Chapter-5

PARABOLIC PATTERNS AND HELICOIDAL ARCHITECTURE OF CORNEA AND LENS OF HILL STREAM FISH, *Acrossocheilus hexagonolepis*

INTRODUCTION

The ocular refractive structures (cornea and lens) play extensive and varied role in the visual system in addition to their normal function of refraction of light. They regulate the quality and quantity of light entering the Retina by acting as interference filters, reducing reflection from the eye surface, helping the reflection of specific wavelengths from regions behind the receptive cells and assisting in selective light absorption (Bernhard *et al.*, 1965; Somiya, 1976; Dey and Dakhar, 1992). The eyes of vertebrates follow the same general plan. They are less flattened, fluid filled hollow organ and consist of normal complement of six oculomotor muscles. Its wall is composed of three general layers variously modified in different regions of the eyeball to perform different functions (Munz, 1971).

The photo-environment in which fish live is probably more varied when compared with that of any other vertebrates. Some of them are inhabitants of clear streams water and the surface of water bodies, where penetration of light is quite high. Some are bottom dweller or inhabitants of turbid water bodies in poor illumination, while some change from an environment of very low light levels to that with a very high intensity. Due to their exposure to very different light conditions fish adapt themselves to a great extent, which give rise to diversity in the structures and physiology of visual system. Like any other ocular structures (cornea and lens) also show chemical specialization as well as structural modifications in response to photo-adaptation (Lythgoe, 1971; Kennedy and Milkman, 1956; Somiya, 1976; Dey *et al.*, 1994).

Fish lenses are spherically shaped with high dioptric power. Spherical lenses have fascinated scientists since an early attempt to understand how light is
refracted by nearly spherical raindrops to produce rainbows. Spherical lenses can be subject to spherical aberration or any of five other primary aberrations characteristics of optical systems. However, spherical or chromatic aberration, or both, could adversely affect the image quality of spherical fish lenses.

Unlike terrestrial vertebrates, the principal refractive structure in the fish eye is the lens, since little refraction takes place at the cornea whose refractive index is approximately equal to that of water. Hence in fish, specializations in the dioptic apparatus in response to photo-adaptation is expected more in a lens than in cornea. However structural specialization in response to optical phenomena other than refraction has been described in some fish cornea (Lythgoe, 1971).

The lens of fishes is spherical or nearly spherical and crystalline with high refractive index. The focal length of these lenses has been described as a constant ratio (Matheson’s ratio) of focal length to lens radius of c.2.55: 1 (Walls, 1942; Pumphery, 1961). The refractive index is thought to drop continuously and parabolically, according to Matthiessen, (1980) from the center to the periphery so as to produce a lens with little or no spherical aberration.

Some of these optical phenomena correlated with the ultra-structural features of cornea and lens have been carried out in the past (Farrell et al., 1973; Lythgoe, 1971; Bernhard et al., 1965). It is noteworthy that the special optical phenomena reported in ocular refractive structures are related to the photo-environment to which the eye adapted. Therefore, a transmission electron microscopy study has been carried out on the cornea and lens of a hill stream fish, *Acrossocheilus hexagonolepis*, which is exposed to a very high intensity of light for most of the time.

AIMS AND OBJECTIVES

The aims and objectives of this study are:

1. To study and explain the parabolic pattern arrangement of the fibrils of the cornea of *A. hexagonolepis*. 
2. To study and explain the helicoidal arrangement of the macrofibrils of the lens of *A. hexagonolepis*.

3. To know the functional significances of the cornea and lens of *A. hexagonolepis*.

**MATERIAL AND METHODOLOGY.**

**Material:**

Cornea and lens were excised from the eye of fish purchased from local suppliers, and fixed in 3% glutaraldehyde prepared in 0.1 M Na-cacodylate buffer (pH 7.2) for 4hr at 4°C. The samples were post-fixed in 1% OsO₄ in 0.1 M Na-cacodylate buffer (pH 7.2), dehydrated in graded acetone to propylene oxide and embedded in Araldite. Pieces of plastic embedded samples were reoriented and ultrathin sections (500-600 Å) were cut on a Lkb ultramicrotome, ultratome V. The sections were mounted on copper grids, stained in aqueous uranyl acetate and lead citrate and examined with a JEE 100X transmission electron microscope (Jeol). The cornea and lens from six individual fish were used in the investigation.

**Methodology:**

**Transmission Electron Microscopic (T.E.M) studies:**

A. **Specimen Block Preparation for TEM:**

The resin blocks were prepared by the method of Hyat, 1981 as follows:

1. **Fixation (Primary fixative):** Fresh cornea, lens or retina are excised from the eye of the animal and were fixed in 3% Glutaraldehyde primary fixative by immersion process at 4°C for 4 hours.

2. **Washing:** The tissues were washed in 0.1 M Sodium Cacodylate buffer. The tissues were left overnight in this buffer at 4°C. Final trimming of the tissues to appropriate size was done while the tissues were inside the buffer.
3. **Post Fixation (Secondary Fixative):** The tissues were washed with double-distilled water and kept in it 15 min at 4°C and post-fixed in 1% Os O4 (Secondary fixative) in 0.1M Sodium Cacodylate buffer (Ph7 2) for 2 hours at 4°C.

4. **Dehydration:** Dehydration was carried out by giving two changes of 15 min each to different acetone grade to propylene oxide (Glauert, 1974).

5. **Clearing the tissues from acetone:** To facilitate infiltration, the tissues were kept in Epoxy propane as it can completely remove the traces of water (in case of incomplete dehydration). Two changes with Epoxy propane of 30 mins each were carried out at room temperature.

6. **Infiltration:** Infiltration was carried out at the room temperature with the liquid resin, Araldite CY212, with which Embedding of tissues was carried out following the method of Glauert and Glauert (1958).

**B. Ultrathin Sectioning of Specimens for TEM:**

Pieces of plastic embedded samples were reoriented and ultrathin sections (500-600 Å) were cut on a LKB ultramicrotome, ultratome V following the method of Reid (1974).

**C. Preparation of Ultrathin Sections:**

Under optical microscope, the area to be examined under TEM was selected and the blocks were further trimmed. The sections were lifted using the matted surface of the grid, which affords of the sections firmly.

**D. Staining Ultra thin Sections:**

A double staining method using uranyl acetate and lead citrate was routinely followed (Glauert, 1977). The metal grids carrying the sections were placed down onto the stain, kept in dark for 10 to 15 mins. Then the grids were washed in 2 lots of 50% ethanol and 2 lots of double distilled water with continuous agitation. These were then dried carefully on a filter paper. A few ml of lead citrate was taken in a watch glass and the grids were places onto the stain for 10 mins. Each grid was washed carefully in 0.1 N Na OH sol. and then in 2
lots of double distilled water. The grids were then dried and stored in a grid box and examined with a JEE 100 X transmission electron microscope (Jeol).

E. Electron Microscope Photography:

Depending, on the speed of the films (DIN/ASA) used, the conditions for right light and exposure time was standardized and set for exposure. Following exposure, the prints were developed in Agfa 100 standard photo paper at 20°-24°C for about 2 mins. These were fixed in Agfa 301 fixer for 30 mins. The prints were then washed well in running water and dried in a glazer.

OBSERVATION

The transmission Electron Microscopy of oblique sections of cornea of Acrossocheilus hexagonolepis revealed the existence of patterns resembling parabola at certain regions. The corneal fibrils are arranged in parallel in each plane and with planes parallel to the corneal surface. While the parallel arrangement of the fibrils is distinct in the first few layers, adjacent to the corneal surface, the direction of orientation of the fibrils is always in one direction (Fig. 5.1). Interestingly, the parabolic patterns were restricted only to oblique sections with their marked absence from section cut precisely normal to the corneal surface.

Figure: 5.1. Transmission electron micrograph of the cornea of *A. hexagonolepis*.
The parabolic pattern of cornea is seen in localized sites. The cornea is relatively thin, transparent structure consisting of two curved and fairly parallel surfaces. The oblique section of the lens shows helicoidal arrangement of micro fibrils. The macrofibril consists of parallel bundles of some crystalline components of the lens, which constitutes the micro fibrils, measuring approximately 0.03µm in diameter. Their cross-sectional outline was very variable (Fig. 5.2) rotation of planes of micro fibrils in helicoidal lens appears to be anti-clockwise looking away from the observer. The lens of the fish is spherical which can be subjected to spherical aberration. The lens protrudes through the pupil. The lens of fish is of high quality as it is protrudes out.

Figure: 5.2. Transmission electron micrograph of the lens of *A. hexagonolepis*.

**DISCUSSION**

The developmental sequence is common to all vertebrates’ lenses; the ultimate shape and refractive index distribution of the lens varies considerably among species. The primary reason for this variation is the fact that the cornea of the vertebrate eye acquires refractive significance when the eye is in air but not when the eye is in water (Sivak, 1980).

The cornea is a relatively thin, a vascular, transparent structure consisting of two curved and fairly parallel surfaces. Its refractive function is dependent on
the existence of media of unequal refractive index in front and in back, a condition met when the eye is in air but not in water. Clearly, the aquatic habitat of ancestral vertebrates limited the original function of the cornea to that of a transparent window.

In aquatic vertebrates the lens is usually spherical and protrudes through the pupil ensuing wide field of view because of the absence of corneal refraction. Since the lens is the only refractive elements of an aquatic eye, present-day fishes or other aquatic vertebrates (e.g. sirenians, cetaceans) are said to possess a spherical crystalline lens of high refractive index. The refractive index is thought to drop continuously and parabolically, according to Matthiessen, (1880) from the center to the periphery so as to produce a lens with little or no spherical aberration.

The transmission electron microscopy of lens of Acrossocheilus reveals that it is constructed of micro fibrils. Micro fibrils are reported in arthropod cuticles (Rudall, 1965), and are also known in -keratin of Hair (Filshie and Rogers, 1961) and the oothecal protein of tortoise beetles (Atkins et al., 1966). As in arthropod cuticles, the bundle of micro fibrils of the lens constitutes the macro fibrils, with highly variable cross-sectional outline. The helicoidal changes of direction shown by arthropod macrofibrils in a section cut normal to the layers where some macro fibrils are cut in longitudinal sections, some in transverse and intervening ones sectioned obliquely (Neville, 1975) were also shown by macro fibrils of the lens in the present study (Fig.5.3). In this context it is worth-mentioning that collagen in vertebrates eye (Jakus, 1964) and cortical proteins of fish oocytes (Gotting, 1965) are reported to show parabolic patterning in some localized sites.

Spherical lenses of fish have fascinated scientists since early attempts to understand how light is refracted by nearly spherical raindrops to produce rainbows. Spherical lenses can be subject to spherical aberration or any of five other primary aberrations characteristics of optical systems (Fincham, 1959).
The fish lens is of very high quality (Fernald, 1988), particularly when compared with a glass bead of uniform refractive index. This is especially interesting because in most fish, the lens protrudes through the pupil, so the iris cannot restrict images to its central area. Therefore, to be useful, the lens must be of a high quality throughout, unlike lenses found in air-living vertebrates, in which a pupil restricts vision to near the central axis.

The parabolic pattern observed in the oblique section of cornea of Acrossocheilus is also seen in localized sites, it has been suggested that the parabolic patternning of arthropod helicoids plays in reflecting or transmitting circularly polarized light. The system, which reflects left circularly polarized light, transmits right circularly polarized light (Neville 1975). The Helicoidal theory of lamellate cuticle proposed by Bouligand (1972) was explained by Neville and Luke (1971b) diagrammatically as in (Fig.5.3). Since the parabolic pattern of cornea and helicoidal changes of direction shown by macro fibrils in the lens of Acrossocheilus has similarity with that of the helicoids arthropod cuticle (Neville, 1975) it is reasonable to suggest that the parabolic patternning of cornea and lens play the similar role of transmitting or reflecting circularly polarized light. Detail studies will be required to know how this structural
specialization is related to the photo-adaptation of the hill stream fish *Acrossocheilus hexagonolepis*.

**SUMMARY**

1. The cornea of the hill stream fish, *Acrossocheilus hexagonolepis* appears to have a helicoidal structure, as revealed by parabolic patterning in oblique section examined by transmission electron microscopy.

2. The oblique section of the lens showed a helicoidal arrangement of the macrofibril, which consist of macrofibrils.

3. The helicoidal architecture and arrangement of the micro and macrofibrils are similar to that reported in optically active cuticles of arthropods, which are known to transmit or reflect, right to left circularly polarized light