CONTINUING MEDICAL EDUCATION

VITILIGO

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The late Pandit Jawaharlal Nehru once expressed his hope that drugs would be found to combat the three major maladies of the Indian population, namely, leprosy, tuberculosis and vitiligo.[1] The incidence of vitiligo in the Indian population is thought to be about 3% compared to about 1% of world population.[1] There is more need for research on vitiligo since very little is known about it and since it affects a large population of India. Vitiligo occurs in both sexes and in all races of man and in other species. The onset of the disorder could be any time from childhood to senescence. In this short article, this problem is reviewed in relation to the present understanding of its etiology and the knowledge on melanogenesis in general. The reader is referred to earlier reviews[1-4] on this topic. The recent review[4] was quite exhaustive on this topic.

In vitiligo, apart from the absence of melanin and of identifiable melanocytes, the vitiliginous epidermis appears to be normal. The exception to this conclusion is the recent observation that vacuolated keratinocytes have been observed in normally pigmented peri-lesional skin of vitiligo patients.[5] Most of the vitiligo patients are otherwise generally healthy, though there are certain disorders more often associated with it.[4]

Aetiopathogenesis

Vitiligo is not an infectious disease. Its aetiology is far from clear although many theories have been put forward. The major theories could be listed as, (1) genetic theory, (2) melanocyte self destruction theory, (3) neural control theory, and (4) autoimmune hypothesis. Evidence for and against the above theories is briefly described below :

Genetic theory

El-Mofty[6] in his book on vitiligo and psoralens collected evidence indicating that 35.25% of the patients of vitiligo had familial history. Lerner[1] obtained similar figures from his vitiligo patients. In monozygotic twins,[7] vitiligo occurred at the same time and location suggesting genetic basis for the disorder. But this is contradicted by the case of another monozygotic twin where only one of them was affected.[8] It is thus fair to conclude that factors other than or in addition to genetic factors may be involved for vitiligo.[9]

Melanocytes self destruction hypothesis

This hypothesis predicts that toxic intermediates of melanogenesis may bring about the destruction of pigment cells and thus cause depigmentation.[10] Most phenols, or catechol derivatives of natural (including melanin precursors) or synthetic origin in a proper concentration inhibit tyrosinase activity and thus the production of melanin. The same substances can cause lethal changes in the melanocytes.[11] Men exposed to p-tertiary butyl phenol develop depigmentation resembling vitiligo[12] not only in places of contact with this compound but also in places not exposed to its direct contact. Melanocyte self destruction theory was further

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supported by the recent observation that actively melanizing melanoma cells release an agent into the culture media which inhibits proliferation of not only of like cells but also fibroblasts as tested by the decrease in the rate of DNA synthesis or cell number. Exogenous tyrosinase added to the medium can mimic the effect of endogenous tyrosinase activity.[13] Frequent changes of the medium of actively melanizing melanoma cells decrease the degree of inhibition of growth of cells suggesting that release of an agent by actively melanizing melanoma cells into the medium was responsible for the cytotoxicity of this medium for cells.Tyrosine, 3, 4 dihydroxyphenylalanine (dopa), and dopachrome, the metabolites involved in melanin synthesis are cytotoxic.[14,15] Tyrosine decreases the growth rate of melanotic cells at 5 mM, while cytotoxic effects of dopa or dopachrome are seen at concentrations above 10^{-5}M. In normal and vitiligo human skin, the levels of tyrosine and dopa are in the range of 1-2 mM and 10-20 μM respectively and do not reach toxic levels.[16] In addition, under in vivo conditions, these metabolites when made by the melanocytes will be localised in the melanosomes. It therefore is unlikely that toxic metabolites of melanin precursors could accumulate sufficiently to be cytotoxic to melanocytes. A slightly modified hypothesis was put forward by Burn.[17] According to this hypothesis, vitiligo might be due to the inhibitory action on tyrosinase by a phenolic derivative which may be a degradative product of a normally occurring component in melanin synthesis. This component inhibits tyrosinase and thus melanin synthesis. As a consequence, melanocytes are destroyed. Some localised and temporarily limited event may precipitate this process. Riley[18] suggested that an abnormal phenolic metabolite is produced in individuals with vitiligo gene and when its level reaches a critical level in some region of the dermis, melanocytes are destroyed. However, such metabolites are yet to be discovered in vitiligo skin. Thus, in the absence of any positive evidence so far that melanocytes could be destroyed at physiological concentrations of melanogenic intermediates the question remains open.

Neural hypothesis

Both pigment forming melanocytes and neuronal cells are derived from the neural crest and both utilise tyrosine to produce their products, melanin and catechol amines respectively. Lerner[1] suggested that hypopigmentation may be caused by an excess of norepinephrine or some other catecholamine released in increased amounts at the peripheral nerve endings. Most phenol or catechol derivatives inhibit tyrosinase activity and thus production of melanin, and cause lethal changes in melanocytes.[11] Degenerative changes in the terminal portions of peripheral nerves in vitiliginous areas were observed in support of the above theory.[19]

Recent ultrastructural studies had indicated anatomic contact between nerve fibres and melanocytes.[20] The exchange graft experiments of Orentreich[21] where pigmented skin transplanted to vitiligo skin becomes vitiligenous and vitiligo skin transplanted to normally pigmented skin becomes pigmented indicate that the skin lesion in vitiligo resides deeper in the skin. However, contrary results were obtained by others.[22-24] In view of this and in the absence of direct evidence for a primary neural defect in human vitiligo skin, this theory is difficult to be accepted. In addition, the weight of clinical observations among neurological disorders is against the neural hypothesis as the causative factor for vitiligo.[4,25]

Autoimmune theory

According to this theory, autoimmunity is the cause of vitiligo. Autoimmunity is a process where the defence mechanism of the body goes hay-wire. If fails to distinguish between self and non-self and the body produces antibodies

against its own antigens. This could happen if a primary disturbance in the immune system results in autoimmunisation with the formation of autoantibodies against some antigen(s) of the melanocyte. As a result, melanogenesis may be inhibited or melanocyte may be destroyed. Alternatively, some injury to melanocytes may result in the release of an antigenic substance so that antibody formation occurs either against the melanogenic process or the antibodies become cytotoxic to melanocytes.

The incidence of vitiligo is higher in patients with autoimmune diseases as compared to its incidence in the general population.[1,26-39] The development of vitiligo at the site of physical trauma known as Koebner phenomenon may be explained as due to release of antigens of injured melanocytes into the blood and production of antibodies against them. Some features of chemically induced depigmentation where areas unexposed to the depigmenting agents also get depigmented suggest an immune pathogenesis.[12]

Immunological studies

Direct evidence for the involvement of the immune system in vitiligo is sparse. The existence of antibodies to melanin in the serum of 26 patients with vitiligo was claimed,[40] but others[41] failed to confirm this observation. Hertz et al[37] reported by immunofluorescent complement fixation technique, the existence of circulating antibodies to melanocytes in the serum of two patients with vitiligo, muco-cutaneous candidiasis, alopecia universalis and multiple endocrine deficiencies. Betler et al[42] confirmed the observation of Hertz et al[37] in one patient with vitiligo. However, Howantiz et al[43] analysed serum samples of a large number of persons with various types of vitiligo and other disorders of pigment cells for the prevalence of complement fixing antibodies to melanocytes. Their results indicate that these antibodies do not occur in persons with common vitiligo but these are present in a substantial number of individuals with chronic muco-cutaneous candidiasis. It appears as though antimelanocyte antibodies are associated with chronic muco-cutaneous candidiasis rather than with vitiligo. A recent study by Naughton et al[44] had demonstrated unequivocally in the serum of majority of vitiligo patients, the presence of antibodies to surface antigens of melanocytes grown in cell culture according to the method of Eisinger et al.[45]

However, loss of pigment in the skin but not from hair often occurs in vitiligo,[45a] and this cannot be explained by the autoimmune theory.

The existence of antibodies to melanocytes in the serum of patients with vitiligo even if confirmed in future, is by itself not enough evidence for the autoimmune theory of vitiligo. It will have to be shown that the antipigment or antimelanocyte antibodies are cytotoxic to pigment cells or inhibit melanin synthesis. In the absence of this vital information the autoimmune theory of vitiligo remains unsubstantiated.

All the theories for the aetiology of vitiligo suggest that melanocytes are absent in the vitiligo macule since they are destroyed by different mechanisms in different theories. Yet no microscopic evidence exists for the actual dissolution and disruption of melanocytes in the vitiligo skin. This is a serious drawback of all these theories.

Existence of inactive melanocytes in vitiligo

Although identifiable melanocytes are absent in vitiligo patches,[46] indeterminate cells or alpha-dendritic cells are observed in vitiligo macules[47] and the work of Mishima et al[48] suggested a dynamic inter-relationship between melanocytes and indeterminate cells. According to this hypothesis, the indeterminate cells are inactive melanocytes and they increase in pro-

portion to the disappearance of active melanocytes in vitiligo. This hypothesis was supported by many observations. (1) Forty eight hours after a single ultraviolet radiation exposure of skin, an increased number of melanocytes with a corresponding decrease in the number of indeterminate cells was observed.[49] (2) The study of indeterminate cell numbers in repigmented areas of vitiligo suggests that they can be changed into active melanocytes.[50] (3) Kukita[51] identified the indeterminate cells in the melanocytic portion of hair melanin in white hair from vitiligo subjects. (4) Presence of tyrosinase activity and synthesis of melanin from tyrosine by vitiligo skin homogenates suggest the presence of melanocytes[73] and since there was no melanin sythesis in vitiligo skin, the tyrosinase activity observed in vitiligo skin may be from the indeterminate cells. (5) Jimbow and Uesugi[48a] had shown that repeated exposure of the trunk and plantar skin of adult mouse to ultraviolet light (280-315 μm) resulted in the activation and proliferation of precursor melanocytes and they become 3, 4-dihydroxyphenylalanine positive. This could not have resulted by the migration of melanocytes from either dermis, sweat glands or from anywhere.[48b] Bleehen et al[52] found that melanocytes were replaced by indeterminate cells and Langerhans cells in depigmented skin by chemical agents. In view of these observations it may be assumed that the major defect in vitiligo may not be the destruction and thus loss of melanocyte but rather inhibition of melanin synthesis and conversion of active melanising melanocytes to inactive melanocytic or alpha dendritic cells or indeterminate cells. Thus, whether melanocytes were destroyed or their active melanising ability is lost in vitiligo remains to be resolved one way or the other.

The brief review of the existing theories for the aetiology of vitiligo shows that the information is far from complete. Basically, the defect in vitiligo is lack of melanin synthesis either because the melanocyte is lost or because it is not making melanin. We do not know adequately the various factors that regulate this synthesis, what is known briefly is reviewed below.

Melanin synthesis and its control

Human skin colour : Normal human skin colour is mainly due to melanin. Melanin pigment in the skin is located in a very small

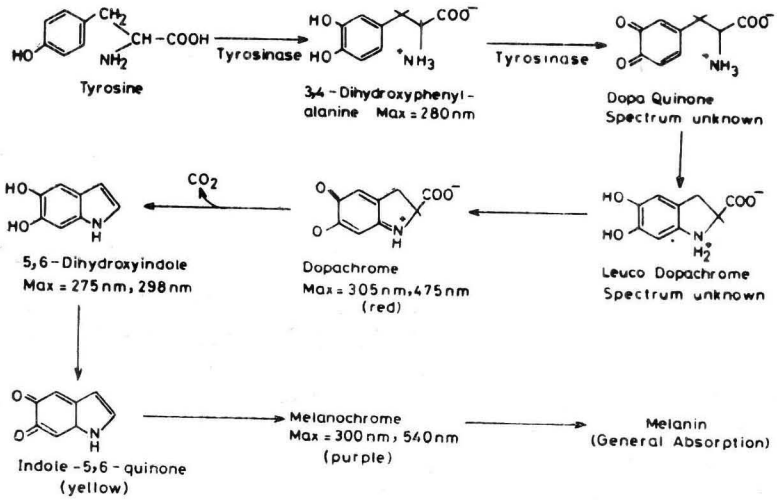

Fig. 1. Schematic representation of eumelanin biosynthesis.

granule called the melanosome.[53] The melanosomes are formed in the melanocytes. They are transferred into the keratinocytes directly by phagocytosis of dendrites containing melanosomes of melanocyte.[54-58] These are distributed throughout the epidermis by the outward movement of the keratinocytes and thus contribute to the colour of the skin. Racial differences in the colour among humans are not due to quantitative differences in the number of melanocytes in the skin but apparently due to differences in the number of melanosomes, their size and distribution within the keratinocytes.[57] For instance, the melanosomes in Negroid skin are numerous,[58] longer and wider than those in Caucasoids and unassociated.[59] These differences are responsible for the Negroid skin to be darker than the caucasian skin.

Melanins : There are two major classes of integumentary melanins. The black-brown ones are called eumelanins and the yellow to red melanins are called the pheomelanins. Melanin is the most conspicuous absorber of the visible

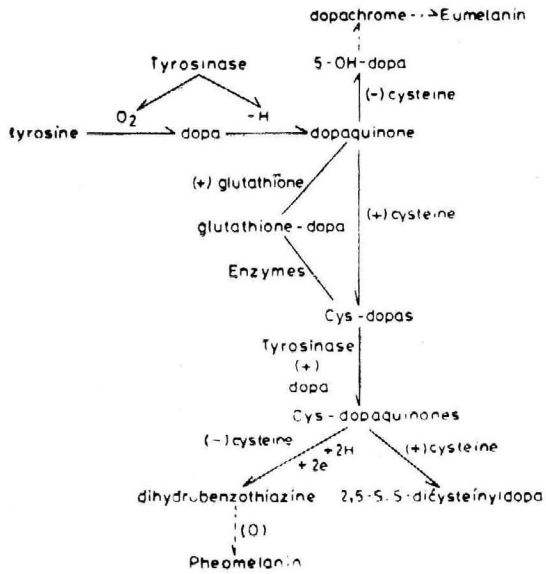

Fig. 2. Schematic representation of pheomelanin biosynthesis.
(+) = Presence (+) = Absence

and longwave ultraviolet radiation and of free radicals in skin. Melanins thus act as a filter for biologically harmful radiations from the sun.[90-92] The biosynthesis of eumelanins [60-63,63a,64] and pheomelanins are described in figs. 1 & 2. Eumelanins are almost insoluble in all solvents while pheomelanins are soluble in dilute alkali. A protein matrix is a prerequisite for eumelanin formation in the melanosome while pheomelanin granules develop without an organised protein skeleton.[64]

The chemistry of melanogenesis

The early stages of mammalian melanogenesis involve the conversion of tyrosine to 3, 4 dihydroxyphenyl-alanine (dopa) and its further oxidation to dopaquinone. These reactions are catalysed by tyrosinase[65-68] (E.C.1.14.18.1). The first one is called cresolase activity and the later as dopa oxidase activity. The cresolase activity of tyrosinase obtained from many sources has a characteristic lag, is inhibited by excess tyrosine, and has an essential requirement of 3, 4-dihydroxyphenylalanine as a hydrogen donor.[68-77] These properties disappear when the human skin tyrosinase is partially purified.[78] Further studies on the tyrosinase from human skin and from murine melanoma revealed that these properties can be modulated by pH or pH and tyrosine (Chaya and Ramaiah, Tripathi and Ramaiah—unpublished observations) and may have important implications in the regulation of melanin synthesis. The partially purified enzyme could be inhibited by a protein which is present in the cytosol as well as in the melanosomes[78] and by various dialysable factors in the skin homogenate.[73] In addition, recent reports indicate that there is an additional enzyme involved in the conversion of dopa-chrome.[79] Moreover, factors were isolated which could accelerate or block conversion of 5, 6 dihydroxyindole to

indole quinone.[80] The role of these factors on melanin synthesis is yet to be clearly established.

Approaches to the problem

Whether the lack of melanin in vitiligo skin is indeed due to lack of melanocytes, or due to inhibition of melanin synthesis and consequent conversion of melanocytes to inactive melanocytes is an important question to answer before a clear understanding of vitiligo and thus its cure is accomplished. A most recent report[93] identified a hypothalamic factor from bovine hypothalamus which specifically stimulates growth of melanocytes in vitro. It could as well be that such a factor may also be present in the hypothalamus of human beings and alteration of its level or its modification for some reason may lead to the decrease or elimination of melanocytes from the epidermis. Answers to these querries can now be obtained with the recent successful developments of methods for culturing of melanocytes in vitro.[45,93] In the absence of melanin synthesis, the melanocytes cannot be unequivocally identified by the existing methods. In tissue culture, in the presence of cholerotoxin and phorbol ester, melanocytes were shown not only to multiply but also synthesise melanin. Under these conditions, or in the presence of the hypothalamic factor which stimulates melanocyte multiplication, the inactive melanocytes if present could be stimulated to synthesize melanin and thus be identified. These experiments are already in progress in our laboratory.

Treatment of vitiligo

To bring back colour into the white skin of vitiligo, Indians and Egyptians[81] are known to have used extracts of the plant BaVaches (*Psoralea corylifolia L*). The seed extracts were used both orally and topically according to the ancient Indian literature. Fahay and Abushady[83] had isolated three cyrstalline compounds identified as 8 methoxypsoralen, isoamylaneoxypsoralen and 5-methoxypsoralen. El Mofty[6] was the first physician to treat vitiligenous lesions with psoralens and sunlight and to report successful repigmentation in 30-35% of the cases treated. These compounds belong to the class of furocoumarins, certain isomers of which are called psoralens. Their structure is shown in fig. 3. The mechanism of their action

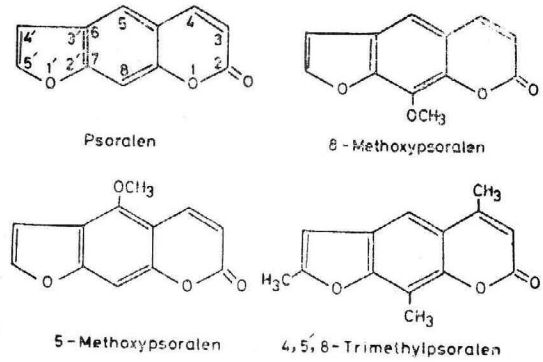

Fig. 3. Structure of psoralen and its derivatives.

is not clear. The photoexcited psoralen molecules are known to form cross links with pyrimidine and purine bases in DNA and appear to precede other cellular or vascular changes.[84] The other treatments worthy of mention include skin grafting[85] if the vitiligo patches are few and stationary, and corticosteroids.[86-89].

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