4.1 HISTOMORPHOLOGICAL STUDY:

The histomorphological observations of human lens epithelium showed that the central zone of normal lens epithelium was characterized by uniform spreadout of polygonal cells. The cells of the germinative zone were smaller and frequently showed karyokinetic activity. At the equatorial zone, the cells were densely packed in meridional rows. Our results confers with earlier work (Tripathi and Tripathi, 1983a).

In the central zone of different types of cataractous lens epithelial layer, several abnormalities were observed. The extent of abnormality and number of the abnormal cells varied in different types of cataracts. The most common change found in all types of cataractous lens epithelium was cytoplasmic vacuoles. Among the four types of cataracts studied, mature cataract (MC) and cortical cataract (CC) showed highest degree of vacuolisation compared to nuclear cataract (NC) and posterior subcapsular cataract (PSC). This observation coincides with the previous report, (Vasavada et al., 1991). Presence of superimposed nuclei was an unique feature that we observed in cataractous lens epithelium, though it is a well known fact that the cells of the central zone do not mitose. This feature was found to be more prevalent in MC and PSC, while in NC and CC, such incidence was quite less. It indicates increased mitotic activity. Previously, Vasavada et al., (1991) (in human) and Thampi, (1994) (in rat UV cataract) had reported similar observations. While Vani, (1995) had shown multilayering of the lens epithelium in selenite cataract.
In case of NC lens epithelium the distribution pattern of the cells was almost identical to the normal epithelium. Nuclei were in the centre of the cells and distance between two adjacent cell nuclei was also same. The characteristic change found was the presence of pyknotic nuclei, which were rarely observed in other cataracts. Pyknosis of the nuclei is an expression of the changes occurring within the nuclei of the epithelial cells. Owing to abnormal gene information, the protein synthesis would decrease which otherwise is known to be highest in the epithelial layer compared to other parts of the lens (Zigman, 1991). The biochemical studies undertaken showed a decrease in the total protein level of NC lens epithelium. Therefore biochemical results also conferred with histomorphological result.

In CC lens epithelium, the nuclei of the cells varied in shape from oblong, elliptical, spherical to lobed. In most of the cells, nucleus was decentralised which was not the condition in case of NC, PSC and the normal lens epithelium. Moreover, uneven distance between adjacent cell nuclei was observed in semithin sections of the lens epithelium. This may be due to loss of the cells. Karim et al., (1987) and Konofsky et al., (1987) have also observed increased distance between the adjacent cell nuclei in cataracts involving the lens cortex in contrast to those involving the nucleus of the lens. Moreover, Okuma et al., (1991) found that the percentage of decrease in the mean cell density was greater in the central zone than in the germinative zone and suggested that the decrease of the cells in the central zone was due to degeneration and loss of the cells while
the decrease of the cells in the germinative zone was due to reduced mitotic activity.

The PSC lens epithelium showed enlarged nuclei and faintly stained cytoplasm. Same characteristic was observed by Philipson and Fagerholm, (1981). Otherwise the distribution pattern of the cells was like that of the normal ones.

The highest abnormalities, in terms of extent and number, were observed in MC lens epithelium. Complete destortion of distribution pattern of the cells could be seen. The cytoplasm was loaded with extensive large vacuoles. Small vacuoles were also present in nuclei. In case of nuclei, various changes were observed like degenerating nuclei, superimposed nuclei, few enlarged nuclei and increased distance between adjacent cell nuclei, etc. MC is characterised by complete opacification of the whole eye lens and appears total white in colour. As mentioned above the observation of lens epithelium of MC showed all the different characteristics routinely observed in various kinds of cataracts like NC, CC and PSC. Thus it can be hypothesized that the MC is an exaggerated form involving all the other three types of cataracts.

4.2 ULTRASTRUCTURAL STUDY:

The ultrastructural study of the different types of cataractous lens epithelium showed various cellular variations. Among the four types of cataracts studied, least changes were observed in NC lens epithelium. Most of the cells were normal having round or oblong nuclei with uniformly dispersed chromatin
material in the nucleoplasm. The cytoplasm of such cells contained free ribosomes, golgi complex, mitochondria, rough endoplasmic reticulum, etc. Cell junctions appeared as small, dense plaques on the cytoplasmic aspects of the plasma membranes. The abnormal cells showed decreased number of different cell organelles. This may affect the physiology of the cell. Vacuoles could be seen in cytoplasm of all abnormal cells. This may alter the normal integrity of the cytoplasm. Apical cell border was observed to be disrupted at few places. Cytoplasm appeared to be granular. In the nuclei of few cells, condensed chromatin matter was observed. Probably due to this we observed pyknotic nuclei in light microscopic study. Earlier workers (Kuwabara, 1975; Kobayashi and Suzuki, 1975; Rafferty and Goossens, 1978; Perry et al., 1979) have also shown condensation of chromatin in nuclei and granulation of cytoplasm. They suggested that these changes are due to age and thought to be involved in etiology of cataract development. The mean age of NC lens epithelium in this study was older compared to other cataracts. Therefore observed abnormalities may be the senile changes.

Detachment of epithelium from the capsule at many places was the unique observation found only in CC. Altered homeostasis could be the reason for it. This finding is similar to that of Farnsworth et al., (1983). They observed the separation of the basal lateral cell borders and a disruption of the normal continuous relationship between the capsule and basal epithelial cell surface. The role of ribosomes and endoplasmic reticulum in protein synthesis and that of mitochondria in energy synthesis is well established. We observed decrease in
the number of these organelles which obviously leaves the cells with deteriorated protein and energy synthesising mechanisms. Our observation is corroborated by the findings of Okuma et al., (1991). Moreover, the biochemical results in this study show significant decrease in the level of total proteins compared to normal and NC lens epithelium. Therefore biochemical results and ultrastructural results coincides with each other. Other cytoplasmic observation includes presence of pinocytotic vesicles and membrane bound debris which indicates degenerating state of the cells. The membrane bound debris could be the secondary lysosomal bodies. The nuclei of the CC lens epithelial cells were observed to be of varied shapes. Many of them were found to be the degenerated ones. It may be the reason for increased distance between adjacent cell nuclei as observed in semithin sections of the epithelium. Fragmented nuclei could also be seen. It may be speculated that cells of CC could be dying through necrotic and apoptotic mechanisms because the structural characteristics of these cells are similar to those reported by Singh and Anand, (1994).

Most of the PSC lens epithelial cells were normal having oblong nuclei and clear cytoplasm. The abnormal cells were quite few with vacuoles and degenerating cell organelles in the cytoplasm. Due to the presence of enlarged cells, as we observed in flat preparations and semithin sections of the epithelium, our ultrastructural results noted straightening of the cell boundaries in such cells in contrast to the folded membrane generally found in the normal cells. This finding confers with Robison et al., (1990) (in sugar cataract).
The most pronounced structural changes were observed in MC lens epithelium. There was complete loss of integrity of the cell cytoplasm. It was characterised by extensive vacuolisation, degenerating cell organelles and decreased number of the cell organelles. Nuclei were found to be in different shapes. The apical cell membrane was disrupted. The cells in such state, indicates altered cell function and/or cell metabolism. Such cells are prone to die.

4.3 BIOCHEMICAL STUDY:

4.3.1 Proteins:

The cellular architecture and transparency of the lens is believed to be the result of a spatial order of the lens proteins. Normal lens comprises of water (65%) and organic matter (35%), the nature of the latter being structural proteins. The structural water soluble proteins (α, β and γ-crystallins) and the insoluble proteins (albuminoid) account for most of the dry weight of the lens (Rink et al., 1982). The crystallins are very important for the transparency and the refractive power of the normal lens.

In this investigation, it was observed that in comparison to the normal lens there was a decrease in total proteins in all types of cataractous lens. This observation is a clear manifestation of decrease in the soluble proteins of the lens as there was an increase in the insoluble proteins. Three different reasons can be put forth to explain the decrease in the level of total proteins of the epithelium: (a) decreased crystallin synthesis; (b) leakage of intact crystallin polypeptides
into aqueous humor and (c) increased breakdown of protein molecules by oxidation of sulfhydryls.

Amongst the four types of cataracts studied, least decrease in total proteins was observed in NC, though it's mean age (74 years) is older than other three types of cataracts. This indicates that ageing might be responsible for such decrease. However, it is difficult to obtain normal lens epithelium of such an old age. Otherwise, it would have been possible to find out whether depletion in the protein content is due to ageing or due to the development of cataract. The histomorphological study has shown the presence of pyknotic nuclei in the NC lens epithelium. This indicates hampered DNA synthesis which may lead to decrease in crystallin synthesis and thus decrease in the total protein content.

The level of total proteins decreased the most in MC followed by CC. Histomorphological and ultrastructural studies have shown the presence of fragmented nuclei and disrupted cell membranes in these cataracts. The phenomenon of leakage of the intact crystallin polypeptides through damaged cell membrane cannot be denied as Augusteyn (1981) has reported leakage of low molecular weight proteins from the lens. Moreover, Watanabe & Shearer, (1989) presumed that damaged plasma membrane provides passage for the leakage of polypeptides. Contrary to that in case of NC, incidence of disrupted cell membrane was rarely observed. Thus leakage through damaged cell membrane might be the reason for decreased protein in MC & CC. The observed
degenerating nuclei in MC & CC, indicates decreased protein synthesis, which may be the other factor for depleted level of proteins.

Insolubilisation of soluble proteins, which appeared to be more pronounced in MC followed by CC & NC+PSC, might lead to loss of integrity of cytoplasm. The ultrastructural results of cell cytoplasm of these cataracts coincide with the insolubilisation of proteins.

NC+PSC is a mixed type of cataract. It's protein values were not significantly different from NC and CC, while compared to normal and MC it showed significant difference. Since the cell membrane of most of the cells in this category were found to be like normal cells, the chances of leakage of the polypeptides are very less. On the other hand, pyknotic nuclei, which were observed in histomorphological study, probably indicates the depletion of protein level due to altered chromatin material.

4.3.2 Sulfhydryls (SH):

The sulfhydryl groups are crucial for lens transparency. They play various roles like maintenance of protein structure and function, in transport processes, Na⁺-K⁺ exchange and organic ion transport. Lenticular sulfhydryls are found to exist in two forms: Protein bound sulfhydryl (PBSH) and Non-protein bound sulfhydryl (NPSH).

In the present study, compared to normal, all types of cataractous lens epithelium showed significant decrease in the level of total sulfhydryl (TSH) and
NPSH. While decrease in PBSH was only significant in CC and MC. Therefore, depleted level of TSH is the manifestation of decline in both PBSH and NPSH. It is believed that GSH accounts for majority of the NPSH (Giblin et al., 1985) which protects membrane SH (Epstein and Kinoshita, 1970) and protein thiols (Kinoshita and Merola, 1973). Due to loss of NPSH, PBSH cannot withstand the oxidative environment. The results showed loss of NPSH accompanied by decrease in PBSH in all cataracts. However, compared to normal such decrease was found to be significant in CC and MC only. It indicates that PBSH in CC and MC is more damaged compared to NC and NC+PSC. Moreover, the sulfhydryl-groups can modify the proteins in such a way that proteins become crosslinked by disulfide bond and aggregate to a very large extent and ultimately become insoluble. The results of insoluble proteins showed significant increase in CC and MC, which is corroborated by decreased PBSH. Thus increased insoluble protein level reported in this study of cataractous lens epithelium could be due to either disulfide cross-linking of proteins (PSSP) or mixed disulfide forming protein bound GSH, i.e. PSSG. In NC, the degree of PBSH loss was less pronounced which confers with nonsignificant increase in insoluble proteins compared to normal ones.

Altered plasma membrane integrity and increased leakage could be the other reason for the loss of sulphhydrils. SH oxidation and protein insolubilization are believed to be associated with structural deformation (Cooper et al., 1986). Our results of ultrastructural studies revealed extensive structural abnormalities in CC and MC compared to NC and NC+PSC.
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4.3.3 Glutathione (GSH):

Mammalian ocular lens is known for its higher concentration of GSH, the highest being in the epithelium (Reddy et al., 1990). The sulfhydryl group of GSH is responsible for much of the biological activities of glutathione (Megaw, 1984). The following are the major functions of GSH:

(a) Maintenance of the transparency of lens by acting as an antioxidant (Giblin et al., 1981).
(b) Detoxification of xenobiotics (Saneto et al., 1982).
(c) Protection of critical thiol groups involved in cation transport and permeability (Epstein and Kinoshita, 1970a,b).
(d) Reduction of lenticular protein disulfide links (Rathbun, 1980).

The concentration of GSH decreases steadily with age (Lou et al., 1990) and in the course of the formation of all types of cataracts (Reddy, 1971). The mechanism for such depletion is not known but is probably due to decline in GSH related enzyme activities, mixed disulfide formation involving GSH and other factors including hereditary predispositions and exposure to environmental influences like radiation or diet.

The present study has shown significant decline in the level of GSH in all types of cataractous lens epithelium compared to the normal ones. The highest decline was observed in MC followed by CC and both of these are even significantly low compared to NC and NC+PSC, which were not significantly
different from each other. As GSH is one of the factors responsible for maintaining protein thiols in reduced form, any decrease in GSH level would be followed by decrease in reduced protein thiols, which leads to insolubilization of the proteins. The data of insoluble proteins of CC, NC+PSC and MC confirm this. In case of NC, though there was a significant decline in GSH, insoluble protein level was not diminished significantly compared to normal. The explanation of this can be: (a) protein thiols become oxidized only when the level of GSH in the lens drops below the critical level (Reddy, 1990), and (b) ageing which causes steady depletion of GSH (Reddy, 1971) as the samples used for NC were of the higher age group (68 years).

Moreover, diminished GSH synthesis would also affect GSH level. In fact our results of \( \gamma \)-glutamylcysteine synthetase (\( \gamma \)-GCS), an enzyme known to initiate the GSH synthesis, also showed significant decreased activity in all types of cataractous lens epithelium. Therefore, inhibition of GSH synthesis could be another reason for decrease in GSH level. Glutathione reductase (GR) is an enzyme which converts oxidised glutathione (GSSG) to reduced glutathione (GSH). Decrease in the activity of GR, as observed in this study, would hamper reduction of oxidised glutathione and thus contribute to diminished level of GSH.
4.3.4 \(\gamma\)-Glutamylcysteine synthetase (\(\gamma\)-GCS):

Looking at the vitality of GSH for the maintenance of lens transparency, the anabolism and catabolism of GSH also deserve great importance because alteration in these processes can damage the lenticular transparency.

Two enzymes, \(\gamma\)-GCS and glutathione reductase (GR), are well known for their role in formation of two peptide bonds between three amino acids (L-glutamate, L-cysteine and glycine (Sethna et al., 1982/83) during GSH biosynthesis. The first step of GSH biosynthesis, which is catalyzed by \(\gamma\)-GCS forms L-\(\gamma\)-glutamylcysteine by linking L-glutamate and L-cysteine.

\[
\text{L-glutamate} + \text{L-cysteine} + \text{ATP} \xrightarrow{\gamma\text{-GCS}} \text{L-\(\gamma\)-glutamylcysteine} + \text{ADP} + \text{pi}
\]

Step 1: \(\gamma\)-Glutamylcysteine synthetase (\(\gamma\)-GCS) reaction.

The second step, which is catalyzed by glutathione synthetase (GS), forms \(\gamma\)-Glutamylcysteinylglycine (Glutathione, GSH) by linking L-\(\gamma\)-glutamylcysteine and glycine.

\[
\text{\(\gamma\)-glutamylcysteine} + \text{glycine} + \text{ATP} \xrightarrow{\text{GS}} \text{\(\gamma\)-glutamylcysteinylglycine}
\]

Step 2: Glutathione synthetase (GS) reaction.
It is stated that reaction catalyzed by \( \gamma \)-GCS govern the rate of GSH synthesis (McMinn and Ottaway, 1976). Hence \( \gamma \)-GCS is known as the regulatory enzyme of GSH anabolism. Therefore GSH synthesis rate can be determined by analysing the \( \gamma \)-GCS activity.

All cataractous lens epithelium have exhibited lowered \( \gamma \)-GCS activity with a parallel decrease in GSH level. Earlier work has shown correlation between diminished \( \gamma \)-GCS and declined GSH levels in cataractous lenses (Sadasivudu et al., 1981). In this study, we found highest \( \gamma \)-GCS activity in NC and lowest in MC among all types of cataracts. Moreover difference in the activity of \( \gamma \)-GCS in NC & NC+PSC compared to CC & MC was significant. This difference in the activity of the anabolic enzyme in the above mentioned cataracts support the fact that the defence mechanism involving GSH is more active in NC & NC+PSC, which counteract the damaging and unbalancing effects encountered in the lens epithelium. The higher activity of \( \gamma \)-GCS in NC and NC+PSC compared to CC & MC is ought to show high levels of GSH and that is evident from the data of GSH levels.

**4.3.5 Glutathione reductase (GR):**

Glutathione reductase (GR) is well known for its ability to keep the cellular concentration of reduced GSH high by catalyzing the conversion of oxidized glutathione (GSSG) to the reduced form (GSH). GR possesses flavin adenine dinucleotide (FAD), a prosthetic group, which transforms GSSG to GSH.
Glutathione reductase (GR) reaction

The present results show significantly diminished activity of GR in all types of cataractous lens epithelium compared to the normal ones. Moreover NC+PSC and NC show higher activity of GR compared to CC and MC. This higher activity of GR implies that there is an attempt to protect the lens epithelium from oxidative damage by regenerating GSH from its oxidized form (GSSG).

The decreased activity of GR in CC and MC evokes changes in the lens epithelium as GR provides reducing capacity for the formation of DNA (Holmgren, 1981; Vani, 1995; Thampi, 1994). Thus from the above inferences, it may be speculated that the diminished of GR activity could affect two major constituents of the lens - proteins and GSH, leading to the accumulation of GSSG and H₂O₂ which is toxic to the lens epithelium.

4.3.6 γ-Glutamyl transpeptidase (γ-GTP):

γ-GTP, an enzyme of γ-glutamyl cycle is of major importance in glutathione metabolism. It initiates GSH degradation. It catalyzes 3 types of reactions: (a) transpeptidation in which the γ-glutamyl moiety is transferred to an acceptor, (b) auto-transpeptidation, in which the γ-glutamyl moiety is transferred to GSH to form γ-glutamyl GSH and (c) hydrolysis, in which the γ-glutamyl moiety is transferred to water. GSH, GSSG, 5-substituted glutathione
and other γ-glutamyl compounds are substrates for this enzyme (Meister and Anderson, 1983). The GSSG which is shown to pass through the cell membrane (Srivastava and Beutler, 1968) is a substrate for γ-GTP which degrades the GSH (Rathbun and Wicker, 1973). Since the major site of active transport is the capsule epithelium, it might be expected that γ-GTP if involved in this process is localized here. γ-GTP was later found to be a membrane bound lipoprotein and concentrated within the capsule epithelium (Reddy and Unakar, 1973).

Of all the enzymes involved in the γ-glutamyl cycle, only γ-GTP reacts effectively with glutathione S-transferase (GST). This together with mercapturic acid pathway which degrade conjugated GSH, form the only known system which may remove GSH from the lens. The degradation of GSH by γ-GTP is able to transport amino acid into the tissue (Reddy, 1979).

In this study, we found significant loss of γ-GTP activity in all the four types of cataractous lens epithelium compared to normal. However, loss of enzyme activity in MC was highest and exhibited significantly lower γ-GTP activity compared to NC, CC and NC+PSC. Moreover, neither NC nor CC was observed to be significantly different from NC+PSC. At the same time the difference between NC and CC was significant, the lowest activity being in the NC.

The results indicates that γ-GTP activity in cataractous lens epithelium decreases which means that the degradation of GSH may also decline. Since the
GSH degradation is coupled with amino acid transportation, protein synthesis might also be affected which confers with our protein data which were observed to decline. Therefore, we presume that γ-GTP activity may not be responsible for GSH decrease in cataractous lens epithelium.

4.3.7 Glutathione S-transferase (GST):

GST catalyzes detoxification of xenobiotics where conjugation of electrophiles and sulfur of glutathione occur which is the initial step in the mercapturic acid pathway.

In lenticular epithelium, GST activity was found to decrease in all types of cataracts compared to normal. There has been a report on the diminished GST activity in human cataractous lenses (Rao et al., 1983). However, in this study decrease in activity of GST was significant in CC and MC compared to normal, which indicates inhibited detoxifying mechanism. This loss in activity should result in accumulation of GSH in the cataractous lens epithelium, but the opposite was observed. The reduction of GSH level can be explained on the basis of decreased GSH synthesis and/or increased lens epithelial leakage in all types of cataractous lens epithelium, being significant in CC and MC.

With respect to removal of xenobiotics and detoxifying function of GST, the activity of the enzyme in cataractous lenses is more than sufficient to remove any potential cataractogenic compound via mercapturic acid pathway. This enzyme was interjected between GSHS and γ-GTP in the glutamyl cycle, because
of wide range of substrate specificity of GST compared to γ-GTP (Tate and Meister, 1974). In addition to conjugation with electrophiles, the metabolic pathway promotes destruction of hydroperoxides by means of glutathione peroxidase. If this pathway is the one which performs the vital protective function, failure of any of the component of the same would be expected to severely affect lenticular epithelial integrity.

4.3.8 Superoxide dismutase (SOD):

SOD, catalase and glutathione peroxidase (GSH-Px), these three enzymes are known to occupy prominent position in the defense system of the lens. They are involved in protection against harmful oxidants such as superoxide radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and the hydroxyl radical (OH$^-$) which are derived from the univalent reduction of oxygen.

In the lens, the major activity of SOD is in the epithelium (Bhuyan and Bhuyan, 1978). It catalyzes the conversion of superoxide anion, a powerful oxidant, into less toxic H$_2$O$_2$. The H$_2$O$_2$ formed is eliminated by catalase or GSH-Px. It is also stated that elevated H$_2$O$_2$ may inhibit SOD (Vani, 1995). We found decreased activity of SOD in cataractous lens epithelium compared to normal. Moreover, the observed activity of SOD in different types of cataracts were significantly different from each other. The highest loss was observed in MC. The diminished activity may explain the severe oxidative stress created by H$_2$O$_2$. 

97
4.3.9 Catalase:

Elevated level of hydrogen peroxide (H$_2$O$_2$) in aqueous humor may impair lens membrane permeability and contribute to cataract formation. In a significant number of cataract patients, H$_2$O$_2$ level in lens and aqueous have been found to increase by an average of three-fold and in some patients, more than five-fold compared to normal (Ramachandran et al., 1991; Bhuyan and Bhuyan, 1992). Increased levels of H$_2$O$_2$ cause inhibition of SOD activity and this inhibition is potentiated by the inhibition of the activity of catalase.

In this study, in CC and MC lens epithelium, catalase activity was observed to be reduced significantly compared to normal and NC+PSC. The decrease in the activity in NC was less pronounced compared to CC and MC. This shows that due to inhibition of the activity of the catalase in CC and MC, H$_2$O$_2$ might have increased which could cause the inhibition of SOD.

4.4 A COMPARATIVE DISCUSSION:

Cellular changes are manifestations of the biochemical changes occurring within the cell (Huang et al., 1990). In this study we found structural and biochemical changes in all different types of cataractous lens epithelium. The observed degenerative and atrophic changes could bring about malfunctioning of the lens epithelium which would disturb the normal metabolism of the lens and cause opacification. Histomorphological and ultrastructural study of the different types of cataracts studied revealed that pronounced epithelial structural damage occurred in CC and MC, highest being in the latter one. While
in PSC, the structural changes were not so degenerative. The structure of NC lens epithelium was almost similar to normal epithelium.

In case of the nucleus of the epithelial cell, individual differences could be found in different types of cataracts. In NC, though the nuclei were observed to be normal, few pyknotic nuclei were observed. It indicates DNA damage. Ageing has been considered to be one of the factors for such damage (Rattan, 1989). In PSC most of the nuclei were normal while few were enlarged. The most striking and unique character of PSC was presence of superimposed nuclei which shows increased mitotic activity of the cells. Such condition was also observed in MC while in NC and CC it's occurrence was very less. CC exhibited highly degenerative and fragmented nuclei. Condensation of chromatin was very common. Such nuclear changes were reported by Broglio and Worgul, (1985) in irradiated lenses. Moreover, assay of single lens epithelial cells isolated from patients with cataract suggested that about half the cells had a significantly greater level of DNA single strand breaks than controls (Kleiman and Spector, 1993).

Integrity of the cell cytoplasm is important for normal cell functioning. In our study extensive loss of integrity of epithelial cell cytoplasm was observed in CC and MC. Both types of cataracts showed extensive vacuoles; degenerated cell organelles like mitochondria, endoplasmic reticulum, golgi body, etc., membrane bound vesicles (which could be secondary lysosomes); pinocytotic vesicles and granules in the cytoplasm. But in the NC lens epithelium, clear
cytoplasm having normal appearing cell organelles, was the feature of most of the cells. However, vacuoles in the cytoplasm and intercellular space could also be observed occasionally. Moreover, in PSC lens epithelium, cytoplasmic characteristic of most of the cells were found to be normal while cytoplasm of few abnormal cells cytoplasm were found to be loaded with degenerative cell organelles. Faintly stained cytoplasm was frequently observed.

The cell boundaries are important for maintenance of shape and internal environment of the cell, since it regulates inward and outward flow of various constituents necessary for regulation of normal cell metabolism. In MC and CC, we observed disrupted cell borders. Moreover detachment of the basal cell border from the capsule was very frequent in CC. Disturbance to transport mechanisms located in cell membrane can cause alteration in the cell homeostasis. In case of PSC, we didn’t observe disrupted cell borders but in NC such incidence were quite few.

In general the distribution pattern of the epithelial cells was almost normal in NC and highly uneven in CC and MC. The extent of such irregularity in PSC was less, compared to CC and MC. Moreover decreased cell height and cell shrinkage were dominant features of CC. Due to observed epithelial degenerative changes we found increased distance between adjacent cell nuclei in CC, which would be due to cell loss. It also indicates decreased cell density. Our observation confers with that of Karim et al., (1987). They observed decreased cell density in cortical cataract compared to other types of cataracts.
(NC, PSC and MC) observed by Vasavada et al., (1991). Morphological changes like cell shrinkage, condensation of chromatin, collapse of nucleus and mitochondria are the characters indicating dying cell (Singh and Anand, 1994). We speculate that the epithelial cells of CC might be at high risk of cell death compared to NC and PSC.

Like morphology (structure), the bio-chemistry of the lens epithelial cells show individual differences in different types of cataracts. All the biochemical parameters studied were observed to decline. However, the extent of reduction is variable.

It is well established that during cataractogenesis there is decrease in soluble and total protein and an increase in the insoluble proteins. Same pattern was observed in all types of cataractous lens epithelium as compared to normal in our study and this observation coincides with Bhat et al., (1991). MC showed highest reduction in soluble and total proteins beside elevated insoluble proteins. This pattern of change in protein level was less marked in CC compared to MC. But both were significantly different from NC, which showed least changes in protein level. NC+PSC, which is a mixed type of cataract differs significantly from MC.

GSH, is vulnerable to oxidative stress (Spector, 1995). In addition to GSH synthesis, transport of intact GSH from the circulation and aqueous humor is essential for maintaining GSH levels in the lens, particularly in the epithelium (Mackic et al., 1996).
In the present study compared to normal we observed significant decline in GSH level in all types of cataractous lens epithelium - highest being in MC and least in NC. The difference of GSH level in different cataractous lens epithelium were significant except NC and NC+PSC. The diminished GSH level could be due to following reasons:

1. Ageing (Lou et al., 1990)
2. Decrease in GSH synthesis
3. Decrease in GR activity
4. Increase in GSH degradation
5. Decrease in transportation of systemic GSH.

Among all cataracts, GSH level in MC declined significantly compared to NC though their mean age (70 and 68 respectively) was almost same in this study. It indicates that in NC depletion of GSH level could be due to ageing rather than cataract. Moreover, CC also exhibited significantly low GSH level compared to NC. While NC+PSC was not significantly different from NC. Moreover in CC and NC+PSC mean age (54 and 58 respectively) was also largely different from NC, which again indicates age dependent diminution of GSH level in NC cataract.

This study showed depletion in γ-GCS (an enzyme known to be the rate limiting factor of GSH synthesis) and GR (an enzyme that catalyzes conversion of GSSG into reduced GSH) in all cataractous lens epithelium compared to
normal. Moreover, elevation in the activity of $\gamma$-GTP and GST (an enzyme of $\gamma$-glutamyl cycle) can increase the GSH degradation. Our observation showed decline activity of both enzymes in all cataractous lens epithelium which indicates that $\gamma$-GTP and GST may not be the major factor for observed GSH depletion. Moreover, molecular studies have indicated the presence of the recently cloned facilitative RcGshT GSH transporter (Yi et al., 1995) in the lens (Kannan et al., 1995). The RcGshT transporter was found in both the lens cortex and the epithelium (Kannan et al., 1996). Recently, the expression of another GSH transporter, i.e. the Na-dependent GSH co-transporter has been demonstrated only in the epithelium (Kannan et al., 1996) and its presence at the basolateral side has been suggested (Mackic et al., 1996). Our histomorphological and ultrastructural observation revealed disrupted cell boundaries. Such damage may disturb the transport mechanisms. Thus transportation of GSH from systemic circulation into lens through lens epithelium may decline and ultimately contribute to diminished GSH level.

Decrease in the activity of above mentioned GSH related enzymes and altered cell borders were observed to be highest in MC followed by CC and lowest in NC. In case of NC+PSC it was observed that it was not significantly different from NC.

Moreover, GSH is known to account for majority of the NPSH (nonprotein bound sulfhydryl). Decrease in GSH level coincide with decreased NPSH level which again contribute in reduction of TSH (total sulfhydryl) level.
Under such circumstances PBSH (protein bound sulphhydrils) cannot withstand the oxidative insult. Therefore, the diminished PBSH was found to be accompanied by NPSH depletion in all the cataractous lens epithelium compared to normal.

It is now well established that $\text{H}_2\text{O}_2$ severely affects critical cell functions. SOD, catalase and glutathione redox cycle are involved in detoxification of various toxic substances. Activity of SOD was observed to decline significantly. But in case of catalase, though diminished activity was observed in cataractous lens epithelium compared to normal, we didn’t observe significant differences among NC, CC and NC+PSC. While in MC it was significantly different from other three types. It indicates that though catalase was active, it couldn’t prevent the epithelium from the damaging effects of oxidative stress. Our observation confers with Giblin et al., (1990). They suggested that fully active catalase was not able to resist the cytotoxic effects, if the cells have inhibited GR activity. In our study we observed decreased GR activity in the cataractous lens epithelium. However, individual differences were observed in the levels of decreased GR activity.