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# Sunlight and Melanin Pigmentation*

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## 1. INTRODUCTION

When viewed from the perspective of photobiology, melanin pigmentation of human skin can be described in two categories: the first, *constitutive* or *intrinsic skin color,* and the second, *facultative* or *inducible skin color* (Quevedo *et al.*, 1974). Constitutive skin color designates the genetically determined levels of cutaneous melanin pigmentation in accordance with the

* This study was supported by Grants USPHS CA-21208 and CA-05003.



K. C. Smith (ed.), *Photochemical and Photobiological Reviews*

genetic programs of the cells in the absence of direct or indirect influences (e.g., solar radiation, hormones, or other environmental factors). Facultative skin color characterizes the increase in melanin pigmentation above the constitutive level and arises from the complex interplay of solar radiation and hormones upon the genetically endowed melanogenesis of the individual. The facultative skin color change brought about by solar radiation is commonly referred to as "suntan."

## 2. CONSTITUTIVE MELANIN PIGMENTATION

### 2.1. Biology of Melanin Pigmentation

The melanin pigmentary system of the human skin is based on two cell types: dendritic melanocytes and nondendritic keratinocytes. The melanocytes synthesize specialized organelles called melanosomes within which the pigment melanin is contained. Melanosomes are transferred to the keratinocytes and transported by these cells to the epidermal surface or stratum corneum. The production and the transfer of these chromoprotein-carrying organelles, the melanosomes, is a complex process involving the structural and functional organization of both melanocytes and keratinocytes. This functional unit, consisting of one melanocyte and approximately 36 keratinocytes, is known as the *epidermal-melanin unit* (Fitzpatrick and Breathnach, 1963; Hadley and Quevedo, 1966; Frenk and Schellhorn, 1969) (Fig. 1). Information gathered within the past five years indicates that the functional activity of the multicellular epidermal-melanin unit, rather than the melanocyte alone, is the focal point for the determination of skin color. This unique symbiotic relationship results in a uniform and wide distribution of pigment granules throughout the entire epidermis, although the melanocyte population of the epidermis is no more than 10–25% of the population of the keratinizing basal cells (Szabo, 1967*a*). The structural basis of normal melanin pigmentation of mammalian skin depends on the following factors: (1) the formation of pigment granules, the melanosomes; (2) the melanization of melanosomes, involving the synthesis of the enzyme tyrosinase and the enzymatic oxidation of tyrosine into melanin; (3) the movement of the melanosomes from the protoplasmic mass, the perikaryon, into the dendrites of the melanocytes; (4) the transfer of these melanosomes into the keratinizing epidermal cells, the keratinocytes; (5) the incorporation of melanosomes by these cells either as single discrete particles or as melanosome complexes; (6) the degradation of the melanosomes within keratinocytes; and (7) the rate of exfoliation of keratinocytes. Although each of these factors plays an important role in understanding the nature of hyperpigmentation arising from the complex interplay of light and the tanning ability of the individual, only factors such as the formation, melanization, and transfer of melanosomes will be discussed (Fig. 1).

### 2.1.1. Formation of Pigment Granules

The melanocyte synthesizes melanosomes within which the pigment melanin is contained. Four stages of melanosomal development are currently recognized (Fitzpatrick *et al.*, 1967, 1971*a,b*; Toda and Fitzpatrick, 1971; Quevedo *et al.*, 1974; Jimbow and Kukita, 1971; Jimbow, *et al.*, 1971; Jimbow and Fitzpatrick, 1974; and reflect the degree of its melanization.

*Stage I* describes a spherical, membrane-delineated vesicle that

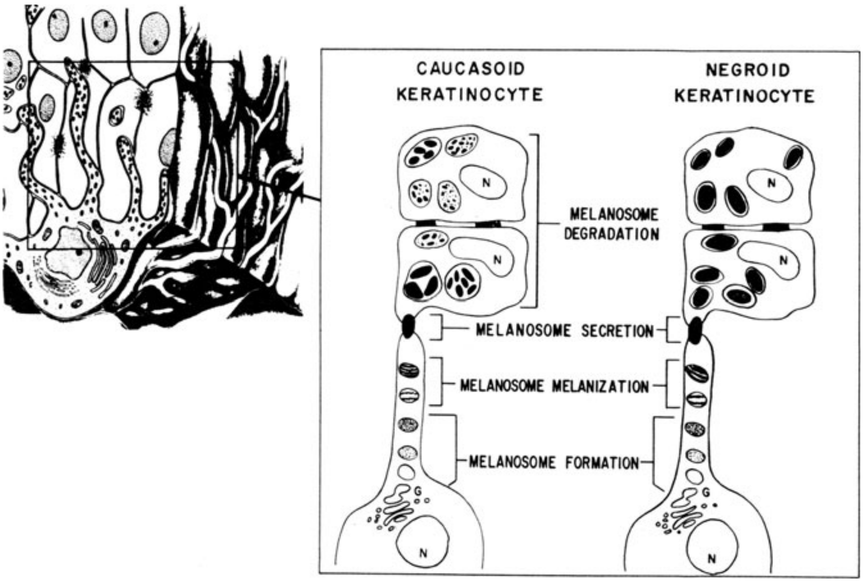


Fig. 1. Epidermal melanin unit illustrating the structural and functional basis of normal melanin pigmentation of skin. Only four processes involved in melanogenesis are illustrated: formation of melanosomes, melanization of melanosomes, secretion and transfer of melanosomes, and degradation of melanosomes. The left half of the figure shows a melanocyte with its branched dendrites in contact with several keratinocytes. The right half of the figure shows an epidermal melanocyte-keratinocyte unit of a Caucasoid and a Negroid subject. Note the difference between melanosomes in the Negroid and Caucasoid keratinocytes. In the Negroid keratinocyte, the melanosomes are discrete; in the Caucasoid keratinocytes, groups of two or more melanosomes are aggregated within membrane-limited, lysosome-like organelles, and melanosomes often show evidence of degradation.

contains tyrosinase and (or) filaments that have a distinct 10-nm (100-Å) periodicity. The formation of melanosomes in this initial stage of development is regulated by the Golgi apparatus, the Golgi-associated endoplasmic reticulum, and rough endoplasmic reticulum. A stage I melanosome is believed to contain tyrosinase, structural proteins, membranes, and possibly certain auxiliary enzymes. It is speculated that polypeptides of the enzyme tyrosinase are synthesized on ribosomes and that the phospholipid-containing proteins are fashioned into membranes by the Golgi complex.

*Stage II* describes an oval organelle in which numerous membranous structures become evident, with or without cross-linking, and show a distinct 10-nm periodicity.

*Stage III* reflects an oval organelle in which melanin synthesis has begun through the enzymatic oxidation of tyrosine. Melanin accumulation occurs on the inner membrane, and the 10-nm periodicity evident earlier in stage II becomes partially obscured.

*Stage IV* reflects the end stage of development of melanosomes in which the deposition of melanin has resulted in the obliteration of the internal structure of melanosomes in stages II and III. The organelle is electron opaque and shows electron-lucent bodies at its periphery (Jimbow and Fitzpatrick, 1974*a*).

#### 2.1.2. Melanization of Melanosomes

Melanin synthesis takes place within the melanosomes (Seiji *et al.*, 1963*a,b*; Fitzpatrick *et al.*, 1967; Moyer, 1966). The copper-containing enzyme tyrosinase, present in these organelles, catalyzes the oxidation of both monohydric (tyrosine) and dihydric phenols (3,4-dihydroxyphenylalanine) to orthoquinones. Molecular oxygen acts directly as the hydrogen acceptor in the reactions catalyzed by tyrosinase. In contrast to melanins of plant origin, which are generally described as *catechol melanin* in type, the mammalian melanins are *indole* in type and are composed basically of indole-5,6-quinone units. According to the classic Raper–Mason scheme of enzymic melanin formation from tyrosine and 3,4-dihydroxyphenylalanine (dopa) critically reviewed by Mason (1967), melanin is thought to be formed from tyrosine → dopa → dopa-quinone → dopa-chrome → 5,6-dihydroxyindole → indole-5,6-quinone → melanin through the polymerization of indole-5,6-quinone units (a homopolymer of indole-5,6-quinone linked through a single bond type). However, several chemical studies of natural melanins (Nicolaus, 1968; Swan, 1963, 1964; Robson and Swan, 1966; Hempel, 1966) using $^{3}H$-labeled and $^{14}C$-labeled precursors have revealed that mammalian melanins are not composed entirely of indole-5,6-

quinone units, but are complex polymers (a heteropolymer or a random polymer) consisting of several different monomers that may be coupled by various bond types of dopa-quinone, dopa-chrome, 5,6-dihydroxyindole, and 5,6-dihydroxyindole-2-carboxylic acid at various oxidation levels held together by a variety of bond types. Pyrrole units, carrying carboxyl groups, in addition to phenolic or quinonoid groups or both, are also present, and some of them represent carboxylated terminal units. The redox state of melanin polymer is equally important. The polyquinonoid melanin can have oxidized (quinonoid), as well as reduced (phenolic) forms of indole-5,6-quinone. These remarks relate only to the synthesis of the black–brown melanin (eumelanin) and much less is known about the yellow and red pigments in mammals (phaeomelanins) which differ from eumelanin. Eumelanin is insoluble in almost all solvents and is resistant to chemical treatments, whereas pheomelanin is soluble in dilute alkali (Duchon *et al.*, 1968; Fitzpatrick *et al.*, 1971*a,b*). It is believed that dopa-quinone formed from the oxidation of tyrosine by tyrosinase interacts with the sulfur-containing amino acid, cysteine, to form cysteinyldopa from which phaeomelanin is derived by pathways yet incompletely understood (Fitzpatrick *et al.*, 1971*b*, Quevedo *et al.*, 1974).

### 2.1.3. Transfer and Dispersion of Melanosomes

We have discussed the fact that the epidermal melanocytes are in symbiotic relationship with the epidermal keratinocytes. The importance of this symbiotic relationship can be well recognized when one examines the distribution of melanosomes after their transfer into keratinocytes in various races (Mottaz and Zelickson, 1967; Klaus, 1969). Electron microscopic observations of human epidermis obtained from people of various racial backgrounds have revealed that melanosomes occur within keratinocytes as discrete granules (nonaggregated form) or as aggregates of two or more discrete granules within membrane-limited bodies (Hori *et al.*, 1968; Szabo *et al.*, 1969; Toda *et al.*, 1972; Wolff and Konrad, 1971; Olson *et al.*, 1973). In Caucasoids and Mongoloids, the melanosomes are almost always found in groups (Fig. 1) and resemble the membrane-limited vacuoles that have been identified as phagolysosomes (Mishima, 1967*a,b*; Hori *et al.*, 1968). In the melanosome complexes of fair-skinned Caucasoids, the melanosomes are loosely packed and there is some granular substance located between them. In Mongoloids and Orientals, the groups of melanosomes are usually very tightly packed, with little or no substance between the melanosomes. After UV irradiation, the keratinocytes of Mongoloids and Caucasoids usually still contain melanosome complexes

(Szabo *et al.*, 1969). However, the number of groups increases, and there is a tendency toward increased numbers of melanosomes inside the melanosome complexes. The keratinocytes of Negroids (African and American) and Australoids (Szabo *et al.*, 1969; Mitchell, 1968; Toda *et al.*, 1972) contain mostly single melanosomes, and only occasionally does one find groups and doublets of melanosomes inside keratinocytes (Fig. 1). This aggregation or nonaggregation of melanosomes in keratinocytes appears to be a size-dependent phenomenon (Toda *et al.*, 1972; Wolff and Konrad, 1971) inasmuch as ellipsoidal melanosomes smaller than $0.6 \times 0.3$ nm in size are usually arranged in groups of two or more and show evidence of degradation. Unlike those of Caucasoids and Mongoloids, the melanosomes of Negroids and Australian aborigines are larger ($0.7–0.8 \times 0.3–0.4$ nm), and they usually do not form such aggregated complexes within the keratinocytes but are found as single, discrete bodies (Toda *et al.*, 1972; Olson *et al.*, 1973; Wolff and Konrad, 1971; Wolff *et al.*, 1974). Thus, variations in the size of melanosomes and the distribution pattern of melanosomes in the keratinocytes significantly influence the color of the skin. When melanosomes are aggregated and are few in number and small in size, they will contribute less to the scattering and absorption of impinging light than when they are singly dispersed, greater in number, and large in size (Table 1).

### 2.2. Normal Skin Color and Human Racial Color Differences

The most obvious difference between the various human races is the variation in the color of the skin. The factors that determine the skin color of normal skin include: (a) a reflection coefficient of skin surface; (b) absorption coefficient of epidermal-cell and dermal-cell constituents; (c) scattering coefficients of various cell layers; (d) thickness of the individual cell layers (stratum corneum, epidermis, and dermis); (e) the concentration of UV light and visible light absorbing components such as proteins (keratin, elastin, collagen, lipoprotein), melanin, nucleic acid, urocanic acid, carotenoids, hemoglobin (reduced and oxidized), and lipids; (f) the number and spatial arrangement of melanosomes and melanocytes; (g) the number and spatial arrangement of blood vessels and the relative quantity of blood cells (reduced and oxidized hemoglobin) flowing through the vessels. Pigmentation of the skin, as viewed clinically, is principally related to the variation in the content of melanin in the epidermis. If melanin pigment were absent from the skin, as in Vitiligo, the color of skin in all races would appear to be milk-white.

It appears therefore, that the color of skin is determined by the func-

**TABLE 1. Relationship between Constitutive Skin Color and Size and Distribution Pattern of Melanosomes in Habitually Exposed Skin**

| Skin color | Size of melanosomes | Melanization of melanosomes | Tyrosinase activity in melanocytes | Distribution of melanosomes in epidermal keratinocytes | Approximate number of melanosomes per basal keratinocyte[a] |
|---|---|---|---|---|---|
| Heavily pigmented skin of African and American Negroes and Australian Aborigines | 0.7–0.8 μm × 0.3–0.4 μm | Fully melanized, predominantly in stage IV | Marked | Single, non-aggregated | 400 ± 35 |
| Moderately pigmented skin of Mongoloids (American Indians, Orientals) | 0.5–0.7 μm × 0.2–0.4 μm | Moderately melanized stages III and IV | Moderate | Mixed, non-aggregated as well as aggregated | 250 ± 50 |
| Moderately pigmented skin of Caucasoids (East Indians, Italians, Egyptians) | 0.5–0.7 μm × 0.2–0.4 μm | Moderately melanized stages III and IV | Moderate | Predominantly aggregated | 200 ± 5 |
| Lightly pigmented skin of Caucasiods (fair-skinned Americans, British, French, Germans, etc.) | 0.4–0.6 μm × 0.2–0.4 μm | Partially melanized stages II and III | Weak | Predominantly aggregated | 100 ± 50 |

[a] Based on random calculations of 50 keratinocytes of basal layer.

tional state of pigment-producing cells, the melanocytes. One of the most obvious questions to ask is how the function of melanocytes is related to phenotypical coloration, a coloration determined genetically (constitutive) or produced by environmental factors (facultative). On the macroscopical level one can qualitatively assign color grades (e.g., black, brown, olive, moderately fair, very fair, freckled, etc.) based upon the quantity of melanin in the epidermis. At the level of light microscopy, when skin biopsies are examined, one can also distinguish the variations in skin color by the presence of more or less melanin in the skin (Gates and Zimmerman, 1953). The color of human skin derives from the visual impact of the total melanin content of the epidermis and is influenced by the reflection, absorption, and scattering of the impinging radiation on the surface of the skin. But the racial origin or the background of an individual cannot be ascertained by the mere visual color of the skin based on its melanin content. A representative of the Mediterranean race or an Asiatic Caucasoid may be labeled as "white" during winter but as "colored" by the end of a sunny summer. It is also true that neither a numerical count of melanocytes nor any other histologic characteristic as seen by light microscopy in the paraffin-embedded skin sections could reveal the racial origin of the skin specimens. The epidermal melanocyte system of various human races has been investigated by Staricco and Pinkus (1957), Szabo (1954, 1959, 1967*a,b*), Mitchell (1963), and Toda *et al.*, (1973). These investigators compared the melanocyte density of single representatives of various "colored" groups with the average melanocyte frequency of white Caucasians and observed that the colored races do not have more melanocytes than white Caucasians. Careful studies of human skin, particularly of the unexposed regions of the body, have revealed that racial differences in skin color are not due to differences in the number and distribution of melanocytes but are due to characteristic differences in the rate at which melanosomes are produced by melanocytes and transferred and distributed in keratinocytes (Fitzpatrick *et al.*, 1965, 1971*a,b*; Toda *et al.*, 1973; Pathak, 1967; Pathak *et al.*, 1971; Quevedo *et al.*, 1974).

Racial color differences can be recognized at the ultrastructural level and involve: (a) differences in the localization or distribution pattern of melanosomes in the keratinizing malpighian cells, either in the aggregated or in the nonaggregated form, or as a combination of aggregated and nonaggregated forms; (b) variation in the number of melanosomes in the epidermal melanocytes and keratinocytes; (c) differences in the size of melanosomes; (d) differences in the degree of melanization of melanosomes; and (e) differences in the degradation of melanosomes due to variations in the hydrolytic activity of these organelles. Variations in the hydrolytic activity of the melanosomes can influence the degradation of melanosomes. The lighter skin color of Caucasoids may result from the degradation of melanosomes, a phenomenon that has been observed to occur within keratinocytes (Hori *et al.*, 1968; Szabo *et al.*; Toda *et al.*, 1974).

## 3. FACULTATIVE MELANIN PIGMENTATION: ACTION OF LIGHT

Solar radiation profoundly influences skin color. Increased melanin pigmentation which occurs after exposure of human skin to sunlight or to UV light from artificial sources is familiarly known as "tanning." Tanning of the skin involves two distinct photobiologic processes: (1) immediate tanning (IT), sometimes referred to as immediate pigment darkening (IPD) reaction, and (2) delayed tanning (DT) (Fig. 2). The biophysical, biochemical, and ultrastructural bases of these two processes will be reviewed briefly with special emphasis on the effects of single and multiple exposures to UV radiation on (a) changes in the number of melanocytes (Figs. 3, 4); (b) synthesis of melanosomes, i.e., the number of melanosomes and their size; (c) melanization of melanosomes; and (d) the transfer of melanosomes to keratinocytes; concomitant changes in keratinocytes concerning (e) the number melanosomes transferred; and (f) the distribution pattern of melanosomes within the keratinocytes.

### 3.1. Immediate Tanning Reaction

IT can best be seen in pigmented individuals or in the previously tanned areas of fair-skinned individuals. IT can be induced both by long-wave UV (315–400 nm) and visible light (400–700 nm). UV-A (315–400-nm) radiation is more effective in the induction of IT than is visible radiation (Pathak, 1967; Pathak *et al.*, 1962*a,b*; Pathak and Stratton, 1969; Jimbow and Fitzpatrick, 1975; Jimbow *et al.*, 1974*a,b*, 1975*a,b*). UV-B radiation (290–315 nm), the sunburn-producing spectrum, does not stimulate IT as effectively as UV-A radiation. This selective induction of IT by UV-A is related to the depth of penetration and absorption of this radiation at the dermo–epidermal junction. The skin begins to be hyperpigmented with 5–10 min of midday summer sun exposure and can be maximally pigmented with 1 h of irradiation. When the skin is withdrawn from exposure to light, the hyperpigmentation fades rapidly within 30–60 min, and thereafter the color usually fades gradually, so that after 3–4 h the irradiated areas are barely hyperpigmented. Sometimes, however, after prolonged sun exposure, 90–120 min, skin may remain hyperpigmented for as long as 36–48 hr, after which time newly synthesized melanin (new melanogenesis or DT) begins to hy-

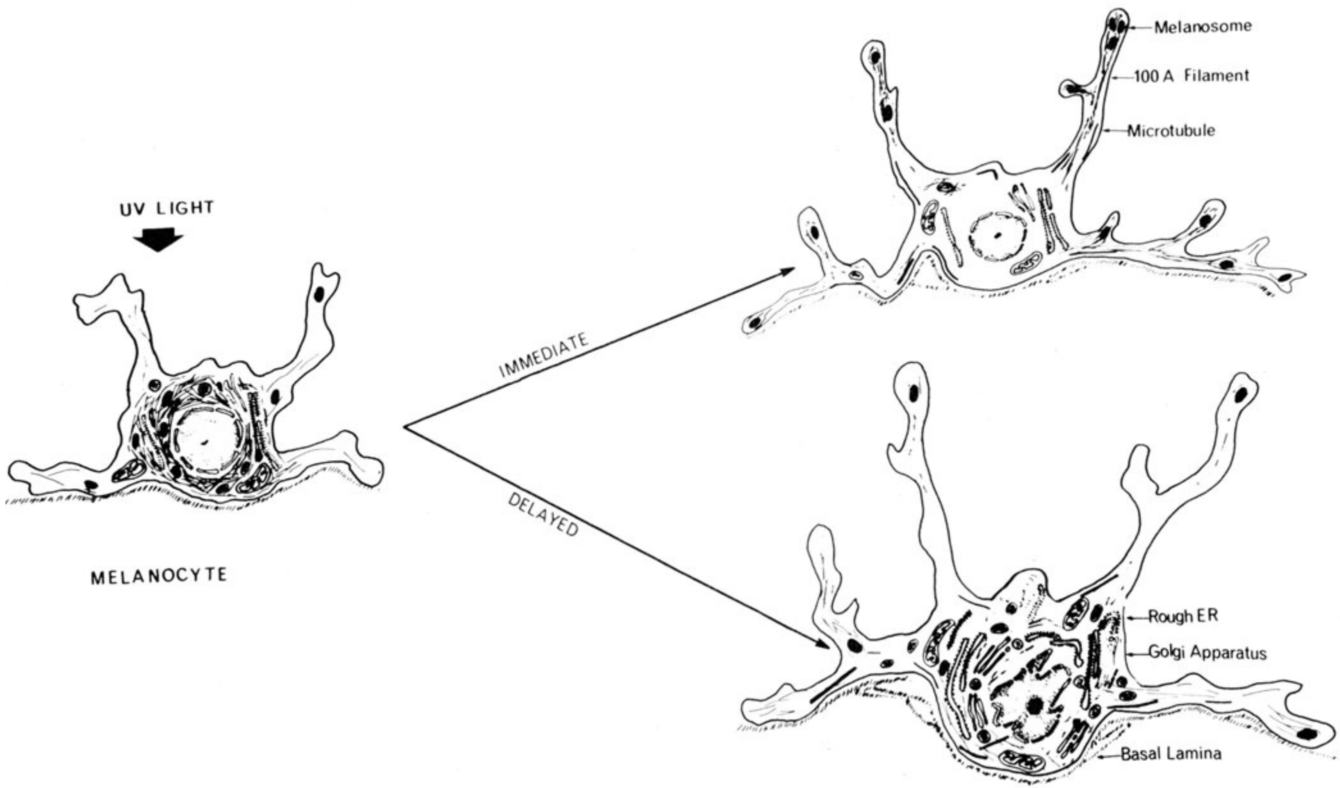


Fig. 2. Two photobiologic processes in melanin pigmentation stimulated by sunlight are illustrated: immediate tanning (IT) and delayed tanning (DT) reaction. The left side of the figure shows a melanocyte from the unexposed skin. This melanocyte shows a round or oval nucleus, few melanized melanosomes, and dense aggregates of fine filamentous structures around the nucleus. Top right shows a melanocyte after induction of IT. Melanocytic thin 10-nm filaments are hardly seen in the perinuclear area; they can be seen in the dendritic processes. The melanosomes are closely intermingled with 10-nm filaments. Lower right shows a hypertrophic melanocyte with well-developed dendrites after DT.

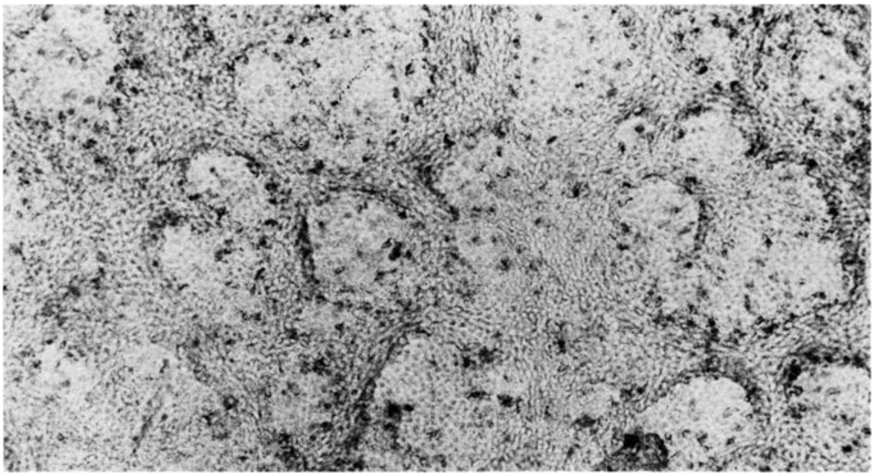

Fig. 3. A low-power view of dopa-incubated epidermal preparation from the unexposed Caucasoid skin. Biopsy was initially incubated in 2N NaBr, epidermis was then split from the dermis and reincubated in dopa solution (Starrico and Pinkus, 1957). The perikaryon of the melanocyte is small and the dopa reaction is weak.

perpigment the skin. The residual hyperpigmentation of IT is due to the redistribution of the existing melanosomes within the keratinocytes. Inasmuch as the IT reaction is a rapid phenomenon and can be induced in a matter of a few minutes, it appears that IT results from changes in melanosomes already existing in the melanocytes and keratinocytes of skin and is brought about by a combination of several of the events described below.

### 3.1.1. Photooxidation of Preformed Melanin

An immediate photooxidation of already existing melanin polymer occurs through the generation of semiquinonelike free radicals in melanin (Pathak, 1967; Pathak and Stratton, 1968). As stated earlier, the indole-5,6-quinone units in the melanin polymer can exist in different stages of oxidation. The comparatively reduced state of melanin is evidenced by a brown or lightly tanned color of the skin, and the comparatively oxidized form can be recognized as a dark brown or black color. One of the most important properties of melanins is their stable, free radical character which is ascribed to the semiquinonoid form of 5,6-dihydroxyindole that is stabilized by resonance throughout the highly conjugated polymer. The free radical content depends upon the degree of melanization and oxidation (Mason *et al.*, 1960; Blois *et al.*, 1964; Pathak, 1967; Stratton and Pathak, 1968; Pathak and Stratton, 1969). Prior to irradiation the lightly tanned skin

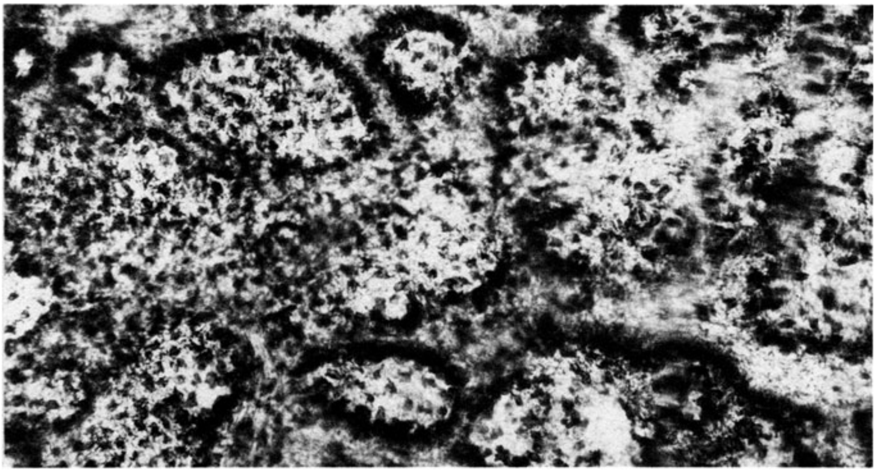

Fig. 4. A low-power view of dopa-incubated biopsy preparation at day 5 after exposure to UV radiation (150 mJ/cm$^2$, 290–320 nm). The intensity of the dopa reaction is markedly increased, the perikaryon of the melanocyte is hypertrophic, and there is marked arborization of the dendritic process.

exhibits a weak electron paramagnetic resonance signal characteristic of melanin free radicals. Immediately after irradiation with UV and visible light, the color of the skin changes to dark brown or black and one can detect a significant increase in the semiquinonelike free radicals in the melanin polymer, suggesting that an immediate oxidation reaction is also occurring in the polymer. Furthermore, the unexposed skin of fair individuals contains melanosomes which are partially melanized and can be recognized in stages II and III of their development. After induction of IT, however, one can often detect melanosomes in stages III and IV (highly melanized form). The unexposed skin of darkly pigmented individuals (e.g., Mongoloids, Negroids, and pigmented Caucasoids) already contains melanosomes in stages III and IV. Their melanosomes are mostly in stage IV when the IT reaction is induced.

### 3.1.2. Changes in the Distribution Pattern of Melanosomes in the Epidermis

In the melanocytes of unexposed skin, the melanosomes are usually aggregated around the nucleus and are rarely seen in the dendritic processes (Figs. 2, 5, 6). After the induction of IT, however, the melanosomes become prominent in the dendritic processes (Figs. 2, 7, 8; Jimbow *et al.*, 1973, 1974*a*). There is also a definite change in the number and dispersion pattern of melanosomes in keratinocytes after the IT reaction. A random count of melanosomes in keratinocytes with nuclei located in the basal layer of four subjects showed a statistically significant increase in the number of melanosomes per keratinocyte after the IT reaction. These findings suggest that during the IT reaction there was a rapid transfer and redistribution of pigmented melanosomes from the melanocytes to the keratinocytes. It is equally possible that this redistribution and variations in the melanosomal number discussed above reflect changes in the sol-gel property of the cytoplasm and also an increment in the negative charge of the melanosomes due to the absorption of radiant energy.

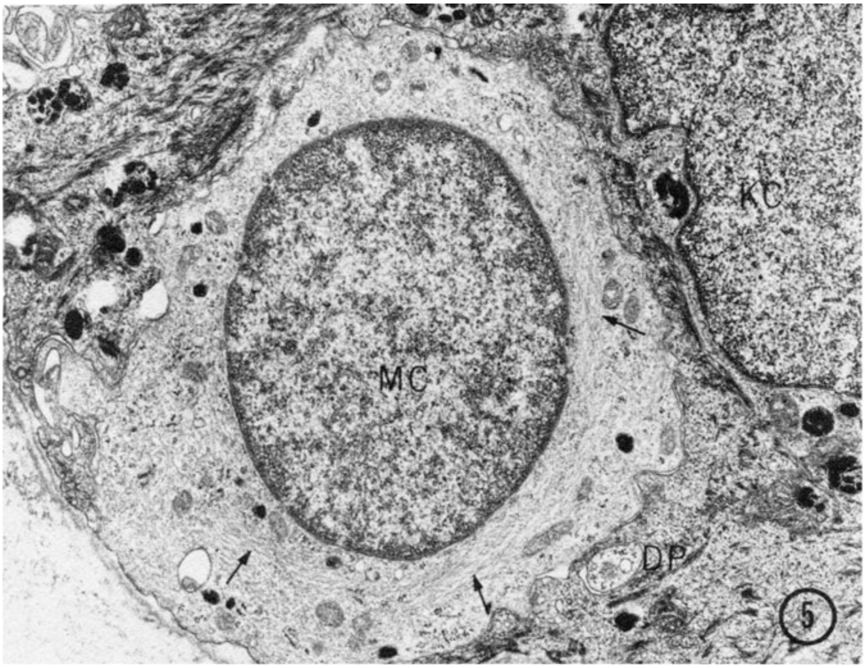


Fig. 5. An electron micrograph of unexposed skin of buttock from a Mongoloid subject. The melanosomes are mostly in melanizing stages (stages II and III). Dense aggregates of 10-nm filamentous structures (see arrows) around the oval nucleus can be seen. On the right side of the figure is a keratinocyte (KC); DP = dendritic processes, MC = melanocyte. (×10,200).

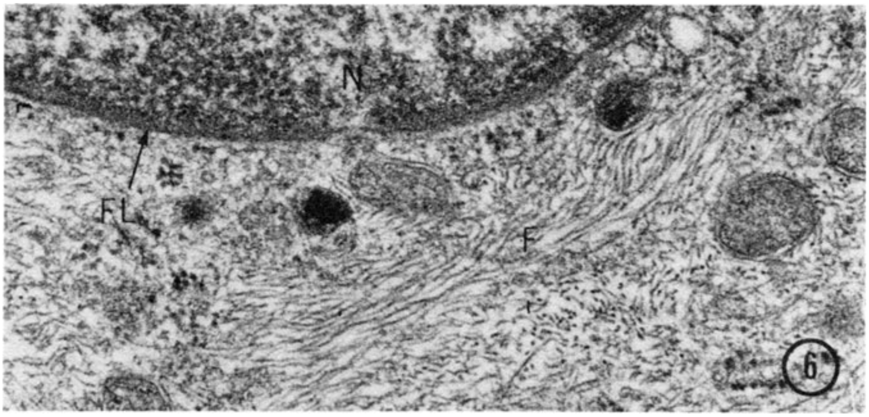


Fig. 6. A higher-power view of a portion of the melanocyte shown above. Around the nucleus, an aggregate of 10-nm filaments (F) and melanosomes in stages I–III can be seen. FL = fibrous lamina of the nucleus (N).

### 3.1.3. Changes in the Distribution Pattern of 10-nm Melanocytic Filaments

Recent observations of Jimbow and Fitzpatrick (1975*a*) and Jimbow *et al.* (1973, 1975*a*) have indicated that human melanocytes contain 10-nm-diameter filaments. These melanocytic filaments, and probably microtubules also, play a prominent role in the IT reaction and provide a motive force for rapid movement and transfer of melanosomes from melanocytes into the keratinocytes (Figs. 2–8). This hypothesis is based on the following observations. Prior to the induction of IT, the melanocytes from the habitually nonexposed regions of the body in the skin of Caucasoids, Mongoloids, and Negroids exhibit: (a) few melanosomes, (b) numerous 10-nm-diameter filaments, and (c) a few microtubules (25–27 nm in diameter) characteristically aggregated around the nuclei. *Per contra,* significant changes in the distribution pattern of these organelles in IT involved (a) prominence of dendritic processes laden with 10-nm-diameter filaments; (b) translocation of melanosomes from the perikaryon to dendritic processes which forms a concourse of melanosomes in the bundles and a meshwork of microfilaments; (c) a few microtubules in the extended processes of dendrites; and (d) a concomitant increase in the number of melanosomes in the keratinocytes (Jimbow and Fitzpatrick, 1975; Jimbow *et al.*, 1973, 1975*a*).

Thus, IT reflects changes in the existing melanosomes and does not involve the new synthesis of melanosomes. The most noticeable changes include (a) photooxidation of melanin, (b) marked change in the distribution pattern of melanocytic filaments and microtubules as well as melanosomes characterized by shifting and dispersion of these filaments and tubules from the perinuclear area to the dendritic processes of melanocytes, and (c) a recognizable decrease in the number of melanosomes in the perikaryon accompanied by an increase in the number of melanosomes in the keratinocytes.

### 3.2. Delayed Tanning Reaction and Hyperpigmentation of the Skin

Delayed tanning is a process which involves the production, transfer, distribution, and, to a limited extent, degradation of melanosomes (Jimbow *et al.*, 1974*a,b,* 1975*b*). The degree of melanin pigmentation that occurs following exposure of human skin to solar radiation varies to a certain

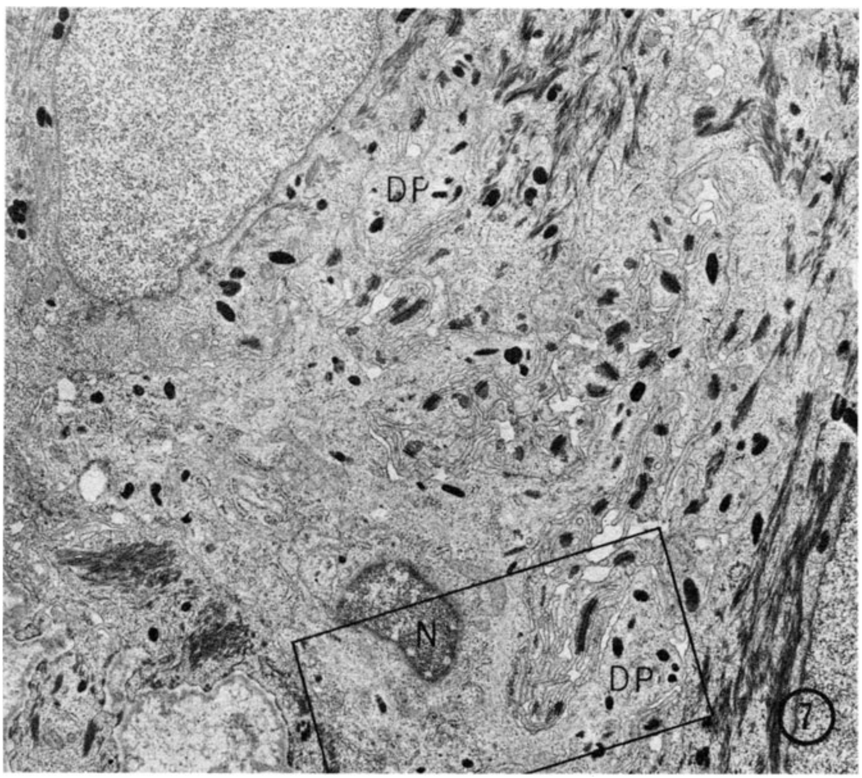


Fig. 7. A melanocyte from Mongoloid buttock skin after the IT reaction. The specimen was taken from the same subject shown in Figs. 5 and 6. The dendritic processes (DP) of the melanocytes are well developed and extend into the keratinocytes. The melanosomes become less aggregated in the perinuclear area (N = nucleus) and are now prominently seen in the dendritic process. The melanocyte contains more melanized melanosomes than those shown in Fig. 5 before exposure.

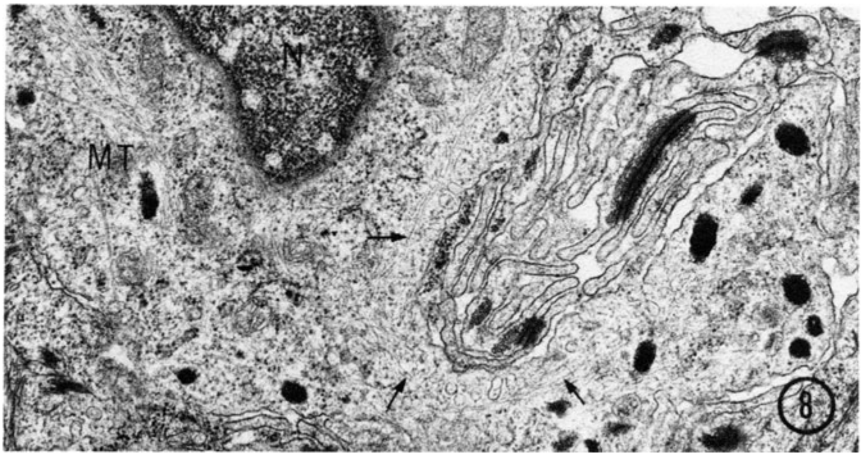


Fig. 8. High-power view of the portion of the melanocyte shown in a rectangular box in Fig. 7. The nucleus (N) is indented and shows nuclear pores. The 10-nm filaments appear to be stemming from perinuclear area to the tip of dendrites (see arrows). Microtubules (MT) are located in the periphery of the cytoplasm.

extent with the total dose of solar radiation received, but more importantly it is regulated by the genetically controlled functional capacity of the epidermal melanin unit of the individual. Genes control the structure of the melanosomes, the level of tyrosinase activity, the polymerization process of the indolequinone and other intermediates, and the development of the dendrites that transfer the melanosomes to the keratinocytes. Solar radiation or UV radiation from artificial sources influences the genetically controlled normal melanin pigmentation (facultative color) of the skin in one or more of the following ways: (a) an increase in the number of functional melanocytes (dopa-positive) as a result of proliferation of melanocytes, and also possibly the activation of the dormant or resting melanocytes (Figs. 3, 4); (b) hypertrophy of the melanocytes and increased arborization (branching) of the dendrites of melanocytes; (c) augmentation of melanosome synthesis manifested by an increase in the number of melanosomes both in the melanocytes and in malpighian cells (keratinocytes) (Figs. 8, 9). The number of fully melanized melanosomes (stage IV) is increased both in the melanocytes and the associated pool of keratinocytes. Even the number of early and intermediate stage (partially melanized, stage I and II) melanosomes is increased; (d) an increase in tyrosinase activity due principally to the synthesis of new tyrosinase in the proliferating melanocytes; (e) an increase in the transfer of melanosomes from melanocytes to keratinocytes as the result of increased turnover of keratinocytes; (f) an increase in the size of melanosomes and also an increase in the size of the melanosome complex. This is, however, greatly dependent on the racial complexion and genetic background of the individual. These observations are illustrated in Figs. 9 and 10 and summarized in Table 2.

Pigmentary responses in individuals who are exposed to sun can be grouped into the following five categories:

I. (Easy burn and no tan): People who sunburn very easily and do not show visually recognizable evidence of tanning (e.g., very fair skin, red hair, blue eyes, freckled skin, people with Celtic background—Irish and Scottish).

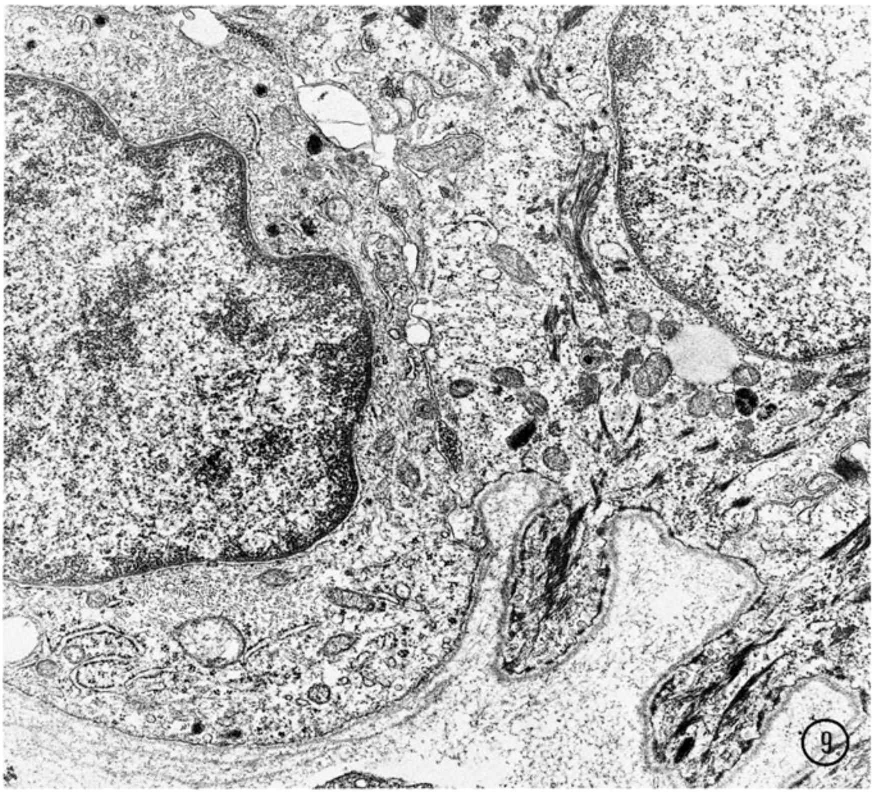


Fig. 9. An electron micrograph of a melanocyte and a keratinocyte from an unexposed Caucasoid back skin prior to UV irradiation. On the left side of the figure one can see a melanocyte with few melanosomes in stages II and III of their development. Notice the perinuclear distribution of 10-nm filaments. The keratinocyte (right side) shows few melanosomes which are in aggregated or complex forms.

Fig. 10. An electron micrograph of a Caucasoid back skin during the delayed tanning (DT) reaction induced by UV-A radiation (315–400 nm). The specimen was taken from the same subject shown in Fig. 9. There are three melanocytes laden with numerous, highly melanized (stage IV) melanosomes. Note the increased number of transferred melanosomes in the keratinocytes. The melanosomes are either singly dispersed or are aggregated and forming complexes. Both UV-B (290–315 nm) and UV-A plus 8-MOP can induce similar changes (see Table 2).

II. (Easy burn and slight tan): People who sunburn easily and tan slightly (e.g., moderately fair skin, people with blond hair, blue-green, or hazel eyes).

III. (Burn and then tan): People who burn moderately in the beginning, acquire a tan readily, and then do not generally burn on subsequent sun exposures (e.g., "brunette skin," olive skin, medium color skin).

**TABLE 2. Changes in Epidermal Melanin Unit in Delayed Tanning Reaction after Repeated Exposures to UV-B, UV-A, or UV-A plus Oral 8-Methoxypsoralen (8-MOP)**

| Nature of observations | Nonexposed skin | Exposed skin after UV-B, UV-A, or UV-A plus oral 8-MOP |
|---|---|---|
| *Macroscopic* | | |
| Degree of visual pigmentation | + | Increased with UV-B ++, UV-A +++, UV-A plus 8-MOP ++++; |
| Onset of visual pigmentation | Continuous at steady state | UV-A within 48 h<br>UV-B within 72 h<br>UV-A plus 8-MOP within 96-120 h |
| Degree of erythema reaction | None | Minimal with UV-A, moderate with UV-B, most with UV-A plus 8-MOP, but minimal with UV-A plus trimethylpsoralen. |
| *Light microscopic* | | |
| Melanin granules | Barely visible in fair Caucasoids but easily visible in Negroids and Mongoloids | Increased in all races; most with UV-A plus 8-MOP |
| Number of melanocytes | About 700–950/mm$^2$ in unexposed skin and 1000–1300/mm$^2$ in habitually exposed skin; no racial differences in population density | Increased 2–3 times within 7–10 days; most remarkable increase in UV-A plus 8-MOP |
| Perikaryon of melanocytes | Small | Markedly enlarged |
| Dendrites of melanocytes | Poorly developed | Prominent and marked arborization |
| Dopa reaction | Weak | Strong; UV-A plus 8-MOP > UV-A; UV-A $\geqq$ UV-B |
| Tyrosine reaction | Barely detectable or absent | Increased and easily detectable in all races |
| *Electron microscopic* | | |
| *In melanocytes*: | | |
| Number of melanosomes | Few | Markedly increased |

*Continued*

TABLE 2. Continued.

| Nature of observations | Nonexposed skin | Exposed skin after UV-B, UV-A, or UV-A plus oral 8-MOP |
|---|---|---|
| Melanization of melanosomes | Predominantly unmelanized in fair-skin (stages I–III); melanizing forms (stage III–IV) in dark skin | Melanized melanosomes (stages III and IV) markedly increased. In UV-A and UV-A plus 8-MOP most of the melanosomes are fully melanized; in UV-B melanosomes are in various stages of melanization |
| Size of melanosomes | Small (400–500 nm in long axis) Caucasoids; large in Negroids (500–700 nm in long axis) | Some increased; some are 700–800 nm even in Caucasoids |
| Distribution of melanosomes | Perinuclear and rarely in dendrites | Variable (diffused in perikaryon and dendrites); more prominent in dendrites particularly with UV-A or UV-A plus 8-MOP |
| 10-nm (100 Å) filaments | Dense, perinuclear aggregation | Diffusely scattered in perikaryon and dendrites |
| Golgi apparatus | Poorly developed | Well developed, marked increase in size and number |
| Rough endoplasmic reticulum | Poorly developed | Well developed |
| Microtubules | Perinuclear | Perinuclear and in dendrites |
| *In keratinocytes*: | | |
| Distribution pattern of melanosomes | Aggregated (melanosome complexes) in Caucasoids, nonaggregated in Negroids and Australoids, and mixed (aggregated and single) in Mongoloids. | Slightly altered (more single and aggregated melanosomes) in Caucasoids and Mongoloids; in Negroids nonaggregated form |
| Ratio of nonaggregated versus aggregated form of melanosomes | Predominantly aggregated in Caucasoids; nonaggregated in Negroids | Increased ratio of nonaggregated form, particularly with UV-A plus 8-MOP |
| Number of melanosomes per melanosome complex | 5–6 melanosomes in Caucasoids | Fewer, 3–4 melanosomes in Caucasoids; most prominent decrease UV-A plus 8-MOP |

TABLE 2. Continued.

| Nature of observations | Nonexposed skin | Exposed skin after UV-B, UV-A, or UV-A plus oral 8-MOP |
|---|---|---|
| Autophagic vacuoles in melanocytes and keratinocytes | Absent or very rare | Usually seen in UV-B and UV-A plus 8-MOP treated skin |
| Lipid droplets | Absent or very rare | Usually seen in UV-B and UV-A plus 8-MOP treated skin |

IV. (No burn and good tan): People who do not burn readily but tan substantially; their eyes and hair are most likely dark (e.g., pigmented Caucasoids, Orientals, and others).

V. (Never burn and markedly tan): Markedly pigmented people (African and American Negroes, Australian Aborigines) who generally never burn but get profusely dark skinned after sun exposure.

### 3.3. Action Spectrum for Melanogenesis

Wavelengths shorter than 320 nm, which cause sunburn (erythema), are considered to stimulate melanogenesis or delayed tanning most effectively (Blum, 1955, 1959). The action spectrum for sunburn induced by exposure to sunlight has a maximum at 300–307 nm; that for sunburn induced by UV radiation from artificial sources has a maximum at 250–254 nm (8-h response). The erythema effectiveness gradually decreases at 270–280 nm (a distinct trough is seen at 280 nm due to absorption by proteins of the stratum corneum) and shows a distinct rise at 290–293 nm. The erythema effectiveness subsequently decreases rapidly at 313–320 nm). Wavelengths longer than 320 nm are weakly erythemogenic (Pathak and Epstein, 1972; Ying *et al.*, 1974). It requires nearly 800–1000 times more energy to produce a minimally perceptible erythema (20–30 J $cm^{-2}$) at 320–400 nm than is required to produce a similar degree of erythema reaction at 290–300 nm (about 20–30 mJ $cm^{-2}$). If the erythema reaction were directly related to melanogenesis, the findings discussed above would suggest that the maximum efficiency for melanogenesis should be at 250–254 nm, followed by slightly less response at 290–315 nm, and long-wave UV and visible radiation would be least effective in the stimulation of melanogenesis. It is, in fact, quite the opposite. Germicidal radiation is significantly less melanogenic than UV-B (290–315 nm) or UV-A (315–400 nm) radiation. The pigmentation produced by 254 nm is less intense and of shorter duration than that produced by UV-B or UV-A radiation (Parrish *et al.*, 1972). Several hundred multiples of the minimal erythema dose (MED) exposures of UV-C radiation ($\lambda$ < 280 nm) will not produce any blistering reaction nor any intense pigmentation response, while as little as 3–6 times the MED exposure to 290–315-nm radiation may cause an intense erythema and pigmentation reaction. For example, an exposure to 254-nm radiation equivalent to 30 × MED will produce a maximum of grade 2+ pink erythema response and a minimal tan (grade +, light brown tan). On the other hand, an exposure to 297-nm radiation equivalent to 2–5 × MED will produce a grade 2+ pink erythema response and a moderate tan (grade ++, medium brown tan). An exposure dose of 10 × MED at 297 nm can produce a grade +++, deep brown tan. In recent studies (Ying *et al.*, 1974; Jimbow *et al.*, 1974*a,b*, 1975*b*; Willis *et al.*, 1972), it was observed that UV-A (315–400 nm) was less erythemogenic and induced less intracellular degenerative change than UV-B. UV-A was found to be more effective in the induction of new melanogenesis than was UV-B. For a long time it has been generally believed, and more or less firmly stated in the dermatologic literature, that melanogenesis (DT) is optimally initiated by UV light of the so-called erythema spectrum (i.e., by UV-B). As early as 1962, Pathak *et al.*, showed that irradiation of human skin with long-wave UV radiation (UV-A), and to a limited extent visible light, would not only stimulate IT but also new melanogenesis. Subsequent studies by Langner and Kligman (1972) and Willis *et al.*, (1972) reemphasized the profound stimulation of melanin pigmentation by UV-A. In fact, UV-A appears to be more effective in the induction of new melanogenesis than UV-B radiation (Jimbow *et al.*, 1974*a,b*, 1975*b*). Thus, the generally held concept of the initiation of melanogenesis only by UV-B should be modified; long-wave UV light (UV-A) must be included in the melanogenic spectrum. It must be stressed, however, that if one were to estimate the quantum efficiency for melanogenesis by UV-B and UV-A, it would be apparent that UV-B is more efficient in the induction of melanogenesis than is UV-A. It requires approximately a minimum of 50–100 mJ $cm^{-2}$ to stimulate melanogenesis by UV-B, whereas a minimum of 10–12 J $cm^{-2}$ of UV-A is required to stimulate melanogenesis. It would appear, therefore, that the experimentally observed marked stimulation of melanogenesis by UV-A, both by Jimbow *et al.*, (1974*a,b*, 1975*b*) and Langner and Kligman (1972), is due to the fact that the less energetic photons associated with UV-A radiation (about 70–

80 kCal mol$^{-1}$) cause less cellular degeneration than does UV-B (about 95–100 kCal mol$^{-1}$). Differences in the depth of transmission and absorption of UV-A and UV-B radiation within the epidermis are also important factors in the differential activation of the epidermal melanocytes. In fair-skinned individuals, most of the impinging UV-B radiation (about 75–80%) is absorbed by the nonviable multicellular layer of the stratum corneum. About 10–15% is absorbed by the viable cells of the malpighian layer (keratinocytes), and about 7–10% will transmit through the dermo-epidermal junction and be absorbed in the papillary dermis (Pathak and Epstein, 1971; Pathak and Fitzpatrick, 1974). It is the absorbed radiation at the dermo-epidermal junction where the melanocytes are localized that stimulates or activates melanogenesis. UV-A radiation, on the contrary, can penetrate deeper through the dermo-epidermal junction. Nearly 20–35% of the impinging radiation will penetrate through the epidermis and reach the hairbulb region. It appears that the better stimulation of melanogenesis by UV-A than by UV-B is due to the selective activation and proliferation of melanocytes both at dermo-eipdermal junction and in the hairbulb. Many of the ultrastructural changes reflecting formation, melanization, transfer, and degradation of melanosomes that take place in skin following repeated treatments with either UV-B, UV-A, or UV-A plus 8-MOP (8-methoxypsoralen) are listed in Table 2. For details concerning the stimulation of pigmentation by UV-A plus 8-methoxypsoralen, a potent photosensitizing agent of skin, the reader is referred to the articles by Pathak *et al.* (1974) and Pathak and Fitzpatrick (1974).

Visible radiation (400–700 nm) and infrared radiation ($\lambda > 750$ nm) are extremely weak in the induction or stimulation of melanogenesis. A single exposure in the range of 25–30 J cm$^{-2}$ of visible light will not stimulate melanogenesis. Repeated exposures of skin to either visible radiation or to infrared radiation may stimulate some melanogenesis but most of the stimulation is secondary to the effects of heat resulting from prolonged absorption of the radiant energy.

### 3.4. Effect of Single and Multiple Exposures of UV Radiation on Human Melanocytes

Photobiologic processes that lead to hyperpigmentation of skin following a single exposure to UV-B or UV-A are different from those that result from multiple exposures to UV-B or UV-A radiation. The increased melanin pigmentation after a single exposure to either UV-B or UV-A radiation primarily reflects changes in the functional activity of the melanocytes, whereas the hyperpigmentation induced by multiple exposures to either UV-B or UV-A reflects changes not only in the functional activity of the melanocytes but also the numerical changes in the epidermal melanin units (i.e., in the melanocytes and the associated pool of keratinocytes). A single exposure to either UV-B or UV-A causes none or minimal change in the number of functional melanocytes, but reveals an increment in the synthesis, melanization, and a transfer of melanosomes (Pathak *et al.*, 1965; Jimbow *et al.*, 1974*a,b*, 1975*b*). Multiple exposures to either UV-A or UV-B cause a marked increase in (a) the number of melanocytes; (b) the number of melanosomes synthesized; (c) the degree of melanization of the melanosomes as a result of an increase in tyrosinase activity; (d) the number of melanosomes transferred to the keratinocytes; (e) a distinct alteration in the size of some of the melanosomes (some of the newly synthesized melanosomes are larger in size than those in the unexposed skin); and (f) alteration in the distribution pattern of melanosomes in keratinocytes as a result of a change in the size of some of the melanosomes.

## 4. PHOTOPROTECTIVE ROLE OF MELANIN

In pigmented peoples, there exists a unique light absorbing and filtering system that minimizes the impact of photons on the vulnerable viable cells of the epidermis. The "white" population possesses a much less protective neutral density filter known as melanin, while the albino skin has virtually no neutral density filter, and more UV light penetrates such skin. In people with absence of melanin or in people who sunburn easily and tan poorly, skin will develop, early in life, abnormal changes caused by sunlight: wrinkling, keratoses, telangiectasia, and skin cancer (Fitzpatrick *et al.*, 1974). The onset of the cancerous or other abnormal changes is directly related to the degree of sun exposure and the latitude at which the person resides (i.e., sun intensity times the duration of exposure), and is inversely related to the amount of melanin in the skin. Among races with dark skin, in which melanin pigment effectively filters UV radiation, there is very little skin cancer. This photoprotective role of melanin is attributable to its presence in the particulate and nonparticulate form in the epidermis. In the stratum corneum most of the melanin is usually in the nonparticulate, amorphous form, although in certain individuals who are heavily pigmented, one does find a few melanosomes scattered randomly in the nonviable, horny cells. Most of the particulate forms of melanosomes are believed to be degraded due to the presence of hydrolytic activity associated with the melanosome complexes or in the outer membrane of the discrete organelles. Melanin present in the melanosomes is nondegradable (Hori *et al.*, 1968). The rest of the viable cells of the epidermis contain melanin-laden melanosomes in the particulate form.

The photoprotective role of melanin is accomplished by the following physical and chemical properties of the biochrome (Pathak and Fitzpatrick, 1974):

**a.** Melanin absorbs UV and visible radiation and can act as a neutral density filter. Melanins isolated from human hair, melanoma, and other biologic materials show high absorption without any characteristic peaks or absorption bands in the UV, visible, and near-infrared region (200–2400 nm). This absorption increases in the shorter wavelengths in the UV spectrum and appears to be due to highly conjugated system in the polymer.

**b.** Melanin-laden melanosomes attenuate the impinging radiation by scattering; this scattering involves any process that deflects electromagnetic radiation from a straight-line path and results in the attenuation of radiation. This increases the total absorbing path through which the UV radiation must pass. For particles with the dimensions of the order of wavelength of light in the UV spectrum (0.3 $\mu$m), the impinging light may be scattered according to the Rayleigh relation (scattering is inversely proportional to the fourth power of the incident light). Maximum scattering occurs when the wavelength of light approaches the diameter of the particle. For particles larger in size than the wavelength of the incident light (e.g., melanosomes which are 0.3–1.0 $\mu$m in size), the scattering relationship is quite complex, and more incident light will be scattered in the forward direction than in the backward direction.

**c.** Melanin absorbs the radiant energy in the UV and visible spectra and dissipates the absorbed energy as heat. In this regard it is of interest to point out the hypothesis of McGinnes and Proctor (1973) that melanin in the cell may serve as a device by which it may convert the energy of the excited states into heat by a phenomenon known as photon-photon conversion. This hypothesis implies that melanin polymer can act as an amorphous semiconductor in which coupling of phonons (i.e., vibrational modes of the melanin polymer) to its excited electronic states plays a role in the dissipation of energy absorbed from the impinging radiation.

**d.** Melanin can also utilize the absorbed energy and undergo immediate oxidation through the generation of semiquinonoid free radicals (Pathak and Fitzpatrick, 1974).

**e.** Melanin, as a stable free radical, with its ability for oxidation and reduction, can act as a biologic electron exchange polymer and minimize the impact of the impinging photons on the other vulnerable cell constituents (e.g., DNA) (Pathak and Fitzpatrick, 1974). The free radicals in melanin are quite stable and the unpaired electrons seem to be limited to localized regions of the polymer or are stabilized by a large number of resonance structures. Because of the unpaired electrons in melanin it may in effect serve as a one-dimensional semiconductor, where any bound protons serve as electron traps. A free flow of charge in the form of electrons is then possible through the melanin (Longuet-Higgins, 1960; Pathak and Stratton, 1968; McGinnes *et al.*, 1974). It is known that UV irradiation increases the spin concentration in biological tissues such as human skin. Trapping of free radicals which could disrupt the metabolism of living cells is thus feasible in presence of stable free radicals in melanin polymer.

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