STUDIES ON CERTAIN PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS OF VISION IN SOME BIRDS

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This humble endeavour of mine is

dedicated to my parents.

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I, Bendang Ao, hereby declare that the subject matter of this thesis is the record of work done by me, and that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge, to anybody else, and that this thesis has not been submitted by me for any research degree in any other University or Institute.

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General Introduction

Birds have been defined as "wings guided by eyes" or as "eyes powered by wings". The eye, or more appropriately, vision, assumes great importance given the nature or mode of living of birds. We have to consider the fact that they usually have to focus on distant objects, and this necessarily requires visual acuity or sharpness, which in turn must be due to a certain type of arrangement of their visual apparatus.

Of all the senses, vision is unique in the sense that it provides an animal a detailed map of its surroundings in terms of millionths of a second. Vision or seeing begins with the absorption of perceptible light, which is a form of radiant energy, by pigments in the receptor cells of the retina. The ultimate source of energy for all life is solar radiation. When a photon strikes and interacts with particles of matter, it sends an electron into a higher energy level or excited state (Hoar, 1983).

"Light" is a narrow band within the broad electromagnetic spectrum, which extends from the cosmic and gamma rays with wavelengths of only a ten-billionth of a centimetre to the radio waves, which may be miles in length. The wavelengths of light extend from 380 to 760nm with extreme limits of 310 to 1050nm in very intense artificial sources. Photoreception in all animals is almost covered by the extreme human range (Hoar, 1983).

The visual process basically involves three steps: an optical stage, a transduction stage and a physiological stage. The first stage involves the projection of an image of an object of the outside world on the retina with the help of the cornea and the lens. The second stage involves the absorption of photons by

the photosensitive visual cells to generate electrical signals and the third stage involves the analysis of these primary signals. There may possibly be a fourth stage marking the conscious awareness of visual display. Vision is thus complex and integrated process of reflection, refraction, selective absorption and a psychomatic process to see the objects

A detailed anatomy of the eyes as well as its adaptation has been described by Walls (1942). The overall shape of the avian eye is always asymmetrical. The eyeball is flat, globose or tubular and is connected to the brain by the optic nerve. It has three usual layers viz., (i) an outer sclerotic coat with a transparent front – the conea (ii) an inner lining of vascular pigmented choroid, continuous with the ciliary body and the iris in front. In the centre of the iris is the pupil, through which light enters to be focussed by the lens onto the innermost layer and (iii) the retina, which contains the light- sensitive cells i.e., the rod and cone cells.

The cornea is the firm transparent front part of the outer sclerotic coat of the vertebrate eye, covering the iris and the pupil. It bulges slightly - its curved surface bending the light rays passing through it. The corneal curvature is absolutely regular so that the avian eye does not suffer from corneal astigmatism (Rochon- Duvigneaud, 1943). Its thickness is generally greater in larger birds, but there is no direct linear relationship with gross body size, with values ranging from 110μ to 936μ (Rochon- Duvigneaud, 1943). Its histological structure is fairly similar in all cases. The strongly curved cornea coupled with the rather flattened anterior surface of the lens results in a comparatively deep anterior

chamber to the eye. Within this, there is abundant aqueous humour, which together with the vitreous humour helps to keep the eyeball distented.

Chemically, the cornea is chiefly composed of proteins and carbohydrates. According to Maurice and Riley (1970), the nature of cellular and extra-cellular proteins is so different that each anatomical layer must be considered separately. (i) The corneal epithelium: the cells are shed constantly from the outer surface and replaced from the rapidly dividing basal layer (Maurice and Riley, 1970). Electron microscopic studies by Jakus (1964) revealed the presence of well-organised tonofibrils in the cytoplasm of the flattened epithelial cells. A high level of soluble proteins has been reported in the epithelium. (ii) The stroma: it is chiefly composed of proteins, a greater part of which is collagen organised into fibrils (Maurice and Riley, 1970). The arrangement of the collagen fibrils in regular parallel patterns has been proposed to depend on reactions with the soluble proteins like chondroitin sulfate (Mathews, 1965) (iii) The endothelial layer: it consists of a single of cells 2- 5μ thick and is not easily analysed (Maurice and Riley, 1970). It secretes a basement membrane called Descemet's membrane, which is several times thicker than itself and probably continues to grow throughout life (Salzman, 1912). It is extremely resistant to chemical and enzymatic actions because of the unusual association of proteins with high concentrations of carbohydrates (Maurice and Riley, 1970).

The cornea owes its transparency to the precise spacing and arrangement of the collagen fibrils that make up its substance (Lythgoe, 1979), but the exact biochemical mechanism of the transparency is not clear.

The lens on the other hand plays a vital role in the process of image formation and accommodation. The lens, according to Campbell (1967) is a transparent, crystalline, biconvex disc enclosed in a highly elastic non- cellular capsule of varying thickness, which is formed by the single layered epithelial cells below it. The substance of the lens consists of a series of ribbon- like fibres, which arise from the equatorial region and are actually greatly elongated epithelial cells. The fibrillar cells proceeds from the equator towards the lens centre. The transparent lens possesses a regularity of of cellular arrangement very similar in some ways to that of a cornea (Kuck, 1970).

Crystallins are the principle soluble proteins in the lens, and can be subdivided into four immunologically distinct families, commonly called as α, β, γ and δ crystallins. All families are found in all vertebrates except δ crystallins, which is a characteristic of birds and reptiles. The chief non- soluble lens protein is albuminoid (Krause, 1933). According to Waley (1965), it is a mixture and can be extracted to give glycoproteins and that growth and differentiation of the lens is controlled by influencing the synthesis of albuminoid by the glycoproteins. The short- range order of crystallin proteins accounts for eye lens transparency (Delaye and Tardieu, 1983).

The chief function of the lens in vision is accommodation. Kuck (1970) observed that "the sole function of the lens is to refract the image- bearing light beam in a controllable fashion, while, itself, remaining perfectly transparent". The focal length i.e., the distance between the lens and the object can be varied by changing the curvature of the lens, so as to focus the image at the retina.

In all birds the lens is very soft although it has a harder core, and consequently retains considerable powers of accommodation throughout life. Pumphrey (1916) discussed the actual process of accommodation on birds. Cursory observations reveal the extreme speed with which focal changes occur and the act of accommodation is also accompanied by a rapid, strong and sometimes transient pupillary contraction. This does not normally shorten the focus of the eye, but results in a considerable increase in its depth of focus. The muscles, which are involved in accommodation therefore, appear to be functionally synergic with the *sphincter iridis*, which contracts the pupil (Ronald Pearson, 1971).

The avian retina is constructed according to the general vertebrate plan. It is the innermost light- sensitive layer .Its relative thickness is varied and by no means proportional to the overall size (Ronald Pearson, 1971). The thicker condition is generally found in the passerines and falconiformes.

The retina contains the visual cells i.e., rods and cones, and in the aves, the cones are most numerous in diurnal species, and the rods in nocturnal species. The fine structure of these photosensitive cells has been studied in the pigeon by Cohen (1963a,b), and in chicken by Morris and Shorey (1967), Matsuka (1967a,b), and Pedler (1969). Cohen (1963a) concluded that the outer segment of both the rods and cones consists of flattened saccules enclosed within a membrane – the continuity of saccules and the cell membranes occuring along the entire length of the outer segments in the cones, but only at the base in case of the rods.

Moreover, the external segment of the rod cells is broader than those of cones in the chicken.

In the chicken and pigeon, in addition to the rods, there exists three types of cones i.e., single cones of two types viz., type I and II, and twin or double cones made up of a principal cone and a accessory cone. In pigeon, Morris and Shorey (1967) distinguished between two types of single cone cells in both of which the internal segment is shorter than those of principal cones. At the level of the outer limiting membrane, each is separated from its neighbour by intervening Muller cells and, as in the accessory cones, the nuclear region is connected to the synaptic body by a narrow fibre. The synaptic bodies of two independent cones are sometimes closely associated with the outer plexiform layer, and only separated by a thin layer of Muller cell cytoplasm.

Cones of type I are distinguished from those of type II by the presence of electron – dense oil droplets in the apical region of the inner segment, and by the greater number of mitochondrial cristae. The nucleus is also more ventral in type I cones.

As mentioned earlier, the principal cone is closely related to the accessory cone so that the two types together form a double cone structure. These two are not separated from each other at the level of the outer limiting membrane, as in the case of other sensory cells. The nucleus of the accessory cone is more scleral in position than that of the principal cone, and it is connected to the synaptic body by a narrow fibre. It also has a paraboloid in the enlarged vitreol region similar to

that of the rods. A further distinguishing feature is that the synaptic body of the accessory cone is smaller, and is partly surrounded by that of the principal cone.

The external segment of the rod cells in the chick is broader than those of cones (Morris and Shorey, 1967). The mitochondria comprising an ellipsoid are closely packed, elongated, and have densely packed cristae. A paraboloid is situated vitreal to the mitochondria in the inner segment and a long cytoplasmic cylinder joins the inner segment and the nuclear region. The paraboloid granules of both the rods and accessory cones have been suggested to be glycogen bodies by Morris and Shorey (1967).

In a typical avian retina there are usually one or more regions of the retina where the concentrations of cones exceed that found elsewhere, and are termed as "areae". Within the areae, there are usually steep- sided depressions called "foveae", at the bottom of which the cones attain their closest packing (1,000,000 per mm^2 in the larger members of the falconiformes). In many species of birds like Hirundinidae, Sternidae etc., there is a second smaller fovea at the posterior or temporal border of the retina, separated from the central fovea by some 6mm. An area is, therefore, a place of maximum optical resolution. The function of the central fovea is less obvious but Pumphrey (1961), suggested that refraction from its sloping side enables the eye to be locked to a given object and increases the eye's sensitivity to movements of that object.

There are three principle types of area- fovea arrangements according to Duijm (1958) viz., (i) a single area, which may or may not be foveate, lying close to the optic axis, called 'area centralis' (found in many graminivorous species) (ii)

the area is extended into a horizontal band within which the fovea can assume the form of a trough (in many water birds) (iii) there can be two areas, both of which are foveate (falconiformes, hirudinidae, alcidinidae and trochiliformes). It is possible that they are used in binocular stereoscopic vision (the central fovea is close to the optic axis, and the lateral fovea is so placed that the image of an object ahead can, with a slight degree of convergence be formed on the temporal fovea of both eyes simultaneously during free flight and in hunting of prey (Ronald Pearson, 1971).

In higher vertebrates, the image on the retina is formed by altering the curvature of the lens, while in fishes it is done by altering the distance between the lens and the retina. In addition to accommodation, several other curious devices have been summarised in fishes by Munz (1971). These mechanisms lead to similar results, but do not require any active mechanism (Walls, 1942).

The retina thus, above all, preserves optical resolution – any failure would compromise the acuity with which the animal can see (Richard, 1986).

One of the most common features of light (photopic) and dark (scotopic) adaptations is the change of the concentrations and movement of visual pigments in addition to modifications in neuronal interactions (Munz, 1971). Retinal pigment migration is rapid in fishes, anurans and birds, but slow and slight in turtle and crocodiles. It is absent in snakes and mammals. Alternative and better mechanisms for the control of retinal illumination have progressively developed during the evolution of vertebrates e.g. – pupillary response in fishes by way of variable pupil diameters, where the iris responds directly to light intensities, while

in higher forms, the response is mediated via nervous reflex arcs (Mc Cauley, 1971).

The phenomenon of pigment migration has been studied in many invertebrates and all groups of sub- mammalian vertebrates. Highnam and Hill (1977) reported that hormones regulate pigment migration in crustaceans, while Goldschmidt and Bernard (1974) postulated that in insects it might be dependent on nerves. The avian eye resembles that of lower vertebrates in the pigment movements that result from changes in the amount of incident light. A decrease in light intensity results in retraction of the pigment within the epithelium so that it lies at the extreme tip of the rod and cones (Ronald Pearson, 1971).

The epithelial cells contain particles or crystals of reflective substances, which, in fishes, have been found to be guanine (Walls, 1942). In addition, melanin is present in the same cells and migrates normally, occluding the tapetum.

Vision is a complex phenomenon of photochemical events ultimately leading to image formation with the help of visual pigments, which are carotenoid in nature. These pigments of animals are either dissolved in the tissue fats or are combined chemically with specific proteins. Visual pigments have now been investigated in representatives of each major phylum with highly specialised eyes. In all cases, the active pigment is an aldehyde of vitamin A called "retinal" combined with a protein called "opsin". Vitamin is an alcohol, and often referred to as "retinol". Retinal is also called "retinene", but less frequently.

There are two types of retinals i.e., $retinal_1$ or retenene1 (R_1), which results oxidatively from vitamin A_1 and $retinal_2$ or $retenene_2$ (R_2) resulting oxidatively

from vitamin A_2. Vitamin A_2 differs from A_1 in having an extra double bond between atoms 3 and 4, and this bestows on it a longer wave absorption maxima (Clayton, 1971). $Retinal_1$ can be oxidatively converted to $retinal_2$ (Hoar, 1987). By combining R_1 or R_2 with various opsins (proteins) found in rods and cones, it is possible to generate a variety of visual pigments differing in the wavelengths of maximum absorption. For example, the ones most commonly found in nature are, rhodopsin (R_1 + rod opsin), porphyropsin (R_2 + rod opsin), iodopsin (R_1 + cone opsin), cyanopsin (R_2 + cone opsin).

The photosensitive pigments on bleaching by a sufficient amount of light energy, splits into their constituent parts, and the retinals change into a different isomeric form (cis to trans) and are reduced to vitamin A, which diffuses from the receptor cells into the pigment epithelium. The vitamin A is converted enzymatically to the (11-) cis isomer, returned to the rod outer segment, and oxidised back to retinal, which combines with opsin to form the original form again. Similar photochemical processes take place in the cornea.

Only a small group of animals have been found to possess colour vision. Among the vertebrates, the faculty of hue discrimination has been found in primates, birds, lizards, frogs, turtles and teleost fishes. It is associated with bright light vision, foveae with rich area of cones, and eyes with good mechanism for accommodation (Hoar, 1983). Mc Cauley (1971) has postulated that three different types of cones are required for colour discrimination.

Much work has been done on avian eyes (Lashely, 1916; Rochon-Duvigneaud, 1943; Tansley, 1965; Pumphrey, 1961) regarding their structure.

Bowmaker (1979), Reuss and Olcese (1986), Chen and Goldsmith (1984), Chen *et al.* (1984), Goldsmith (1986) etc have worked on some important physiological and biochemical aspects such as visual pigments, oil droplets in the retina, activation by light, UV receptors in the retina, spectral classes of cones. But, in spite of this, much remains to be done. For example, little attention has been given to the physiology and biochemistry of vision, apart from works by Bowmaker and Martin (1985), Thomas and Rawal (1986), Raghuvarman (1980), Deb and Raghuvarman (1994), Dey, *et al.* (1994) etc. Moreover, studies with particular reference to different ecological niches (aerial, terrestrial and aquatic) are yet to be done comprehensively. The other components of the eye such as the acid mucopolysaccharides – a basic constituent of the cornea and the lens, ascorbic acid, fluorescent compounds etc. have not been studied thoroughly or exhaustively in birds in general and Indian birds in particular. Keeping this in view, a study on certain physiological and biochemical aspects of vision of some birds from different ecological niches i.e., the terrestrial domestic chicken, *Gallus domesticus* and the aerial Indian blue rock pigeon, *Columba livia intermedia* (Strickland) has been undertaken.

The purpose of this research is to obtain more information by physiological and biochemical methods, on the hitherto unknown aspects of vision in the two birds from different ecological niches or habitats i.e., the domestic chicken, *Gallus domesticus,* and the Indian Blue rock pigeon, *Columba livia intermedia* (Strickland), and thus, lead to a better understanding of the phenomenon of avian vision.