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The melanocyte system and keratinization

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Synopsis—The factors producing skin colour are mentioned together with methods of estimating the redness of the skin due to the haemoglobulin contained in the dermal vasculature, and the pigmentation of the skin due to the melanin contained in the epidermal cells.

The histochemistry of the melanocyte in respect to its dopa oxidase activity is described together with more recent findings of hydrolytic enzymes in these cells. The possible action of the melanocyte population of the epidermis in determining the type of keratin produced by the epidermal cells is discussed.

Skin colour

The colour of the skin is dependent on three main factors–the red of blood contained in the skin vasculature, the black of melanin pigment produced by a group of cells known as melanocytes, and the natural yellowish colour of the epidermis and dermis. The colour contribution due to blood varies according to the state of the capillaries and the degree of oxygenation of the blood. Thus dilated capillaries tend to make the skin red whilst their constriction causes pallor. The degree of oxygenation of the blood alters the colour from the bright red of fully oxygenated blood in dilated capillaries to the blue of slowly flowing, deoxygenated blood in dilated blood vessels. It will be readily appreciated that all variations between these two extremes can occur.

Brown-black melanin imparts the brown tinge to the skin in varying

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degrees, from virtually nothing in the nordic races to almost jet black in certain Negro races. The degree of skin pigmentation is not related to numerical differences in the melanocyte population as a heavily pigmented Negro has the same number of melanocytes per unit area from a given site of skin as a white man. These racial differences in coloration must therefore be due either to greater production of pigment by the melanocytes, or to a decreased rate of destruction, or both.

The pigment is produced by specialized dendritic cells, known as melanocytes, situated in the basal layer of the epidermis. It is then passed into the adjacent epidermal cells by the dendritic processes of the melanocytes. This transfer of the melanin granules to epidermal cells has been termed "cytocrine activity" (1). The pigment tends to accumulate in the upper part of the epidermal cells above the nucleus, and thus protects this vital structure from the effects of solar radiation.

In white skin there is virtually a complete absence of pigment immediately above the lowest layers of epidermal cells. This sudden reduction cannot be entirely accounted for by a dilution effect due to the division of the melanin-containing epidermal cells with a consequent decrease in the individual cell pigment load. In dark skin, melanin can be readily detected in the upper region of the epidermis and sometimes even as high as in the keratin layer. It is thought that in fair skin the pigment becomes rapidly reduced, and this results in a lightening of the colour of the melanin granules. The rapid phase of pigmentation of fair skin following exposure to sunlight is thought to be due to the photodynamic reoxidation of this reduced pigment, whereas the tanning that appears two or three weeks later is considered to be due to increased melanin production effected by actual stimulation of the melanocytes.

In man both the skin and the hair are pigmented, and it is probable that the melanocyte population of the hair follicles, and of the intervening epidermis, constitute a single cell population. In animals having a heavy fur covering, the hair may or may not be pigmented but the underlying epidermis is usually devoid of pigment. This is because the overlying hair affords adequate protection from solar radiation and therefore epidermal pigmentation is not required. Pigmentation of the hair does increase the protection afforded by the keratin of the hair fibres, but the main functions of hair colour in animals is camouflage and secondary sexual characteristics.

The depth at which the melanin is situated in the skin is important as this causes changes in its apparent colour. Thus pigment within the

epidermis appears as increasing shades of brown to black, whereas the same pigment deep in the dermis appears blue. The blue naevus and mongolian spot are examples of this apparent blue colour of deeply situated melanin.

The determination of natural skin colour

The accurate matching, precise determination and recording of skin is difficult in dermatological practice. Photographic methods, using strictly controlled colour temperature lighting, with the emulsion developed under standardized conditions together with colour charts photographed alongside the subject, often fail to give satisfactory comparisons.

A comparator method has also been used in which the skin is illuminated at the same time as a series of standard colours and the disc rotated until visual matching is obtained.

Probably the best method is by the use of reflectance photometry; the apparatus employed for this purpose is manufactured by Evans Electroselenium Ltd. The principle of this technique depends on the absorption of rays of a selected wavelength directed on to the skin surface. The wavelength of the incident radiation is determined by interposing filters between the light source and the skin. The degree of absorption is estimated by measuring the light reflected back from the skin by means of a selenium cell. The current generated in the cell is recorded by a sensitive galvanometer. The greater the quantity of the rays absorbed by the skin, the less radiation will be reflected back to the selenium cell and consequently the lower will be the galvanometer reading. For the determination of red due to blood in the skin, red light is suitable because in this region of the spectrum a good haemoglobin has a good maximum absorption.

However, the maximum absorption by melanin occurs in the uv region of the spectrum and therefore the determination of the degree of brownness due to melanin was unsatisfactory when visible light was used with any of the filters supplied with the original apparatus. We therefore modified the photometer for use with uv rays. A uv light source in the form of an "overrun" projection lamp (Philips A1 – 186) with a 1 mm Chance OX1 filter was substituted for the original light source to produce a radiation having a wavelength of around 3,600 Å. These rays are readily absorbed by melanin and because the selenium cell is sensitive to this range of uv light this gave a much more sensitive method of measuring the brown pigmentation of the skin (2).

A photographic technique was also developed in which a uniform radiation of the skin with uv rays was attained by a battery of four uv lamps (Osram 125 MEW/V) having a total output of about 14 watts at a wavelength of about 3,650 Å. The area of skin under investigation was photographed through an OX1 1 mm filter at a standard distance and exposure, and the emulsion processed to a given gamma. Densitometry readings of the negative, compared with a simultaneously photographed control, were made with a radiological densitometer. This method gave comparable results with those obtained by reflectance photospectometry.

THE NATURE OF SKIN PIGMENT

Melanin, as already mentioned, is formed by specific epidermal cells known as melanocytes. The only criterion of a melanocyte is that it produces melanin, and at present the only satisfactory method for its histological demonstration is by incubating epidermis in dihydroxyphenylalanine (dopa). It is remarkable that this compound should be more readily acted upon by the enzymes of the melanocyte than the postulated natural substrate, tyrosine, which is thought to be the true precursor of melanin. The reason given for this anomaly is that *in vitro* the melanocyte is unable to accomplish the first stage of transforming tyrosine into "dopa" and if, therefore, "dopa" is presented to the cell the rest of the metabolic pathway towards melanin formation proceeds to completion.

As mentioned above, the precursor of melanin is thought to be tyrosine, and by a series of reactions, some enzymatic oxidations by the enzyme tyrosinase and some autoreductions, this amino acid is transformed into the brown-black pigment, melanin. In the final product this is closely associated with protein to form melanin granules, which are then passed into adjacent epidermal cells.

Another type of melanin has also been described, known as pheomelanin (3). This is the yellow-coloured pigment which is thought to be responsible for the yellow skin colour of the Oriental races and also for the yellow colour in the hair of "agouti" varieties of several animal species. Some authorities consider that the production of pheomelanin involves similar metabolic pathways to those of melanin synthesis and that possibly it is derived from the same precursor, tyrosine. A difference between these two melanins is shown by their solubilities in sodium hydroxide. It has also been suggested that pheomelanin represents a further oxidation product of melanin.

Other aspects of melanocyte function

The definition of a melanocyte is that it is a cell that forms melanin and is able to oxidize dihydroxyphenylalanine to a black pigment. It has also been shown that dendritic cells could be detected at higher levels in the epidermis than the basal layer. These cells situated in the upper regions of the epidermis were, however, dopa negative. Therefore other methods had to be used for their demonstration and the gold impregnation techniques were probably the most reliable for their detection. Because these so-called high-level melanocytes were dopa-negative it was considered that they were melanogenically effete and had no further function. They were carried passively upwards with the general movement of the epidermal cells, and were finally exfoliated with the keratin layer.

Recently we have used various enzyme techniques on human and animal skins and were surprised to find that although these high level cells were inactive in respect to dihydroxyphenylalanine, nevertheless they were very active in respect to other substrates. The first enzyme to be detected was adenosine triphosphatase (5). Later we obtained evidence that they also exhibit acid phosphatase and sulphatase activity. It would, therefore, appear that while they no longer produce pigment they almost certainly perform other functions at higher levels in the epidermis.

During the process of keratinization the epidermal cells, or keratinocytes as they are sometimes called, become transformed into a stable, chemically-resistant fibrous protein known as keratin. This unreactive material, together with its contained lipids, acts as the first barrier against physical and chemical insults to the skin in particular, and to the body as a whole. The process of keratin formation appears to be a two-fold process. One is cellular cytolysis in which the nucleus and other components of the cell are removed before the occurrence of the second stage of molecular keratin polymerization. Thus, the physical nature of the final keratin layer can be modified to the particular site requirements of the animal depending on the relative amounts of the cellular material removed before keratinization (6).

It is quite obvious that the horny layer on our palms and soles is different from that of other regions, but it can be shown that more subtle differences also exist between other skin sites. It is difficult to explain the mechanism whereby these regional differences in keratinization are brought

about, but one possible means of affecting this would be by the action of these high-level dendrocytic cells.

It is beyond reasonable doubt that the autolysis of the cell protoplasm prior to keratinization is due to hydrolytic enzymes released in the upper layers of the epidermis at the level of cells known as the granular layer. In skin diseases where there is no organised liberation of these enzymes, none of the cell contents is removed, and the whole of the cell, including its nucleus, becomes involved in the process of keratinization. Such a state of affairs exists in the skin disorder, psoriasis.

Palmar and plantar keratinization occupy a position midway between the keratin produced in psoriasis and that, for example, produced on our backs. Here much of the cell contents, except the nucleus, escape autolysis and consequently the keratin layer is more solid and stable than that formed on the back. It is important to mention that although the keratin layer is different this does not necessarily imply that the actual keratin molecules forming the horny layer differ from each other.

As we have detected three distinct enzyme systems in melanocytes other than their dopa oxidase activity, it would perhaps seem reasonable that this other hydrolytic activity might influence the mode of keratinization of the epidermal cells.

The ATPase activity is probably related to motility of the cells. It is most unlikely that it is the type of ATPase associated with ion transfer through cell membranes, and is probably the type associated with contractile protein. This would suggest that the dendritic cells move actively up within the epidermis and are not passively carried upwards by the general movement of the epidermal cells.

Acid phosphatase and sulphatase are both lysosomal types of hydrolytic enzymes, and both would be capable of causing cellular hydrolysis if they were transferred into the epidermal cell in a similar manner to that in which the melanin granules are transferred into epidermal cells of the basal region. It is also possible that any effects of these high-level dendrites could be due to an induction or activation of hydrolytic enzymes rather than to an actual transfer of such an enzyme into epidermal cells.

Although the precise mechanism cannot be directly demonstrated, the circumstantial evidence points strongly towards the involvement of the high-level dendrocytes. Thus in plantar skin where there is relatively little hydrolysis, it is extremely difficult to demonstrate ATPase active dendritic cells, whereas in normal epidermis where there is a much greater hydrolysis of the epidermal cells they are readily detectable in considerable numbers.

In psoriasis, which is associated with the absence of organised hydrolytic enzyme activity, ATPase cells are virtually absent in the lower layers of the epidermis and those in the upper part of the epidermis are abnormal in that they show weak ATPase activity and are without dendrites. Acid phosphatase and sulphatase have not been demonstrated *within* dendritic cells although they are profusely scattered throughout the psoriatic epidermis.

Comparative studies on lower mammals also support the hypothesis that the dendritic cells have an influence on the type of keratin produced by the epidermal cells.

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DISCUSSION

DR. L. GOLBERG: A substantial proportion of the population are taking phenathiazines in one form or another, or are exposed to things like chloroquin and it is now suggested that all new drugs should be screened for their ability to combine with melanin as a protective measure against the possibility of damage to the retina. In the skin we have another situation where there is melanin and the possibility of combination with it; we know that molecules of this sort also tend to be selectively accumulated in lysosomes and there would be a tendency for such substances to accumulate in the skin. On numerous occasions there have been clinical descriptions of skin staining developing, say, from the administration of phenathiazine, but to what extent are there minimal degrees of change in skin pigmentation which are not so striking as to draw themselves to the attention of the physician, but yet do affect the shade of the skin?

THE LECTURER: Lysosomes are at the present time one of our interests and much of this work on sulphatases and the other acid hydrolases are in point of fact directed to the lysosomal activity of the skin. There is good reason for believing that the skin hydrolases are actually of lysosomal origin. Although the electromicroscopists have for years stoutly denied that they have been able to detect lysosomes in epidermal cells, they are beginning to change their minds. They have now definitely stated that these bodies are present in high level melanocytes, and more recently a paper has been published in which they have admitted seeing them in the epidermis from cases of atopic eczema, but they still deny having seen them in normal skin. However, I feel sure they must be there, at least in the sense that the lysosome is only a concept of containing active hydrolytic enzymes within protective envelopes. The fact that their pictures do not show the usual correct anatomical size or shape of envelope does not

necessarily mean they do not exist.* Dr. Riley (in my department) has been engaged in work on skin photosensitivity and has shown that these reactions are probably due to damage to skin lysosomes with the consequent liberation of lysosomal enzymes. If the skin is pretreated with lysosomal stabilizers, such as chlorpromazine, these reactions can be prevented. I think that further work along these lines with lysosome stabilizers will ultimately show that many cutaneous reactions are due to lysosome damage. Chloroquin is, I believe, a lysosome stabilizer, and it has been used clinically in dermatology for many years as protection against sunlight. At first it was thought to act by increasing the screening effect of the 'keratin' layer. This is possible, but as it is a lysosomal stabilizer' it would be capable of protecting the skin because of this property.

DR. I. M. GIBSON: You mentioned *Phenergan* as a lysosomal stabilizer. I believe it is an antihistamine – is there a long and complex series of reactions involved, or is this simple direct action?

THE LECTURER: As far as I know it is a simple direct action and is an effect of *Phenergan* in addition to its action as an antihistamine. Dr. Rees, a colleague of mine, has shown that the toxic effects of various liver poisons could be protected against by giving *Phenergan* and other compounds, such as quinine. He considered that they stabilized the outer cell membrane, and the endoplasmic reticulum. These actions, I think, are quite divorced from any antihistaminic effect.

*Since the reading of this paper an article has been published by R. L. Ohlson, *J. Invest Dermatol.* **46**, 431, (1966) in which he has demonstrated acid phosphatase activity to be concentrated within bound structures of human epidermal cells. He suggests that these structures may be lysosomes.