Vision, the most precious gift of god, is beyond any terms of scientific explanations. The fundamentals of the functional architecture of the human organ of vision may be outlined as a spherical eyeball, housing an optical apparatus that produces inverted and reduced images of the outside world on a layer of nerve cells consisting the sensory apparatus. The optical apparatus of the eye, thus, plays a significant role in the sense of interpreting the world. This apparatus consists of two refracting elements, the cornea and the crystalline lens.

1.1 THE CRYSTALLINE LENS:

The crystalline lens of the eye has been referred to variously as an organ, a suborgan or a tissue which represents a specialized and important sole function to transmit and refract the light reaching the eye on the photoreceptor layer of the retina. In the eye, the lens is placed between iris and vitreous body. The lens is suspended in a stable position in the visual axis by zonular ligaments that extend from the equatorial lens capsule to the ciliary epithelium.

The lens is formed by a sequestered group of modified ectodermal cells that give rise to a highly organised, transparent, refractive and cellular organ. The lens continues to grow throughout the lifespan of the animal and its growth rate which is highest in young animals, declines with age (Cenedella, 1989). The lens is unique in having no blood or nerve supply. Owing to its avascular status the lens is dependent upon the aqueous humor for its nourishment, which also acts as a sink to remove its metabolic wastes. The metabolites from the aqueous humor passively diffuse into the lens and the end products of metabolism from
within the lens diffuse out into the aqueous, and is removed from the anterior chamber by the canal of Schlemm and Uveoscleral route.

The mammalian eye lens has a common general plan, gross appearance and function but still considerable differences exist among the species of this class (Prince, 1956; Walls, 1963). The shape of the lens, which is dependent on the habitat of the animal, varies in different species from perfectly spherical to flattened forms. The man and spiny anteater have the flatest lenses in mammalia (Duke-Elders, 1958; de Jong, 1981). The anterior surface of which faces the cornea and has a less spherical form than the posterior surface facing the vitreous.

The human lens consists of the following 3 parts (Fig. 1):

1. Lens capsule
2. Lens epithelium
3. Lens fibers

1.1.1 Lens Capsule:

The lens capsule, resistant enclosure of the lens, is one of the thickest basement membrane in the body. It is highly elastic and transparent. It consists of collagen and glycoproteins secreted by the lens epithelial cells (Dische and Borenfreud, 1954). Its thickness varies at different regions of the lens (Table 1).
Fig. 1: Diagramatic cross-section of human lens

CZ: Central Zone, 
EZ: Equator zone, 
LN: Lens nucleus, 
GZ: Germinative zone, 
C: Capsule, 
LC: Lens cortex, 
E: Epithelium
TABLE - I

Age-Dependant variations in the Thickness of the Human Lens

<table>
<thead>
<tr>
<th>Capsule</th>
<th>Age</th>
<th>14 days</th>
<th>2-5 years</th>
<th>15 years</th>
<th>35 years</th>
<th>56 years</th>
<th>71 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior pole</td>
<td>1</td>
<td>6</td>
<td>8</td>
<td>9</td>
<td>14</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Anterior max</td>
<td>2</td>
<td>8</td>
<td>12</td>
<td>14</td>
<td>21</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>Equator</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>14</td>
<td>17</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Post max</td>
<td>4</td>
<td>18</td>
<td>18</td>
<td>23</td>
<td>23</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>Posterior pole</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>2-3</td>
</tr>
</tbody>
</table>

[Reproduced from Tripathi & Tripathi (1983) for explanation]

Electron microscopic studies have shown the lamellar nature of the capsule. Each lamella contains fine filaments. Linear patches of the filaments having a periodicity of 600Å are present in the anterior and the equatorial regions of the capsule suggesting their relation to epithelial activity (Tripathi and Tripathi, 1983; Spencer, 1985). The lens capsule continues to grow throughout most of the life; growing to accommodate the increasing volume of lens. The lens capsule contains enzymes (Wortman and Becker, 1956), ATP and glycolytic intermediates (Dische and Ehrlich, 1955) but cannot be considered to have independent metabolism. The lens capsule depends on contact with lens epithelium and fibers for metabolic supplies. It is a non-cellular basement membrane that provides insertions for the zonular fibers and thought to have an important role in molding the shape of the lens during accommodation. However, more recent experiments reveal that decapsulation produces no change in the equatorial diameter and increases the antero-posterior diameter only slightly.
The capsule is a non-selective permeable membrane to electrolytes, some dyes and smaller plasma proteins. Larger molecules of albumin and globulin cannot pass through. It also prevents the passage of bacteria and inflammatory cells into the lens. It is essential for the maintenance of structural integrity of lens and plays an important role in filtration process between inner and outer environments of the lens.

1.1.2 Lens Epithelium:

The lenticular epithelium, consists of monolayer of polygonal cuboidal epithelial cells underlying the anterior capsule, is derived from the original lens vesicle cells that did not differentiate into primary fibers. The epithelium does not extend to the posterior side of the lens because the cells originally located there have elongated into primary fibers. The height of these cells is approximately 5-8 μm and an average width of 13 μm (Brown and Bron, 1987). Measurements of cell density of human non-cataractous lens epithelium range from 3,900 cells/mm² to 5,780 cells/mm² (Jacobson and Sater, 1988; Fagerholm and Philipson, 1981; Guggenmos-Holzmann et al., 1989). Interestingly, women have a higher average epithelial cell density (5,780 cells/mm²) than men (5,009 cells/mm²) (Guggenmoos-Holzmann et al., 1989). On the basis of the proliferative kinetics, the monolayered epithelium has been divided into following three zones (Fig. 1):

1. Central zone
2. Germinative zone
3. Equatorial zone
In the central zone, the cells appear as polygonal in meridional section. The cells have round or oval nuclei. The basal surface is smooth but firmly attached to the capsule at various points, so that during accommodation, the epithelium and capsule can move together and do not slide (Farnsworth et al., 1976). The cells are joined laterally by desmosomes and junctional complexes located near the apices of the highly interdigitated cell membranes. The desmosomes of the lens epithelia are strongest points of attachment between the cells. These keep the cells of epithelium in close apposition during accommodative shape changes. Gap junctions are present along the membranes of lens epithelial cells and their presence is consistent with the need for cell-to-cell communication and passage of intercellular materials in the lens. Hemidesmosome like structures have also been described at the interfaces of the epithelium with the capsule and the fibers (Porte et al., 1975).

Cells at the central zone are mitotically inactive but do divide when podded to do so by any toxic chemicals or any mechanical or hormonal trauma (Maisel et al., 1981; Tripathi and Tripathi, 1982; Worgul, 1982). They are, as Muggleton-Harris states, “a true collection of aged cells”.

In the germinative zone, the epithelial cells are smaller and more cylindrical. The cells are joined by a large number of lateral interdigitations as observed in the central zone, the apices are connected to the underlying fibers by desmosomes and gap junctions. The nuclei is spherical and present in the centre or towards the cell apex. Nuclei undergoes karyokinetic activity. The cytoplasm
seems to contain many more organelles, than the cells of the central zone. The divided young cells in germinative zone migrate to the equator of the lens.

Near the equator, the cells become columnar in shape and assume a pyramidal shape. The basal portion being wider than the apex, they tend to be arranged in meridional rows. The cytoplasm of the cells in this zone shows an increase in the number of multivesicular bodies and ribosomes. Microtubules are also pronounced in these cells. Cells in the meridional rows elongate by sending a basal process posteriorly beneath the capsule and the apex anteriorly beneath the epithelium and subsequently undergo differentiation to form the fiber cells. Differentiation of the fibers involve cell elongation, organelle loss, formation of specialized cell junctions and fiber cell denucleation (Lovicu and McAvoy, 1992).

Rather than shed the cells by exfoliation like other tissues, the lens incorporates all of its cells within itself that are ever produced. The lens is thus an unique organ which records the effects of aging and growth within itself.

Ultrastructural studies of lens epithelial cells show them to be typical, rather normal cells with all the usual organelles present. The nuclei of the epithelial cells are often indented or lobed. Chromatin is dispersed and nuclear pores are prominent (Alcala and Maisel, 1985). The cytoplasm of the epithelial cells contains free ribosomes, polyriboosomes, smooth as well as rough endoplasmic reticulum, golgi figures, centrioles and small vesicles. Mitochondria are an exception in epithelial cells in the sense that they are smaller in size, have indistinct cristae and twisted in appearance. These organelles are concentrated more towards the apex of cell, i.e. between nucleus and the cell membrane adjoining the cortical fibers. The epithelial cells are also
rich in various cytoskeletal elements such as actin, myosin, vimentin, microtubules, spectrin and α-actinin (Ramaekers and Bloemendal, 1981; Benedetti et al., 1981; Alcala and Maisel, 1985) which are found scattered in cytoplasm. The actin and intermediate filaments are aggregated along the apical surface of cells, specially at epithelial/ fiber junctional interface. Microtubules are more abundant in germinative zone where they take part in cell division (Hogan et al., 1971; Kuwabara, 1968; Ramaekers and Bloemendal, 1981). Recently, lens epithelial cells have been shown to possess a network of polygonal arrays of actin combining microfilaments. It is hypothesized that the function of the polygonal arrays is to stabilize the lens epithelial cell during the process of accommodation (Rafferty and Scholz, 1984; 1989; Rafferty et al., 1990; Yeh et al., 1986).

Biochemically, the lens epithelial cells are the most active cells in the lens as majority of the pumping sites for osmoregulation of the lens are located in the anterior epithelial cell layer. Moreover, the epithelium contains high ATP levels and it is the region of the lens where most of the energy requiring processes occur (Kinsey et al., 1973). The concentration of various enzymes such as glutathione reductase, glutathione peroxidase, superoxide dismutase and glutathione-S-transferase, in the lens is highest in epithelial layer. These enzymes are parts of an elaborate defense system of the lens against oxidative damage. One third of the Na+/K+ - ATPase activity of the lens which plays important role in ion transportation, is located in the capsule - epithelium (Palva and Palkama, 1976). More than 90% of protein synthesis of the lens occurs in the epithelium
and outer cortical fibers of the lens. Glutathione (GSH), which is important for maintenance of lenticular transparency in the sense that it protects the lens environment against oxidative damage, is very high in epithelium compared to cortex and nucleus of the lens. GSH level is known to decrease in almost all types of experimental cataractogenesis. Thus a study of GSH metabolism appears to be a relevant parameter in understanding changes occurring in the epithelium during cataract formation.

The lens epithelium is responsible for following important functions:

- Growth of the lens by mitosis, fiber cell formation and protein synthesis.
- Active transportation of ions and metabolites across the lens capsule.
- Maintenance of lens viability.
- Control and repair of lens damage caused by photochemical reactions, oxidation, radiation, trauma and other injuries (Straatsma et al., 1991).

1.1.3 Lens Fibers:

At the lens equator, the epithelial cells, *in vivo*, differentiate into elongated fiber cells of cortex and the differentiation process is characterised by specific biochemical and morphological changes. Lens fiber is a popular term because of morphological appearance of cells. As additional fibers are produced, older ones are internalized and thus the fibers make up the bulk of the lens nucleus and cortex. All the lens fibers taper slightly from equator to anterior and posterior poles. It probably takes nearly 3 months for a cell of germinative zone to differentiate into a mature fiber.
The lens has remarkably high protein content (35%) of the lens wet mass (Racz et al., 1979), 1% lipid, 1% inorganic ions and rest is water. The tightly packed nature of the lens fibers, the arrangement of lens proteins and the regulation of ion and water balance all play significant role in maintaining the outstanding clarity of the normal lens. The development of larger aggregates of protein, appearance of vacuoles and destortion of lens structure all lead to increased light scattering and the clinical observation of cataract.

1.2 CATARACT:

Morphologically a cataract is any disturbance of the optical homogenity of the crystalline lens causing impairment of vision. According to Seddon et al. (1991), cataract is an opacity of lens that reduces the best corrected visual acuity to 20/30 or even worse. Such opacity can be small or large, localized or involves the whole lens. Cataract is the most common cause of blindness all over the world. According to the World Health Organization (WHO) statistics, there are about 45 million blind people in the world; of which 17 million (40%) are blind due to cataract (WHO, 1979; Dawson and Schwab, 1981). There are 12 million blind people in India (NPCB Report, 1992).

Many classification schemes have been proposed for cataract. Of these, the lens opacities classification system (LOCS) proposed by Chylack et al. (1988; 1989; 1993) is the one most often used and it is found to be reliable and reproducible. According to LOCS-I, depending upon the location of the opacities, there are three major types of cataracts (Fig. 2):
Fig. 2: Diagramatic cross-section of human lens showing location of opacity

C : Capsule, 
NC : Nuclear cataract, 
PSC : Posterior subcapsular cataract 
E : Epithelium 
CC : Cortical cataract
1. Nuclear cataract
2. Cortical cataract
3. Posterior subcapsular cataract

It is now clear from the study of whole fiber mass of the respective cataractous lenses that these cataracts are formed through very different mechanisms. Nuclear cataract is characterised by the accumulation of insoluble proteins which appear to be derived from the soluble proteins. Cortical cataract is characterised by the altered electrolytes and water level (Augusteyn, 1981). While the Posterior subcapsular cataract is known to develop due to abnormal differentiation of epithelial cells at equatorial zone of the lens (Philipson and Fagerholm, 1981). When the entire lens including nucleus and cortex becomes opaque due to the continuous progression of any type of cataract, the condition is termed as mature cataract.

1.3 CATARACT AND EPITHELIUM:

The role of the lens epithelium, in maintaining the homeostasis of underlying fiber cells is very well known. A number of studies suggest that injury to the lens epithelium plays an important role in cataractogenesis (Worgul et al., 1989; Hightower and McCready, 1992; 1993; Hightower et al., 1994).

Several animal studies have been carried out stating close association of structural or functional epithelial alterations and cataract development. UV radiation has been reported to have deleterious effects on the lens epithelium which includes DNA damage (Spector et al., 1990), alteration in synthesis of proteins (Andley et al., 1990), decreased epithelial cell survival (Andley et al.,
enhanced prostaglandin synthesis (Andley et al., 1994), increased sodium and calcium ion concentration (Hightower et al., 1993), increased lipid peroxidation (Hightower et al., 1994) altered actin organization and lowered activity of Na, K-ATPase and catalase (Sidjanin et al., 1993; Zigman et al., 1995).

X-irradiated cataract produces dose dependent nuclear fragmentation in cells of the germinative zone, transitional zone and meridional rows of the lens epithelium (Worgul and Merriam, 1980). Since these cells are postmitotic and differentiating, one may conclude that they are undergoing interphase death. Disruption of the plasma membrane is also evident (Broglio and Worgul, 1982).

Selenite, a strong oxidizing agent, can impair lens calcium homeostasis by catalyzing the oxidation of membrane sulfhydryl groups in the lens epithelium. Therefore selenite induced cataract involves elevation of calcium in the lens nucleus leading to activation of proteolytic enzyme, calpain, which could result in proteolysis of β-crystallins, causing abnormal interaction of the crystallins, insolubilization of the proteins and light scattering (Shearer et al., 1992). Suppression of mitosis and nuclear fragmentation are the sources of the early changes in the epithelium, recorded histologically. As a result of decreased prophase and fall in DNA synthesis, it has been concluded that selenite may cause a block in the S-phase of the cell cycle in the lens epithelial cells (Huang et al., 1990).

In the galactose cataract model, the mitotic figures of the lens epithelium increase for at least a three day period and the pattern changes after 7 days of galactose treatment. However, if the feeding continues for several days a
A multilayered epithelial plaque develops over the monolayered anterior epithelial cells (Gona, 1984; Gona and Gorelli, 1985).

**TABLE 2**

**Human Lens Epithelial cell density reported by various groups**

<table>
<thead>
<tr>
<th>Type of Cataract</th>
<th>Mean Cell density (cells/mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Nuclear cataract</td>
<td>4850</td>
</tr>
<tr>
<td>2 Cortical cataract</td>
<td>-</td>
</tr>
<tr>
<td>3 Posterior subcapsular cataract</td>
<td>-</td>
</tr>
<tr>
<td>4 Nuclear cataract + Cortical cataract</td>
<td>4385</td>
</tr>
<tr>
<td>5 Nuclear cataract + Posterior subcapsular cataract</td>
<td>-</td>
</tr>
<tr>
<td>6 Cortical cataract + Posterior subcapsular cataract</td>
<td>-</td>
</tr>
<tr>
<td>7 Mature cataract</td>
<td>4152</td>
</tr>
<tr>
<td>8 Hypermature cataract</td>
<td>3604</td>
</tr>
</tbody>
</table>

While changes in the lens epithelium have been studied extensively in experimental cataracts, there are few reports on human cataractous lens epithelial changes. The epithelial cell density, was found to be dependent on type of cataract (Konofsky et al., 1987; Karim et al., 1987; Vasavada et al., 1991) (Table 2). Konofsky et al. (1987) showed that the cell density of nuclear cataractous epithelium (4850 cells/mm²) was higher than nuclear cataract + cortical cataract (4385 cells/mm²) which, in turn showed higher cell density.
than mature (4152 cells/mm²) and hypermature cataract (3604 cells/mm²). In cases of advanced clouding of the lenses (i.e. in the mature and hyper mature manifestations of cataracts), progression of the clouding appeared to be paralleled by a decrease in the cell density. In Posterior subcapsular cataract, the cell density (4547 cells/mm²) (Vasavada et al., 1991) was found to be lower and higher than reported nuclear and cortical cataract respectively.

From the histomorphological point of view, the human cataractous lens epithelium revealed a variety of abnormal irregular cells and cell patterns. Vasavada et al. (1991) believed that various morphological abnormalities are peculiar to type of the cataract. The cytoplasmic vacuoles, nuclear shrinkage and capped nuclei are the characteristics of the Nuclear cataractous epithelium. In the mixed type of cataract which includes nuclear and posterior subcapsular opacities, varied nuclei size was frequently observed. The distances between nuclei become greater and cellular foci are found to be present in the epithelium of older lenses. This has emphasized the significance of reduction and cytological changes of epithelial cells in old and cataractous lenses. Decrease in the number of cells and cellular activity of lens epithelium are important causes of senile cataract (Kuwabara, 1979).

At the ultrastructural level, the lens epithelium of the age-related cataract showed a variation in the number of abnormal cells and the degree of abnormality. Overall decrease in the cytoplasmic organelles were noted with decreased number of golgi apparatus and rough endoplasmic reticulum, but an increase in the number of mitochondria were observed. The cytoplasm was
granular, nuclei were oblong with slight indentations and clumped chromatin (Straatsma et al., 1991; Perry et al., 1979).

1.4 AIM OF THE STUDY:

Till date surgery is the only cure for cataract. But due to post operative complications, ophthalmic researchers are thinking more about the preventive remedies. For that one must have detailed knowledge about the mechanisms of cataractogenesis. Extensive research has been done to understand the mechanisms of cataract progression but still there are several questions remaining to be solved especially regarding the epithelial layer. Though, importance of epithelium for the internal lens environment and cytoarchitecture is well explained and well documented, little attention has been given to it.

The aim of this study embodies the following aspects in normal and different types of cataractous lens epithelium, which would enable us to understand the role being played by the epithelium in mechanisms of different types of cataract development. Moreover this study would also be helpful to assess the differences existing between the epithelium of different types of cataracts.

I. Histomorphological study

II. Ultrastructural study

III. Biochemical study