Effect of Ultraviolet on the Distribution Pattern of Microfilaments and Microtubules and on the Nucleus in Human Melanocytes

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INTRODUCTION

Increased melanin pigmentation which occurs after exposure of human skin to sunlight or to ultraviolet light (uv) from artificial sources is familiarly known as "tanning." Tanning of the skin involves two distinct biological phenomena (1) immediate tanning (IT), (sometimes called immediate pigment-darkening reaction), and (2) delayed tanning (DT). IT is optimally produced by both long uv (320–380 nm) and visible light (400–700 nm), and DT is optimally stimulated by exposure to the so-called sunburn spectrum (290–320 nm) and to a lesser extent by exposure to longwave uv and visible radiation (λ > 320 nm).

IT can best be seen in pigmented individuals or in the previously exposed (tanned) areas of fair-skinned individuals. The skin begins to be pigmented within 5–10 min of midday summer-sun exposure and becomes maximally pigmented after 1 hr of irradiation. The pigmented areas, when withdrawn from exposure to light, fade rapidly within the first 30 min, and thereafter the color usually fades gradually, so that after 3–4 hr, the irradiated areas are barely hyperpigmented. Sometimes, however, after prolonged sun exposure between 90 and 120 min, residual hyperpigmentation may be visible for as long as 24–36 hr, after which time newly synthesized melanin (new melanogenesis) begins to hyperpigment the skin.

Until recently, IT was said to result from changes in melanosomes already existing in the melanocytes and keratinocytes and involved (a) photooxidation of preformed melanin, through the generation of semiquinonelike free radicals in the melanin polymer (1, 2), (b) changes in distribution pattern of melanosomes in basal and suprabasal keratinocytes of the epidermis, and (c) no apparent increase in the number of melanosomes (1, 3). On the other hand, DT was described as resulting from new synthesis of melanosomes and from changes in the melanocytes and keratinocytes (3). It involved (a) an increase in number of functioning melanocytes as a result of proliferation of melanocytes or of activation of melanocytes from the inactive states, or of both, (b) an increase in tyrosinase activity and an increase in the rate of melanization, and (c) an increase in the transfer

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of melanosomes into keratinocytes as a result of the increased synthesis of melanosomes within melanocytes (2, 3). Compared with the relatively well-known mechanism of DT, little is known about IT, particularly concerning functional changes in melanocytes during IT reaction.

This study of caucasoid, mongoloid, and negroid skin was directed to (a) characterization of ultrastructural events in melanocytes before and after sun exposure of the skin, and (b) the delineation of the relation of these events to the changes in the four biological processes of melanin pigmentation: viz., formation, melanization, transfer, and degradaton of melanosomes. In this paper we shall cover only the newer concepts concerning the photobiology of IT. Data pertaining to DT will be published elsewhere.

MATERIALS AND METHODS

Ultrastructural changes in melanocytes and keratinocytes during IT were studied on six adult volunteers, among whom were four caucasoids, one mongoloid, and one negroid. The site of biopsy specimens before and after irradiation involved (a) unexposed skin of buttock, and (b) habitually exposed (tanned) skin of forearm (lateral, as well as medial aspects).

A. Induction of IT

Immediate tanning reactions were induced in three different ways, using two light sources:

1. Buttock skin under artificial light (xenon arc). The gluteal areas of four subjects (two caucasoid, one mongoloid, and one negroid) were exposed to long-wave uv and visible radiation (340–640 nm) emitted by a 150-W xenon arc lamp. Wave-lengths shorter than 340 nm and longer than 640 nm were filtered out by insertion of appropriate Corning glass and dichroic filters in the path of the collimated light beam. The radiation dose impinging on the skin surface was 21.25 mW.sec/cm² which was delivered in 20 min. Immediately after irradiation, skin biopsy specimens of the unexposed (control) and exposed regions were obtained by Thiersch’s operation (excision of skin above the subpapillary layer of dermis) under a local block anesthesia using the sterile solution of lidocaine (Xylocaine) without epinephrine. In all, eight biopsy specimens were obtained from the four subjects.

2. Forearm skin under sunlight. The dorsal antibrachial areas of two caucasoids and the volar antibrachial areas of one caucasoid and one mongoloid were irradiated for 30 min to summer sun at midday. Each subject had two sites for sun exposure: (a) Site 1 received the whole spectrum of unfiltered solar radiation (λ > 290 nm), (b) Site 2 received filtered light of 340–640 nm. Control sites were left covered with black papers and highly reflecting aluminum foils during the period of sun exposure. Skin biopsy specimens from the unexposed (control) and sun-exposed sites were obtained immediately after irradiation by Thiersch’s operation.

B. Electron Microscopy

The tissues obtained by Thiersch’s biopsy were prefixed at room temperature for 2 hr with a mixture of 2.5% formaldehyde and 2.5% glutaraldehyde made in 0.1 M cacodylate buffer, pH 7.2. The tissues were then postfixed for 2 hr with 2% osmium tetroxide made in 0.1 M cacodylate buffer, pH 7.4, at room tempera-
EFFECT OF UV ON HUMAN MELANOCYTES

The fixed tissues were stained with saturated uranyl acetate made in 0.1 M veronal buffer, pH 7.2, for 20 min at room temperature. They were then dehydrated in increasing concentrations of alcohol, embedded in epoxy resins, and ultrasectioned by LKB microtome attached with a glass or diamond knife. The sections were stained again with lead citrate and examined under a Siemens Elmiskop I-A microscope.

RESULTS

A. Ultrastructural Characteristics of Buttock Skin Before and After Induction of IT by Long-Wave UV and Visible Light (340–640 nm)

1. Nuclei and nucleoli in melanocytes. In the buttock skin of the unexposed, fair-skinned (relatively hypopigmented) caucasoid subjects, the nuclei were usually round or oval (Fig. 1), whereas in the dark-skinned caucasoids, mongoloids, or negroids, the nuclei were relatively more indented. The nucleoli in the melanocytes were usually less prominently seen in all races. The heterochromatin was characteristically visible along the periphery of the nuclei and rarely seen around the nucleoli (Figs. 1 and 4). Exposure of the gluteal skin in all the subjects after long-wave uv and visible irradiation caused definite changes in the ultrastructure of the nuclei and nucleoli. When compared with the unexposed region, the nuclei became more indented and the nucleoli became more prominent (Figs. 3 and 5). The random counting of nucleoli from 50 melanocytes with nuclei revealed a definite increase in number of nucleoli after induction of IT (Table 1). The distribution pattern of heterochromatin in the nuclei, however, remained unchanged. The aggregation of the heterochromatin around the nucleoli showed no remarkable changes after IT.

2. Microfilaments and microtubules in melanocytes. Two ultrastructurally different filamentous organelles, one microfilaments and the other microtubules, were recognizable in the melanocytes of both the unexposed and the exposed skin. The microfilaments were about 100 Å in diameter. Microfilaments, measuring about 50 Å in diameter, which have been reported in various tissues, could not be seen (4–8). These 100-Å microfilaments were more numerous than the microtubules, which measured about 250–270 Å in diameter and exhibited a center of low density with a characteristic hollow appearance.

a. Microfilaments and Microtubules before IT. The microfilaments were abun-
dantly present in the melanocytes of the unexposed skin of all subjects and were characteristically located around the nuclei. These microfilaments were rarely seen along the periphery of the cytoplasm and in the dendritic processes (Figs. 1 and 2). They usually aggregated and formed bundles of filaments that were nearly parallel or slightly intermingled with each other (Fig. 2). The bundles of these microfilaments ran long or short courses along the nuclei and virtually formed a ring around the nuclei. They occupied an extensive area of the cytoplasm appearing to push the other organelles (e.g., mitochondria, endoplasmic reticulum, and melanosomes) toward the periphery of the cytoplasm. This perinuclear aggregation of microfilaments was particularly prominent when the nuclei were round or oval (Fig. 2). Dispersion of the melanosomes was rarely seen within these bundles of microfilaments. This particularly perinuclear distribution of microfilaments is the distinguishing feature of a melanocyte in the unexposed region of the skin. Melanocytes that had indented nuclei (e.g., in the unexposed skin of pigmented
FIG. 1. A low-power view of melanocytes (MC) of unexposed buttock skin from a fair-skinned caucasoid. The melanocytes contain relatively large, round or oval nuclei with little indentation. They contain few dendritic processes. Very few melanized melanosomes can be seen. Around the nuclei of the melanocytes, dense aggregates of fine filamentous structures of microfilaments (arrow) can be seen. Melanized melanosomes can be seen in small number in nonaggregated or aggregated states (×4740).

FIG. 2. A high-power view of a melanocyte encircled in Fig. 1. A fine network of microfilaments with a parallel orientation or a slight intermingling can be seen around the nucleus. The microtubules running a short course can be seen (arrow). Melanosomes are in an unmelanized stage of development (×43,200).

FIG. 3. Buttock skin of the subject shown in Figs. 1 and 2 after irradiation with longwave uv and visible light (340–640 nm) emitted by a xenon arc lamp. In contrast to the unexposed skin, the melanocyte of the irradiated skin contains prominently visible, well-developed dendrites. The nucleus (N) becomes more indented. The mitochondria become more prominent and enlarged. The bundles or networks of microfilaments are now prominently visible along the periphery of the cytoplasm and in dendritic processes (arrow). They are less prominent around the nucleus. The melanosomes in the keratinocytes also appear to be increased in number. The lipid droplet (LD), which is hardly observed in an unexposed buttock skin, can also be seen (×18,205).
**FIG. 4.** A normal buttock melanocyte of a heavily pigmented caucasoid subject before irradiation. The melanocyte contains a large oval nucleus and a small nucleolus (NN) that is not associated with heterochromatin. Around the nucleus, the bundles or networks of microfilaments, which run long or short courses, can be seen (arrow). Melanosomes (48) in various developmental stages are abundantly present (×17,500).

**FIG. 5.** A melanocyte from the buttock skin after irradiation with long-wave uv and visible light. The biopsy was obtained from the same subject shown in Fig. 4. The nucleus of the melanocyte (N) becomes more indented. Microfilaments that were seen in an aggregated form around the nucleus before exposure are hardly seen in the perinuclear region. The bundles or fine meshworks of microfilaments are now seen in the dendritic processes (arrows). The number of melanosomes (30) are greatly decreased. The melanosomes are mostly in the fully melanized stage (Stages III and IV) (×10,450).

**FIG. 6.** A high-power view in a portion of dendritic processes of the melanocyte. The biopsy was obtained from the same caucasoid subject shown in Figs. 4 and 5. The bundles of microfilaments that run relatively long courses can be seen in the center of dendrites. The melanosomes are closely intermingled with or encircled by the fine networks of microfilaments (×35,400).
TABLE 1
Effect of Longwave UV Plus Visible Light (λ > 320 nm) on Nucleoli and Nucleoli-Associated Heterochromatin of Melanocytes

![Graph showing the effect of exposure on melanocytes](image)

| Site: FOREARM | BUTTOCK |
|--------------|---------|
| Exposure: 30 min. | 20 min. |
| λ > 320 nm | λ > 320 nm |

*Based on calculations of 50 melanocytes with nuclei.

U = Unexposed
E = Exposed

caucasoids, mongoloids, and negroids) also showed abundant bundles of microfilaments around the nuclei (Fig. 4) and inside the indented portion of nuclei. Cross sections of these bundles of microfilaments showed that each contained an aggregate of 20–30 microfilaments. Occasionally, however, a large bundle with as many as 236 microfilaments was observed.

The microtubules were less prominent as compared with the microfilaments in the unexposed buttock skin. These microtubules were also present around the nuclei and a few in the periphery of the cytoplasm and in the dendritic processes. In contrast to microfilaments, the microtubules did not form any bundles and did not run a long course (Fig. 2).

b. Changes of Microfilaments and Microtubules after IT. In IT reactions, stimulated by long-wave UV and visible light, the most noticeable change was observed in the distribution pattern of microfilaments and microtubules. The microfilaments, which were characteristically located around the nuclei of the unexposed skin, were rarely seen around the nuclei of the exposed melanocytes. They were prominently seen shifting to the periphery of the melanocytes and in the dendritic processes...
The aggregates or bundles of nearly parallel microfilaments were invariably seen streaming from the center of the cytoplasm toward the periphery and extending to the dendritic processes (Figs. 3 and 5).

The dendritic processes, which were highly prominent in the exposed skin, contained bundles of nearly parallel microfilaments. These filaments were often seen extending in long courses in the center of the dendritic processes (Fig. 5). In the dendritic processes of the exposed skin, a convergence of the melanosomes and the microfilaments could also be seen. They appeared to follow a course from the perinuclear region to the tip of the dendrites. The melanosomes appeared to be dispersed within bundles of the parallel microfilaments (Fig. 6). The melanosomes appeared to be encircled by the fine network of microfilaments. Melanosomes, not only in stage IV (highly melanized stage) but also in stages II and III (un-melanized and melanizing stages) could be seen in these evenly oriented, parallel bundles of microfilaments. Such a dispersion of melanosomes in the early stages of development in the dendrites could not be detected in the number of specimens examined from the unexposed regions of the body, including those that were obtained from highly pigmented subjects. In the tip of the dendrites, however, the microfilaments were also prominent. These microfilaments were intricately interlocked with each other, forming a fine “meshwork.” They did not show any direct attachment or contact with the outer membrane of the melanocyte. The melanosomes, in various stages of development, were abundant and were aggregated within the meshwork of microfilaments.

The microtubules, rarely seen in the unexposed skin, were more frequently seen after IT reaction. They were characteristically located along the periphery of the cytoplasm or in the dendritic processes. Contrary to the distribution pattern of microfilaments, microtubules were mostly seen as isolated tubules along the periphery of the dendrites. Occasionally in the protruding area of the cytoplasm, several (three to five) microtubules were seen intermingling with each other. Melanosomes were not seen in association with microtubules even after IT.

3. **Melanosomes in melanocytes before and after IT**.

   a. Melanization of melanosomes. The unexposed buttock skin of fair-skinned caucasoids contained very few melanosomes. Most of them were usually in Stages II and III of their development (Figs. 1 and 2). After induction of IT, however, we could more often detect melanosomes in Stages III and IV of their development. The melanocytes of unexposed skin of mongoloid, negroid, and pigmented caucasoid, however, contained many melanosomes in stages III and IV (Fig. 4). After induction of IT, many melanosomes predominantly in Stage IV were seen (Figs. 5 and 6). These changes in the degree of melanization of melanosomes suggest either a phenomenon of photooxidation of melanin or an increase in the melanin formation through tyrosinase-mediated oxidation of melanin precursors.

   b. Distribution pattern of melanosomes. In the unexposed skin, the melanosomes in the melanocytes of caucasoid, mongoloid, and negroid subjects were mostly aggregated around the nuclei, usually beyond the bundles of microfilaments. These melanosomes were rarely seen in the dendritic processes of melanocytes. After induction of IT, however, the melanosomes became prominently visible in the dendritic processes or along the periphery of the cytoplasm of melanocytes (Figs. 2, 5, and 6).

4. **Melanosomes in keratinocytes**. Depending upon the degree of skin pigmentation, the melanosomes in the unexposed skin were seen in varying numbers, pri-
TABLE 2

Number of Melanosomes per Basal Keratinocyte* Before and
After Immediate Tanning of Skin

| Site     | Exposure: 20 min. \( \lambda > 320 \text{ nm} \) | Exposure: 30 min. \( \lambda > 320 \text{ nm} \) |
|----------|-----------------------------------------------|-----------------------------------------------|
| BUTTOCK  | ![Graph](image1)                               | ![Graph](image2)                               |
| FOREARM  | ![Graph](image3)                               | ![Graph](image4)                               |

*Based on calculations of 50 keratinocytes with nuclei

Marly in the basal and suprabasal cell layers (Fig. 1). In the negroid skin, the melanosomes were predominantly seen as single particles distributed randomly in the cytoplasm of the keratinocytes. In fair-skinned caucasoids, melanosomes were few in number, and were predominantly seen in an aggregated pattern. In pigmented caucasoid skin and in mongoloid and oriental skin, melanosomes were distributed in a mixed pattern of either an aggregated or a nonaggregated form. After induction of IT, there was no recognizable change in their size and distribution pattern (i.e., they remained either in aggregated or in nonaggregated form). There was no definite change in the dispersion pattern of melanosomes in the keratinocytes. They were sometimes seen in the perinuclear or supranuclear areas, and at times they were seen randomly dispersed in the whole cytoplasm of the keratinocytes. The most noticeable change after IT was, however, the number of melanosomes per keratinocyte. A random count of melanosomes in 50 keratinocytes with nuclei located in the basal layer of four subjects was done before and after induction of IT. As can be seen in Table 2, each subject showed a statistically significant
increase \( P < 0.005 \) in the number of melanosomes per keratinocyte after the IT reaction. Because of sample variations, it was difficult to establish whether there was any change in the number of melanosomes in the keratinocytes above the basal layer.

B. Ultrastructural Characteristics of Habitually Exposed Forearm Skin Before and After Induction of IT by Sunlight (290–700 nm)

1. Before the IT reaction. The control skin biopsies of forearm, before irradiation revealed the following ultrastructural characteristics:

Unlike the unexposed buttock skin, the nuclei of the melanocytes were indented in all the subjects examined. The heterochromatin was more prominently aggregated around the nucleoli as well as in the periphery of the nuclei than those in the buttock skin (Figs. 7 and 11).

In contrast to the melanocytes of the unexposed buttock skin, which characteristically showed dense perinuclear aggregation of microfilaments, the microfilaments and the microtubules of the forearm skin were found around the nuclei, as well as in the whole cytoplasm (Figs. 7, 8, and 12). Some microfilaments were in nearly parallel stacks and some were arranged at random in a meshwork of filaments. All of these filaments ran short courses and were intermingled closely with well-developed organelles (mitochondria, endoplasmic reticulum, and melanosomes) (Figs. 8 and 12).

The melanocytes contained a relatively large number of melanosomes, mostly in stages III and IV of their development. These melanocytes also had prominent dendrites, well-developed Golgi apparatus, large mitochondria, and abundant free ribosomes (Figs. 8, 11, and 12).

The melanocytes were distributed not only in the center of the cytoplasm but also in the periphery and in the dendritic processes (Fig. 11).

The keratinocytes of forearm skin contained more melanosomes than did the buttock skin in the same subjects \( P < 0.005 \); Tables 2 and 4). These melanosomes were distributed either in the perinuclear or in the supranuclear areas. They were distributed in either nonaggregated or aggregated states according to their genetic background (Fig. 11).

2. After the IT reaction. The forearm skin after irradiation and induction of the IT reaction with filtered sunlight (340–640 nm) showed all the essential changes that were described in the buttock skin after induction of the IT reaction with long uv and visible light (340–640 nm). These ultrastructural changes included (a) shape and size of nuclei and nucleoli (Figs. 9 and 13); (b) an aggregation pattern of heterochromatin (Table 3); (c) a predominant aggregation of microfilaments and microtubules associated with melanosome dispersion (Figs. 10 and 14); and (d) an increase in the number of melanosomes per keratinocyte (Table 4).

There was, however, an additional ultrastructural change when the skin of forearm was irradiated with whole-spectrum sunlight (290–700 nm) that included the sunburn-producing spectrum (290–320 nm) (Figs. 15 and 16). This ultrastructural change was of a great interest and significance and was related to a specific change in the distribution pattern of nucleoli-associated heterochromatin. This ultrastructural change appears to be brought about by the damaging effect of the sunburn spectrum (290–320 nm).
FIG. 7. A low-power view of a melanocyte in forearm skin obtained from a caucasoid subject before IT. The melanocyte contains relatively many melanosomes in various developmental stages. These melanosomes are located mostly in the center of the melanocytes. The dendritic processes (DP), however, possess very few melanosomes compared with the center of the cell. The melanocyte has also large mitochondria and several lipid droplets. The dense aggregates of microfilaments (arrow) can be seen around the nucleus (×4720).

Fig. 8. A high-power view of the melanocyte encircled in Fig. 7. The bundles of microfilaments (arrow) can be seen around the nucleus (N). The diffusely scattered microfilaments can also be seen abundantly in the periphery of the cytoplasm. DP = dendritic process of melanocyte (×19,100).

FIG. 9. A low-power view of the forearm melanocyte obtained from the same subject shown in Figs. 7 and 8, after irradiation by sunlight with a filter (λ > 340 nm). The melanocyte contains a prominently indented nucleus (N) and well-developed dendritic processes (DP). These dendritic processes, which were aggregated with a few melanosomes before IT (see Fig. 2), contain more melanosomes than does the perikaryon of the melanocyte (×6680).

FIG. 10. A high-power view of the dendritic process of the melanocyte shown and encircled in Fig. 9. A large number of melanosomes in various developmental stages are seen in the dendritic process. Along these melanosomes, the meshworks and bundles of microfilaments are aggregated (arrows) (×25,530).
**Fig. 11.** A low-power view of the forearm skin in a mongoloid subject before IT. The melanocyte (MC) contains many melanosomes in various developmental stages. The dendritic processes (DP) do not contain many melanosomes as compared with the perikaryon. (x4675).

**Fig. 12.** A high-power view of forearm skin obtained from the same subject shown in Fig. 11. Besides many melanosomes in various developmental stages, abundant microtubules (MT) and microfilaments (MF) can be seen. These two organelles show cross-linking among the melanosomes and mitochondria, and do not reveal any specific distribution pattern as shown in Figs. 1, 4, and 7 (x30,900).

**Fig. 13.** A low-power view of the forearm skin in the same mongoloid subject shown in Figs. 11 and 12, after irradiation by whole-spectrum sunlight. The melanocyte (MC) hangs down far from the epidermis into the dermis and contains a long dendritic process (DP) that extends into the epidermis. No remarkable changes in the distribution pattern of melanosomes in keratinocytes can be seen after IT (see Fig. 11) (x4950).

**Fig. 14.** A high-power view of a serial section of the dendrite shown and encircled in Fig. 13. Along the dendritic process, many microtubules (arrows) can be seen together with microfilaments. These microtubules run a long course and the melanosomes in the various developmental stages are dispersed around these tubules (x28,000).
In the unexposed skin of the buttock, as well as of the forearm, the heterochromatin was prominently visible around the periphery of the nuclei, but much less prominent around the nucleoli. Likewise, in the skin irradiated with long-wave UV and visible radiation (340–640 nm), the heterochromatin pattern remained unchanged (Table 1). In contrast, however, when the irradiation was carried out with the whole spectrum of sunlight (including the sunburn spectrum), the heterochromatin was conspicuously visible in the aggregated form around the nucleoli (Table 3) and was also noticeable around the periphery of the nuclei (Figs. 15 and 16). No other changes such as plaque or spots of nucleoli suggestive of pathological alteration were seen.

**DISCUSSION**

The results presented in this study appear to reveal three important phenomena during the IT reaction: (1) changes in the distribution pattern of microfilaments...
and microtubules; (2) changes in the number of melanosomes transferred into keratinocytes; and (3) changes in nuclei and nucleoli-associated heterochromatin. Since IT can be induced in a few minutes with sunlight and light from artificial sources, these ultrastructural changes are brought about rapidly. The significance of each of these three findings is discussed as follows:

A. Role of Microfilaments and Microtubules in IT

The roles of microfilaments and microtubules have been extensively examined with respect to cell-shape changes, cytokinesis, axonal growth, tubular-gland formation in the oviduct, morphogenesis in salivary epithelium, tail resorption in tunicate metamorphosis, invagination during gastrulation, smooth-muscle contraction, cardiac-muscle contraction, and many other processes. It has been suggested that the bundles or meshwork of such filaments or tubules represent a contractile machinery for a broad spectrum of cellular movements and developmental processes (4, 5). Based on the effects of such drugs as cytochalasin B and colchicine, Spooner and Wessells have further proposed that microfilaments contract in a purse-string fashion to narrow one end of certain cells, and that the sensitivity of these drugs implied the presence of some sort of a contractile role of the microfilaments (5, 6). Recent observations of McGuire et al. also revealed that cytochalasin B reverses and prevents melanin-granule dispersion caused by dibutyryl cyclic AMP and theophylline in the melanocytes of Rana pipiens (7). They believed that microfilaments, which were abundant in epidermal melanocytes of frog skin, were structural elements responsible for melanin-granule dispersion. Wikswo and Szabó suggested,
TABLE 4

| Number of Melanosomes per Basal Keratinocyte* Before and After Immediate Tanning of Skin |
|-----------------------------------------------|
| Before exposure | After exposure |

* Based on calculations of 50 keratinocytes with nuclei

Site: FOREARM

Exposure: 30 min. >290 nm

in the tissue-culture study of melanocytes and keratinocytes of guinea pigs, that microfilaments maintained the morphogenesis and movement of melanocytes and keratinocytes. They did not, however, examine the effect of microfilaments on the transfer of melanosomes (8).

Our present study suggests that microfilaments and microtubules in human melanocytes are responsible organelles in the immediate tanning reaction induced by sunlight and long-wave uv plus visible lights (340–640 nm). Inasmuch as the IT reaction is a rapid phenomenon and can be induced in a matter of a few minutes, we believe that the microfilaments and microtubules can provide a motive force for the streaming and the rapid transfer of melanosomes into the keratinocytes. This suggestion is based on the following conclusions:

1. The prominence of dendritic processes laden with microfilaments and microtubules (after IT).

2. The shifting and dispersion of microfilaments and microtubules from the perinuclear regions to the periphery of the cytoplasm and the dendritic processes.
3. The changes characterized by movement of melanosomes from the perikaryon to the dendrites.
4. A convergence of melanosomes and bundles of microfilaments from the perikaryon into the dendritic processes.
5. A remarkable decrease in the number of melanosomes in the perikaryon accompanied by an increase in the number of melanosomes in the dendrites.
6. An increase in the transfer of melanosomes manifested by an increase in the number of melanosomes per keratinocyte.

The relative roles and the relationship between microfilaments and microtubules in the transfer of melanosomes, however, are not well understood in this study and remain to be investigated.

B. Changes in the Number of Melanosomes Transferred

Hyperpigmentation of the skin in IT can be due to the changes in one or more of the following four major processes involved in melanin pigmentation: (a) increase in the formation of melanosomes and melanocytes, (b) increase in the rate of melanization in melanosomes, (c) increase in the transfer of melanosomes from melanocytes to keratinocytes and (d) alterations in the degradation of melanosomes in keratinocytes.

Pertinent to these four processes is the new finding concerning a remarkable increase in the transfer of melanosomes from melanocytes to keratinocytes. In our earlier studies and in the present study, it was established that there is no apparent increase in the number of melanocytes and melanosomes, as well as no alteration in the degradation of melanosomes (2, 3). It would thus appear that hyperpigmentation of skin in IT involves: (a) a rapid transfer of melanosomes resulting in their increase in the keratinocytes; (b) an increase in the rate of melanization of melanosomes, either due to photo-oxidation of melanosomes or due to the enzyme-mediated oxidation of DOPA. The rapid transfer of melanosomes appears to be brought about by the active, motive force of microfilaments and microtubules.

C. Changes in Nucleoli and Nucleoli-Associated Heterochromatin

Changes reflecting either an increase in size (or volume) or an increase in the number of nucleoli can be ascertained by frequent examination of many sections of nucleoli in a certain number of melanocytes. Tables 1 and 3 show the results of such an attempt and give a relationship between the number of nucleoli and nucleoli-associated heterochromatin in 50 melanocytes. We suggest that an increase in size (or volume) of nucleoli in melanocytes immediately after irradiation appears to be more probable than in increase in the number of nucleoli. This probability is further strengthened by the realization that the biopsies were obtained immediately after a short (20–30 min) exposure period and that there was no evidence of any mitotic figures in the cells. Furthermore, repeated examinations of many sections of IT skin biopsies could not reveal any recognizable changes in the number of nucleoli per melanocyte. The increase in size of the nucleoli after irradiation was observed not only with the whole spectrum of sunlight but also with the filtered sunlight (340–640 nm) and with long-wave uv and visible radiation from xenon arc lamp. Based on these findings, we suggest that the changes in the size of nucleoli is not a wavelength-dependent phenomenon.

It was suggested that an increase in the size or volume of nucleoli may reflect an increase in RNA and protein synthesis in the nucleoli, or, alternatively, it may
indicate a kind of blockade of transport of nucleolar products resulting from cellular damage (9). Montgomery et al., in a study of uv effect on Chang liver cells, observed a rapid enlargement of nucleoli and the formation of plaques or spots. They suggested that such changes reflected destruction of nucleoli (10). It is also known that nucleoli enlarge in rapidly growing embryonic cells and other cells that are actively engaged in protein synthesis (11).

We suggest that the remarkable increase in an aggregation of nucleoli-associated heterochromatin is dependent on the effect of short-wave uv radiation (290–320 nm), inasmuch as an aggregation of heterochromatin could not be seen in biopsy specimens obtained from sites irradiated with long-wave uv and visible radiation (340–640 nm). The heterochromatin change around the nucleoli may reflect alterations in the chemical or physical properties of DNA, RNA, or chromatin (DNA–protein complex) or some cross-linking of macromolecules (12).

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