CHAPTER IV

PIGMENT MIGRATION
INTRODUCTION

Vision is a special somatic efferent sense and for maximum efficiency of vision it is necessary for the eyes to be adapted to various stages of illumination. This adaptation is aided by the movement of screening pigments and the migration of screening pigment in the eyes is a characteristic feature. The pigment of the visual system is melanin, produced in the melanaphores, the best known of all pigment cells or chromatophores (Bagnara et al. 1978). The term melanin is a generic one that actually includes the black or brown eumelansins and the phaeomelansins that are yellow or red in colour (Prota, 1980). Melanin is the structural pigment of the skin, hair, many feathers, scales and eye.

The biological significance of melanin, is that they absorb light throughout the visible spectrum as well as in the U-V spectrum range. Melanin granules prevent reflection of transmitted light (Lythgoe, 1979). This pigment is the essential component in the retinal layer preventing reflection. The retinal pigment granules, melanin in the pigment epithelial cells exhibit distinct movements during light and dark adaptations and thereby help in the process of visual adaptations in various photic levels.

Several different methods of dark and light adaptations are known; some methods are characteristic of particular animal
groups, although a particular species normally possesses more than one method. The various adaptive methods can be divided into three classes: (1) optical regulation of the light reaching the visual pigment (melanin) through pupil; (2) absorption by the visual pigment; and (3) neural process (Lythgoe, 1979).

The melanin granules of the pigment epithelium and the rods and cones themselves frequently move in response to changes in light intensity in such a way that particular cell types are shielded from unwanted light. The changes are collectively known as retinomotor or photomechanical movements. Phylogenetically they appear to be older than the pupillary movements and usually one or the other mechanism is well developed in a particular class of animal (Lythgoe, 1979).

Retinomotor movements are best developed in birds, fishes and frogs, the phenomenon was first studied separately by Boll (1877) and Kuhne (1877) and has been reviewed by Blaxter (1970) and Ali (1971,1975). Though the underlying mechanisms are not known but both the rods and cones and the pigment granules within the epithelial cells of the pigment epithelium may move in response to changing light intensity. The myoids and ellipsoids of rods and cones contract and elongate in response to change in light intensity. This movement is almost coincident with the migration of pigment granules within the cells of the pigment epithelium (Lythgoe, 1979). In
dark adapted state the pigment granules within the epithelial cells surrounding the rods and cones move to the back of the retina and the cones move in the direction of the retinal pigment epithelium. At the same time, the rod myoids may contract resulting the movement of rods away from the pigment epithelium. During light adapted state the pigment granules are dispersed fairly evenly through the pigment epithelium and the rod myoids expand so that pigment granules surround the rod outer segments (Anctil et al., 1980; Klyne and Ali, 1980).

The movements of the rods, cones and retinal pigment granules are not exactly synchronized and in some cases either pigment migration or rod and cone migration proceeds in the absence of the other. The movements during dark adaptation are slow and require times of the order of an hour to complete (Walcott, 1975) while the light adaptation is much faster and proceeds rapidly. The most familiar components of light and dark adaptations are, changes in concentration of visual pigments and modifications in neuronal interaction (Munz, 1971).

The neural process is probably the most important and least understood mechanism involved in dark and light adaptations. During the complete state of full light adapted to the fully dark adapted retina, the neural organization of the retina changes. The state of dark adaptation involves rods, much summation and without any colour vision while the light
adaptation involves cones, little summation and colour vision. The initial stages of adaptations are extremely rapid completing in less than one fifth of a second. The later stages of adaptation proceed more slowly. Perhaps the whole neural organization from brightness to darkness takes place within about 30 mins. (Lythgoe, 1979) In spite of pronounced positional changes in the retinal pigment and visual cells in dark and light there is difference in the results in photochemical movement due to photic stimulation in teleosts (Ali, 1959, 1964; Detwiler, 1943; Arey, 1916).

In this connection, it is of interest to note that Kakcheyev (1943) formulated the hypothesis of nervous control in the process of dark adaptation. Ali (1964a) suggested that pigment migration may be influenced by hormones. Bagnara and Hadley (1969) believes that in all probability intermedin is the agent in melanophoric response in the skin of fishes. A number of suggestions as to the involvement of ions in the pigment migration have been proposed (Freeman et al. 1968). According to Veerdonk (1962) and Novales (1959) transmembrane ion induces pigment migration through intermedin.

Further, retinomotor responses can be influenced by colchicine (Anctil et al. 1979), cyclic AMP (Bitensky et al. 1973) and 5-hydroxytryptamine (Kato et al. 1982; Allen and Burnside, 1986).
Effect of photopic and scotopic states on neurosecretion and its role on pigment migration has been suggested in insects (Veron, 1973; Dey, 1980). Although various roles of neurosecretion in fish have been described but reports on the relationship between light and dark states and neurosecretion are lacking.

Keeping above in view, the effect of colchicine, cAMP and 5-HT in the pigment migration in the retina and effect of light and dark on neurosecretory cells have been investigated in *Cyprinus carpio* and *Clarias batrachus*.

However, the above experiments could not be performed in marine fish, pomfret, due to non-availability of live ones.
MATERIALS AND METHODS

Studies on Light and Dark Adaptations

To study the movement of retinal pigment granules in light- and dark-adapted states, two groups of experimental fishes were selected. One group has been kept in sunlight for about 3 hrs. for light adaptation. After that period, the fishes are decapitated and eyes fixed in 10% buffered formalin. Routine histological preparations are carried out for paraffin embedding methods and 7-8 μ thick sections are cut for microscopic preparations.

The other group of fishes has been dark-adapted for the same period as the light-adaptation experiment. The eyes are similarly fixed and after necessary preparations 7-8 μ microtome sections were cut. All preparations were carried out in dark and the eyes were kept in light-tight vials since in some enucleated dark-adapted eyes pigment migration may take place when exposed to light (Ali, 1964).

Effect of Light and Dark on Neurosecretion

To examine the influence of light and dark on the neurosecretory system of Cyprinus and Clarias, the fishes are adapted in light and dark for about 3 hrs.
After the required period of adaptations the specimens were decapitated and fixed in alcoholic Bouin's fixative for 24 hrs and a hole was done on the skull for the penetration of the fixative. After that the brain was taken out and again fixed in the fixative for another 24 hrs. Routine histological methods were followed and 10 μ thick sections were cut. The sections were stained in paraldehyde-fuchsin-one-step trichrome (Gabe, 1966).

**Effect of 5-HT (Sigma), cyclic AMP (Sigma) and Colchicine**

To study the effect of 5-HT, cAMP and colchicine on retinal pigment (melanin) on the eyes of *Cyprinus* and *Clarias*, each of the above drugs was administered intraocularly by microsyringe in dark adapted animals only.

Concentrations of 5-HT and cAMP being 0.8 mM each and the concentration of colchicine being 0.3 mM. Each of the substances were dissolved in cold-blooded ringer solution.
OBSERVATIONS

The position of the pigment granules during light- and dark-adaptations in carpio and catfish are shown in Figs. 50, 51, 52 and 53 respectively. However, all the parts of retina do not respond equally.

Effects of Light and Dark on Neurosecretory System

It has been observed in both the dark adapted fishes (carpio and catfish), a large accumulation of compact and purple neurosecretory substances in the neurosecretory pericarya. (Figs. 54 and 55).

Conversely, in the light adapted fishes, a significant reduction of the neurosecretory materials has been observed presumably due to axonal transport (Figs. 56 and 57).

Effect of 5-HT, cAMP and Colchicine

Light microscopic studies of paraffin sections of dark adapted eyes reveals that in all cases almost complete migration (dispersion) of retinal pigment granules similar to controlled light adapted eyes. It seems that pigment dispersion is more intense in the treated eyes (Figs. 58, 59, 60, 61, 62 and 63) and total masking of the visual cells could be seen.
EXPLANATION TO FIGURES

Fig. 50 Light adapted (control) retina of *Cyprinus carpio*, showing pigment dispersion and movement of cones away from the pigment epithelium x 500.

Fig. 51 Dark adapted (control) retina of *C. carpio*, showing pigment concentration and exposed rod outer segment x 500.

C, cone, r, rod, PC, pigment epithelium, P, pigment, ros, rod outer segment.
EXPLANATION TO FIGURES

Fig. 52  Light adapted (control) retina of *Clarias batrachus* x 500.

Fig. 53  Dark adapted (control) retina of *C. batrachus* x 500.

(Other details as in Figs. 50 and 51)
EXPLANATION TO FIGURES

Fig. 54  Sagittal section through the diencephalon of dark adapted *Cyprinus carpio*, showing large accumulation of neurosecretory materials in the neurosecretory cells x 1250.

Fig. 55  Sagittal section through the diencephalon of dark adapted *Clarias batrachus*, showing large accumulation of neurosecretory materials in the neurosecretory cells x 500.

NSC - Neurosecretory cell.
EXPLANATION TO FIGURES

Fig. 56  Sagittal section through the diencephalon of light adapted Cyprinus carpio, showing reduced neurosecretory contents in the cells x 500.

Fig. 57  Sagittal section through the diencephalon of light adapted Clarias batrachus, showing reduced neurosecretory contents in the cells x 500.
EXPLANATION TO FIGURES

Fig. 58  Effect of 5-HT on dark adapted retina of *C. carpio*, showing pigment dispersion similar to light adapted state x 500.

Fig. 59  Effect of 5-HT on dark adapted retina of *C. batrachus*, showing pigment dispersion similar to light adapted state x 500.

Fig. 60  Effect of cAMP on dark adapted retina of *C. carpio*, showing pigment dispersion similar to light adapted state x 500.

Fig. 61  Effect of cAMP on dark adapted retina of *C. batrachus*, showing pigment dispersion similar to light adapted state x 500.

Fig. 62  Effect of colchicine on dark adapted retina of *C. carpio*, showing pigment dispersion similar to light adapted state x 500.

Fig. 63  Effect of colchicine on dark adapted retina of *C. batrachus*, showing pigment dispersion similar to light adapted state x 500.
DISCUSSION

The basic physiology of the photoreceptor system has been adapted in many different ways to varied habits and habitats. The two basic adaptive features being (1) the changes in length and shape of photoreceptors (rods and cones) that are brought by changes in the lighting conditions of the environment (Ferrero et al., 1979); and (2) the dispersion or concentration of pigment granules (Ali, 1975b).

The arrhythmic eye, which performs well over a wide range of light intensities, has several adaptive mechanisms associated with activity during both day and night. These are primary devices for controlling the amount of illumination which reaches the photosensitive cells. The maximum available light should impinge on the receptor during the night, but during the day when illumination is adequate the significant problem is the resolution of pictures, that is, the retinal elements or small groups of the cells must be excited separately by different points from the picture during the day, but at night acuity is sacrificed for sensitivity and light is collected from many angles to excite the receptor cells. This adaptive mechanism depends on several photomechanical or retinomotor responses involving rapid changes in the pigment distribution and the action of contractile elements in the iris and/or the retina (Hoar, 1987).
Adomian and Sjostrand (1975) on the basis of their observation of elongation of microtubules in the cat fish retina, concluded that microtubules must be assembled and disassembled in connection with myoid elongation and shortening. Burnside (1976,1978) and Warren and Burnside (1978) demonstrated the role of microtubules and microfilaments in retinomotor responses. The authors found actin- and myosin-like filaments are responsible for cone contraction of some marine teleosts. Disappearance of the microtubules following colchicine induced block of cone elongation suggests that microtubules mediate cone elongation through a sliding mechanism (Warren and Burnside, 1978). While Anctil et al., 1979), have shown that rod contraction is mediated by microtubules but not elongation and suggested that there may exist inter- and intraspecific differences in retinomotor (rod versus cone) mechanism in lower vertebrates. However, Walls (1942) commented on the confusion surrounding mechanisms controlling photomechanical movements.

The retina respond to light and signals its presence and pattern to the brain through the optic nerve. The signals from individual rods, cones or retinal cells are not transmitted to the brain in isolation from its neighbours but the signals from neighbouring visual cells may interact with each other, they may be added together so that their sensitivity is the sum of their receptive areas or they may inhibit each other
(Pirenne, 1967). However, it is likely that the reception of a single photon by a vertebrate rod is potentially enough to trigger a response (Ashmore and Falk, 1976). There are topographical differences within the retina itself where some types of rod or cone may respond by moving while others do not even within a same species (Wall, 1942; Tansley, 1965). Neural summation seems to be less developed in cones (Wall, 1942) and some predator fishes have also adapted their cone for low light intensities (Munz and McFarland, 1977).

Inherent, in the transmission of information is that various salient features of the pattern are abstracted by the neural retina and transmitted inwards to the brain. Exactly how this is done is presently an active area of research (Lythgoe, 1979).

Among the vertebrates, retinal pigment migration is rapid and extensive in the teleosts, anurans and birds while it is slow and less marked or slight in turtles and crocodilians and is absent in snakes and mammals (Walls, 1942). During dark the melanin pigment granules contract and move away towards the back of the retina, thus exposing the rods and cones; in light the pigment granules disperse through the receptor layer and outer layer of the retina and surrounds the rod tip.
But, the mechanism by which the pigment granules alter their position in response to photic stimulation is not clearly understood and a number suggestions have been put forwarded. For example, Kinoshita (1963) has discussed the role of electro-chemical changes causing melanin migration in fish melanophores; ionic exchange between cell exterior and interior (Lerner and Takahashi, 1956) and the importance of intracellular Ca$^{2+}$ level in pigment migration has been suggested by Ishibashi (1957). Role of microtubules in pigment aggregation and dispersion in of the scale/fish has also been proposed by Wakswo and Novales, 1969).

As for neuroendocrinological control of pigment migration in fishes, it has been suggested that pigment migration is either controlled by nerves or hormones or both (Fujii, 1969). The two hormone hypothesis of Enami (1955), assuming two antagonistic principles, e.g. melanocyte stimulating hormone (MSH) which disperses melanin and melanocyte concentrating hormone (MCH) has not been accepted universally. Baker (1963, 1968a) suggested that MSH is solely responsible for both pigment concentration and dispersion in fish melanophore. In all probability intermedin is the actual hypophyseal agent involved in melanophoric response in fishes (Bagnara and Hadley, 1969), though Chavin (1956) emphasized the role of ACTH in melanophoric response of fishes.
Even, the light sensitivity of the diencephalon in blinded minnows was first reported by von Frisch (1911), indicating the role of nervous control of pigment migration. Teleosts possess both nervous as well as hormonal control over their skin melanophores, where the former plays the major role (Davson, 1970). But Osborn (1938) has shown that in cat fish both hormonal and nervous factors are responsible for pigment dispersion. According to Parker (1948) the melanophore activity is under the control of double innervation where concentrating is adrenergic and dispersion is cholinergic. Ali (1964a) in his experiment, restrained and anaesthetized goldfish and then exposed one eye to bright light. The rods and cones of the dark adapted eye did not move, but the retinal pigment melanin expanded partially. This led Ali to suggest that pigment migration may be influenced by hormones.

The nervous control in melanin dispersion and aggregation is mediated by the release of transmitter substance by nerves in the skin of fishes (Fujii, 1969).

On the basis of the above, it can be assumed that there could be some relationship between visual adaptation and neurosecretory mechanism.

As for the general significance of neurosecretion, Scharrer's concept (1952b), that neurosecretory cells represent a connecting link between the nervous system and endocrine glands,
is widely accepted. Their essential role is to transmit stimuli received from the nervous system to endocrine glands (Gabe, 1966). The neurosecretory cells respond to stimuli despite their glandular activity and it seems reasonable that neurosecretion plays an essential part in maintaining equilibrium between the organism and its surroundings. There is also a general relationship between hypothalamo-neurohypophyseal neurosecretion and general adaptation (Gabe, 1966). The secretion is elaborated also during alarm stimuli (Ames and van Dyke, 1952).

In fish, relationship between neurosecretory peptides and osmoregulation-salt balance and reproduction (Perks, 1969) has been established. Depletion or accumulation of neurosecretory products in the neurohypophysis during stress has been observed (Leatherland, 1967). Maetz (1963) pointed out the importance of neurosecretion to avoid stress in fish. However, reports on the relationship between vision and neurosecretion are lacking.

Keeping this in view an attempt has been made in the present investigation and a distinct effect of light or dark on the neurosecretory system has been observed. In the dark adapted fish there is a large accumulation of neurosecretory products whereas in the light adapted ones there is considerable reduction of the material in the hypothalamic region of the brain. During light adaptation, presumably axonal transport of neurosecretory materials take place. It may be due to the fact that during
dark the rate of discharge of the neurosecretory material is much slower while in light adapted forms synthesis of neurosecretory material is slower but discharge is faster. Thus, it can be suggested that the accumulation and discharge of the materials in response to photopic and scotopic stimulation is for visual adaptations.

Generally, there are two basic types of neurosecretory products. One class includes those neurotransmitters with a low molecular weight such as catecholamines (dopamine, adrenaline and nor-adrenaline) or biogenic amines like 5-hydroxytryptamine (5-HT) or serotonin. The other class is characterized by compounds of relatively high molecular weight such as proteins or peptides (neuropeptides).

It is well established that catecholamines and the biogenic amines like 5-HT are released instantaneously for rapid physiological phenomena or adaptations. It has also been shown that light adaptation occurs at a very faster rate in animals while dark adaptation is a slower process. Thus in any attempt to study the effect of neurosecretion in pigment migration in the vertebrate eye, the involvement of catecholamines or biogenic amines, like 5-HT seems to be important.

With this view, the effect of 5-HT on the migration of pigment granules of retina in fish eye has been tested. It has been found following the administration of 5-HT that it induces complete pigment dispersion like light adapted state in the dark
adapted eyes of both the fishes. In this connection it can be mentioned that the role of 5-HT in inducing light adapted condition in the dark adapted retina of green fish is effected by the release of dopamine by 5-HT. These observations strongly suggest that 5-HT induced dopamine, acts as extracellular messenger directly inducing light adapted cone retinomotor movement (Allen and Burnside, 1986). Kato et al. (1982) has also shown Ca\(^{2+}\) dependent 5-HT stimulates dopamine release in carp retina. However, 5-HT has been reported to have melanin aggregating action (Scheline, 1963; Scott, 1965).

In addition to this role of a physiological activator such as cyclic 3',5'-adenosine monophosphate (cAMP) has been tried. We have observed that cAMP triggers pigment migration as in light adapted state in dark-adapted eyes. It has been reported that cAMP activates the physiological process characteristic of a particular e Hector cell which also acts as a regulatory agent in all animal cells (Bonner, 1971; Robison et al., 1971) and as intracellular messanger (Robison et al., 1971). cAMP also triggers the specific response of the cell (Vander et al., 1980). Level of cyclic nucleotide such as cAMP has been shown to differ between light- and dark-adapted retina of ground squirrel (Citellus tridecemlineatus) by DeVries et al., (1982). According to Bagnara and Hadley (1969), the effect of intermedin is mimicked by cAMP by expanding melanophores in amphibians (Bagnara and Hadley, 1969).
Further, effect of an alkaloid, colchicine has been studied in the present investigation. Pigment dispersion in the dark adapted eye is induced similar to light light-treated state. Wilson and Novales (1969) found that colchicine disperse melanosomes of Fundulus scale. Colchicine inhibits cone myoid elongation and rod myoid contraction in trout retina (Anctil et al., 1979). The important cytoskeletal elements, microtubules are disrupted by colchicine (Margulis, 1973). The microtubules are important motile processes concerned with intracellular transport of nerve cells, the flow of pigment granules in chromatophores and the secretion of glands (Hoar, 1967).

It seems that the visual system and the neurosecretory system as well, is effected by dark and light condition. These together respond to photic stimulation in a co-ordinated manner, since vision is a special exteroceptive sense (Puchanan, 1957), postulated that neurosecretory materials are discharged under the influence of exteroceptive or visceral impulses and is transported through the hypophyseal portal system to the anterior hypophysis where it contributes to the synthesis and release of anterior lobe hormone. (Palay, 1953)

A photoreceptor is a light trap converting radiant energy into nerve impulses. The metabolic machinery such as mitochondria and associated organelles, in addition to, routine activities of the cell, assemble or generate chromoproteins and produce
transmitters which effect the synapse. The photoreceptive cells are metabolically very active, for chromoproteins are subject to destruction by light (Young, 1970) and transmitters must be steadily passed into synaptic vesicle (Hoar, 1987).