Pigment Cell Biology: An Historical Review

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Colors decorate, but also affect, all members of the animal kingdom. Bees and other insects are attracted by the exquisite colors of flowers in their search for nectar to make honey. The eyes of the tiny hummingbird are especially sensitive to red colors so they can find flowers with the high energy nectars they require. Colors have delighted humans since prehistoric times. Primitive man used different colored pigments as ceremonial decorations, as signs of mourning or hostility, and as decorations on their bodies to advertise their status. They left us proof of their fascination with colors in the cave paintings found throughout the world. Every tribe and ethnic group in the world makes exquisitely colored artifacts, such as masks, banners, and spiritual symbols, as part of its cultural heritage.

Some of the most brilliant and striking uses of colored inks are found in the illuminated religious and biblical manuscripts laboriously prepared by hand in the dark ages. The Renaissance gave rebirth to the use of color in paintings and frescos. Modern western and artists and painters like Monet and other French impressionists captured the very essence and glimmer of color and sunlight in their paintings. Other artists have used color to establish moods. Johannes Itten, a leading proponent of the color theory in the Bauhaus school of art established in Wiemer, Germany, divided colors into warm and cool shades. He proposed that the manner in which the two types are combined affects peoples' perception of them. That colors add so much to our lives is given banal proof in the difference between a black-white and color television sets.

It should be no wonder then that in the writings from the most ancient times, scholarly people have speculated on why and how humans could be as black as Australian aborigines and Ethiopian warriors or as white as albinos. The shades of brown, tan, yellow-brown, red-browns observed in skin or the jet black, browns, yellows, auburns, reds and whites observed in hair are indeed a remarkable array of colors.

Through the ages, people of different skin colors have been refered as gods or enslaved as inferiors. Social and economic forces have attached cultural baggage to skin color not intended by nature. Biologically the pigment system is an important part of the skin, mucous membrane, eyes, ears, meninges, and other organs. We will review briefly the history and growth of our understanding of the pigment system. We arbitrarily have divided our review into five temporal periods: earliest studies prior to 1900; introduction of the scientific method for the study of pigmentation, 1900–1950; the period of 1950 to 1975; and the modern era from 1975 to the present. The last section presents a partial list of important questions that remain topics for study in the future. Hundreds of workers have made important contributions to this subject. We cannot name all of them or cite even one work from each in this brief review. We have attempted to acknowledge as many investigators as possible, and have included the work on melanocytes most frequently in *The Journal of Investigative Dermatology*.

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Supported in part by National Institutes of Health Grants AM25252, 1R 29 R38954-01, NIOSH 2-R01-OHO 2091-03, and the Ahlers Foundation, Cincinnati, Ohio.

THE EARLIEST STUDIES: PRE-1900

Some of the earliest scientific writings on the source of skin color and pigment were published in the 17th century by Malphigii for whom the epidermis was named. (This part of the manuscript was excerpted from SN Klaus: Some early scientific studies of skin color[1].) Malphigii was interested in how the skin perceived the sensation of touch. He separated the dermis and epidermis by allowing a piece of skin obtained from a black Ethiopian soldier to remain at room temperature overnight. He observed that between the inner layer (dermis) and outer covering (epidermis), there was a black mucous-like substance. He believed he identified the cause for dark skin. His observations were made without the use of a microscope.

John Hunter in the 18th century fixed tissues and was able to dissect the pigment layer from the skin. This seemed to be definitive proof that a pigment was present within the epidermis and caused the black coloring of an individual's skin. The source of the pigment was unknown. Many investigators believed that it was an accumulation of bile.

The function of melanin was not known. Many scientists noted that dark skin seemed to heat more rapidly when exposed to sunlight than white skin. Benjamin Franklin showed that dark colored cloths laid upon fresh snow on a bright sunny day melted the underlying ice crystals more rapidly than light-colored cloth. It was known that black-skinned individuals normally inhabited tropical regions and that white individuals came from the north. It seemed perverse to Franklin that nature would endow individuals living in the tropics with a type of skin which was inappropriate for the environment. Everard Home resolved this enigma with a simple but elegant experiment. He exposed both his hands to the sun. One was covered with a black cloth, the other was left bare. He measured the temperature of his skin. He found that the black cloth did indeed elevate the temperature of his skin a few degrees. However, the exposed hand became sunburned. He concluded that pigment did indeed cause a slight warming of the skin but protected it from the non-thermal, i.e., scorching, effects of the sunlight. Thus was born the idea that melanin was a sunscreen which prevented sunburn, a concept which persists to the modern age and is only now undergoing reconsideration.

INTRODUCTION OF SCIENTIFIC METHOD: THE SCIENCE OF PIGMENTATION FROM 1900–1950

The Names of Pigment Cells Terminology for the pigment cell system was poorly defined before 1950. It was not known which cells were capable of synthesizing melanin. Melanophages, now known to be macrophages laden with melanin, were not distinguished from dermal melanocytes, i.e., true pigment cells found, for example, in Mongolian spots. By 1950, there was a consensus that melanin producing cells should be called melanoblasts, and non-melanin producing cells called melanophores. This terminology was changed after 1950 [2].

The Embryonic Origin of Pigment Cells In 1934, G. P. Du-Shane published the first evidence that melanoblasts in the axolotl came from the neural crest. Because lower vertebrates had a duplicate pigmentary system in their skin, one in the dermis and the second in the epidermis, the applicability of this observation to mammals was debated. By 1940, Mary Rawles had published definitive results from experiments in which the neural tube was exterpolated and explanted that convinced all skeptics that melanocytes in vertebrates also were neural crest derivatives [3]. That concept is still regarded as dogma today.

0022-202X/89/$03.50

The Melanin Producing Cell: Melanoblast Bruno Bloch, a Swiss dermatologist, in 1917 described the dopa (dihydroxy phenylalanine) reaction for the identification of melanoblasts. A positive dopa test was considered proof that a cell was capable of synthesizing melanin. Bloch and others thought dopa was the primary substrate for the production of melanin and was delivered by the blood. Pierre Masson later found that melanin reduced silver ions, especially if it was dissolved in an ammonium nitrate solution. These early investigators used unfixed, often very thick sections. The techniques did not produce high resolution and the results were difficult to interpret. Prior to 1950, everyone agreed that melanin was found in greatest quantity in the basal layer of the epidermis. It was the general impression that basal keratinocytes, as well as melanoblasts, synthesized melanin. That melanoblasts were dendritic and were exclusively responsible for the production of melanin was not unequivocally agreed on until the work of Becker was published. Becker stained paraffin-embedded tissues which could be cut into relatively thin (5 μm) sections. He was able to delineate dopa positive pigment cells from keratinocytes more precisely. He concluded that melanin was transferred from melanocytes into surrounding keratinocytes. These points are now universally accepted. Billingham found that melanocytes were capable of dividing within the skin [4]. Most investigators believed the Langerhans cell to be a dying melanocyte. However, Merkel cells were considered to be an entirely different cell type.

The Melanin Pathway Bruno Bloch and others thought that dopa was the primary substrate for melanin. H. S. Raper identified the enzyme tyrosinase in several plants. The plant enzyme converted tyrosine as well as dopa to melanin. However, human epidermis was not known to contain the tyrosinase enzyme. Thus Raper's work was considered not to be applicable to human skin. Billingham later showed that human skin did indeed contain a tyrosinase enzyme [3]. He also suggested that tyrosine, not dopa, was probably the primary substrate for melanin. Raper also described the basic steps in the conversion of tyrosine to melanin through which tyrosine is first oxidized to dopa, to dopaquninone, and then through a series of intermediates which finally condense into the polymer called melanin. Mason modified details of the pathway, but the general outlines of the synthesis proposed by these two investigators remain valid today.

The Melanin Molecule Mason in 1948 divided the types of melanin into animal, plant, and synthetic forms. Melanin visualized by light microscopy appeared granular. The fact that pigment was contained within melanosomes was unknown. The two types of pigments, eumelanin and phaeomelanin, were not distinguished. Whether synthetic and natural melanin were similar was debated. Melanin was considered to be a polymer [2,3]. Whether it was composed of identical subunits (homopolymer) or a potpourri of different compounds (heteropolymer) was heatedly disputed. Harold Bloom suggested that melanin was a very poor sunscreen and that most of the protection against sunlight was provided by the thickness of the stratum corneum [2,3]. This point has been re-emphasized today.

The Transfer, Degradation, and Excretion of Melanin That melanin was synthesized exclusively by epidermal melanocytes was not appreciated until the 1930's. Because basilar keratinocytes had so much melanin within their cytoplasm, originally it was thought that they were capable of synthesizing this pigment. This latter idea was abandoned and the concept of transfer of pigment became universally accepted [5]. Melanin could be found in epidermal keratinocytes, but also in dermal melanophores, lymph nodes, and so on. It was thought that sunlight caused rapid transit of pigment from the basilar layer of the epidermis to the stratum corneum from where it was excreted by desquamation. Most investigators also thought that melanin normally was transported via melanophores to lymph nodes, liver, and spleen, and eliminated in the urine or via the intestinal tract. It is surprising that little more is known today about the process of melanin degradation and excretion.

Regulation of Melanocyte Function and Proliferation Sunlight was an obvious stimulant of pigment cell function because it produced tanning. Tanning from melanin synthesis was observed 3–5 d after exposure to sunlight and was distinguished from immediate pigment darkening (the Mierowsky phenomenon). Immediate pigment darkening occurred within hours after light exposure, occurred in cadavers, and could be induced by heat as well as light filtered through window glass (UVA spectrum; 320–400 nm) [2,3]. Mechanical injury, radiation of all types, and heat all enhanced pigmentation. Investigators recognized that diseases of the adrenal and pituitary gland were associated with hyperpigmentation, although they could only speculate about the mechanisms for enhanced pigmentation. It was suggested that the loss of sodium chloride associated with Addison's disease (adrenal insufficiency) accelerated the oxidation of vitamin C in the skin. The loss of vitamin C could enhance melanin formation. Stephen Rothman showed that the skin contained many proteins with sulfhydryl side chains which inhibited the oxidation of tyrosine to melanin. He also showed that sunlight depleted the epidermis of sulfhydryl containing compounds. He suggested that melanin formation was regulated by this mechanism [2,3].

Diseases of Pigmentation Vitiligo was thought by S. W. Becker to be a functional impairment of melanocytes, not a destruction of the cell. Malignant melanoma was poorly described and not differentiated from the benign Spitz nevus (juvenile melanoma). Piebaldism was not well understood. It was noted that only one of the Dionne quintuplets, all five of whom were thought to be monozygotic, had a moderately large congenital nevus. Thus, congenital nevi were thought not to be genetically determined.

Melanocyte Function: Cell/Cell Interactions Melanin was known to be an oxygen scavenger but not a great sunscreen. However, it was recognized that blacks had fewer skin cancers. Epidermal pigment cells clearly transferred melanin into keratinocytes, but the biochemical signals causing this transfer were not known. As noted above, Langerhans cells were considered to be dying pigment cells.

1950–1975: INTRODUCTION OF MOLECULAR AND CELLULAR BIOLOGY INTO PIGMENTATION

The Names of Pigment Cells In the 1950's, the terminology of the pigment cell system changed slightly. A melanoblast, formerly a pigment producing cell, was redefined as a precursor cell that can differentiate into a mature cell capable of synthesizing melanin. The mature cell was renamed a melanocyte [6]. Melanophore was a term reserved for dermal pigment cells that are found only in lower vertebrates and which were responsible for the rapid color changes exhibited by these animals [7]. Melanophages were defined as dermal macrophages laden with melanin [8,9].

The Embryologic Origin of Melanocytes It was shown that the neural crest, especially the cranial portion, was the embryologic anlage for many mesenchymal tissues of the head and neck, i.e., the bones, cartilage, and muscles of the face, as well as leptomeninges and portions of the cardiac conduction system. These data provided a hypothetical basis for understanding complex pigmentary syndromes such as the multiple lentigines syndrome. In addition, work by Beatrice Mintz elegantly demonstrated that melanocytes migrating from the neural crest followed dermatomal pathways [10].

Melanin Producing Cells: Melanocytes The newly named melanocyte was the cell responsible for the synthesis of all melanin [5,6,11,12]. That epidermal pigment cells synthesized melanin throughout life, whereas dermal melanocytes, uveal, and retinal pigment epithelial cells did not, was first recognized in the 1950's. Thomas Fitzpatrick proposed the concept of the epidermal melanin unit, i.e., one melanocyte providing melanin to about 35 keratinocytes [6,13].

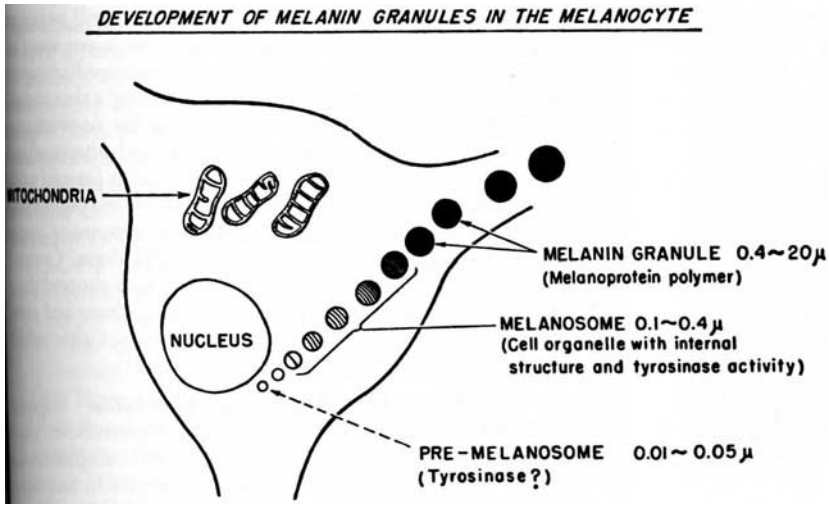

Figure 1. Diagram showing development of melanin granules in the melanocyte. Melanin granules are distinct cytoplasmic constituents of the melanin forming cell and have their own characteristic enzyme (tyrosinase). The term ''melanosome'' is proposed for the distinctive, enzymatically active particle which is the site of melanin formation and is located within the cytoplasm of the melanocyte [14].

By electron microscopy it was recognized that melanin was packaged in melanosomes that had a very precise substructure. M. Seiji first proposed the term melanosome for this cytoplasmic organelle (Fig 1) and suggested that melanosomes were formed by the fusion of vesicles from the smooth endoplasmic reticulum and the Golgi apparatus [14]. He suggested that tyrosinase was synthesized in the rough endoplasmic reticulum. The tyrosinase containing vesicle fused with particles and other vesicles and became activated. That epidermal pigment cells always were adjacent to the epidermal basal lamina and were free of desmosomal attachments was also noted by electron microscopy. Other ultrafine details of the nuclei, mitochondria, and cellular substructure were described.

Attempts to determine whether the enzymes of the glycolytic (Embden-Meyerhoff) and the Krebs citric acid cycle were present in melanosomes were impeded by the problem of separating melanosomes and mitochondria [2,3,15]. The electron microscope permitted investigators to monitor the separation process. Although glycolytic oxidative enzymes were not found in melanosomes, other enzymes like acid and alkaline phosphatases, which are characteristics of lysosomes, were found. Melanosomes may be one type of lysosome.

George Szabo completed studies that showed that the difference between dark and lightly colored skin was the quantity of melanin. In black skin, melanocytes form numerous large melanosomes. In white skin, pigment cells produce smaller and partially melanized melanosomes. However, white skin could be induced to produce larger and more fully pigmented organelles by exposure to sunlight [5,16].

Langerhans cells were still relegated to the garbage heap as dying melanocytes.

The Melanin Pathway A. B. Lerner first showed that one enzyme could catalyze the first two steps in the melanin pathway (Fig 2); that is, one enzyme could function both as tyrosine hydroxylase and dopa oxidase [17]. For simplicity, this enzyme was called tyrosinase despite its dual function. Lerner and T. B. Fitzpatrick isolated the enzyme and showed that dopa was a necessary cofactor to accelerate the oxidation of tyrosine to dopa. Eventually, multiple forms of tyrosinase were identified (Fig 3) [18].

That tyrosine, not dopa, was the normal substrate [19] for the ptroduction of melanin in man was shown by clinical observations on children with the disease phenylketonuria. Phenylalanine competes for the binding site of tyrosine and inhibits the function of tyrosinase. Children with phenylketonuria are albinoid and have very light colored hair. Removal of phenylalanine from their diet and substitution with tyrosine not only improves their health, but also returns the color of their skin and hair to normal.

New intermediary steps in the conversion of tyrosine to melanin were identified during this time [19]. The Raper-Mason pathway became longer and more complex. Oxidation of tyrosine to melanin was found to require release of at least one molecule of CO_2 and the removal of four electrons from tyrosine.

Melanin Polymer The structure of melanin was still unproven, although most investigators suggested that the pigment was a heteropolymer formed by condensation of many intermediary metabolites in the melanin pathway. Mason argued strongly that melanin was a homopolymer [20]. Giuseppi Proto and his co-workers in Italy distinguished two pigments, eumelanin and phaeomelanin. Eumelanin is black, brown, or yellow in color and is formed exclusively from tyrosine. In contrast, phaeomelanin is a red pigment and is formed from two amino acids, tyrosine and cysteine. Cysteine condenses with dopaquinone to form cysteinyl dopa. Cysteinyl dopa is the precursor for phaeomelanin. Later, Prota showed that in all human melanin there was a mixture of both phaeo and eumelanin [27]. Blacks formed predominantly eumelanin, Celtic individuals a larger proportion of phaeomelanin.

Melanin Transfer, Degradation, and Excretion Microfilaments composed of actin and myosin and microtubules which formed the cytoskeleton of dermal melanophores in amphibian skin are required for the translocation of melanosomes in and out of dendrites. In human pigment cells, 10 nm intermediate filaments appear to be responsible for the transfer of melanosomes from the perinuclear region to the tip of the dendrites. Intercellular transfer of melanosomes is accomplished by phagocytoses of the tip of the dendrites containing melanosomes by the adjacent keratinocytes (Fig 4) and later digested so that the melanosomes can disperse through the cytoplasm of the keratinocyte [22]. Many large melanosomes are found in keratinocytes of black skin [23]. Smaller melanosomes are retained in the cytoplasm of the melanocytes in white skin.

Regulation of Melanocyte Differentiation and Growth In the 1950's, Aaron Lerner and Kazuo Shizume isolated alpha-MSH (13 amino acid) from bovine pituitary glands and determined its structure [24], Later, Teh Lee [25] isolated a second pigmenting hormone, beta-MSH (17 amino acid). Both of the hormones induced rapid alterations of skin color in amphibians [26,27]. This was due to translocation of melanosomes

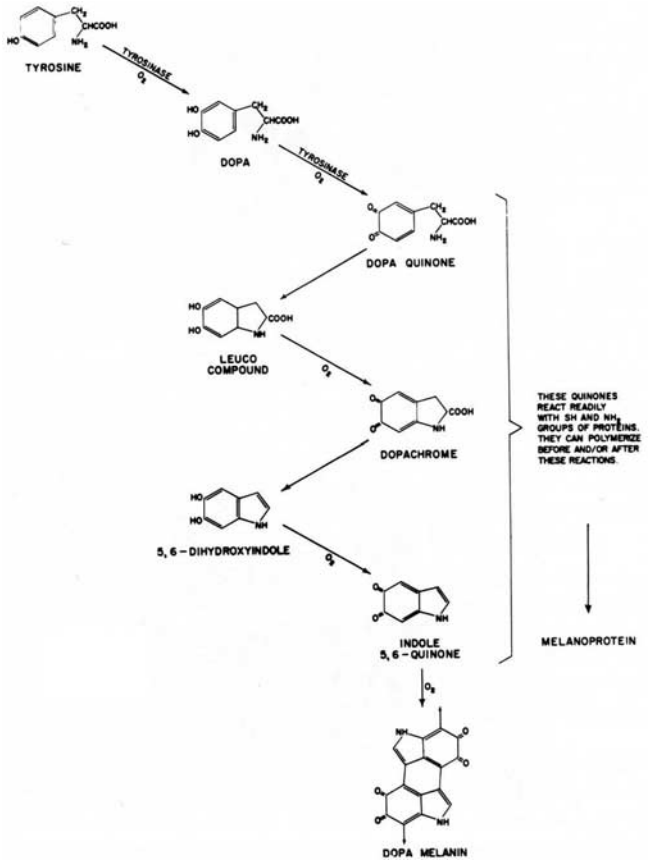

Figure 2. Enzymatic oxidation of tyrosine to melanin [17].

within dermal melanophores. M Hadley showed that MSH could also increase melanization by epidermal pigment cells in these same animals. MSH, and to a lesser degree ACTH, injected into humans for weeks or months did stimulate an increase in pigmentation, although MSH molecules were far less potent in humans than in the amphibian system [28,29].

Lerner also isolated melatonin from the pineal glands [35]. It rapidly reversed the effects of MSH on dermal melanocytes in frog skin [30]. However, melatonin, named for its ability to lighten amphibian skin, had no effect on human skin color. It does have critical importance for the regulation of pituitary function, and its secretion is regulated by the intensity of ambient sunlight. Julius Axelrod won the Nobel Prize in the early 1970's for his work on on pineal function and its synthesis of melatonin and other hormones.

Richard Snell showed that the various MSH molecules had very different potencies in different animal species. He also demonstrated that thyroxine, androgens and estrogens could increase pigmentation. The sex hormones had particularly potent effects on sexually related skin such as the genitalia, areola, and nipples.

Glenda Wong first observed that the cultures of murine Cloudman S91 melanoma cells obtained from the American Tissue Type Collection could respond to MSH [31]. Wong and Pawelek, using the S91 Cloudman melanoma line, demonstrated that MSH bound in the membrane of the cells activated the enzyme adenylate cyclase which formed cyclic AMP (cAMP). cAMP, an intercellular messenger, activated a second enzyme, protein kinase. Thereafter, an unidentified series of events occurred which caused transcriptional and translational events resulting ultimately in activation of tyrosinase. Pawelek postulated that the new tyrosinase activity was principally it due to activation of preformed enzyme [31]. Currently, it is thought that MSH both activates preformed enzyme as well as induces *de novo* synthesis.

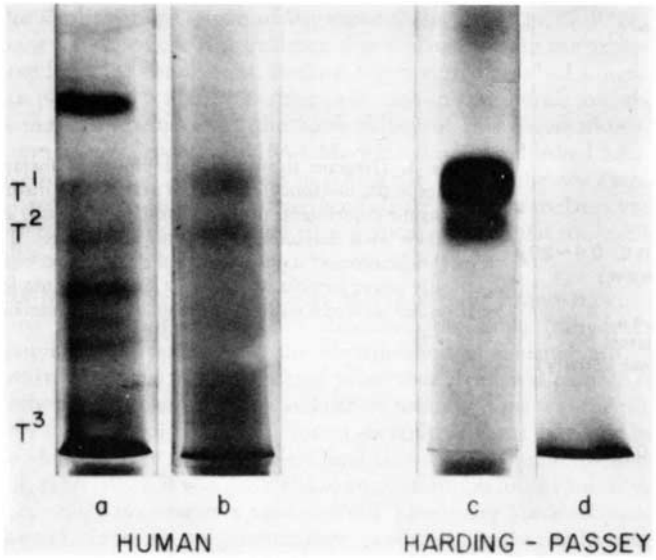

Figure 3. Patterns of proteins (*a*) and active tyrosinase (*b*) extracted from human metastatic malignant melanoma. Patterns of activity of soluble (*c*) and macromolecular (*d*) tyrosinase derived from Harding-Passey mouse melanoma as shown by acrylamide-gel electrophoresis. As oriented here, the enzyme migrated from the bottom (cathode) to the top (anode) of the gel [18].

Pawelek observed that Cloudman melanoma cells grew more slowly in the presence of MSH. He proposed that cAMP was a double signal, i.e., it increased tyrosinase activity but decreased cellular proliferation. He noted that in many of his mutant melanoma cell lines, cAMP was not necessarily a signal for either function. This work brought pigmentation to a molecular level [32,33].

Diseases of Pigmentation A major advance of clinical significance was the classification of melanomas into nodular, superficial spreading melanomas and lentigo maligna by G. McGovern and Wallace Clark [34]. Later, a prognostic index (Clark levels) was proposed and found to correlate accurately with the clinical course of patients with melanomas. This has been partially superceded by the system described by Alexander Breslow and measures the thickness of a melanoma with an ocular micrometer. The Breslow system as a single parameter is a somewhat more accurate indicator for predicting the five-year survival of patients with melanoma or recurrence rates. Clinicaly, it seems that the best information is a recombination of both the Clark level and the Breslow thickness. Vitiligo was shown to be a destruction in the elimination of the melanocytes from the depigmented areas of skin [36]. Psoralens were recommended for treatment of vitiligo [55]. Some types of oculocutaneous albinism were proven to be due to defects in the tyrosinase enzyme (absence of, or aberrant processing of the enzyme), not due to a loss or lack of melanocytes (Fig 5) [37]. In contrast, piebaldism [38] was found to be due to a genetically determined absence of melanocytes from the unpigmented patches [36].

The Function of Melanocytes; Cell/Cell Interactions Little work was done on this topic until the 1980 s. However, Inga Silberberg published her first article implicating the Langerhans cell in contact dermatitis in guinea pigs. The concept was rejected by many pigment cell biologists who considered the cell an effete melanocyte. The relationship of melanocytes and Langerhans cells remained a dilemma [39].

1975–1987: THE MODERN ERA

Names of Pigment Cells No changes in the terminology have occurred in the last thirty years [40].

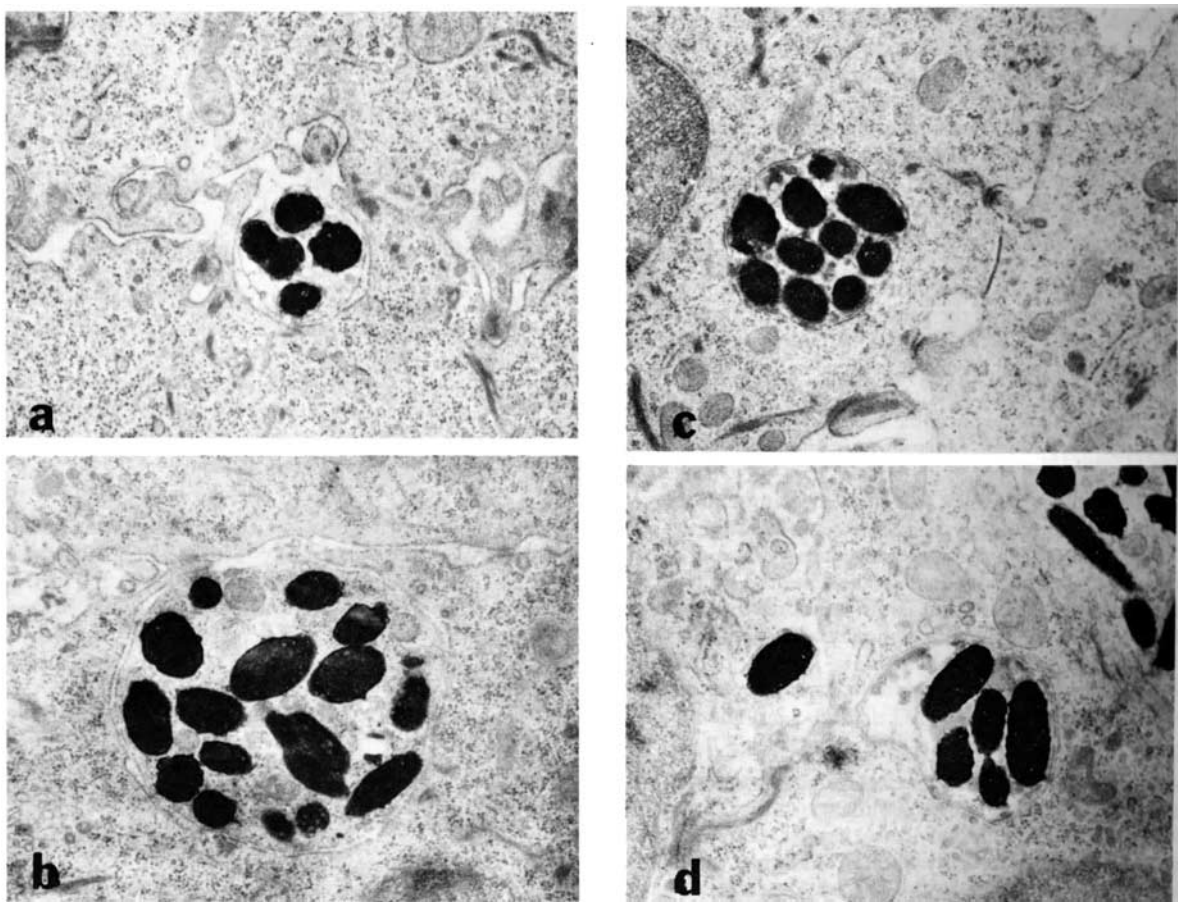

Figure 4. Cytoplasmic projections of the cortical cell extend from the cell body and wrap around the tip of the melanocyte dendrite *(a)* which is later pinched off and completely enclosed within the cortical cell cytoplasm (*b*). The pinched-off portion of the dendrite exists for a period of time in the cortical cell cytoplasm enveloped by its own membrane and that of the cortical cell (*c* and *d*) [22].

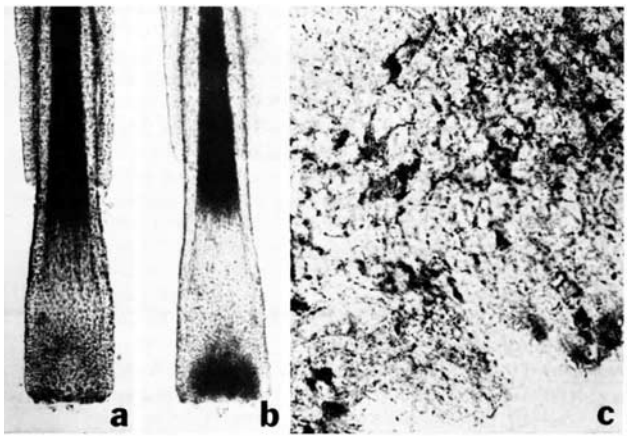

Figure 5. *a:* Human albino hair root, control, *b:* Human albino hair root, after incubation in tyrosine solution for 24 h; pigmentation is confined to region of lower matrix where melanocytes are normally present, *c:* Pigmented dendritic cells in unstained albino epidermis after incubation in tyrosine [37].

The Embryonic Neural Crest Origin of Melanocytes From the work of Karen Holbrook, it is now recognized that in humans melanocytes begin to migrate from the neural crest as early as 2.5 weeks post-fertilization. The cells are first found in the dermis and arrive in the epidermis around 8–10 weeks. Melanosomes and small amounts of melanin can be found by electron microscopy shortly thereafter [40]. The cells do not actively produce melanin until after birth except in some areas like the genitalia and nipples [41]. At birth these sites are deeply pigmented. Presumably this pigmentation is due to the steroidal stimulation of sex specific skin induced by hormones from the mother.

The eyes also have a full complement of pigment cells at birth [41]. Those in the retinal pigment epithelium come from the primitive forebrain, not the neural crest. They are replete with melanin by 8–10 weeks of gestation. Melanin in the retinal pigment is now thought to be the embryologic inducer for normal development of the fovea and optic nerves. Lack of melanin in the eyes during the first trimester is thought to be the cause of the ocular dysfunction and foveal hypoplasia diagnostic of oculocutaneous albinism.

Choroidal melanocytes which come from the neural crest in humans do not initiate melanin formation until the third trimester of gestation and may continue to produce melanin and cause darkening of the iris of an individual until early adulthood [41].

Melanin Producing Cells: Melanocytes Melanosomal formation and tryosinase transport were described in more detail (Fig 6) [42,43]. Melanosomes containing phaeomelanin are round and have an irregular substructure. Eumelanosomes are oval in shape and have a very orderly substructure. All pigment cells can produce either type of melanosome or melanin, although the switch mechanism for this has not been well identified.

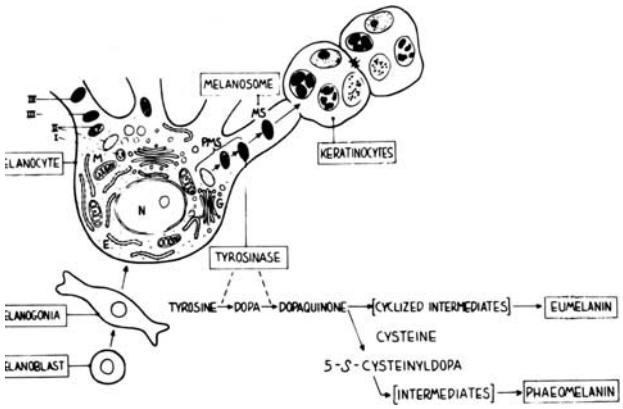

Figure 6. Diagram summarizing major events in the early development of melanocytes, their subsequent differentiation, and interaction throughout life. *G*: Golgi apparatus; *E:* endoplasmic reticulum; *N:* nucleus; M: mitochondria; *PMS:* early melanosome; *MS:* fully melanized melanosome; *I, II, III,* and *IV:* stages in melanosome development, shown in *upper left corner* of dendritic process [43].

Tyrosinase is translated on ribosomes of the rough endoplasmic reticulum. The core tyrosinase molecule is transported to the Golgi apparatus where its carbohydrate moieties are modified. At the trans face of the Golgi apparatus, the tyrosinase is already capable of giving a positive dopa reaction, i.e., synthesizing melanin. In the Golgi apparatus, tyrosinase is glycosylated. Complete glycosylation seems critical for translocation of tyrosinase into Golgi-derived coated vesicles and for fusion with premelanosomes formed from the smooth endoplasmic reticulum. Fusion of the two vesicles puts active tyrosinase into the melanosome [41]. Tyrosinase converts tyrosine to dopa very slowly at pH of 6.8 or above. Recent evidence indicates that at lower pH the reaction is much more rapid. Results of preliminary work indicate that the melanosomal pH may be significantly lower than previously thought. Thus, tyrosinase activity may be determined in part by the pH of the local environment within the cytoplasmic organelle.

The source of melanocytes in hair bulbs is mysterious. Telogen hairs normally fall out and are replaced by anagen hairs which retain their normal color. At older age, such hairs become gray. Some melanocytes are shed within the hair itself. Do melanocytes dedifferentiate and remain in the dermis or epidermis until the new hair grows? Or is there a pool of melanoblasts residing in the dermis near the hair bulb ready to repopulate the new hair? This is a persisting enigma. It is interesting that one patient with silver-white hair received electron beam for mycosis fungoides. He lost hair in those areas exposed to radiation. When the hair regrew, it was pigmented. This observation suggests that a pool of primitive melanoblasts residing within the skin and was activated by the X radiation.

By the late 1970s it became abundantly clear that Langerhans cells and pigment cells were different and the former had unique immunologic functions. By 1987, new data suggested that pigment cells interacted closely with both the Langerhans cell and keratinocyte [44,45].

A major advance was the serendipitous discovery by M. Eisinger that normal human melanocytes grew rapidly and vigorously in the presence of phorbol esters and cholera toxin [46,47]. Other investigators have used extracts from various tissues, e.g., the hypothalamus, and growth factors such as basic fibroblast growth factor rather than phorbol ester to grow melanocytes in culture.

Melanin Pathway The intermediary steps in the conversion of tyrosine to melanin were more precisely defined [48]. A second enzyme was identified, namely, dopachrome oxido reductase. This enzyme acted as a dopachrome conversion factor as well as an indole blocking factor. It converted dopachrome to 5,6-dihydroxyindole and in the absence of tyrosinase inhibited the oxidation of dihydroxyindole to indolequinone. In addition, tyrosinase has been shown to be active in a third reaction involving the oxidation of 5,6-dihydroxyindole to 5,6-indolequinone, which subsequently formed melanin. The existence of other non-enzymic regulators such as Ca^{++} and other factors within the melanin pathway have been postulated in order to explain the multiple types of albinism (9 types) in which patients have adequate quantities of active tyrosinase enzyme but form melanin very slowly.

Multiple glycosylated forms of tyrosinase have been identified and are capable of giving a positive dopa reaction. The smaller molecule (68 kd) is thought to be a precursor of the final mature and active molecule, an 81.2-kd protein. Several investigators have indicated that they have isolated and are working on identifying the sequence for the tyrosinase gene.

The Melanin Molecule It is now agreed that melanin is a heteropolymer. However, its precise structure, if constant, is not known. The question whether animal, plant, and synthetic melanin are similar remains to be determined. Phaeomelanin is rapidly degraded by ultraviolet light and releases free oxygens [49]. Eumelanin is more photostable.

Melanin Transfer, Degradation, and Excretion Culture of melanocytes indicates that they are very active cells synthesizing melanin and moving their dendrites hither and there. The cells are mobile *in vitro* and can be induced to migrate also *in vivo*. For example, repigmentation of patients with vitiligo involves migration of melanocytes primarily from hair follicles. Keratinocytes also are active partners in melanin transfer. In tissue culture, it is clear that keratinocytes are very actively phagocytic. They will phagocytize polystyrene beads or isolated melanosomes. Thus it is likely that the keratinocyte and epidermal pigment cell communicate actively during the melanin transfer process. Signals for this are unknown.

Regulation of Melanin Formation MSH is the classic melanotropic hormone [50]. It is produced by the synthesis of a large peptide, proopiomelanocortin, in the pituitary. The latter peptide has 256 amino acids and is cleaved in the pituitary into a variety of bioactive compounds including α-MSH, ACTH, β-lipotrophin, enkephalins, and endorphins [56]. Beta-MSH probably is not a biologically relevant molecule in humans [51,52].

It is unclear how physical types of energy, for example, electron beams or electromagnetic irradiation (UV light) [53], stimulate melanin formation or pigment cell growth. It is known that keratinocytes store 7-dehydrocholesterol. Recent work indicates ultraviolet light photoisomerizes 7-dehydrocholesterol into cholecalciferol. This latter compound activates the enzyme phospholipase A_2 and causes liberation of arachidonic acid. Cholecalciferol is later converted into vitamin D. Arachidonic acid is converted to prostaglandins and leukotrienes or other metabolites.

Recently a vitamin D receptor has been isolated from normal human melanocytes. The receptor binds specifically 1,25-dihydroxy vitamin D3. The dihydroxylated vitamin D and 25-hydroxy vitamin D_3 suppress tyrosinase activity of human melanocytes growing in the very artificial media containing phorbol esters, cholera toxin, and other agents. What effect vitamin D has on epidermal pigment cells in normal human skin is not clear. Many investigators have suggested 1,25 dihydroxy vitamin D_3 is involved in regulation of melanin synthesis. It has also been suggested that melanin content determines the amount of UV required for optimal vitamin D_3 synthesis in the epidermis [51].

Arachidonic acid stimulates epidermal proliferation and melanin synthesis. This effect is mimicked by oleic acid, an indicator that fatty acids have a direct effect on epidermal pigment cells. That the effects of arachidonic acid are partially, albeit significantly, blocked by indomethacin suggests that some of the observed results are mediated by prostaglandins. The proliferation of epidermal pigment cells induced by

ultraviolet light is also blocked by indomethacin. It seems that prostaglandins affect pigment cells in the epidermis. Application of PGE to murine skin stimulates both proliferation and melanization [51].

In vitro, PGE is one of the most potent activators of tyrosinase in murine Cloudman melanoma cells, more so than α-MSH. However, it retards proliferation. Prostaglandin E does increase intracellular cyclic AMP. This and other extensive data confirm that cAMP cannot be both a signal for melanization as well as control of proliferation [57]. PGE retards cell growth of Cloudman cells. PGE may be producing its effect by holding cells in the G2 phase of the cell cycle. Others have shown that it is in G2 phase of the cell cycle that the MSH receptor is expressed. Possibly *in vivo* a noxious agent stimulates PGE formation which pushes cells into G2 and initiates melanogenesis. In G2, MSH receptors would be unveiled. MSH would then enhance the stimulatory effect and cause production of melanin. Anthony Thody has suggested that beside the pituitary gland, the epidermis might be a site for MSH synthesis.

It is now known that alpha-MSH is an antagonist for interleukin 1, a critical early activating signal for the immune response. Interleukin 1 in the hypothalamus induces fever by activating formation of prostaglandin E. In the liver, interleukin 1 causes release of acute phase reactants. For lymphocytes, interleukin 1 is necessary for initiation of immune response. Alpha-MSH blocks all of these effects. Every cell in the epidermis, keratinocytes, melanocytes, and Langerhans cells, synthesize interleukin 1 and/or an interleukin 1-like molecule called ETAF. Application of alpha-MSH to the skin abrogates its immune functions. This MSH induced block is reversible by interleukin 1/ETAF, an indication that within the skin there is a competitive interchange between MSH and interleukin 1 [58]. Conversely, ETAF totally blocks the melanogenic effect of MSH on Cloudman melanoma cells. The normal enhancement of melanization and tyrosinase activity induced by MSH is completely reversed by low concentration of ETAF [59]. This further suggests that cells of the epidermis must have a very intricate signaling system.

Diseases Identification of the pathophysiology [54] and the treatment of pigmentary disorders lags far behind our knowledge of the cellular and molecular biology of the pigmentary system.

Function of Melanocytes; Cell-Cell Interactions We believe melanocytes are one of the three cells in the epidermal tripod. The three cell types interact closely. Most inflammatory processes such as ultraviolet light, psoralens plus UVA (PUVA), the oxidation of arachidonic acid to prostaglandin and leukotrienes, the oxidation of pro-carcinogens by the aryl hydrocarbon hydroxylase system — each of which of which is a potent melanogenic agent — also produce toxic Oxygens. We suggest the melanocyte function is to remove these toxic oxygens from surrounding cells and convert them into an inert molecule called melanin. Melanin itself can be and is an oxygen scavenger and a sunscreen. But the more important aspect is the ability of the melanocyte to remove the mutagenic and carcinogenic toxic oxygen from the surrounding keratinocytes and Langerhans cells. Melanin in dark skin is only an indicator of the effectiveness of this process. We suggest that the melanocyte-keratinocyte-Langerhans cell tripod must work closely together for optimal homeostasis in the epidermis.

THE FUTURE: SOME UNRESOLVED PROBLEMS

The following problems need to be resolved in the future: 1) Identification and isolation of the MSH receptor and the role of MSH in melanocyte function; 2) Identification and isolation of the prostaglandin receptor; 3) Identification and isolation of the epidermal thymocyte activating factor (ETAF) receptor; 4) Determination of the mechanisms by which ETAF inhibits the response of melanoma cells (i.e., increase melanin synthesis) to melanogenic agents such as MSH; 5) Precise identification of the mechanism for the transcription of enzymes and structural proteins needed for melanosomal formation and translocation of tyrosinase from the Golgi apparatus into the pre-melanosome; 6) The role of PGE in the control of cellular proliferation and alteration of melanocyte cell cycle, and the implications of these effects on tyrosinase stimulation; 7) The role of vitamin D in melanization; 8) The role of pigment cells in epidermal homeostasis in response to noxious agents; 9) Determination of the structure(s) of melanin; 10) Production of better treatments for pigment cell disorders based on the cellular and molecular biology of the system, i.e., melanoma, vitiligo, etc; 11) Determination of the mechanism for degradation of melanin within the epidermis; 12) Identification of other regulatory factors in the melanin synthetic pathway which might explain disorders such as albinism.

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