melanocytes. Summarized briefly, no significant change in melanosomal size or distribution was noted in long-term PUVA-treated skin. This does not exclude cytogenetic changes caused by PUVA in melanocytes, but it does show that if such changes occur they are not manifested in the morphology of the

pigment cells nor in their products. This is in contrast to observations following topical psoralens plus UVA (21, 12, 24), but the reason for this discrepancy is not clear. The failure to induce significant morphological changes with oral psoralens is perhaps due to a smaller quantity of psoralen reaching the melanocyte under conditions of systemic photochemotherapy.

On the other hand, in order to achieve a therapeutic effect, the smaller amount of psoralen reaching the skin via the systemic route has to be compensated by a higher dose of UVA delivered to the skin. At present, a study is under way to investigate whether the difference in melanocyte responses observed by Toda et al. (21) and by us is due to the differences in the psoralen concentration in the skin or whether other, still unknown, mechanisms are at work.

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