MELANOGENESIS: THE MECHANISM OF SKIN PIGMENTATION

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The colour of a man's skin always arouses interest; you notice it at once and immediately draw conclusions, often false, which place him in some ethnic group. Blemishes, brought about either by excessive, patchy pigmentation or by its opposite, causing large decolourized blotches, give rise to great concern and mental distress. Of recent years the racial distribution pattern of skin colouring has given rise to special legislation. And sporadic social upheavals can be traced to the prejudice inherent in our attitude towards this dermatological characteristic.

Fortunately, however, the concern of this article is only with the biochemical processes in the epidermis which result in pigmentation, as well as with the disturbances which may arise in the normal functioning of these processes. The whole elaborate performance is admirably described and considered from every angle by various writers in a special number of the *Journal of Investigative Dermatology*[1] reporting the proceedings of a symposium held at Brook Lodge, Michigan, USA, in March 1958. The writer freely acknowledges his indebtedness to this fertile source of information in presenting this condensed account.

The burden of performing the intricate physicochemical operations for the production of skin pigment is borne by a highly specialized epidermal cell, the *melanocyte*. Its function is to make and distribute that special substance,

melanin, which gives the dark tint to the skin in coloured races and which constitutes the carefully cultivated, cosmetically acceptable, tan in fair-skinned individuals. This it achieves in response to various stimuli and inherited trends under the curbing and guiding influence of other physiological processes.

Ordinarily, sunlight supplies the necessary energy for initiating the pigmentation process when this is required. The ultra-violet light (UVL) division of the solar spectrum, itself a mere fraction of the vast electro-magnetic spectrum in the universe, is the operative dispenser. The wave-lengths of this portion of the spectrum are conveniently measured in terms of Angström (Å) units. One Angström unit (Å1) is the ten-millionth part of a millimetre (10^{-7} mm.) in length.

Ultra-violet light rays are non-ionizing;[2] that is to say they do not cause the ejection of an electron from the atom through which they pass. Only those rays which are absorbed have any effect and the amount of energy expended in the atom or molecule is equal to that absorbed. In the epidermal cells this absorbed energy is transformed into photochemical processes, manifold in nature, of which the ones which concern us in this article are either erythema-producing or pigment-forming. Selective action, either erythema-producing or pigment-forming, is shown by rays of different wave-lengths within the confines of the UVL band.[3] Briefly, there are 3 main groups of wave lengths of UVL, according to Meyer,[4] viz.:

Group A: Long wave-length group, from Å 3,900 to Å 3,200 present in sunlight and the light from carbon arc-lamps; this band does not cause erythema; pigmentation is slow in developing.

Group B: Medium wave-length group, from Å 3,200 to Å 2,800, present in sunlight, and the light from carbon arc-lamps and mercury vapour lamps; produces erythema.

Group C: Short wave-length group, Å 2,800 to Å 1,800; not present in sunlight or the light from carbon arc-lamps; but present in that from some types of mercury vapour lamps; does not produce erythema; pigmentation is early and of a greyish lustreless tint.[4]

Other effects of certain wave-lengths of UVL, i.e. carcinogenic, histamine-producing, bactericidal, etc. hardly fall within the scope of this article.

Zierz distinguishes 2 kinds of pigmentation, viz.:

1. Pigmentation produced by wave-lengths below Å 3,150. This is slow in developing and is called 'indirect' pigmentation because it follows on erythema and fades with subsequent desquamation.

2. Pigmentation due to wave lengths from Å 3,150 to Å 4,000; this he calls 'direct' pigmentation since it comes on fairly soon (about 1 hour) and is more lasting. It is supposed to be due to the oxidization of already existing, pre-formed melanin.

THE MELANOCYTE

The melanocyte is derived from the primitive neural crest, from which it emigrates in uterine life to settle in certain selected sites, viz. (1) the basal layer of the epidermis and mucous membranes at the dermo-epidermal junction, (2) the corresponding site in hair bulbs, (3) the uveal tract of the eye, and (4) the meninges of the brain, notably the leptomeninges.

Sometimes the melanocytes fail to reach their destination in the skin. In some racial types, chiefly mongolian and negroid, they fall just short of it and form large masses, usually in the lumbosacral region—the so-called mongolian spot, or smaller agglomerations halt in the upper dermis to constitute the blue naevus of Jadassohn. The former usually disappears in early life; the latter persists, but is not associated with malignancy. The proper setting for the epidermal melanocyte is the basal layer. Every 4th or 5th cell of this layer is a melanocyte and since, at times, the dark granules of melanin are concentrated round the nucleus, leaving the cytoplasm free, they were named 'clear cells'. Unlike the ordinary basal cell, the keratinocyte, the melanocytes possess numerous fine dendritic tubules, which spread out amongst the prickle cells of the Malphigian layers and convey to them the pigment, melanin. Thus, the melanocyte is really a secretory cell and its function is not only to manufacture the deeply pigmented granules of melanin but to supply the other epidermal cells with this protective substance. Melanin granules, in the form of dust-like particles, are found not only within the cells, but also lying free in the corneal layer in dark-skinned individuals. In

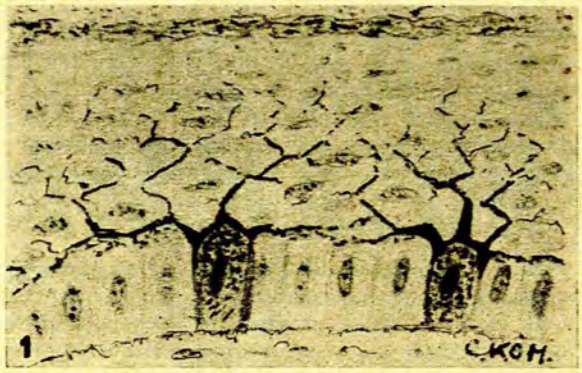


Fig. 1. Semi-diagrammatic. Two melanocytes are shown in the basal layer of the epidermis with their dendrites conveying granules of melanin. In some stages of melanin synthesis the granules are colourless.

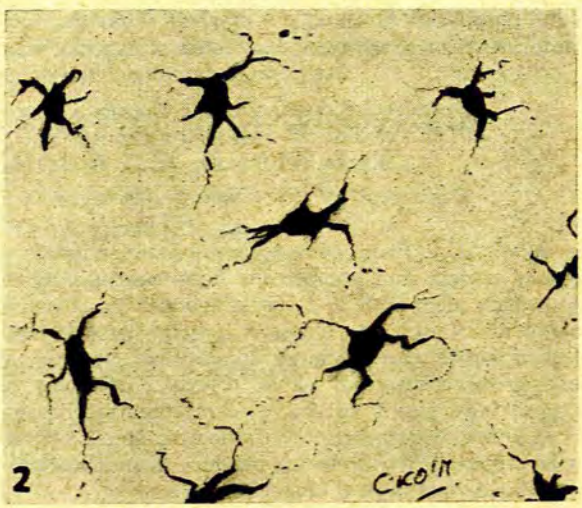


Fig. 2. Semi-diagrammatic. A horizontal section of epidermis at the level of the basal layer showing the syncytial arrangement of melanocytes.

fair-skinned people they accumulate after exposure to sunlight or as the result of other stimuli.

The distribution of the melanocyte population throughout the epidermis varies in density, being more marked in exposed areas such as the forehead than, say, on the inner aspect of the thigh. The total number has been estimated at the figure 2×10^9 with an average of 1,560 per sq. mm. The following figures illustrate this varying density in different regions of the body; they are given in mean numbers per square millimetre:[1]

Forehead	2,010 ± 210
Neck	1,400 ± 220
Thigh	1,000 ± 70
Prepuce	2,100 ± 280
Dorsum of foot	1,420 — 2,840 (two estimates)

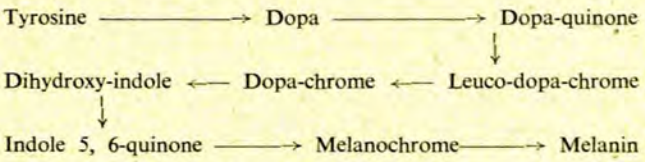
Melanocytes are more numerous on the epidermal ridges than in the corresponding valleys and, although individual variations doubtless exist, on the whole the total number is much the same in all races. The white man has as many as the dark man. He actually has more on his forehead than the negro has on the inside of his thigh. The albino has as many as either but their functional capacity is negligible. It is the differences in activity of the melanocytes rather than variations in total numbers, whether due to racial, individual or physiological factors, that make up the diverse gradations in skin pigmentation. The entire epidermal melanocytic system should be visualized as a close network of branching cells situated on the horizontal plane of the dermo-epidermal junction and forming an intricate lace-like pattern that can only be seen after special staining.

FUNCTION OF MELANOCYTES

The production of melanin by the melanocytes is the result of highly specialized and complicated intracellular activity. Much of our knowledge on the point has been gained by studies of mammalian and frog skin. Much remains still to be learnt. It is known, however, that enzymes play a dominant part. The increased pigmentation of the skin following exposure to sunlight is not due to an increase in the number of melanocytes but to an increase in melanin production by those already present. Three factors are chiefly concerned, viz. (1) a foundation substance, *tyrosine*, an amino-acid from which melanin is synthesized, (2) an enzyme, in this instance *tyrosinase*, which sparks off the process, and (3) an exciting stimulus, which normally in sun tanning is a band of UVL rays, its exact place in the UVL spectrum not having yet been accurately determined. We could compare these 3 factors roughly with the petrol, the sparking system and the battery of a motor-car.

Both tyrosine and tyrosinase are normally present in the melanocyte, the former in molecular form in the cytoplasm and the latter situated on the surface of the so-called melanin granules. Melanin itself is a protein conjugate formed by the union of a quinioid polymer, *indole 5, 6-quinone* with protein. It is the final stage of a complex chain of metabolic events. One of the intermediate stages, the second in the series, is a substance called *dihydroxy-phenylalanine*, dopa for short. Bloch was one of the first to experiment with the substance. He found that when sections of human skin were immersed in a solution of dopa at pH 7·4 the melanocytes and their processes were stained black. The reaction is not a specific one, because leukocytes, under the same conditions, show similar darkening. The so-called dopa reaction is a landmark in the development of the histochemistry of the skin and is still a serviceable laboratory test.

Starting with the amino-acid tyrosine, which, with the enzyme tyrosinase, is a fundamental prerequisite, the successive steps in the production of melanin are shown in the following diagram; for the sake of clarity the structural formulae are omitted:



Oxygen, together with tyrosinase, is necessary for the completion of the synthesis and is supplied by the cell itself. Some of the changes or reactions occur rapidly, others slowly. Some are certainly enzymatic, others may not be. And it is believed that oxidation is an essential process, for some of the stages at least.

The elaboration of melanin from its precursor tyrosine does not proceed in a constant automatic manner. On the contrary, it is subject to controls and checks and is necessarily dependent on the availability of tyrosine and tyrosinase. In the albino there is no lack of melanocytes or tyrosine; but there is an inherited shortage of tyrosinase. We might compare the position of the albino in this respect to that of a motor-car in which the engine is in working order and an adequate supply of petrol is available but the ignition system is faulty or absent. Certain chemicals, too, by combining with the copper in tyrosinase deprive this enzyme of an essential, thus halting the course of events at its beginnings and so seriously interfering with the production of melanin. Furthermore, certain substances—hormones and such like—exert through the circulation a degree of remote control over the complex happenings of melanogenesis.

Leaving out of consideration, for the moment, the initiating effect of UVL radiation or other stimulus, there are other operative factors which can be classified into two main categories, viz.:

(A) Substances produced in remote parts of the body and subsequently brought in contact with the pigment-forming cells; these substances determine the *distribution* pattern of melanin granules within the melanocytes.

(B) Factors dependent on intracellular, enzymatic activity determining the *amount* of melanin.

EFFECT ON MELANOGENESIS BY HORMONES AND OTHER SUBSTANCES

Experiments on frogs and marine species showed that both removal of the pituitary gland and injection of its extracts exert a dominant influence on skin pigmentation. Injection of an extract of hog's pituitary has a darkening effect on frog's skin. In humans the same effect is produced. Recently two hormones, alpha and beta melanotic stimulating hormones (MSH), have been isolated from hog's pituitary gland. Chemically, they belong to the class of polypeptides. Similar hormones have been isolated from the pituitary gland of other mammals, though certain structural differ-

ences exist. It seems certain, then, that a melanotic stimulating hormone (MSH) is secreted by the pituitary which has the property of darkening the skin by its action on melanocytes. But the action of MSH does not proceed uncontrolled. Removal of the adrenal glands or suppression of their function by disease, as in Addison's disease for instance, leads to bronzing of the skin through uncontrolled pigmentation.

It is believed that the adrenals exert an antagonistic effect to the pituitary and that the balance between these reciprocal functions determines the degree of skin pigmentation at any time.[6] The clinical phenomenon of increased pigmentation, in conditions where the normal functioning of the adrenals is interfered with, makes this belief more than a reasonable assumption. Sex hormones, too, in a still unexplained manner, have a certain stimulating effect on melanin production. Pillsbury[6] records, for instance, that eunuchs fail to tan when exposed to ultra-violet light unless they are given male sex hormones at the same time. The increased pigmentation during pregnancy is probably the result of a direct stimulation of the MSH function of the anterior pituitary by circulating hormones.

The production of melanin seems to be a protective mechanism, though at first sight it is difficult to see how particles of 1 μ size, with measurable spaces between the particles, could form an effective barrier against UVL wave-lengths of much smaller dimensions. The response follows exposure to such stimuli as sunlight, ionizing radiations like X-rays, and inflammatory processes in the skin itself, such as dermatitis, lichen planus and discoid lupus erythematosus. But what is not known for certain is how exactly, say, UVL stimulates the process of melanogenesis. It is suggested that it does so rather by suppressing inhibitors, since the enzyme tyrosinase is said not to function optimally under the usual physiological conditions. Although, like other epithelial cells, the melanocytes multiply and are cast off or form large aggregates, in both simple and malignant conditions, the chief factor in increased pigmentation, it must be repeated, is not an increase in the number of melanocytes so much as an increase in the amount and distribution of melanin.

INTRACELLULAR, ENZYMATIC FACTORS AFFECTING MELANOGENESIS

Each individual melanocyte has within itself the 3 necessary units for the elaboration of melanin, viz.: (1) coarse so-called melanin granules, which form the foundation, as it were, on which the final pigment is built, (2) tyrosine, the amino-acid, which through a series of changes eventually becomes the dark substance melanin, and (3) the enzyme tyrosinase, which is the sparking-off stimulus that starts the whole intricate chemical process. Tyrosinase belongs to a group of copper-containing enzymes which catalyse the oxidation of both mono- and dihydric phenols. In mammals it catalyses the hydroxylation of tyrosine to dopa and the further hydroxylation of dopa to dopa-quinone. Both of these steps are essential preliminaries to the sequence of events ending in melanin.

A further indispensable condition is the reduction of the copper in tyrosinase from the cupric to the cuprous state. It follows, then, that any substance, and there are many, which by forming bonds with the copper prevents this reduction acts as an inhibitor and holds up the whole process of melanogenesis from the very start. Mercury, gold, BAL, sulph-hydryl groups, etc. unite avidly with copper. They are all therefore inhibitors of pigmentation. Thus is explained the hypopigmentation noted in patients under treatment with BAL and the rationale of the use of ammoniated mercury for the treatment of excessive pigmentation. Similarly, in a roundabout way, the reason for the hyperpigmentation sometimes noted in arsenic therapy becomes clear. Arsenic unites with sulph-hydryl groups readily, before they get a chance to form bonds with the copper in tyrosinase, and this removal of the potent inhibitor sulph-hydryl leaves the field clear for melanogenesis to proceed apace. It is noteworthy that these two commonly encountered metals have directly opposite effects. Mercury decreases pigmentation, arsenic increases it, both by playing antagonistic roles *vis-à-vis* the copper atoms in tyrosinase. Hydroquinone is another inhibitor of tyrosinase activity. Its monobenzyl ether is met with in the rubber industry, where contact with it is a well-known occupational hazard for negro workers in the USA, causing bleaching of the hands.

The utter dependence of melanin synthesis on enzymatic intracellular activity is beautifully illustrated in the condition phenylpyruvic-oligophrenia. Sufferers from this rare condition tan poorly and are subject to eczema and other defects. The background is a breakdown in the normal synthesis of tyrosine brought about in the following manner: Phenylalanine, an essential amino-acid, is the precursor of tyrosine. It is not synthesized in the human body but is ingested in the food. An enzyme phenylalaninase, normally present in the liver, hydroxylates phenylalanine to tyrosine. But in phenylpyruvic-oligophrenia there is a congenital absence of this enzyme, or some alteration in its quality, so that there results a superabundance of phenylalanine and a corresponding deficiency of tyrosine, the ground substance for the synthesis of melanin. About 10% of the ingested phenylalanine is synthesized to tyrosine by means other than the liver, so that the serum shows normal values; but there remains a superfluity of phenylalanine, and the organism attempts to get rid of it by excreting it as pyruvic acid and other similar compounds in the urine, which is coloured red in such circumstances by the addition of ferric chloride. Meanwhile the enzyme, tyrosinase, has a greater affinity for phenylalanine than for its usual partner, tyrosine; thus the production of melanin is sabotaged at the very first stage.

THE EFFECT OF THE INTRACELLULAR DISTRIBUTION OF MELANIN GRANULES

The tolerance of the negro skin for sunlight is due, not only to the presence of larger, coarser granules of melanin in the melanocytes themselves, but also to the presence of these granules in other cells of the epidermis and even in the corneal layer, which is itself thicker than in the white-skinned person. The melanin granules, it will be remembered, are conveyed to the other epidermal cells through the dendritic processes of the melanocytes and move to the surface of the skin with the continual growth of the epidermis. Furthermore, the pigmentation following, for instance, exposure to sunlight, may be more apparent than real, for the same effect may be produced either by an actual increase in the number of melanin granules within the cell.

or by a rearrangement of the granules without any increase in number. When the melanin granules are widely dispersed throughout the cytoplasm an impression of increased darkening is caused, but when the same number are clumped together on the nucleus the opposite effect is produced.

If a solution of MSH is added to frog's skin the movement of the melanin granules outwards from the nucleus can be seen. MSH therefore causes darkening of the cell by the dispersion of the granules which renders the cell more opaque. Other substances that have a similar dispersal effect are: ACTH, caffeine, apresoline, mesantoin, progesterone. Recently a substance that has a contrary effect, that is, clumping the melanin granules, with a consequent lightening of the cell, has been isolated from the pineal glands of cows. This hormone, probably originating from

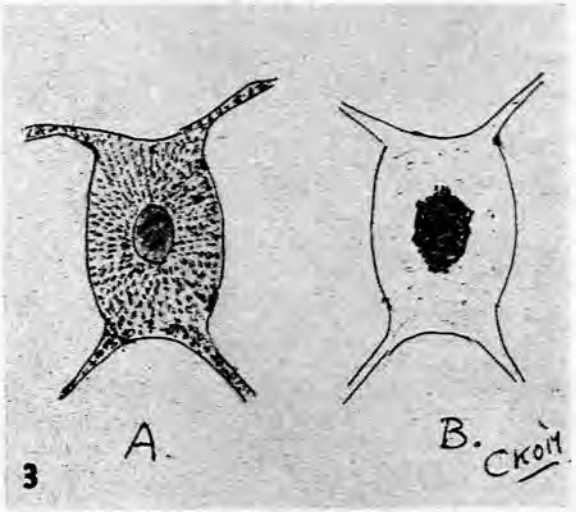


Fig. 3. Diagrammatic.

A. A melanocyte in which the granules are dispersed throughout the cell protoplasm, rendering it dark and almost opaque. Action of MSH.

B. The same cell, in which the melanin granules are clustered on the surface of the nucleus. The cell is clear. Action of melatonin.

the amino-acid tryptophane, has been named melatonin. It has been found in human pineal glands as well as in peripheral nerves, where it may possibly have something to do with the development of vitiligo. Other lightening agents causing clumping of the granules on the nucleus are nor-adrenaline, adrenaline, acetylcholine, serotonin, diamox. None of these are as powerful in effect as melatonin. The members of these two antagonistic groups are set out as follows:

Substances darkening the cell by dispersal of granules	*Substances causing lightening of the cell by clumping of granules*
MSH both alpha and beta (pituitary)	Melatonin (pineal)
ACTH	Nor-adrenaline
Caffeine	Adrenaline
Apresoline	Acetylcholine
Mesantoin	Serotonin
Progesterone	Diamox
and others	Tri-iodothyronine and others

RÉSUMÉ

The complete process of pigment formation may be conveniently condensed in the following statements:

1. The synthesis of the skin pigment melanin is carried out by the melanocyte, which is situated in the basal layer of the epidermis; it migrates from the neural crest to the skin and other sites during intra-uterine life.

2. The number of melanocytes per sq. mm. of skin varies according to the site, being most numerous in the exposed areas; the average number is 1,560.

3. Increased pigmentation is presumably a normal protective response to agents such as sunlight, UVL, X-rays, inflammatory processes, etc. Accompanying thickening of the corneal layer, as in negroes, gives increased protection.

4. The amino-acid tyrosine is the foundation on which the structure of melanin synthesis is built. The enzyme tyrosinase is an indispensable factor. Lack of either of these or interference with their functions leads to pigmentary disorders. Oxygen is necessary in some stages.

5. A hormone secreted by the anterior pituitary gland MSH stimulates melanin production. The adrenals have an antagonistic effect.

6. Increased pigmentation is not so much due to an increase in the number of melanocytes as to an increase in the amount of melanin formed and to the intracellular arrangement of the melanin granules.

7. MSH and other substances cause dispersion of the granules; melatonin, a secretion of the pineal gland, and other substances, e.g. adrenaline, have an opposite effect. Dispersal of granules darkens the melanocyte; clumping of granules make it less opaque.

SUMMARY

The role of the melanocyte in the synthesis of melanin is discussed and the importance of the enzyme tyrosinase is stressed. Mention is made of the various factors, both intracellular and extracellular, which speed up or hinder the process. A comparison is made between those substances which darken the melanocytes by dispersing the melanin granules and those which lighten them by clumping the granules on the cell nucleus.

The writer re-affirms his special indebtedness to the various contributors to the special number of the *Journal of Investigative Dermatology*[1] dealing with this subject. The fruits of their laborious researches are here presented in abridged form to the general medical reader.

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