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INCREASED SIZE OF STAGE II AND III MELANOSOMES DURING PUVA THERAPY

Kristian Ree

Institute of Pathology, Electron Microscopical Laboratory, the National Hospital, Oslo, Norway

Abstract. Four different theories have been presented to show how early melanosomes are formed and at what stage in melanosome formation melanization starts. In the present investigation the perikaryon of melanocytes have been studied and the longitudinal and the transverse diameter of stage I–IV melanosomes estimated before and after PUVA therapy in 5 patients. An increase in the longitudinal diameter of stage II and III melanosomes was found, whereas no increase in stage I and IV melanosomes was detected. No increase in vesiculo-globular bodies was detected in stage I and II melanosomes after PUVA therapy, while in stage III melanosomes vesiculo-globular bodies appeared to have increased in number of PUVA therapy. It is concluded that during stimulated melanogenesis—as seen after PUVA therapy—an unknown mechanism is activated, causing increased amounts of structural proteins to be incorporated into each stage II melanosome. The vesiculo-globular bodies do not appear to take part in this process.

Key words: Coated vesicles; Melanogenesis; Melanosomes; PUVA; Vesiculo-globular bodies

PUVA therapy causes tanning of the skin by inducing a stimulation of melanogenesis. Melanogenesis is, however, a multistage process which usually involves both an increase in the number and size of melanocytes and an increased synthesis of melanosomes and melanin (2).

Melanosomes are formed in the melanocytes by the assembly of 1) structural proteins, 2) tyrosinase, 3) "membranes", and 4) possibly auxiliary enzymes and may be ontogenetically classified into stages I–IV (Figs. 1–4) (2). Several different theories have been presented to explain how melanogenesis is initiated (2, 6), how early melanosomes are formed and at what stage of melanosome formation melanization starts (2).

In the present investigation the perikaryon of epidermal melanocytes was photographed and studied before and after PUVA therapy in 5 patients and the longitudinal and the transverse diameters of all stage I–IV melanosomes estimated. The purpose of the study was to establish whether the stimulated melanogenesis observed after PUVA therapy alters the size of the melanosomes during any of the developmental stages I–IV and to correlate the results to current theories on melanosome formation. An increasing body of evidence supports the theory that tyrosinase is brought to the melansomes by coated vesicles (CV) and that these vesicles are visualized in the melanosomes as vesiculo-globular bodies (VGB) (2, 3, 10). VGB have been suggested to participate both in the process of melanization as well as in the organization of the protein matrix of the melanosomes (4). A qualitative judgement of CV and VGB was therefore performed before and after PUVA therapy. A theory is presented as to how melanosomes are formed during stimulated melanogenesis.

MATERIALS AND METHODS

Patients

Five male caucasian patients (patients E. M., E. B., E. L., O. J. and K. H.) with psoriasis vulgaris covering 30–40% of their body surface were selected for the study. They all had type II–III skin, had never previously received PUVA therapy and had not received UV-irradiation for at least 3 months. All 5 petients had blue eyes. Before starting PUVA therapy a biopsy from a skin area not clinically affected by psoriasis was taken on the gluteal region 10 cm below the patient's left iliac crest. The biopsies were fixed and processed for electron microscopy as described below. Screening laboratory tests were examined and found to be normal.

PUVA therapy

The therapy regimen was in accordance with that used by Wolff et al. (12) using Sylvania FR 90T12 lamps (Waldmann 4000 whole body irradiation unit) (1) and 8-methoxy psoralen (Meladinin, Nyco, Oslo). The lamps were tested before each treatment with a Waldmann PUVA meter (1) giving an exposure time of between 40 and 60 minutes to achieve a dose of 10 J/cm^2. The patients received therapy three times a week. Twenty minutes after completing a 10 J/cm^2 irradiation a post-treatment biopsy (skin uninvolved by psoriasis) was taken at a distance of 2 cm from the pre-treatment biopsy. At the time

Table I. *Mean longitudinal and transverse diameters (in nanometers) of stage I, II, III, and IV melanosomes before and after PUVA therapy in 5 patients*

The results are based upon the following numbers of single measurements in each individual case: Melanosome type I: 9–33; type II: 10–44; type III: 126–326; type IV: 5–43

Melanosomes, stage		Patient E. B.		Patient O. J.		Patient K. H.		Patient E. M.		Patient E. L	
		Before	After	Before	After	Before	After	Before	After	Before	After
I	Longitudinal	165 ±50[b]	225 ±65	170 ±40 NS	180 ±65	175 ±55 NS	180 ±45	170 ±45 NS	210 ±75	165 ±60[d]	225 ±60
	Transverse	130 ±30[c]	175 ±50	150 ±35 NS	155 ±60	165 ±50 NS	165 ±30	125 ±30 NS	155 ±45	125 ±35[d]	180 ±35
II	Longitudinal	175 ±40[d]	275 ±85	150 ±45[c]	205 ±45	190 ±60[¶]	210 ±45	160 ±50[c]	240 ±85	185 ±55[c]	265 ±85
	Transverse	135 ±25 NS	130 ±35	125 ±25[a]	150 ±40	140 ±60 NS	140 ±40	125 ±30 NS	135 ±40	125 ±35 NS	145 ±40
III	Longitudinal	175 ±55[a]	205 ±80	170 ±45[d]	205 ±70	190 ±65[c]	220 ±70	190 ±70[d]	225 ±75	180 ±50[d]	240 ±85
	Transvere	120 ±30 NS	130 ±30	135 ±35 NS	135 ±35	140 ±30 NS	145 ±30	125 ±35 NS	135 ±35	135 ±35[d]	150 ±40
IV	Longitudinal	180 ±50 NS	180 ±70	180 ±50 NS	185 ±75	160 ±50 NS	175 ±70	210 ±85 NS	190 ±70	180 ±75 NS	235 ±85
	Transverse	120 ±15 NS	125 ±25	125 ±25 NS	120 ±35	115 ±25 NS	135 ±40	140 ±30 NS	130 ±35	110 ±45 NS	155 ±40

Student's *t*-test: NS = not significant; [a] $p<0.05$; [b] $p<0.02$; [c] $p<0.01$; [d] $p<0.001$.

of sampling the patients were just becoming free of their psoriasis (after 2½–3 months' therapy) and were about to start maintenance therapy. At this time the total UVA dosage for patient E. M. was 80 J/cm², E. B. 66 J/cm², E. L. 52 J/cm², O. J. 107 J/cm² and K. H. 145 J/cm².

Transmission electron microscopy

Lidocaine chloride 2% was injected subcutaneously and 10×5 mm boat-shaped biopsy samples were cut free with a scalpel and forceps and transferred to 2.5% glutaraldehyde in Sørensen's phosphate buffer (pH 7.3) at room temperature. The samples were trimmed and cut perpendicular to the skin surface into 2 mm cubes. After 4 hours the biopsies were rinsed three times in Tyrode solution (pH 7.3) and postfixed at 4°C for 2 hours in 1% osmium tetroxide in Tyrode solution (pH 7.3). The specimens were rinsed in Tyrode solution three times and dehydrated in a graded series of ethanol (30% for 15 min, 60% for 15 min, 90% for 20 minutes and 100% for 30 min, ×2) and in propylene oxide (30 min, ×2). The specimens were left overnight at 4°C in a 1:1 mixture of propylene oxide/Epon (Epon 812, Ladd, Burlington, Vermont). The mixture was replaced by Epon and 4 hours later the specimens were orientated and embedded in flat embedding moulds. The blocks were hardened for 3 days at 60°C. Silver-grey ultrathin sections were cut with a diamond knife and mounted on 75-mesh Formvar-coated copper grids. The sections were post-stained with uranyl acetate and lead citrate (9) and viewed in a Jeol 100 B transmission electron microscope.

From each of the 5 control and test biopsies, 3–5 blocks (blocks with satisfactory orientation) were chosen for semi-thin sectioning, 2 μm sections were cut perpendicular to the skin surface, stained with Toluidine blue 1% and examined under the light microscope. Random areas of the epidermis (above a dermal papillae) were trimmed out for thin sectioning. The sections used in the present study were the same as those used to study Langerhans cells previously reported (8). The ultrathin sections included the stratum basale, stratum spinosum, stratum granulosum and stratum corneum. Each block was sectioned 3–4 times and 50 μm were trimmed away between each ultrathin sectioning and different areas of the epidermis sectioned each time. From each ultrathin section, one grid (out of 3–5) was chosen at random. The first section on the grid observed in the electron microscope to be completely unobstructed by grid bars was surveyed for the presence of melanocytes. Whenever a melanocyte was observed to be sectioned through its nucleus the perikaryon was photographed part by part at a primary magnification of ×20 000. The pictures were photographically enlarged to ×60000. The magnification of the electron micrographs was checked using a magnification calibration grid. The melanosomes were classified into stages I–IV according to the definition outlined in the captions to Figs. 1–4. Every melanosome observed on the electron micrographs was thus classified and measured with a ruler with a precision of 0.5 mm. The measurements were carried out by a person not knowing the scope of the study. A qualitative judgement of the number of CV in the cytoplasm and VGB in the melanosomes was performed before and after PUVA therapy. A total of 258 electron micrographs were examined in the overall control group and 182 in the test group, giving a total number of measurements as listed in the legend to Table I. In the case of melanosome stage III the 20 largest measurements were extracted

Table II. *Mean longitudinal and transverse diameter (in nanometers) of the 20 largest stage III melanosomes before and after PUVA therapy in 5 patients*

The calculations are in each case based upon the 20 largest values chosen among 126–326 single measurements (Table I)

	Patient E.B.		Patient O.J.		Patient K.H.		Patient E.M.		Patient E.L.	
	Before	After	Before	After	Before	After	Before	After	Before	After
Longitudinal	230 ±45[a]	320 ±60	245 ±30[a]	360 ±40	285 ±40[a]	360 ±35	315 ±35[a]	365 ±45	255 ±45[a]	420 ±45
Transverse	150 ±25 NS	160 ±25	190 ±30 NS	200 ±20	190 ±30 NS	180 ±20	190 ±35 NS	180 ±15	180 ±30[a]	225 ±30

Student's *t*-test: NS=not significant; [a]$p<0.001$.

from the raw data in Table I and compiled in Table II. The results of the study were analysed statistically using mean, standard deviation and Student's *t*-test.

RESULTS

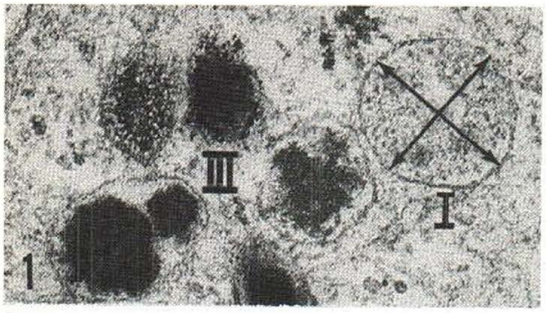
Melanosomes in all stages of development could be observed in the perikarya of melanocytes both, before and after PUVA therapy (Figs. 1–4). The relative frequencies of the different stages are listed in the caption to Table I. It is seen that stage III melanosomes were most frequently observed. Very few stage II melanosomes with only longitudinal filaments were seen, the overall majority of stage II melanosomes exhibited transverse cross-striations as depicted in Fig. 2.

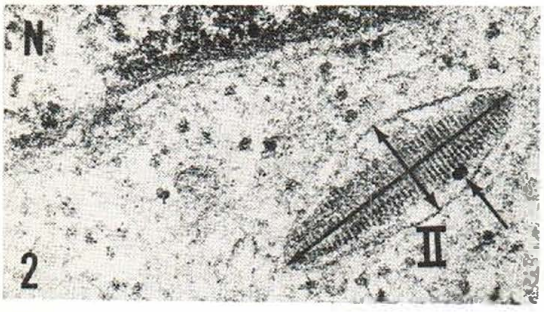
Difficulties sometimes arose as how to classify atypical melanosomes that did not fit into the general classification outlined in the captions to Figs. 1–4. Spherical melanosomes were sometimes seen containing few filaments concomitantly with electron-dense material (Fig. 5). Although these melanosomes had some of the characteristics of stage I melanosomes they were classified as stage III due to their content of electron-dense material. Melanosomes were also seen with a well developed cross-striated matrix and a granular, electron-dense longitudinal structure beside the matrix (Fig. 6). These melanosomes had most of the characteristics of a stage II melanosome, but were classified as stage III due to their content of electron-dense material. A rather similar type was also seen showing heavy electron densities in half the melanosomes and moderate electron densities in the other half (Fig. 7). Longitudinal striations were seen in the electron-dense part and transverse striations in the more electron-translucent parts (Fig. 7). These melanosomes were classified as stage III. Longitudinal striations were also observed in moderately electron-dense melansosomes (Fig. 8). These were classified as stage III. The classification applied in the present study thus resulted in stage III melanosomes as being the most heterogenous group.

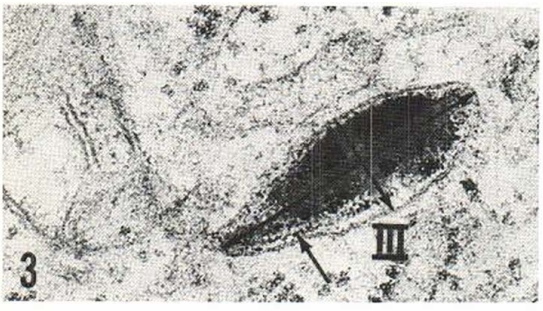
An increase in both the longitudinal and the transverse diameter of stage I melanosomes was observed after PUVA therapy in 2 of the patients, while for the others no change in the size of stage I melanosomes was detectable (Table I). For stage II and III melanosomes an increase in the longitudinal diameter was evident after PUVA therapy in all 5 patients. The transverse diameter of stage II and III melanosomes was unchanged in 4 of the patients and increased in one. The size of stage IV melanosomes was not found to change after PUVA therapy in the present study. CV were seen in increased number in the cytoplasm of melanocytes after PUVA therapy and in some melanocytes they were particularly numerous (Fig. 9). VGB were generally not seen within stage I and II melanosomes, but one or two could sometimes be observed both before and after PUVA therapy (Figs. 2 and 5). In stage III melanosomes VGB were frequently observed and they appeared to have increased in number after PUVA therapy. From Table II it is seen that by selecting the 20 largest stage III melanosomes for analysis a significant increase in the longitudinal diameter of these melanosomes was registered after PUVA therapy.

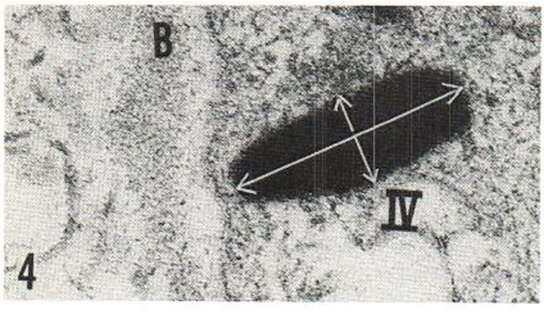
DISCUSSION

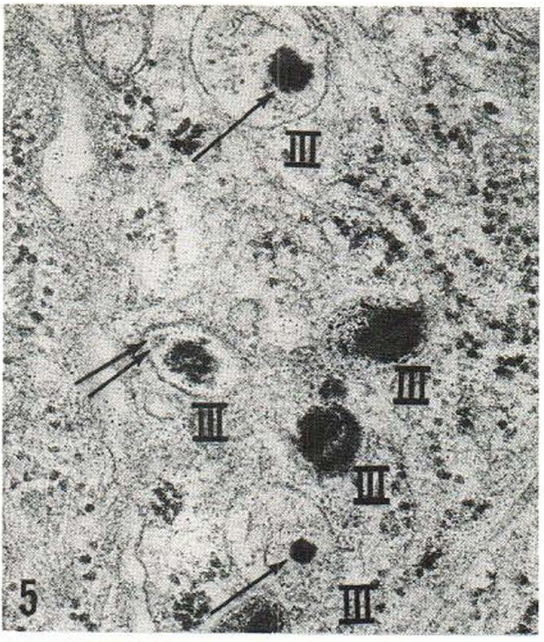
The size, shape, number and colour of melanosomes is under genetic control (2, 4). Non-genetic factors, such as UV irradiation of PUVA therapy,

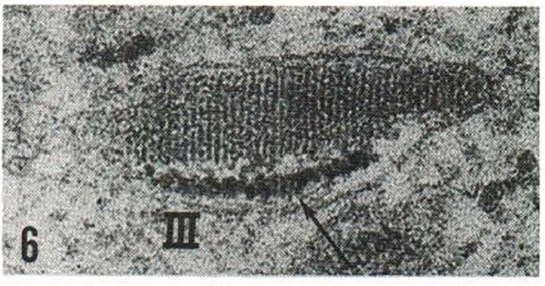












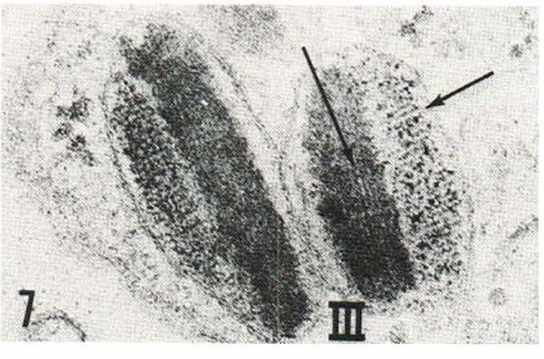


Fig. 1. Cytoplasm of a melanocyte showing a vesicle containing filamentous material (I). This structure was defined as a stage I melanosome and was used for diameter measurements (lines with two arrowheads). To the left different varieties of stage III melanosomes are seen (III) all of which were used for diameter measurements (see Table I and II). (Patient E. L. after PUVA therapy.) ×80000.

Fig. 2. Melanosome exhibiting transverse cross-striations (II). This structure was defined as a stage II melanosome and was used for diameter measurements (lines with two arrowheads) (see Table I). A 40 nm electron-dense body is seen close to the melanosome matrix (arrow). This structure represents a vesiculo-globular body. Nucleus (N). (Patient E. L. after PUVA therapy.) ×80000.

Fig. 3. Melanosomes containing faintly visible longitudinal striations (arrow) covered by electron-dense material (III). This structure was defined as a stage III melanosome and was used for diameter measurements (lines with two arrowheads). (See Table I and II.) (Patient E. L. after PUVA therapy.) ×80000.

Fig. 4. Uniformly electron-dense melanosome seen close

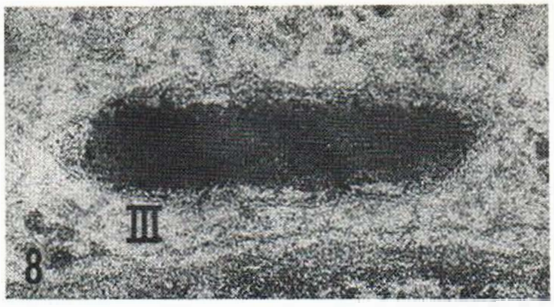


Fig. 8. Moderately electron-dense melanosome containing longitudinal striations and a coating membrane. This melanosome was classified as stage III. (Patient E. L. after PUVA therapy.) ×120000.

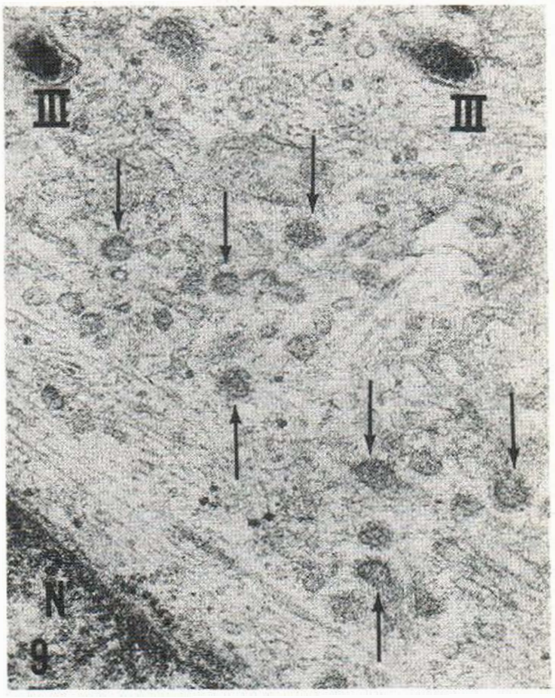


Fig. 9. Numerous moderately electron-dense vesicles observed in increased numbers in the cytoplasm of melanocytes after PUVA therapy. The vesicles were coated by diffusely outlined moderately electron-dense material and were defined as "coated vesicles" (arrows). Nucleus (N). Melanosomes stage III (III). (Patient O. J. after PUVA therapy.) ×60 000.

may, however, increase the size of fully melanized stage IV melanosomes (5, 7, 11, 13, 14, 15). The fact that we failed in the present study to demonstrate any increase in the size of stage IV melanosomes after PUVA therapy may be explained by the experimental procedures applied: 1) while previous investigators have studied stage IV melanosomes either present in the dendritic parts of the melanocytes or present in the keratinocytes, we have in the present study focused on the developmental stages present in the perikaryon of the melanocytes; 2) during stimulated melanogenesis an increased transport of melanosomes takes place from the perikaryon to the tips of the dendrites (2), which may have influenced the range of melaosomes present in the perikaryon; 3) to obtain a precise classification of melanosomes into stages (Figs. 1–4) it was decided to restrict to stage IV only those melanosomes that were uniformly electron dense. These melanosomes were more frequently observed without a coating membrane (Fig. 4).

The present study establishes that during PUVA therapy an increase in the size of stage II and III melanosomes takes place. Stage II melanosomes are composed mainly of structural proteins and membranes. Thus it appears that during stimulated melanogenesis, increased amounts of structural proteins and membranes are incorporated into each melanosome. As we did not find any increase in VGB in stage II melanosomes after PUVA therapy we postulate that VGB probably do not participate in the process of incorporating structural proteins

to the basement membrane (B). This structure was defined as a stage IV melanosome (IV) and was used for diameter measurements (lines with two arrowheads). (See Table I.) (Patient E. M. before PUVA therapy.) ×80 000.

Fig. 5. Spherical vesicles containing some filaments concomitantly with electron-dense material (arrows). These atypical melanosomes were classified as stage III due to their content of electron-dense material (III). Three other stage III melanosomes are also depicted (III), one of which contains vesiculo-globular bodies (double arrows). (Patient E. L. after PUVA therapy.) ×80000.

Fig. 6. Melanosome containing a well developed matrix with cross-striations. Close to the matrix a granular, more electron-dense longitudinal structure is seen (arrow). This melanosome was classified as stage III due to its content of electron-dense material. (Patient E. L. after PUVA therapy.) ×120 000.

Fig. 7. Two melanosomes, one-half of each containing electron-dense material and the other half, less electron-dense material. The electron-dense material exhibits longitudinal striations (arrow), while the less electron-dense material exhibits transverse cross-striations (arrow) partly covered by electron-dense granules. These melanosomes were classified as atypical stage III melanosomes. (Patient E. L. after PUVA therapy.) ×120000.

into the melanosomes. Two independent mechanisms are probably brought into action during stimulated melanogenesis: one mechanism induces an increased production of structural proteins, while the other induces an increased production of coated vesicles containing tyrosinase. The coated vesicles are probably not programmed to fuse with any particular developmental stage of the melanosomes, but may fuse by chance with any melanosome. This theory could explain the formation of the atypical melanosomes depicted in Figs. 5–7 and why some stage II melanosomes increase in size before melanization starts.

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K. Ree, M.D.
Institute for Pathology
Rikshospitalet
Oslo 1
Norway