THE MELANOCYTE: CYTOLOGY AND CYTOCHEMISTRY*

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Ultraviolet radiant energy has been known for decades as a highly effective agent for stimulation of melanin formation in human skin, yet little is known of the specific biochemical mechanism underlying this effect. This gap in our knowledge has prevailed despite a considerable knowledge of the nature and action of ultraviolet on pure chemical systems and, in addition, a fairly comprehensive knowledge of the metabolic sequence leading to melanin formation in human skin.

One of the direct or indirect targets of ultraviolet exposure of skin is the melanocyte or melanin-forming cell. We will therefore review briefly the cytology of the melanocyte, the biochemical pathway of melanin as it occurs in the melanocyte and the present status of our knowledge of the biochemical basis of suntanning.

CYTOLOGY OF MELANOCYTES

Origin of Melanocytes

The melanocyte is a specialized cell, distinctive from all other cell types, characterized by two or more dendritic processes (Fig. 1) and containing, in its pigmented form, myriads of light or dark brown cytoplasmic granules. The melanocyte is derived from the melanoblast which arises in the neural crest and the outer layer of the optic cup and migrates during neonatal life to three principal sites: the skin (the epidermal-dermal junction of the skin and mucous membrane, and the hair bulb), the central nervous system (mainly the leptomeninges), and the eye (the uveal tract and the retina). Because of similarities of origin, cytology and cytochemistry, melanocytes may be grouped together as the melanocyte system (Fig. 2).

The Melanocyte as a 'Secretory' Cell

Melanocytes form a horizontal network at the plane of the dermo-epidermal interface and are integrated closely with the epidermal cells, establishing contact by means of their numerous cytoplasmic processes. When viewed in whole mounts of epidermis obtained by enzymic separation of the epidermis from the dermis, this network of cells gives the appearance of a fish net (Fig. 3). By this close contact with the epidermal cells, the melanocytes are able to transfer melanin granules into neighboring epidermal cells. This ability of melanocytes to transfer granules into another cell has been termed *cytocrine* by Masson (1). Similar cytocrine melanocytes occur in the upper portion of matrix of the hair bulb, and these pigment cells transfer melanin granules to the cortical cells of the growing hair. Studies of the fine structure of hair melanocytes and cortical cells with the electron microscope by Birbeck *et al.* (2), support the Masson idea of melanocytes as cytocrine cells. Melanocytes were found to contain a compact cluster of ergastoplasmic membranes proximal to the papilla and mitochondria located distal to the nucleus. This type of cell polarity is that of a secretory cell. Sagittal sections of the hair follicle at different levels revealed that a portion of the cytoplasmic process of the hair melanocyte appeared to be phagocytized by the cortical cell, then the cell wall of the process disappeared and the melanin granules were seen to be dispersed throughout the cytoplasm of the cortical cells.

The Role of Melanocytes in Protection Against Ultraviolet

As the epidermal cells move outward to become the stratum corneum, the melanin granules contained within them are carried along and appear in the stratum corneum not as granules but as fine, irregular, pigmented particles. In the skin of negroid peoples the stratum corneum is regularly flecked with these dust-like melanic particles. The stratum corneum of caucasians is not ordinarily melanized except during a rather variable period after exposure to ionizing and ultraviolet radiation. The protective role of the melanized corneal layer of negroid skin against

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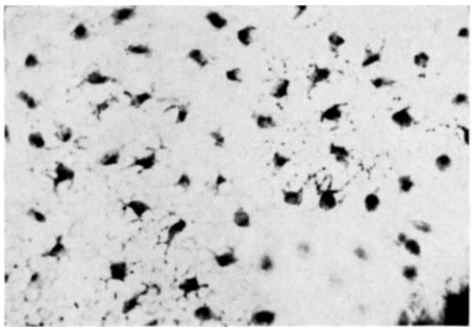


FIG. 1. Human white skin, whole mount of epidermis, separated from dermis by incubation in crude trypsin (see Ref. 5). Note large cell body of melanocyte and numerous cytoplasmic (dendritic) processes. (dopa reaction)

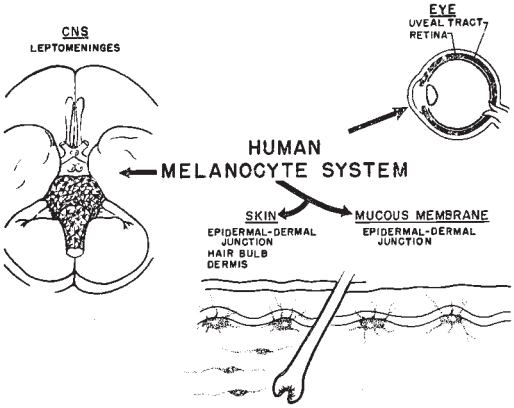


FIG. 2. Human Melanocyte System. Dermal melanocytes occur normally only in embryonic life but may be seen frequently in Asiatic newborn in the sacral area (so-called Mongolian spot). Melanocytes in the mucous membrane are ordinarily non-melanized in caucasians, but frequently melanized in asiatics and negroid peoples.

ultraviolet has been established by the studies of Thomson (3). He obtained layers of stratum corneum from *unexposed* skin of negroids and caucasians by cantharides blisters. *Although the thickness of the stratum corneum was approximately the same in both racial groups, the negroid corneum was clearly more melanized.* Thomson placed the sheets of corneum on a photographic plate and exposed them to ultraviolet and demonstrated the greater ultraviolet filtering ability of the negroid corneum. Thus the greater tolerance of negroid skin to ultraviolet exposure was found to be related to the degree of melanization of the stratum corneum. It had been stated previously that the relative resistance of negroid skin to ultraviolet radiation was due to a greater thickness of the stratum corneum compared to caucasians (4). Thomson's data would suggest that melanization rather than thickness of the corneum explains the differential responses of negroid and caucasian skin to ultraviolet exposure. Albino or vitiligo skin can develop increased resistance to ultraviolet radiation by graded exposures, presumably by thickening of the stratum corneum (Fig. 4). Thus, melanin formation is not an essential but an accessory factor in ultraviolet protection. On the basis of our present knowledge, it is not possible to assign relative values to the stratum corneum and melanin as biological ultraviolet filters.

Total Number, Frequency and Distribution of Epidermal Melanocytes

The application of quantitative cytological methods to the pigment problem has supplied

FIG. 3. Human white skin, face, whole mount of epidermis. The melanocytes form a continuous syncytium at the dermal-epidermal interface. (Dopa reaction).

FIG. 4. Human white skin, forearm, vitiligo. Note sharp demarcation of non-melanized vitiligo skin with normal melanized skin. A few isolated melanocytes remain in the vitiligo skin. The non-melanized vitiligo skin contains minimal or no melanin yet the vitiligo area can develop "tolerance" to ultraviolet light by graded exposures and thickening of the stratum corneum. When vitiligo skin is first exposed, however, marked erythema develops while the normal melanized skin may show little or no erythema.

FIG. 5. Human white skin, face, whole mount of epidermis, dopa reaction. The mean number of melanocytes per square millimeter averages 2310 ± 150. There is no rete ridge pattern and the basal cell layer is relatively smooth. Compare with Fig. 6.

FIG. 6. Human white skin, upper extremity, whole mount of epidermis, dopa reaction. The mean number of melanocytes per square millimeter on the upper extremity averages 1160 ± 40. Note the well-developed rete ridge pattern as compared with Fig. 5.

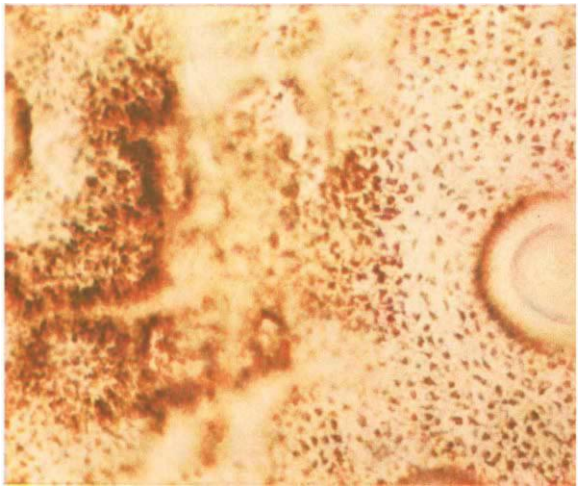


Figure 3

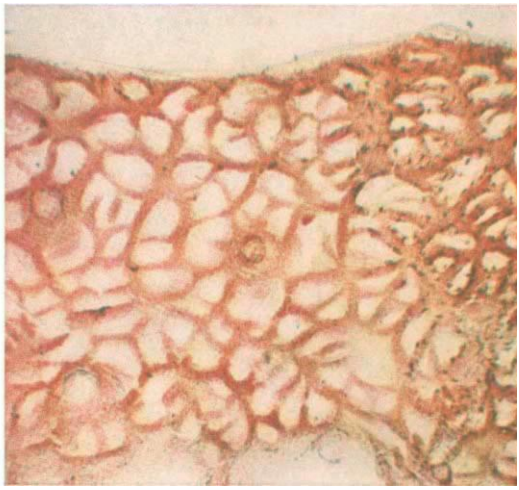


Figure 4

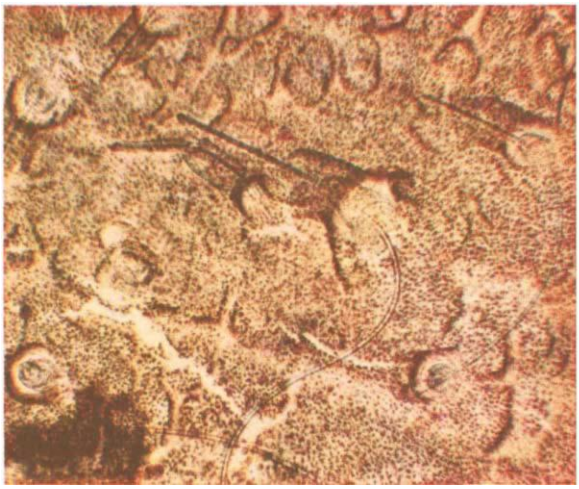


Figure 5

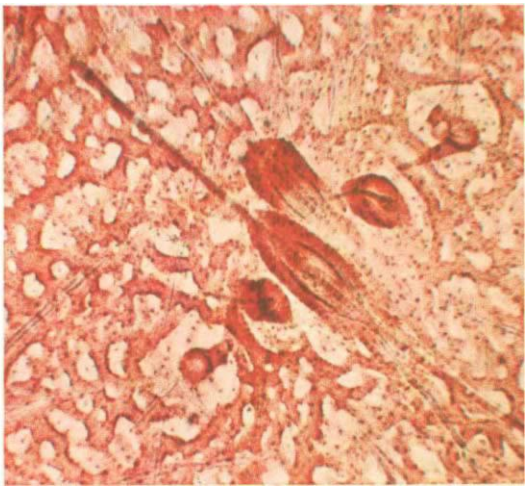


Figure 6

quinone, with protein. The polymerization and coupling occur on the surface of a subcellular cytoplasmic particle, the *melanin granule*. The quininoid polymer is derived from the amino acid, *tyrosine*, by a chemical reaction catalyzed by an aerobic oxidase, *tyrosinase*. Since tyrosinase is attached to the melanin granule, melanin synthesis is precisely localized to the cytoplasm of the melanocyte.

Tyrosinase is one of a large group of copper-containing oxidases that catalyze the oxidation of both mono- and dihydric phenols to ortho-quinones. In mammalian tissue tyrosinase catalyzes the hydroxylation of tyrosine to dihydroxyphenylalanine (dopa) *and* the oxidation of dopa to dopa quinone (Fig. 10). The mechanism of action of tyrosinase is not clearly understood. Mason (8) has recently presented a plausible hypothesis of tyrosinase action. In the presence of the dihydric phenol, dopa, two *cupric* atoms in tyrosinase are reduced to *cuprous* atoms. In the absence of dopa or other reducing systems, hydroxylation as catalyzed by tyrosinase does not occur. Thus the initial step in the activation of tyrosinase is a reduction of the enzyme from the cupric to the cuprous state.

Dopa, in addition to acting as a substrate, can serve as an activator for the reduction of the cupric to the cuprous state of tyrosinase. *In vivo*, tyrosinase may thus exist as a reduced enzyme active towards both tyrosine and dopa, or, because of a high prevailing oxidation potential, may exist in an oxidized form in which tyrosinase is inert but activable towards tyrosine and readily active towards dopa.

Cytochemical Demonstration of Melanin Formation

Most of the existing knowledge regarding melanin formation in human skin has been obtained by the use of cytochemical technics utilizing melanogenic substrates, tyrosine and dopa (9). The use of dopa as a substrate has certain limitations since it is an unstable intermediate in the metabolic pathway of tyrosine to melanin and is readily oxidized to melanin by enzymes or oxidizing systems in addition to tyrosinase. Tyrosine, however, is oxidized to melanin only by tyrosinase contained on cytoplasmic melanin granules. However, the conversion of *tyrosine* but not dopa to melanin is characterized by a variable 'lag' period. In the presence of low concentrations of tyrosinase this lag period is markedly prolonged and the enzyme is not detectable by cytochemical technics utilizing tyrosine as a substrate in certain loci of melanocytes, *e.g.*, epidermal melanocytes. Since there is no lag period with dopa as a substrate, tyrosinase in epidermal melanocytes is readily demonstrated when skin slices are incubated in dopa but not tyrosine. In other areas, particularly the hair melanocytes, tyrosinase is present in relatively high levels and the enzyme is readily detected with both tyrosine and dopa.

An apparent weakness of the cytochemical tyrosinase reaction using either tyrosine or dopa

FIG. 9. Primary malignant melanoma, dorsum foot. a) Histologic section H & E × 100. b) Radioautograph of same section incubated in tyrosine-2-C^{14}. Note intense deposition of silver grains indicating sites of conversion of labeled tyrosine to labeled melanin. Note the melanocytes in the stratum corneum, especially in (b). The radioactivity indicates that melanocytes containing enzymically active melanin granules have migrated to the stratum corneum. This occurs in hyperpigmentation associated with increased melanocyte proliferation rather than an increased rate of melanogenesis.

FIG. 13. Tyrosinase reaction in ultraviolet irradiated and unirradiated normal human skin. a) Melanocytes in human skin exposed *in vivo* to ultraviolet radiant energy and incubated in tyrosine dissolved in a phosphate buffer at pH 6.8. Slices of irradiated skin incubated in phosphate buffer alone showed a complete absense of melanin formation in the melanocytes similar to those in (b); paraffin sections ×1025. b) Slices of unirradiated portion of skin, same donor as in (a), that have been incubated in tyrosine phosphate buffer under the same conditions as in (a). Note complete absence of melanin formation. Tyrosinase in normal melanocytes of the epidermal-dermal junction is present in low concentrations and therefore fails to convert tyrosine to melanin during the period of the incubation because of a long lag period, *or* may be present in concentrations high enough to convert tyrosine to melanin but is inhibited or exists as an apoenzyme.

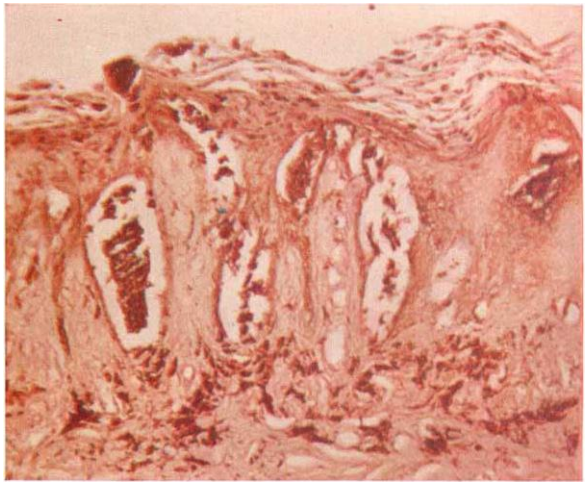


Figure 9a

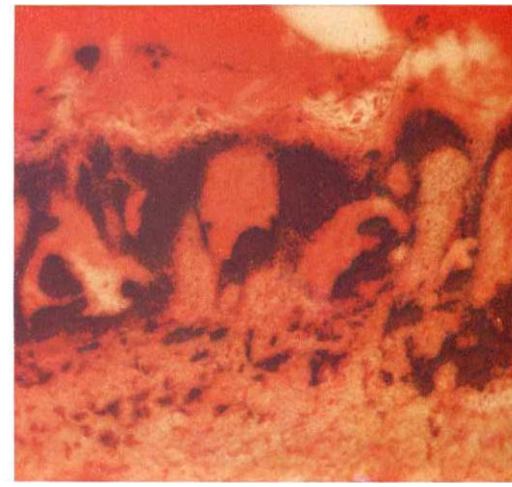


Figure 9b

Figure 13a

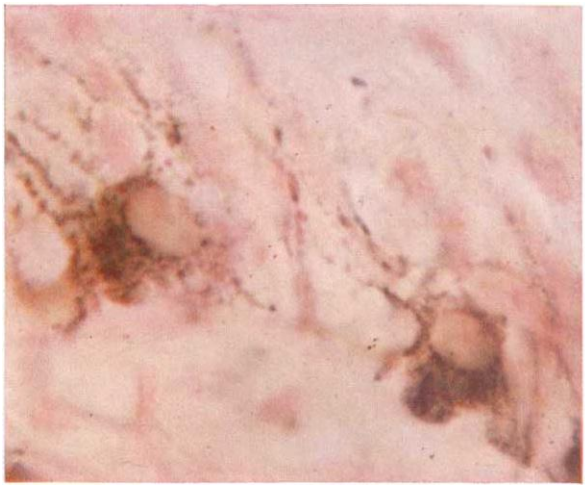
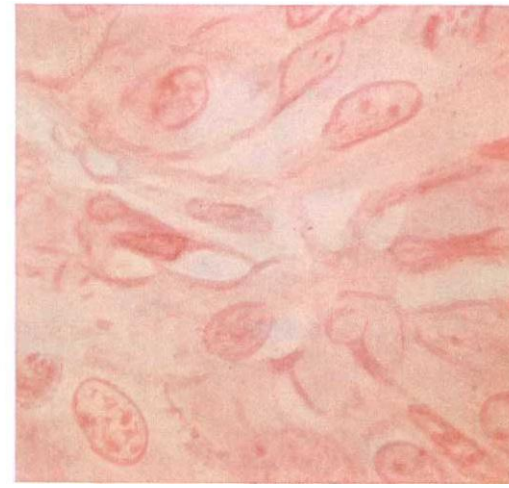


Figure 13b



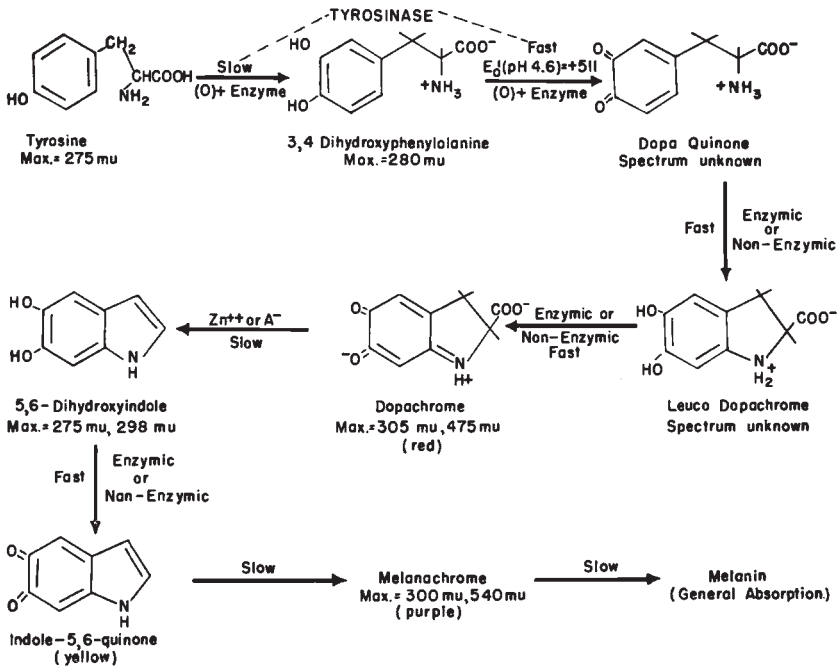


FIG. 10. Metabolic pathway of tyrosine to melanin catalyzed in the first two steps by a single enzyme complex, tyrosinase. The conversion of tyrosine to dopa is characterized by a 'lag' period whose duration varies *inversely* with the level of tyrosinase activity. This lag period is also shortened in the presence of dopa.

as an analytical tool is the difficulty in judging the extent of the reaction in heavily melanized pigment cells. The use of labeled melanogenic substrates, *e.g.*, C^{14} labeled tyrosine, has circumvented this problem and provides for the measurement of tyrosinase activity in fully melanized melanocytes (Fig. 11). The formed radioactive melanin is detected, not by color change, but by the density of the silver grains produced on a photographic emulsion (10). A large number of radioautographs have been prepared using this method of melanoblasts and melanocytes from a variety of sources. Three types of patterns appear to exist in melanoblasts and melanocytes. (See Table 1.)

Type I. Tyrosinase and "Dopa Oxidase" Activity

Type II. "Dopa Oxidase" Activity

Type III. No Activity Towards Tyrosine or Dopa

Type I activity exists in normal human hair bulb melanocytes and is readily detected by histochemical methods utilizing both C^{14} labeled tyrosine and non-radioactive dopa. Type II activity is present in the melanocytes at the epidermal-dermal junction. Type III activity is found in adult retinal pigment epithelium, vitiligo, and dermal melanocytes present in the Mongolian spot.

Since little is known regarding the factors that regulate tyrosinase activity *in vivo*, it is not possible to explain the mechanism of the three types of tyrosinase activity. High levels of tyrosinase concentration would show both tyrosinase and 'dopa oxidase' activity (Type I). Lower levels of tyrosinase or partial inhibition of the enzyme would result in a type II pattern (no tyrosinase but dopa oxidase activity). Absence of both tyrosinase and dopa oxidase activity would suggest complete inhibition (as in vitiligo) or absence of the enzyme (as in albinism).

The various factors that determine the *rate* and *amount* of melanin formation include: 1) the availability of the melanin precursor, tyrosine; 2) the rate of tyrosinase synthesis; 3) the presence of factors that activate tyrosinase (*e.g.*, dopa); 4) the presence of naturally-occurring inhibitors of the tyrosine-melanin pathway (*e.g.*, phenylalanine and sulfhydryl groups) (Fig. 12). An additional factor limiting melanin forma-

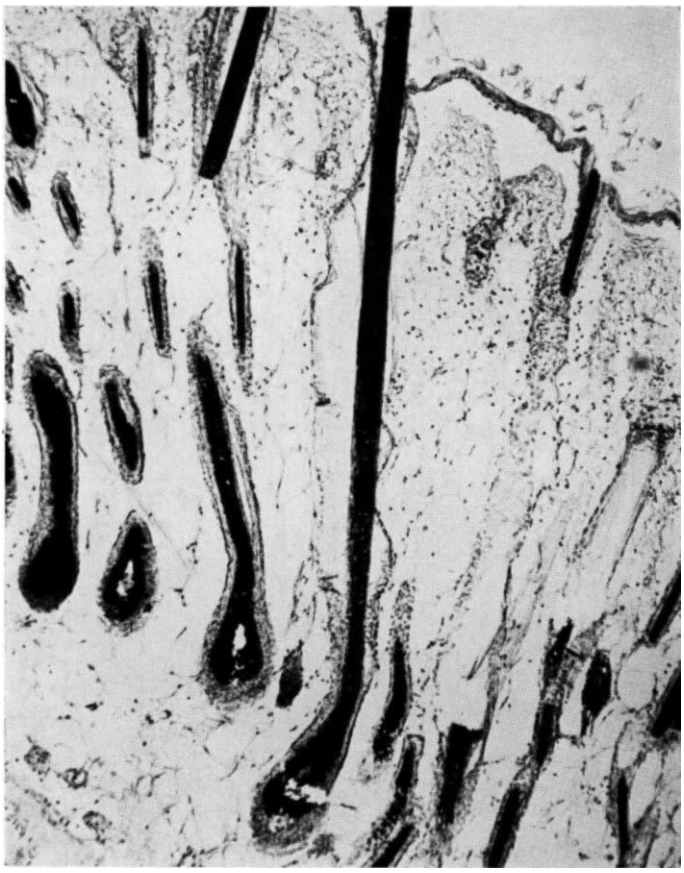


FIG. 11A.

FIG. 11. Hair bulbs, C-57 black mouse in anagen VI. a) H & E × 400, b) Radioautograph × 400, lithium carmine. Note deposition of silver grains in the hair bulbs only indicating the sites of radioactive melanin. Slices of skin were incubated in tyrosine-2-C^{14}.

tion not included in the four mentioned above is the *inverse relationship of tyrosinase activity to melanization of the melanin granule* (11). Manometric and radioautographic studies of three types of melanin granules (embryonic chick retinal pigment epithelium, Harding-Passey and B-16 experimental mouse melanomas, and the hair bulbs of intense brown and black mouse genotypes) have shown that *brown* melanin granules (10 day old chick retinal pigment epithelium, Harding-Passey mouse melanoma and the brown mouse genotype) contain high levels of tyrosinase activity while *black* fully-melanized granules (17-day retinal pigment epithelium, B-16 mouse melanoma, and the black mouse genotype) contain decreased amounts of tyrosinase activity. It has been possible to show that *in vitro* melanization of brown granules (Harding-Passey mouse melanoma) with high intrinsic activity results in a decrease in the reaction velocity. A melanocyte may therefore be heavily pigmented and yet have little or no tyrosinase activity, as in the *adult* chick and mammalian retinal pigment epithelium.

SUN-TANNING

The melanin pigment response to sunlight (or ultraviolet) exposure is said to be of three types: *melanin darkening*, *melanin migration*, and *melanin formation* (4).

Melanin darkening appears within a few minutes after exposure to relatively long wave lengths (3000–4200 A) with a maximally effective action spectrum of 3400 A. This response is said to be oxygen-dependent as it is abolished if

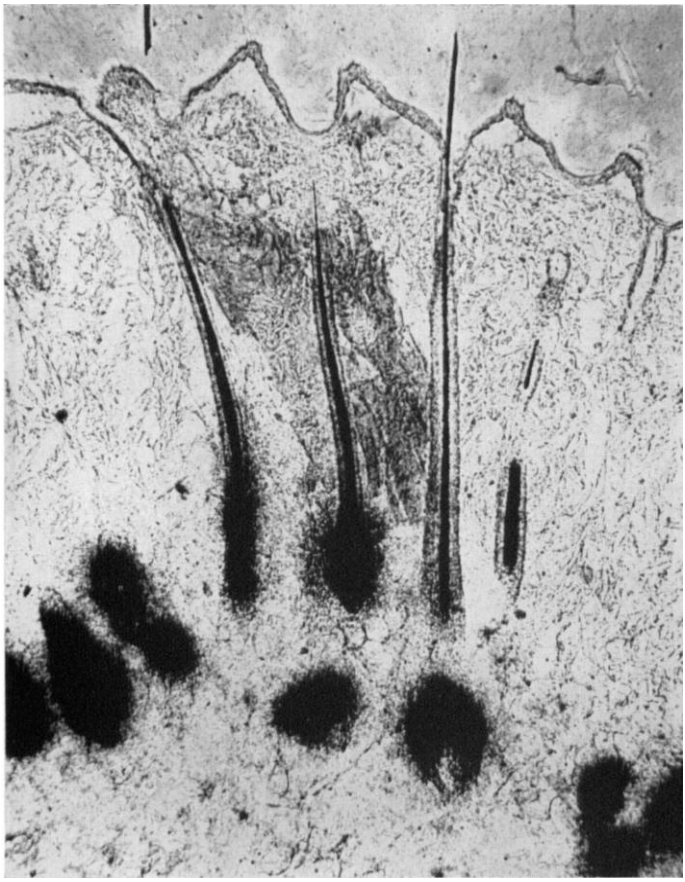


FIG. 11B.

the skin is blanched by pressure applied with quartz plate. It is doubtful that this technic decreases oxygen levels significantly. No data are available to refute the notion that what is observed as melanin darkening may in fact be new melanin formation. However, the rapidity of the response makes it highly unlikely that melanin synthesis could occur.

Several days after exposure to sunlight or other ultraviolet light sources, melanocytes are observed to be "dendritic", and this has been interpreted as *melanin migration*. Similar dendritic melanocytes are seen in many types of melanin hyperpigmentation, such as Addison's disease and post-inflammatory pigmentation. An epidermal melanocyte that is producing melanin appears dendritic because it is fully packed with newly-formed melanin granules both in the perikaryon and in the dendrites.

Melanin formation following exposure to ultraviolet is the result of increased melanogenesis in the existing melanocytes and not melanocyte proliferation. The time sequence of melanin formation following ultraviolet exposure has been observed by reflectance spectrophotometry. According to the reflectance studies of Edwards and Duntley (12), using the Hardy recording spectrophotometer, melanin formation begins after two days, reaches a maximum after 19 days, and ceases after one month. The skin does not return to its initial melanin content, however, until nine and one-half months later. The action spectra for melanin formation is 2800–3100 A. The maximum effective wave lengths in this range have not been determined.

Following sunlight or ultraviolet light exposure there is a marked increase in the level of tyrosinase activity in epidermal melanocytes (Fig. 13) (13). Neither the maximum effective wave lengths nor the time sequences of tyrosinase

TYROSINASE AND "DOPA OXIDASE" ACTIVITY IN MELANOBLASTS AND MELANOCYTES
(Histochemical and Radioautographic Studies)

	SKIN	HAIR BULB OR FEATHER	RETINAL PIGMENT EPITHELIUM	UVEAL TRACT	PATHOLOGIC
Tyrosinase (+) Dopa-oxidase (+)		HUMAN, Black, Brown, Blonde, Red hair during Anagen MOUSE, Black, Brown, Yellow hair during Anagen III-VI	Black Mouse Embryo (9 day to Newborn) Chick Embryo Wh. Leghorn (4th-7th day) Rh. Is. Red (4th-7th day) Black Australorp (4th-8th day) Human fetus (5 month)		Malignant melanoma Blue Nevus Cellular Blue Nevus Junctional nevi (during proliferative phases, especially in children)
Tyrosinase (−) Dopa-oxidase (+)	Epidermal-dermal junction			Human Chick embryo	Dermal Pigmented Nevi Dermal Melanocytosis (Mongolian spot) Junctional Nevi
Tyrosinase (−) Dopa-oxidase (−)			Adult Black mouse Embryonic and adult albino mouse Adult Human		Vitiligo Albinism (skin and hair bulb) Oculodermal melanocytosis

TABLE 1

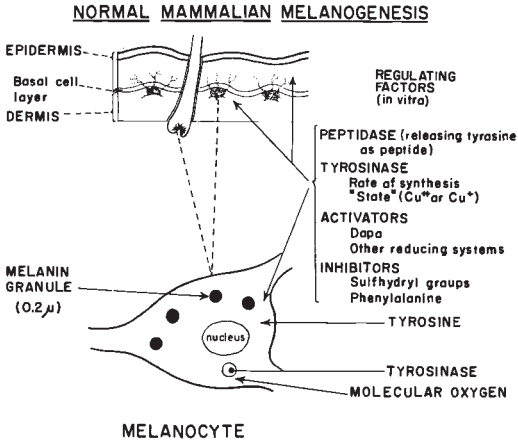


FIG. 12. Factors regulating mammalian melanogenesis. Recent tracer studies with O^{18} have shown that molecular oxygen is utilized in the hydroxylation of tyrosine. (Mason, 1956).

activation by ultraviolet are known. Furthermore, the biochemical mechanisms underlying this activation are not clearly understood. Several hypotheses have been advanced (14), and these are based on the rather limited knowledge of the various factors regulating melanin formation. 1) Trace amounts of dopa are formed by ultraviolet irradiation of tyrosine solutions; thus ultraviolet may provide catalytic amounts of dopa which activate tyrosinase (*vide supra*), 2) the level of inhibitory sulfhydryl groups (15) is reduced following ultraviolet irradiation of skin. However, it has not yet been possible to activate tyrosinase in human epidermal melanocytes by incubation in various chemicals that bind sulfhydryl groups, 3) the redox potential decreases appreciably after ultraviolet irradiation. Low redox potentials are known to favor tyrosinase activity (14). 4) The erythema resulting from ultraviolet irradiation may elevate the skin temperature, and an increase in temperature is known to accelerate *in vitro* melanin formation. However, melanin formation occurs readily with air or water-cooled ultraviolet light sources in which there is little or no elevation of skin temperature. It is apparent that the biochemical basis of suntanning is not known, and none of the hypotheses thus far advanced is clearly established.

SUMMARY

One of the major direct or indirect targets of ultraviolet exposure of skin is the melanocyte, or melanin-forming cell. The melanocyte is a highly specialized secretory cell which is derived from the neural crest and is localized in the skin, eyes and central nervous system. In the skin, melanocytes form a horizontal network at the plane of the dermo-epidermal interface and are integrated closely with the epidermal cells establishing contact by means of their numerous tenuous cytoplasmic processes.

Melanocytes contribute melanin granules to epidermal cells and exhibit the cell polarity of secretory cells. As epidermal cells move outward, the melanin granules in them are carried along and are seen as melanic particles in the stratum corneum. The melanized stratum corneum of negroid peoples and of caucasians who have been exposed to solar radiation acts as a biological filter of ultraviolet radiant energy. In addition, there is a thickening of the stratum corneum after exposure to ultraviolet. The relative resistance of negroid skin to ultraviolet induced erythema is related to the degree of melanization of the stratum corneum rather than a greater thickness of the stratum corneum. The stratum corneum is approximately equal in caucasian and negroid skin.

Quantitative studies of the regional distribution of melanocytes have revealed a marked individual and regional variation. Two to three times as many melanocytes are found on the face as on the thigh, for example. There is no significant difference in the number of melanocytes per square millimeter in negroid and caucasian skin. The variations of skin color in different races and in most disorders of melanin hyperpigmentation are related to an increased rate of melanin formation rather than melanocyte proliferation.

Melanin is a protein-conjugate formed by the coupling of a quininoid polymer, indole-5,6-quinone, with protein. The polymerization and coupling occur on the surface of a subcellular cytoplasmic particle, the melanin granule. The quininoid polymer is derived from the amino acid, tyrosine, by a chemical reaction catalyzed by an aerobic oxidase, tyrosinase, which is attached to the melanin granule.

Tyrosinase activity in the melanocyte is controlled by a series of checks and balances most of which are not clearly defined, *in vivo*. The level of enzyme activity and the degree of melanization appear to be important factors in the rate of melanin formation.

The biochemical basis of suntanning has not been clearly established. Ultraviolet can activate tyrosinase present in the melanocytes of the dermo-epidermal junction, but the action spectrum and the time sequences are not yet determined.

REFERENCES

1. Masson, P.: Pigment Cells in Man, in Biology of Melanomas, N. Y. Academy of Sciences, **4:** 15, 1948.
2. Birbeck, M. S. C., Mercer, E. H. and Barnicot, N. A.: The structure and formation of pigment granules in human hair, Expt. Cell Research, **10:** 505, 1956.
3. Thomson, M.: Relative efficiency of pigment and horny layer thickness in protecting the skin of Europeans and Africans against solar ultraviolet radiation. J. Physiol., **127:** 236, 1955.
4. Blum, H. F.: The physiological effects of sunlight, Physiol. Rev., **25:** 483, 1945.
5. Szabo, G.: The number of melanocytes in human epidermis. Brit. M. J., **1:** 1016, 1954.
6. Staricco, R. J. and Pinkus, H.: Quantitative and qualitative data on the pigment cells of adult human epidermis. J. Invest. Dermat. **28:** 33, 1957.
7. Billingham, R. E. and Medawar, P. B.: A study of the branched cells of mammalian epidermis, with special reference to the fate of their division products. Philosophical Trans. B., **237:** 151, 1953.
8. Mason, H. S.: Structure and functions of the phenolase complex. Nature, **177:** 79, 1956.
9. Fitzpatrick, T. B. and Kukita, A.: Tyrosinase activity in vertebrate melanoblasts and melanocytes. (In press) in Pigment Cell Biology, Academic Press, 1958.
10. Kukita, A. and Fitzpatrick, T. B.: Demonstration of tyrosinase in melanocytes of the human hair matrix by autoradiography, Science, **121:** 893, 1955.
11. Seiji, M. and Fitzpatrick, T. B.: Unpublished data.
12. Edwards, E. A. and Duntley, S. Q.: Pigments and color of living human hair. Am. J. Anat., **65:** 1, 1939.
13. Fitzpatrick, T. B., Becker, S. W., Jr., Lerner, A. B. and Montgomery, H.: Tyrosinase in human skin: Demonstration of its presence and its role in human melanin formation, Science, **112:** 223, 1950.
14. Lerner, A. B. and Fitzpatrick, T. B.: Biochemistry of melanin formation, Physiol. Rev., **30:** 91, 1950.
15. Rothman, S., Krysa, H. F. and Smiljanic, A. M.: Inhibitory action of human epidermis on melanin formation. Proc. Soc. Exper. Biol. & Med., **62:** 208, 1946.