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THE INFLUENCE OF PUVA TREATMENT ON THE SUBSTRUCTURE OF NEVOCELLULAR NEVI IN PSORIATIC PATIENTS

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Abstract. The influence of PUVA treatment on junction A-cell nevi in psoriatic patients has been analysed. After long-term PUVA therapy the nevocytes display an obvious increase in melanosome synthesis. The melanosomes are clearly polymorphous, without any predominant configuration. The structural organization of the melanosomes is profoundly aberrant. Formation of autophagosomes is conspicuous. The cytoplasmic filaments are displaced peripherally. No convincing signs of malignancy are observed electron microscopically. Light microscopical findings of a bridging proliferation of nevocytes between junction nests is a precautionary observation, however.

Key words: Cytoplasmic filaments; Melanosome; Nevus; PUVA treatment

Within the epidermis lying over the lamina basalis and in the epidermo-dermal border area, melanin-synthesizing cells occur either singly as melanocytes or are aggregated into cell nests when benign and are then designated pigmented cell nevi. These two cell types are regarded as having differing embryological origins (8, 11, 15), though some authors (3, 6) believe them to be identical cell types. The submicroscopic morphology of normally functioning melanocytes has been analysed many times (2, 9, 17, 21), whereas the ultrastructure of nevocytes appears to be less frequently reported (6, 14). The cells forming the pigmented cell nevi have submicroscopic structure similar to that of melanocytes. The melanosomes of both cell types are roughly comparable and the particularly rampant dendritic processes of melanocytes have been reported to resemble pseudopodic cytoplasmic processes (6) or virtual dendrites (4) but with an impaired capacity to penetrate and transfer.

The melanosomes of the normal melanocytes are ellipsoid in stages II-IV. From being spherical in stage I they become ellipsoid in stage II and display several filaments having a distinct periodicity. In stage III the filamentous structure is partially obscured by electron-dense material. In stage IV, the melanosomes, still ellipsoid, are heavily melanized and are therefore very electron dense. In normal melanocytes the size of melanosomes ranges between 700–900×150–300 nm, whereas the melanosomes of nevocytes (A-cells) measure 400–600×100–200 nm (15). The melanosomes, both of normal melanocytes and of nevocytes (A-cells), often seem to be located at the periphery of the cytoplasm and many are enclosed by a unit membrane. A phototoxic pigmentary response provoked by photosensitizers reveals an increase in melanosomal size (19) without any increase in the number of melanosomes when induced by topical psoralens and UVA. Systemic photochemotherapy with PUVA, however, does not induce significant changes in melanocytes and does not alter the distribution pattern within melanocytes.

Among the non-specific cell organelles a difference between melanocytes and nevocytes has been observed (6) namely that the endoplasmic reticulum is absent or, more probably, poorly developed in nevocytes, i.e. in UV-unstimulated cells.

The transfer mechanism of melanosomes from melanocytes to keratinocytes has been considered to be the result of cell organelle secretion (1), but epidermal cell culture indicates that the process is merely a type of cytophagocytosis (16). A corresponding process of cytophagocytosis of the pseudopodic extrusions of nevus cells does not appear to have been investigated.

Pigmented cell nevi may consist of various cell types, reflecting their topography and divided by Miescher & von Albertini (12) into A-, B- and C-types. The A-cells are epithelioid are located superficially and are found in both junction and compound nevi. Histochemically they display tyrosinase activity, in contrast to the spindle-

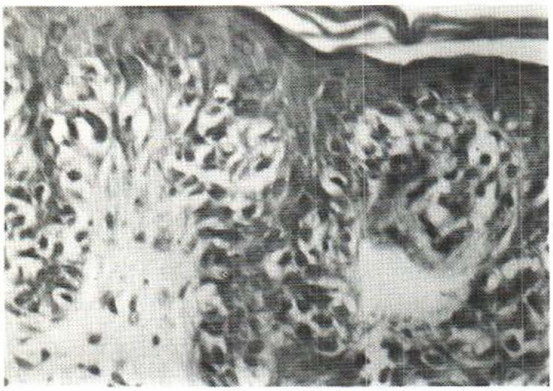


Fig. 1. Light microscopical appearance of junctional nevus from PUVA-treated psoriatic patient, showing the bridging proliferation of nevocytes. ×200.

shaped C-cells. B-cells have the capacity to synthesizie melanin, though under normal circumstances they do not. Thus A- and B-cells are both capable of forming the specific cell organelle, the melanosome, although at different stages of development in A- and B-cells, thus reflecting differing degrees of melanization (stages I–IV (18) and of the internal submicroscopic organelle differentiation (5).

Ultraviolet exposure at varying wavelengths, with or without the interference of psoralen compounds, produces a similar pattern of changes. As regards the melanocytes, an increase in melanogenesis is induced with a change of melanosome pattern and a switch from aggregated to non-aggregated presence is also distinguishable in keratinocytes. The melanocytic dendrites show prolific arborization. Both in the dendrites and otherwise throughout the cytoplasm, ubiquitous melanosomes are observed in different developmental stages. Those found in the keratinocytes are more numerous and larger and form a greater proportion of single melanosomes (7, 19).

MATERIAL METHODS

Twenty-one nevi were excised from 18 patients (18–48 years of age) kept on maintenance PUVA therapy for the control of psoriasis. The nevi were taken exclusively from the backs and from the lateral surface of the upper arm. They measured 0.3–0.6 cm in diameter. None of the tumours showed any clinical signs of malignancy. The mean dose of PUVA was 620 joules/cm^2, the highest dose being 2 100 joules/cm^2. The excisions were divided four routine histological and electron microscopical analyses.

The specimens for electron microscopy were fixed in 2% glutaraldehyde buffered with cacodylate buffer at pH 7 at 4°C for 6 hours. Post-fixation was carried out in 2% OsO_4 buffered with cacodylate buffer at pH 7 for 2 hours. The specimens were rinsed in the buffer solution and dehydrated in increasing concentrations of acetone and embedded in Spurr epoxy resin. Ultrathin sections examined in a Philips 400 electron microscope.

RESULTS

Light microscopy

In several routine light microscopical analyses, junction or compound nevi without signs of malignancy were found. The nevi consisted predominantly of A-cells rich in melanin. B-cells were seen only occasionally.

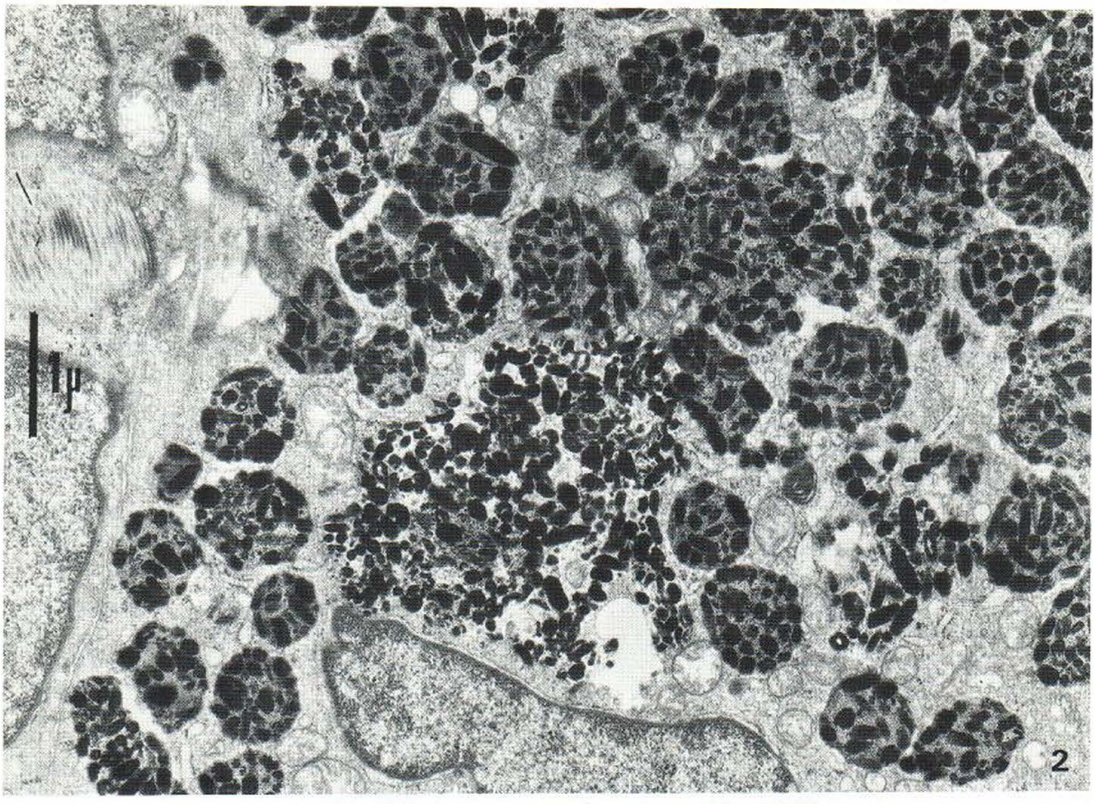
The melanocytes observed in juxta-position to the nevi exhibited very long dendrites, rich in melanin. Some nevi, however, of both junction and compound type and consisting mainly of A-cells, differed considerably from the usual structural architecture. Out of photonic microscopical view they translocate through a linear atypical bridging proliferation of nevocytes within the epidermo-dermal junctional area almost or even completely discplacing the basal epidermal cells. By light microscopy these proliferating nevocytes had an almost normal configuration.

In some cases, however, these cells are not conforming to A-cells, but displayed nuclear abnormalities and a tendency to pagetoid growth. Transformed nevi of this type are not dealt with in the present investigation but will be published separately (Fig. 1). Subepidermally, in juxtaposition to the nevi, small perivascularly arranged lymphocytic infiltrates were observed. More or less numerous melanophages were present.

Electron microscopy

The nuclei of the nevus cells and cytoplasm were often irregularly delineated (Fig. 2) and sometimes displayed villous projections which occasionally penetrated the lamina basalis. In such nuclei the

Fig. 2. Survey electron micrograph of part of nevocyte during PUVA treatment, showing cytoplasmic abundance of polymorphous melanosomes and autophagosomal structures. Endoplasmic reticula are present. ×16 560.
Fig. 3. Electron micrograph, displaying the pronounced polymorphism of melanosomes (*M*). Nuclear envelope (*NE*) appear considerably thickened. Cytoplasmic fibrils indicated by arrows. ×57 960.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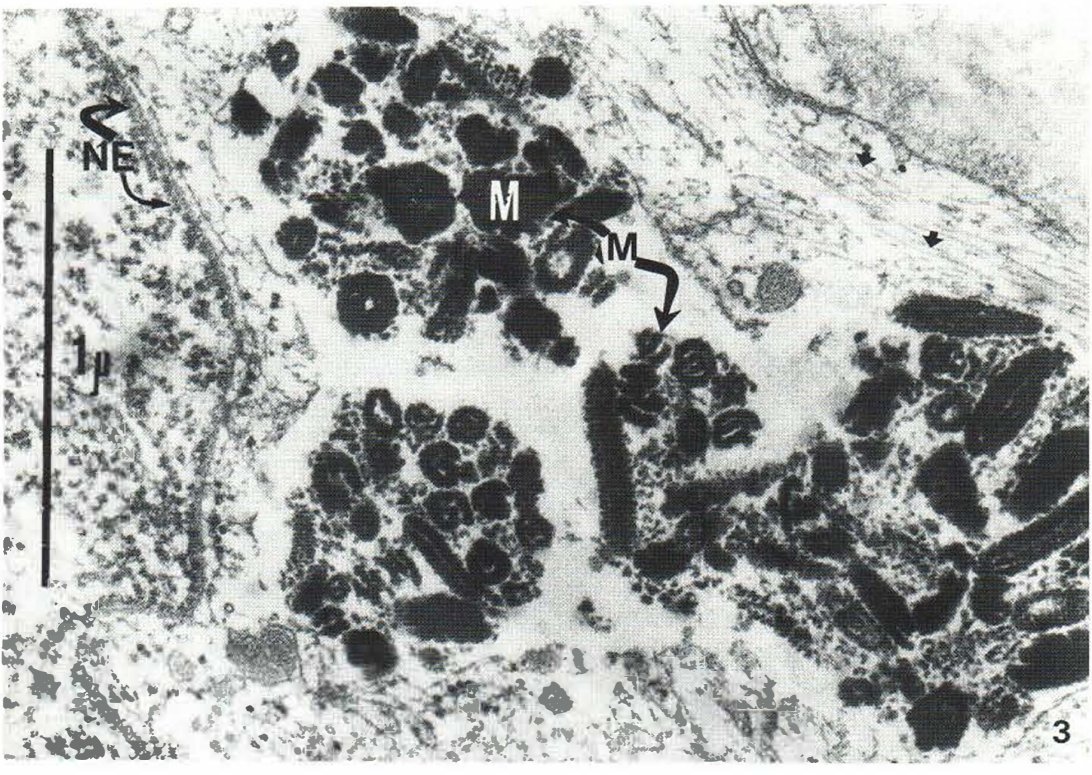
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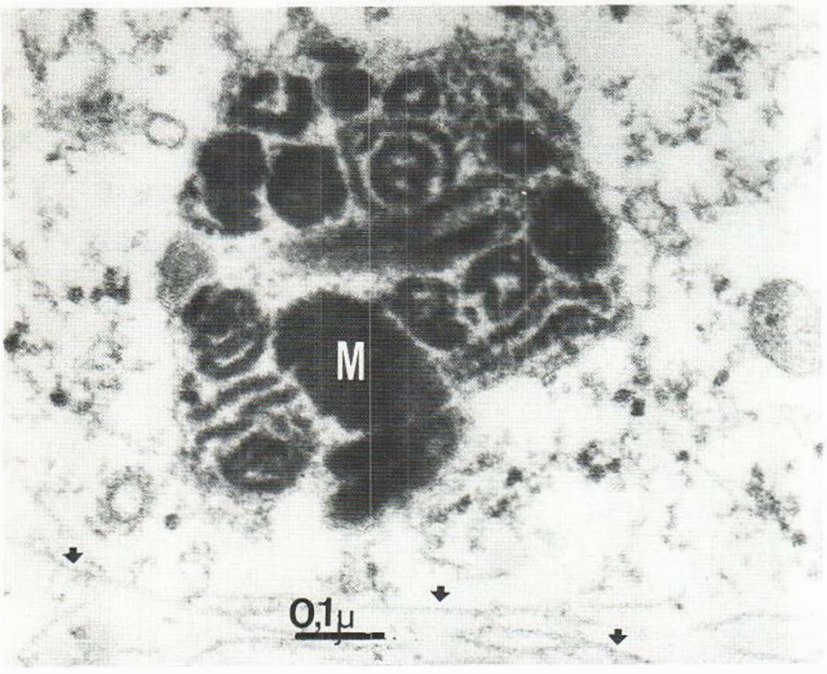

Fig. 4. High magnification of aberrant melanosomes. ×117 000.

chromatin was located mainly peripherally, adhering closely to the innerface of the nuclear envelope. The nucleoli sometimes appeared in numbers, but otherwise singly. The nuclear envelope was greatly thickened and had a predominantly amorphous structure, but here and there with indicative outer and inner leaflet. The thickness of the nuclear envelope was remarkably constant, measuring about 250 Å. Protruding from the outer leaflet, microfibrillar-like structures appeared to merge with the cytoplasm. The nuclear pores were not prominent (Fig. 3).

The cytoplasm contained relatively abundant, mostly normally configurated mitochondria, although some were observed to be swollen, presenting a pseudo-myeloid internal structure. However, some nevocytes contained mitochondria with an amorphous, fairly high electron scattering material and often more or less vacuolarly degenerated. There was an abundance of Golgi apparatuses. They appeared well developed and had very many vesicles, with a pre-eminence of the larger type. They were located mostly in the vicinity of the abundant melanosmes. The endoplasmic reticulum was well developed and ribosomes, both free and in polysomal configurations, were numerous. Normally shaped microtubules were also frequent. Centrioles were occasionally seen often in close proximity to the Golgi complex.

Careful analyses of the ubiquitous melanosomes of the irradiated A-cells revealed a remarkable polymorphism in size, shape and internal structure (Figs. 2, 3). The size varied widely, measuring 300–600×110–210 nm. The normal characteristic shape of longitudinally sectioned melanosomes, reminiscent of a rugby-football or ellipsoidal structure was seldom seen. The paucity of melanosomes in stages I–II was evident. The degree of melanization was considerable but showed marked variations. A categorization into normal, abortive, granular and lamellar melanosomes could not be applied to the present material due to the so obviously aberrant morphology.

The melanosomes were mostly located more centrally than peripherally and were very numerous. Within the cytoplasm of irradiated nevocytes, cytoplasmic fibrils occur, but the great abundance of melanosomes, whether singly or in clumps, translocated the fibrils mostly to the cytoplasmic periphery. Within some nevocytes, however, melanosomes were also ubiquitous in the more or less dendrite-like processes, definitely exceeding the number of melanosomes in non-irradiated nevocytes.

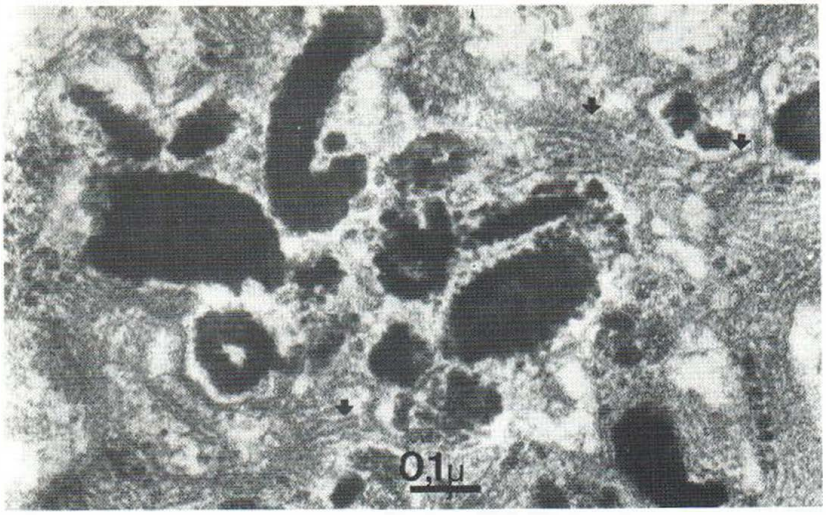

Fig. 5. Fish-hook-like and ring-shaped melanosomes. ×93 600.

The melanosomes were found individually dispersed in nevocyte cytoplasm, though more often they appeared in polymelanosomal configurations which may be either free or membrane-bound. Usually the single melanosomes were observed without a limiting structure, though very infrequently they can be enclosed in a sac of unit membrane. The presence of such an envelope appears to be more pronounced in certain nevocytes. Aggregates of mostly strongly melanized melanosomes were frequently found in cytoplasm of irradiated nevocytes. The aggregates vary considerably in size, as seen in sections (Figs. 2, 3, 4). They may contain between 3 and up to 40 melanosomes. Some of the clumps of the melanosomes were enclosed by a unit membrane and were mostly round or ovoid in appearance. Between the clustered melanosomes numerous small, strongly electron scattering particles measuring 7–15 nm were randomly dispersed. Within such clumps, melanosomes were decorously and sometimes extortionately undergoing degradation, indicating an autophagosomal property (Fig. 4).

The melanosomal structure in irradiated nevo-

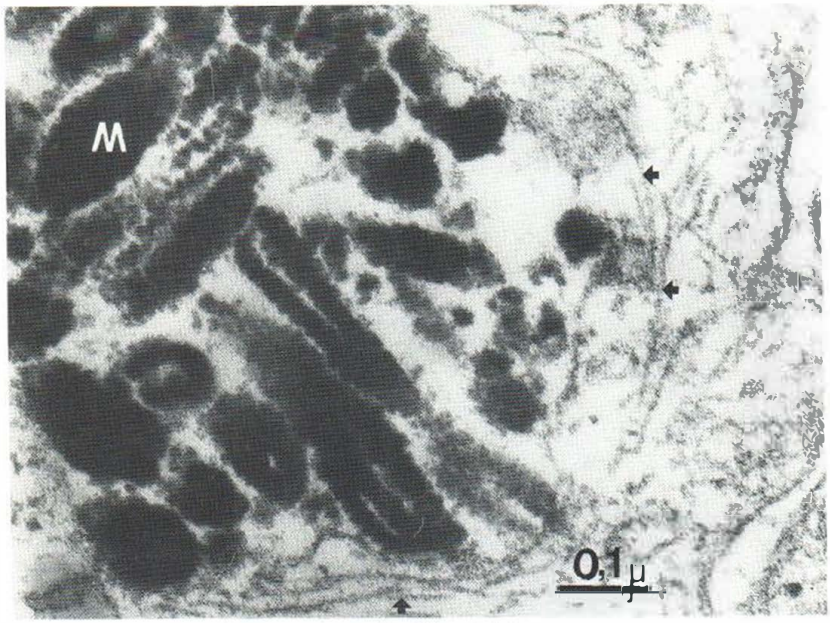

Fig. 6. Variations in melanosomal structure. ×117 000.

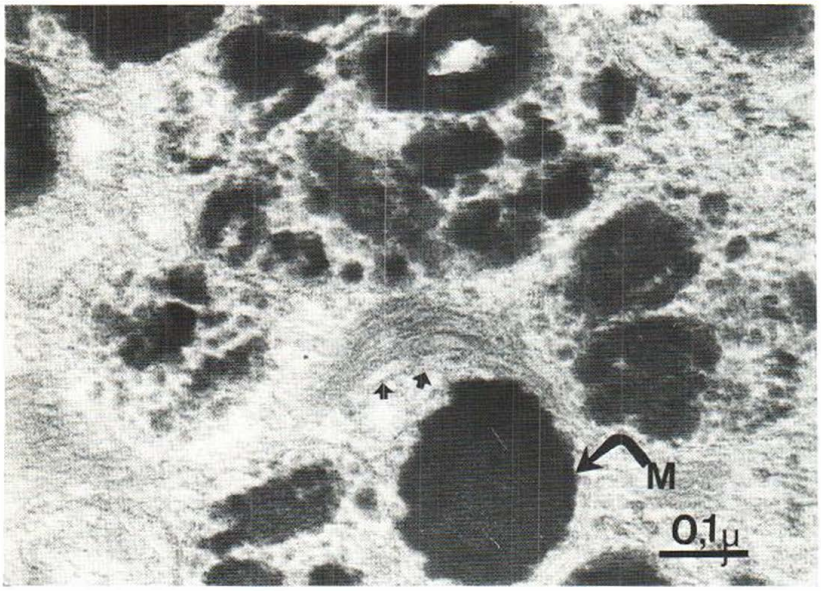

Fig. 7. Cross-sectioned melanosomes with an internal tubular substructure. ×117 000.

cytes was usually highly abnormal. They could not be categorized and subdivided into types according to established terminology. Their diversity was remarkable. Sometimes, however, nevocytes occurred which concomitantly contained normally differentiated melanosomes in various stages, although mostly highly melanized. Abnormal melanosomes longitudinally sectioned revealed a mostly cigar-shaped or more less ellipsoidal outline, though sometimes also rectangular (Figs. 5, 6). The internal structure of melanosomes thus sectioned consisted of closely packed, longitudinally extended profiles without obvious cross-linking structure reflecting pathological procreation suggesting a tubular structure measuring 25–30 Å (Fig. 8).

The melanosomal outline often displayed a unilaterally polar fimbriated divarication. Melanosomes sectioned lengthwise also exhibited a deficiency of procreation, exhibiting only a peripheral, high electron scattering structure often revealing periodicity and a central, more or less hollow core.

When sectioned obliquely the melanosomes

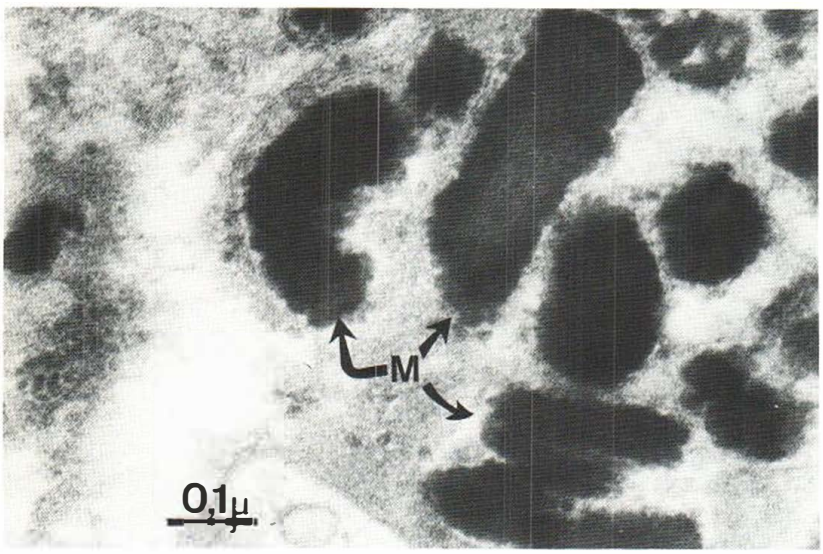

Fig. 8. Longitudinally sectioned melanosome with an internal tubular structure. ×117 000.

could present a "forceps"-like, sometimes divaricating or even more blurred outline. Occasionally such configurations enclosed an extended profile showing periodicity located singly, centrally, or connected with the convergent part of the "forceps"-like structure (Figs. 3, 6). Some of the melanosomes additionally had a comma-shaped or fish-hook-like outline with or without a central profile (Figs. 5, 8).

Cross-sectioned melanosomes were predominantly round, though slightly facetted or multiangular forms occurred. Some had a homogeneous apperance, occasionally with a less electron-scattering centre. The most frequent appearance was a more or less regular ring-shape (Figs. 3, 4, 5, 7). Very often the ring-shaped melanosomes had another concentric circular structure of high electron density. Sometimes even two internal annular structures, centrally connected, occurred (Fig. 4). Repeatedly, central highly electron scattering verticils were present within the ring-shaped melanosomes (Fig. 3). The paracrystalloid pattern observed in longitudinally sectioned melanosomes was also present and then of even more tubular appearance and of the same diameter as earlier mentioned (Fig. 7).

DISCUSSION

Even though this is not an investigation into the frequency of nevocellular nevi in psoriatic patients, the collecting of the present material has shown that the occurrence of nevi of this type is lower than among a normal population.

Light microscopically the investigated nevi are often accompanied by a more or less pronounced inflammatory, lymphocyte-dominated infiltration. The significance of this infiltrate seems uncertain but is nonetheless remarkable.

The cells of those nevi are predominantly of the A-type. Light microscopically the A-cells of the present material do not reveal signs or marks of atypical morphology. No appreciable increase in mitotic activity could be observed. A remarkable proliferation of melanin synthesizing cells can sometimes be observed propagating in the basal layer of the epidermis, suggesting a connection between two or more nevus junction nests. The biological significance of this undoubted pathological bridging proliferation as being a sign of enhanced cellular activity and its relation to possible early malignant transformation is beyond the scope of this study and will be further analysed.

Submicroscopically there was marked evidence of stimulation by UV-light, causing evident hypertrophy of the Golgi apparatuses and endoplasmic reticula of the granular type. Centrioles were frequently present. The obvious occurrence of centrioles credentially indicates a prelusion of a mitotic activity. Scanty information concerning the ultrastructure of malignant, pigment-producing cells indicates an increased occurrence of centrioles.

The cytoplasmic filaments usually abundant in normal melanocytes and in non-irradiated nevocytes were observed in the present material, though less frequently and also peripherally displaced. This might imply an altered functional activity influencing the intracellular melanosomal movement.

The findings concerning cytoplasmic filaments are similar to the results about distribution the pattern of cytoplasmic filaments during UV-mediated melanin pigmentation and the role of 100 Å filaments according to Jimbow & Fitzpatrick (10). However, in UV-stimulated nevocytes, no obvious elongation of cytoplasmic processes occurs and there is no apparent transportation of melanosomes.

The irradiated nevocytes generally contain numerous melanosomes, in comparison with non-irradiated cells. In neoplastic, malignant-transformed nevocytes, melanosomes are superabundant compared with benign melanin-synthesizing cells. However, malignant cells, though presenting a considerable polymorphism of melanosomes, usually provide a certain predominating type. In the present material the melanosomes reveal a striking polymorphism of various outlines, always without predominance of any certain type. This undoubted redundancy in the synthesis of melanosomes having an aberrant shape is an expression of UV-simulation, but without signs of malignant transformation. The synthesis of melanosomes, as judged by their accumbency to the Golgi apparatuses and endoplasmic reticula, seems to follow normal pathways, although terminating in aberrantly outlined forms. The degree of melanization of the polymorphous melanosomes seems fairly high. Consentaneous to the UV exposure, the melanosomes are abundant. They are clearly aggregated into clumps, with very numerous organelles. These presumably represent autophagosomes. Within such configurations, various stages of degradation of melanosomes are ob-

served. Some autophagosomes are to a great extent filled with a granular material. Clumping of a similar kind is a more characteristic feature of keratinocytes but also occurs occasionally in malignant transformation of melanocytes/nevocytes of malignant melanomas. Judging from the present material, with its enhanced melanosome synthesis, there is additonally a lack of transfer mechanism of the organelles, resulting in the formation of autophagosomes.

The ultrastructural analyses have thus not revealed any convincing evidence of malignant transformation of nevocytes due to ultraviolet influence. The light microscopical observations of bridging proliferation between junctional nevus cell nests is, however, a precautionary observation. This amphibologic phenomenon will be further studied submicroscopically.

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