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

BENDANG AO

THESIS SUBMITTED IN FULFILMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY IN ZOOLOGY

OF

NORTH - EASTERN HILL UNIVERSITY
1999

This humble endeavour of mine is

dedicated to my parents.

THE NORTH – EASTERN HILL UNIVERSITY

May, 1999

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.

This is being submitted to the North – Eastern Hill University for the degree of Doctor of Philosophy in Zoology.

(Head of Department) 2/6/99
Head Deptt. of Zoology,
School of Life Sciences,
NEHU., Shillong- 22.

(Joint Supervisor) 2/6/99
Deptt. of Zoology,
School of Life Sciences
Shillong- 22.

(Supervisor) 2/6/99
Dr. A. Raghu Varman
Department of Zoology
North- Eastern Hill University
Shillong.

(Candidate) Bendang 2/6/99

ACKNOWLEDGEMENT

First of all, I would like to convey my gratitude to my chief supervisor Prof. A. Raghuvarman, Dean, School of Life Sciences. I am indebted to him for his exemplary guidance, wise counsel, and also for inculcating in me a perpetual thirst for knowledge.

I also thank my co- supervisor Dr. B. Kharbuli, Dean of Students' Welfare, for being always there when needed, and also for making my life as a Research Scholar hassle free.

I would like to put on record my thankfulness to the previous Head of Department Prof. (Mrs.) V. Tandon, and the present Head Prof. B.K. Sharma, for the use of laboratory and instrumentation facilities.

I am very grateful to my lab. mates, Miss. Sentimenla, and especially Mr. S. R. Hajong, who has been deeply involved in my work right from the beginning. He has also been a good sounding–board, off whom I could bounce off new, crazy ideas at all odd times.

I would be remiss in my duty if I did not acknowledge the help and good wishes of my colleagues, Dr. S. Lyngdoh, Mr. B. Mukhim, Mr. Upal Sengupta, Mr. Imliwati, Dr. Babu John, Mr. Akumenba Jamir, Mr. T. Temjen, and a host of others too numerous to mention here.

The kindness of Dr. Sudip Dey, of the Electron Microscope Laboratory, RSIC, NEHU, Dr. Delamere, of the Department of Ophthalmology, University of Louisville, Kentucky, USA, and Dr. Wyss, of F. Hoffmann – La Roche AG, Switzerland, in generously presenting me the reprints of their works is gratefully acknowledged. They were of immense help to me.

The excellent photographic work of Mr. Bijoy Das is also much appreciated, as were his critical comments on some of my photographic 'excursions'.

I also thank Prof. Pardeshi Lal, Head of the Department of Zoology, Nagaland University, for his good wishes and helpful suggestions.

Lastly, I thank my family members, who have been very supportive of me and my work.

Shillong.

Dated. 2/06/99

Bendang

(BENDANG AO)

CONTENTS

Lists of Figures: Photoplates, Tables and Charts:

activity.

Plate 14: Similar preparation in *Columba livia intermedia.*

Plate 15: Whole mount preparation of corneal endothelium of *Gallus domesticus* without Na^+ and K^+ in the incubating medium showing inhibition of enzyme activity.

Plate 16: Similar preparation *in Columba livia intermedia.*

Plate 17: Whole mount preparation of corneal epithelium of *Gallus domesticus* without Ouabain in the incubating medium showing enzyme activity.

Plate 18: Similar preparation in *Columba livia intermedia.*

Plate 19: Whole mount preparation of corneal epithelium of *Gallus domesticus* with Ouabain in the incubating medium showing inhibition of enzyme activity.

Plate 20: Similar preparation *in Columba livia intermedia.*

Plate 21: Whole mount preparation of corneal epithelium of *Gallus domesticus* without Na^+ and K^+ in the incubating medium showing inhibition of enzyme activity.

Plate 22: Similar preparation in *Columba livia intermedia.*

Plate 23: T.S. of lens of *Gallus domesticus* showing ascorbic acid as dark granules.

Plate 24: T.S. of cornea *of Gallus domesticus* showing ascorbic acid as dark granules.

Plate 25: T.S. of lens of *Columba livia intermedia* showing ascorbic acid as dark granules.

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 retenenel (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.

Acid mucopolysaccharides

INTRODUCTION:

Glycosaminoglycans are a large family of heterogenous polysaccarides widely distributed in animal tissues (Hascald, 1981). They combine with proteins to form proteoglycans. Recent work has indicated that, in addition to the physiochemical properties of the proteoglycans, glycosaminoglycans may have specific biological functions conferred upon them by specific sequences within the carbohydrate chain (Carney, 1994).

The term "mucopolysaccharide" was coined by Meyer (1938) to include all those substances with similar physico-chemical properties, isolated from connective tissues. Later on the terms "glycosaminoglycans", "glycoproteins" and "mucoproteins" were in vogue, but they failed to distinguish between bacterial polysaccharides and antibiotics containing amino sugars.

Mucopolysaccharides come under two main classes – those containing uronic acid i.e., the acid mucopolysaccharides (AMPs) and those that are neutral. The AMPs may be further sulphated (e.g. Chondroitin sulphates) or non-sulfated (eg. hyaluronic acid). According to Jagues (1977), the terms AMPs and SMPs (sulphated mucopolysaccharides) appear to provide an adequate description and have the further advantage of continuous use.

According to Kennedy and White (1983) the term "mucopolysaccharide" was introduced to describe 2-amino-2-deoxyhexose- containing polysccharide materials of animal origin occurring either as free polysaccharides or as their protein derivatives. But since glycosaminoglycans always come within the mucopolysaccharide category irrespective of the way in which the term has been

used, they were described widely as "acid mucopolysaccharides" on account of their highly cationic nature. Now, it is known that the glycosaminoglycans are attached covalently to proteins. Thus, AMPs in actual terms mean the glycosaminoglycans of a proteoglycan plus (sometimes) a few amino acid units.

Chemically, the cornea, which is the firm transparent part of the outer sclera of the eye, is chiefly composed of proteins and carbohydrates. The cornea has three layers – a multi-layered epithelium composed of squamous cells of different shapes and sizes, a stroma of modified connective tissue fibres lying in a mucoprotein ground substance (Willmer, 1966), and an endothelium. According to Maurice and Riley (1970), the nature of cellular and extra-cellular proteins of the cornea are so different that each of the anatomical layers must be considered separately. Electron microscopic studies by Jakus (1964) have revealed the presence of well – organised tonofibrils in the cytoplasm of the flattened epithelial cells.

The particular anatomical position of the cornea necessitates, among other things, the reactions occurring in it to operate at temperatures below that of the blood. It must also share the flexibility with the outer layers of the skin and perhaps, the epithelial lining of the lungs and air passages. Maurice and Riley (1970) reported that 94% of the stroma is composed of proteins – the greater part of which is collagen organised into fibrils. The size and arrangement of the fibrils in regular parallel patterns is essential for the transparency of the cornea (Lythgoe, 1979). This arrangement of the fibrils has been proposed to depend on

reactions with the soluble protein e.g. Chondroitin sulphate proteins (Mathews, 1965).

The chemical composition of the various layers and the biochemical reactions that take place in them do not appear to differ in any major respect from those in other tissues of the body. However, the mucopolysaccharide components of the connective tissues present a distinctive pattern, with a resident transport mechanism in the endothelium, whose mode of action is still not clear, but which may prove to be unusual.It is further suggested that the acid polysaccharides are distributed along a fibrous protein, attached only by a short length of the polysaccharide chain, whereby the remainder of the chain remains free, which can combine with the cationic sites of the collagen fibrils by virtue its high negative charge. All of the glycosaminoglycans carry a high negative charge at neutral pH.

It has not yet been possible to make a comparable analysis of the glycosaminoglycan chains attached at the specific loci on the protein cores of proteoglycans. However, it is clear that variations can exist within a population of glycosaminoglycan chains in the number of repeating units, the extent of sulfation, the distribution of sulphated residues along the polysaccharide chains and the number and distribution of iduronate residues (Kennedy, 1979; Roden, 1980)

The lens is composed of a series of ribbon-like fibres, which arise from the equatorial region and are actually greatly elongated epithelial cells. The fibrillar cells proceed from the equator towards the lens centre in a regular arrangement very similar in some ways, to that of a cornea (Kuck, 1970). The lens has soluble

and non-soluble proteins. According to Piatigorsky (1984) all four families of soluble proteins called α,β,γ and δ crystallins are found in all vertebrates, except δ-crystallins, which are characteristic of birds and reptiles only. Each class is composed of multiple polypeptides with its own primary structure (Basaglia, 1989). The chief non-soluble lens protein is 'albuminoid' (Krause, 1933), which according to Waley (1965) is a mixture, and from which glycoproteins can be extracted. The growth and differentiation of the lens is controlled by influencing the synthesis of albuminoid by the glycoproteins. According to Delaye (1983) the short-range order of crystallin proteins accounts for eye lens transparency.

MATERIALS AND METHODS:

The eyes, enucleated from live animals, were fixed in 10% buffered formalin or Bouin's fluid until required.

For histochemical studies routine paraffin embedding technique was used, followed by cutting of 7 - 8μ thick sections by microtome. The sections were stained with Toluidine blue (Humason, 1971) for the detection of mucopolysaccharides.

This was based on the metachromatic reaction given by a cationic dye e.g. Toluidine blue, which according to Curran (1964), is able to survive alcoholic degradation. But, this demonstration of mucopolysaccharides in cornea is extremely dependent on the state of hydration, as was found in the present study, by treating some sections with ethanol. This confirms the propositions of Cejkova and Brettschneider, (1969) and Cejkova and Balkova, (1974).

For biochemical examinations, the method as described by Dietrich *et al* (1977) was followed.

EXTRACTION:

Previously defatted (by acetone) and dried tissues (100 μg – 1 gm) were suspended in 20 ml of 0.05 M Tris-HCl buffer, of pH 8. To this mixture, 10 mg of trypsin was added, then a few drops of toluene were added to layer the surface, and incubated at 37°C for 24 hours. After incubation, conc. NaOH was added till the pH of the mixture was brought to 11 and maintained for 6 hours at room

temperature. Then the pH was brought to 6 by the addition of HCl and then the mixture was centrifuged.

To the resulting supernatant, 0.1 ml of 2M NaCl and 2 volumes of ethanol were added and kept overnight at 5°C. The mixture was centrifuged and the precipitate was collected and dried and again resuspended in 1ml 0.05M sodium acetate, pH 6.5 along with 1 mg each of DNAse and RNAse. The solution was incubated for 24 hours at 37°C with a layer of toluene.

After incubation, 0.1 ml of 2M NaCl and 2 volumes of ethanol were added to the solution and kept overnight at 5°C. It was then centrifuged and the precipitate so collected was dried and dissolved in 0.5 ml of water, heated at 100°C for 2 minutes.

CHROMATOGRAPHY:

The extracted AMP (acid mucopolysaccharide) was hydrolysed with 6N HCl 100°C for 10 – 12 hours and then evaporated to dryness. The dried residue was then dissolved in 0.5 ml of water and spotted on Whatman No. 1 paper and ascending paper chromatograms run using butanol, acetic acid and water in the ratio of 4:1:1 (v/v) as solvent (Giri and Nigam, 1954).

The chromatogram was developed with silver nitrate (0.1ml of saturated solution in 20ml of acetone) and sodium hydroxide (0.5gm in 25ml of rectified spirit) as suggested by Trevelyan *et al (1950).* The chromatogram was then washed in 6N ammonium hydroxide for 10 minutes and then washed in running water and dried at room temperature.

Plate - I

Fig 1, 2, 3, 4

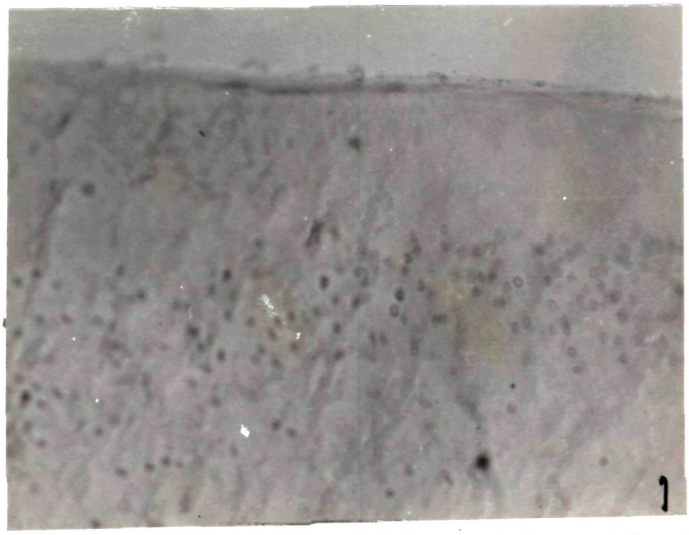
Plate 1. T.S. of lens of Gallus domesticus. 500x

Plate 2. T.S. of cornea of Gallus domesticus. 500x

Plate 3. T.S. of lens of Columba livia intermedia. 500x

Plate 4. T.S. of cornea of Columba livia intermedia. 500x

In what are to these sections?


1


2


3

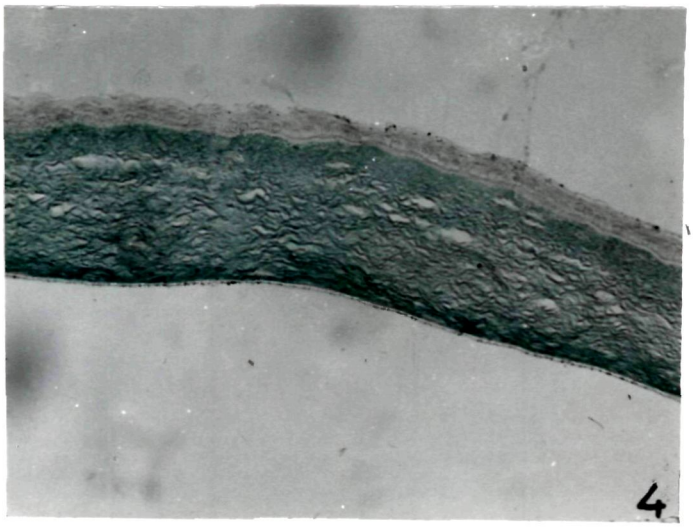
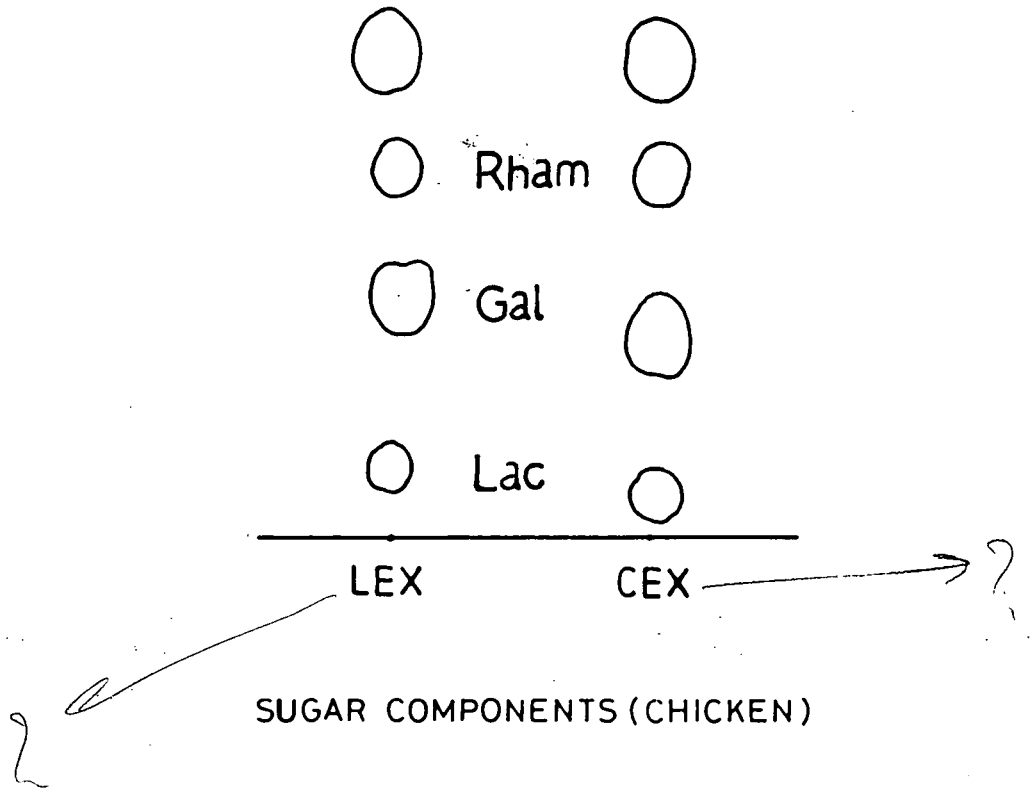

4

Fig. 1. Ascending paper chromatogram of the sugar components of the lens and corneal mucopolysaccharides of the chicken, *Gallus domesticus.*

Rham = Rhamnose
Gal = Galactose
Lac = Lactose



SUGAR COMPONENTS (CHICKEN)

Standards also should be on the same paper.

Table 1. Results of histochemical tests carried out in the eyes of *Gallus domesticus* and *Columba livia intermedia.*

Stains and tests	Reference	Cornea			Lens	Retina	
		Ept.	Stroma	Endo.		V.C.L	Neural & *glial cell layer*
Mallory's triple connective stain	Pantin, 1946	Blue	Blue	Blue	Orange	Liliac	Light blue to violet
Masson's trichro me stain	Gurr, 1956	Green	Green	Green	Green	Violet	Violet
Delafield's Haematoxylin	Carltan. 1947	Violet	Violet	Violet	Pink	Pink	Light to deep violet
Biuret test	Serra, 1946	++	++	++	++	+	+
Millon's test	Baker, 1956	+	++	+	++	++	++
Xanthoprotein test	Peors, 1961	+	++	+	++	++	++
Nile blue	Llile, 1956	++	+	++	++	+	+
Sudan III	Baker, 1956	++	+	++	++	+	+

+ Moderately positive ++ Intensely positive

Table 2. Results of staining reactions for the detection of acid muco-polysaccharides carried out in the eyes of *Gallus domesticus* and *Columba livia intermedia.*

Stains & tests	Reference	Cornea	Lens		Retina	
			Cortex	Medulla	Visual cell layer	Neural & ganglion cell layer
Basic fuchsin	Stemplen, 1962	Deep red	Deep red	Red	Purplish violet	Light blue to purple
Aldehyde fuchsin	Cameron & Steele, 1959	Purple	Purple	Light purple	Violet	Violet
Toluidine blue	Pearse, 1961	Violet	Light violet	Bluish	Bluish	Violet

ELECTROPHORESIS:

The samples were applied as streaks on Whatman No.1 paper strips using 0.1M phosphate buffer of pH 6.6 at $6V/cm^2$ for 8 hours. After removal from the electrophoretic apparatus, the paper strips were dried at room temperature and stained with Toluidine blue (0.04% in 80% acetone). The staining of the strips was followed by 2 – 3 rinsings in 0.1% acetic acid and 2 – 3 times in water. The strips were then dried at room temperature.

OBSERVATIONS:

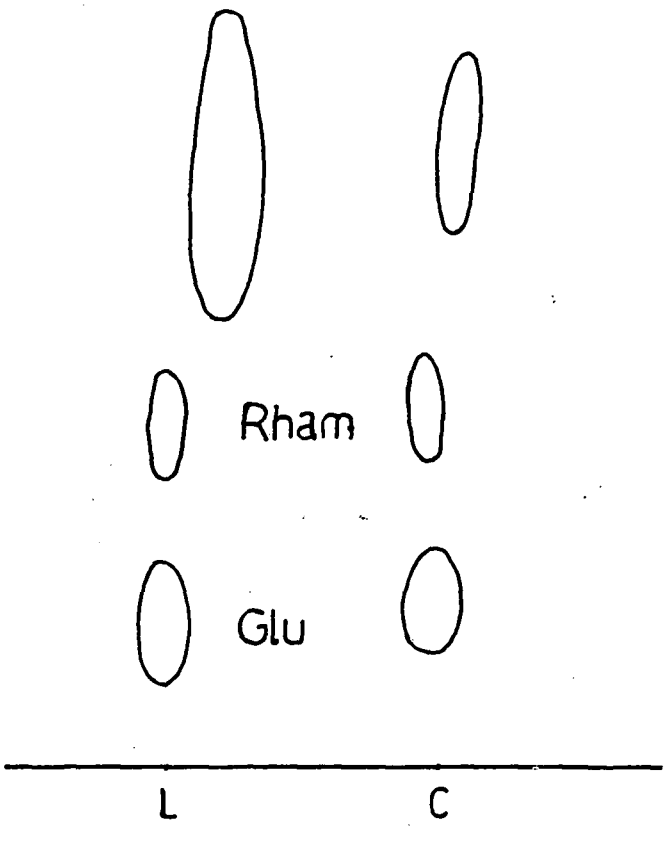
Examinations of the corneal histological sections of the chicken and the pigeon revealed the stroma as externally lined by epithelium and internally by an endothelium, like in other vertebrates. In chicken, the stroma revealed that it is formed of fine collagen fibrils along the plane of the cornea, while in the pigeon, the stroma is more loosely or tightly arranged. The sections showed very clear demarcations on staining with Toluidine blue (Plates1&2). The lens sections similarly showed clear regions on staining with Toluidine blue (Plates 3&4).

The retinal visual cell layer, on the other hand, showed a purplish – violet colour, and the other neuronal and glial cells exhibited varying shades of light blue, green and purple colour (Plates 1 - 4).

The corneal, lenticular and retinal layers, when subjected to Biuret, Millon's and Xanthoproteic tests, gave intense positive reactions indicating the presence of proteins. Similarly, when treated with Nile blue and Sudan III for lipids, they reacted positively (Table 1).

Fig. 2. Ascending paper chromatogram of the sugar components of the lens and corneal mucopolysaccharides of the pigeon, *Columba livia intermedia.*

Rham = Rhamnose
Glu = Glucose.



SUGAR COMPONENTS (PIGEON)

Standards also should be on the same paper

For the detection of polysaccharide complex, corneal sections, when treated with 0.5% basic fuchsin and aldehyde fuchsin respond positively in both birds, showing purple colour. The corneal stoma upon staining with Toluidine blue become purple in colour (Plates 2 & 4) showing metachromasia (Table 2) The reactions indicate the presence of AMPs, which are mostly sulphated.

Similarly, on treatment with aldehyde and basic fuchsin, the lens tissue of the two birds gave positive reactions i.e., purple colour, but the reaction in the cortical part was more intense as compared to the medullary part. The cortical parts are slightly metachromatic while the medulary zones are orthochromatic (Table 2). The lens, thus, contains very little AMPs.

On the other hand, the retinal layers were seen to respond variously – the neuronal and glial cells staining deeply showed a light blue to purple or green colour, while the visual cell layers were purplish –violet in colour. On staining with aldehyde fuchsin, the retinal layers responded by giving a faint violet colour. The visual cell layers of the retina responded orthochromatically but the neuronal and glial cell elements yielded metachromasia thus indicating the presence of AMPs, mostly in the neuro-glial layers of the retina.

CHROMATOGRAPHY:

The chromatographic analysis of AMPs of the cornea and lens of the eyes of the two birds indicated the presence of three sugar components in the cornea and lens of the pigeon i.e., glucose, rhamnose and one which could not be identified, while in the case of chicken, four sugar components were seen viz.,

Fig.3. Electrophoretic movement patterns of standard mucopolysaccharides.

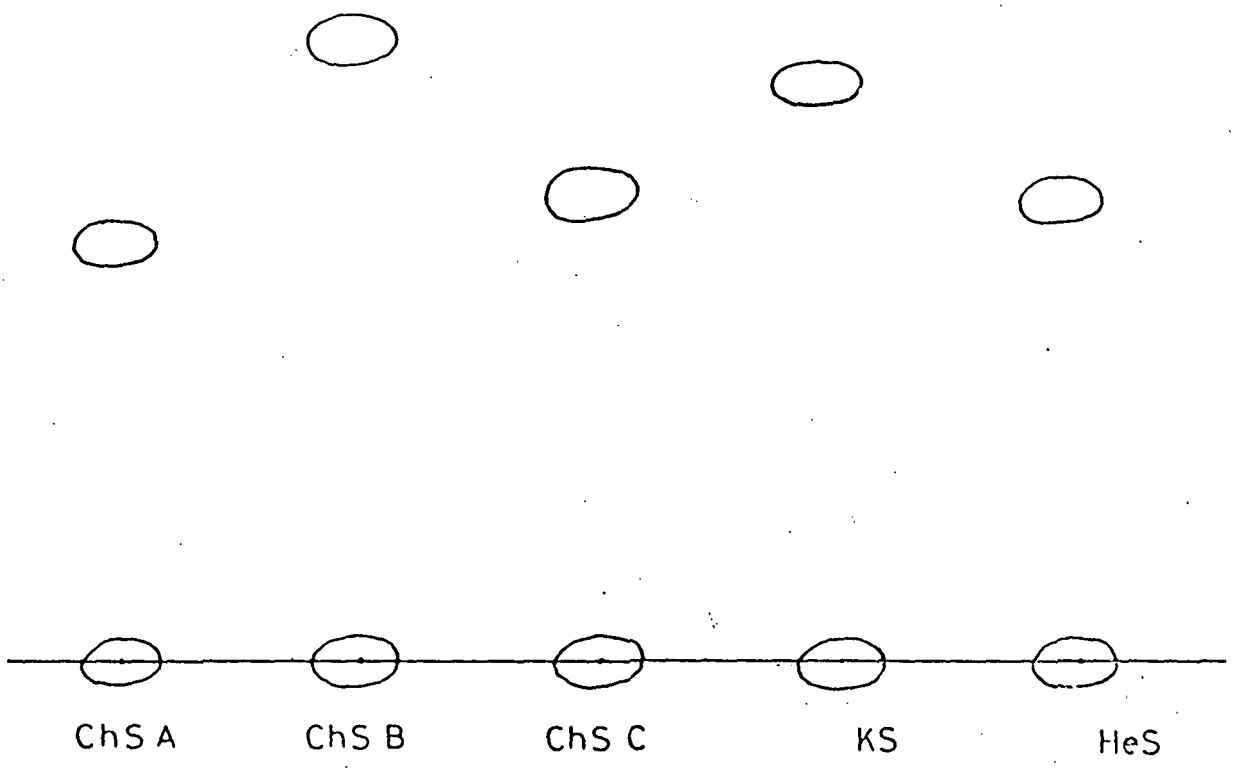
ChS A = Chondroitin sulphate A/ Chondroitin 4 – sulphate.

ChS B = Chondroitin sulphate B/ Dermatan sulphate.

ChS C = Chondroitin sulphate C/ Chondroitin 6 – sulphate.

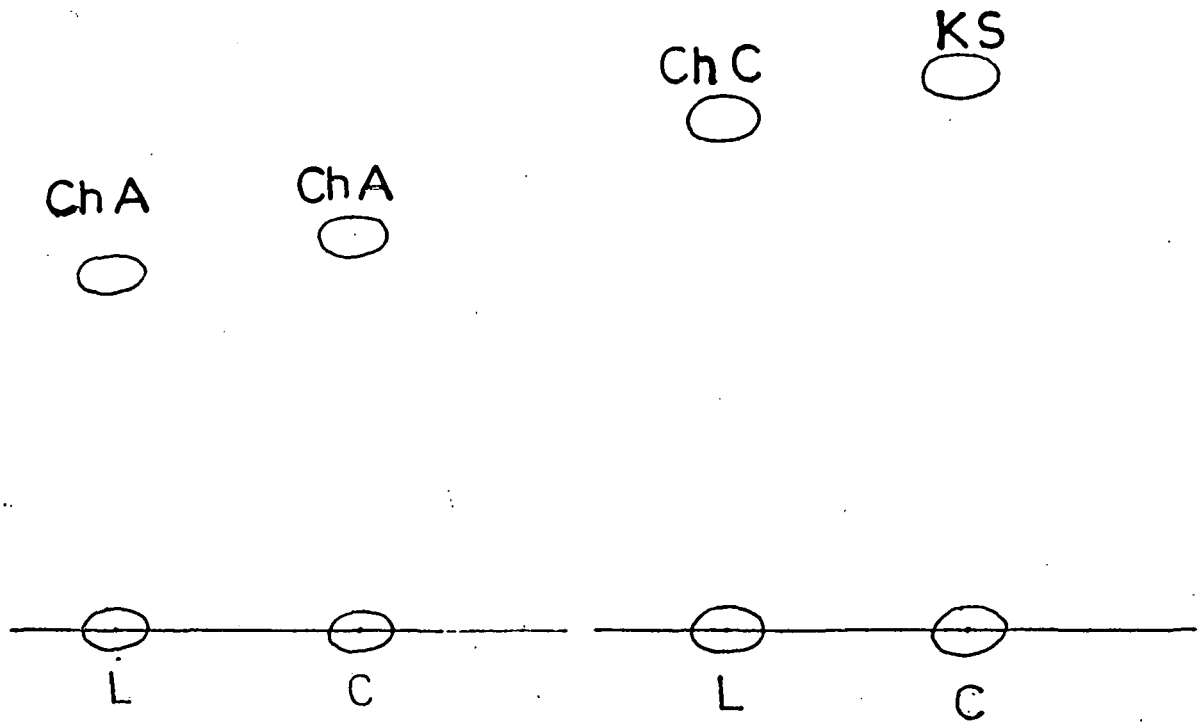
KS = Keratan sulphate.

HeS = Heparan sulphate.



STANDARD AMPs

Fig.4. Electrophoretic movement patterns of the crude mucopolysaccharide extracts from the cornea and lens of *Gallus domesticus* and *Columba livia intermedia*.



AMPs (CHICKEN)

AMPs (PIGEON)

Both standards & samples should be on same paper.

glucose, rhamnose, lactose and one component which could not be identified (Figs. 1 & 2)

ELECTROPHORESIS:

The electrophoretic mobilities of the crude extracts of the AMPs from the corneae and lenses of the two birds, when compared with several standard AMPs in different buffer systems, indicated that the major components of the AMPs belong to the group of sulphated AMPs (Figs. 3 & 4).

DISCUSSION:

AMPs are composed of amino sugars with a uronic acid, and may have sulfate radicals (sulphated mucopolysaccharides). Thus mucopolysaccharides are of the following types: -

1.Non-sulfated mucopolysaccharides

e.g. Hyaluronic acid

2.Sulfated mucopolysaccharides

(i) Chondroitins

(a) ChS A / Ch-4-S

(b) ChS B / Dermatan Sulphate

(c) ChS C / Ch-6-S

(ii) Heparitins

(a) Heparitin Sulphate A

(b) Heparatin Sulphate B

(c) Heparatin Sulphate C

(iii) Keratins

Keratan Sulphate / Keratosulfate

(iv) Heparins.

Heparins although belonging to the group of mucopolysaccharides, are usually absent in connective tissues. However, according to Jaques (1975), in some biological situations, the same functions exercised by Heparins in some cells or species seem to be performed by chondroitins in other cells or species (e.g. In basophilic leucocytes and mast cells).

The amino sugars of these heteropolysaccharides are glucosamine (in heparins, heparatins, keratosulfates and hyaluronic acid), galactosamine (in chondrotins), uronic acid i.e., chiefly iudoronic acid (in heparins, heparitins C and D and chondroitin B), and glucoronic acid (in other compounds except keratosulfates where it is replaced by galactose). With the possible exception of hyaluronic acid, the connective tissue polysaccharides are all synthesised by their parent cell as components of proteoglycans (Roden, 1980).

"Glycoprotein" is a term often used to apply to any macromolecule which contains carbohydrate and protein, and in such loose areas of use, the term really applies to molecules which, if properly classified, come under the headings of glycoproteins, proteoglycans and carbohydrate-protein complexes (Kennedy and White, 1983).

Glycoproteins contain a protein chain, which consists of about 200, or so amino acid units, which are any of the 20 naturally, occuring L-α -amino acids. Covalently attached to this protein back bone and pendent to it are the carbohydrate parts of the molecule, consisting of hetero-oligosaccharide chains, which are usually branched and can contain neutral monosaccharides and basic monosaccharides (Kennedy and White, 1983),

Proteoglycans also contain a backbone of proteins, but the carbohydrate residue takes the form of linear chains possessing regular alternating monosaccharides involving acidic monosaccharides (D-glucoronic acid /L-iduronic acid) and basic monosaccharides (2-amino-2-deoxy-D-galactose and 2-amino-2-deoxy-2-glucose). The basis units are usually N-acetylated and some

times N- sulphated, and the acidic units are some times O-sulphated. This results in the chains being strongly acidic, a factor recognised in their names of "acid mucopolsaccharides". The systematic name for these chain is "glycosaminoglycans" (Kennedy and White, 1983).

A factor distinguishing glycoproteins from proteoglycans, is the number of carbohydrate units per length (or molecular weight) of protein backbone, with protein being predominant in glycoprotein, and carbohydrates in proteoglycans (Kennedy and White, 1983). A full and definitive discussion of the nomenclature of glycosaminoglycans and glycoproteins has been published by Kennedy (1979a).

The carbohydrate components of proteoglycans are glycosaminoglycans. These compounds are the only source of hexuronic acids in animals and occur in nearly all parts of mammalian bodies and to a lesser extend in fish and bacteria. They are amongst the essential building blocks of the macromolecular framework of connective and other tissues (Kennedy and White, 1983).

One of the principal functions of connective tissues is to support and bind together the organs and bones - the functional part of the body. Though the connective tissues assume different forms in different parts of the body, like the collagen fibres, cell membranes, the extracellular fibrils, the extracellular amorphous ground substance which surrounds the collagen and elastin fibres, cartilages and bones, there is a fundamental similarity in the components in the sense that all these materials contain proteoglycans, and therefore, glycosaminoglycan chains.

It has become increasingly apparent that connective tissues have many physiological functions in addition to being a supporting medium. In tissues, the protein chains of the proteoglycan chains and the collagen fibres lie side by side and the glycosaminoglycan chains of the proteoglycans interact with the collagen to give non-covalent binding effect. Glycosaminoglcans and proteoglycans in fact, interact with a variety of molecules *in vitro*, and some of which may represent functions *in vivo* (Kennedy, 1979a).

Mucopolysaccharides have been detected in many vertebrate and invertebrate tissues and various authors have also studied their biological significance. For example, they have been isolated from the aorta of man (Barnes and Partridge, 1968; Berenson *et al., 1966*), pig (Wagh and Roberts, 1972), cow (Radhakrishnamurthy and Berenson, 1973), horse and sheep (Robert, Robert and Robert, 1970), elephant (McCullagh *et al.* 1973) and chicken (Keelay *et al.* 1969). Bowes et al (1958) and Wolff *et al* (1971) have reported on the presence of mucopolysaccharides in the skin of cattle, Timple et al (1969) and Borel *et al* (1970) on rabbit, Enomoto et al (1966) on cartilages of fishes, Denizod (1970) on the ampullary sense organs of some weakly –electric fishes, Janado and Dunston (1972) and Vittier *et al* (1972a,b) on bovine nasal cartilages, Di Benedetta *et al* (1969) and Margolis and Margolis (1970) on the brain of rat.

Kobayashi and Pedrini (1973), suggested that mucopolysaccharides have a major role in structural organisation of intercellular matrix, and may be involved in electrostatic and steric interaction with other macromolecule of the matrix such as collagen and elastin, while Jeanloz (1970) proposed that they may also have

some functions in controlling the metabolism of cells and movement of metabolites on the basis of their rather specific chemical structures.

Rubin and Howard (1950) have proposed that the calcification of bones and cartilages are associated with mucopolysaccharides. Another characteristic property of mucopolysaccharides is the selective association or binding with small inorganic cations, especially H^+, Na^+ and Ca^{++} and also with cationic groups of macromolecules (Mathews, 1959; Oosawa, 1971). In this regard Farber and Schubert (1957) have shown that, in chondroitin sulphate, the percentage binding of calcium is greater than that of sodium, and Urist et al (1968) have also found a small preference for binding calcium and sodium in chondroitin sulphate. Thus, Mathews (1975) has suggested that these substances act as a store for calcium in cartilage tissue and that is why they have specific roles in tissue calcification.

Other important roles of mucopolysaccharides in "Water-binding" and maintenance of tissue osmotic pressure have been reported by Ogston and Wells (1972) as well as Wells (1973b). According to Ogston (1966a) the effects of mucopolysaccharides on tissue osmotic pressure is not only by influencing the water balance, but also by introducing excess swelling pressure which is balanced by an internal structural resistance. They also attributed to the mucopolysaccharides, a role in maintaining mechanical flexibility and elasticity of tissues.

Works on invertebrate mucopolysaccharides are scanty and too far in between, when compared to the extensive amount of work done on vertebrates. Some of the works have been done by Hunt and co-workers (1970) on marine

snails, where they detected a glucan sulfate-peptide component from mucin. Mustafa and Kamat (1970) have reported the occurrence of mucopolysaccharides in brain ganglia and imaginal discs of the housefly, *Musca domestica;* Ashhurt and Costin (1971) in the locust. Mathews (1975) detected a chondroitin sulphate-like substance from the cranial cartilage of *Locusta opalescens.* They have also been reported in arthropods, especially hyaluronic acid. They have been detected in the salivary glands of various insects (Vadgama and Kamat, 1971) and in the dermal glands (Baldwin and Salthouse, 1959). Some interesting roles of mucopolysaccharides, especially in arthropodan cuticles has been reported by Meenakshi and Scheer (1959) and Sundara Rajulu (1969), in terms of the calcification of the cuticle of *Hemigrapsus mudus* and *Cingalabolous bungioni* respectively, while Krishnan (1965) has suggested that the acid mucopolysaccharides may be associated with –S-S- bonding of the cuticle in the scorpion *Palaemoneus swammerdami.*

Sannasi (1969) considered the extreme flexibility of the inter- segmental cuticles of the queen of *Odontotermis obesus* to be due to the occurrence of acid mucopolysaccharides. He based his conclusions on the fact that acid mucopolysaccharides are said to possess a high water binding capacity (Ogston, 1966a,and Katchalsky, 1964).

Various workers have also reported pathogenic role of mucopolysaccharide. Matalon *et al* (1974a), Hall *et al* (1978), Neufeld and Fratantoni (1970), McKusick *et al* (1978) etc have elucidated on inborn errors of mucopolysaccharide metabolism and /or mucopolysaccharide storage disorders.

Matsuoka *et al* (1982) have observed that mucopolysaccharides are responsible for dermal thickening in Acromegalic patients. Wolf *et al* (1982) have reported that age related decreased synthesis of proteochondroitin sulphate in the costal cartilage of old rats is by a mechanism or mechanisms involving a reduced activity of xylosyltransferase.

According to Shahnaz and Glaser (1982), there exists an association in the accumulation of glycosaminoglycans (GAG) and fibronectin. This association is affected by dimethyl sulfoxide and may help in further studies and treatment of the genetic disease, mucopolysaccharidoses, caused due to increased accumulation of GAG.

Various workers have reported the occurrence of mucopolysaccharides and their significance in vision in ocular tissues of various vertebrates and invertebrates. For instance, Moczar and Moczar (1973) and Anseth (1961a) found mucopolysaccharides in the corneal stroma of squids and in twelve vertebrates including teleosts respectively. The squid cornea, unlike the analogous tissue of vertebrate eyes is a direct continuation of the skin. Balazs (1965) has thoroughly reviewed the biochemistry of the cornea and other tissue. Deb and Raghuvarman (1994) have observed that glycosaminoglycans are essential for the maintenance of corneal structure and function.

The other ocular tissues where mucopolysaccharides have been reported are the vitreous body (Balazs, 1965; Berman and Voaden, 190; Bleckmann, 1984), aqueous and ciliary body (Cole, 1970; Schachtschabel *et al.* 1977), interstitial matrix surrounding the photoreceptor cell of the cattle (Berman and

Bach, 1968; Berman, 1969), inter-photoreceptor matrix of vertebrates (Rolich, 1970), and sclera of ox (Robert and Robert, 1967).

Mucopolysaccharides have also been reported in the compound eyes of some insects such as *Periplanata americana, Belastoma sp.* (Dey, 1976), *Musca domestica*, *Apis cerena indica* (Dey, 1980), *Paelemon sp.*, *Limunus polyphenus* (Dey, Raghuvarman and Michael, 1978).

Thus, keeping the above account in view, it can be assumed that AMPs in the ocular tissues, like in other vertebrates also play some vital role in the visual aspect or life of birds, and in the light of the present findings, it can be assumed that some role of AMPs certainly exists in the visual processes of the domestic chicken *Gallus domesticus* and the Indian blue rock pigeon *Columba livia intermedia* (Strickland).

The cornea being the only important external structure of the eye has drawn special attention of various workers in terms of its physiology and adaptation in regard to vision. It is already known that the bulk of cornea of the vertebrate eye is taken up by the stroma which functions as a supporting structure, and is adapted for the transmission of a high percentage of incident light of visible wavelength (Maurice, 1969).

Anseth and Fransson (1970) have found that during chick corneal development, the occurrence of highly sulphated KS (Keratan sulfate) is associated with the rise in the transparency of the stroma, and they have also suggested that stromal transparency is correlated with the presence of the normal proportions of KS and ChS A. Payrau *et al.* (1967) observed that the transparency

of the cornea is based on the state of hydration of the tissue. They based this on the fact that the corneal stroma of most vertebrates, including mammals, birds and teleosts absorb water where ever free water is accessible.

In contrast, Maurice and Riley (1970) contented that oedema of cornea leads to disorganisation of its structure and less transparency, but dehydration does not appear to have serious optical effects.

Moczar *et al.* (1969a,b) and Moczar and Moczar (1970, 1972) supposed that high content of mannose in the structural protein is responsible for the non-swelling of elasmobranch cornea, while other workers like Hedbys (1961, 1963) are of the view that the dehydration properties of corneal stroma to be due to AMPs. They explained the mechanisms of corneal swelling on the basis of electrostatic repulsion between acidic groups of these electron molecules. It is now known that the pH value is a decisive factor for taking up of water by the cornea (Cejkova and Brettschneider, 1969). The protein polysaccharide complex provides more stable and specific configuration within the molecules than electrostatic linkages could (Maurice, 1969, 1972).

Further, authors like Anseth and Fransson (1969) have demonstrated the synthesis of AMPs by corneal epithelial and stromal cells, and that they are important in maintaining the corneal structure in relation to its environment (Deb, 1990). They are known to act as selective ion barriers (Jeanloz, 1970). Moreover, the protective role of AMPs in the cornea has been demonstrated by Robert and Robert (1967).

Maurice and Riley (1970) stated that the three layers of the cornea are largely self-sufficient with regard to their glucose metabolism and energy supplies, while, in the case of synthetic processes, there may be some dependence on the enzyme systems of other layers for intermediate metabolites, but this has not been demonstrated conclusively.

Similarly, volumes of work have been done and well documented on crystallin proteins and their role in lens transparency by many workers like Morener (1894), Spector (1964, 1965), Spector *et al* (1968), Kuck (1970) etc. Kuck (1970), Kinoshita (1965), and Rawal and Rao (1978, 1980, and 1983) have described various biochemical aspects and cataract formation in lens. Ziegler and Sidbury (1977) have worked on the evolutionary conservation of lens proteins. Basaglia and Callegarini (1987b, 1988) as well as Basaglia (1989) have worked on the species specificity of crystallins in fishes along with other correlations such as environmental conditions, visual acuity and age. But relatively less work has been done on glycoproteins of vertebrates in general and avian lens in particular.

As has been mentioned earlier, the chief function of the lens in vision is accommodation (Kuck, 1970). Accommodation means the distance over which the lens can maintain focus at the retina, and which involves two factors i.e., deformability and refractivity. The former depends upon the configuration of lens fibres and the means used to weld them into a medium which is optically homogenous, but which, however, still consists of discrete fibres which can slide over each other in the process of stretching or contracting. Deformability thus involves the properties of glycoproteins, the cement lubricant substance located at

the ... surface (Jakus, 1964). Refractivity on the other hand, is dependent on the lens proteins: the soluble crystallins and the albuminoid (Maurice, 1970), which gives a relatively high refractive index to the lens.

Kuck (1970) and Krause (1938) have found that the lens contains very little glycoprotein. Bellows (1944) believed that this was extracellular and would serve as a cement substance between individual fibres and promote the loose bonding necessary for the optical functions of the lens. So, inspite of their small quantity (150 – 200 µg/g wet weight in lens cortex and 90µg/g in lens nucleus of cows 1.5 – 2.5 years old:), the importance of glycoproteins cannot be overlooked.

The lens cortex contains much of the glycoproteins as compared to the nucleus, which has been shown in both young and old cattle and rabbits, leading to the conclusion, as suggested by Morener's experiment, that it is localised at the interfibrillar surfaces, rather than within the lens (Kuck, 1970). The lens glycoprotein appears to belong to the group of sialoglycosaminoglycans and contains glucosamine, galactose, glucose, mannose, fucose and derivatives of neuraminic acid. Kuck (1970) has shown that the equatorial cortex not only has more glycoproteins, but also is relatively richer in neuramic acid, when compared to the polar cortex. Moreover, electron-microscopic observations have revealed that the equatorial cortex is more affected as compared to the polar cortex, when lenticular aberrations are induced by prolonged starvation in the catfish *Heteropneustes fossilis* (Bloch), in addition to loss of connection between the fibres (Deb *et al.* 1990). The abundance of hydrophilic type of glycoproteins at

the equator is believed to be related to ... n rate of growth and differentiation (Dische, 1965a).

In the retina, Rohlich (1970), Marchase *et al* (1982) and Verner et al (1987) have reported the occurrence of mucopolysaccharides. In the present study, metachromatic reactions have been observed in the retinal layers of the two birds, thereby indicating the presence of mucopolysaccharides in the retina.

Thus, on the basis of the above reports, similar roles can be adduced to the bird corneal, lenticular and retinal glycoproteins following their detection.

An important point that is worth a mention, is that of the role of vitamin A in vision, because it has been shown to be involved in the synthesis of sulphated mucopolysaccharides. Wolf and Varandani (1960) have reported that deficiency of this vitamin in the culture of cartilage tissues blocks the synthesis of mucopolysaccharides. It is presumed that retinol or an active form of it or retinoic acid releases an enzyme or enzymes required for the synthesis of mucopolysaccharides.

Adenosine triphosphatase

INTRODUCTION:

ATPase is one of the most important of the approximately thirty enzymes in the plasma membrane because of its role in ion transfer across the cell membrane (DeRobertis *et al,* 1970). It has also been shown to be intimately associated with the phenomenon of photoreception (Langer, 1964, 1974; Weber and Schorrath, 1971; Drujan and Ali, 1972; Bownds *et al.* 1972) and there are firm indications that ATPase has an important role in phosphorylation.

A very important step in the visual process is nervous stimulation or excitation, which is an outcome of the photolysis of a rhodopsin molecule by a quantum of light. The exact mechanism is yet to be unravelled, and three main hypotheses have been proposed to explain this mechanism viz., (1) Enzyme hypothesis (2) Solid state hypothesis and (3) Ionic hypothesis.

The enzyme hypothesis postulates that rhodopsin is a proenzyme that gets converted into an active enzyme upon absorption of light and thereby catalyses an electrochemical process which lead to visual excitation. In this regard, McConnel and Scarpelli (1963) have claimed that rhodopsin is an ATPase, with retinene a co-factor, activated by light. But Bonting and Bangham (1967) negated this claim by stating or proving that neither Mg^{++} - activated ATPase or Na^{+} - K^{+} - activated ATPase can be identical with rhodopsin.

The solid state hypothesis holds that a solid state of energy transfer by resonance or photoconduction might take place following light absorption by the rod outer segments. However, Hagins and Jennings (1960), failed to find any evidence in support of the energy transfer resonance in frog rods.

While, according to the ionic hypothesis, there is movement of ions across the photoreceptor membrane following visual excitation similar to that occurring in a neuron. In other words, this hypothesis holds that the maintenance of ionic gradients across the photoreceptor membrane is important for visual excitation and that ATPase regulates this gradient of ions. Hagins *et al* (1962) have observed that the action of light on photoreceptor cells produces a local inflow of current within 7μm from the site of light absorption. This receptor current consists of an influx of Na^+ ions into the rod outer segments, due to a local light – induced increase in Na+ permeability as well as an efflux of K^+ ions.

Evidences in support of this hypothesis have been put forward by quite a few workers on a number of animals – Stieve (1965) in the photoreceptor cells of *Limulus*, Eguchi (1965) on cray fish, Hagins (1965) on squid, Brown et al (1969) on barnacle, Fulpius and Baumann (1969) on honey bees etc. They all basically state that, as a direct consequence of an increased Na^+ permeability through the photoreceptor membrane, light- stimulated potentials have been observed.

Sekoguti (1960) as well as Bonting *et al* (1963) have detected the presence of Na^+- K^+ - activated ATPase in the rod outer segments which lends further credence to the ionic hypothesis, since this enzyme has been shown to be actively involved in the process of repolarization of nerve, muscle and electric organs. They found a high enzyme activity in cattle retina, retinae, choroid, sclera, iris and vitreous humour of man and cat, and also found that the enzyme activity is stimulated by the addition of K^+ in the Na^+ - containing assay medium (Bonting *et al*, 1963, 1964).

The presence of Na^+ and K^+ - activated ATPase has been reported in the corneal endothelium, while Maurice and Riley (1970), Tervo and Palkama (1975) and Midelfart (1987) have also reported its activity in the epithelium and nerve fibres of the stromal tissues of the cornea. Its presence in the ciliary body has been reported by Riley (1964), Palkama and Uusitalo (1970) and Harkonen *et al* (1972), and in the lens by Kinoshita *et al* (1961), Kinsey *et al* (1965), Harris and Becker (1965), and Palva and Palkama (1974). Mac Gregore and Matschinsky (1986) reported on the altered ATPase activity in pathogenic retina.

A universal characteristic of Na^+- K^+- ATPase is the specific inactivation by ouabain and other cardiac glycosides (Stekhoven and Bonting, 1981; Anstee and Bowler, 1984). The ouabain-sensitive Na^+ - K^+ -ATPase has been identified in the active Na^+ - K^+ transport systems of a variety of biological membranes. Recently, Ali - Robai (1993) has shown that the ouabain sensitivity of Na+ - K^+ - ATPase from different tissues of the grasshopper *Poekilocerus bufonius* is different and may also be insensitive to it- the insensitivity to ouabain being probably due to tissue- dependent differences in the isozyme patterns available in the same animal. Another interesting report is by Harvey *et al* (1983), who reported that in insects feeding on plants rich in K^+ content, the Na^+ - K^+ - ATPase is ouabain- insensitive.

The ouabain - sensitive Na^+ - K^+ - ATPase is an enzyme that catalyses Na/K exchanges, maintaining constant with its activity, the gradients of these ions across the plasma membrane of a wide variety of cells (Lechene, 1988).

Available information shows that a good amount of work on ATPase has been done in many animals ranging from insects to higher vertebrates. However, its activity in avian ocular tissues, especially in chicken and pigeon is lacking. An attempt has therefore been made to localise the ouabain – sensitive Na^+ - K^+ - activated ATPase enzyme system in the cornea and lens of the two birds, by a specific histochemical method i.e., the Wachstein – Meisel lead trapping technique (1957), as modified by Mc Clurkin (1964), and optimised and made highly specific by Palkama and Uusitalo (1968, 1970). The effects of the omission of each of the activators Na^+, K^+ and Mg^{++} have been investigated with special reference to the epithelial localisation.

MATERIALS AND METHODS:

The birds are decapitated, the eyes removed and immersed in the pre-incubating solutions. The lenses and the corneas are then carefully extracted and kept separately in the cooled pre- incubating media for cleaning. Ouabain is used as a specific inhibitor of Na^+ - K^+- activated ATPase system and to act as a control medium.

Pre- incubation media:

Solution A: 0.02M Tris – HCl buffer (pH 7.2)

Solution B: 3x 10^{-4}M ouabain added to the above (for its inhibitory effect on the enzyme).

Fixation medium:

2.5% glutaraldehyde diluted in Tris – HCl buffer (pH 7.2)

Rinsing medium:

Tris – HCl buffer and 0.33M sucrose solution.

Incubation medium:

The incubation medium contains 3mM Tris- ATP as substrate, 3mM Pb $(NO3)_2$ as precipitating agent, 3mM $MgSO_4$, 70mM NaCl and 70mM KCl as activating agents. All these are dissolved in Tris – HCl buffer (pH 7.8)

Stain:

Ammonium sulphide (1:100)

Mounting medium:

Glycerol jelly

The tissues were first kept in the pre- incubation media for 1 hour at 4°C, then fixed in 2.5% glutaraldehyde for 20 – 30 minutes at 4°C and then rinsed for 4 hours at 4°C. This was followed by incubating in the incubation medium for 30 minutes at 37°C. Prior to incubation, care was taken to see that the pH of the medium is adjusted to 7.2.

After incubation, the tissues were rinsed in 0.33 sucrose solution, stained in ammonium sulphide and mounted in glycerol jelly.

CONTROL STUDIES:

The effect on enzyme activity of the cornea and the lens due to the omission of some other essential co-factors from the incubation medium were also investigated. The following co-factors were omitted from the medium:

(1) Na^+ and K^+ (2) Na^+, K^+ and Mg^{++} (3)Na^+ (4) K^+ (5) Mg^{++} (6) ATP (7) Pb

EFFECT OF TEMPERATURE:

The tissues were preheated to 60°C - 65°C for 1hour in order to exclude the artifactal precipitate due to non-enzymatic hydrolysis of ATP.

OBSERVATION:

Na^+ - K^+ - ATPase activity was observed in the epithelial cell membranes of the corneas and the lenses of the two birds i.e., *Gallus domesticus* and *Columba livia intermedia* (Strickland). The positive reaction was indicated by a dark brown colour, while no reaction was visible inside the cell. Here it is worthwhile to note

Plate - II

Fig 5

Plate 5. Whole mount of lens of *Gallus domesticus*, incubated without ouabain in the incubating medium, showing enzyme activity. 500x

6

Plate 6. A similar preparation of the lens *Columba livia intermedia*, showing enzyme activity. 500x.

7

Plate 7. Whole mount of lens of *Gallus domesticus*, with the addition of $3x10^{-4}$ M ouabain, demonstrating the inhibition of enzyme activity. 500x

8

Plate 8. A similar preparation of the lens of *Columba livia intermedia*, showing the inhibition of enzyme activity. 500x

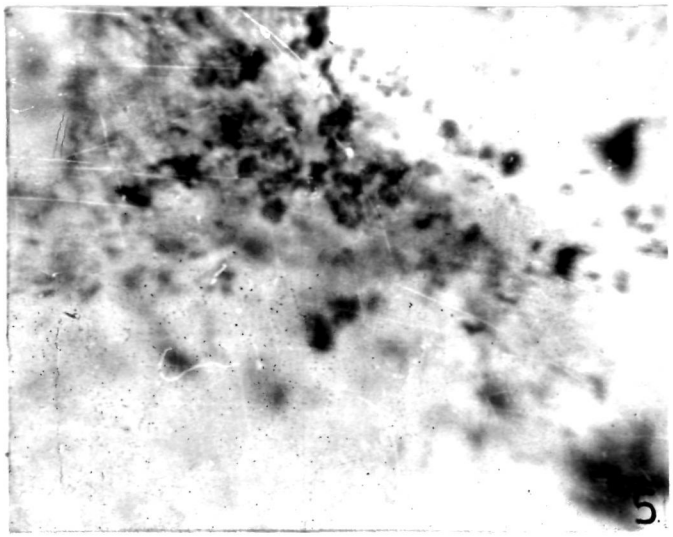
9

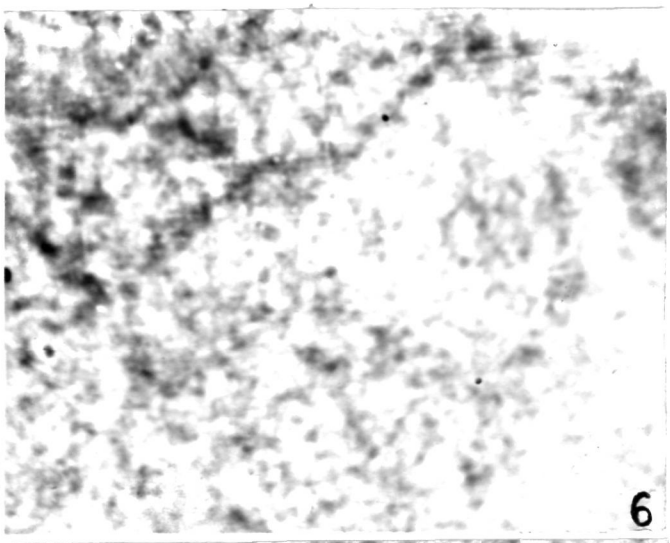
Plate 9. Whole mount of lens of *Gallus domesticus* without the activators Na^+ and K^+ in the incubating medium showing the inhibition of enzyme activity. 500x

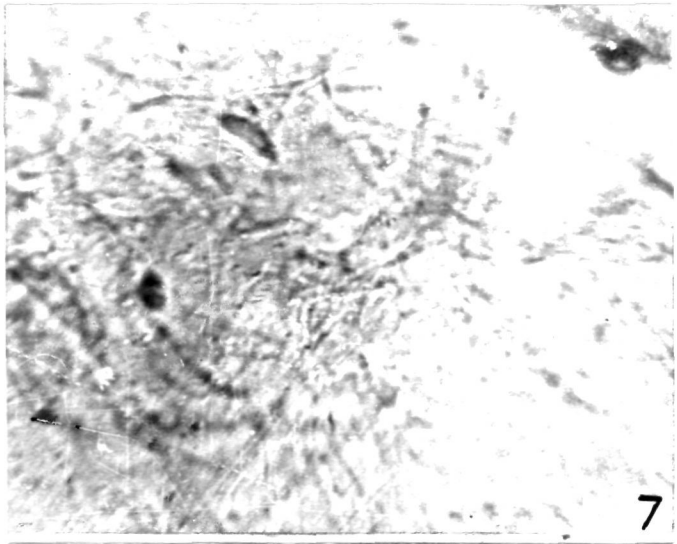
10

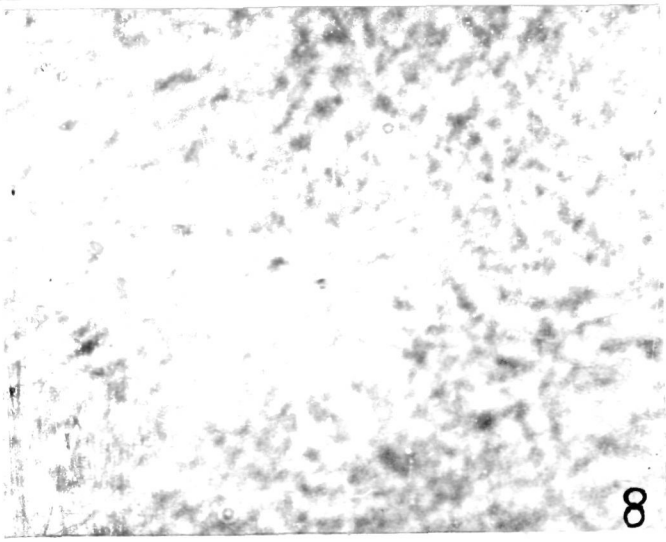
Plate 10. A similar preparation of the lens of *Columba livia intermedia*, showing the inhibition of enzyme activity. 500x

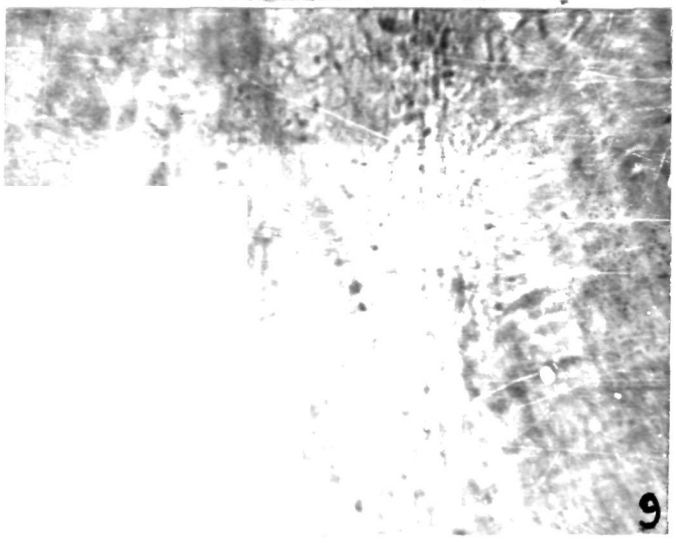
not Comming with Color photos


5


6


7


8


9

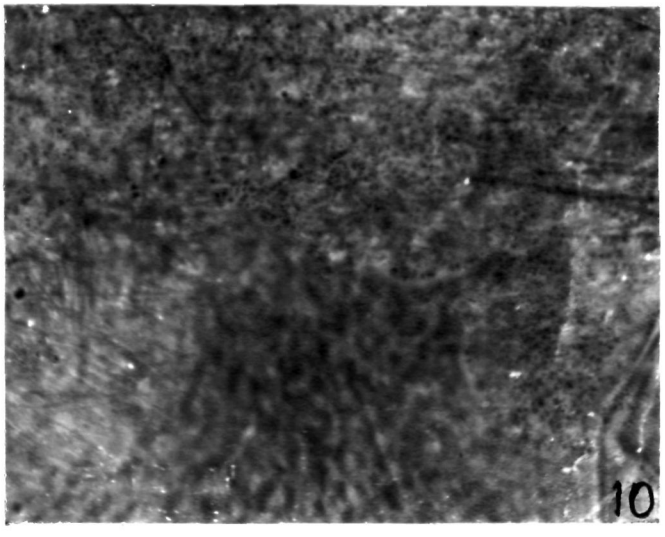

10

Plate 11. Whole mount of corneal endothelium of *Gallus domesticus*, incubated without ouabain in the incubating medium, showing enzyme activity. 500x

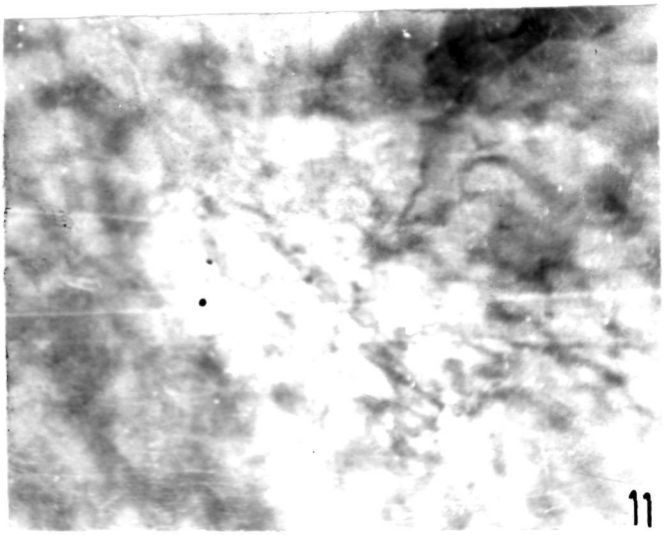
Plate 12. A similar preparation of the corneal endothelium of *Columba livia intermedia* showing enzyme activity. 500x.

Plate 13. Whole mount of corneal endothelium of *Gallus domesticus*, with the addition of $3x10^{-4}$ M ouabain, demonstrating the inhibition of enzyme activity. 500x

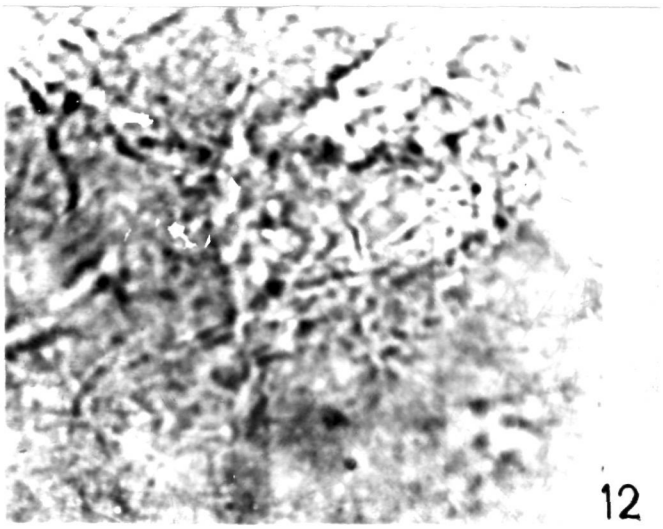
Plate 14.A similar preparation of the corneal endothelium of *Columba livia intermedia*, showing the inhibition of enzyme activity. 500x

Plate 15. Whole mount of corneal endothelium of *Gallus domesticus* without the activators Na^+ and K^+ in the incubating medium showing the inhibition of enzyme activity. 500x

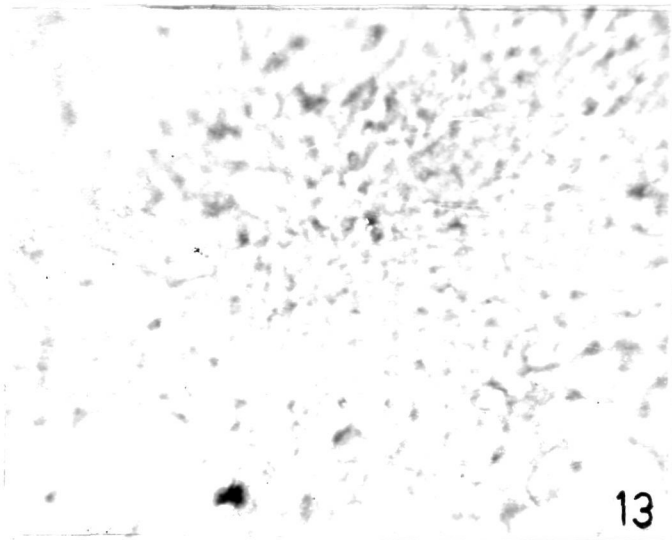
Plate 16. A similar preparation of the corneal endothelium of *Columba livia intermedia*, showing the inhibition of enzyme activity. 500x



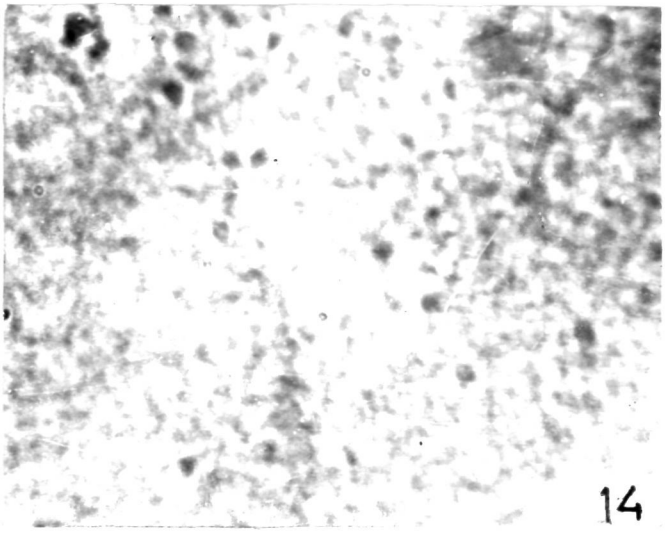
11



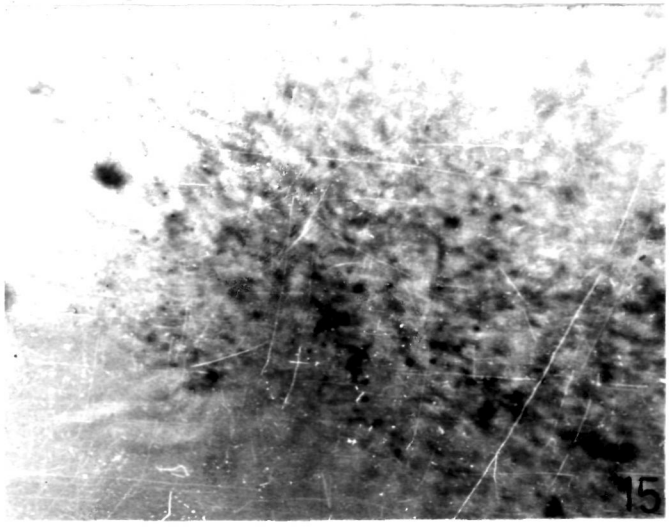
12



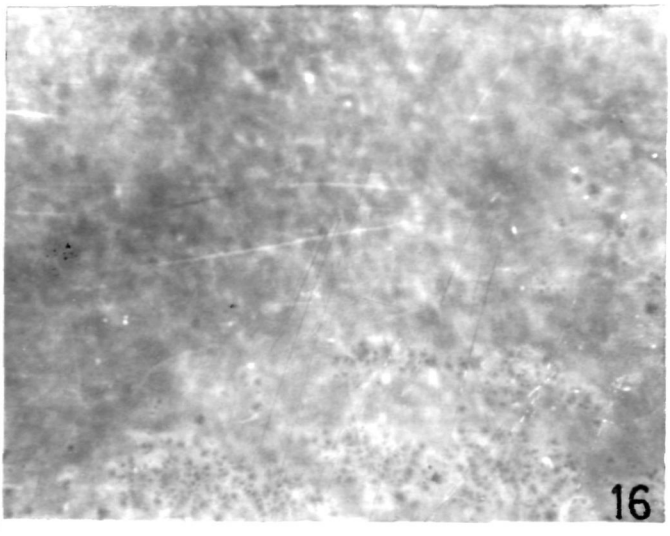
13



14



15



16

Plate 17. Whole mount of corneal epithelium of *Gallus domesticus*, incubated without ouabain in the incubating medium, showing enzyme activity. 500x

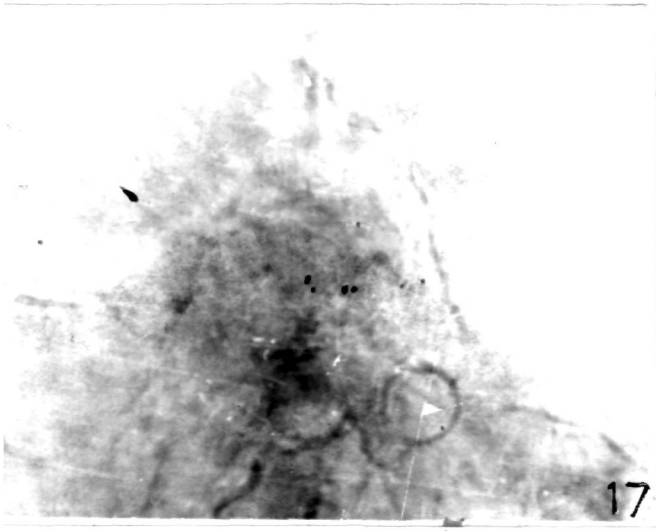
Plate 18. A similar preparation of the corneal epithelium of *Columba livia intermedia* showing enzyme activity. 500x.

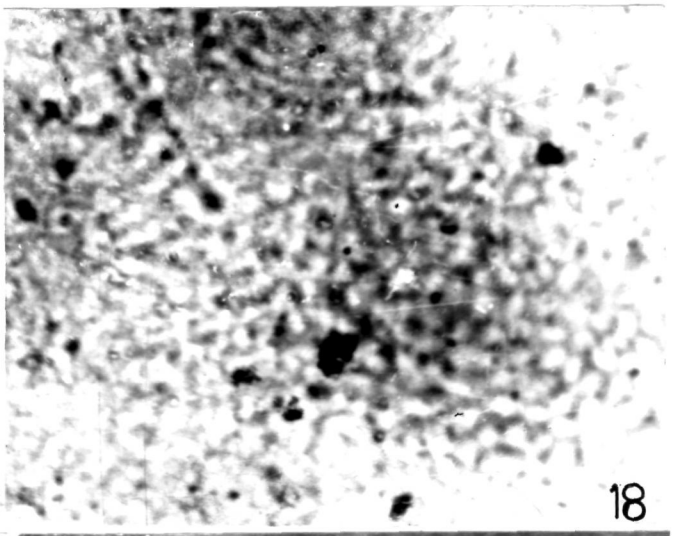
Plate 19. Whole mount of corneal epithelium of *Gallus domesticus*, with the addition of $3x10^{-4}$ M ouabain, demonstrating the inhibition of enzyme activity. 500x

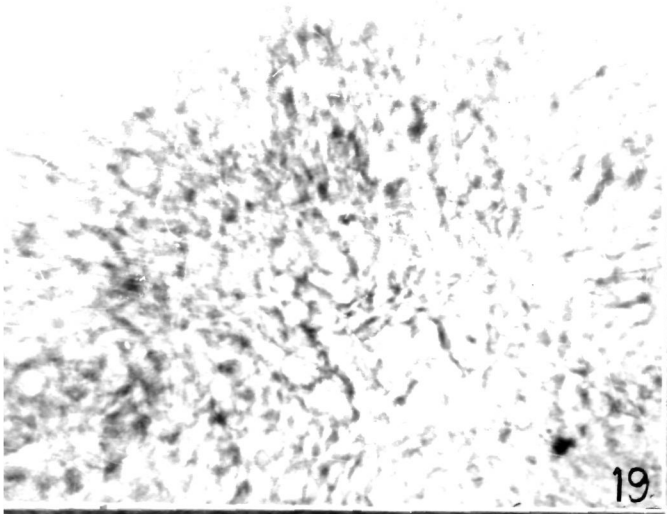
Plate 20.A similar preparation of the corneal epithelium of *Columba livia intermedia*, showing the inhibition of enzyme activity. 500x

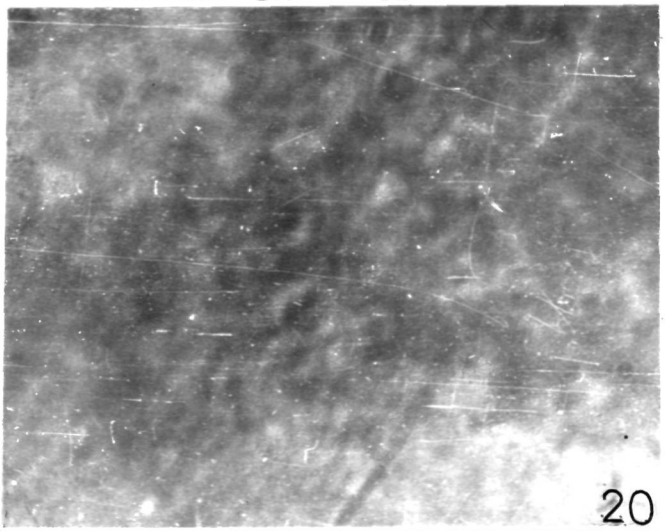
Plate 21. Whole mount of corneal epithelium of *Gallus domesticus* without the activators Na^+ and K^+ in the incubating medium showing the inhibition of enzyme activity. 500x

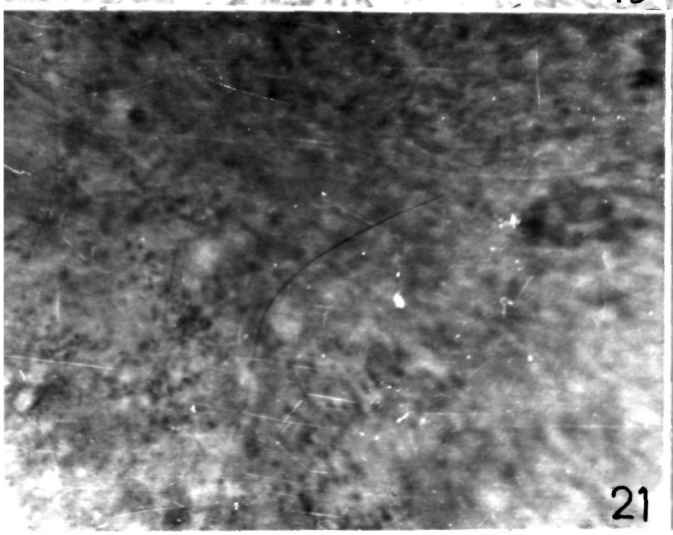
Plate 22. A similar preparation of the corneal epithelium of *Columba livia intermedia*, showing the inhibition of enzyme activity. 500x

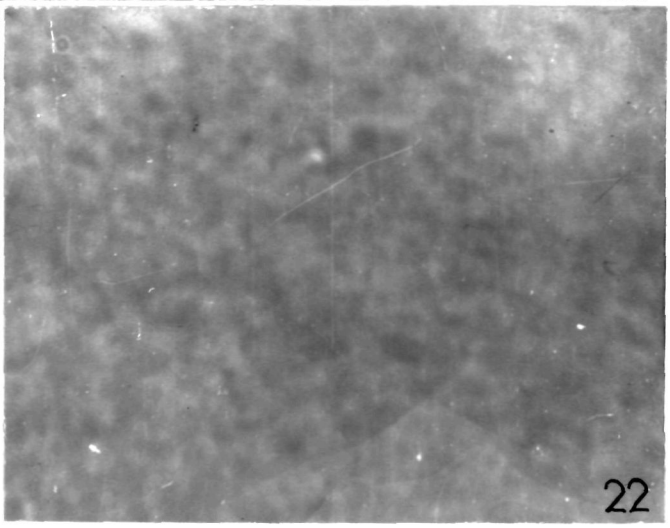

17


18


19


20


21


22

that a light brown cytoplasmic colour was seen as well as a light staining of the nucleus when the concentration of Mg^{++} or K^{+} was not optimal, but this is due to background staining and thus, not indicative of any enzyme activity.

A further confirmation of ATPase activity was by the addition of ouabain in the pre – incubating medium (solution B) which resulted in the absence of the characteristic brown colour.

Lens:

In the lens, the enzyme activity is restricted only to the epithelial cell borders that lie immediately below the capsule (Plates 5 & 6). In the whole mount preparation when pre- incubated in solution A. But this activity was not seen when incubated in the presence of ouabain (Solution B). The capsule as well as the lens fibres does not show any enzyme activity.

The results of the control experiments i.e., without the activators Na^{+} and K^{+} are shown in Plates 9 & 10.

Cornea:

A similar situation to the above was seen in the cornea i.e., on pre-incubation in solution A, a positive reaction could be seen in the corneal endothelial cells (Plates 11 & 12) and epithelial cells (Plates 17 & 18). But very little or no reaction was seen when pre- incubated in solution B i.e., with ouabain (Plates 13, 14, 19 & 20). The stroma, in both cases showed negative results, thus confirming the enzyme activity in the epithelial cells.

These findings thus, support the theory of Tormey and Diamond (1967), that the active transport site is between the adjacent cells, as has been observed in both the cornea and the lens.

DISCUSSION:

The ouabain – sensitive, Na^+, K^+ – ATPase is an enzyme that catalyses Na /K exchange, maintaining constant with its activity the gradients of these ions across the plasma membrane of a wide variety of cells (Lechene, 1988). Similarly, Bonting (1970) has stated that the Na^+ - K^+- activated ATPase enzyme system occurs in nearly all cell membranes over which a gradient for Na^+ and K^+ exists.

The presence of this enzyme in numerous organ tissues has been well documented. For instance, Hendler *et al* (1971) have found that the outer medulla of the kidney consists of, or contains the highest concentrations of Na^+ - K^+ - ATPase. Schmidt and Dubach (1971) have observed its activity, particularly in the peritubular cell membranes of the ascending limb of the Henle's loop, which agrees with its postulated role in active cation transport.

Bonting *et al* (1964a) found a major part of the Na^+ -K^+ -ATPase activity to be localised in rod outer segments. Frank and Goldsmith (1965) also reported similar findings in isolated rod outer segments of pig retinas. In addition, this activity is also high in the membranes of excitable cells such as brain, nerve, muscle and the electric organ of the electric eel, as well as in the Na^+ - transporting tissues.

The high activity of this enzyme in rod outer segments led Bonting and Bangham (1967) to formulate their " cation channel" hypothesis for the visual process. They assumed that the enzyme is not only located in the rod outer segment membranes, but also in the rod sac membranes, and which has been previously detected histochemically by Scarpelli and Graig (1963).

The ouabain- sensitive Na^+-K^+- ATPase has been found to be involved in the active Na^+-K^+ transport system of a variety of biological membranes (Skou, 1965; Bonting *et al*, 1963). It has also been found that concentration of Na^+ and K^+ are required for the maximal stimulation of the ATPase activity (Lehninger, 1970). The enzyme is especially inhibited by cardiac glycosides and ouabain. ATPase has been considered as a Na^+-K^+ "pump enzyme", based on the fact that Na^+ -K^+ ATPase leads to hydrolysis of ATP, and resulting in the movement of Na^+ and K^+ against their electrochemical gradients (Skuo, 1972). This hydrolysis of ATP occurs in the intracellular side using OH^- from the interior and H^+ from the exterior, liberating phosphate (Mitchell, 1961). It has been postulated that the ATP-ATPase –enzyme –substrate complex may act as a true carrier mechanism that binds internal sodium and releasing it outside the membrane. A similar but reverse mechanism is postulated for potassium. According to Lehninger (1970), in the absence of an active cation pump, the cell would swell and burst due to internal osmotic pressure.

The Na^+ - K^+ activated ATPase in the corneal epithelium, endothelium and the nerve fibres of the stromal layers is presumed to play some part in the transport mechanism. According to Maurice and Riley (1970), corneal hydration and thickness is controlled by the low permeability of the surface membranes, and by an active pump mechanism that actively pumps out the fluid that enters, and a cation- dependent ATPase system is one of the pump mechanisms involved in the regulation corneal water balance. But there is a great deal of disagreement over

the precise location and character of the mechanisms controlling corneal hydration (Tervo and Palkama, 1975).

The exact role of Na^+- K^+- ATPase in the corneal epithelium is still open to question because according to authors like Maurice (1965b), the corneal epithelium plays no role in pump mechanisms, but rather, offers a high resistance to the movement of all ionic substances.

In the rabbit corneal epithelium, an active inward Na^+- transport mechanism to the stroma has been identified. Zadunaisky and Lande (1971) have also reported a Na^+ - dependent mechanism for pumping chloride ions from the aqueous humour to the tear film in frog corneas. Moreover, Klyce et al (1973) have shown that the rabbit corneal epithelium not only pumps sodium ions from the tear film towards the stroma, but also chlorides in the opposite direction, when stimulators like epinephrine and cAMP are present. However, it seems possible that definite variations between different species may exist vis-à-vis the pumping mechanisms of the corneas (Ehlers, 1973).

The endothelium on the other hand, is more permeable to ions than the epithelium (Maurice, 1961), and is directly concerned with the transport mechanism. Maurice (1972) considered the endothelium to be the chief site for the active pumping process. He, furthermore asserted that the permeability of the endothelium also seems to influence the corneal water balance, thus leading him to advance the "pump leak" hypothesis, which proposes that the endothelium pumps water and sodium into the anterior chamber against hydrostatic pressure, which causes a passive leakage of water and electrolytes through the endothelium

towards the stroma. In this regard, Friedman (1973) proposed that the corneal water balance is exclusively based on a passive mechanism i.e., the osmotic properties of the tear film and the permeability of the endothelium would be the main regulatory systems.

The endothelial pumping mechanism needs sodium and bicarbonate (Dikstein and Maurice, 1972; Hodson, 1971), and the absence of these activators causes a fall in the trans-endothelial potential difference (Fischbarg, 1973). Davson (1949) proposed that the endothelium is essential in maintaining normal corneal thickness and transparency. It is known that changes in ionic balance causes swelling of the cornea, and in this regard, Langham and Kostalnik (1965), have demonstrated that ouabain causes a decrease in the corneal Na^+ - K^+ - ATPase activity and obviates the temperature reversal phenomenon. Thus, it seems that the cation pump aids the process of maintenance of ionic balance.

The presence of Na^+ - K^+ - ATPase activity in fish cornea, may be an indication of many different metabolic functions, and also that the active cation transport system seems to possess many properties similar to epithelial Na^+ - K^+ - ATPase (Bonting, 1965, 1970; Bonting et al., 1963).

In the lens, Na^+- K^+- ATPase has been found to have many basic metabolic functions (Bonting, 1965). Kinsey and Reddy (1965) as well as Bonting (1965) have suggested that Na^+- K^+ activated ATPase is mainly concerned with cation transport. Furthermore, Bonting (1965) assumes that one of these ions (potassium) is required for protein synthesis of the lens. The histochemical localisation of the Na^+ -K^+- ATPase on the epithelial cell membrane supports the

general opinion that the active cation transport mechanism of the lens is located in the epithelium, and that ion movement through the capsule is caused by passive diffusion (Bonting, 1965; Kinsey and Reddy, 1965). The energy required for the active cation transport is supplied by the high-energy phosphate bond mainly derived from anaerobic glycolysis (Kinoshita *et al.*, 1961; Mandel and Klethi, 1962).

The cation pump thus regulates the normal volume of the lens by actively excreting sodium from it. Conversely, when the enzyme of the membrane is inhibited or denatured, the potassium concentration of the lens is decreased, with a consequent increase of sodium. This results in an increase of water content of the lens, which leads to loss of transparency.

The Na^+- K^+- ATPase enzyme system appears to play a similar role or roles in bird ocular tissues as in other higher vertebrates. Moreover, ATPase in the epithelial layers of the cornea and lens of birds also requires Na^+, K^+ and Mg^{++} for activation, and its sensitivity to ouabain appears to fulfill all the criteria of the enzyme as described by Skuo (1964).

Since mucopolysaccharides also have a role in ionic balance as already discussed in the previous chapter, it might be pertinent to study the relationship between the enzyme activity and mucopolysaccharides.

Ascorbic acid

INTRODUCTION:

It is well established that the visual process is largely influenced by energy in the photoreceptor organs of animals. The generation of energy in biological systems is chiefly accomplished through oxidation – reduction reactions. This energy generation in vertebrate photoreceptors, particularly in the lens is affected by the process of reduction i.e., removal of hydrogen, and for which, a reducing factor should essentially be present in the visual system. (Goldschmidt, 1924)

Several authors like Glick *et al* (1936), Rosner *et al* (1938), Henkes (1946) etc have reported the presence of two such reducing agents viz., ascorbic acid and glutathione in the lens of some vertebrates. Pierie (1946), Pfister and Paterson (1977) have also reported the presence of ascorbic acid and glutathione in the corneal layers. Workers like Heath (1962), Heath and Fiddick (1965b) have reported the occurrence of ascorbic acid in the retina of several vertebrates. The occurrence of ascorbic acid in the vitreous body has been reported by Balazs (1961), Reddy and Kinsey (1960), Naumann (1959), Krueger *et al* (1959), while its presence in the aqueous humour has also been reported in rabbit by Podesta and Baucke (1938); in ox by Podesta and Baucke (1938), Johnson (1936); in man by Muller and Duschke (1934) and Purcell, *et al* (1954); in monkey by Kinsey and Jackson, (1949); in guinea pig by Muller and Buschke, (1934), Podesta and Baucke (1938); in sheep by Vladesco and Stefanesco, (1939); in cat by Langham (1950), and in rat by Muller and Buschke (1934). The presence of ascorbic acid has also been reported in the lens of rat, dog, guinea pig, rabbit, sheep, frog, pig, man, cow, and horse. It has also been reported in the compound eyes of the

honey bee, *Apis cerana indica* and the house fly *Musca domestica* (Dey and Raghuvarman, (1984 a, b).

The oxidation – reduction role of ascorbic acid in the calf corneal epithelium has been reported by Anderson and Spector (1971), and by Kulhman and Resnick (1959) in rabbits. Pierie (1946) has reported that the concentration of ascorbic acid is approximately 1.8μ moles per gram molecular weight of the cornea of ox. The high concentration of ascorbic acid in corneal epithelium indicate a preponderance in cells rather than in body fluids (Maurice and Riley, 1970).

Ascorbic acid, otherwise known as vitamin C is an antiscorbutic, water – soluble and heat labile compound. According to Heath and Fiddick (1970), in biological systems, apart from the free form, it is also found in the bound form or ascorbigen, and also as complexes with other macromolecules like proteins and nucleic acids (Chinoy and Buch, 1977).

Following the detection of high levels of ascorbic acid in the various ocular tissues of vertebrates, its importance in the visual function of animals has been the subject of close scrutiny or study by various workers, like Pierie (1965), Kinoshita (1964), Henkes (1946), Rawal and Rao (1977) etc, who have worked on different aspects like light – reactions in the eye, and also its distribution or percentage in different animals inhabiting different ecological niches and habitats. The importance of ascorbic acid in the visual physiology of vertebrates such as amphibians (*Rana hexadactyla*, *Bufo melanostictus,* birds *(Columba livia)* and mammals*(Cavia porcellus,Tephozous longimanus)* have been studied by Rawal

and Rao (1977).They have observed that animals like the amphibians living in well illuminated areas contain low levels of ascorbic acid in the lens, while those living in shaded or less lighted areas have a comparatively higher amount. Amongst mammals,the nocturnal bats show comparatively higher amounts of ascorbic acid in the lens. In higher forms ascorbic acid shows degradation except in birds,where it is lower than in amphibians and fishes but higher than in reptiles and mammals.

According to Sharma(1989),ascorbic acid is unusually high in the primate eye. The ascorbic acid level is higher in the lens and aqueous humour in diurnal mammals than nocturnal ones. Irrespective of the wide variations in the concentration of ascorbic acid between tissue and between species, the level has been found to be greatly reduced in the aging lens of all the species investigated so far. For instance, the rat and the cow differ in the lens-size, life span and average ascorbic acid content, but the animals show similar drops in ascorbic acid concentrations in the aging lens (Kuck, 1961)

However, a decrease in the normal levels of ascorbic acid in tissues is also due to other factors like physiological stress, pollution, infection and diseases and seasons (Chatterjee and Pal, 1975; Mauck, *et al.*, 1978; Lewin, 1974; Agarwal and Mahajan,1980).

Some recent works by Prabhakaran *et al* (1992) show that ascorbic acid has a role in the glycation and cross-linking of isolated lens crystallins. This view has also been validated by Chen *et al* (1998). Dreyer *et al.* (1993), have worked on the metabolism of ascorbic acid in biological system. Moreover, the effect of

ascorbic acid on active sodium transport and on iron uptake and storage in lens epithelial cells have been discussed by Hou *et al* (1998) and Goralska, *et al* (1998). Padh (1990) has worked on the cellular functions of ascorbic acid and has shown that it is involved in numerous biological functions.

In biological systems, apart from the free form, ascorbic acid is also found in the form of ascorbigen or bound form (Heath and Fiddick,1970 ;Guha and Gupta,1983),as complexes with other macromolecules (Chinoy and Buch, 1977) and also as co-factors and enzymes (Anderson and Spector, 1971). Therefore, an assay of free ascorbic acid alone, in tissue may lead to inaccurate inference (Isherwood and Mapson,1962)

Keeping the above mentioned views and findings in mind, histochemical and biochemical studies have been undertaken in the cornea and lens of the domestic chicken, *Gallus domestica* and the Indian blue rock pigeon, *Columba livia inntermedia* (Strickland)

MATERIALS AND METHODS:

The specimens were procured from the market and kept in the laboratory for one week. . The chicken used for the experiment is the broiler chicken (White leghorn) and the pigeon is the Indian blue rock pigeon, which is abundantly found in Shillong.

Histochemical method:

The histochemical test employed is the one according to Bacchus (1950). The tissues are separated from the animal and immersed in 5% silver nitrate with two drops of acetic acid per ml at 56°C for 30 minutes in darkness. The tissues are then washed in several changes of distilled water for 30 minutes and then treated with sodium thiosulfate for a further 30 minutes. The tissues are then washed in distilled water and transferred to 70% alcohol. This is followed by dehydration, clearing and infiltration-all in dark or subdued light. Sections are treated according to normal histological procedures and stained with toluidine blue and counter stained with eosin.

Biochemical method:

For the biochemical test, a modified method of Chinoy et al.(1976) has been followed .

As has been mentioned earlier, in biological materials, ascorbic acid not only exists in free form (AA), but also in a bound form which is also known as ascorbigen(ASG)(Guha and Paul .1936). It is also well known that ascorbic acid

is continually acted upon by a number of oxidising enzymes, including a specific peroxidase which catalyses the formation of its free radical (FR)-monodehydroascorbic acid (MDHA) (Gurevich,1963). Further, Chinoy (1967,1969), has reported that endogenous as well as exogenously added ascorbic acid forms a complex with macromolecules, in living systems.

Isherwood and Mapson (1962) have suggested that the actual concentration of ascorbic acid in a tissue represents the excess formed in synthesis, over that used in metabolism. Accordingly, they are of the view that any study regarding the concentration of ascorbic acid in its free form alone would lead to inaccurate inference. Taking all these into concentration, the estimation of ascorbic acid in the tissues studied has been done by the simultaneous determination of (i) free form of ascorbic acid (AA)(ii) bound form or ascorbigen (ASG) (iii) its enzymatic utilisation (AAU) and (iv) its complexing with other macromolecules (AA-MM complex).

Reagents:

Metaphosphoric acid (HPO_3) :

3% W/V solution (0.275M)

15% W/V solution (1.375M)

These solutions are refrigerated at 3°C.

Buffer solutions:

Buffer A (0.5M):10.55gm of citric acid dissolved in 1N NaOH and the volume made up to 100ml with a pH of 4.8.

Buffer B (1.5) : 31.65gm of citric acid dissolved in 3N NaOH and the volume made up to 100ml with a pH of 4.8.

Buffered HPO3 :

Three volumes of 3% HPO_3 were mixed with one volume of buffer A(pH 3.6).The buffered HPO_3 was prepared fresh for every experiment.

Standard ascorbic acid solution :

10mg of ascorbic acid was dissolved in glass double- distilled water, saturated with CO_2. Then the volume made up to 100ml and stored in an amber-coloured bottle at 3 - 5°C. Standard ascorbic acid solution was always prepared fresh.

Standard dye solution :

10mg of 2, 6- dichlorophenol- indophenol (BDH) was dissolved in de-ionised water at 80°C, cooled and the volume made up to 200ml.

Standard curve :

Ascorbic acid solutions of concentrations ranging from 10µg to 100µg/ml were prepared from the stock by diluting with CO_2- saturated glass double distilled water to the required concentrations.

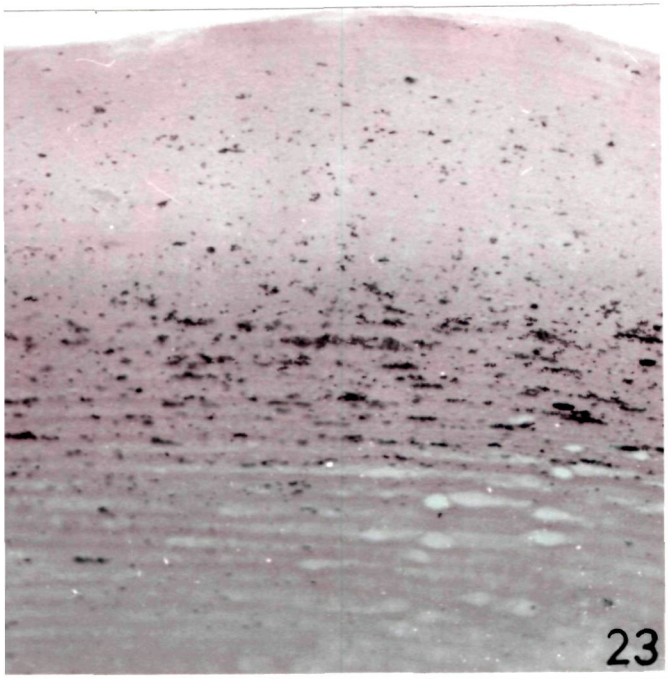
1ml aliquots of each ascorbic acid solution was mixed with 1ml of buffered HPO_3 (pH 3.6) solution- the pH remained stable following the addition

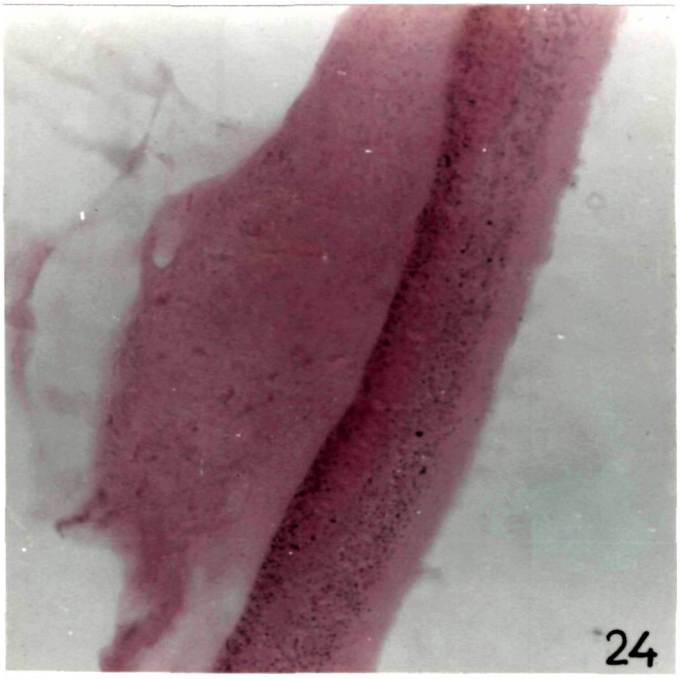
Plate 23. Lens of *Gallus domesticus* showing ascorbic acid as dark granules. 500x

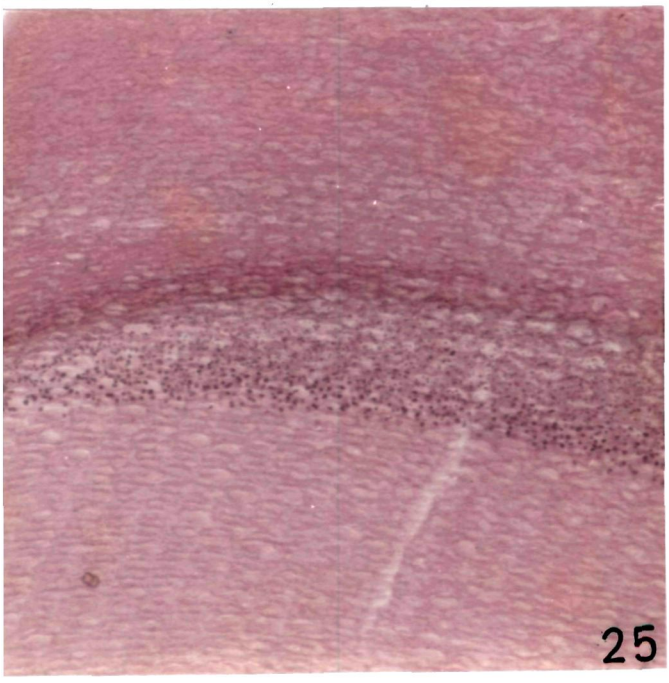
Plate 24. Cornea of *Gallus domesticus* showing ascorbic acid as dark granules. 500x

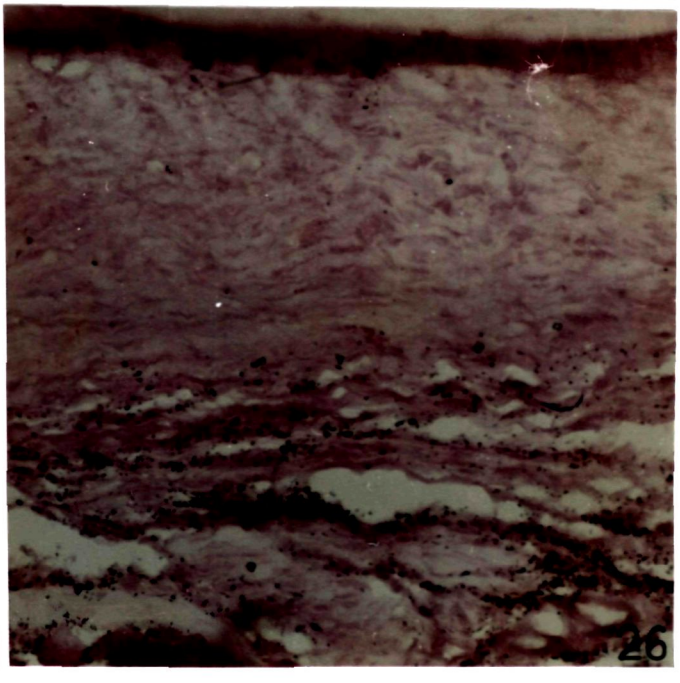
Plate 25. Lens of *Columba livia intermedia*, showing ascorbic acid as dark granules. 500x

Plate 26. Cornea of *Columba livia intermedia*, showing ascorbic acid as dark granules. 500x


23


24


25


26

of ascorbic acid due to high buffering capacity, and 5ml of the standard dye solution, and the reading taken in a Beckman (DU 640) spectrophotometer at 500nm. The readings were taken for each ascorbic acid solution, and a graph was prepared by plotting the values.

Extraction of materials :

The eyes were carefully dissected out and the cornea and lens carefully separated, weighed and separately homogenised in 1- 2ml of CO_2- saturated glass double distilled water with a pinch of purified silica. The contents were then transferred to test tubes. The mortar and pistil were rinsed with cold CO_2 saturated glass double distilled water thus making up the volume to 12ml and then divided into three parts of 4ml each for analysis.

(1) Determination of free ascorbic acid (AA) :

4ml of cooled buffered HPO_3 was added to 4ml of the original homogenate. After thorough mixing, it was filtered and 2ml of the filtrate was diluted with 5ml of glass double distilled water and the spectrophotometer adjusted to zero to compensate for the turbidity factor. Then 5ml of the standard dye solution was added to another 2ml of the remaining filtrate and the reading noted.

The concentration of ascorbic acid in 1ml of the original extract was calculated as follows-

As 2ml of each aliquot contains 1ml of the original homogenate, the concentration of ascorbic acid per gram fresh weight of the material is given by-

$$A = \frac{a \times V}{W} \times 100$$

Where A = ascorbic acid content of the samples in μg/g fresh weight.

a = ascorbic acid in μg/ml of the original homogenate.

V = total volume of the original homogenate.

W= weight of the sample taken for analysis (in mg)

(2) Determination of ascorbigen (ASG)

2ml of 15% HPO_3 was added to 4ml of the original homogenate and the mixture heated in a water bath at 75°C for 15 minutes for hydrolysing ascorbigen. After cooling, the mixture was buffered by adding 2ml of 1.5M citric-NaOH buffer (pH3.6), thus increasing the volume to 8ml. The mixture was then filtered and the reading noted as before.

The concentration of ascorbic acid in 2ml of the buffered hydrolysed extract was determined from the standard graph. Then, the value of free ascorbic acid in 1ml of the homogenate, which had been previously determined, subtracted from it to obtain the ascorbic acid equivalent of ascorbigen in 1ml of the homogenate.

Ascorbigen content per gram fresh weight of tissue was calculated as follows-

$$B = \frac{V(b-a)}{W} \times 1000$$

Where, B = ascorbic acid equivalent of ascorbigen in μg/g fresh weight of the sample.

V = total volume of the original homogenate.

b = ascorbic acid (μg) in 2ml of buffered hydrolysed extract.

a = ascorbic acid (μg) per ml of original homogenate.

W= weight of the sample taken for analysis (in mg)

Now, to the remaining 4ml of the original homogenate, 4ml of ascorbic acid solution (100μg/ml) is added and incubated at 30°C ± 2°C with thorough shaking for 10 – 20 minutes and filtered.

(3) Determination of ascorbic acid utilisation (AAU)

3ml of buffered HPO_3 was added to 3ml of the filtrate and the reading noted. The ascorbic acid content of 2ml of aliquot of the buffered solution (i.e. 0.5ml of the original homogenate) was determined from the standard graph. The value of ascorbic acid in μg, thus obtained, was multiplied by 2 to obtain the value of ascorbic acid per 1ml of the original homogenate left unutilised after incubation.

The calculation of AAU per gram from weight is as follows-

$$C = \frac{V(a + 100) - 2c}{W} \times 1000$$

Where, C = ascorbic acid (μg) utilised per gram from weight during a given period of incubation.

a = ascorbic acid (μg/ml) of the original homogenate.

c = amount of ascorbic acid (μg) left over in 2ml of the buffered–incubated solution (i.e. 0.5ml of the original homogenate)

W= weight of the sample taken for analysis (mg)

(4) Determination of AA-MM complex

From the remaining 5ml of filtrate, 4ml was taken and 2ml of 15% of HPO_3 was added. The mixture was hydrolysed, cooled and buffered with 2ml of buffer solution B i.e., 1.5 M citric acid-NaOH buffer (pH3.6), thus bringing the total volume to 8ml. The reading was noted as before.

The ascorbic acid content in the aliquot was estimated from the standard graph and multiplied by 2. Subtracting the value of ascorbic acid left over in 1ml of the original homogenate before hydrolysis from the above value, gives the amount of ascorbic acid released by hydrolysis of AA-MM (μg/ml).

The amount of ascorbic acid complexing with macromolecules per gram fresh weight is calculated as follows –

$$D = \frac{2V(d-c)}{W} \times 1000$$

Where, D = ascorbic acid (μg) released from the complex per gram fresh weight of the tissue

V = total value of the original homogenate

d = ascorbic acid (μg) in 2ml of the incubated hydrolysed buffered solution (i.e., in 0.5ml of the original homogenate)

c = amount of ascorbic acid (g) left over in 2ml of the buffered-incubated

solution (i.e., 0.5ml of the original homogenate).

W = weight of the sample taken for analysis.

OBSERVATION:

The principle of the histochemical tests for the detection of ascorbic acid in biological systems, is based on the fact that silver nitrate reduces ascorbic acid in tissue sections and produces a characteristic pattern of black granules scattered in the region, where ascorbic acid is present. Barnett and Bourne (1941), on the basis of available evidences have stated that in vertebrates it is "justifiable to assume that the reactions observed are unlikely to be due to reducing substances other than ascorbic acid".

Barnett and Fisher (1943) raised some doubts or had some misgivings about the efficiency of this method, but their doubts were answered by Bourne (1944) who has corroborated the earlier findings of Barnett and Brown (1941).

In the present study, the corneal epithelium and endothelium of both *Gallus domesticus* and *Columba livia intermedia* (Strickland), gave positive reactions i.e., dark ascorbic granules could be seen, but no reaction has been observed in the corneal stroma (Plates 24 & 26). However, the granules have been found to be more dense in the case of *Gallus domesticus*. This has been validated by the biochemical evidence (Figs. 5 - 8). The lens, on the other hand show the granules being predominant on the anterior portion. Further more, the granules are seen to be denser in *Gallus domesticus* (Plates 23 & 25) This has also been detected biochemically (Figs. 5 - 8).

Fig.5. Histogram of free ascorbic acid (AA) in the lens and cornea of *Gallus domesticus* and *Columba livia intermedia.*

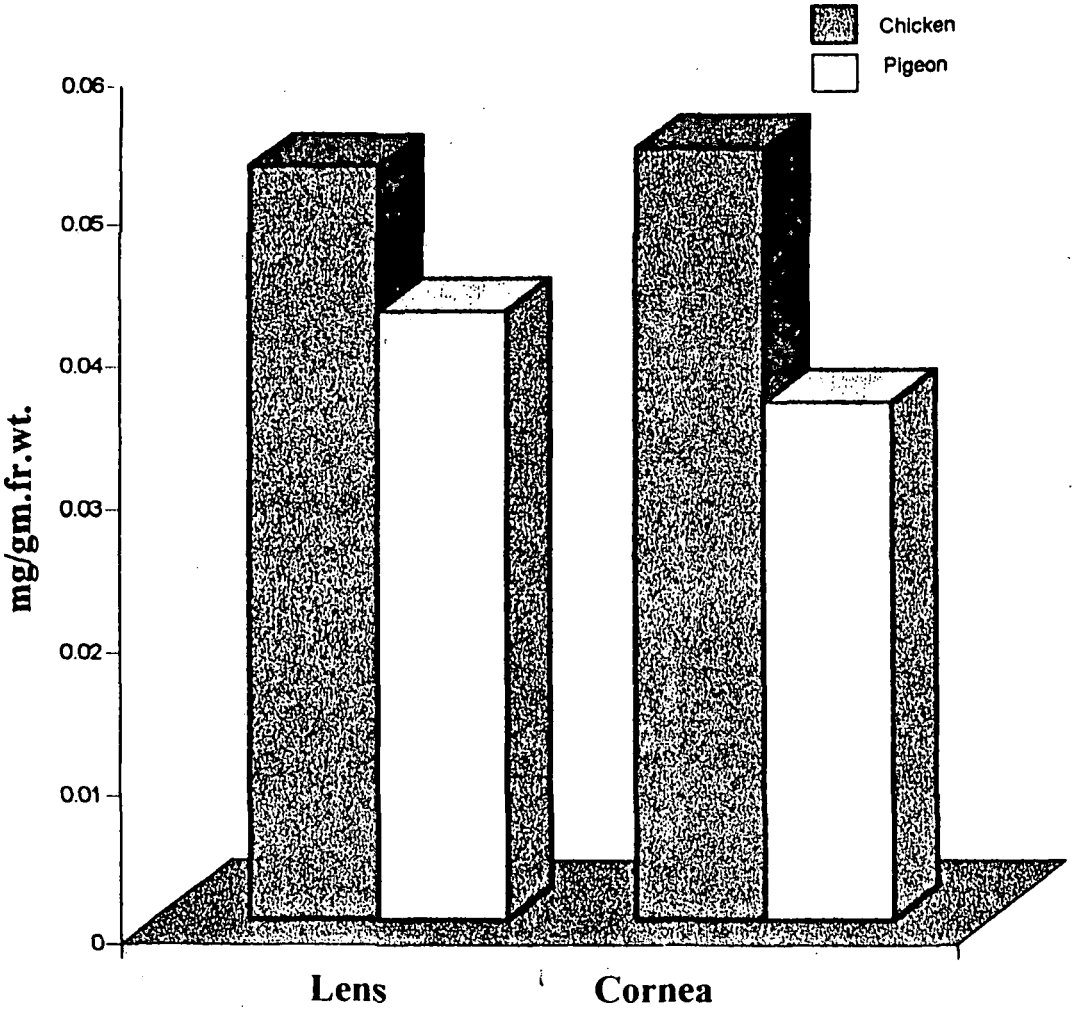

Chicken
Pigeon
0.06
0.05
0.04
0.03
0.02
0.01
0
mg/gm.fr.wt.
Lens
Cornea

Fig.7. Histogram of ascorbic acid utilisation (AAU) in the lens and cornea of *Gallus domesticus* and *Columba livia intermedia.*

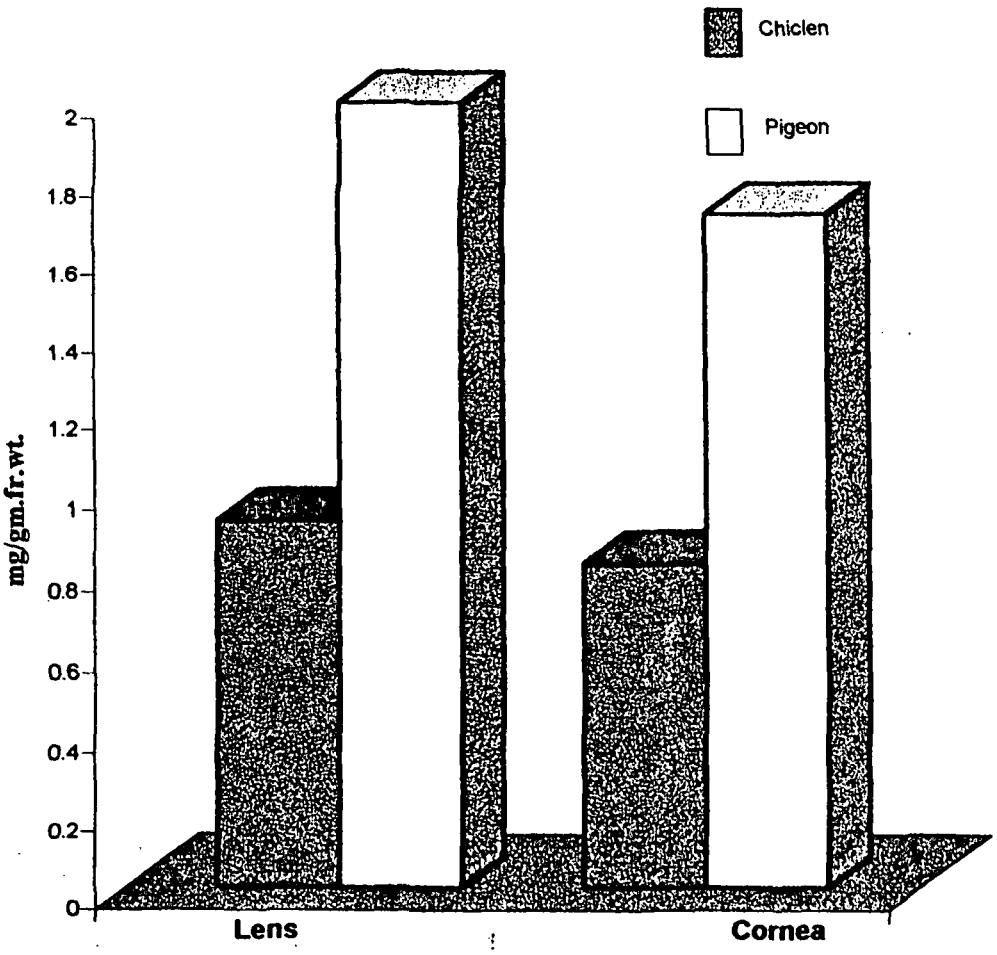
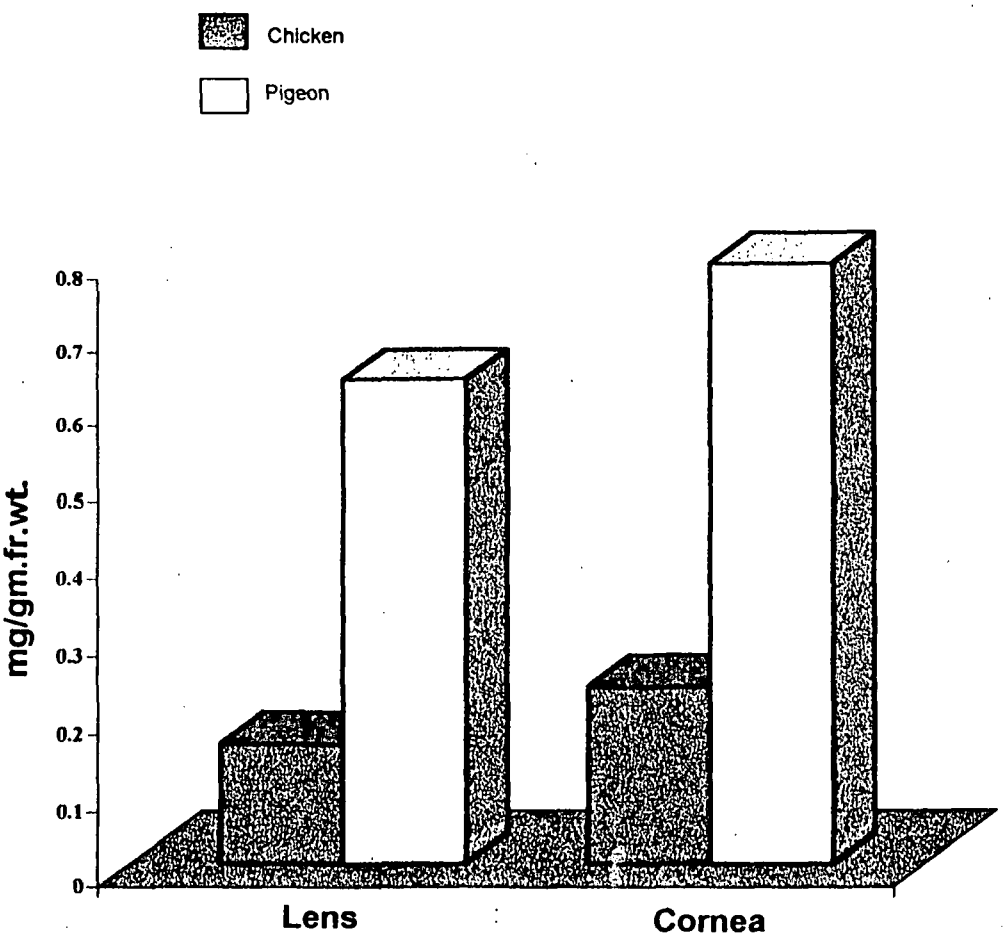

Chiclen
Pigeon
mg/gm.fr.wt.
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Lens
Cornea

Fig.8. Histogram of complexed form of ascorbic acid (AA - MM) in the lens and cornea of *Gallus domesticus* and *Columba livia intermedia.*


Chicken
Pigeon
mg/gm.fr.wt.
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Lens
Cornea

The histochemical detection has been further substantiated or confirmed by biochemical estimation of ascorbic acid (turnover) in the cornea and lens i.e., the free form (AA), bound form or ascorbigen (ASG), its utilisation (AAU) and its complexing with other macromolecules (AA- MM) (Figs. 5 - 8).

The auto-oxidation of ascorbic acid is a major stumbling block or source of error when it comes to studying ascorbic acid concentration in any biological system. Chinoy *et al.* (1976) have devised a fairly simple method to prevent this auto-oxidation. They have used glass-distilled water saturated with CO_2 for the extraction and preparation of ascorbic acid solutions. They have found that auto-oxidation of ascorbic acid is rapid at 30°C, but when the glass double distilled water is cooled to 3°C-5°C, the rate of auto-oxidation slows down and is effective upto 180 minutes. But when the glass distilled water is saturated with CO_2, the auto-oxidation is completely checked at both 30°C as well as 3°C - 5°C.

Another possibility of error in the estimation of ascorbic acid is with regards to the dye 2,6-dichlorophenol indophenol. According to Karrer, (1950), 2,6-dichlorophenol indophenol solution is very unstable at low pH (which is characteristic of indophenol dyes), decomposing to give quinones and aminophenols. Chinoy *et al.* (1976) have further reported that unbuffered HPO_3 rapidly decolourises the dye – the rate of decolourisation being proportional to the concentration of HPO_3 present in the system. They could effectively stabilise the dye by buffering it with citrate-NaOH buffer at a pH of 3.6.

For the determination of ascorbigen (ASG) and AA-MM complex, hydrolysis was carried out at 75°C for 15 minutes, which would normally lead to a loss of ascorbic acid. But this was checked by the use of 15% metaphosphoric acid in the system. The addition of 15% HPO_3 is preceded by the addition of buffer solution, which effectively brings the pH level to 3.6 when mixed in the ratio of 1:1.

All the interfering substances other than ascorbic acid were checked by carrying out the estimation in a strong acidic medium. Thus, it can be concluded that the dye reducing property observed in the corneal and lens homogenates of the two birds, is most likely to be due to the presence of ascorbic acid in these tissues.

DISCUSSION:

No scientifically satisfactory description or explanations about the physiological role of ascorbic acid have been put forward till date. The presence of ascorbic acid in all eukaryotic organisms suggests fundamental roles, even though they are not clearly and completely understood (Seib and Tolbert, 1982).

However, the detection of ascorbic acid in the cornea and lens of *Gallus domesticus* and *Columba livia intermedia* (Strickland), in the present investigation, confirms the earlier findings of ascorbic acid levels in the eyes of other vertebrates by various authors, as has been already mentioned at the beginning of this chapter. But compared to the amount of work done on ascorbic acid of lens, not much has been done on the corneal tissues.

Ascorbic acid is one of the most important sugar acids and a very unstable compound, readily undergoing oxidation to dehydroascorbic acid, and all higher species appear to employ ascorbic acid as a co-factor in certain specific reactions (Lehninger, 1972). It has been postulated that monodehydroascorbic acid, a stable and free radical anion, is the intermediate in the oxidation of ascorbic acid by a metal ion. Stability of this radical anion and its conversion to dehydroascorbic acid and ascorbic acid helps to explain the antioxidant role that ascorbic acid plays in biological systems (Seib and Tolbert, 1982).

The role of ascorbic acid in collagen formation has been reported by Mayes, (1988). Ascorbic acid is also essential for the formation of intercellular cement, and since imperfect formation of cement substance leads to defective synthesis of connective tissue, the role of ascorbic acid assumes great importance.

Harper et al. (1979) reported that imperfect formation of the cementing substance is caused by the failure of collagen to synthesise, which is due to a deficiency in the enzyme, proline hydrolase, which converts proline to hydroxyproline.

Electron microscopic observations have revealed cellular atrophy and damage of nerve cells due to hypovitaminosis C (Sulkin and Sulkin, 1975). This has also been recently corroborated by Malik et al. (1995).

Insects, invertebrates, fishes and certain bats and birds cannot synthesise ascorbic acid (Chatterjee, *et al.* 1975), and consequently the eye takes up ascorbic acid by an energy-dependent active transport mechanism (Nicola et al. 1968; Sharma et al.; 1964). Interestingly, Omaye *et al.* (1982) have also reported that, ascorbic acid is also taken up by several tissues by an energy-dependent and Na+-sensitive process, and which, according to Cole (1970) might also, possibly play some role in the active transport of ascorbate across the ciliary epithelium.

Pirie (1946) has shown that in ox and rabbit, the ascorbic acid content is higher in the corneal epithelium than the stroma, which originates endogenously, and it has been observed by Pfister and Paterson (1977) as well as Pfister *et al.* (1978) that ascorbic acid reduces the ulceration of cornea following alkali - induced burn in rabbit.

An important role of ascorbic acid i.e., the oxidation of NADPH has been established by Anderson and Spector (1971). The oxidation of NADPH to NADP is accomplished through ascorbic acid and glutathione oxidation-reduction system with consequent production of hydrogen peroxide in calf cornea and the process is catalysed by two enzymes-dehydroascorbic acid reductase and glutathione

peroxidase. The NADPH is produced by the hexose monophosphate shunt during the process of glucose metabolism in the corneal epithelium of bovines and rabbits which has been studied by Kinoshita *et al.* (1955) and Kinoshita (1964), and Kuhlman and Resnich (1959). Another important function of ascorbic acid is the inhibition of 3,5-cyclic AMP phosphodiesterase and thereby stimulating ion transport in the corneal epithelium and other ocular tissues of frogs and rabbits (Buck and Zadunaisky, 1975). They have found that ascorbic acid increases the short circuit current of the isolated cornea of the rabbit and frog and also that, the inhibitory effect of ascorbic acid on the 3,5-cyclic AMP phosphodiesterase activity causes an increase in the cyclic AMP level in the corneal epithelium. This increase in cyclic AMP content in the corneal epithelium possibly enhances active ion-transport across the cornea.

McLaren (1970) has observed that the lens has a higher concentration of ascorbic acid in the cortex than the nucleus and that, the level falls rapidly in a state of deficiency. In this regard, Horning (1975) has suggested the association of ascorbic acid with the metabolism of the lens. Verma *et al.* (1979) reported that in rats, ascorbic acid prevents light - induced damage to the lens cation pump *in vitro.* Heath (1962) has also observed that the concentration of ascorbic acid is higher in the lens than in the aqueous humor and that it is necessary for maintaining the normal level of ascorbic acid in the aqueous and vitrous humuors of the cornea.

Kinsey and Jackson (1949) relates the high concentration of ascorbate in the eye with the regression of the hyaloid system and it thus, might account for

the interspecies differences. According to Sharma (1989), the high concentration of ascorbic acid in the ocular tissues is maintained by an active transport of ascorbate from the plasma across blood or aqueous barriers. The relatively higher content of ascorbic acid in the eyes than other tissues is a good indication of its importance in vision and the difference in ascorbic acid concentration between the eye lens and other tissues may be due to an efficient mechanisms operating in the eye which assures retention or uptake of this vitamin in the eye than in other organs. Hughes *et al.* (1971), have observed that the brain and ocular lens retain a high ascorbic acid level even in the absence of a dietary source of this vitamin, while other tissues show total depletion. They observed this by maintaining guinea pigs in an ascorbic acid free diet for 14 days, followed by its estimation in various tissues.

Hughes *et al.* (1971) have further suggested that the function of ascorbate may be of vital importance to the eye and thus leading to maintenance of its level as long as possible. In this connection, it is worthwhile to mention the findings of Kinsey (1947) as well as Barany and Langham, (1955) who stated that the lens as well as the aqueous humor of various animals retains a high concentration of ascorbic acid by a mechanism of active transport from the blood.

The lens maintains a high level of reducing agents such as ascorbate and glutathione to maintain a high energy demand. This high concentration of ascorbic acid suggest that in addition to some co-enzymatic functions, ascorbate may also modulate some non-enzymatic metabolic reactions in the lens. Its most probable action is the prevention of unwanted reactions initiated by O_2 and free

radicals, either photochemically or under ambient non-photochemical conditions. Sharma (1989) has also suggested that ascorbate terminates the propagation of various free radical reactions in oxidation, photolysis and radiolysis.

Some biochemical reactions involving glutathione and ascorbic acid, which may be relevant to, lens metabolism has been revealed by Kinoshita (1964). It is known that the pentose phosphate pathway is the main source of energy of the lens where $NADP^+$ is made available for the enzymes of the pathway through the respiratory link between ascorbic acid and glutathione. Rawal and Rao (1977) suggested that the relatively high concentration of ascorbic acid and glutathione in the normal lens of some forms is due to their high energy demand and is significant for the maintenance of lens transparency as suggested by Daisley (1955).

In this context, it is noteworthy to mention that in our study, the level of free ascorbic acid and ascorbigen in the pigeon have been found to be significantly lower than in the chicken and consequently its utilisation and complexing with other macromolecules is higher than in chicken. This difference in ascorbic acid turnover between the two species may be inferred to be due to adaptation to different ecological niche or environments.

In the present study, while studying the utilisation of ascorbic acid, it has been observed that a portion of the ascorbic acid of the aliquot incubated for ascorbic acid utilisation, form complexes, presumably with macromolecules instead of being oxidised. This bound ascorbate, can however be recovered by

hydrolysing with HPO_3. This complexing ability of ascorbic acid is responsible for the formation ascorbigen or bound form of ascorbic acid.

Complexing of ascorbic acid with other macromolecules have been reported from time to time (Chinoy, 1967, 1969; Chinoy *et al.* 1971, 1973, 1974). Bonner (1957) has reported that ascorbic acid is acted upon by a number of enzymes, which oxidise ascorbic acid directly or indirectly, and over and above this oxidation, it forms complexes with macromolecules, such as proteins and nucleic acids. Szent-Gyorgyi (1960) has suggested that such complexing may lead to the formation of charge transfer complexes, which have been reported to occur in biological systems very frequently, and that they actively take part in the energy transfer process.

Taking into consideration all the above mentioned reports and correlating the findings of the present study, it can be reasonably assumed that ascorbic acid might be equally significant in the visual processes of the two birds studied.

Pigment migration

INTRODUCTION:

Vision is a special somatic efferent sense. It is necessary for the eyes to be adapted to various stages of illumination for maximum efficiency. A characteristic feature of this adaptation is the movement and migration of screening pigments in the eye.

The best known of all pigments of the visual system is "melanin", produced in the melanophores, which are pigment cells, or chromatophores (Bagnara et al. 1978). The term "melanin" is a generic name that actually includes the black or brown "eumelanins" and the "phaeomelanins" i.e., those that are yellow or red in colour (Prota, 1980). Melanin is the structural pigment of the skin, hair, many feathers, scales and eyes.

The biological significance of melanin is that, they absorb light throughout the visible spectrum as well as the UV spectrum or range. The melanin granules prevent reflection of transmitted light (Lythgoe, 1979). The retinal pigment granules in the pigment epithelial cells exhibit distinct movements during light and dark adaptations, and thereby help in the process of visual adaptation in various photic levels. Several different methods of visual adaptations are known – some are characteristic of particular animal groups though a particular species normally possesses more than one method. According to Lythgoe (1979), the various adaptive methods can be divided into three classes: (i) optical regulation of the light reaching the visual pigment (melanin) through the pupil (ii) absorption by the visual pigment and (iii) neural processes.

The melanin granules as well as the rods and cones themselves, move in response to changes in light intensity in such a way that particular cell types are shielded from unwanted light. These changes are collectively known as "retinomotor" or "photochemical" movements. According to Lythgoe (1979) these movements appear to be phylogenitically older than pupillary movements, and usually one or the other mechanism is well developed in a particular class of animal. The phenomenon of retinomotor movements was first studied, independently by Boll (1877) and Kuhne (1877) and has been reviewed by Blaxter (1970). It is known that retinomotor movements are best-developed in birds, fishes and frogs. The rods and cones as well as the pigment granules within the epithelial cells of the pigment epithelium may move in reponse to changing light intensities, although the underlined mechanisms are not known. The myoids and ellipsoids of the rods and cones contract or elongate in response to changes in light intensities, and these movements are almost coincident with the migration of pigment granules within the cells of the pigment epithelium (Lythgoe, 1979).

During light-adapted state, the pigment granules are dispersed fairly evenly through the pigment epithelium and the rod myoids expand so that the pigment granules surround the rod outer segments (Anctil et al., 1980; Klyne and Ali, 1980). In dark-adapted state, the pigment granules within the epithelial cells surrounding the rods and cones move to the back of the retinal pigment epithelium. Simultaneously, the rod myoids may contract resulting in the movement of the rods away from the pigment epithelium. These movements of the rods, cones and retinal pigment granules are not exactly synchronised, and in

some cases either pigment migration or rod and cone migration proceeds in the absence of the other. Movements during light adaptation are much faster than the movements during dark adaptations, which are slower and require times to the order of an hour to complete (Walcott, 1975).

The most familiar components of light and dark adaptations are changes in concentration of visual pigments and, modifications in neural 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 fully light- adapted to the fully dark- adapted retina, the neural organisation of the retina changes. The state of dark adaptation involves rods, much summation and no colour vision, while light adaptation involves cones, little summation and colour vision.

The time duration for the different stages of adaptations is different – the initial stages of adaptations being extremely rapid, taking less than one-fifth of a second for completion, while the later stages of adaptation proceed more slowly. Perhaps the whole neural organisational changes (from brightness to darkness and vice-versa) take place within a time span of thirty minutes (Lythgoe, 1979). In teleosts, inspite of pronounced positional changes in the retinal pigment and visual cells during light and dark conditions, there is a difference in photochemical movements due to photic stimulation (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) also suggested that hormones might influence pigment

migration. Bagnara and Hadley (1969) believed that in all probability intermedin is the agent involved in melanophoric response in the skin of fishes.

The involvement of ions in the migration of pigments has been suggested by a number of workers (Freeman *et al.*1968; Veerdonk, 1962; and Novales, 1959). According to them, transmembrane ions induce pigment migration through intermedin. In addition, the involvement of colchicine, cyclic AMP and 5-Hydroxytryptamine in retinomotor responses have been reported by Anctil *et al.* (1979), Bitensky *et al.* (1973) and Kato *et al.* (1982) and Allen and Burnside, (1986). Veron (1973) and Dey (1980) have suggested the effect of photopic and scotopic states on neurosecretion and their roles in pigment migration in insects. Deb (1990) has also attributed photopic and scotopic states on the phenomena of neurosecretion and pigment migration in the case of fishes.

Although various roles of neurosecretion in birds have been described, reports on the relationship between light and dark states and neurosecretion are lacking. Keeping the above points in view, the effect of 5-HT, colchicine and cyclic AMP on pigment migration in the retina, and the effect of light and dark conditions on neurosecretory cells have been investigated in *Gallus domesticus* and *Columba livia intermedia* (Strickland).

MATERIALS AND METHODS:

STUDIES ON LIGHT AND DARK ADAPTATIONS:

Two groups of experimental birds were selected. One group was kept in sunlight for three hours for light adaptation, while the other group was kept in darkness for the same period for dark adaptation. After the completion of three hours the birds were decapitated and the eyes removed and fixed in 10% buffered formalin or Bouin's fluid until required. Then routine histological preparations were carried out for paraffin embedding method and 7 - 8μ thick sections cut for microscopic preparations and study.

In the case of the dark -adapted eyes, all the preparations were carried out in light- proof vials, since pigment migration might take place when exposed to light (Ali, 1964).

EFFECT OF LIGHT AND DARK ON NEUROSECRETION:

Two groups of birds were light- and dark-adapted for three hours each. After the required period of adaptation, the birds were decapitated, holes drilled on the heads and fixed in alcoholic Bouin's fluid for twenty four hours for penetration of the fixative. Then the brain was taken out and again fixed in the fixative for another twenty four hours. This was followed by routine histological methods and 10μ thick sections cut. The sections were stained in paraldehyde-fuchsin- one step trichrome (Gabe, 1966).

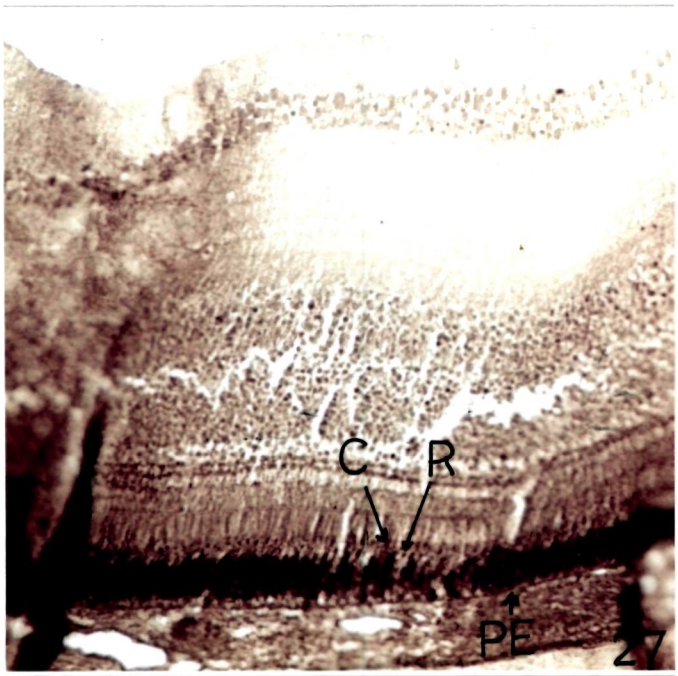
Plate 27. Light – adapted retina of *Gallus domesticus.*

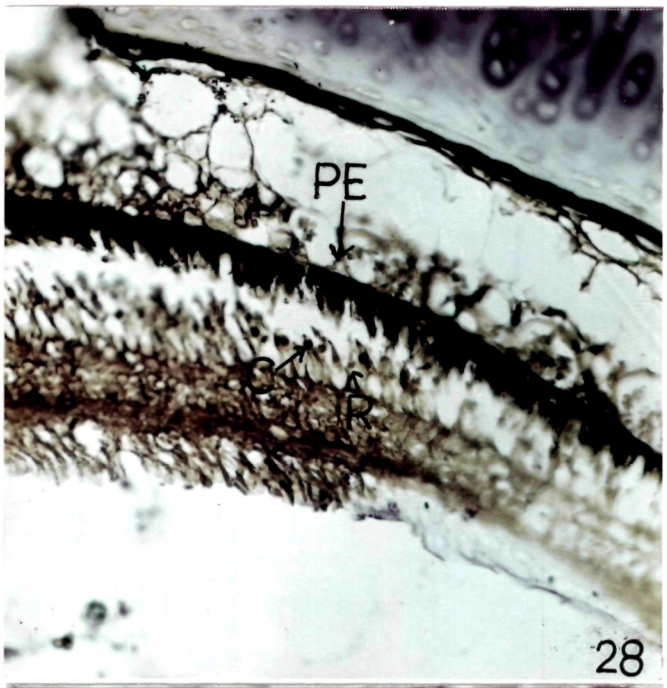
Plate 28. Dark – adapted retina of *Gallus domesticus.*

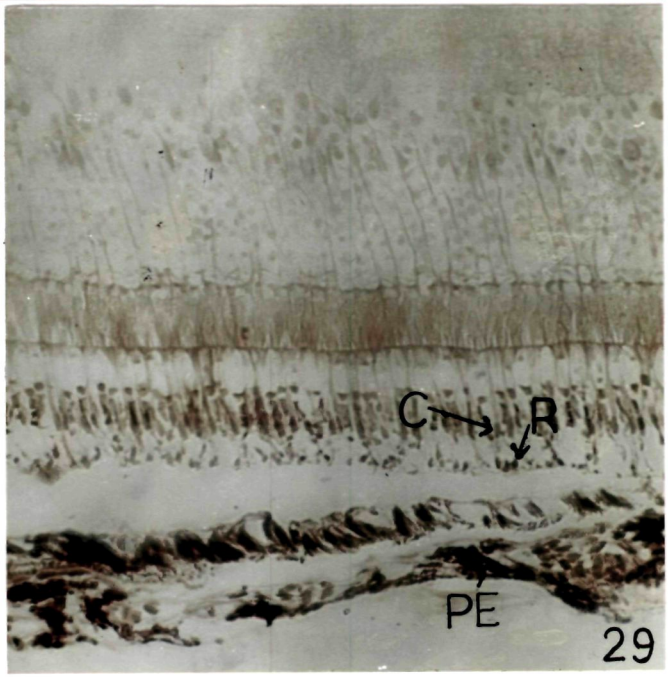
Plate 29. Light – adapted retina of *Columba livia intermedia.*

Plate 30. Dark – adapted retina of *Columba livia intermedia.*

R = Rod
C = Cone
PE = Pigment epithelium.


C
R
PE
27


PE
C
R
28


C
R
PE
29

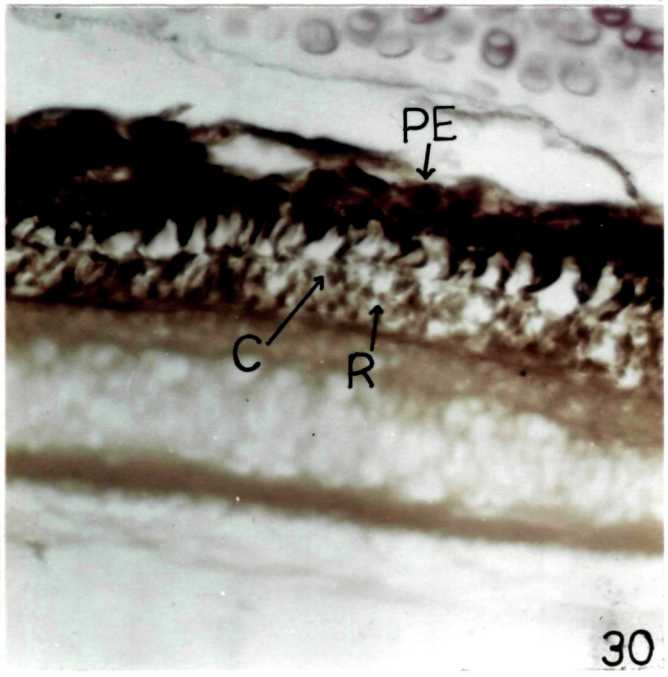

PE
C
R
30

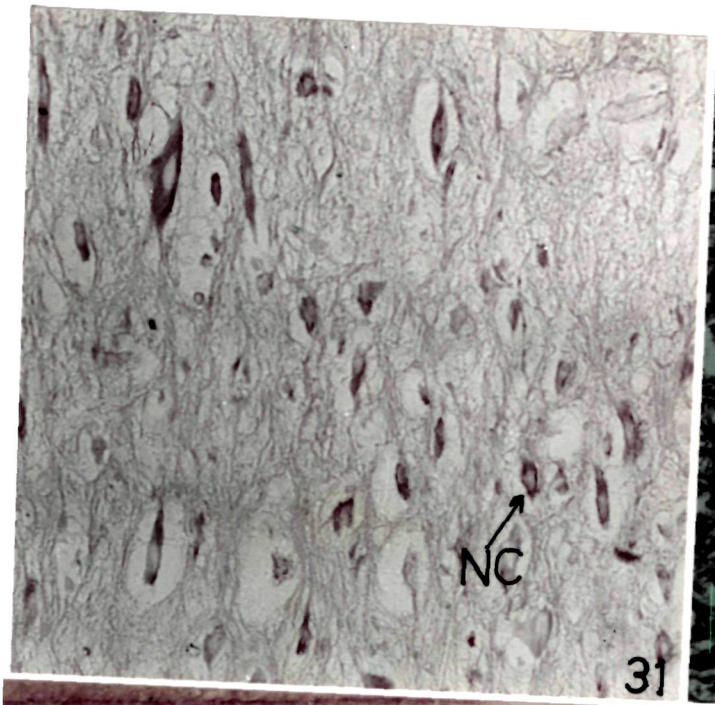
Plate 31. Light – adapted optic lobe of *Gallus domesticus,* showing reduced content of neurosecretory cells. 500x

Plate 32. Dark – adapted optic lobe of *Gallus domesticus,* showing increased content of neurosecretory cells. 500x

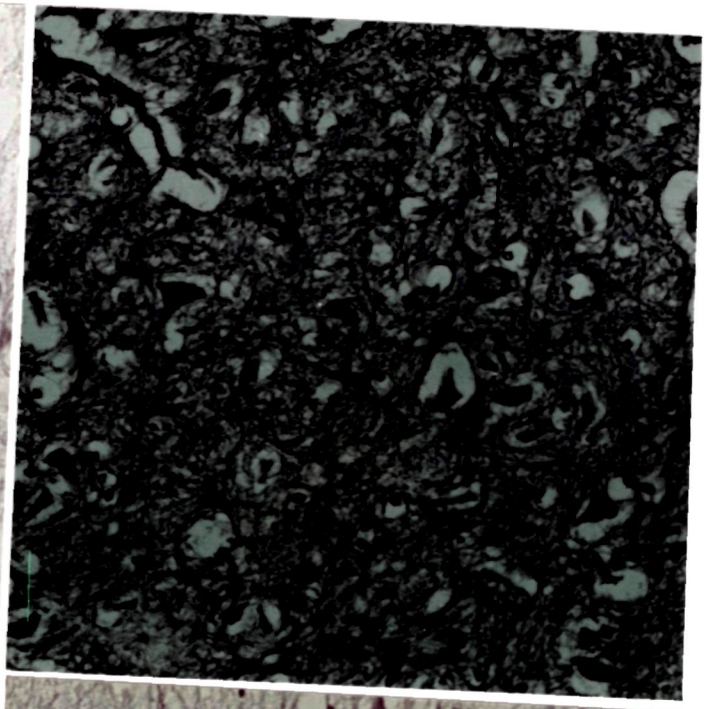
Plate 33. Light – adapted optic lobe of *Columba livia intermedia,* showing reduced content in the neurosecretory cells. 500x

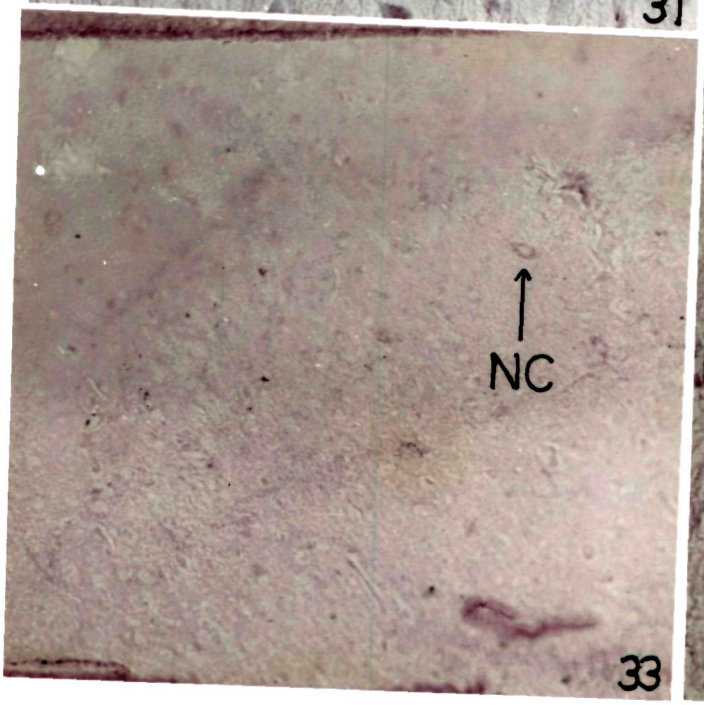
Plate 34. Dark – adapted optic lobe of *Columba livia intermedia,* showing increased content in the neurosecretory cells. 500x

NC = Neurosecretory cells.

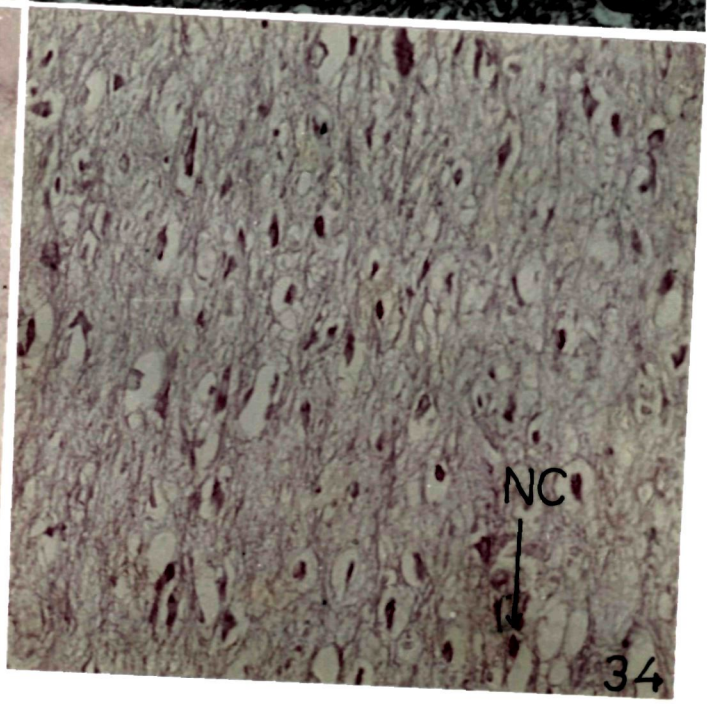


31





33



34

EFFECT OF 5-HT, cAMP AND COLCHICINE:

Each of the above mentioned drugs were injected intra-ocularly by microsyringe to dark-adapted animals only, to study their effect on retinal pigment (melanin) migration. The concentrations of 5-HT and cAMP are 0.8mM each while the concentration of colchicine is 0.3mM. Each of the drugs were dissolved in cold-blooded Ringer's solution.

OBSERVATIONS:

The positions of the pigment granules during light and dark adaptations in *Gallus domesticus* and *Columba livia intermedia* (Strickland) are shown in the photoplates numbered 27 – 30 respectively.

EFFECT OF LIGHT AND DARK ON NEUROSECRETORY SYSYTEM:

In both the dark adapted bird species, a large accumulation of compact and purple neurosecretory substances in the in the neurosecretory pericarya has been observed (Plates 32 & 34).

Conversely, in the light-adapted birds, a significant reduction of neurosecretory materials has been observed, presumably due to axonal transport (Plates 31 & 33).

EFFECT OF 5-HT, cAMP AND COCHICINE:

Light microscopic studies of paraffin sections of dark-adapted eyes revealed that in all cases, almost complete migration (dispersion) of retinal

Plate 35. Dark – adapted retina of *Gallus domesticus*, injected with 5 – HT, showing pigment dispersion similar to the light – adapted state. 500x

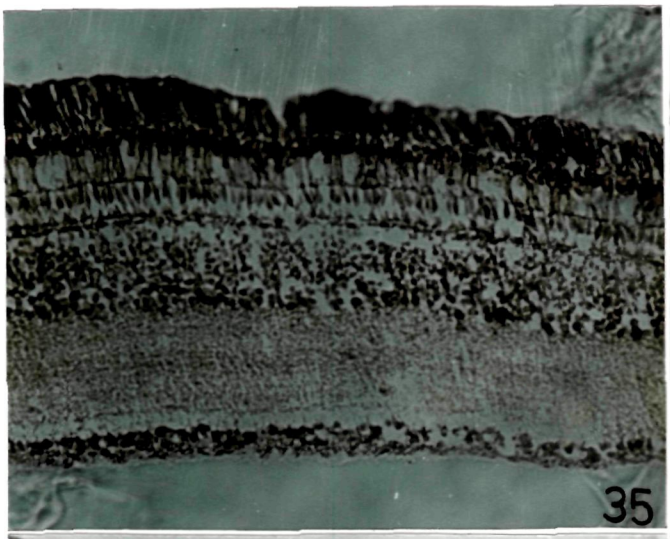
Plate 36. Dark – adapted retina *Columba livia intermedia*, injected with 5 – HT, showing pigment dispersion similar to light – adapted state. 500x

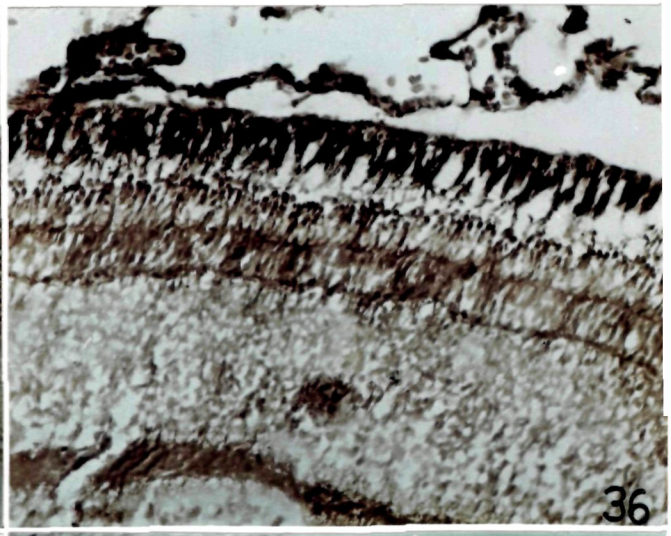
Plate 37. Dark – adapted retina of *Gallus domesticus*, injected with cAMP, showing pigment dispersal similar to light - adapted state.500x

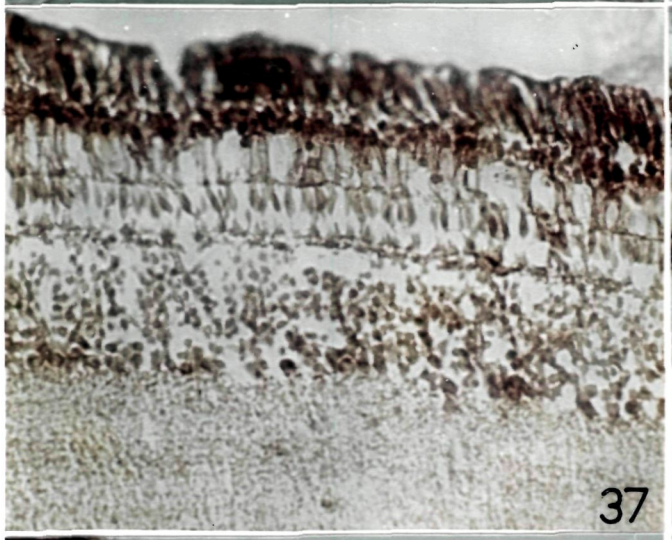
Plate 38. Dark – adapted retina of *Columba livia intermedia*, injected with cAMP, showing pigment dispersal similar to light – adapted state. 500x

Plate 39. Dark – adapted retina of *Gallus domesticus*, injected with colchicine, showing pigment dispersal similar to light – adapted state. 500x

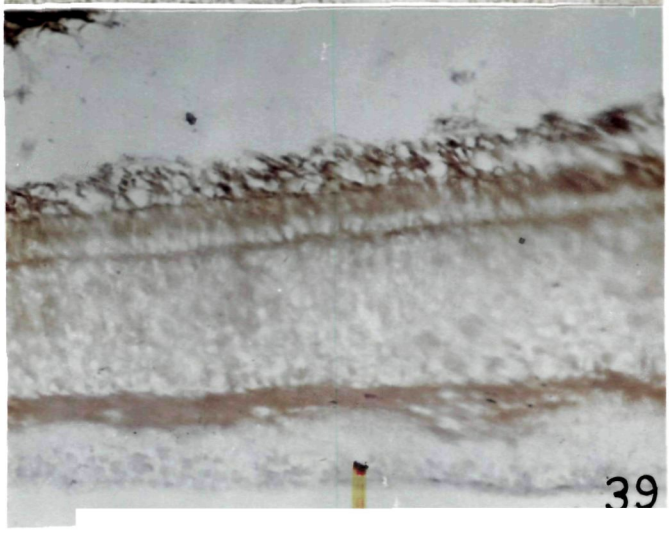
Plate 40. Dark – adapted retina of *Columba livia intermedia*, injected with colchicine, showing pigment dispersal similar to light – adapted state. 500x

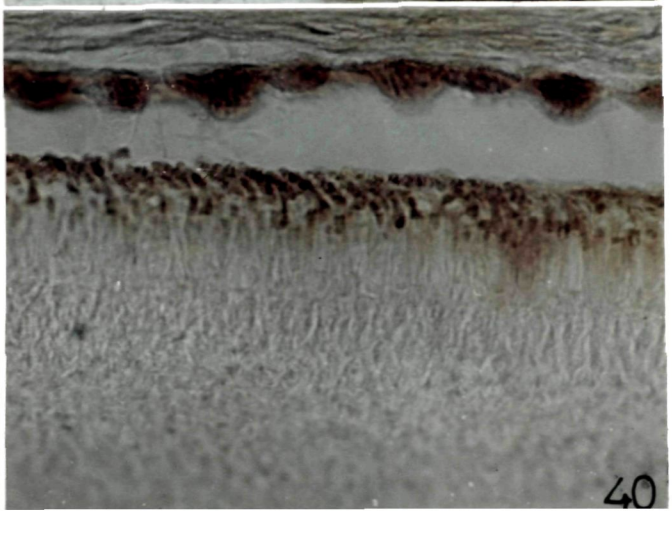

35


36


37


38


39


40

pigment granules similar to controlled light adapted eyes occurs i.e., after treatment with 5-HT, cAMP and colchicine. It appears that pigment migration is more intense in the treated eyes (Plates 35 - 40), and total masking of visual cells can be seen.

DISCUSSION:

The arrhythmic eye, which performs well over a wide range of light intensities, has several adaptive mechanisms associated with activity both during day and night. These adaptive mechanisms are the primary devices, which control the amount of illumination reaching the photosensitive cells. 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 (i) the changes in length and shape of photoreceptors that are brought about by changes in the lighting conditions of the environment (Ferrero *et al.* 1979) and (ii) the dispersion or concentration of pigment granules (Ali, 1975b).

At night , acuity is sacrificed for sensitivity and light is collected from many angles to excite the receptor cells. During darkness, the maximum available light should impinge on the receptors, but during the day, when illumination is adequate, another significant problem arises with regards to resolution of pictures, because, the retinal elements or small groups of cells must be excited separately from different points of the object. These adaptive mechanisms depend on several photo- mechanical or retinomotor responses involving rapid changes in the pigment distribution, and the action of contractile elements in the iris and the retina (Hoar, 1987).

Warren and Burnside (1978) demonstrated the role of microtubules and microfilaments in retinomotor responses. They found that actin- and myosin- like filaments are responsible for cone contraction of some marine teleosts. The disappearance of the microtubules following colchicine - induced block of cone

elongation suggests that mi[illegible]iate cone elongation through a sliding mechanism (Warren and Burnside, 1978). Adomian and Sjostrand (1975), on the basis of their observations of the elongation of microtubules in the catfish retina, concluded that microtubules must be assembled and disassembled in connection with myoid elongation and contraction. In this connection, Anctil *et al.* (1979), have shown that rod contraction is mediated by microtubules, but not elongation, and suggested that there may exist inter- and intra- specific differences in retinomotor (rods versus cones) mechanism in lower vertebrates. Walls (1942), has commented on the confusion surrounding the mechanism that controls photochemical movements.

Different light conditions induce responses in the retina, which then signals the patterns to the brain via the optic nerve. The signals from the individual cells are not transmitted in isolation from its neighbours to the brain, but all the signals from neighbouring cells may interact with each other, to be added up, so that their sensitivity is the sum of their respective 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 rods or cones may respond by moving, while others do no move, even within the same species (Walls, 1942; Tansley, 1965). According to Walls (1942) neural summation seems to be less developed in cones, and in this connection, Munz and McFarland (1977) have reported that some predator fishes have also adapted their cones for low light intensities.

Inherent in the transmission of information is that, various salient features of the patterns are abstracted by the neural retina, and transmitted inwards to the brain. Exactly how this is done is an active area of research (Lythgoe, 1977).

According to Walls (1942), among the vertebrates, retinal pigment migration is rapid and extensive in teleosts, anurans and birds, while it is slow and less marked or slight in turtles and crocodilians, and is absent in snakes and mammals. During dark, the rods and cones are exposed due to the movement of pigment granules towards the back of the retina, while during light the pigment granules are dispersed through the receptor and outer layer of the retina, and surround the rod tip.

Despite the fact that studies on pigment migration started as long back as 1891 in compound eyes of arthropods (Exner, 1891), the mechanism by which pigment granules change their position in response to photic stimulation is yet to be clearly understood. Several workers have put forward a number of suggestions. For example, Lerner and Kakahashi (1956) have discussed the role of ionic exchange between cell exterior and interior with regards to pigment migration; Ishibashi (1957) suggested the importance of intracellular calcium ion level in pigment migration; Kinoshita (1963) put forward the view that electro- chemical changes cause melanin migration in fish melanophores; Wiksow and Novales (1969) commented on the role of microtubules in pigment aggregation and dispersion in the scale of fishes; Fujii (1969) has suggested that pigment migration is either controlled by nerves or hormones or both. Enami (1955) proposed a two hormone hypothesis, assuming two antagonistic principles, for

example, melanocyte stimulating hormone (MSH) which disperses melanin and melonocyte concentrating hormone (MCH), but this has not been accepted universally. Baker (1963, 1968a) has suggested that MSH is solely responsible for both pigment concentration and dispersion in fish melanophores. In all probability intermedin is the actual hypophyseal agent involved in melanophoric responses in fishes (Bagnara and Hadley, 1969), though Chavin (1956) emphasised the role of ACTH in melanophoric response of fishes.

The role of nervous control of pigment migration was indicated, when Von Frisch (1911) first reported the light sensitivity of the diencephalon in blinded minnows. Davson (1970) has shown that teleosts possess both nervous as well as hormonal control over their melanophores, where the former plays the major role. But Osborn (1938) has shown that in catfish both hormonal and nervous factors are responsible for pigment dispersion. According to Ali (1964a), hormones may influence pigment migration. He based this on the findings from his experiment on gold fishes. He restrained and anaesthetised gold fish and then exposed one eye to bright light, while the other was closed. The rods and cones of the dark-adapted eye did not move, but the retinal pigment melanin expanded partially. In this regard Fujii (1969) also stated that melanin dispersion and aggregation in the skin of fish is by nervous control via the release of transmitter substances. On the basis of the above-mentioned works, it can be assumed that there is a close relationship between visual adaptation and neurosecretory mechanisms.

According to Scharrer's (1952b) widely – accepted concept, the neurosecretory cells represent a connecting link between the nervous system and the endocrine glands. Their essential role is to transmit stimuli received from the nervous system to the endocrine glands (Gabe, 1966). The neurosecretory cells respond to stimuli despite their glandular activity, and it is logical that neurosecretion has an important role in maintaining equilibrium between an organism and its environment or surroundings. According to Ames and Van Dyke (1952), the secretion is also elaborated during alarm stimuli.

It is well established that there is a relationship between neurosecretory peptides and osmoregulation – salt balance and reproduction in fishes (Perks, 1969). Leatherland (1967) has showed the importance of neurosecretion in times of stress, but reports on the relationship between vision and neurosecretion in birds are lacking.

An attempt has therefore been made in the present investigation to see the effect of light and darkness on the neurosecretory system. In dark–adapted birds, there is a large accumulation of neurosecretory products in the hypothalamic region (optic lobes) of the brain, while, in light – adapted birds, there is a considerable reduction of the material, which may be presumably due to axonal transport of neurosecretory materials. During darkness, the rate of discharge of neurosecretory materials is slower while in light – adapted state, synthesis of neurosecretory material is slower but discharge is faster. Thus, accumulation and discharge of the materials in response to photopic and scotopic stimulation may be suggested to be for visual adaptation.

It is well established that there are two basic types of neurosecretory products. One class includes those neurotransmitters with low molecular weights such as catecholamines (e.g. Dopamine, adrenaline and nor-adrenaline) or, biogenic amines (e.g. 5-HT or serotonin). The second class comprises compounds of relatively high molecular weights such as proteins or peptides (neuropeptides). It is also known that the biogenic amines are released instantaneously for rapid physiological adaptational phenomena, and as has been mentioned earlier, light adaptation occurs at a faster rate than dark adaptation, which is a slower process. Thus, a study of the involvement of catecholamines or biogenic amines in neurosecretion and pigment migration is important.

Keeping this in view, the effect of 5-HT on the migration of pigment granules in the retina of the two birds has been tested and it has been found that, following intraocular administration of 5-HT to dark adapted birds, a complete pigment dispersal similar to that of light adapted states has occurred. These reversal is effected by the release of dopamine by the 5-HT which acts as an extracellular messenger that directly induces light adapted cone retinomotor movement (Allen and Burnside, 1986). 5-HT has been reported to have melanin aggregating action by Scheline (1963) and Scott (1965). Kato *et al.* (1982) have shown that Ca^{++}- dependent 5-HT stimulates dopamine release in carp retina.

Apart from 5-HT, the role of cyclic 3,5-adenosine monophosphate (cAMP) as a physiological activator has been studied in the present investigation. It has been observed that cAMP triggers pigment migration similar to light adapted state when injected into dark-adapted eyes. Bonner (1971) and Robinson

et al. (1971) reported that cAMP activates the physiological process characteristic of a particular effector cell, which also acts as a regulatory agent in all animal cells. It also triggers the specific response of the cell (Vander *et al.* 1980). It has also been reported to act as intercellular messenger by Robinson *et al.* (1971). While Bagnara and Hadley (1969) have reported that cAMP mimics the effect of intermedin by expanding melanophores in amphibians. The levels of cyclic nucleotides such as cAMP have been shown to differ between light- and dark-adapted retinas of the ground squirrel, *Citellus tridecemlineatus* (De Vries *et al.*, 1982)

In addition, the effect of colchicine, an alkalloid, has also been studied in the present investigation. On injection of colchicine to eyes of dark-adapted birds, pigment granule dispersion similar to that in a light adapted eye ensues. Wiksow and Novales (1969) found that colchicine disperses melanosomes of the scales of *Fundulus* and Anctil *et al.* (1979) reported that colchicine inhibits cone myoid elongation and rod myiod contraction in trout retina. Moreover, Margulis (1973) reported that the important cytoskeletal elements – the microtubules are disrupted by colchicine. According to Hoar (1987), the microtubules are important motile processes concerned with intracellular transport in nerve cells, the flow of pigment granules in chromatophores and the secretion of glands.

Thus, it appears that the visual system as well as the neurosecretory system is affected by dark and light conditions, and together respond to photic stimulation in a co-ordinated manner. Buchanan (1957) postulated that since vision is a special exteroceptive sense, the neurosecretory materials are

discharged under the influence of exteroceptive or visceral impulses, and are transported through the hypophyseal portal system to the anterior hypophysis. In the hypophysis they contribute to the synthesis and release of anterior lobe hormone (Palay, 1953).

A photoreceptor is a light trap that converts radiant energy into nerve impulse. The photoreceptor cells are metabolically very active because the metabolic machinery such as mitochondria and associated organelles, in addition to routine activities of the cells, generate chromoproteins and produce transmitters which affect the synapse. The chromoproteins are subject to destruction by light (Young, 1970), and the transmitters must be steadily passed into synaptic vesicles (Hoar, 1987).

Fluorescent compounds

INTRODUCTION:

Pigmented compounds are found in animals in all levels of phylogeny. These pigments are biochemically extremely varied, ranging from the almost ubiquitous melanins, carotenoids and pteridines to the less common quinones and flavins (Needham, 1974; Kennedy, 1979). An interesting feature of some of these pigments is that they possess the characteristic property of fluorescence (Matsumoto, *et. al.*, 1960). An example of a fluorescent compound is the pterine – type also known as pteridine.

Fluorescent compounds absorb and convert high frequency light energy to lower frequencies. The fluorescent compounds originate in pigment cells or chromatophores, which are particularly prevalent among cold – blooded vertebrates with principal pigmentary activities of scattering or reflecting light Bagnara, *et. al.*, 1978). According to Lythgoe (1979), they may also act as filters. The pigment cells or chromatophores are usually bright in color, which have been actually shown to be imparted by the pteridines, either solely or together with other kinds of pigments such as carotenoids (Matsumoto, 1965a; Hama, 1963; Obika and Bagnara, 1964).

The term "pteridine" is derived from the Greek word "pteros", which means wings or feathers. These pigments are so named because of their discovery in the wings of butterflies for the first time by Hopkins (1889). Pteridines constitute a variety of compounds having the molecular structure of pyrimido [4, 5 – b] pyrazine (Purmann, 1940). According to Wieland and Schopf (1925) and Schopf and Reichert (1941), each pteridine has a common structure of either 2-

amino- 4- hydroxypteridine or 2, 4- dihydroxypteridine, and is customarily called "pterine" or "lumazine" respectively. Purman (1940) reported that pteridines have a structural similarity with that of purines, imidazo [4, 5- d] pyrimidine. Albert (1957) and Cresswell *et al.* (1965) have established the chemical similarity of pteridines and purines. The metabolic similarity between pteridines and purines has been reported by Weygand and Weldschmidt (1955), Aaronson and Rodriguez, (1958), and McNutt, (1964). Other workers like Weygand *et al.* (1964), Kidder and Dewey (1968) and Sugiura and Goto (1968) have shown that naturally occuring pteridines are synthesised from purine nucleosides.

Authors like Buchanan *et al.* (1948); Albert (1954), Weygand *et al.* (1961) and Stackhouse (1966) have shown the close relationship between purines and pteridines with regards to the fundamental synthetic pathway and general chemical properties such as, solubility, chromatographic behavior and susceptibility to enzymes.

Numerous pteridine derivatives have been reported in the biological system, ever since the discovery of the chemical structure of some naturally occuring pteridines like leucopterines, xanthopterines, iso- xanthopterines etc.

Bagnara and Taylor (1970), reported that pteridines are localised in "pterinisomes" (pteridine containing organelles) and in carotenoid vesicles, on the basis of electron microscopic studies. These are deposited in the crystals that make up the reflecting plates (Bagnara *et al.* 1988).

According to Bagnara (1966), the colour of pteridines range or vary from white (leucopterines) to yellow (sepiapterines), or to red (dorsopterines). The

sepiapterines comprise yellowish sepiapterine and isosepiapterine, while the red dorsopterines include dorsopterine, isodorsopterine and neodorsopterine. The colourless leucopterines are generally divided into two groups i.e., (i) blue or violet- blue and (ii) violet fluorescent. They include biopterines, rana- chrome 3, xanthopterine and isoxanthopterine (Fujii, 1969). Bagnara (1983) has suggested that the vastly different pigment cells are related to each other due to their similar origin from the neural crest, and can transform from one kind to another, particularly in fishes and amphibians due to their common origin.

As has been mentioned earlier, pteridines as well as their derivatives are reported to perform a variety of functions in the biological system. They are located in the brightly coloured pigment cells of the skin, scales, pigment epithelium- choroid- layer and peritonium. They are also found in miniscule amounts or as traces in tissues devoid of pigment cells (Hama *et. al.* 1960a; Obika, 1963; Matsumoto, 1965a, b; Matsumoto and Obika, 1968; Matsumoto *et. al.* 1969). Most of the investigations on pteridines have been concentrated on animal skin pigmentation, and reports on the occurrence of pteridines in the vertebrate ocular system are scarce.

Pteridines have been reported in the eyes of some insects such as *Drosophila*, *Calliphora, Ephestia* etc (Gregg and Smucker, 1965; Viscontini and Stierlin, 1961, 1962, 1963). Pirie and Simpson (1946) reported a pteridine compound in the choroid of *Squalus acanthius*, which they suggested, was xanthopterine. A fluorescent pteridine product has also been reported in the primate lens, while the presence of pteridines in the lenses of bovines and rabbits

as well as the cornea and retina of bovine, has been reported by Cramer- Barthels (1962). Deb (1990) has also reported it in three fishes, *Cyprinus carpio, Clarias batrachus* and *Stromateus argenteus*. In birds, Oliphant (1988) has detected pteridines in the iris.

An investigation has therefore been carried out on the cornea and lens of the two birds, to ascertain the occurrence of pteridines and their possible functions.

MATERIALS AND METHODS:

CHROMATOGRAPHY:

Paper chromatography has been used for the separation and identification of pteridines from the corneae and the lenses of the two birds. Paper chromatography has been chosen because it is simple yet yields excellent separation (Matsumoto *et al.* 1971).

Chromatography was carried out with Whatman No.1 paper, in dark or dim light because they are photolabile and that light might cause the decomposition of sepiapterine, biopterine and most of 6- substituted pteridines into 6- carboxypterine and other unidentified pteridines.

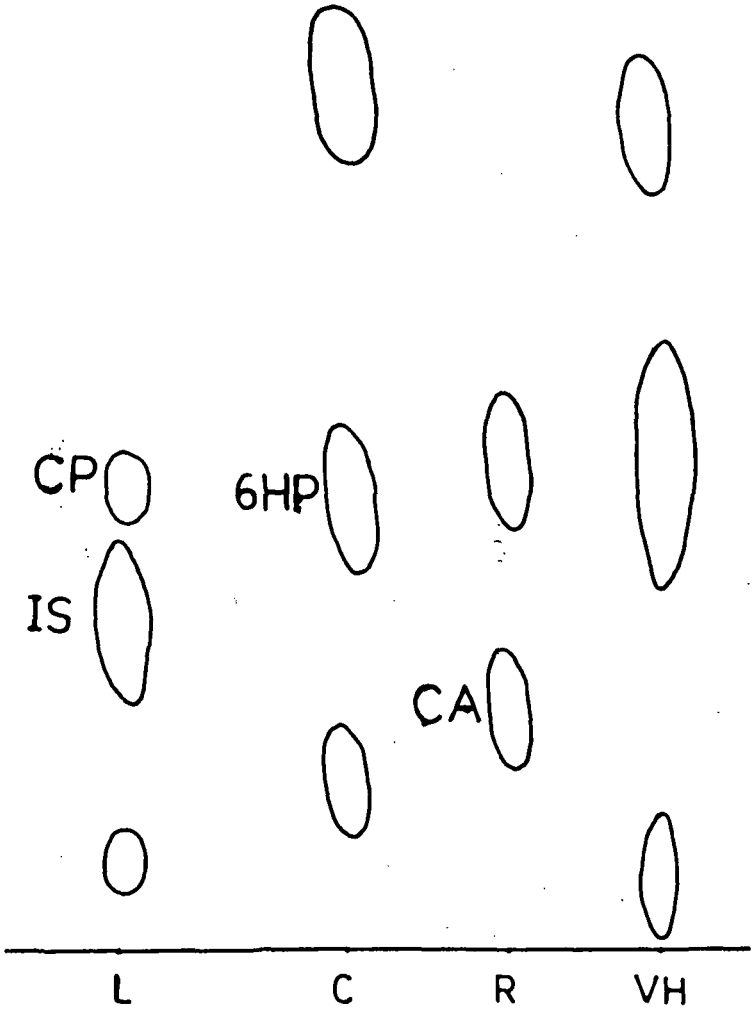
Fresh tissue samples were directly applied on to the chromatographic paper and run in solvent, or, the pteridines were extracted using any of the many methods devised so far like the squash technique (Hadorn and Mitchel, 1951), Quick extraction method (Matsumoto *et al.* 1971), Awapara's method of extraction with ethanol, extraction with trichloroacetic acid (TCA), extraction with HCl etc. In the present study the squash technique has been used.

SQUASH TECHNIQUE (Hadorn and Mitchel, 1951):

After dissection, the tissue samples (corneae and lenses) were squashed directly on the chromatographic paper by firmly pressing between two clean microscopic slides. The squashed samples were then dried with an electric dryer

Fig. 9. Chromatogram showing the fluorescent compounds in the lens (L), cornea (C), retina (R) and vitreous humour (VH) of *Gallus domesticus.*

CP= Cyprino – purple
6 HP = 6 – Hydroxypteridine
IS = Isoxanthopterine
CA = Carboxypteridine

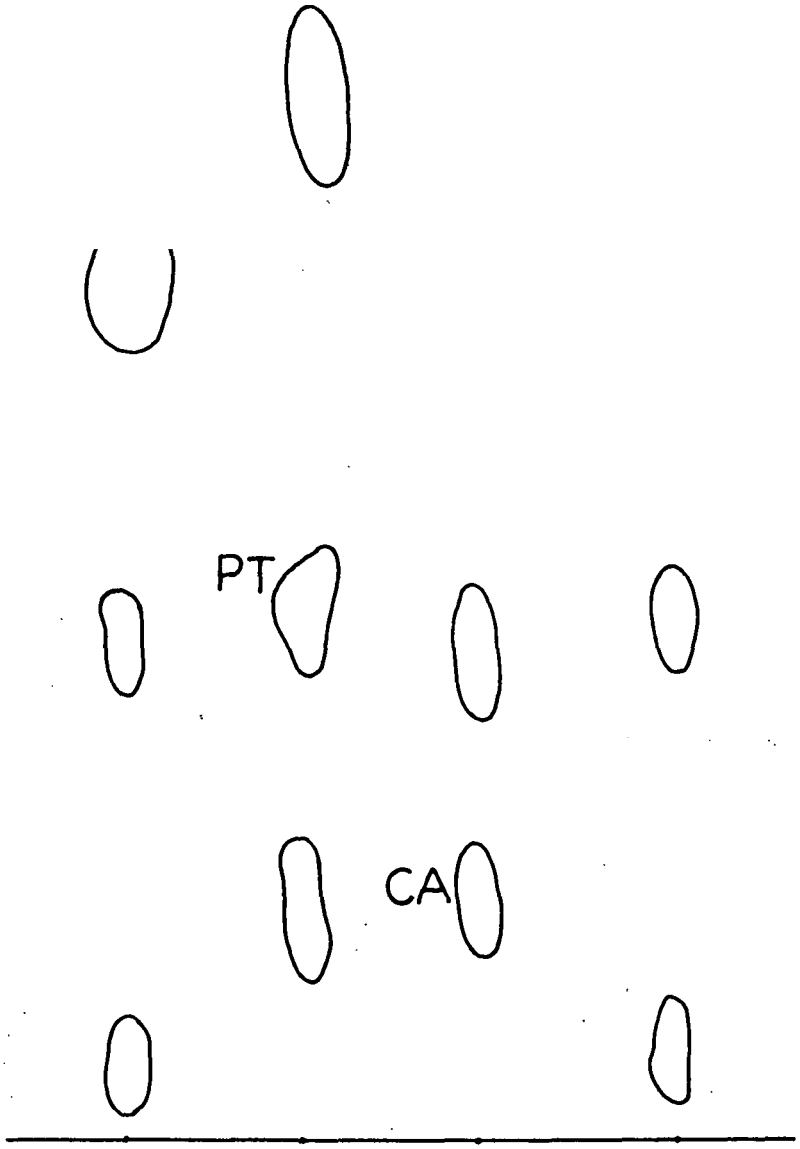


FLUORESCENT COMPOUNDS (CHICKEN)

Standards 9

Fig. 10. Chromatogram showing the fluorescent compounds in the lens (L), cornea (C), retina (R) and vitreous humour (VH) of *Columba livia intermedia.*

CA = Carboxypteridine
PT = Pterine



FLUORESCENT COMPOUNDS (PIGEON)

Standards ?

and the chromatogram run in the solvent made up of n- propanol and 1% or 7% ammonia in the ratio of 2:1 v/v.

The positions of the pteridines were determined by examining the chromatogram under UV lamp and the Rf values measured.

OBSERVATIONS:

It has been observed that the corneae of the two birds contains highly photolabile fluorescent pteridines. The chicken cornea contains 6– Hydroxymethyl pteridine and two unidentified compounds, and the lens contains isoxanthopterine, cyprino– blue and one unidentified compound (Fig.5). The pigeon cornea is seen to contain carboxypteridine, pterine and one unidentified compound, and the lens contains three unidentified compounds (Fig.6).

However, all these pteridines which have been identified in the two birds are only on the basis of their Rf values and hence tentative. A point to be noted here is that some photo- stable yellow fractions have been observed in both the birds, which could probably be carotenoids, as has been described by McFall-Ngai *et al.* (1986).

DISCUSSION:

Pteridines appear to be of universal occurrence in living organisms (Hoar, 1986). Pteridines and their derivatives perform myriad functions in the living system. For instance, an important vitamin, folic acid, is a pterine- containing compound, and another important vitamin, riboflavin, seems to be closely related to the pteridines in its biosynthesis (Forrest, 1962). According to Handschumacher and Welch (1960), the conjugated pteridines are known to function as principal therapeutics for acute leukemy. They may also act as co-enzymes in the enzymatic synthesis of inosinic acid, serine, and methionine (Blakley, 1969). Pteridines are reported to be associated with lipid synthesis (Kidder and Dewey, 1963), and other physiological functions (Ziegler and Ziegler, 1965). Kaufman (1959) and Matsubara *et al.* (1966) have suggested that certain reduced pteridines such as sepiapterin, dihydrobiopterin and tetrahydrofolic acid take part in the hydroxylation of phenylalanine to tyrosine. Pteridines also play a significant role as potential regulators of cellular events (Frost and Bagnara, 1979).

However, apart from all the functions cited above, one of the most important roles of pteridines is their functioning as pigments in the biological system. In this context, it is noteworthy to mention that, in vertebrates, pteridine synthesis is an autonomous xanthopore event (Bagnara, 1983).

Bagnara *et al.* (1988) have shown that iridiophores contain pteridines. Iridiophores are cells that are involved in the phenomenon of pigmentation, by virtue of their utilizing crystallin deposits of purines in organelles called reflecting

platelets. These produce the structural colours by means of their orderly arrangement within the cell. Thus according to Menter *et al.*, (1979), the reflecting platelets may be elements used in reflection, light scattering, diffraction and interference.

Pteridines have been detected in the eyes of certain insects (Gregg and Smucker, 1965); in the integumentary pigment cells of lower vertebrates including fishes (Kaufman, 1959a; Matsumoto *et al.* 1968, 1969; Ziegler 1964; Matsumoto, 1965a,b; Hama *et al.* 1965), in amphibians (Gunder, 1955; Hama and Obika, 1960; Obika 1963; Bagnara, 1961; Richards and Bagnara, 1967) and in reptiles (Hama and Fukada, 1964).

Rauen and Stamm (1952), as well as Uyeda and Rabinowitz (1963) have observed that one of the most distinguishing features of pteridines is their fluorescence under UV irradiation. Depending upon concentration, pH and molecular weights, the pteridine compounds give off fluorescence under different wavelengths. This characteristic property of fluorescence of the pteridines has been used for their detection. The pigments protect the lens from near UV wavelengths by converting the light to a less harmful wavelength by fluorescence (Kuck Jr., 1970). In this connection Walls and Judd (1933a,b) stated that the yellowish pigment is an effective intraocular filter for blue light which prevents the highly dispersive violet rays of the spectrum and thus increases visual acuity. It has been suggested that the fluorescence compounds in young human lens is meant for protection of the retina against UV light.

The total light emitted in a fluorescent system always contains less energy than the light absorbed, but it is possible for the emitted light to be brighter than the incident light of long wavelength (Clayton, 1971). The energy for emission of light in fluorescence comes from light of shorter wavelengths (Lythgoe, 1979). McFall-Ngai *et al.* (1986), have suggested that lens pigmentation increases visual acuity by way of reducing chromatic aberrations, glare and any scattering that may be caused by shorter wavelength visible light.

The significance of pteridines as screening pigments in compound eyes has been suggested by a number of workers like Goldsmith (1958a,b), Langer and Thorell (1966) etc. Nearly all of the pigments are so- called "cut-off" filters in the sense that they absorb wavelengths shorter than a certain value, while the longer wavelengths are transmitted or reflected (Lythgoe, 1979).

Cremer-Bartles (1962) described a photosensitive fluorescent substance, apparently a pteridine that occurs in the cornea, lens and retina. This substance takes up phosphate, thus leading to the hypothesis that it might be a photodynamic substance, in a system by which light energy is trapped in the lens for the useful purpose involving phosphate transfer. He has also shown that the compound is synthesised in the lens when incubated in light, but decreases when incubated in darkness.

Kuck Jr. (1970) and Matsumoto *et al.* (1971) have shown that lens fluorescence varies widely in various species, and also within the same species. Despite their common occurrence in a variety of species, a more or less specific

pteridine pattern is exhibited by each species in which each component is clearly defined.

According to various reports, pteridines are suggested to play a significant role in the visual system of fishes. But, as already mentioned, studies of pteridines in other vertebrate eyes, especially birds, have not been fully carried out. Thus, further investigations on the pteridines compounds of vertebrates in general and birds in particular are required.

General discussion

In the present investigation, certain aspects of the ocular system of the chicken, *Gallus domesticus* and the Indian blue rock pigeon, *Columba livia intermedia* (Strickland) have been studied histo-chemically as well as bio-chemically, and some interesting observations have been made.

In the corneae and lenses of the two birds, an important component – the protein – polysaccharide complex, "acid mucopolysaccharide" has been detected. Mucopolysaccharides had been previously detected in various tissues and a number of roles ascribed to them. In the ocular tissues, the corneal mucopolysaccharides assume great significance on account of their polydisparity and chemical heterogeneity, which is unique amongst all connective tissues (Maurice and Riley, 1970).

Moczar and Moczar (1973) have shown that the evolutionary changes of macromolecules in cornea in relation to differentiated connective tissues. Smelser (1963) studied the hydrophilic nature of mucopolysaccharides in carp and mammalian cornea. Mayer *et al.* (1953), studied the polydisparity and chemical heterogeneity of mucopolysaccharides in ox cornea. Robert et al. (1965b) isolated and elucidated the chemical nature of the various fractions of mucopolysaccharides in calf and rabbit cornea. Volumes of work on the relationship between mucopolysaccharides and macromolecular composition and swelling nature of cornea, have been done on a number of vertebrates and invertebrates by a number of workers like Robert and Schillinger (1967), Moczar and Moczar (1970, 1973), Moczar et al. (1969a, b), Cejkova and Bolkova (1970, 1973, 1974). The swelling properties of the cornea of some vertebrates in relation

to glucosamine and galactosamine ratio might be the critical factor in corneal swelling.

In the lens, the protein- polysaccharide complex is present at the interfibriler surface (Kück Jr. 1970). They control the growth and differentiation of cell fibres in different parts of the lens. The mucopolysaccharides also serve as interfibriler cement substances in the lens, which is necessary for optical function (Bellows, 1944).

In view of this intimate relationship between acid polysaccharides and proteins as well as hexosamine synthesis in developing tissues, it may help to understand the role of polysaccharides and glycoproteins in determining the different structures of connective tissues (Maurice and Riley, 1970). Murray (1988) has suggested that glycosaminoglycans are the structural molecules of collagen, serving as lubricants and protective agents. They transport minerals and trace elements, and are also responsible for cell to cell attachment and communication. They also appear to serve as receptors, carriers for macromolecules, regulators of cell growth and have effects on protein synthesis and intra- nuclear functions.

Glycosaminoglycans are polyanions, because they have acidic sulfate or sulfate groups of uronic acid in their structure. Many of their functions result from this particular character. In addition, the binding between glycosaminoglycans and other extracellular macromolecules is electrostatic in character because of its polyionic nature. This binding contributes significantly to the structural organization of the connective tissue matrix.

In the present investigation, various aspects of bird vision have been experimented on, and as such, an exhaustive study of the mucopolysaccharides was not possible to be carried out. But, it is reasonable to suggest that mucopolysaccharides in ocular tissues may play some significant role in the visual physiology of birds. Further, it would be of significance to find out if the functions of the mucopolysaccharides are mechanical or optical or both, along with the relationship between vitamin A and mucopolysaccharide synthesis.

The study of the enzyme, adenosine triphosphatase (ATPase) was carried out in the cornea and lens of the two birds, because the enzyme is known to enhance the metabolism to supply energy for visual purpose through cation transport, which also maintains the ionic balance and has many metabolic functions (Bonting, , 1970). A Na^+ - K^+ - activated ATPase was found in the cornea and lens of both the birds. The role of this enzyme in cation transport of cell membranes is well established. Skuo (1965) and Dikstein and Maurice (1969) have shown that this enzyme maintains the water balance or hydration of the ocular tissues thus maintaining transparency. It is thus highly probable, that this enzyme might have an important role in the visual process through cation transport by way of influencing osmoregulation.

It would be interesting to study the relationships, if any, between ATPase and mucopolysaccharide synthesis because it is known that the ATPase system is responsible for glucose transfer.

Another important aspect of the present investigation is the study of ascorbic acid in the cornea and lens of the two birds. As has been already

mentioned, ascorbic acid might play an important role in energy generation of bird photoreceptors. The role of ascorbic acid in collagen synthesis is well established (Mayes, 1988), and collagen is an essential constituent of the cornea where the mucopolysaccharides remain in close association with collagen. Evidences for and against ascorbate stimulation of sulfated glycosaminoglycans are found in literature. Human skin fibroblasts in tissue culture produce more sulfated polysaccharides in the presence of ascorbate. Lavietes (1971) has also shown that the presence of ascorbate in the culture medium does affect the distribution of chondroitin sulfate that is synthesized. Therefore, the role of ascorbic acid in mucopolysaccharide synthesis seems a worthwhile line of further research.

The migration of the retinal pigment, melanin, in response to various factors has also been studied, and it has been observed that intraocular administration of 5-HT, colchicine and cyclic AMP to dark-adapted eyes stimulates pigment migration similar to light adapted state. This may be due to stimulated dopamine release, by acting as intercellular messengers and disruption of microtubules respectively. In addition, a close relationship between neurosecretion and pigment migration in response to various adaptational states or photic stimuli has been observed. But, pending further detailed studies, the precise nature or factors responsible for this cannot be advanced.

Another aspect that has been looked into in the present investigation is about the fluorescent substances in the eyes of the two birds. Different fluorescent compounds could be seen with respect to the lens and cornea of the same bird, as

well as in between the lens and cornea of the two birds. The presence of the fluorescent compounds in the eyes of the two birds is significant in terms of visual phenomenon because they serve as screening pigments to filter out the harmful UV rays.

Taking all these into consideration, it can be concluded that acid mucopolysaccharides (AMPs), ascorbic acid, Na^+ - K^+ - activated ATPase, fluorescent compounds and neurosecretion all have significant roles to play in the visual system of birds, but further research work is required for completely understanding the phenomenon of vision, especially with regards to species from different ecological niches.

References

Aaronson, S. and Rodriguez, E. (1958). Relationship between purines and folic acid vitamins in the micrococcus, *Gaffkya homari. J. Bact. 75,* 660 – 665.

Adomian, G. E and Sjostrand, F. S (1975). Morphological basis for the photomechanical response in the photoreceptors of the vertebrate retina. *Proc.33rd Ann.Meetings Electron Microsc. Soc . Amer.* 476-477.

Agarwal, N. K. and Mahajan, C. L. (1980). Comparative tissue ascorbic acid studies in fishes. *J. Fish Biol. 17(2),* 135 – 142.

Al–Robai, A. A. (1993). Different ouabain sensitivities of Na^+/K^+ - ATPase from Poekilocerus bufonis tissues and a possible physiological cost. *Comp. Biochem. Physiol.* Vol. 106B, No. 4, pp. 805 – 812.

Albert, A. (1954). The pteridines. In "L. Zechmeister, Fortschritte der Chimie organischer Naturstoffe" Springer-Verlag, Berlin, Heidelberg, New York.

Albert, A. (1957). Transformations of purines into pteridines. *Biochem. J. 65,* 124 – 127.

Ali, M. A. (1959). The ocular structure, retinimotor and photobehavorial response in juvenile Pacific salmon. *Can. J. Zool. 37,* 965 – 996.

Ali, M. A (1964). Retinimotor responses in the enucleared eye of the brown bullhead, *Ictalurus nebulosus* and the goldfish, *Carassius auratus. Rev. Can. Biol. 23(1),* 55 – 66. Ali, M. A. (1964a). Retinomotor responses of the goldfish *Carassius auratus* to unilateral photic stimulation. *Rev. Can. Biol.* 45 – 53.

Allen, D. M. and Burnside, B. (1986). Dopaminergic regulation of cone retinomotor movement in isolated teleost retina: II. Modulation by α-aminobutyric acid and serotonin. *J. Neurochem. 46(4),* 1022 – 1031.

Ames, R. G. and Van Dyke, H. B. (1952). In "Neurosecretion" (Ed. Gabe, M.) Pergamon Press, London, New York and Toronto, 1966.

Anctil, M.; Ali, M. A and Couillard, P (1979) . Cone myoid elongation and rod myoid contraction are inhibited by colchicine in trout retina. In "Ultrastructural correlates recepetors"(Ferrero,E.; Anctil,M and Ali,M.A .). *Rev.Can.Biol. 38,*249-264.

Anctil, M.; Ali, M. A.and Couillard, P. (1980). Cone myoid elongation and rod myoid contraction are inhibited by colchicine in trout retina. *Experientia 36,* 574 –575.

Anderson, E. I and Spector , A (1971) . Oxidation –reductions involving ascorbic acids and the hexoses monophosphate shunt in the corneal epithelium . *Invest. Ophthalmol. 10 (1)* , 41-53.

Anseth, A. (1961a). Studies on corneal polysaccharides III. Topographic and comparative biochemistry. *Exp. Eye Res. 1,* 106 –115.

Anseth, A. and Fransson, L. A. (1969). Studies on corneal polysaccharides VI. Isolation of dermatan sulphate from corneal scar tissue. *Exp. Eye Res. 8,* 302 – 309.

Anseth, A. and Fransson, L. A. (1970). The importance of the polysaccharides for the normal functioning of the corneal stroma. *Acta. Ophthal. 48,* 455 – 464.

Anstee, J. H and bowler, K. (1984) Techniques for studying Na^+/K^+ - ATPase. In "Measurement of ion transport and metabolic rate in insects" (Eds. Bradley, T. J. and Miller, T. A.), pp. 187 – 220, Wiley, New York.

Arey, L. B. (1916). Movements in the visual cells and retinal pigment of the lower vertebrates. In "Ocular structure, retinomotor and photobehavorial responses of juvenile Pacific salmon by Ali, M. A., *Can J. Zool. 37*, 965 – 996.

Ashhurt, D. and Costin, N. (1971). Insect mucosubstances 1. The mucosubstance of developing connective tissue in the locust, *Locusta migratoria. Histochemie. J. 3*, 279 – 295.

Ashmore, J. F and Falk, G (1976). Absolute sensitivity of rod bipolar cells in a dark adapted retina. *Nature (Lond.) 263*, 248 - 249.

Bagnara, J. T,; Frost, S. K. and Matsumoto, J. (1978). On the development of pigment patterns in amphibians. *Amer. Zool. 18*, 301 – 312.

Bagnara, J. T. (1961). Chromatotrophic hormone, pteridines and amphibian pigmentation. *Gen. Comp. Endocr. 1*, 124 – 133.

Bagnara, J.T. (1966). Cytology and cytophysiology of non-melanophore pigment cells. *Int. Rev. Cyto. 20*, 173 – 205.

Bagnara, J.T. (1983). Developmental aspects of vertebrate chromatophores. *Amer. Zool. 23*, 465 – 478.

Bagnara, J.T. and Hadley, M.E. (1969). The control of bright colored pigment cells of fishes and amphibians. *Am. Zoologist 9*, 465 – 478.

Bagnara, J.T. and Taylor, J.D. (1970). Difference in pigment containing organelles between color forms of the red-backed salamander, Plethodon cinereus. *Z. Zellforsch. 106,* 412 – 417.

Bagnara, J.T.; Frost,S.K.and Matsumoto,J. (1978). On the development of pigment patterns in amphibians. *Amer. Zool. 18,* 301 –312.

Bagnara, J.T.; Kreutzfeld, H.L.; Fernandez, P.J. and Cohen, A.C. (1988). Presence of pteridine pigments in isolated iridophores. *Pigment Cell Res. 1,* 361 – 365.

Baker, B.I (1968a). Unpublished data. Chromatophores and pigments. In "Fish Physiology" (Eds. Hoar, W.S. and Randall, D.J.) Vol. III. Academic Press, New York, London.

Baker, B.I. (1963). Effect of adaptation to black and white backgrounds on the teleost pituitary. *Nature, 198,* 404.

Baker, J.R. (1946). The histochemical recognition of lipids. *Quart. J. Micros. Sci. 87,* 441 – 470.

Balazs, E.A (1961) . In " The structure of the eye"(Ed. Smelser, G.K) pp. 293-310. Academic press,

Balazs, E.A. (1965). Amino sugar containing macromolecules in the tissues of the eyes and ear. In "The Amino sugars" (Eds. Balazs, E.A. and Jeanloz, R.W.), Vol. 2A, pp. 401 – 460. Academic Press New York.

Baldwin, W. and Salthouse, T. (1959). Dermal glands and mucin in the molting cycle of *Rhodnius prolixsus.* Stal. *J. Insect Physiol.*

Barany, E.H. and Langham, M.E. (1955). In "Biochemistry of the eye" (Ed. Graymore, C.N.) pp. 146. Academic Press, London and New York, 1970.

Barnes, M.J. and Partridge, S.M. (1968). Isolation and characterization of glycoprotein from human thoracic aorta. *Biochem. J.109*, 883-896.

Barnett, S.A. and Bourne, G. (1941). The distribution of ascorbic acid (Vit. C) in the early stages of the developing embryo. *J. Anat. 75*, 251 – 264.

Barnett, S.A. and Fisher, R.B. (1943). Experiments on the silver nitrate method for the demonstration of ascorbic acid (Vit. C). *Brit. J. Expt. Biol. 20*, 134 – 15.

Basaglia, F. (1989). Hemoglobins and soluble proteins of the eye lens of the Italian barbs, *Barbus barbus plebejus* and *B. meridionalis* (cypriformes, teleosti). *Comp. Biochem. Physiol. 93B*, 707 – 710.

Basaglia, F. and Callegirini, C. (1988). Biochemical characteristics of red mullet of the central Mediterranean. *Comp. Biochem. Physiol. 88B*, 731 – 736.

Basaglia, F. and Callegirini, C. (1987b). Soluble eye lens proteins of Italian Ictalurids. *Comp. Biochem. Physiol. 88B*, 219 – 221.

Bellows, J.G. (1944). "Cataract and anomalies of the lens". Mosby, St.Louis.

Berenson, G.A., Radhakrishnamurthy, B.; Fiskin, H.F., Dessauer, H. and Arquembourg, P. (1966). Individuality of glycoproteins in human aorta. J. *Artereosclerosis Res. 6*, 214-223.

Berman, E.R. (1969). Mucopolysaccharides (glycosaminoglycans) of the retina: Identification, distribution and possible biological roles. *Bibl. Ophthalmol. 79*, 5 – 31.

Berman, E.R. and Bach, G. (1968). The acid mucopolysaccharides of cattle retina. *Biochem. J. 108*, 75 – 88.

Berman, E.R. and Voaden, M. (1970). The vitreous body. In "The biochemistry of the eye" (Ed. Graymore, C.N.) pp. 373 – 471. Academic Press, London, New York, 1970.

Bitensky, M.W.; Keirns, J.J and Wagner, R.C (1973). Cyclic AMP and photoreceptor functions. In "Biochemistry and physiology of visual functions" (Ed.Langer, H.) Springer –Verlag, Berlin, Heidelberg, New York.

Blakley, R.L. (1969). In "Biochemistry of folic acid and related pteridines". North-Holland research Monographs, Frontline of Biology, 13. North-Holland Publishing Co. Amsterdam, London.

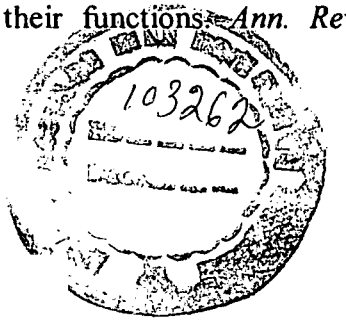
Blaxter, J.H.S. (1970). In: Ecology of vision". (Lythgoe.J.N.). Clarendon Press, Oxford, 1979.

Bleckmann, H. (1984). Glyccosaminoglycan metabolism of cultured fibroblasts from bovine vitreous body. *Grafe's Arch. Clin. Exp. Ophthalmol. 222*, 90 – 94.

Boll, F. (1877). In "Ecology of vision"(Ed. Lythgoe, J.N.) pp.75. Clarendon Press, Oxford, 1979.

Bonner, J.T. (1971). Aggregation and differentiation in cellular slime molds. *Ann. Rev. Microbiol. 25*, 75 – 92.

Bonner, W.D. (1957). Soluble oxidases and their functions. *Ann. Rev. Plant Physiol. 8*, 427 – 452.



Bonting, S.L. (1965). $Na^+ - K^+$ activated adenosine triphosphatase and active cation transport in the lens. In "Symposium on the lens" (Ed. Harris, J.E.) pp. 347 – 363. C.V. Mosby and Co., St. Louis, 1965.

Bonting, S.L. (1970). Sodium – potassium – activated adenosine triphosphatase and cation transport. In "Membranes and ion transport" (Ed. Bittar, E.F.) Vol. 1, pp. 257 – 363, Dawson and Goodall.

Bonting, S.L. and Bangham, A.D. (1967). On the biochemical mechanisms of the visual process. *Expl. Eye Res. 6,* 400 – 413.

Bonting, S.L.; Carvaggio, L.L. and Hawkins, N.M. (1963). Studies on Na^+ - K^+ - activated adenosine triphosphate, VI. Its role in cation transport in the lens of the cat, calf and rabbit. *Arch. Biochem. Biophys. 101,* 47 – 55.

Bonting, S.L.; Carvaggio, L.L.; Candy, M.R. and Hawkins, N.M. (1964). Studies on Na^+ - K^+ - activated ATPase XI. The salt gland of the herring gull. *Arch. Biochem. 106,* 49 – 65.

Borel, J.P.; Desanti, M; Clogne, M. and Bochet, C. (1970). Constitution de 6 glycoprotein associess un collagene dans le derme de lapin. C. R. *Acad. Sci. (Paris) 270,* 2714-2716.

Bourne, G. (1944). Intracellular localization of vitamin C. *Nature, 153,* 254 – 255.

Bowes, J.H.; Elliot, R.G. and Moss, J.A. (1958). The extraction of soluble protein from skin by alkaline solution. In "Recent advances in gelatine and glue research" (Ed. Bowes, J.H.) pp. 71 – 75. Pergamon Press New York.

Bowmaker, J.K. (1979). Visual pigments and oil droplets in the pigeon retina, as measured by microspectrophotometry, and their relationship to spectral

sensitivity. In "Neural mechanisms of behavior in the pigeon" (Eds. Grandre, A.M. and Mazwell, J.H.) pp. 287 – 305. Plenum, New York.

Bowmaker, J.K. and Martin, G.R. (1985). Visual pigments and oil droplets in the penguin, *Spheniscus humboldti. J. Comp. Physiol. 156*, 71 – 77.

Bownds, J.H.; Dawes, J., Miller, J. and Stahlman, M. (1972). Phosphorylation of frog photoreceptor membrane induced by light. *Nature, New Biol. 237*, 125 – 127.

Brown, H.M.; Meech, R.W.; Koike, H. and Hagiwara, S. (1969). Current voltage relations during illumination. Photoreceptor membrane of a barnacle. *Science, 166*, 240.

Buchanan, A.R. (1957). Functional neuro-anatomy. Lea and Febiger, Philadelphia.

Buchanan, J.M.; Sonny, J.C. and Deluva, A.M. (1948). Biological precursors of uric acid II. The role of lactate, glycine and carbon dioxide as precursors of the carbon chain and nitrogen atom 7 of uric acid. *J. Biol. Chem. 173*, 81 – 98.

Buck, M.G. and Zadunaisky, J.A. (1975). Stimulation of ion transport by ascorbic acid through inhibition of 3,′ 5′ – cyclic AMP phosphodiesterase in the corneal epithelium and other tissues. *Biochemica et Biophysica Acta.* 251-260.

Campbell, W.F. (1967). The dioptric mechanism of the eye. In "The physiological basis of medical practice"(Eds. Best, C. and Taylor, N.) pp. 329 – 356. Scientific Book Agency, Calcutta, 1967.

Carney, S.L. (1994). "Proteoglycans". In "Carbohydrate Analysis – a practical approach" (Eds. Chaplin, M.F. and Kennedy, J.F.). Oxford University Press, Oxford, New York, Tokyo.

Cejkova, J. and Bolkova, A. (1970). Effect of fixatives on acid mucopolysaccharides (AMPs) in the experimentally hydrated rabbit cornea. *Ophthal. Res. 1*, 273 – 278.

Cejkova, J. and Bolkova, A. (1973). Acid mucopolysaccharides in non-swelling dogfish corneas. *Ophthal. Res. 5*, 362 – 370.

Cejkova, J. and Bolkova, A. (1974). Differences in hydration characteristics of corneas in various animal species. *Albrecht V Graefes Arch. Klin. Exp. Ophthal. 190*, 358 – 360.

Cejkova, J. and Brettschneider, I. (1969). The effect of hydration of the rabbit cornea on the histochemical demonstration of acid mucopolysaccharides. *Histochemie 17*, 327 – 336.

Chaterjee, I.B.; Majumdar, A.K.; Nandi, B.K. and Subramaniam, N. (1975). *Ann. N.Y. Acad. Sci. 258*, 24.

Chatterjee, G.C. and Paul, D.R. (1975). Metabolism of L-ascorbic acid in rats under administration of mercury: Effects of L-ascorbic acid supplementation. *Int. Vit. Nutr. Res. 45*, 285 – 292.

Chavin, W. (1956). Chromatophores and pigments. In "Fish Physiology" (Eds. Hoar, W.S. and Randall, D.J.) Vol. III. Academic Press, New York, London.

Chen, D.M. and Goldsmith, T.H. (1984). Appearance of a Purkinje shift in the developing retina of chick. *J. Exp. Zool. 299*, 265 – 271.

Chen, D.M.; Collins, J.S. and Goldsmith, T.H. (1984). The ultraviolet receptor of bird retinas. *Science 285*, 337 – 340.

Chen, W.H.; Lu, S.H.; Dutt, K.; Smith, A.; Hunt, D.M. and Hunt, R.C. (1998). The relative ability of glucose and ascorbate to glycate and crosslink lens protein *in vitro*. *Exp. Eye Res. 67.* No. 1.pp 95.

Chinoy, J.J. (1967). Studies on cellular and molecular aspects of floral induction – a review. Indian *J. Plant Physiol. 11*, 202 – 220.

Chinoy, J.J. (1969). A new concept of flowering on the basis of molecular and sub – molecular events occuring in shoot apex and the leaf of wheat. *Indian J. Plant Physiol. 12*, 67 – 80.

Chinoy, J.J.; Gurumurti, K. and Singh, Y.D. (1973). Inductive effect of low dose of gamma radiation on the biosynthesis of ascorbic acid and sulfhydril compounds. *Indian J. Plant Physiol. 16*, 42 – 49.

Chinoy, J.J.; Singh, Y.D. and Gurumurti, K. (1971). Effects of water stress on paramagnetic behaviour. Ascorbic acid turnover and enzymic activity in *Cicer arirtinum. J. Ind. Bot. Soc. 50*, 462 – 472.

Chinoy, J.J.; Singh, Y.D. and Gurumurti, K. (1974). Biosynthesis of ascorbic acid and mobilisation pattern of macromolecules during water stress in germinating *Cicer* seedlings. *Biologia Plant*, 16, 301 – 307.

Chinoy, N.J.; Singh, Y.D. and Gurumurti, K. (1976). Colorimetric determination of ascorbic acid turnover in plants. *Indian J. plant Physiol. 19*, 122 – 130.

Chinoy,N.J and Buch, H.P (1977) . Metabolic significance of ascorbic acid in human semen . *Indian. J.Exp.Biol. 15*, 921-923.

Clayton, R.K. (1971). "Light and living matter" Vol. 2, pp. 243, McGraw-Hill, New York.

Cohen, A.L. (1963a). Fine structures of the receptors of the pigeon. *Exp. Eye Res. 2*, 88 – 97.

Cohen, A.L. (1967b). Vertebrate retinal cells and their organisation. *Biol. Rev. 38*, 427 – 459.

Cole, D. (1970). Aqueous and ciliary body. In "Biochemistry of the eye" (Ed. Graymore, C.N.) pp. 105 – 171. Academic Press, London and New York, 1970.

Cramer-Bartels, G. (1962). A light sensitive fluorescent substance in bovine and rabbit lenses. *Exp. Eye Res. 1*, 443 – 448.

Cresswell, R.M.; Maurer, H.K.; Strauss, T. and Brown, G.B. (1965). Purine N-oxides XIV. A total synthesis of pyrimidine N-oxide, a pteridine 1-N-oxide, and xanthine 3-N-oxide. *J. Org. Chem. 30*, 408 – 410.

Curran, R.C. (1964). The histochemistry of mucopolysaccharides. *Int. Rev. Cytol. 17.*

Daisley, K.W. (1955). In "Biochemical studies on the vertebrate lens" (Eds. Rawal and Rao) *Vaidya B-Sciences XX(2)*, 172 – 178, 1977.

Davson, H. (1949). Some considerations on the salt content of fresh and old ox corneae. *Brit. J. Ophthal. 33*, 175.

Davson, H. (1970). A text book of general physiology. Vol.2. J. and A. Churchill, London, 1970.

Deb, M.K. (1990). Ph.D Thesis. N.E.H.U.

Deb, M.K. and Raghuvarman, A. (1994). Glycosaminoglycan fractions in the cornea of two fishes. *Curr. Sci. 67*, 1020.

Deb, M.K.; Dey, S.; Varman,A.R. and Michael, G.R. (1990). Scanning electron microscopic studies on lenticular aberrations due to prolonged starvation in a catfish, *Heteropneustes fossilis* (Bloch). *Vision Res 30(7),* 1017 – 1019.

Delaye, M. and Tardieu, A. (1983). Short – range order of crystallin proteins accounts for eye lens transparency. *Nature, Vol. 302* pp. 415 – 417.

Denizot, J. P. (1970). Comparative histochemical analysis mucopolysaccharides in the ampullary sense organs of weakly-electric fish. *Gnathnemus petersu* (Mormyridae), *Gymnotir cerapo* (Gymnotidae) and *Gymonarcus niloticus* (gymnarchidae). *Histochemie 23(1),* 82-90.

DeRobertis, E.D.P.; Nowinski, W. and Saez, F.A. (1970). The plasma membrane. In "Cell biology". pp. 150. W.B. Saunders and Co. Philadelphia, London and Toronto

Detwiler, S.R. (1943). Vertebrate photoreceptors. In "Ocular structure, retinomotor and photobehavorial responses of juvenile Pacific salmon , by Ali, M.A., *Can. J. Zool. 37*, 965 – 996.

DeVries, G.W.; Cohen, A.I.; Lowry, O.H. and Ferrendelli, J.A. (1982). Cyclic nucleotide levels in light adapted and darkadapted ground squirrel (*Citellus tridecemlineatus*) whole eyes. *Vision Res. 22(10),* 1237 – 1240.

Dey, S. (1976). Acid mucopolysaccharides in the lens cuticle of insects. M.Sc. Dissertation, N.E.H.U.

Dey, S. (1980). Ph.D. Thesis. N.E.H.U.

Dey, S.; Deb, M.K.; Varman, A.R. and Dkhar, B. (1994). Intra – and intercellular specialisation of photoreceptor cells and orientation of pigment bodies as a physiological adaptation to dim light within a cat fish, *Clarius batracus. Cytobios 79*, 151 – 159.

Dey, S.; Raghu Varman, A. and Michael, R.G. (1978). Acid mucopolysaccharides in the lens cuticle of the fresh water prawn, *Macrobrachium birmanicum* (Crustacea) and the marine horse shoe crab, *Limulus polyphemus* (Xiphosura) and their physiological significance IV. *All India Symp. On Comp. Animal Physiol. Endocrinol.*

Dey,S and RaghuVarman,A (1984a) . Ascorbic acid in the compound eye of the honey bee, *Apis cerena indica, . Indian Biologist XVI (1) and (2),* 45-48.

Dey,S and RaghuVarman,A (1984b). Ascorbigen in the compound eye of the house fly, *Musca domestica. Curr. Sci. 53,* 1264-1265.

Di Benedetta, C.; Brunngraber, E.G.; Whitney, G; Brown, B.D. and Aro, V. (1969). Compositional patterns of sialofuco hexosaminoglycans derived from rat brain glycoproteins. *Arch. Biochem Biophys. 131*, 404 – 413.

Dietrich, C.P.; Sampaio, L.O. and Toledo, O.M.S. (1977). Preparation of a crude AMP fraction from tissues with proteolytic enzymes. In "Methods of biochemical analysis" (Ed. Glick, D.) Vol. 24, pp. 284 – 285. Wiley, New York.

Dikstein, S. and Maurice, D.M. (1969). In "Biochemistry of the eye" (Ed. Graymore, C.N.) pp. 1 – 103. Academic Press, London and New York, 1970.

Dische, Z. (1947). A new specific colour reaction of hexuronic acid. *J. Biol. Chem. 167*, 189 – 198.

Dreyer, R. and Rose, R.C. (1993). Lacrimal gland uptake and metabolism of ascorbic acid. *Proc. Soc. Exp. Biol. Med. 202(2)*, 212 – 216.

Drujan, B.D. and Ali, M.A. (1972). ATP metabolism in light and dark adapted retinae. In "*VI*th *Int. Congr. Photobiol., Bochum.* 1972. Book of Abstracts (Ed. Wschenk, G.O.) pp. 208.

Duijm, M. (1958). On the position of a ribbon – like central area in the eyes of some birds. *Archs. Neural. Zool. 13*, 128 – 145.

Eguchi, E. (1965). Rhabdome structure and receptor potentials in single crayfish retinula cells. *J. Cell Comp. Physiol. 66*, 441.

Ehlers, N. (1973). In vitro studies of trans- and intra- epithelial potentials of the cornea. *Exp. Eye Res. 15*, 553.

Enami, M. (1955). Melanopore concentrating hormone (MCH) of possible hypothalmic origin in catfish, *Parasilurus. Science 121*, 36 – 37.

Enomoto, N.; Nakagawa, H.; Matsuda, K. and Tomiyasu, Y. (1966). Sialic acid in the external mucous substance and the cartilage of fishes. *Bull. Japan Soc. Sci. Fisheries 32*, 442-445.

Exner,S (1891). Die Physiologic der facettierten Augen Von Krebsen und Insekton. Liepzig.

Farber, S.J. and Schubert, M. (1957). The binding of cations by chondroitin sulphates. *J. Chem. Invest. 38*, 249 – 264.

Ferrero, E. ; Anctil,M and Ali,M.A (1979) . Ultrastructural corelates of retinomotor responses in inner segments of vertebrates photoreceptors . *Rev.Can.Biol. 38*,249-264.

Fischbarg, J. (1973). Active and passive properties of the rabbit corneal epithelium. *Exp. Eye Res. 15*, 615.

Forrest, H.S. 91962). In "Pteridines: Structure and metabolism". Florkin and Mason, 4, 615 – 641.

Frank, R.N. and Goldsmith, T.H. (1965). Adenosine triphosphatase activity in the rod outer segments of the pig's retina. *Arch. Biochem. Biophys. 110*, 517 – 525.

Freeman, A.R.; Connel, P.M. and Fingerman, M. (1968). An electrophysiological study of the red chromatophore of the prawn, *Palaemonetes* : Observations on the action of red pigment – concentrating hormone. *Comp. Biochem. Physiol. 26*, 1015 – 1025.

Friedman, M.H. (1973). Unsteady aspects of corneal thickness control. *Exp. Eye Res. 15*, 645.

Frost, S. K. and Bagnara, J.T. (1979). Alluprinol-induced melanism in the tiger salamander, *Ambystoma tigrinum nebulosum. J. Exp. Zool. 209*, 455 – 466.

Fuji, R. (1969). Chromatophores and pigments. In "Fish physiology" (Eds. Hoar, W.S. and Randall, D.J.) Vol. III. Academic Press, London and New York, 1969.

Fulpius, B. and Baumann, F. (1969). Effects of sodium and calcium ions on slow and spike potentials in single photoreceptor cells. *J. Gen. Physiol. 53*, 541.

Gabe, M. (1966). "Neurosecretion". Pergamon Press, London, New York and Toronto, 1966.

Gire, K.V. and Nigam, V.N. (1954). Circular paper chromatography of carbohydrates. *J. Indian Inst. Sci. 36*, 49 – 63.

Goldschmidt, M. and Bernard, G.D. (1974). In "General and Comparative Physiology" (Ed. Hoar, W.S.) 3rd ed. pp. 381. Prentice – Hall of India, New Delhi, 1987.

Goldsmith, T.H. (1958a). The visual system of the honey bee. *Proc. Natl. Acad. Sci. 44*, 123 – 126.

Goldsmith, T.H. (1958b). On the visual system of the bee, *Apis mellifica. Ann. N. Y. Acad. Sci. 74*, 223 – 229.

Goldsmith, T.H. (1986). Interpreting trans – retinal recordings of spectral sensitivity. *J. Comp. Physiol. A. 159*, 481 – 487.

Goralska, M.; Harned, J.; Fleisher, L.N. and McGahan, M.C. (1998). The effect of ascorbic acid and ferric ammonium citrate on iron uptake and storage in lens epithelial cells. *Exp.Eye Res. 66(6)*. pp. 687.

Gregg, T.G. and Smucker, L.A. (1965). Pteridines and gene homologies in the eye colour mutants of *Drosophila hydei* and *D. melanogaster. Genetics 52,* 1023 – 1034.

Guha, B.C. and Paul, J.C. (1936). In "Ascorbic acid: Chemistry, metabolism and uses" (Eds. Seib, P.A. and Tolbert, B.M.) pp. 179 – 197. *Amer. Chem. Soc. Washington, D.C.*, 1982.

Gunder, I. (1955). Quantitative Untersuchungen uber die Entwicklung der pterine und des Riboflavines in der Haut und in den Augen Von Bufo bufo. *Z. Naturf. 106,* 173 – 177.

Gurevich, A.A. (1963). Catalytic action of peroxidase in the induced reaction of 0- dinitrobenzene by ascorbic acid. *Dokl. Biol. Sci. 145,* 674 – 677.

Hadorn, E. and Mitchell, H.K. (1951). Properties of mutants of *D. melanogaster* and changes during development as revealed by paper chromatography. *Proc. Nat. Acad. Sci. USA. 37,* 650 – 665.

Hagins, W.A. (1965). Electrical signs of information flow in photoreceptors. *Cold Spr. Harb. Symp. Biol. 30,* 403.

Hagins, W.A. and Jennings, W.H. (1960). Energy transfer with special reference to biological systems. *Disc. Farady Soc. 27,* 180 – 190.

Hagins, W.A.; Zonana, H.V. and Adams, R.G. (1962). Local membrane current in the in the outer segment of the squid photoreceptors. *Nature, 194,* 844 – 847.

Hall, C.W. ; Liebaers, I.; DiNatale, P. and Neufeld, E.F. (1978). Enzymic diagnosis of the genetic mucopolysaccharide storage disorders. *Methods Enzymol. 50*, 439.

Hama, T. and Fukada, S. (1964). In "Pteridine chemistry" (Eds. Pfliederer, W. and Taylor, E.C.) pp. 495 – 505. Pergamon Press, Oxford.

Hama, T. and Obika, M. (1960). Pteridine synthesis in the amphibian neural crest cell. *Nature (Lond.) 187*, 326 – 327.

Hama, T.; Goto, T.; Tohnoki, Y. and Hiyama, Y. (1965). The relation between pterines and chromatophores in the Medaka, *Oryzias latipes. Proc. Jap. Acad. 41*, 305 – 309.

Hama, T.; Matsumoto, J. and Obika, M. (1960a). Localisation of pterines in the goldfish skin melanophores and erythropores. *Proc. Jap. Acad. 36*, 217 – 222.

Hama,T. (1963). The relation between the chromatophores and pterin compounds. *Ann. N.Y. Acd. Sci. 100*, 977.

Handschumacher, R.E. and Welch, A.D. (1960). In "The nucleic acids" (Eds. Chargaff, E. and Davidson, J.N.) pp. 453 – 526, Academic Press, New York and London.

Harkonen, M.; Palkama, A. and Uusitalo, R. (1972). Functional dependence of the ciliary epithelium ATPase activity and and intraocular pressure on the autonomic nervous system. *Acta Physiol. Scand. 86*, 327 – 341.

Harper, H.A.; Rodwell, V. and Mayers, P.A. (1979). Review of physiological chemistry. pp. 702. Lange Medical Publication. Los Altos. California.

Harris, J.E. and Becker, B. (1965). Cation transport of the lens. *Invest. Ophthalmol. 4,* 709 – 722.

Harvey, W.R.; Cioffi, M. and Wolfersberger, M.G. (1983). Chemiosmotic potassium ion pump of insect epithelia. *Comp. Physiol.* 13, 163 –175.

Hascald, V.C. (1981). Biology of carbohydrates (Eds.Ginsburg,V.and Robbins, P.) Vol. 1, pp. 1 – 49. Wiley, New York.

Heath, H. and Fiddick, R. (1965b). In "Biochemistry of the retina" (Ed. Graymore, C.N.) pp. 14.Academic Press, London and New York.

Heath,H and Fiddick,R (1970) .In " Biochemistry of the eye"(Ed.Graymore ,C.N) . Academic press, London and New York.

Hedbys, B.O. (1961). The role of polysaccharides in corneal swelling. *Exp. Eye Res. 1,* 81 – 91.

Hedbys, B.O. (1963). Studies on the hydration on the corneal stroma. pp. 1- 20. *Akademisk. Lund.*

Hendler, E.D.; Torelli, J. and Epstein, F.H. (1971). The distribution of sodium – potassium – activated adenosine triphosphate in the medulla and cortex of the kidney. *J. Clin. Invest. 50,* 1329.

Highnam, K.C. and Hill, L. (1977). In "General and Comparative Physiology" (Ed. Hoar, W.S.) 3rd ed. pp.281. Prentice – Hall of India, New Delhi, 1987.

Hoar,W.S (1983). In "General and comparative physiology" 3rd Ed. Prentice – Hall of India, New Delhi, 1987.

Hodson, S. (1971). Evidence for bicarbonate – dependent sodium pump in corneal endothelium. *Exp. Eye Res. 11,* 20.

Hopkins, F.G. (1889). In "Experiments in physiology and biochemistry" (Ed. Kerkut, G.A.) Vol. 4, pp 289 – 346. Academic Press, London and New York, 1971.

Horning,D. (1975). Distribution of ascorbic acid metabolites and analoques in man and animals. *Ann.N.Y. Acad. Sci. 258,* 103 –117.

Hou, Y.I.; Pierce, W.M. and Delamere, N.A. (1998). The influence of ascorbic acid on active sodium transport in cultured rabbit non – pigmented ciliary epithelium. *Investl. Ophthalmol. Vis. Sci. 39(1).* pp. 143.

Hughes,R.E.; Hurley,R.J. and Jones,P.R.(1971).Retention of ascorbic acid by the guinea pig eye lens. *Exp.Eye Res. 12,* 39 – 43.

Humason, G.L. (1971). Anima Tissues Techniques. W.H. Freeman and Co. San Fransisco.

Hunt, S.; Grant, M.E. and Liebovich, S.J. (1970). Polymeric collagen isolated from marine snail (*Buccinum undatum*) connective tissue. *Experimentia, 26,* 1204 – 1205.

Isherwood, F.A. and Mapson, L.W. (1962). Ascorbic acid metabolism in plants. *Annu. Rev. Plant Physiol. 13,* 529 – 530.

Ishibashi, T. (1957). Effect of the intracellular injection of inorganic salt on fish scale melanophore. *J. Fac. Sci. Kokkaido Univ., Ser VI(13),* 449 – 454.

Jakus, M.A. (1964). Ocular fine structures. Little Brown and Co. Boston.

Janado, M. and Dunstone, J. (1972). A glycoprotein from bovine nasal cartilage. *J. Biochem. (tokyo) 71,* 635-643.

Jaques, L.B. (1977). Determination of heparin and related sulphated mucopolysachharides. In "Methods of biochemical analysis". 24, 206.

Jeanloz, R.W. (1970). Mucopolysaccharides of higher animals. In "The carbohydrates" (Eds. Pigman and Herb), Vol. 2B pp. 589 – 625. Academic Press, New York.

Karrer, P. (1950). Organic Chemistry. Elsevier, New York, 1950.

Katchalsky, A. (1964). Polyelectrolytes and their biological interactions. *Biophys. J. 4*, 9 – 41.

Kato,S. ; Teranishi,T.; Kuo,C.H and Negishi,K (1982). 5-HT stimulates tritium-labelled dopamine release from the fish (*Cyprinus carpio*) retina . *J.Neurochem. 39(2),* 493-498.

Kaufman, S. (1959). Studies on the mechanism of enzymatic conversion of phenylalanine to tyrosine. *J. Biol. Chem. 234*, 2677 – 2682.

Keeley, F.W., La Bella, F. and Queen, G. (1969). Dityrosine in a non hydroxyproline, alkali soluble protein isolated from chick aorta and bovine ligament. *Biochem. Res. Commun.34,* 156-161.

Kennedy, G.Y. (1979). Pigments of marine invertebrates. *Adv. Marine Biol. 16,* 309 – 381.

Kennedy, J.F. (1979). Proteoglycans –Biological and Chemical aspects in Human life. Elsevier, Amsterdam.

Kennedy, J.F. and White, C.A. (1983). Bioactive carbohydrates. In "Chemistry, Biochemistry and Biology" pp. 183. Ellis Harwood Limited. Halstead Press, New York, Brisbane, Chichester, Toronto.

Kidder, G.W. and Dewey, V.C. (1963). Relationship between pyrimidine and lipid biosynthesis and unconjugated pteridine. *Biochem. Biophys. Res. Commun. 12,* 380 – 283.

Kidder, G.W. and Dewey, V.C. (1968). A new pteridine from Tetrahymena. *J. Biol. Chem. 243,* 826 – 833.

Kinoshita, H. (1963). Electrophoretic theory of pigment migration within fish melanophore. *Ann. N.Y. Acad. Sci. 100,* 992 – 1003.

Kinoshita, J.H. (1964). Annual review selected topics in ophthalmic biochemistry. *Arch. Ophthal. 72,* 554.

Kinoshita, J.H. (1965). Cataracts in galactosamia. *Invest. Ophthalmol. 4,* 786 – 799.

Kinoshita, J.H.; Kern, H.L. and Merola, L.O. (1961). Factors affecting the cation transport of calf lens. *Acta Biochem. Biophys. 47,* 458 – 466.

Kinoshita, J.H.; Marurat, T. and Helfant, M. (1955). The pathways of glucose metabolism in corneal epithelium. *Science, 122,* 72.

Kinsey, V.E and Jackson,B (1949) . In" Biochemistry of the eye" (Ed Graymore , C.N) , Academic Press, London and New York.

Kinsey, V.E. and Reddy, D.V.N. (1965). Studies on the crystalline lens XI. The relative role of epithelium and capsule in transport. *Invest. Ophthalmol. 4,* 104 – 116.

Kinsey,V.E. and Jackson,B.(1949). In "Biochemistry of the eye"(Ed.Graymore, C.M.) pp.150 –170. Academic Press, London and New York, 1970.

Kinsey,V.E.(1947). In "Biochemistry of the eye". (Ed. Graymore, C.N.) pp.150 – 170. Academic Press, London and New York, 1970.

Klyce, S.D.; Neufeld, A.H. and zadunaisky, J.A. (1973). The activation of chloride transport and Db cyclic – AMP in the cornea of the rabbit. *Invest. Ophthalmol. 12,* 127.

Klyne,M.A.and Ali, M.A.(1980). Retinomotor responses: role of microtubules. *Mikroskopie 36,* 199 – 210.

Kobayashi, T.K. and pedrini, V. (1973). Proteoglycan – collagen interactions in human costal cartilage. *Biochem. Biophys. Acta. 303,* 148 – 160.

Krause, A.C. (1933). In "Biochemistry of the eye" (Ed. Graymore, C.N.) pp. 183 – 254. Academic Press, London and New York, 1970.

Krishnan, N. (1965). Occurrence of acid mucopolysaccharides in the cuticle of the scorpion, *Palaemoneus swammerdami. Curr. Sci. 34(4),* 120 – 121.

Krueger, R.; Kuchle, H.J and Kalfhaus,G.(1959).In "Biochemistry of the eye" (Ed. Graymore, C.N.) Academic Press, London and New York.

Kuck, J.E.R. (1970). Chemical constituents of the lens. In "Biochemistry of the eye" (Ed. Graymore, C.N.) pp. 183 – 254. Academic press, London and New York, 1970.

Kuck, J.F.R. Jr. (1970). Chemical constituents of the lens. In "Biochemistry of the eye" (Ed. Graymore, C.N.) pp. 183 – 254. Academic Press, London and New York, 1970.

Kuhne,W. (1877). In "Ecology of vision"(Ed. Lythgoe, J.N.). Clarendon Press, Oxford, 1979.

Kulhman,R.E and Resnick, R.A (1959) . The oxidation of C14 –labeled glucose and lactate by the rabbit cornea . *Arch. Biochem. Biophys. 85*,29.

Langer, H. (1964). Der phosphatstoff – weehsel des facet temauges im dunkeln und im Licht. *Helgolander Wiss. Meeresunters 9,* 251 – 252.

Langer, H. (1974). Activitatwen von ATPasen im Fliegen – Auge. In "25 Coll. Ges. Biol. Chem. Mosbach. 1974" (Ed. Jaenicke, L.) pp. 29 – 32. Springer, Berlin.

Langer, H. and Thorell, B. (1966). The functional organisation of the compound eye (Ed. Bernhard, C.G.) Pergamon Press, Oxford.

Langham, M.E (1950). In " Biochemistry of the eye" (Ed, Graymore, C.N) Academic Press, London and New York.

Langham, M.E. and Kostalnik, M. (1965). The effect of ouabain on the hydration and adenosine triphosphatase activity of the cornea. *J. Pharmacol. Exp. Ther. 150*, 398.

Lashely, K.S. (1916). The colour vision of birds. The spectrum of the domestic fowl. *J. Animal Behaviour, 6*, 1 – 26.

Lavietes, B.B. (1971). Kinetics of matrix synthesis in cartilage cell cultures. *Exp. Cell Res. 68*, 43 – 48.

Leatherland, J.F. (1967). Structure and function of the hypothalamo – hypophysel complex and associated ependymal structure in the fresh water eel, *Anguilla anguilla* L. (Ph.D. Thesis, univ. Leeds) In "Fish Physiology" (Eds. Hoar, W.S. and Randall, D.J.) Vol. II. Academic Press, New York and London, 1969.

Lechene, L. (1988). Biological role of the $Na^+ - K^+$ pump. In "The Na^+, K^+ - pump. Part B: Cellular aspects" (Eds. Skou, J.C.; Norby, J.G.; Maunsbach, A.B. and Esmann, M.), pp. 171 – 194. Alan R. Liss, New York.

Lehninger, A.L. (1970). In "Biochemistry". Worth Publishers Inc. New York, 1970.

Lerner, A.B. and Takahashi, Y. (1956). Hormonal control of melanin pigmentation. *Hormonal Res. 12*, 303 – 320.

Lewin, S. (1974). Recent advances in the molecular biology of vitamin C. In "Vitamin C" (Eds. Brich, G.G. and Parker, K.J.) pp. 221 – 252. Applied science Publishers Ltd. London.

Lythgoe, J.N. (1979). In "Ecology of vision". Clarendon Press, Oxford, 1979.

Mac Gregore, L.C. and Matschinsky, F.M. (1986). Altered retinal metabolism in diabetes II. Measurement of (sodium – potassium) ATPase and total sodium and potassium in individual retinal layers. *J. Biol. Chem. 261(9)*,4042 – 4058.

Malik, A.; Kojima, M. and Sasaki, K. (1995). Morphological and biochemical changes in lenses of guinea pigs after vitamin C deficient diet and UV- β radiation. *Ophthalmic Res. Vol. 27.* No.4.

Mandel, P. and Klethi, J. (1962). Pathways of adenosine triphosphate production by calf lens homogenates. *Nature (London), 195*, 306.

Marchase, P.B.; Karo, L.A.; Kelly, C.M. and McClay, D.R. (1982). A possible role for ligation and the phosphoglycoprotein it binds in calcium – dependent retinal cell adhesion. *J. Cell Biochem. 18(4),* 461 – 468.

Margulis, L. (1973). Colchicine – sensitive microtubules. *Int. Rev. Cytol. 34*, 333 – 361.

Margulis, R.K. and Margukis, R.U. (1970). Sulphated glycoproteins from rat brain glycoproteins. *Biochem. 9*, 4389 – 4396.

Matalon, R.; Arbogast, B. and Dorfman, A. (1974a). Deficiency of ChS N – acetylgalactosamine 4 – sulphate sulphatase in Moroteaux – Lamy syndrome. *Biochem. Biophys. Res. Commun. 61*, 1450.

Mathews, M.B. (1959). Macromolecular properties of isomeric chondroitin sulphates. *Biochem. Biophys. Acta. 35*, 9 – 17.

Mathews, M.B. (1975). Connective tissue – macromolecular structure and evolution. Springer – Verlag, Berlin, Heidelberg, New York.

Matsubara, M.; Katoh, S.; Akino, M. and Kaufman, S. (1966). Sepiapterine reductase. *Biochem. Biophys. Acta. 122*, 202 – 212.

Matsumoto, J. (1965a). Role of pteridines in the pigmentation of chromatophores in cyprinid fish. *Jap. J. Zool. 14*, 45 – 91.

Matsumoto, J. (1965b). Studies on fine structure and cytochemical properties of erythropores in swordtail, *Xiphophorus helleri*, with special reference to their pigment granules (pterinosomes). *J. Cell Biol. 27*, 493 – 504.

Matsumoto, J. and Obika, M. (1968). Morphological and biochemical characterisation of goldfish erythropores and pterinosomes. *J. Cell Biol. 39*, 233 – 250.

Matsumoto, J.; Bagnara, J.T. and Taylor, J.D. (1971). Isolation and separation of pteridines from animals. *Exp. Physiol. Biochem. 4*, 289 – 363.

Matsumoto, J.; Kajishima, T. and Hama, T. (1960). Relation between the pigmentation and pterine derivatives of chromatophores during development in the normal black and transparent scaled types of goldfish, Carassius auratus. *Genetics 45*, 1177 – 1189.

Matsumoto, J.; Kajishima, T. and Taylor, J.D. (1969). The pterinosomes: a sub-cellular unit in brightly coloured pigmentation of lower vertebrates. *VIth Int. Pigment Cell Conf. Seattle, Washington, USA. Abstr. J. Invest. Derm. 54*, 92.

Matsuoka, L.Y.; Wortsman, J.; Kupchella, C.K.; Engi, A. and Dretrich, J.E. (1982). Histochemical characterisation of the cutaneous involvement of acromeagaly. *Arch. Intern. Med. 142(10)*, 1820 – 1823.

Matsusaka, T. (1967a). The fine structure of the basal zone of the pigment epithelial cells of the chick retina. *Exp. Eye Res. 6*, 38 – 41.

Matsusaka, T. (1967b). Lamellar bodies in the synaptic cytoplasm of the accessory cone in the chick retina. *J. Ultrastructure Res. 18*, 55 – 70.

Mauck, W.L.; Mehrle, P.M. and Meyer, F.L. (1978). Effects of polychlorinated biphenyl archov. 1254 on growth, survival and bone development in brook trout, *Salvelinus fontanalis. J. Fish Res B. Can. 35*, 1084 – 1088.

Maurice, D.M and Riley, M.V (1970). In "Biochemistry of the eye" (Ed. Graymore,C.N) . Academic Press, London and New York.

Maurice, D.M. (1961) In "The structure of the eye" (Ed. Smelser, G.K.) pp. 381 – 391. Academic Press, New York and London.

Maurice, D.M. (1968b). In "The Eye" (Ed. Davson, H.) 2nd ed. Academic Press, New York and London.

Maurice, D.M. (1969). The cornea and sclera. In "The eye" (Ed. Davson, H.) 2nd ed. Vol. 1, pp. 489 – 600. Academic Press, New York.

Maurice, D.M. (1972). The location of the fluid pump in the cornea. *J. Physiol. 221*, 43.

Maurice, D.M. and Riley, M.V. (1970). In "The biochemistry of the eye" (Ed. Graymore, C.N.) pp. 1 – 95. Academic Press, London and New York.

Mayes, P.A. (1988). Metabolism of important hexoses. In "Harper's Biochemistry" (Eds. Murray, R.K.; Granner, D.K.; Mayes, P.A. and Rodwells, V.W.) 21st ed. Prentice – Hall International Inc.

Mc Clurkin, I.T. (1964). A method for the cytochemical demonstration of sodium – activated adenosine triphosphatase. *J. Histochem. Cytochem. 12*, 645 – 658.

Mc Cullagh, K.G.; Derouette, S. and Robert, L.(1973). Elephant aorta tissue II. Amino acid analysis, structural glycoprotein and antigenicity. *Exp. Mol.Pathology. 18*, 202-213.

McCauley, W.J. (1971). In "Vertebrate Physiology" pp. 347. W.B. Saunders Co. Philadelphia, London, Toronto.

McConnel, D.G. and Scarpelli, D.G. (1963). Rhodopsin: an ATPase. *Science, 139*, 848.

McFall-Ngai, M. ; Crescitelli, F.; Childress, J. and Horwitz, J. (1986). Pattern of pigmentation in the eye lens of the deep sea hatchet fish, *Argyropelecus affinis* Garman. *J. Comp. Physiol. A. 159*, 791 – 800.

McKusick, V.A.; Neufeld, E.F. and Kelley,T.E. (1978). The mucupolysaccharide storage diseases. In "The metabolic basis of inherited disease, 4th ed. (Eds. Stanbury, J.B. ; Wyngaarden, J.B. and Fredrickson, D.S.) pp. 1282. McGraw – Hill, New York.

McLaren, D.S. (1970). Nutritional aspects of the eye. In "Biochemistry of the eye" (Ed. Graymore, C.N.). Acedemic Press , London and New York, 1970.

McNutt, W.S. (1964). In "Pteridine chemistry" (Eds. Pfleider and Taylor). pp. 427 – 441, Pergamon Press, Oxford.

Meenakshi, V.R. and Scheer, B.T. (1959). Acid mucopolysaccharides of the crustacean cuticle. *Science, 130*, 1189 – 1190.

Menter, D.G.; Obika, M.; Tchen, T.T. and Taylor, J.D. (1979). Leucophores and Eridiophores of *Fundulus heteroclitus*: biophysical and ultrastructural properties. *J. Morph. 160*, 103 – 119.

Meyer, K. (1938). *Cold Spring Harbour Symposia on Quantitative Biology 6*, 91.

Midelfart, A. (1987). Visualisation of Ouabain binding in bovine cornea. *Acta. Ophthalmol. 65(4)*, 424 – 429.

Mitchell, R. (1961). *Nature, 791*, 144.

Moczar, E. Moczar, M. (1972). The phylogenetic evolution of the corneal stroma. Distribution of carbohydrates in the macromolecular complexes of the

primate Baboon (*Cynocephalus papio papio*) corneal stroma. *Comp. Biochem. Physiol. 43B*, 261 – 270.

Moczar, M. and Moczar, E. (1973). Phyllogenetic evolution of the cornea. Macromolecular composition of the corneal stroma of the squid, *Sepia officinalis. Comp. Biochem. Physiol. 45B*, 213 – 223.

Moczar,E. and Moczar, M. (1970). Glyvannes lies' a' la fraction insoluble de la cornee de rousette (*Seylliorhinus canicula* L.) *European J. Biochem 13*, 28 – 36.

Moczar,E; Moczar, M. and Payrau,P. (1969a). Influence des interactions proteine – glycanne sur le potential d' hydration du stroma corneen. *C.r. hebd. Seanc. Acad. Sci., Paris 268 D*, 3091 – 3093.

Moczar,E; Payrau. P. and Robert, L. (1969b). Distribution of carbohydrates in the soluble and unsoluble fractions of the stroma of fish corneas. *Comp. Biochem. Physiol. 30*, 73 – 82.

Morenor, T. (1894). Untersuchungen der protein substanzen in den leichtbrechenden Median des Auges 1. *Ztschr. Physiol. Chem. 18*, 61.

Morris, V.B. and Shorey, C.D. (1967). An electron microscope study of the types of receptors in the chick retina. *J. Comp. Neurol. 129*, 313 – 340.

Mrinal, K. Deb and A. Raghu Varman (1994). Glycosaminoglycan fractions in the cornea of two fishes. *Curr. Sci. Vol. 67*, No. 12 pp. 1020.

Muller,H.K and Duschke,W. (1934). In"Biochemistry of the eye" (Ed.Graymore,C.N.). Academic Press, London and New York.

Munz, F.W. (1971). The visual pigments of epipelagic and rocky – shore fishes. *Vision Res. 4,* 441 – 454.

Munz, F.W. (1971).Vision: Visual pigments. In "Fish Physiology" (Eds. Hoar, W.S. and Randall, D.J.) Vol. V. pp. 1 – 26. Academic Press, London and New York, 1971.

Munz,F.W and Mc Farland, W.N (1977). In" Ecology of vision"(Ed. Lythgoe,J.N) Clarendon Press, Oxford, 1979.

Murray, R.K. (1988). In "Harper's Biochemistry" (Eds. Murray, R.K.; Granner, D.K.; Mayes, P.A. and Rodwell, V.W.) 21st ed. pp. 589 – 606. Prentice-Hall International Inc.

Mustafa, M. and Kamat, D. (1970). Mucopolysaccharide histochemistry of *Musca domestica* 1. A report on the occurance of a new type of KOH – labile alcianophilia. *Histochemie. 21,* 54 – 63.

Naumann, H.N (1959) . In " Biochemistry of the eye" (Ed. Graymore,C.N). Academic Press, London and New York.

Needham, A.E. (1974). The significance of zoochromes. Springer – Verlag, Heidelberg.

Neufeld, E.F.and Fratantoni, J.C. (1970). Inborn errors of mucopolysaccharide metabolism. *Science, 169,* 141. New York, London

Nicola, A.F.; Clayman, M. and Jhonstone, R.M. (1968). *Gen. Comp. Endocrinology. 11,* 332 – 337.

Novales, R.R. (1959). The effect of osmotic pressure and sodium ion concentration on the response of melanophores to intermedin. *Physiol. Zool. 32*, 15 – 28.

Obika, M. (1963). Association of pteridines with amphibian larval pigmentation and their biosynthesis in developing chromatophores.

Obika, M. and Bagnara, J.T. (1964). Pteridines as pigments in amphibians. *Science 143*, 485.

Ogston, A.G. (1966a). On water binding. *Federation-Proc. 25*, 986 – 989.

Ogston, A.G. and Wells, J.D. (1972). The osmotic properties of sulfoethyl – sephadex. A model for cartilage. *Biochem. J. 128*, 685 – 690.

Oliphant, L.W. (1988). In "Advances in pigment cell research" (Ed. Bagnara, J.T.) pp. 65 – 82. A. R. Liss, New York.

Omaye, T.S.; Tillotson, J.A. and Sauberlich, H.E. (1982). In "Ascorbic acid: Chemistry, metabolism and uses" (Eds. Seib, P.A. and Tolbert, B.M.) pp.199 – 222. American Chemical Society, washington, D.C.

Oosawa, F. (1971). Polyelectrolytes. Dekker, New York.

Osborn, C.M. (1938). In "A text book of general physiology" (Ed. Davson. H.) Vol.2. J. and A. Churchill, London, 1970.

Padh, H. (1990). Cellular functions of ascorbic acid. *Biochem. Cell Biol. 68*, 1166 – 1173.

Palay, S.L. (1953). Neurosecretory phenomena in the hypothalamo – hypophyseal system of man and monkey. *Am. J. Anat. 93*, 107 – 141.

Palkama, A. and Uusitalo, R. ((1968). Histochemical demonstration of ouabain – sensitive sodium – potassium – activated adenosine triphosphatase system. *Scand. J. Clin. Lab. Onvest. (suppl) 101*, 48.

Palkama,A. and Uusitalo, R. (1970). Histochemical demonstration of sodium – potassium – activated adenosine triphosphatase activity in rabbit ciliary body. *Annal. Med. Exp. Biol. Fenn. 48*, 49 – 55.

Palva, M. and Palkama, A. (1974). Histochemically demonstrable sodium – potassium – activated adenosine triphsphatase (Na^+ – K^+ – ATPase) activity in the rat lens. *Expl. Eye Res. 19*, 117 – 123.

Payrau, P.; Pouliquen, Y.; Faure, J.P. and Offret, G. (1967). In "Biochemistry of the eye" (Ed. Graymore, C.N.) pp.13. Academic Press, London, New York.

Pedler, C. (1969). Rods and cones – a new approach. *Int. Rev. Gen. Exp. Zool. 4,* 219 – 274.

Perks, A.M. (1969). In "Fish Physiology" (Eds. Hoar,W.S. and Randall, D.J.) Vol. 2. Academic Press, London and New York.

Piatigorsky, J. (1984). Lens crystallins and their gene families. *Cell, 38*, 620 – 621.

Pierie, A. (1946). In "Biochemistry of the eye" (Ed. Graymore, C.N.). Academic Press, London and New York, 1970.

Pirenne,M.H (1967) . "Vision and the eye". 2nd Ed. Chapman and Hall, London.

Pierie, A. (1965). A light-catalysed reaction in the aqueous humour of the eye. *Nature, 205*, 500.

Podesta, H.H and Baucke, J. (1938). In " Biochemistry of the eye" (Ed. Graymore, C.N). Academic Press, London and New York.

Prabhakaran; Malladi and Ortwerth, B.J. (1992). The glycation and cross – linking of isolated lens crystallin by ascorbic acid. *Exp. Eye Res. 55(3),* 451 – 459.

Prota, G. (1980). Recent advances in the chemistry of melanogenesis in mammals. *J. Invest. Derm. 75,* 122 – 127.

Pumphrey, R.J. (1961). In "Biology and Comparative Physiology of Birds". Vol. II (Ed. Marshall, A.J.) pp. 55 – 68. Academic Prress, New York, London.

Purcell, E.F.; Lerner,L.H and Kinsey, V.E (1954) . In " Biochemistry of the eye" (Ed. Graymore, C.N). Academic press, London and New York.

Purmann, R. (1940a). In "Experiments in physiology and biochemistry" (Ed. Kerkut, G. A.) Vol. 4, pp. 289 – 364. Academic Press, London and New York, 1971.

Purmann, R. (1940b). Isolation and separation of pteridines. In "Experiments in physiology and biochemistry" (Ed. Kerkut, G.A.) Vol. 4. pp. 289 – 364. Academic Press, London and New York, 1971.

Purmann, R. (1941). In "Experiments in physiology and biochemistry" (Ed. Kerkut, G.A.) Vol. 4. Academic Press, London and New York, 1971.

Radhakrisnamurthy, B. and Berenson, G.S. (1973). Structure of glycopeptide from a glycoprotein from bovine aorta . *J. Biol. Chem. 248,* 2000-2006.

Raghu Varman, A. (1980). Melanins in the pectin of pigeon and their roles. *Sci. and Cult. 45,* 289 – 290.

Rauen, H.M. and Stamm, C.V. (1952). Beitrage zur Analyse der Pterine. *Hoppe-Seyler's Z. Physiol. Chem. 289,* 201 – 210.

Rawal, U.M and Rao, G.N. (1977). Biochemical studies on the vertebrate lens. 1. Quantitative study of L-ascorbic acid and glutathione. *Vidya B-Science Vol. XX(2),* 172 – 178.

Rawal, U.M. and Rao, G.N. (1978). Biochemical studies on the vertebrate lens. *Comp. Physiol. Ecol. 3(4).* 262 – 264.

Rawal, U.M. and Rao,G.N. (1980). Biochemical studies on the vertebrate lens III. Lactic acid, glucose and fructose. *Comp. Physiol. Ecol. 5(1),* 32 – 34.

Rawal, U.M. and Rao,G. N. (1983). Biochemical studies on the vertebrate lens. VI. Amino acids. *Comp. Physiol. Ecol. 8(2),* 109 – 112.

Reddy, D.V.N and Kinsey, V.E (1960). In " Biochemistry of the eye" (Ed. Graymore, C.N). Academic Press, London and New York.

Reuss, S. and Olcese, J. (1986). Magnetic field effects on the rat pineal gland: Role of retinal activation by light. *Neurosci. Lett. 64,* 97 – 101.

Richard H. Masland (1986). The functional architecture of the retina. *Scientific American.* Vol. 225. No. 6, pp. 102 – 111.

Richards, C.M. and Bagnara, J.T. (1967). Expression of Pteridines in neural crest tranplants between *Pleurodeles* and *Axolotl. Dev. Biol. 15,* 334 – 347.

Riley, M.V. (1964). The sodium – potassium adenosine triphosphatase of rabbit ciliary epithelium. *Exp. Eye Res. 3,* 76 – 84.

Robert, A.M., Robert, B. and Robert, L. (1970). In "Chemistry and molecular properties of intracellular matrix."(Ed. Balays, E.A.) pp.237-244. Academic Press, New York.

Robert, L. and Robert, B. (1967). In "Proteids of the biological fluids" (Ed. Peetres, H.) Vol. 15 pp. 143. Elsevier, Amsterdam.

Robert, L. and Schillinger, G. (1967). Etude sur la composition chimique des cornees de poissons Elasmobranches et Teleosteens. *Archs. Ophthal. (Paris). 27*, 813 – 818.

Robert, L.; Robert, B.; Parlebas, J.; Zweibawm, A. and Oudea, P. (1965b). In "Biochemistry of the eye" (Ed. Graymore, C.N.) pp. 1 – 103. Academic Press, London and New York, 1971.

Robinson, G.A.; Butcher, R.W. and Sutherland, E.W. (1971). Cyclic AMP. pp. 531. Academic Press, New York.

Rochon – Duvigneaud. Cited in "The Avian Brain" (Ed. Ronald Pearson).

Roden, L. (1980). Structure and metabolism of connective tissue proteoglycan. In "The biochemistry of glycoproteins and proteoglycan" pp. 267 – 371 (Ed. Lennarz, W.J.), Plenum Press, New York and London.

Roderick K. Clayton (1971). Vision in higher animals. In "Light and living matter Vol. 2. The biological part". McGraw – Hill Book Company.

Rolich, P. (1970) The interphotoreceptor matrix: Electron microscopic and histochemical observation on the vertebrate retina. *Exp. Eye Res. 10(1)*, 80 – 86.

Ronald Pearson (1971) In "The Avian Eye".

Rosner, L.; Farner, C.J. and bellows, J.G. (1938). In "Biochemical studies on the vertebrate lens 1. Quantitative study of L – ascorbic acid and glutathione. Rawal and Rao, (1977). *Vidya B- Science XX(2)*, 172 – 178.

Rubin, P.S. and Howard, J.R. (1950). *Conference on metabolic interactions, Trans. 2nd Conf. Col. 2* pp. 155.

Salzman, M. (1912). The anatomy and histology of the human eyeball in the normal state – its development senescene (Tr. Brown, E.V.L.) Chicago University Press.

Sannasi, A. (1969). Ph.D thesis, Madras University, Madras.

Scarpelli, D.G. and Graig, E.L. (1963). The fine localisation of nucleoside triphosphatase. *J. Cell Biol. 17*, 279 – 288.

Schachtschabel, D.O.; Bigalke, B. and Rohen, J.W. (1977).Production of glycosaminoglycans by cell cultures of the trabecular meshwork of the primate eye. *Exp. Eye Res. 24*, 71 – 80.

Scharrer, E. (1952b). "Neurosecretion" (Ed. Gabe,M.) Pergamon Press, London, New York and Toronto, 1966.

Scheline,R.R.(1963). Adrenergic mechanism in fish: Chromatophore pigment concentration in cuckoo wrasse, *Labrus ossifagus* L. *Comp. Biochem. Physiol. 9,* 215 – 227.

Schmidt, U. and Dubach, U.C. (1971). Na^+ - K^+ - stimulated adenosine triphosphatase: Intracellular localisation within proximal tubule of the rat nephron. *Ofluegers. Arsh. Eur. J. Physiol. 330*, 265.

Schopf, C. and Reichert, R. (1941) In "Experiments in physiology and biochemistry" (Ed. Kerkut, G.A.) Vol. 4, pp. 289 – 346. Academic Press, London and New York, 1971.

Scott, G.T. (1965). Physiology and pharmacology of colour change in the sand floundler, *Scopthalmus aquosus. Limnol. Oceanog. 10*, R 230 – R 246.

Seib, P.A. and Tolbert, B.M. (1982). "Ascorbic acid: Chemistry, metabolism and uses". American Chemical Society, Washington, D.C.

Sekoguti, Y. (1960). On the ATPase activities in the retina and rod outer segments. *J. Cell Comp. Physiol. 56*, 129 – 136.

Sengupta,P.N.and Guha,B.C. (1938).In "Ascorbic acid: Chemistry, metabolism and uses" (Eds. Seib, P.A. and Tolbert, B.M.) American chemical Society, Washington, D.C., 1982.

Sharma, V.K. (1989). Vitamin C protects eye lens from radiation damage. *Sci.Report 26*, 291 – 303.

Skuo, J.C. (1964). Enzymatic aspects of active linked transport of Na^+ and K^+ through the cell membrane. *Progr. Biophys. Mol. Biol. 14*, 137 – 166.

Skuo, J.C. (1965). Enzymatic basis for active transport of Na^+ and K^+ across cell membranes. *Physiol.Rev. 45*, 596 – 617.

Skuo, J.C. (1972). The relationship of the Na^+ - K^+ - activated enzyme system in the transport of sodium and potassium across the cell membrane. *Bioenergetics, 4*, 203.

Smelser, G.K. (1963). Corneal hydration-comparative physiology of fish and mammals. *Invest. Ophthal. 1*, 1 – 11.

Spector, A. (1965). The soluble proteins. *Invest. Ophthalmol. 4(4)*, 579 – 591.

Spector, A.; Wandel, T. and Li, L.K. (1968). The purification and characterisation of the highly labelled protein fraction from calf lens. *Invest. Ophthalmol.*

Stackhouse, H.L. (1966). Some aspects of pteridine biosynthesis in amphibians. *Comp. Biochem. Physiol. 17*, 219 – 235.

Steive, H. (1965). Interpretation of the generator potential in terms of ionic process. *Cold Spr. Harb. Quant. Biol. 30,* 451.

Stekhoven, F.S. and Bontinf, S.L. (1981) Transport adenosine triphosphatase: properties and functions. *Physiol Rev. 61*, 1 – 76.

Sugiura, K. and Goto, M. (1968). Biosynthesis of pteridines in the skin of the tadpole, *Rana catesbeiana. J. Biochem. Tokyo, 64*, 657 – 666.

Sulkin, N.M. and Sulkin, D.F. (1975). Tissue changes induced by marginal vitamin C deficiency. *Ann. N.Y. Acad. Sci. 258*, 317 – 328.

Sundara Rajulu, G. (1969). Presence of acid mucopolysacchsarides in the cuticle of a millepede, *Cingalobolus bungnioni. Curr. Sci. 38*, 345 – 346.

Szent – Gyorgyi (1960). "Introduction to submolecular biology". academic Press, New York.

Tansley, K. (1931). The regeneration of visual purple with special reference to dark adaptation and night blindness. *J. Physiol. 71,* 442 – 458.

Tansley, K. (1965) In "Vision in vertebrates". Chapman and Hall, London.

Tervo, T. and Palkama, A. (1975). Electron microscopic localisation of adenosine triphosphatase (Na^+ – K^+ – ATPase) activity in the rat cornea. *Exp. Eye Res. 21,* 269 – 279.

Thomas, I. And Rawal, U.M. (1986). Blood-aqueous-lens barriers in vertebrates. A biochemical study of proteins and glutathione. *Proc. 7th National Symp. Of India on Society of Life Sci. Gulbaarga, India.*

Timple, R.: Wolff, I. And Wleiser, M. (1969). Acidic structural proteins in connective tissue. I. Solubilization and preliminary characterisation. *Biochem. Biophys. Acta. 194,* 112-120.

Trevelyan, W.E.; Proctor, D.P. and Harrison, J.S. (1950). Detection of sugars on paper chromatograms. *Nature, 166,* 444 – 445.

Urist, M.R.; Speer, D.P.; Ibsen, K.J. and Strates, B.S. (1968). Calcium binding by chondroitin sulphate. *Calcif. Tissue Res. 2,* 253 – 261.

Uyeda, K. and Rabinowitz, J.C. (1963). Fluorescence properties of tetrahydrofolate and related compounds. *Analyt. Biochem. 6,* 100 – 108.

Vadgama, M. and Kamat, D. (1971). The occurance of hyaluronic acid and / or hyaluronidase – labile substances in the salivary gland cells of insects with diverse feeding habits. *Acta. Histochem. 40,* 108 – 115.

Van de Veerdonk, F.C.G. (1962). Mechanism of hormone action in colour change in amphibia. *Gen. Comp. Endocrinol. 2,* 623 – 624.

Vander, A.J.; sherman, J.H. and Luciano, D.S. (1980). Human physiology, 3rd ed. pp. 724. McGraw – Hill, New York.

Verner, H.H.; Rayborn, E.M.; Osterfield, A.M. and Hollyfield, J.G. (1987). Localisation of the proteoglycans within the extracellular matrix sheath of cone photoreceptors. *Exp. Eye Res. 44(5),* 633 – 642.

Veron, J.E.N (1973). The physiological control of the chromatophores of *Austrolestus annulosus* (Odonata). *J. Insect Physiol. 19,*1689-1703.

Vittur, F., Pugliorella, M.C. and De Bernard, B.(1972a).Some properties of properties of proteoglycan of pre-osseous cartilage. *Biochem. Biophys. Acta. 257*, 389-397.

Vladesco, R and Stefanesco,H (1939) . In " Biochemistry of the eye" (Ed, Graymore, C.N), Academic Press, London and New York.

Von Frisch, K. (1911). In "Fish Physiology" (Eds. Hoar, W.S. and Randall, D.J.) Vol. III. Academic Press, New York, London.

Wachstein,M. and Meisel, E. (1957). Histochemistry of hepatic phosphatase at a physiologic pH. *Am. J. Path. 27*, 12 – 23.

Wagh, P.V. and Roberts, B.I. (1972). Purification and characterization of a glycoprotein from the internal region of porcine aorta. *Biochemistry 11*, 4222-4227.

Walcott, B. (1975). In "Ecology of vision" (Ed. Lythgoe, J.N.) Clarendon Press, Oxford, 1977.

Walls, G.L. (1942). "The vertebrate eye and its adaptive radiation". Carnbrook Inst. of Sci. Bloomfield Hills, Michigan.

Walls, G.L. and Judd, H.D. (1933). The intraocular colour filters of vertebrates. *Br. J. Ophthal. 17*, 641 – 676.

Warren, R.H and Burnside ,B (1978) . Microtubules in cone myoid elongation in the teleost retina. *J.Cell Biol. 78*,247-259.

Wells, J.D. (1973b). Salt activity and osmotic pressure in connective tissues. 1. A study of solutions of dextran sulphate as a model sysytem. *Proc. Soc. 183B*, 399 – 419.

Weygand, F.; Simon, H.; Dahms, G.; Keil, K.D. and Millauer, H. (1964). Pterine aus 2, 4, 5-triamino-6-hydroxypirimidin und Zucken. *Chem. Ber. 97*, 1002 – 1023.

Weygand, F. ; Simon, H.; Dahms, G.; Waldschidt, M.; Schliep, H.J. and Wacker, H. (1961). Uber die Biogenese des Leucopterins. *Angew. Chem. 73*, 402 – 407.

Wieland, H. and Schopf, C. (1925). In "Experiments in physiology and biochemistry" (Ed. Kerkut, G.A.) Vol. 4, pp. 289 – 364. Academic Press, London and New York, 1971.

Wiksow, M.A. and Novales, R.R. (1969). In "Fish Physiology" (Eds. Hoar, W.S. and Randall, D.J.). Vol. III. Academic Press, London and New York.

Willmer, E.N. (1966). Physiology of vision. In "Physiological basis of medical practice" (Eds. Best, C. and Taylor, N.) pp. 267. Scientific Book Agengy, Calcutta, 1967.

Wolf, B.; Gressner, A.M.; Nevo, Z. and Greiling, H. (1982). Age related decrease in the activity of U.D.P.- xylose core protein xylosyltransferase (Ec. 2.4.2.26) in rat costal cartilage. *Mech.Ageing Dev. 19(2)*, 187 – 190.

Wolf, G. and Varandani, P.T. (1960). Studies on the function of vitamin A in the mucoploysaccharide biosynthesis. *J.Biol. Chem. 225*, 995 – 1008.

Wolff, I.; Fuchswans, W.;Weiser, M.; Furthmayer, H. and Timple, R. (1971). Acidic structural proteins of connective tissue. Characterisation of their heterogenous nature. *Eur. J. Biochem. 20*, 426-431.

Young, R.W. (1970). Visual cells. *Sci.Am. 223(4)*, 81 – 91.

Zadunaisky, J.A. and Lande, M.A. (1971). Active chloride transport and control of corneal transparency. *Amer. J. Physiol. 221*, 1837.

Zhou, L.L.; Higginbottom, E.J. and Yue, B.Y.J.T. (1998). Effects of ascorbic acid on levels of fibronectin, laminin and collagen type I in bovine trabecular meshwork in organ culture. *Curr. Eye Res. 17*. No. 2. Pp. 211.

Ziegler, H. and Ziegler, I. (1965). In "Experiments in physiology and biochemistry" (Ed. Ketkut. G.A.) Vol. 4, pp. 289 – 345. Academic Press, London and New York, 1971.

Ziegler, I. (1964). In "Pteridine chemistry" (Eds. Pfleiderer, W. and Taylor, E.C.) pp.485 – 492. Pergamon Press, Oxford.

Ziegler, J.S. and Sidbury, J.B. (1977). Studies on lens proteins from the smoooth dogfish: Evidence for evolutionary conservation in vertebrate β-crystallins. *Ophthalmic Res. 9(2)*, 92 – 98.

BIODATA

NAME: BENDANG AO

SEX: MALE

NATIONALITY: INDIAN

EDUCATIONAL QUALIFICATION: M.Sc

SPECIALIZATION IN M.Sc.: ENTOMOLOGY

PAPERS / ABSTRACTS PUBLISHED/ACCEPTED :

Abstract titled "Sex – linked inheritance of cuticular polymorphism in the integument of the various castes of the weaver – ant *Oecophylla smaragdina* (L)". Presented in the National Symposium on Fifty Years of Zoology, University of Calcutta, 1999.

Abstract titled "Retinal pigment migration in response to various physiological states in the eyes of *Gallus domesticus*". Accepted for presentation in the National Symposium on "Trends in Environmental Biology", conducted by the Department of Zoology, N.E.H.U., Shillong, Meghalaya, 1999.

SYMPOSIA, CONFERENCES AND WORKSHOPS ATTENDED:

Workshop on UV – Visible Spectrophotometry conducted by the Regional Sophisticated Instrumentation Centre, N.E.H.U., Shillong

'International Conference on Radiation Biology: DNA Damage, Repair and Carcinogenesis'and ' Indo-German Satellite Symposium on Molecular Biology of Radiation Damage and Repair', conducted by the Department of Biochemistry, N.E.H.U