

## Melanin Pigments in Human Pineal Gland

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**Abstract.** Masson-Fontana staining confirmed the presence of melanin pigments in the human adult pineal gland. In 1-10 years age group, the pigments were present within the pinealocytes. In 11-20 years age group also, the pigments were in the pinealocytes only. In 21-30 year age group, the pigments were in the pinealocytes and appeared in the stroma in the areas of glial fibre predominance. In 31-40 years age group onwards, the pigments were present, in addition to pinealocytes, in the stroma among the fibres. As age advanced, the amount of extracellular pigments gradually increased, extracellular pigments were more concentrated, and the extracellular pigments were more clearly seen. There was no gender difference in the amount of melanin pigments. The background comparative anatomy also is discussed.

**Key words :** melanocyte, melanin, pinealocytes, pineal gland

### Introduction :

Melanocytes are melanin-pigment synthesizing pigment cells derived from neural crest and widely distributed in vertebrates. They are stellate cells with long processes with numerous dark brown or black granules of melanin in their cytoplasm. Their function is generally, to prevent light from reaching adjacent cells. In humans, they are present in the epidermis and its appendages, oral epithelium, some mucous membranes, uveal tract of eye ball, parts of middle and internal ear, and parts of leptomeninges in the base of brain. The cells of retinal pigment epithelium, neurons in locus ceruleus and substantia nigra also synthesize melanin. Melanins are high molecular weight polymers, attached to a structural protein, to form melanoproteins, and in the humans, there are two classes, the brown-black eumelanin and red-yellow pheomelanin, both derived from a substrate tyrosine (Dyson 1995).

Pineal gland (epiphysis cerebri) was once considered to be a phylogenetic relic, a vestige of a dorsal third eye, and of little functional significance; the mammalian pineal is now regarded and accepted as an endocrine gland of major regulatory importance, modifying the activity of the adenohypophysis, neurohypophysis, endocrine pancreas, parathyroids, adrenal cortex, adrenal medulla, and gonads. (de Vries and Kappers 1971; Klein 1978; Haulica and Coeulescu 1981; Reiter 1983, 1985, 1987; Malendowicz 1985; Dyson 1995).

The pineal in the big brown bat is pigmented

and intensified with constant darkness (Bhatnagar and Hilton 1994). Pigmented cells in the cat pineal gland show a preferential localization at the ventral surface of the pineal gland near its distal end and the pineal pigment is melanin (Calvo *et al.* 1992). Presence of pigment cells is a constant characteristic in the adult dog pineal gland; the pigment is melanin (Calvo *et al.* 1988). Embryo ovine pineal gland has pigment cells containing melanin (Regodon *et al.* 1998). Pineal glands of neonates consist of cords of dark, nucleated cells, which are frequently pigmented (Min *et al.* 1987). In the human adult, melanin pigments gradually accumulate within the parenchymal cells with increasing age in males, whereas in females, the maximum pigmentation is noticed in 30-40 year age group and then there was a fall (Tapp and Huxley 1972). The present study was done to find whether or not the human adult pineal gland showed gender difference and age changes in the amount of melanin pigments.

### Materials and Methods :

Forty pineal glands were collected from South Indian subjects (31 males and 9 females) who were accident deaths, within five to six hours after death during autopsy. There were no histological postmortem changes. Age of the subjects ranged from one to eighty years. Age groups of the subjects were 1-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80 years.

Pineal glands were removed from the brain

along with the superior colliculus so that the pineal recess of the third ventricle was also included. These were put in Bouin's fluid. After fixation, the specimens were processed for light microscopy. Eight-micron serial sections were cut and stained. Staining methods used were (i) haematoxylin and eosin, (ii) Masson-Fontana method for melanin, and (iii) Mallory's phosphotungstic acid haematoxylin method for neuroglial cells and nerve fibres. The arrangement of the parenchyma and stroma was observed. Melanin pigments were visually studied for location and quantity with regard to gender and age.

### Observations :

Pineal gland had a well defined capsule (piamater) and septa extended from the capsule into the parenchyma dividing it into complete and incomplete lobules. Parenchyma consisted of light and dark pinealocytes and glial cells. Corpora arenacea were a constant feature (*Koshy and Vettivel 2001*). Melanin pigments were present in the pinealocytes and in the stroma. In 1-10 years age group, the parenchyma was highly cellular, predominantly of pinealocytes. Within the pinealocytes, melanin pigments were present. There were no extra cellular pigments. In 11-20 years age group, the parenchyma was highly cellular with the presence of pinealocytes. Pigments were present as in 1-10 year group, within the pinealocytes. In 21-30 years age group, abundant dark melanin pigments were inside the pinealocytes and in the areas of glial fibre predominance. The pinealocytes were not abundant as in 11-20 age group but extracellular pigments were in the transition areas from pinealocytes-predominance to glial fibre-predominance (Fig. 1). In pineal glands of higher age groups, 31-40, 41-50, 51-60, 61-70 years, melanin pigments were seen as clear granules, more among the fibres. The pinealocytes also had melanin pigments within them. The extra cellular pigments were more clearly seen as age advanced. There was no gender difference in the amount of melanin pigments. A gradual increase in melanin, more concentrated extracellularly, occurred as age advanced.

### Discussion :

Pineal gland projects from the roof of diencephalon. A recess of third ventricle extends into its stalk. The pineal was formerly considered a vestigial organ with no function but now it is known to be an active endocrine gland. Its activity is influenced by the daily cycle of light and dark and it is a link between environment and physiology of an organism (individual). It responds to annual changes in day-length and influences gonadal activity in seasonal breeding species but has, though less apparent, significant effect on the reproductive system of other species that breed throughout the year. Its innervation is exclusively via sympathetic fibres that originate in the superior cervical ganglion and enter the cranial cavity accompanying blood vessels supplying the brain (*Fawcett 1994*).

Paired eyes of vertebrates are organs to focus a clear image upon a film of sensory cells known as retina, and these cells convey the impulses to the brain, giving their interpretation of intensity, colour, or movements. In some primitive vertebrates, there are also two different median organs, which serve as receptors for light, although not necessarily to obtain visual images. There are indications, from elasmobranch embryology, that the prevertebrates possess a metameric series of paired visual organs on the roof of the head; most of them rapidly disappear as the lateral eyes become perfect; but two pairs of dorsal eyes still hang on, almost to the cyclostome level. In lamprey, one member of each of these supposed pairs might be seen as a small bulb, attached by a stalk, to the roof of the diencephalon. The anterior one (parietal stalk) does not quite reach, but the posterior (pineal eye) does reach a semitransparent spot in the skin of the head. Possibly, both bodies were present in primitive amphibian, for in frogs, the pineal is found, nearly reaching the skin; yet in some modern reptiles (sphenodon and lizards) a parietal eye is present, with the pineal reduced. Among early vertebrates, the evidence of these organs is simply a foramen in the roof of the skull in ostracoderms, some placoderms, crossopterygians, primitive amphibians, and some of the early reptiles, including Therapsids. It usually lies between the

parietal bones. The parietal eye of the *Sphenodon* is well covered, and no function has been demonstrated, but it contains a retina and a lens; the neurosensory retinal cells synapse with neurons, which go directly down the stalk. In birds, the pineal stalk is reduced but often distinct and with a complicated structure distally. Mammals have a minute epiphysis (pineal organ), which has been suspected to have an endocrine function (Eaton 1960).

In most fish, and amphibians, the pineal organ is a single sac. In the more primitive fish, tail-less amphibians and lizards, there is a second component, the parapineal organ or parietal organ, which arises as an anterior evagination of the pineal organ or as a separate outgrowth of the roof of diencephalon. In frogs, parapineal component lies just beneath the epidermis on the dorsum of the head, where it can be seen. Numerous nerves and nerve endings are found in the pineal organs of lower vertebrates. Pineal organs of lower vertebrates reveal presence of photoreceptor cells that resemble those of the mammalian retina in having a lamellar portion of the apex and a receptor synapse at the base. The most elaborate pineal is found in the primitive lizard, *Sphenodon*. It contains a simple retina, consisting of photoreceptors backed by supporting cells that contain **pigment**, and its parietal component includes a lens-like structure. It thus constitutes a vestigial parietal eye. Probably, the parietal eye of the *Sphenodon* was functional because of the large size of the pineal foramen in the fossil reptiles (Fawcett 1994).

Pineal gland contains cords and follicles of pinealocytes and neuroglial cells among which ramify blood vessels and nerves. Pinealocytes form the pineal parenchyma; neuroglial cells, partially separating the pinealocytes, are like astrocytes. Ultrastructure of human fetal pinealocytes indicates their secretory function in early intrauterine life (Moller 1974). As in adults, they contain all the appropriate organelles together with abundant microfilaments, microtubules, and a few cilia with a 9 + 0 microtubular pattern. Cilia of this type are associated with secretory cells in other endocrine glands (Barnes 1961; Andersen et al. 1970). Extending from the cell body are one or more

processes (Knight et al 1973), which end in terminal buds near blood vessels or ependymal cells of the pineal recess. The terminal buds contain electron dense cored vesicles, which store monoamines and polypeptide hormones (Sheridan and Sladek 1975), release of which requires sympathetic innervation. The polypeptide hormones combine with specific protein carriers, termed neuro-epiphysins (Lukaszyk and Reiter 1975). They are released by exocytosis together with exocytotic debris. When released, the complex dissociates, hormones being exchanged for calcium ions. The calcium-carrier complex so formed is, in the pineal, deposited concentrically around exocytotic debris as corpora arenacea or brain sand. It is often supposed that the pineal gland atrophies with age, corpora arenacea being a sign of atrophy; on the contrary, these corpuscles may indicate continued secretion. There was no evidence of pineal degeneration in the elderly (Wildi and Frauchiger 1965).

The pinealocytes of mammals evolved from the photoreceptor cells of the pineal organ of primitive vertebrates. In the course of their evolution from light sensitive elements to endocrine cells, the region of the cell, specialised for photoreception was lost, together with the sensory nerves connecting it to other regions of the brain. The synaptic ribbons of mammalian pinealocytes may be vestiges of the special synapses that are characteristic of photoreceptors. Whether they have acquired an alternate function, related to secretion, is not known (Fawcett 1994). Pinealocytes of some mammals contain synaptic ribbons, perhaps involved in transmission; vesicles near them contain neurotransmitters such as —aminobutyric acid (Krstic 1976). Similar arrangements of organelles occur in mammalian retinal photoreceptors and simpler submammalian photoreceptors, suggesting that mammalian pinealocytes are derived from photoreceptors (Kappers 1976; Relkin 1976). Transient similarities exist between pinealocytes and retinal photoreceptors in neonatal rats (Zimmerman and Tsi 1975).

Melanin pigments are associated with light and are found in association with photoreceptors. This association, probably, exists with

photoreceptor cells-derived pinealocytes also. It is possible that the pigments, corresponding to those (rodopsin) in photoreceptors and those in the supporting cells in Sphenodon pineal gland, are in the pinealocytes and stroma in the human pineal gland. Increase of melanin pigments is due to the action of pineal indole-melatonin. Melatonin suppresses the melanocyte-stimulating hormone and prevents dispersion of melanin granules. Therefore, the melanin pigments become concentrated in the pineal gland.

The sphenodon pineal has pigments in the supporting cells of the photoreceptors. Mammalian retina has pigment cell layer. Melanin pigments have been shown to be present in animal and human fetal pinealocytes. Correspondingly, the present study, light microscopically, has shown melanin pigments in the human adults. Melanin pigments gradually accumulate within the parenchymal cells with increasing age in males, whereas in females, the maximum pigmentation is noticed in 30-40 year age group and then there is a fall (Tapp and Huxley 1972). The present study shows that there is no gender difference in the amount of melanin pigments, that a gradual increase in melanin pigments, more concentrated extra cellularly, occurs and that melanin pigments are more clearly seen as age advances.

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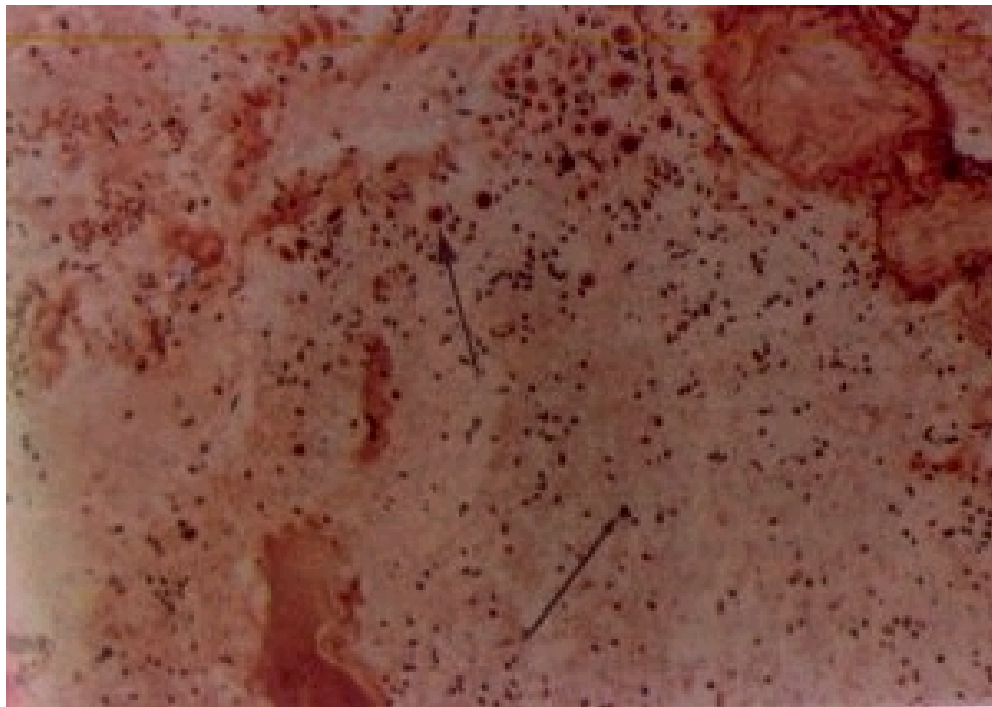
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**Fig. 1**

**Melanin pigments in pineal gland. (Mosson-Fontana, X 400)**

**Melanin pigments in pinealocyte (thick arrow)**

**Melanin pigments in stroma (thin arrow)**