THE PIGMENT MELANIN OF THE SKIN AND HAIR*

By SAMUEL M. PECK, M.D.
New York, N. Y.

IN THE higher vertebrates, especially in man, most of the melanotic pigment is to be found in the ectordermal elements of the skin, where it plays a much more important role than the mesodermal pigment. The pigment building cells (melanoblasts) of the epidermis are the ordinary basal cells, sometimes cells lying directly above them, and cells living at about the basal cell level but showing dendritic processes. Melanoblasts are also found in the hair matrix cells of the bulb, in the epidermal hair sheets, the epidermal parts of the sebaceous glands, the excretory ducts of the sweat and sebaceous glands and in certain mucous membranes.

With the paper of Kolliker in 1860 there began a controversy that lasted for many years as to whether the epidermis built its own pigment or whether it obtained this pigment from the cutis. According to Kolliker, Aebi, Halpern, Riehl and Karg (1860 to 1891), cells from the cutis (connective tissue cells or leukocytes) filled with pigment wandered up to the epidermis and gave up their melanin to it (1). Ehrmann (2), in his comprehensive monograph on pigment in 1896, concluded that melanin was formed in special cells, "melanoblasts," which were not identical with leukocytes, connective tissue cells or epidermal elements. These melanoblasts were of mesodermal origin, and they were capable of wandering into the epidermis and functioning there. By means of their dendrites they transferred their pigment to the other epidermal cells.

Loeb, Mertsching, Garcia, Schwalbe and Post (3), in a series of papers (all published before 1900) based on experimental and embryologic studies, disagreed with the authors mentioned, and maintained that the epidermis was capable of building its own pigment without any help from mesodermally derived elements.

Investigation of the embryology of formation of pigment in the skin of the higher vertebrates, including man, by Meirowsky (4), Wieting and Hamdi (5), Adachi (6), Bloch (7), Miescher (8), Steiner-Wourlisch (9), Dawson (10) and others during the last thirty years has shown that this formation was autochthonous in the epidermis. They all agreed that from the earliest embryonal life the ectodermal

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melanoblasts were seen only at the beginning of pigment formation in the area where pigment was found in postuterine life (7).

The mesodermal melanoblasts of the skin in man are but rudimentarily developed, as in the blue nevus and mongolian spot; but in certain animals, such as the monkeys, guinea pigs, the gray house mouse and the negro fowl, they form an important system of cells. These cells, however, never communicate with the ectodermal melanoblasts, and they have an origin and period of development different from those of the ectodermal cells. They are perhaps akin to those cells that play such an important role in the coldblooded animals. The independence of the development of these two types was especially brought out by Bloch (7) and Steiner-Wourlisch (9).

It seems probable from the investigations of Schmidt (11) on reptiles, and especially from those of Kornfeld (12) (Anuren) that in coldblooded animals, pigment cells of mesodermal origin can wander into the epidermis and function there.

Chemistry of Pigment (Melanin) Formation

Modern investigators of pigment agree that the formation of pigment in the melanoblasts of ectodermal and mesodermal origin is due to the oxidation of a colorless propigment by an enzyme. Fuerth has shown that this melanogen is most probably a cyclic protein component. Fuerth, Przibram, Onslow and others first proposed tyrosine as the melanogen. Free tyrosine has been demonstrated in the circulation of the higher vertebrates, but tyrosinase has been found in insects and coldblooded animals only. There is no positive evidence that tyrosine is the mother substance of melanin in animals, especially in man. In recent years, a number of Italian authors (Angeli, Saccardi, Rondoni, Gallerani, Quattrini and Introzzi) have maintained that pyrrole and its derivatives, such as methylindole and scatole, are also propigments. The fallacy of their conclusions has been brought out by the work of Bloch and Schaaf (7) and of Peck (13).

Bloch (7) has shown that another aromatic amino acid, namely, B-3-4 dioxyphenylalanine, is the probable propigment in warmblooded animals. The main support for this assumption is his discovery of the reaction to dioxyphenylalanine in the melanoblasts. This reaction consists in the change of dioxyphenylalanine into a melanin. Since the reactions take place in sections of skin, Bloch was able to study microscopically the process of the formation of pigment (dopa reaction). He and his school demonstrated that the reaction to dioxyphenylalanine is specific and enzymatic. It is positive only with B-3-4 dioxyphenylalanine and cannot be elicited with tyrosine, epinephrine or other related dioxyphenyl derivatives.

There is a strict parallelism between the reaction to dioxyphenyl-

alanine and the normal formation of pigment. There is no reaction to dioxyphenylalanine in albinotic skin and vitiligo, while an increase reaction is a necessary accompaniment and the first evidence of all processes of pigment formation Bloch (7), Peck (14), Miescher (8). Further pigment is the demonstration of pyrocatechol, the natural deaminization product of dioxyphenylalanine, in the urine of patients with generalized melano carcinoma.

One cannot refute the assumption of the propigment function of dioxyphenylalanine on the ground that its presence has not been demonstrated in the animal body. The deeper understanding of interlocking chemical processes, such as alcoholic fermentation, has shown that an intermediary substance could easily escape analytic detection and yet form a pivotal phase in the course of the enzymatic process.

A great many other criticisms have been raised against Bloch's assumption of a specific dioxyphenylalanine oxidase. They have been based mainly on the fact that there are other catalytic systems of wider distribution that convert dioxyphenylalanine into melanin. Polyphenolase, an enzyme of almost ubiquitous occurrence in living matter, acts on polyphenol derivatives, especially orthodiphenol (pyrocatechol) and its derivatives, such as epinephrine, dioxyphenylalanine, etc., an oxidation that in the presence of amino groups leads to the formation of melanin.

The effects of this enzyme are often overshadowed by a concomitant heat-stable and therefore nonenzymatic mechanism of oxidation, which is attributed to the iron system of the cell. The differentiation between these two catalysts has to be based on careful quantitative studies before one is entitled to refute the enzymatic character of such reactions.

Tyrosinase is another enzyme that can convert dioxyphenylalanine into a melanin. It is found in insects, fungi and higher plants such as potatoes. The mechanism, of the formation of melanin from tyrosine by this enzyme has recently been elucidated by Raper (15). He showed that the first step in the oxidation of tyrosine is the introduction of a second hydroxy group into the benzene ring yielding 3-4 dioxyphenylalanine. The later stages of formation of melanin are due to a less specific mechanism the enzymatic nature of which is doubtful. Tyrosinase also acts on other monophenol derivatives such as paracresol, tyramine, etc. Unless the enzyme is purified, it also oxidizes the substances mentioned because of contamination with polyphenolase. There is no evidence of the identity of either tyrosinase or polyphenolase with dioxyphenylalanine oxidase. The oxidation of dioxyphenylalanine by tyrosinase can certainly not be used as evidence for the identification of this enzyme with the pigment oxidase, since the outstanding fact remains that the dioxyphenylalanine oxidase acts only on dioxyphenyl-

alanine and not on tyrosine (Bloch and Schaaf (7) and our own experiments).

The polyphenol oxidase of the myelogenic cells acts on many polyphenol derivatives and easily oxidizable substances such as epinephrine, pyrogallol and phenylene diamine (Bloch and Peck (16)) as well as on dioxyphenylalanine. In view of the higher specificity of the dioxyphenylalanine oxidase of the melanoblasts, any theory attempting to identify dioxyphenylalanine oxidase with polyphenolase (Oppenheimer (17)) is just as untenable as that of the identity of dioxyphenylalanine oxidase with tyrosinase.

There are a number of proofs for the existence of an individual dioxyphenylalanine enzyme: (1) The dioxyphenylalanine oxidase is extremely labile against the destructive influences of heat and especially of certain poisons as compared to the less specific, more rugged polyphenolase and the nonenzymatic oxidizing catalysts of the cell. (2) Its action is confined to a much narrower hydrogen ion concentration than that of either polyphenolase or tyrosinase. (3) As demonstrated by Bloch and Schaaf (7), the dioxyphenylalanine oxidase is so specific as to be inactive against a substrate showing the slightest change in the chemical constitution. Recently Mulzer and Schmalfuss (18) cast doubt on the specificity of the reaction to dioxyphenylalanine because in their hands 3-4 dioxyphenylethylamine (oxytramine) also gave a positive product of dioxyphenylalanine, it seems that this finding rather corroborates Bloch's theory of the formation of pigment. In the following series of experiments new evidence is presented for the specificity of the reaction to dioxyphenylalanine.

The optical specificity of the action of enzymes has been studied thoroughly in several groups of hydrolyzing enzymes. The problem of optical specificity in oxidizing enzymes has been investigated with tyrosinase. According to Abderhalden and Guggenheim (19), this enzyme is optically selective to a limited degree only, showing some preference for the leverotatory natural substrate.

We (Peck, Sobotka and Kahn (20)) investigated the optical specificity of the dioxyphenylalanine oxidase, and we found that it was strictly specific for the levorotatory natural form of dioxyphenylalanine (21). The dextrorotatory antipode was left untouched by the enzyme (22). Bloch and Schaaf (7) have independently made the same observation.

The formation of melanin from dioxyphenylalanine by crude potato tyrosinase (prepared according to Lichtman and Sobotka (23)) is rapid. It takes place to a considerable degree even when the true tyrosinase is destroyed by heat. The potato preparation acts equally on dextrorotatory and on levorotatory dioxyphenylalanine, as is to be expected in a material rich in polyphenol-oxidizine catalysts.

The enzymes responsible for the

formation of melanin may be summarized as shown in Figure 1.

The Melanoblasts (Pigment-forming Cells)

Pigment (melanin) is found in the human epidermis in the basal and overlying cells as well as in a more bizarre appearing cell with dendritic processes such as condylomas, lichen ruber, molluscum contagiosun psoriasis and penphigus vegetans, as well as in the early stages of melanocarcinomas. In hyperpigmented and acanthotic process they may be found in the layers above the basal cells.

While as a rule it is true that

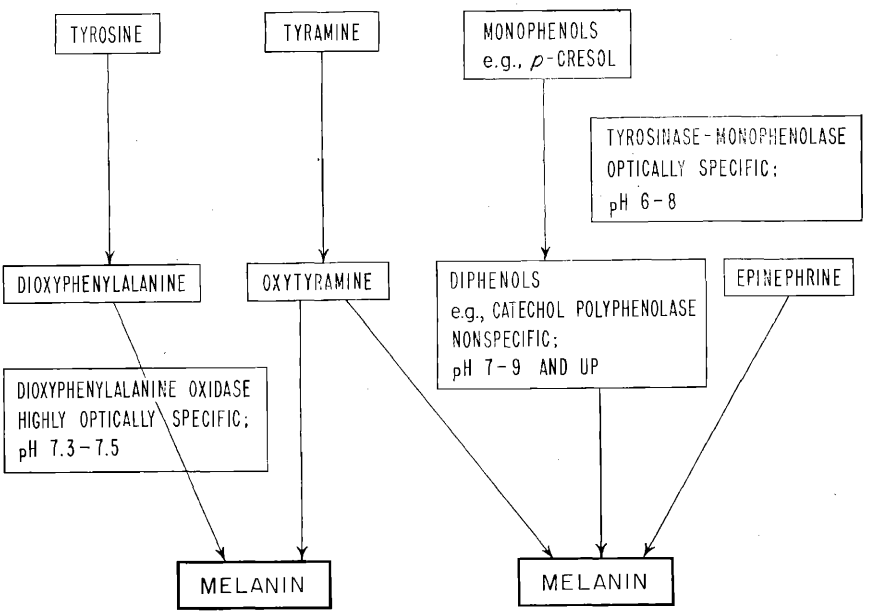

Figure 1.—Enzymes responsible for the formation of melanin

processes. Such dendritic cells are also found in the matrix of pigmented hairs, epidermal hair sheets, sometimes in the epidermal parts of sebaceous glands and in pigmented mucous membranes. They are increased in hyperpigmented processes at the time of pigment formation, especially after exposure to light, x-rays, radium and thorium X. They are well seen in acanthotic dendritic cells are increased at number and therefore better seen in hyperpigmented skins, for reasons not yet clear they are better visualized in acanthoses and in pigmented mucous membranes, even though there is little pigmentation. This may be due to some extent to the fact that the dendritic processes may be obscured by adjoining pigmented cells, and in the relatively pigment-

poor mucous membrane this factor is obviated; or that, as in acanthotic processes, there is a looser type of tissue so that the processes are better seen or better developed. If one examines a skin showing a fairly strong dopa reaction or one that is fairly well pigmented, one is struck by the clarity and the number of dendritic cells, seen in a rete peg cut on a bias. It is possible that in many cases dendrites are given off in a plane which runs parallel to the body surface, so that in the usual section which cuts this plane at right angles the dendrites are poorly visualized. Above all, the state of pigment activity as well as the method employed for its demonstration are of prime importance in the demonstration of dendritic cells.

Many workers (Bloch [7]) have investigated the problem of the nature, origin, anatomy, distribution and biologic significance of the dendritic cell in the human skin, since the original demonstration of them in syphilitic leukoderma and in the normal hair matrix by Riehl (24) (1884). The first thorough investigation of their distribution in the normal white skin was made by Adachi (25) (1903). Recently Becker (26), in a detailed and thorough piece of work, undertook a systematic investigation of the pigment (melanin) of the upper mucous membranes and the skin, with special consideration of the dendritic cells. His investigations showed that dendritic cells were much more frequent in the normal skin and mucous membranes than has been supposed; that they were found in all regions examined—nipple, abdomen, axilla, toe, prepuce, mouth, pharynx and esophagous—and that they varied in size, shape, nature of branching and numerical relation to nondendritic cells in different regions of the skin and mucous membranes and even in the same location in different persons. The dendritic cell in its appearance and variable form may appear very different from the ordinary basal cell. However, all transitions between the most bizarre shapes with long, gnarled branches to basal cells with short stubby processes can be seen (transition forms). The cell body is usually larger than that of a basal cell and may be globular, irregularly oval, club-shaped or polyhedral. The protoplasm is spongy, lacks fibrillation and contains pigment in its cell body as well as in the processes. The processes may be fine and whip-like, or thicker and irregularly formed, extending in the intercellular spaces almost up to the stratum corneum (27). They may run along just below the basal cells, extending under three or four cells, and often curving upward at the end to go up between the basal cells again. From such a long process branches may be given off at right angles, also extending upward. The branches of a dendritic process often end in a Y-form or show at their terminations a button-like swelling. This knoblike or button-like swelling at the end of a process can be seen especially well in dopa

sections. It is perhaps a cross-section of a dendrite which is running at right angles to the section.

According to Masson (28), each dendrite ends in the supranuclear region of a malpighian cell. The dendrites never dip below the epidermiscutis line. Sections in which they seem to do so can sometimes be seen, but serial sections will show that these are artefacts. The processes may spring from any point on the surface of the cells and may be three or more in number and show repeated branching. Often the longest branches spring from the poles of the cell at its longest diameter. Since the dendrites are not all in the same plane and often change their direction, it is unusual to get all the processes in the same section or to be able to follow the dendrites to their final termination. The dendrites often seem to communicate with one another, especially in the dopa sections and sometimes form a swelling at their point of junction.

The nucleus is large, round and/or oval, contains several nucleoli and is often eccentric. Mitotic figures have not been seen in dendritic cells (Bloch (7), Mieschre (29) and Becker (26), except by Masson (28)). The cell lies between the basal cells, usually at the basal cell level, but sometimes it is seen at a slightly higher level, or almost half of the cell body may be below the basal line in the cutis. The whole cell, however, has never been seen below the epidermis.

The dendrites are ordinarily made visible by their impregnation with melanin, although Miescher has seen cells with dendrites in melanomas, when they did not contain any pigment (29). It is a curious fact that the cytoplasm of the dendritic processes does not take the ordinary stains. This is true of the dendritic cytoplasm in lower animals also (Biedermann (30), Ballowitz (31)). In sections treated with silver nitrate the dendrites are still better seen because their pigment content is brought more to the fore by the reduction of the silver (formation of silver melanin) which makes visible the fine pale pigment granules and perhaps propigments.

By far the clearest and truest picture is obtained with Bloch's dopa method which demonstrates the pigment-forming oxydase. With the dopa reaction the dendrites are seen either through the content of granular dopa melanin or, better still and as is usually the case, by the diffuse protoplasmic reaction. This is the only method that clearly demonstrates the cytoplasm, i.e., protoplasm-containing oxidase. In the native as well as in the silver sections the dendrites are demonstrated through their content of pigment granules, which give a picture that is often only outlined by scattered dots, so that it is difficult to trace component parts of the cells (32). With the dopa reaction, however, especially in the diffuse reaction, one obtains a more solid and complete picture. Furthermore, in a strong dopa reaction

nearly every dopa-positive cell may show dendrites, while in the native and silver section very few may be seen.

The striking and often bizarre forms of fully developed dendritic cells, as they are seen between the other pigmented and nonpigmented epidermal cells, may lead them to appear different from the other epidermal cells. They remind one very much of the melanophores of coldblooded animals. Especially in dopa preparations they have a striking resemblance to certain cells of the central nervous system. The peculiar morphologic characteristics of the dendritic cells have led many investigators to occupy themselves with the problem of their origin and function. In the course of time, various theories have arisen about their genesis and function.

It is not necessary in the present state of the problem to discuss the views of the older authors (Schwalbe (7), Rabl (7) and Adachi (25)) who maintained that they are not cells at all but artefacts due to the pigment in the intercellular spaces. That the view was false was clearly indicated by the regularity of the demonstration of the nuclei of the cells.

There are three main theories which still remain for discussion. Most of the investigators who have occupied themselves with this problem support the view that the dendritic cells are closely connected with melanin production. This point of view is supported chiefly by the fact that dendritic cells usually contain pigment and are especially numerous during active pigment production.

Among the supporters of the theory of the pigment function of the dendritic cells there are divergences of opinion about their genesis. Especially the older authors, in analogy to the melanophores of coldblooded animals, considered the dendritic cells as pigment cells of mesodermal origin which had wandered up into the epidermis in embyonal life, and had remained there to function a melanoblasts (Ranvier (7), Kolliker (7), Ehrmann (2) and Del Rio Hortega (33)). Still others (Bloch (7), Miescher (29), Kreibich (34)) considered them as cells of ectodermal nature which are nothing more than special functional phases of ordinary pigment-building basal cells. According to S. W. Becker the melanoblasts of the epidermis do not rise from the ordinary basal cells (26).

The French authors (Masson (28), Pautrier, Levy and Diss (35) and Caudiere (36)) went still further. They separated the dendritic cells completely from the rest of the epidermal cells. The older theory of Masson (19), which was accepted by Pautrier, Levy and Diss, ascribed a metabolic function to the dendritic cells. As "cellules emboceptrices" they have the property of taking propigments and various other substances from the reticuloendothelial cells of the cutis into the epidermis and of carrying away again from the epidermis into the

cutis waste elements or formed products. This function, however, has not been proved. In the more recent papers of Masson (37), the dendritic cells have been classified as nerve cells and have been made identical with the Langerhans cells.

A great deal of confusion has arisen because of the nomenclature used by various authors in writing about dendritic cells. In comparison with the pigment-forming cells of the lower animals they have been called chromatophores or melanophores. Still greater misunderstanding has been caused by their being called Langerhans cells (Masson (28 and 37), Pautrier, Levy and Diss (35), Caudiere (36)), which are epidermal nerve elements and are not melanoblasts. As was shown by Bloch (7), quite apart from the question whether the dendritic cells are closely allied or have their origin from nervous elements, they cannot be identified with the Langerhans cells. This point will be taken up later. The terms in the following studies are those used by the Bloch school; i.e., melanoblasts, pigment-building cells of the epidermis of dendritic or nondendritic form and chromatophores, cells found in the cutis which phagocytose pigment and are not pigment builders. The last-named cells never show a positive dopa reaction, in contradistinction to the melanoblasts of mesodermal origin, which may also be found in the cutis in such conditions as blue nevus and mongolian spots.

Depigmentation by Specific Antioxidants

It was observed by Oettel (38) in 1936 that the peroral administration of hydroquinone to black-haired cats turned the hair gray. Discontinuation of the drug resulted in repigmentation of the hair. In an investigation of occupational leukoderma by Oliver, Schwartz and Warren (39 and 40), Schwartz found that the monobenzyl ether of hydroquinone, contained as an antioxidant in the rubber gloves of the workers, was responsible for the depigmentation. Their experiments as well as our own demonstrated that the depigmentation was due to the action of monobenzyl hydroquinone on the system "dopa"-oxidase dihydroxyphenylalanine ("dopa") (41).

Extensive experiments to study the mechanism of this depigmentation were carried out by Peck and Sobotka (41). In a number of human subjects, both white and colored, the monobenzyl compound was applied in the form of a 50 per cent ointmentor as a 50 per cent ethereal suspension. In a few instances the concentrated powder was used. Leukoderma was produced after incubation periods varying from weeks to months.

Histologic examination of the areas of depigmentation revealed a negative "dopa" reaction and an almost complete disappearance of the melanin. The microscopic picture could not be differentiated from a vitiligo in many instances since the depigmentation was seen to

occur without preceding inflammatory reaction.

A number of guinea pigs were fed approximately 12 grams of monobenzyl ether of hydroquinone over a period of 5 months. This was well tolerated but no pigmentary changes were observed. Local application over a period of months, just as in the human subjects, caused depigmentation. The disappearance of the pigment was accompanied by a negative "dopa" reaction in the affected areas. This observation only applied to the previously pigmented epidermis whereas the hair bulbs were not affected. This was probably due to a lack of penetration of the monobenzyl compound to the melanoblasts in the hair matrix.

Can Pigment Enzyme Activity Be Stimulated in Unpigmented Skin? (42)

The results of our experiments (42) have confirmed the observations of Saxton, Schmekebier and Kelley (43), as well as the earlier work of Loeb (44), Carnot and Deflandre (45) that the transplantation of a black graft into a white skin resulted in the "extension" of the pigment into the surrounding unpigmented host area while a white graft transplanted in a black skin was "invaded" by pigment from the host area. It was perfectly obvious from our histologic studies that the pigment formation in the white host area as well as in the non-pigmented grafts in no way differed from the ordinary mechanism of pigment formation. The concept of the older authors of actual invasion by melanoblasts or other pigment-bearing cells from the surrounding pigmented areas into non-pigmented areas was no more true than when it was proposed years ago for melanin formation in general. Nor was there any support for the conception of Rand (46) that the epidermis of the graft was replaced by the host with its own epidermis so that naturally it finally assumes the same color as the host skin.

It was clearly seen both macro and microscopically that the grafts always remained recognizable. It remained as a definite entity outlined by a scar.

One of us (47), in previous experiments was able to show that the unpigmented epidermis in gray rabbits had the latent power to form melanin. Under proper conditions, such as wound healing and radiant energy, the ordinary basal cells of the epidermis readily assumed melanoblastic function. It was also shown that the relatively unpigmented human epidermis (48), even in total darkness was able to assume very active melanoblastic function under certain inflammatory stimuli which resulted in epidermal regeneration.

We believed that a mechanism similar to the last mentioned would not serve adequately to explain the pigmentary changes noted in our experiments. However, with such an explanation for the pigment formation, in the unpigmented or relatively unpigmented skin of either the host or the graft, an assumption

had to be made that the epidermis of the white skin of the guinea pigs was able to assume pigment formation under stimuli such as radiant energy or as a part of the process of epidermal regeneration such as wound healing.

We exposed the shaved white skin of guinea pigs to ultra-violet light from 3 to 6 minutes at a distance of about 13 inches. The same type and area of skin from which white transplants were made was used. A marked inflammatory reaction with redness and desquamation resulted but no temporary or permanent pigmentation could be demonstrated clinically or histologically.

To study the effect of wound healing as a stimulus to latent pigment formation a white graft was transplanted into a defect in the white skin but again pigment could not be demonstrated. We also allowed wounds to heal spontaneously but no pigment formation could be noted. That such a procedure was capable of stimulating pigment formation in an already pigmented skin could readily be demonstrated in similar experiments on black skin.

Apparently we are at a loss to explain the initiating stimulus which caused the pigmentation to occur. Contact of an unpigmented skin with an already pigmented epidermis was necessary since pigmentation first occurred at points of junction and then slowly extended outward or in the case of a white graft, inward, like ripples in a pond.

It is interesting to speculate on the possible mechanism of the pigment formation. In the modern theory of pigment formation we have a melanoblast which contains an enzyme. This enzyme converts the colorless propigment "dopa" or a closely allied chemical substance through various stages into melanin. The propigment reaches the cell from the circulation. The enzyme is apparently found only in melanoblasts and has never been demonstrated to be transported through the cell membrane and thus convert other cells into melanoblasts.

We would demonstrate by means of the dopa reaction that the ordinary mechanism of pigment formation took place in the newly pigmented areas. We must be led to the conclusion, therefore, that in some way, because of close proximity to pigmented skin, enzymatic action, as far as pigment formation is concerned, is suddenly assumed by the unpigmented epidermis. It is curious that if this enzyme were present in the unpigmented skin it could not be stimulated into activity by radiant energy. Unless our failure to stimulate pigment formation by the use of radiant energy was based on an experimental error or on an error of interpretation, it would seem to prove that the pigment enzyme as we ordinarily find it was not previously present in unpigmented guinea pig skin.

The above experiments are reported because it is of interest to the cosmetic chemist to have an insight into possible avenues of investigation which would lead to formulation of chemical or biologic prod-

ucts to stimulate pigment formation in cosmetic defects resulting from poor pigment formation. The problem is more than academic when hyperpigmentation results in the vicinity of skin grafts by just such mechanisms as have been described. The rim of hyperpigmentation around such grafts often causes serious cosmetic defects.

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