THE SKIN AS A COMMUNITY OF STRUCTURES

E. W. POWELL, M.A., A.R.I.C.*

In part II of his article the author discusses the formation of skin pigments and of hair. These two processes are carried out by cells of two distinct types which respond differently to some topical influences, but are linked by certain common nutritional needs.

PART II

Pigment Formation and Keratin Production in the Skin

The pigments characteristic of the mammalian skin and its associated structures are the melanins, complex polymeric materials giving black, brown and red colours as well as providing the dark absorbing background for certain blues. It is now generally accepted that these materials are formed by the activity of certain specialised cells, the melanocytes. In or close to the basal layers of the epidermis and about the proliferating layer of the hair bulb are situated cells possessing a main body and branches. These are the pigment-forming melanocytes. In the skin, the two activities of keratin production and of the formation of pigment are, therefore, carried out by cells of two distinct types and of differing origins.

Origin and Development of Pigment-producing Cells

During a relatively short period in the growth of the mammalian embryo there is associated with the rudimentary spinal cord a small amount of a tissue termed the neural crest. This is a transitory structure, for the constituent cells disperse and move to positions where they lie at the interface between an epithelium and an underlying tissue. Such migration results in a fairly even distribution of these cells through the epidermis of the human fœtus, and when a hair follicle begins to develop they move down into the dermis with the descending follicle (Zimmerman, 1953). None of these former neural crest cells appear to remain in the upper part of the follicle, and it is of interest that when sweat gland rudiments are formed none of these cells move down from the epidermis with them. In later development, these cells which have moved into the epidermis and hair bulbs can assume the branched form typical of nerve cells. The branches of these cells pass between the epidermal cells and end on them. When active, they produce a substance, dihydroxyphenylalanine, which is related to the noradrenalin formed in other nerve cells, and it is this substance which undergoes oxidation and polymerisation during the formation of melanin. The melanin is passed along the branches of the melanocytes and is deposited between and also enters the epidermal cells.

* 49, Barn Hill, Wembley Park, Middx.

The origin of the melanocytes of the mouse has been beautifully demonstrated by Rawles. Grafts of the presumptive epidermis and dermis of embryo mice were transferred to a suitable part of an incubating chicken's egg when development proceeded in association with the chick (Rawles, 1953). The eggs were those of the White Leghorn and the tissues of the chick develop no melanin pigmentation. The grafts were taken from embryos before and after migration of the neural crest and in addition neural crest material itself was cultivated. The mice used were of a strain which developed black hair, but formed no melanin in the skin. Under the conditions of cultivation, dermis and epidermis with hairs were developed normally, but grafts removed before migration of the neural crest grew white hairs only. Those removed after migration gave rise to black hairs. Moreover, the cultivated neural crest cells were found to migrate in the epithelia of the chick host and assume their normal form and pigmentary activity.

There are many questions remaining to be answered concerning the factors controlling the migration and distribution of the prospective melanocytes, and also concerning their subsequent behaviour. It seems established that these cells undergo growth and division as do other cells, and also that they are included in the outward movement and shedding of the epidermis. Likewise they move outward from the hair bulb in the growth of the hair. During the period of rest of the hair bulb in which a hair club is formed the fate of the melanocytes in that bulb is not known precisely. Later, when the follicle and papilla become active and the complete structure is reconstituted, it is likely that the melanocytes which pigment the new hair arise from precursors in the residue of the previously active hair bulb (Chase *et al.*).

The presence of melanocytes in tissue can be demonstrated by certain staining techniques, and such methods have shown that there are similar numbers per unit area in white and negro human skin. The difference in racial colour in this and other instances is due to differences in the activity of the cells and is genetically controlled. In the condition of albinism, melanocytes are present in the skin, but remain incapable of forming melanin. Difference in activity as shown by the colour of the melanin produced is responsible for the colour pattern in certain guinea-pigs which possess numbers of melanocytes in red areas of skin similar to those in black areas (Billingham & Medawar, 1953).

In common with other living tissues, the pigment-producing cells function through the activity of bio-catalysts, the enzymes. One enzyme associated particularly with the formation of melanin is tyrosinase, which can convert the amino acid tyrosine to dihydroxyphenylalanine, the precursor of melanin. Although emphasis is placed on the particular chemical reactions involving the oxidation and polymerisation of these single amino acids, tyrosine and dihydroxyphenylalanine, melanin is associated with protein in the living

animal. It is not known how far such protein contributes to melanin formation *in vivo* or the continued distribution of melanin to the epidermal tissue.

The importance of inherited factors in the activity of the melanocytes has been stressed, but there are other influences, hormonal and nutritional, for example, on this activity. In the succeeding sections the influence of local treatments and of certain nutritional factors will be discussed in illustration of the differences and similarities of behaviour of the melanocytes in comparison with the keratin-producing cells.

Independence of Pigment Formation and Keratin Production

It has already been noted that normal development of hair takes place in the absence of any potential melanocytes and that the melanocytes of the mouse were able to develop and function normally independently of the keratinising mouse tissues. Many observations of human hair illustrate this degree of independence of pigment and keratin formation. In the human of the white races it frequently happens that the scalp hair in childhood is almost white, but becomes darker particularly in the years of puberty when a general increased activity of the skin structures takes place. In later life, the loss of pigmentation can take place while the hair grows vigorously.

The earliest biological effect of X-rays to be noted was that of hair loss from a site to which they have been applied. The effect has been investigated using different animals, but the most detailed and careful work has been carried out on mice (Chase, 1949). It was shown for mice growing black hair that a dose of radiation could be given which appeared to destroy the activity of the pigment-producing cells while allowing hair growth to continue in the normal manner. Irradiation of skin where the hair follicles were in a resting condition produced the most marked effect. The new hairs growing in that area were grey. This effect is permanent and so reproducible that it has been suggested as a biological standard for radiation. As the amount of radiation to which the area is exposed is increased, temporary epilation as well as loss of melanocyte activity is observed, but a point is reached when hair ceases to be formed and the loss of hair is permanent.

Similar observations have been made with other animals, the hamster, which gives results similar to those obtained with the mouse, the rabbit, the cat and the guinea-pig. As the coarseness of the hair increases, the dose level at which greying occurs approaches that at which loss of hair takes place, and in the human the level at which temporary loss of hair occurs does not lead to loss of pigmentation. In fact, the effect of low levels of X-ray dosage can produce an increase in pigmentation of the skin which is observed, of course, also by exposure to ultra-violet light. Thus, it appears that, in the human, X-radiation at low levels of dosage can stimulate the activity of the melanocytes, but temporary loss of hair occurs before a level

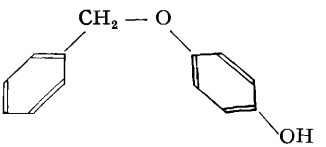
damaging to the melanocytes is reached. In other animals, the melanocyte is inactivated by a level which has no permanent effect on the production of keratin in the form of hair.

There are substances which have an effect on certain tissues similar to that produced by radiation, and some of these radiomimetic substances have been injected into black mice. Among such substances are nitrogen-containing substances related to mustard gas, the so-called nitrogen mustards. Local greying of the hair occurs after injection of suitable levels of these substances and the effect persists through successive generations of hair. It is reported that such mice have retained their white patches for as long as they were observed, which was nearly three years (Boyland, 1952). The mechanism by which radiation and nitrogen mustards produce their effects is not known, but it is known that X-rays can produce free hydroxyl radicals by interaction with water. Free hydroxyl radicals are produced in certain chemical reagents, for example, in a solution containing hydrogen peroxide and ferrous sulphate (Fenton's reagent). Permanent greying of the hair similar to that produced by irradiation and by nitrogen mustards has resulted from the injection of Fenton's reagent into coloured mice. It is possible that free radicals are involved in the effects of ultra-violet radiation, X-rays and the radiomimetic substances on the melanocyte. It has been reported recently that the magnetic properties of living tissue indicate the presence of free radicals (Commoner *et alia*, 1954). This is shown by undenatured protein, but such properties occur in a very marked degree in melanin. Since free radicals can react very readily with each other, these observations may indicate why free radicals such as the hydroxyl radical affect the melanocyte of the mouse so readily.

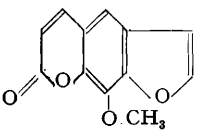
Selective destruction of pigment production has been observed in experiments on the freezing of rat skin (Taylor, 1949). Areas of skin on black rats were cooled *in situ* by contact with solid carbon dioxide or with a special cooling device. The skin was found to survive contact for five seconds with solid carbon dioxide or slower freezing to — 10° C. Hair of normal shape and texture was produced from the areas treated in this way, but it completely lacked pigmentation. A number of generations of rats with white hair were grown, showing that the loss of ability to form pigment was permanent.

Two examples of a selective effect of a drug on pigmentation which have aroused considerable interest have already been reviewed in this journal (Clyman, 1953). In 1936 it was reported that feeding hydroquinone to black rats led to subsequent growth of grey fur and a few years later a derivative of hydroquinone, the mono-benzyl ether, was discovered to be responsible for local loss of skin pigmentation of the arms among Negro workers in a tannery. The skin in those cases appeared to have suffered no other change than the temporary loss of the ability to form melanin. Later

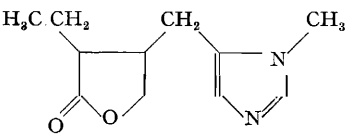
work has shown that sensitisation to the mono-benzyl ether of hydroquinone can arise, and this restricts its application to clinical conditions of excessive pigmentation. Noteworthy are the local effects of topical application of creams or solutions (Denton, *et al.*, 1952) and also the report that, in treating human subjects, the skin is depigmented, but not the hair. The manner in which this compound acts is not known, but it has been suggested that it is converted in the skin to hydroquinone which inhibits the enzyme, tyrosinase. It seems likely that the hair bulbs of the human skin are at too great a depth for an effective local concentration of the ether to be produced by topical application.



Mono-benzyl Ether of Hydroquinone.



8-methoxy-psoralen



Pilocarpine

The second example is of ancient origin, although the identification of the active compound and its preparation in the pure state are recent. From the thirteenth century the Egyptians have used the powdered fruit of a certain plant in the treatment of areas of skin which have undergone spontaneous depigmentation. In this condition of vitiligo, not only the skin but also the hair in that area lacks pigmentation, but in many cases pigmentation could be restored by eating the powdered fruit or applying it topically and exposing the affected area to sunlight. The modern equivalent is oral administration or topical application of 8-methoxy-psoralen and irradiation of the areas with ultra-violet light (Sidi and Bourgeois-Gavardin, 1952). Irradiation is necessary for the success of the treatment. Repigmentation appears first in the hairs, then in the region of skin adjacent to the hair follicle from which the islands of pigmentation spread and join up. Topical application with ultra-violet treatment is successful, and even in a normal white skin inadvertent treatment resulted in a markedly hyperpigmented area (Lerner *et al.*, 1953). So the effect is not peculiar to the condition of vitiligo, but a similar response is given by the normally functioning melanocyte. The mechanism by which the combination of the psoralen and

ultra-violet treatment brings about these changes is not known. Experiments on systems containing tyrosinase, histochemical investigation and attempts to show a hormone-like action have failed to indicate a positive effect of psoralen solutions or irradiated psoralen solutions on these systems. Unsaturated lactones are among the vesicants and have been shown to inhibit certain enzymes containing thiol groups ; even the lactones, the coumarins, related to psoralen will react with thiol groups. Another lactone which has been reported to have the effect of increasing hair pigmentation is pilocarpine (Savill, 1952), an alkaloid which also has known effects on the other epidermal structures, the sweat glands, for example.

Some Conditions Affecting Both Pigment Formation and Keratin Production

Like other functions carried out by living tissues, those of pigment formation and keratin production in the normal animal maintain a certain relation to each other. The changes in the skin which take place in the normal cessation of growth of a hair at the end of a growth cycle affect the hair bulb as a unit and cessation of pigment production occurs too. When a part of the coat of a mouse is in the resting phase, plucking the hairs from that area is sufficient stimulus to initiate the changes leading to reconstitution of an active hair bulb. Both keratin and pigment begin to be produced in step. Where black hairs are formed from a non-pigmented skin, the appearance of black dots on the skin due to pigment formation in the hair bulb indicates that hair growth is taking place although no hair is visible above the surface of the skin. When these mice have their diet markedly reduced in calorific value within a short time of plucking resting hairs, then no appearance of dark spots and no subsequent growth of hair takes place (Loewenthal and Montagna, 1955). Mice have been kept in this condition for two months, a period during which two cycles of hair growth occur on the normally fed mouse, and then the diet has been increased to the normal level. Such an increase in food level results in renewed activity of the hair bulbs in the area plucked two months previously and pigment and keratin formation begin. The phenomena observed in this and other experiments are suggested to be the result of a food-level which is insufficient to produce the energy required for the activity of the hair bulb and maintain the functions essential to the life of the animal. If the reduced level of diet was not fed until reconstitution of the hair bulb and growth on the normal diet are well under way, the effect of restricting the food supply is to retard the growth of pigmented hair, but not to prevent it. It would appear that under these conditions local deposits of fat and glycogen laid down in the period on the normal diet are sufficient to provide energy for the activities of the hair bulb during the whole growth-phase.

The effect of one aspect of nutrition on the pigmentation and growth of human hair has been recognised in many areas of Africa where ill-health and disease due to malnutrition exist. This is the condition of "kwashiorkor," which arises in people living on diets deficient in quality and quantity of protein and is observed particularly in infants and young children. One of the features of this disease is a change in the texture and colour of the scalp hair which is finer, softer and straighter and the pigment has a marked red colour in contrast with the normal jet black. Examination of the histology of the scalp including hair bulbs, follicles and shafts has shown these to be of normal structure (Berte, 1954). It was considered possible that the hair bulbs showed an inability to produce normal pigment and keratin because of an inadequate supply of amino acids.

Investigation of the amino acid composition of a staple foodstuff of some of these people showed that only small amounts of the sulphur-containing amino acids, methionine and cystine, were present and consideration of the change in amino acid composition of the diet, arising in the weaning of an infant from breast milk to such a diet, revealed a marked fall in the supply of these amino acids. The amino acid composition of the hair in cases of kwashiorkor has been compared with the composition of hair from a normal individual, and in one case hair grown during a period of the disease has been compared with hair grown on the same scalp after the subject had been receiving an adequate diet (Bigwood, 1954 ; Bigwood and Robbaza, 1955). The total sulphur and the cystine contents of the hair grown during a period of under-nutrition were lower than the contents of these substances in normal hair. The amino acid analyses available do not allow any conclusions to be drawn about whether this is the only change in composition, but it would appear to be the most outstanding. The effects on the hair observed in kwashiorkor can be observed in black-hooded rats by feeding the local diet of Gambia, where the disease occurs, but experiments on the supplementation of this diet by a protein such as casein plus cystine indicate that this supplementation does not restore the normal condition which is readily obtained by addition of skimmed milk powder. The change in pigmentation and hair growth is not due to a simple lack of sulphur-containing amino acids, but seems related to an adequate supply in a certain form, for example, in a balanced supply of other amino acids.

Investigation of the pigment is limited by the methods at present available for examining the melanins. The pigment in the red hair of kwashiorkor behaves differently on a chromatographic column from the pigment of normal, black, African hair and from that of auburn European hair. However, pigments with a similar behaviour to that from kwashiorkor hair can be obtained from normal hair treated with hydrogen peroxide or exposed to ultra-violet light, that is hair in which the pigment has undergone some oxidation (Nagchaudhuri and Platt, 1954).

The sulphur-containing amino acid, cystine, has been reported to form as much as 18 per cent of the dry, ash-free weight of human hair. The protein associated with the melanin pigment has also been reported to contain cystine and methionine. A further link between the sulphur-containing amino acids and melanin formation lies in the reports of the association of lower concentrations of water-extractable materials containing thiol groups (-SH) in regions of excessive pigmentation compared with regions of normal or reduced pigmentation (Rothman *et al.*, 1953). Furthermore, thiol compounds, such as cysteine, are claimed to protect tissue from the effects of radiation. A solution of cysteine hydrochloride injected beneath the skin of the mouse gave marked protection against a dose of X-radiation which would have caused almost complete destruction of the melanocytes in the hair bulbs. Normal black fur grew from the irradiated area of the protected mice, whereas unprotected mice grew grey hair after receiving a similar dose of radiation (Kulwin, 1953). The action of cysteine in preventing those effects which probably arise from the reactivity of the free radicals produced by irradiation, the inverse correlation between concentration of thiol compounds and pigmentation of the skin, and the similarity of oxidised melanin pigments to that formed in kwashiorkor, give support to the suggestion that thiol compounds might act as regulators of the state of oxidation of the pigment.

The importance of the sulphur-containing amino acids in both keratin and melanin formation is evident, but some doubt exists whether the relation to melanin is direct or whether it is through the interaction of sulphur compounds with another component of the system. Essential to both the formation of melanin and of keratin is an adequate supply of copper in the diet. Copper deficiency in the diet of sheep producing non-pigmented wools reveals itself in the appearance of straight, lustrous wool lacking the marked crimp of the normal fleece, and the wool fibres have a lower tensile strength. Histological examination shows that keratinisation in which the wool structure becomes stabilised in the conversion of combined cysteine to combined cystine takes place over a much greater length of the follicle and the wool may still be in a plastic condition at a distance almost two-thirds the way up the follicle. Keratinisation is normally completed at a level of less than one-third the distance up the follicle. Copper therefore plays an important part in the oxidative process converting thiol to disulphide (Marston, 1946). The association of copper and melanin formation has been shown by the loss of the ability to produce pigment in direct copper deficiency, and also when substances known to combine strongly with copper are present in the diet. Dark-coloured cattle grazed on areas of low copper content in the pasture undergo a marked greying of the hair, and black rats fed upon a copper-deficient diet show a similar change in coat colour. Rats fed phenylthiourea (phenylthiocarbamide), cysteine, thiouracil

or the dithiol, BAL, grew grey hair, but when these substances were omitted from the diet the hair growing subsequently was of the normal black colour. These substances form very stable co-ordination compounds with copper ions and reduce their availability to other systems such as those involved in pigmentation. Similar effects have been noted in human subjects, prolonged treatment with BAL producing grey hair in one case and thiouracil produced areas of depigmentation in a Negro subject (Lerner and Fitzpatrick, 1953). Copper is now known to be an essential part of the enzyme tyrosinase which as stated above plays a major part in melanin formation, and it is possible that the effects of sulphur-containing compounds in the normal skin are mediated through their control of the amount of copper available to enzymes.

Zinc is another metal required for the formation of normal melanin and keratin, and rats fed a diet deficient in this metal grew fur which was soft and woolly, and the portions of fur normally black were light grey in colour (Stirn *et al.*, 1935). Zinc ions are necessary for the activity of certain enzymes so that control of the availability of zinc by thiol compounds might also control the activity of such enzymes.

The process of keratinisation with the conversion of thiol groups to disulphide groups occurs in a region of the hair follicle, the keratogenous zone, above the hair bulb. Histological procedures reveal a high concentration of thiol groups in this region, but in the bulb itself the concentration is much lower. The thiol groups, if present as such in the bulb, are screened in some way, and melanin formation which is influenced by the concentration water-soluble thiol compounds proceeds readily. The state of the thiol groups in the hair bulb is not known : they may be held within a protein inaccessible to chemical reaction and become available only as a process akin to denaturing of the protein takes place.

Summary

The cells responsible for the pigmentation of the skin and hair are of nervous origin, and their particular products which are probably related to a nervous function have become utilised to form melanin. The distinction between these cells and those of epidermal origin which produce keratin is reflected in the distinctive response of the melanocytes to certain conditions which appear to have little effect on keratin formation. These two types of cells, however, have a number of links in common, of which the relations to sulphur-containing compounds and the metals, copper and zinc, have been discussed. There appears some evidence of a system of inter-regulation with the availability of the metals to enzymes being controlled by the sulphur compounds. The metals might also displace each other in various systems and have effects as oxidising catalysts on the sulphur compounds.

The hair bulb, for example, is a system of living cells in which various processes are maintained spatially separated, but the cells depend upon a common environment for their nutrition. Local effects can result from changes in this environment by topical application of certain substances, and examples are available of specific effects upon the cells responsible for pigmentation following such topical treatment.

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