THE ROLE OF MELANOPHORE HORMONE OF THE PITUITARY IN THE COLOUR CHANGES OF COLD AND WARM BLOODED ANIMALS-A REVIEW

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INTRODUCTION

Modern researches have probed deep into the physiology and pharmacology of the pituitary gland. We now know that this tiny body produces an array of hormones and regulates through them not only other endocrines e.g. the adrenal, testes etc. but also controls both independently and through them a variety of other physiologic and metabolic processes operating inside the body.

From its anterior lobe, six separate hormones have so far been isolated and obtained in a highly purified form (Gaddaum & Loraine 1950, Li, 1953). Chemically they are found to be proteins or peptides and of late the amino acid make up of some of them has been established (Levy *et al*, 1955 ; Bell, 1954).

The posterior pituitary principles are two in number—the oxytocin and vassopression. Both these hormones have recently been synthesized—thanks to brilliant researches by du Vignaud and his co-workers (1953) in this field.

Compared to the anterior and posterior lobe principles, the hormone elaborated by the *pars intermedia* or middle lobe of the pituitary has received scant attention. This is due to the earlier belief that apart from blackening fish and frogs for some short duration, this hormone which was variously termed chromatophorotrophic hormone, melanophore hormone or intermedin had got no important assignment, in so far as human beings are concerned. Work undertaken during the last few years have belied the latter idea and active researches have since then been undertaken throughout the world to elucidate the nature, function and chemical constitution of this hormonal principle. In this article we shall primarily deal with investigations designed to elucidate its role in the colour physiology of cold[1] and warm blooded animals including human beings, keeping aside its chemistry and

1. We have confined our discussion to fish and frogs only.

inter - relationship with other hormones notably ACTH to be detailed in a separate communication.

COLOUR CHANGES IN COLD BLOODED ANIMALS

Some cold blooded animals are often found to possess some fine art of camouflaging and thus keeping themselves fit for both offensive and defensive purposes. A frog may be cited as an example. The animal kept in a dark corner changes its colour to black within a short time so that it merges its identity completely with its surroundings. Brought to diffused light, the same animal slowly whitens and after some time assumes a pallor with of course blackspots—now considerably faded—here and there on its skin. Chameleon which is termed "Bahurupi" in our country is so named because it can assume different colours (Bahu means many: Rupi means that having a colour).

MECHANISM OF COLOUR CHANGE IN COLD BLOODED AMIMALS

Scientific investigations solved the mystery of this change. It is now known that colour changes in those animals stem forth from certain alterations in some specialized cells, which interspread their skin and are termed Chromatophores. Various types of Chromatophores exist. They are termed Xanthophores, Erythrophores, Leucophores etc. after the colour of the pigments contained in them. The majority are melanophores, so named because they contain Melanin—a pigment which is either brown or black and in mammalian species constitute practically the whole pigment of the skin. Within each Chromatophore the pigments may either be concentrated approximately at the centre (contracted phase) or may be dispersed widely throughout the cell (dispersed phase) with various intermediate phases in between these two extremes. It is the culmination of these pigmentary alterations that the colour change of the animal is due.

FACTORS REGULATING COLOUR CHANGES IN COLD BLOODED ANIMALS

(a) *Nervous factor.* Investigations as to why such changes occur at all and which are the probable causitive factors were also carried out side by side. Such investigation received a fillip from Brucke's (1852) publication on the colour change of African chameleon. According to him, cutaneous nerves are responsible for keeping the pigment concentrated in the chromatophores, thus maintaining the paleness in the colour of the animal. When such nerves are transacted, the colour cells relax and the pigments spread throughout the interior of the cells, thus darkening the skin. Brucke's nervous interpretation of animal colour change was extended by Lister (1858) who did some useful work on the chromatics of common European frogs. Since animals made blind failed to respond to colour changes, he pointed out the importance of eyes as receptor organs and emphasized that these "are the only channels through which the rays of light gain access to the nervous system so as to induce the colour changes of the skin". Lister's finding was indepedently supported and extended to Pisces and Crustaceans by other investigators (Pouchet, 1875; Von Frisch, 1911). A nervous theory of animal colour changes thus took shape. It has still been kept up by Parker (1950) who has modified it to fit in with later findings. The theory has been a bit strengthened by modern researches of Harris and his group (Harris 1955) about the neural control of the pituitary gland. However in the face of increasing evidences for endocrine participation in the colour physiology, Parker's theory has got few adherents today.

(b) *Endocrine factors—the role of pituitary melanophore hormone.* Side by side evidences were forthcoming which seemed incompatible with the idea of purely nervous control. Thus it was observed that adrenaline, when injected into frogs induced profound colour alterations in them (Corona and Moroni, 1898). This change was duplicated with horned toad (Redfield, 1918). Meanwhile the technique of removal of the pituitary gland (hypophysectomy) was perfected (Smith, 1916) and Atwell (1919) showed that tadpoles so operated was rendered pale but could be restored temporarily to approximately normal dark condition by submitting them to the action of extract made from *pars intermedia.* Swingle (1921) noted that tadpoles became dark when part of the pituitary was implanted in them. The work of the above investigators led Hogben (1924) and his group to undertake a thorough study as to the implication of pituitary in the chromatic changes of lower vertebrates. Basing their results on rigorous experimentation, involving nerve transactions, and/or hypophysectomy of these animals and the implantation of different parts of of the pituitary into them, Hogben and his co-workers came to the conclusions which are indeed revolutionary. According to these group of workers colour changes in lower animals involved direct innervation only to a limited extent, if at all and depend primarily upon fluctuations in the amount of a secretion which originates from the *pars intermedia* and gets released into the blood of the animal concerned. The importance of eyes as a receptor was also stressed by them. In nature, Hogben opined, 'photic stimulation of the eye initiates this process by causing a reflex liberation of the pituitary hormone from the *pars intermedia.* The hormone so liberated, acts on the chromatophores of the skin thus causing a dispersion of the pigments in them and thereby bringing about a change in the colour of the animal'. Hogben's idea was supported by Waring (1942), and later extended by Waring and Landgrebe (1950) who also purified the pituitary principle and showed that administration of a very minute amount of the same causes wide dispersion of the pigments in the chromatophores of intact or hypophysectomized frogs with eventual darkening of their colour. The hormone was named melanophore hormone or Intermedin using the terminology of Zondek (Zondek and Krohn, 1932), who also played a notable part in the purification and assay of this hormone.

Modern tendency is to call this hormone by the name melanocyte stimulating hormone (Lerner, 1954).

TWO TYPES OF COLOUR CHANGES – PHYSIOLOGICAL AND MORPHOLOGICAL

Colour changes discussed so far are temporary. The animals remain dark for a short time and then slowly regain original colour with of course corresponding alterations in the dispersal of the pigments. Besides this another type of change also occurs in them. The latter change is slow and involves not migration but a quantitative change in the pigments of their chromatophores. Secerov (1909) noted this change and termed it 'morphological colour change' to distinguish it from the former which he termed physiological colour change. The term is not ideal but is sufficiently suggestive to warrant the difference between the two types of changes.

FACTORS INFLUENCING MORPHOLOGICAL COLOUR CHANGE
THE ROLE OF MELANOPHORE HORMONE

Environmental changes which elicit physiological colour changes in lower animals are found to evoke morphological colour change also. Thus it was shown that some fish placed in clear glass dishes remained pale and transparent while the same placed in dishes containing sand became dark (Franz, 1910). When fish like *Lebistes Paralichthys* are transferred to black background after a sojourn in white surroundings, an increase of melanophore in their skin was noted (Kuntz, 1915). Summer and Wells (1933) observed similar increase in the number of melanophores and in the amount of melanin pigments in the skin of fish kept under black background ; the same type of fish kept for some time in white background suffered a loss of melanin content with a corresponding degeneration of melanophores of the skin.

A significant relation thus seemed to exist between these two types of colour changes in lower animals. Both were influenced by a change of environments. Oidourne (1936) who studied pigmentary changes in several species of fishes pointed out the relation clearly. According to him 'conditions in the organism which bring about aggregation of pigments within the melanophore do, if maintained, inhibit or retard the developments of melanophores ; while conditions leading to dispersion of such pigments throughout the cells promote the development of melanophores or insure their continued existence". Morphological colour changes, (alteration in pigment) and physiological colour changes (alteration in pigmentary movement), he opined, "are phenomena resulting from a common cause and the neurohumor—he believed in nervous theory—which is instrumental in bringing about pigmentary migration in fundulus etc. probably also brings about the pigmentary change". The common factor responsible was more exclusively pointed out by Osborne (1941) who from his studies on the influence of hypophysectomy upon the melanogenesis of the cat fish (*Ameiurus melas*) concluded that 'the melanin dispersing hormone of the pituitary gland so important in normal colour physiology of the cat fish is also indispensable in the development and maintenance of melanin in melanophores'. Dawes (1941) confirmed him from perfusion experiments in frogs (*Rana temporaria*). A more direct evidence was adduced from this laboratory. Karkun and Mukerji (1953) showed that frogs (*Rana tigrina*) kept continuously dark by administration of a partially purified melanophore hormone from ox-pituitary developed an increase in the total melanin contents of their skin after some time. Further newer pigmentary patches are created on their snouts which persist for a long time after the cessation of injection of the hormone. The role of melanophore hormone with both morphological and physiological colour changes in lower cold blooded animals was thus established beyond doubt.

COLOUR CHANGE IN MAMMALS

In mammalian species, there is, as yet, no evidence that pigment cells exhibit a re-arrangement of their contents and thus influence the colour of the skin-coat. Morphological colour changes in them are also hardly striking. Early in life there is progressive deposition of pigments in their skin and this continues till adult colouration is attained. Depigmentation sometimes occur in old age probably as a result of ageing of the tissues. In the interim period the skin colour is hardly prone to fluctuation except under conditions which may best be termed pathological. It is unfortunate that changes of this type form the main basis of our knowledge regarding pigmentation of mammals. Nevertheless sufficient data are collected and collated together to bring forth an evaluation of the role of different factors influencing pigmentation in mammals.

FACTORS INFLUENCING MAMMALIAN COLOUR CHANGES

Changes in pigmentation in mammals may be initiated by a variety of stimuli. Seasonal changes are too well known to refer to here. In some cases such as the Weasel, the Varying hare, the changes are quite striking. Pigmentation in exposed parts of the body is observed in persons moving to higher altitude and is ascribed to the effect of excessive ultraviolet exposure. Persons receiving heavy metals in drug therapy are occasionally affected and diffused pigmentation is noticed on their skin. In this article, however, we shall confine our attention to pigmentation arising out of hormonal imbalances and try to correlate such pigmentary changes with fluctuation in the melanophore hormone of the pituitary.

COLOUR CHANGES FROM HORMONAL IMBALANCES

It is a common observation that in pregnant females patchy pigmentation appear in different parts of the body particularly in the nipples, the areola and over the linea alba etc. Davis *et al* (1945) found above changes in young women with primary amenorrhoea by oral administration of diethylstilboestrol for some time and duplicated the same changes in castrated male guinea-pigs by parenteral administration of estrogen in them.

The appearance of acne on the face of persons administered testosterone is an oft-repeated clinical observation. Edwards *et al* (1941) evaluated the role of testosterone in pigmentation by studying the effect of cutaneous changes in four male castrates and five eunuchs before, during and after treatment with the male sex hormone. According to him the contents of the melanin in the untreated castrate is slightly subnormal but is accompained by a large amount of melanoid (a probable degradation product of melanin). Treatment with hormone increases both the pigments, particularly the latter.

Progesterone in small quantities darkens frog skin but applied locally to the nipple do not produce hyper-pigmentation in guineapig (Lerner, *et al* 1954).

Thyroid status often determine the skin colour. It is claimed that vitiligo is accompanied with hyper-thyroidism though the reverse is not found true. Antithyroid drugs e.g. phenylthiourea, α-napthyl thiourea when administered to black rats produced depigmentation (Dieke, 1947; Richter *et al,* 1941). Hellerstein (1950) reported a case in which a negro patient treated with thiouracil developed depigmentation in certain areas of his skin.

Increased pigmentation accompanying atropy of the adrenal is a clinical manifestation of Addison's disease. Hyperpigmentation in animals following adrenalectomy has been observed (Ralli & Graef, 1945; Butcher, 1945). In human also total removal of adrenal cortical tissue by surgery produce a clinical picture of Addison's disease with deep hyperpigmentation (Kepler, 1949). Hall *et al* (1953) have shown that increased pigmentation in the skin of patients with Addison's disease is similar in pattern to that found in bilaterally adrenalectomized person and also obtained by prolonged administration of ACTH in human beings.

PARTICIPATION OF PITUITARY IN MAMMALIAN COLOUR CHANGES

THE ROLE OF MELANOPHORE HORMONE

Pigmentary changes are on record in pituitary disorders. Cushing (1932) stated that 'the complexion of an achromegalic, as is well known, noticeably darkens whereas the reverse is true in hypopituitary states in which the skin shows in addition to its other distinctive pecularities, a characteristic pallor'. In hypo-pituitarism, one occasionally finds localized areas of depigmentation, aggravated during menstruation and pregnancy and pituitary underfunction may be the cause of albinism, for signs of deficiency sometimes occur in albino patients (Wolf, 1936). Lerner *et al* (1954) quoted a case in which the chromophobe adenoma of the pituitary of a white male was removed and the pituitary region was subsequently exposed to X-ray irradiation more than once. The patient not only showed signs of hypopituitarism but also developed a sub-normal skin colour.

Experimental evidences about the participation of at least two hormones* in colour physiology are adduced. Thus Vilter (1935) found local hyperpigmentation of the ear of rabbits after implantation of dry extracts of melanophore hormone. Barbarossa (1950) succeeded in creating intense pigmentation in the irradiated nipples of guineapigs, by administering intermedin into them. Fournier *et al* (1938) reported that injections of pituitary extracts rich in melanophore hormone could bring about re-pigmentation in vitiligo. A similar therapy coupled often with X-ray irradiation was tried in a few leucoderma patients by the authors (Shukla, Karkun and Mukerji—unpublished observation) and in few cases with impressive results.

About ACTH another pituitary hormone, it has been consistently observed that persons suffering from rheumatic arthritis develop pigmentation in the body within about a week of administration of the hormone (Sprague *et al*, 1948). The role of ACTH in the development of pigmented nevi was reported (Goldman & Richfield, 1951).

The appearance of increased pigmentation in Addison's disease have already been mentioned (vide Supra). Various explanations were put forward from time to time to explain this phenomenon. An inability of the individual to utilize epinephrine precursors due to the loss of adrenal medullary function was considered to result in the conversion of those materials into excess melanin (Block, 1917). The theory is no longer accepted as valid. Modern tendency is to ascribe this change to excessive pituitary function, consequent to hypofunction of the adrenal. Credit must be given to Sulman (1952) and Johnsson & Hogberg (1952) for independently raising this issue in recent years and thus setting the ball roll in this line of research. Overwhelmed with striking similarity between the physical and chemical properties of melanophore hormone and ACTH, both contended that the two hormones are really identical and that ACTH is the pigmentogenic factor in persons afflicted with Addison's disease, Cushing's syndrome of primary origin, or in those exposed to prolonged stress or pregnancy. Sulman's idea of indentity of the two hormones have been refuted (Morris, 1952 ; Karkun *et al*, 1953,

*Recently Pickford & Kosto (1957) have shown that prolactin stimulates increased production of melanin in the melanophore of hypo-physectomized fundulus. They also noted that prolactin acts synergistically with intermedin. No work has however, been done to elucidate the role of this hormone in mammalinn pigmentation.

'55 & '56 : Deweid *et al*, 1953 and others). Working with cats which have got well defined *pars intermedia* the present authors (Karkun *et al*, 1954) showed that prolonged administration of ACTH brings about stimulation of the *pars intermedia* with changes which is indicative of excess liberation of intermedin. Pigmentation in above cases—we pointed out, is not due to ACTH but is brought about by melanophore hormone, which is released in increasing amounts consequent to overstimulation of the hormone producing cells of the *pars intermedia.* The theory has met with certain criticism (Lerner, *et al*, 1951) though not on well founded arguments; but remains to be critically examined. Certain observations are however on record which may be cited as supporting evidence. Thus (Karkun & Kar, 1954) have found increased phosphatase reaction in the *pars intermedia* cells, consequent to the stimulation of the latter by ACTH. Phosphatase changes are found to occur in protein synthesis (Roche, 1950). The authors thus interpreted these changes to be indicative of increased synthesis of melanophore hormone. Injection of high dose of ACTH evoke pigmentation only after a week while that with melanophore hormone within 7 hours of administration of the hormone (Lerner, 1954). Pigmentation is found in persons put to prolonged stress but not to acute one pointers relevant in this connection.

Whatever may be the mechanism, the view that in Addison's disease, Cushings, syndrome and in persons exposed to prolonged (but not acute) stress and in pregnancy, pigmentation is due to intermedin and not due to ACTH has also been independently supported by Johnsson *et al*, (1953) and later by Lerner (1954). The last investigator actually showed that in normal human being pigmentation could be induced by administration of highly purified melanophore hormone and thus proving beyond doubt that the same hormone do cause pigmentation not only in cold blooded animals but in mammalian species as well.

How far pigmentation created by other endocrine factors described above are of their own creation is not known. It is interesting to recall in this connection certain observations which point out that their action might be at least partly mediated through melanophore hormone. Thus Barbarossa & Pende (1950) noted that injection of estrogen to male and female guineapigs, produced strong pigmentation in the nipples particularly in the female species. A profound hyperplasia of the pituitary with signs of increased secretory activity at the level of the *pars intermedia* was an accompanying change. Frieden (1951) noted that in rats which are thyroidectomized or treated with thiouracil, a great reduction in the intermedin content of the pituitary occurs. Hypothyroidism brought about by administration of goitrogen results in diminished pigmentation of the skin. Surgical ablation of the adrenal as we have already referred to is accompanied with increased pigmentation of the skin. That the *pars intermedia* may be overactive in such animal also is shown by an increasing titre of intermedin as found independently by Johnsson and Hogberg, (1953) in the case of human being and by Shizume & Lerner (1954) with dogs. It thus, seems that in most cases of pigmentation brought about by hormonal agencies, a change either in the histology of *pars intermedia* or in the content of melanophore hormone is an invariable accompaniment. The correlation is suggestive though not conclusive to show that pigmentary changes evoked by the above hormones are mediated by melanophore hormone. The facts emphasized again the importance of this pituitary hormone in mammalian colour physiology.

MECHANISM OF ACTION OF MELANOPHORE HORMONE

What is the mechanism by which this hormone induces migration of skin pigments within the melanophores of the amphibia etc. and/or provokes an increase in the cellular melanin content of the skin of both types of animals (warm blooded or cold blooded)? Definite answers are not yet forthcoming. Based largely on the work of Spaeth (1913, 1916), Gilson (1926), Mathews (1931) and others notably Lerner (1956), we now know that melanin granules remain adherent to a net work of protein fibres within the cytoplasm of the pigment cells. When this protein network is in the gel state, the granules remain concentrated at the centre and the chromatophore is light in colour. By the impact of the melanophore hormone, the protein is reversibly changed into sol state and the pigment granules which still remain attached to the fibres now move out centrifugally with eventual darkening of the cell body. Oxygen must be present for the gel-to-sol conversion of the protein. ATP, oxidative phosphorylation and possibly cytochrome oxidase and sulfhydryl inhibition also may be required (Lerner & Takahashi, 1956). No information is, however, available as to how all these factors are integrated together to bring about this change.

Nothing is known again how dispersion and aggregation of pigments within the chromatophores of amphibia etc., result in increase and decrease respectively of melanin contents as pointed out by Odiourne (1936). Regarding increased synthesis of melanin granules by melanophore hormone, there are reports that the latter augments the activity of the phenolase complex (Kohn, 1953). Our preliminary observation (Karkun, Shukla & Mukerji—unpublished observation) with epidermal skin also supports the above contention. From *in vitro* experiments, however, Lerner failed to note any immediate increase in tyrosinase activity with melanophore hormone (Lerner & Takahashi, 1956). The picture regarding the mechanism by which the hormone provokes an increased synthesis of melanin is thus fluid and remains for prospective future investigators to delineate.

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