Bhaskar Menon Ambady Memorial Oration

SOME INVESTIGATIVE OBSERVATIONS ON PIGMENTARY DISTURBANCES — AN ENIGMATIC CHAPTER IN DERMATOLOGY

B. N. BANERJEE

Pigmentary disturbances of the skin constitute the largest objective finding in dermatological practice. Such disturbances, primary or secondary owe their origin to innumerable etiological factors, starting from superficial contact to deep seated genetically determined biochemical trauma.

Clinically also, the pigmentary changes vary widely in respect to their intensity, extent, disposition and characteristics and may be, in many situations, associated with other textural changes of the skin as well as changes of other structural components, such as blood vessels, nerves, etc.

Literature dealing with the different facets of pigmentary anomalies has piled up almost beyond the scope of one's ready memory and has lately reached the level of molecular biology. In spite of voluminous research explanations regarding the precise mechanisms of pigmentary changes in large many of these conditions remain ill-understood as yet. It is far from my intention to deal here with such developments. I shall only present before you a number of observations as compiled from the works conducted in my department in the last few years by some of my student colleagues in reference to a few commonly encountered conditions in our day-to-day practice. In fact, the observations represent collective data obtained through a sort of team work. I do not claim any part of these works to be exclusively mine but I take pride of my close association with all these studies. Appreciatively I acknowledge the contributions in this regard of my students - colleagues, Dr. A. K. Dutta, Dr. S. B. Mandal and Dr. A. Chatterjee while they worked for their Ph.D. Thesis under my guidance. I also gratefully acknowledge the co-operation we received from the department of Anatomy of our University, particularly to Prof. P. R. Roy, Centenary Professor of Anatomy, Dr. B. C. Roy Post-graduate Institute of Basic Medical Sciences and Dr. A. K. Maiti, Professor of Physiology, University College of Science, Calcutta University.

As you all know, categorisation of pigmentary changes has been most comprehensively made by *Fitzpatrick et al*[1]. The list is a vast one

Presented at the 5th Annual Conference of Indian Association of Dermatologists, Venereologists and Leprologists, Ahmedabad, 1977.
Professor and Head of Department of Dermatology and Venereology
University College of Medicine, Calcutta
Received for publication on 8—3—1977

and it is not an easy job to condense the same. I shall, however, present here somewhat modified classification based on the predominant aetiological background before presenting our own investigative observations in some of these conditions, namely, hypopigmentation in leprosy, pityriasis versicolor, naevus anaemicus and depigmentation in vitiligo.

Basic factors responsible for pigmentary changes of the skin—

(1) Melanisation defect
  (a) Hyper
  (b) Hypomelanosis

(2) Deposits of non melanin elements
  (a) Haemosiderin (Sickle cell anaemia)
  (b) Bilirubin (Jaundice)
  (c) Carotene (carotenaemia)
  (d) Ochre (Ochronosis)
  (e) Other metallic elements.

(3) Vascular anomaly e.g., Naevus anaemicus.

(4) Increased dermal fibrosity (Morphoea, L.S.A.) Scar etc. causes of melanisation defect.

I. GENETIC

Hypomelanosis	Hypermelanosis
Piebaldism, Premature canites Vitiligo, Ash-leaf macule, Albinism. Naevus depigmentosus Phenyl Ketonuria.	Ephelides, Caufe–au–Lait spot, lentigenes, Mongolian spot, Melanocytic Naevus, Naevus of Oto and Incontinentia pigmenti
Syndromes	**Syndromes**
Waardenberg, Fanconi's Chediak-Higashi, Cross-Mckusick-Breen, Tietz, Menke's Kinky hair.	Peutz—Jagher's, Albright Franceschetti – Jadasson Melanism.

II. METABOLIC AND NUTRITIONAL

Hypomelanosis	Hypermelanosis
Chronic protein deficiency, Vit. B_{12} deficiency (only pigment loss in hairs in both cases).	Haemochromatosis, Wilson's disease porphyria, Gaucher's disease, Niemans Pick disease, Kwashiorkor, Pellagra, Sprue, Vit. B_{12} deficiency, Chr. nutritional insufficiency.

III. ENDOCRINAL

Hypomelanosis	Hypermelanosis
Hypopituitarism, Addison's disease Hyperthyroidism	ACTH and MSH producing pituitary and other tumours, Addison's disease, ACTH/ocstrogen therapy, Progestational agents – Melasma, Pregnancy, Nelson's syndrome.

IV. INFLAMMATION AND INFECTION

Hypomelanosis	Hypermelanosis
Pinta. syphilis, leprosy, Tinea Versicolor, Eczematous derm., Pityriasis alba, Psoriasis, DLE Post-inflammatory.	Lichen pigmentosus, Lichen Planus, D. L. E., Lichen simplex chronicus Atopic Derm., Pinta, Syphilis, Erythema dyschromium perstans, Ch. malaria, Ch. Kala-azar, Post – inflammatory.

V. PHYSICAL, CHEMICAL AND PHARMACOLOGICAL AGENTS

Hypomelanosis	Hypermelanosis
Burn (Thermal, UVL and ionizing radiation) Trauma, Hydroquinones Chloroquin (hair only).	UVL – suntanning, Thermal radiation. Ionizing radiation, Trauma of chronic pruritus, Arsenic, Busulfen, Photochemical drugs, Quinacrines, Chlorpromazine, Fixed eruptions, Melanodermatitis toxica, Berlock dermatitis.

VI. MISCELLANEOUS (INCLUDING NEOPLASMA)

Hypomelanosis	Hypermelanosis
Idiopathic guttate, Canites, Sclero derma. **Syndromes :** Vogt – koynagi Alezzandrinis Horner's (Pigment loss in iris) Halo – naevus.	Systemic sclerosis, Ch. hepatic insufficiency, Ch. Encephalitis, Senile lentigo, Catatonic Schizophrenia, Mastocytosis, Malig. Melanoma, Adenocarcinoma with acanthosis Nigricans, Metastatic melanoma and melanogenuria, Beckers melanosis, Periorbital melanosis, Dyskeratosis congenita, Poiklioderma, Riehl's melanosis, Erythrose – peri – buccal Pigmentare of Brocque. **Syndromes** Whipples, Cronkhite – canada, Mendes Decosta.

Our investigative studies, limited as they are, comprised of the following, besides routine clinical recording.

(i) Dermatophysiological
(ii) Biochemical
(iii) Histological
(iv) Histochemical
(v) Neurohistological.

Here, of course, I propose to present in brief our observations based only on the last three parameters and that too in reference to such findings only which appeared to us to be provocative for further follow-on approaches. I may mention here that many of our findings have been published earlier in an assorted way in various journals in India and abroad and naturally I shall try to exclude repetitions as far as permissible.

Precisely speaking, today's presentation of data in reference to processes of hypopigmentation in the relevant clinical entities as mentioned earlier have emerged mostly from histochemical and neurohistological approaches which included studies on :

(i) DOPA-reaction,

(ii) qualitative tissue cholinesterase activity;

(iii) intracutaneous nerve-fibre structures.

Following Table gives the number of patients investigated in each group.

(1) Leprosy —50 (TT)
(2) Pityriasis versicolor—25
(3) Naevus Anaemicus —25
(4) Vitiligo —50

Methodology

(1) Laidlaw's DOPA — oxidase method.

(2) Gomori's acetylthiocholine method for the study of tissue cholinesterase activity.

(3) Romane's Agcl staining method for the study of nerves.

(4) Routine H and E stain.

Fundamentally speaking, the primary objective of study was to assess the status of the cutaneous autonomic nerves and their relationship, if any, in these dermatoses.

Observations

In the lesions of leprosy—variable degree of DOPA—positivity could be observed which appeared to bear a linear co-relation with the degree of clinical hypopigmentation. The hypopigmentation was directly proportional to the DOPA—positivity, an area with pronounced hypopigmentation showing as little as 25% activity in comparison to a normal skin.

Neurohistological findings in the leprosy lesions appeared characteristic-demonstrating degenerative changes at variable levels of nerve bundles and twigs. The changes in the nerves supplying cutaneous autonomic target organs (which were specially looked for) could be seen much more frequently in those cases where the clinical hypopigmentation was relatively pronounced—varying between 88.8% to 100% cases while in the lesions with milder hypopigmentation such involvement of the nerves supplying autonomic target organs varied between 25% to 31.5% of cases.

Tissue cholinesterase activity as observed histochemically was found conspicuously lacking or even absent in cases of leprosy lesions of TT-variety-the relative lack of such activity varied proportionately with degree of clinical hypopigmentation and degenerative changes of the nerve branches supplying the blood vessels.

In pityriasis versicolor : No alteration in the dopa-reaction could be observed. The histochemical picture appeared identical with that of control tissue. There was no change referable to either cutaneous nerves or tissue cholinesterase activity in the skin sections of pityriasis versicolor cases.

In Naevus Anaemicus : Although the clinical hypopigmentation looked very much similar to that of leprosy lesion with pronounced hypopigmentation, no deficiency in the dopa-reaction could be seen. Naturally the histochemical findings in this respect could not be differentiated from the adjacent control skin tissue. Neurohistological findings were non-significant. In none of the sections could any degenerative changes in the nerve structure be detected. Interestingly enough, tissue cholinesterase activity appeared relatively pronounced as compared to control areas.

In Vitiligo: As anticipated, the dopa-reaction was invariably negative. Degenerative changes in the nerves could not be observed uniformly in all the sections of vitiligo lesions. In a limited number of sections taken from vitiligo of pseudosegmentalis type of

SUMMARISED OBSERVATIONS

Diseases under study with No. of cases.	Dopa-reaction	Tissue cholinesterase activity	Peripheral nerve degeneration
1. Hanseniasis (50)	Variable degree of subtotal DOPA negativity.	Conspicuously lacking even absent in many cases.	Very evident
2. Pityriasis versicolor (25)	No difference from control.	No difference from control.	Not present
3. Naevus anaemicus (25)	No difference from control.	Relatively more activity.	Not present
4. Vitiligo (50)	Conspicuous Dopa negativity.	Relatively less activity.	Evident in some cases & suspicious in many more.

more than 5 years duration, some degenerative changes in the nerves presumed to be supplying the autonomic target organs could be observed.

Tissue cholinesterase activity in the sections of vitiligo appeared significantly less compared to adjacent control areas.

Discussion

The current concept of the probable neurochemical mechanism of hypopigmentation as elucidated by Lerner[2] has originally laid the foundation of our approach. Derangement of pigmentation is a characteristic feature in all these disorders under study, but the real mechanism remains an enigma.

The investigative efforts in this study were channelised to screen the primary question whether the clinical hypopigmentation in these disorders is due to any sort of inhibited enzymatic activity involving the conversion process of tyrosine/DOPA to melanin leading to hypomelanosis and if so, whether the relative or absolute DOPA—negativity in any of these diseases bears any relationship with functional and/or morphologic affection of any of the components of cutaneous terminal nerves.

The neurologists concede that the relationship between the structure and function of the complex constituted by the cutaneous nerves and nerve endings is quite confused and disputed. The components of this complex are not fixed immutable structures but are constantly undergoing degeneration and regeneration. They are thus in a dynamic state and capable of adaptation to different circumstances. The proper functioning of the epidermis is also dependent upon the intact dermal nerve supply. Recently an interesting association between the epidermal growth factor (EGF) and the nerve growth factor (NGF) has been postulated. Recent advancements in these fields have been very comprehensively reviewed by *Sinclair*[3].

From the functional view point the post-ganglionic autonomic fibres i. e., sympathetic, can be divided into two groups, cholinergic and adrenergic. In cholinergic fibres acetylcholine is synthesised by the enzyme choline acetylase either within mitochondria or in association with them, and the ester accumulates diffusedly throughout the nerve (*Whittaker*)[4]. Cholinergic fibres also contain acetylcholinesterase which is probably produced by the endoplasmic reticulum (*Brazin et al,*)[5]. In the adrenergic fibres noradrenaline is synthesised from tyrosine. Many other details of the anatomical peculiarities in this respect has been elaborately discussed by *Hillar p*[6].

The vital trophic influences of the autonomic nerves cannot be overlooked besides their direct control over the respective target organs in the skin. The cutaneous autonomic nerves provide singularly important linkage in the neuroendocrinal reactive machinery with distant control in the hypothalmus. This machinery is almost always commissioned for adaptation and for maintenance of total homeostasis in the face of stressful stimuli.

In hypopigmented patches of leprosy, the underlying cause of pigment loss has remained obscure. Various postulations include,

(1) Cellular infiltrate mechanically interfering with the function of the melanocytes ; (2) Neural involvement; (3) Diminished blood supply to the affected part of skin and its appendages; (4) Extra bacillary proliferation of a subcellular self-replicating agent carried by mycobacteria, specifically damaging the melanocytes; (5) Competitive influence of lepra bacilli possessing DOPA—oxide activity being lodged in the melanocytes.

The histopathologic findings under H and E stain led to the conclusion that the degree of clinical hypopigmentation had no correlation with the density of the intradermal infiltrate and hence the theory of mechanical interference appeared untenable.

However, an impressive correlation between the degree of clinical hypopigmentation and the depth of DOPA reaction could be established. Milder the hypopigmentation, greater the depth of dopa-staining. This observation fully corroborates the view of *Nayer and Job*[7] that the hypopigmentation in leprosy lesions is due to hypomelanosis consequent to partial inhibition of melanocytic activity.

Neurohistological changes in respect of leprosy lesions were of degenerative nature, affecting in variable degree the nerve bundles (representing a composite cutaneous nerve consisting of somatic and autonomic components) and the nerve twigs seen as finer fibres running along the course of cutaneous blood vessels and appendageal structures (representing in all probability the sympathetic fibres). It became apparent from the details of the findings that the lesions with pronounced hypopigmentation exhibiting about 25% Dopa-positivity showed degenerative changes in the nerve twigs in a predominant way (88.8% to 100%) while the lesions with mild hypopigmentation showed significantly less involvement of the nerve twigs (25% to 31.5%).

As exhibited histochemically the cholinesterase activity was observed to be relatively absent in the affected territory of the skin. This finding lends substantial support to the suggestion of gross sympathetic hypotonia in the leprosy patches even in the early phase of its evolution. Here is thus an example of a pathologic situation where sympathetic hypotonia is directly correlated with organic involvement. This is quite in contrast to many other dermatoses where sympathetic hypotonia represents only the functional status. It may be recalled that this lack of sympathetic tone is the chief contributor of so many localised sundry manifestations which often time largely aid an early diagnosis of leprosy. Comparative hypo-concentration of cholinesterase in the skin tissue as demonstrated by Gomori's technique might indicate sustained activity of acetylcholine and for that reason overactivity of the parasympathetic system which in turn means relative hypoactivity of sympathetic component. Also, other established parameters of study in leprosy by us and by all other workers unhesitatingly prove that all the

autonomic target organs in the skin supplied by sympathetic flow remain in a stage of paresis and their response to sympathetic stimulation is overwhelmingly inhibited.

In vitiligo, uniform DOPA-negativity in the affected territory of the skin is an established fact. The adjacent control areas in contrast, showed darkly obtained field, particularly at the level of basal layer.

As already exhibited, the shades of brown under Gomori's staining method for detection of cholinesterase activity appeared distinctly less in the vitiligenous areas. This indicates relatively more cholinergic activity. This obviously suggests a sort of peripheral autonomic imbalance possibly associated with operation of some consequential neurochemical agents at the cellular and subcellular levels in the production of depigmentation. The idea is a hypothetical one till now. Although considerable materials (embryological, anatomical, physiological, clinical and experimental) have been advanced in support of this neural hypothesis only a few scattered reports are available regarding the morphologic changes of the peripheral cutaneous nerves, specially the autonomic fibres, in the depigmentated areas. The findings of *Shao Chan-Gen*[8] and E.M. observations of *Breathnach et al*[9] appear highly interesting.

Our observation of comparatively lesser concentration of total cholinesterase in the vitiligenous skin tissue is perhaps the first report of its kind in this respect. This supports the contention of sympathetic hypotonia. The study, of course, needs to be extended to a larger number of cases. The histochemical studies of cholinesterase in its different components, true and pseudo in large series, supplemented by biochemical estimation of the same in the affected tissue has been much emphasised in the last international seminar of Biology of Normal and Abnormal Melanocytes *(Ed. Kawamura et al, University Park Press, Baltimore/Tokyo, 1971).*

This histochemical finding prima-facie supports the postulation of peripheral sympathetic hypotonia in vitiligo, which may or may not have a correlation in the organic involvement of the cutaneous nerves in contrast to that of leprosy.

The mechanism of hypopigmentation in naevus anaemicus appears even more intricating. Whatever may be the real mechanism, it is unassociated with abnormal melanisation. It is known that in the lesions of naevus anaemicus (i) the vascular pattern is normal; (ii) there is no paucity of blood vessels, and (iii) there is no organic defect of the dermal vessels.

In this condition, the presence of sympathetic hypotonia and permanent tonic contractility of the vessels or some sort of defect at the neuro effector junction or a combination of factors have been suggested. An absence of vasodilatation following intradermal infiltration of vasodilatory chemicals, inconsistent flare-reaction of Lewis and non-intensification of pallor following intradermal adrenaline injection have been reported by *Butterworth*[10]. Although these apparently implicate both local and nervous phenomena, the findings of *Fleischer and Zeligman*[11] clearly indicate the intactness of neurons and nervous pathways involved in the mediation of sensory and autonomic axon-reflex reactions; the reactions, however, contrastingly found either depressed or absent in leprosy lesions as a result of defective nervous pathway. In naevus anaemicus although axon-reflex flare reaction is absent, the sweat reaction and the nervous pathway remain intact. Hence, the question is centred around sympathetic hypertonia

and/or some defect of the neuro-effector junction in relation to blood vessels only. In the recent past, *Hitch*[12] *and Hurley*[13] while discussing this issue have emphasised the need of histochemical study in relation to cholinesterase activity and neurohistological study. Nevertheless, seldom has such work been done in these respects. In this sense the present study can reasonably claim priority.

The neurohistological findings obtained in the present study did not show any organic involvement of the cutaneous nerves while the histochemical studies showed dopa-reaction to be normal but cholinesterase activity markedly higher. The last one is an interesting observation hitherto unreported. Analysis of all these parameters indicate a phenomenon of sympathetic hypertonia possibly referable to vascular elements alone and is perhaps the prime factor for the altered physiological status and behaviour of the cutaneous blood vessels in naevus anaemicus. This of course does not exclude the possibility of a defect at the neuro-effector junction, both probably operating in concurrence.

As regards mechanism of hypopigmentation in Pityriasis versicolor several hypothesis have been forwarded, such as (1) prevention of tanning of the affected area by filtering out the UVR by the causative fungus itself, (2) release of some fungal toxins interfering with activity on melanocytes and (3) interference in the oxidation-reduction system of melanin granules. The perfectly normal Dopa-reaction observed in our study rules out the speculation of deleterious effect of hypothetical toxin liberated by the fungus.

The histopathologic and histochemical findings are normal and corroborative of those of *Lewis et al*[14]; *Szabo*[15]; *Gordon*,[16]; *Pinkus and Mehergan*[17] Neither Dopa-reactivity or any neural influence seems to be involved.

It may be concluded that the hypomelanosis, relative or absolute, clinically encountered and histochemically established in the lesion of leprosy and in vitiligo possibly occurs under the influence of neurochemical agents and consequent upon autonomic dysfunction. This perhaps result from organic affection of the autonomic components of the cutaneous nerve fibres, as manifested by simple degenerative changes of the fibres.

REFERENCES

1. Fitzpatrick TB Arndt KA Clark WH et al: Dermatology in General Medicine. McGraw Hill and Co., Blackiston Publication, New York. Ed. 1, 1971.
2. Lerner AB: Vitiligo, Progress in Dermatology, 6 : 1, 1972.
3. Sinclair DC: The nerves of the skin. In the physiology and pathophysiology of the skin. Ed.: Jarrett, A. Vol. II. Academic Press, London and New York, 1973. p. 348.
4. Whittaker VP: In structure and function of nervous tissue, Vol. 3, Biochemistry and disease. Ed.: Boured, G. Academic Press, New York, 1969.
5. Brazin M, Tennison V and Duffg PC: J Cell Biol 31 . 215, 1966.
6. Hillarp NA: Acta Physiol Scand Suppl, 157 : 38, 1959.
7. Nayer A and Job CK: A study of epidermal melanocytes in the hypopigmented patch of leprosy, Indian J Med Res 58 : 187, 1970.
8. Shao Chan, Gen: Changes of the neuroreceptor apparatus of the skin in vitiligo, J Invest Derm 33 : 20, 1959.
9. Breathnach AS; Bor S and Wyllic MA: Electron Microscopy of peripheral nerve terminals and marginal melanocytes in vitiligo, J Invest Derm 47 : 125, 1966.

10. Butterworth T and Walters JD : Observations on pharmacologic response of Vorner's Nevus anaemicus, Arch Derm Syph 66 : 332, 1952.

11. Fleisher TL and Zeligman I : Nevus anaemicus, Arch Derm 100 : 750, 1969.

12. Hitch : Discussion. In : Nevus anaemicus, Arch Derm 100 : 756, 1969.

13. Hurley HJ : Discussion. In : Nevus anaemicus, Arch Derm, 100 : 755, 1969.

14. Lewis GM ; Hipper ME ; Wilson JW ; et al : An introduction to Medical Mycology, Chicago, Year Book Publishers, Ed. 4, 1958.

15. Szabo G : Quantitative histological investigations on the melanocyte system of the human epidermis, In : Pigment cell biology. Gordon, Academic Press, New York, 1959, p. 99.

16. Gorden M : Pigment cell biology, Academic Press, New York, 1959, p. 117.

17. Pinkus H and Mehregan AH : A guide to Dermatopathology, Appleton-century crofts, Educational Divisional Meredith Corporation, 1969, p. 159.

TRUE or FALSE ?

Essential Fatty Acid (EFA) deficiency causes a scaly dermatosis which is reversible by rubbing linoleic acid on to the affected skin.

(Answer Page No. 113)