Although most cells of the body undergo sequential processes of differentiation and aging, the lens cell is perhaps the simplest and most elegant system to study the molecular mechanisms involved in these two fundamental processes. In contrast to the cellular and molecular complexities present in most other tissues, the lens is a much simpler system, comprising of a single layer of epithelial cells that differentiate into fiber cells. Due to very low turn over of proteins in the differentiated fiber cells, our lenses contain proteins that have been made during all ages of our life. The ease of obtaining lens epithelial and fiber cells, plus the relative molecular simplicity of the fully differentiated fiber cells, make the lens one of the best tissues to study the mechanisms that control the fundamental events of cell differentiation and aging.

Differentiation of epithelial cells to lens fiber cells involves the cessation of proliferation, remodeling of cellular architecture, and significant changes in the collection of genes that are expressed, resulting in a restriction of protein synthesis to the high-level expression of a few classes of proteins. In the last five years, it has become evident that differentiation of many cell types involves analogous changes in cellular behavior and a commitment to expression at high levels of cell type-specific genes. Hence, the lens is an excellent system to elucidate the control elements that dictate differentiation in many cell types.
During the later stages of fiber cell differentiation, protein synthesis is essentially terminated and proteolysis is limited. Fiber cells close to the lens center contain aged proteins, while cells close to the lens periphery contain newly synthesized proteins. By carefully peeling away sequential layers of cells, it is possible to obtain proteins of increasing age that date back to before the birth of the organism. Biochemists are just beginning to take advantage of this system to answer fundamental questions on how the aging process changes the nature of proteins.

Based solely on the contribution to understanding differentiation and aging, the lens is indeed an exciting system for study. Nonetheless, it is the transparent properties of the lens and its ability to focus light that present some of the most clinically relevant challenges to scientists involved in eye research.

For most people between the ages of 40 and 50 years, the ability of the lens to focus from distance to near or to accommodate becomes difficult and bifocals are required. The inability to adequately accommodate is known as presbyopia. It is the most common optical disorder of the eye. By understanding the changes in physical properties of the normal lens and its surrounding support structures as a function of age, it may be possible to develop treatments for presbyopia that delay or prevent its onset.

By far, the most serious problem associated with the lens is its loss of transparency. This condition, known as cataract, is the leading cause of blindness in the world. The disorder sometimes occurs in children, but most frequently occurs in adults age 50 and above. To date, there is no
universally accepted pharmacological agent to either inhibit or reverse the progression of lens opacity. The only treatment is surgical removal of the lens, followed by implantation of an artificial lens at the time of surgery or the subsequent use of corrective lenses.

The human age-related cataractous lens is characterized by a lens opacity that is significant enough to decrease the visual acuity of the patient. Although acuity loss is the clinical manifestation of the disorder, the changes in the lens are likely due to the result of a series of biochemical and biophysical events, occurring over many years, that ultimately lead to opacification. The overall objective is to identify markers in the human lens that will clearly differentiate the process of aging from cataractogenesis. Identification and possible quantitation of such markers would significantly aid in understanding the mechanisms that lead to cataractogenesis. This information will help in developing pharmacological agents that can inhibit lens opacification and assist in the early diagnosis of patients who may benefit from cataract therapy. In addition, identifying novel markers for cataract will permit the development of animal model systems that can approximate the biochemical and biophysical events occurring in the human lens. These animal model systems will be valuable in the preliminary screening of possible anticataract drugs.

Following are general categories of possible markers:

- Biochemical markers of the lens epithelium, especially those involving changes in gene expression or mutations in specific genes.
• Lens protein alterations, especially those involving posttranslational modifications.

Age-related cataracts are the most important types of cataract from a public health perspective. Different types of cataract, have characteristic morphologies, yet there is a tendency to generalize about cataracts and their causes.

However very little is known about what, in conjunction with age, initiates the formation of cataracts. The cataractous lens is the end result of processes that occur over many years, even decades. There is no compelling evidence that the factors discussed are the primary cause of cataracts. In fact, the initiation of cataracts is probably the result of varying combinations of these factors, which interact in some complex manner.

The $\alpha$, $\beta$, and $\gamma$ crystallins make up the bulk of the lens mass and are responsible for the larger refractive index of the lens. The $\alpha$ crystallins, comprises of two highly related chains ($\alpha A$ and $\alpha B$), account for approximately 40 percent of lens dry weight. While the families of $\beta$ and $\gamma$ crystallins, comprises of a least nine related proteins, accounting for almost all the remaining dry weight of the lens. The relative contributions of each of the $\alpha$, $\beta$, and $\gamma$ proteins to the total refractile properties of the lens are unknown. In addition, nothing is known concerning the importance of each of the $\beta$ and $\gamma$ species in maintaining normal lens transparency, especially under conditions of metabolic stress. The roles of the $\alpha$, $\beta$, and $\gamma$ crystallins in the possible control of molecular processes that occur during differentiation of the lens epithelial cells also remain undefined.
Recent findings indicate that α crystallins possess molecular chaperone properties that enable them to inhibit nonspecific protein aggregation. This discovery has demonstrated a physiological role as well as a structural role for α crystallin. In addition, significant amounts of αB crystallin have been found in other tissues besides the lens, including the retina, the heart, and the brain. Abnormally large amounts of αB crystallin are also found in some human neurological disorders. Both these observations suggest the intriguing possibility that the αB crystallin may also play physiological roles in other tissues.

This discovery of chaperone activity has provided the impetus for conducting further studies to characterize the structural and biochemical properties of this physiologically important process. The ability to perform structure and function studies has been aided by the techniques of molecular biology. Even though the crystallins make up the bulk of the lens mass and are responsible for the larger refractive index of the lens, little is known about the relative contributions of each of the α, β, and γ proteins to the total refractive properties of the lens. Not much is known about the role of each of the β and γ species in maintaining normal lens transparency, especially under conditions of metabolic stress. Strategies to address these questions need to be developed.

To perform its refractory function, the lens must be transparent; hence, light-scattering elements are precluded. Consequently, the lens is an avascular tissue comprising precisely packed multiple layers of cells. Due to the absence of blood vessels, the lens relies on the epithelial cells to take up solutes and ions from the aqueous humor. The epithelial cells
then must provide all the necessary metabolic requirements to cells in the lens. To accommodate its unique situation, the lens uses a vast array of channels, pumps, and intercellular connections at gap junctions to distribute nutrients, remove metabolites, and maintain the proper ionic balance for both epithelial and lens fiber cells.

The regional distribution of sodium/potassium pumps, channels, and gap junctions throughout the lens likely creates an internal circulatory system that compensates for the lack of the avascular system. A system in which ions and water enter the lens along intercellular clefts, cross fiber cell membranes, flow from cell to cell through gap junction channels toward the surface, and cross surface cell membranes to complete the loop that has been postulated.

The exact reason that the accommodative system fails remain elusive. It has long been suspected that as the lens ages it becomes thicker and can be bendable or can be easily molded, losing its ability to change shape and consequently its ability to accommodate. There is also some evidence to suggest that the muscle controlling the tension of the zonular attachments to the lens capsule may play a significant if not key role in the failure of the accommodative mechanism to respond adequately with increasing age. In addition, there is preliminary evidence that near-vision can actually be restored by physically changing the relationship between the muscle and lens. Lastly, the role of extra lenticular elastic tissues in the ciliary muscle and choroid, in pathophysiology and as therapeutic targets, needs to be explored.
In a somewhat different but related subject, it is known that as the lens ages, the anterior and posterior thickness increases and the equatorial diameter decreases, leading to the paradox that a bigger and a thicker lens is optically less powerful, contrary to the earlier belief of being more powerful.

In younger adults, malfunctioning of the accommodation mechanism has been implicated as a likely cause of adult-onset myopia. Differential accommodation between the two eyes is also likely to be involved in the development of anisometropia, a condition in which the two eyes have different refractive properties.

In view of recent advancements in measuring objectively the aberrations of the human eye in vivo, and that it has been observed that the researchers have a better understanding of the biometry of the aging lens and that they are in a position to accurately model gradient index changes within the lens that could explain the thick lens paradox. Similarly, recent advances in the resolution of a variety of non invasive measuring techniques like high-frequency ultrasound should resolve the geometric uncertainties of the relationship between the ciliary muscle, zonules, capsule, and lens during accommodation and as a function of age. These advances suggest ways to delay or prevent presbyopia.

4.1 Importance of the study

The study undertaken in this piece of work mentions the co-relation of various biochemical changes occurring in normal lens with age or
4.2 Proteins in the Lens

Lens has a higher protein concentration than any other tissue and the concentration in nucleus is higher than that in the cortex. The lens protein contribute 35% of the wet weight of the lens and the crystallins accounts for 80-90% of the soluble proteins. A great deal of progress has been made in characterizing structural changes that occur to lens proteins during the normal aging process. Identifying sites where post-translational modifications occur has been aided by mass spectroscopy. Spectroscopic analysis and two-dimensional gel electrophoresis have permitted testing of the hypothesis that specific post-translational modifications of lens proteins lead to cataract formation. Surprisingly, although many modifications have been identified, none of these have been specifically associated with age-related cataract. Post-translational modifications of lens proteins seem to be a function of normal aging rather than cataractogenesis.

The discovery that α crystallins, a major component of lens fiber and epithelial cells, prevent denaturation and aggregation of proteins in vitro was unexpected. This novel finding suggested a particularly significant role for this important class of proteins, that of a molecular chaperone. Chaperones are proteins that affect protein-protein interactions by stabilizing protein conformations and preventing nonspecific protein aggregation in the face of heat denaturation or other
environmental stresses. Whether the molecular chaperone-like properties of the α-crystallins play a key role in the prevention of protein denaturation and nonspecific aggregation during the aging of the human lens remains to be determined.

The soluble protein of the lens increases with age from $0.281.63 \mu\text{g/mg}$ in 31-40 years age group to $313.06 \mu\text{g/mg}$ in 81-90 years age group. Whereas in the case of insoluble proteins the value increases and the increase is $68.12 \mu\text{g/mg}$ in 31-40 years age group to $85.13 \mu\text{g/mg}$ in 81-90 years age group. But the percentage of insoluble proteins increases from 11.02 to 17.02 in the same groups studied. The amount of soluble protein, insoluble protein and total protein fluctuates within a short margin of 11%, 27% and 14% respectively in the same age groups studied. In the human lens little was previously known about the composition of the insoluble fraction. It has been found that high molecular weight (HMV) protein constitutes a significant fraction of the total protein of the nuclear region and insoluble protein of the whole lens.

4.3 Lens protein fraction

To discuss the modifications of lens protein during cataract formation and to consider the role of oxidation for these modification, a detailed study is presented in the research undertaken. Many theories and researches in past have not been able to distinguish between cortical and nuclear opacities in senile lenses.

The post clinical type of senile cataract is the cortical cataract (Bellows and Bellows, 1975). Histopathologically, cataractous changes in
the lens cortex take a variety of forms such as irregular, entangling of fibers, various swelling and hydropic degeneration, stain irregularities, formation of cytoplasmic granules, cystic space with or without inclusions and water vacuoles, concentric arrangement of membranous lamellar bodies, crystalloid arrays of membranes, and elaboration of complex lipids from degenerating fibers (Creighton et al. 1978, Tripathi and Tripathi et al. 1983). During cataract formation, there is progressive loss of glutathione, inositol and soluble protein, most likely due to abnormal transport and increased permeability (Augusteyn, 1977). A build up of free amino acids in another feature of cataract formation (Takemoto and Azari, 1976).

People with nuclear cataract have a great difficulty in distant vision than near vision. The formation of nuclear cataract is accompanied by a large progressive increase in the amount of insoluble protein in the nucleus, an increase in the soluble proteins (Augusteyn, 1977) and number of oxidative changes involving the sulphur containing amino acids (Truscott and Augusteyn, 1977). The non-sulfide links in the nuclear proteins have shown an increase in the nuclear cataract formation (Kramps et al. 1978). In nuclear cataract, it appears that light-scattering of high molecular weight (HMW) aggregates of protein could be occurring (Garcia-Castineiras et al. 1978). There is also the appearance of a high molecular weight protein fraction which appear to be an intermediate in the formation of water insoluble protein (Gamer and Spector, 1978). Very little was known about what, in conjunction with age, initiates the formation of cataracts. This is, in part, because the cataractous lens is the
end result of processes that occur over many years, even decades. The major initiating factors that have been proposed as a result of epidemiological studies are described. There is no compelling evidence that any of these factors are the primary cause of cataracts. In fact, the initiation of cataracts is probably the result of varying combinations of these factors, which interact in some complex manner.

The modes by which initiating factors affect the lens will be either direct, as in the case of UV exposure, or indirect, by altering levels of metabolites, growth factors, oxidants, antioxidants, auto-antibodies, and so forth. Many investigators have suggested that the initial site of action for these factors is the lens epithelium, where altered epithelium function could affect underlying fiber cells. However, it is unclear if the initial target is the epithelium, the fiber cells, or the surrounding tissues, which secondarily impact the lens.

The major hypothesis proposed to explain age-related cataracts involve either a change in the protein-protein interactions leading to protein aggregation or membrane damage. Processes in the lens thought to affect one or both of these include: oxidative stress; post-translational modification of crystallins; and, more recently, the loss of chaperone-like activity of α-crystallin. These have been considered key mechanisms leading to age-related cataract. A role for oxidative stress in age-related cataracts has been proposed, but the origin of the stress and the manifestation or relevance to any cataract have not been definitely demonstrated. The major posttranslational modifications of lens proteins seem to be a function of normal aging rather than cataractogenesis. A
clear causative association between post-translational modifications of lens proteins and age-related cataract has not been demonstrated.

The results presented in the current study shows both cataract formation and aging are accompanied by a decrease in the solubility of the lens proteins. Multiple extractions were done to solubilize proteins which in the young normal lens are readily soluble. Some of the proteins require strong dissociating conditions to dissolve it fully. As mentioned, different methods used to fractionate lens proteins on the basis of solubility would result in the isolation of many apparently different proteins from similar lenses, and wide variations in estimates of their levels in the lens. We could estimate specific but different varieties of proteins i.e. brown protein fractions and yellow protein fractions. It was also clear from the results that the physical entrapment of soluble proteins in the insoluble fractions and because of lowered solubilities of the proteins, multiple extractions were required to solubilize proteins from both normal and cataractous lenses (Coghlan and Augusteyn, 1977).

In this study there was progressive shift of proteins from the soluble to the insoluble fractions as the color of the lens nucleus intensifies.

The remarkable feature of the observation from urea insoluble fraction was its relationship with the color of the lens. Of particular interest is the appearance of large amounts of urea insoluble protein. Most of the color of the lens is associated with urea insoluble protein fractions. Amongst urea insoluble fraction, the brown protein fraction was more predominant over the yellow protein fraction. These were produced by the
fractionation of the urea insoluble proteins by extraction with urea containing a reducing agent (DTT) yields a bright yellow soluble protein leaving behind a dark brown residue.

The yellow and brown protein fractions are uniquely associated with different types of cataract especially the nuclear cataract and increases with the progression of cataract (Truscott and Augusteyn, 1977). The brown color of the lens in brown cataract is due to very high amount of brown fractions of protein. It contain about seven times higher amount of brown fraction as compared to the yellow fraction of protein. The color of the lens is due to glycation and aggregation of lens proteins.

4.4 Sulfhydryls TSH, GSH, PSH

A highly significant loss of protein sulfhydryls and non-protein sulfhydryls were noted during the inducement of cataractogenesis. In the normal lens the cellular architecture is very regular and its transparency is believed to be the result of a spatial order of the lens proteins (Delaye and Tardieu, 1983).

Changes in the lens sulfhydryls (SH) content during cataract formation have interested numerous investigators. With the evidence of a marked decrease of the SH groups in the cataractous lenses and high molecular aggregation due to SH oxidation has been postulated as the typical factor in the lens protein denaturation (Kinoshita and Merola, 1973). The major part of the lens sulfhydryls (SH) consists of protein sulfhydryls (PSH). The role of sulfhydryls containing compounds in the
maintenance of lens transparency, has been of interest for many years. A lowered content of low molecular weight sulfhydryl compounds has been demonstrated in human cataractous lenses (Harding, 1970) and it has been shown that the cataractous lens proteins contain higher disulphide and lower sulfhydryl contents than the normal lenses.

The lens contains high concentrations of glutathione (GSH). The highest GSH level appears to be in the epithelium; in the rabbit lens, for example there is 64 μmol GSH per gram tissue, which is six times the whole lens concentration. There is a apparently a slow turnover of GSH, Reddy et al. reported a rate of 1.4 % per hour. GSH turnover, essentially the synthetic activity, requires approximately 11% of ATP generated from glycolysis. Enzymes responsible for GSH synthesis (glutathione synthetase and cysteinylglycine synthetase), and for GSH degradation (γ-glutamyltranspeptidase and cysteinylglycine dipeptidase) have been identified and characterized. Glutathione (GSH) is also considered to play an important role in defending the lens against oxidative damage (Reddy, Giblin and Matsuda, 1980). This function implies a close relationship of this peptide with the activity of the hexose monophosphate shunt (HMS) since the pathway generates NADPH which is required for the reduction of oxidized glutathione (GSSG). Under conditions of oxidative stress, shunt activity may be increased in order to maintain high level of reduced glutathione (GSH). GSH is substrate for GSH peroxidase that protects the lens from oxidative damage by metabolizing H₂O₂ which is toxic metabolic of O₂. GSH however, may have an indirect role in the maintenance of normal lens permeability and cation transport by protecting the sulfhydryl.
groups in the membranes of the lens and lens fibres (Epstein and Kinoshita, 1970)

Normal lenses maintain a steady-state concentration of GSH however, this begins to drop in lenses undergoing cataract formation. This has been found to be true in almost all experimental cataract formation. The disappearance of GSH may be due to its diffusion though damaged cell membranes or its formation of GSH-protein mixed disulfides. In any case, the loss of GSH will ultimately affect many changes in lens structure and function.

The GSH content of lens is very low when the cataractous conditions occurs whereas TSH and PSH levels are not found to be affected as severely as GSH. Some studies of the enzymes involved in the GSH metabolism thus reveals that the fall in the content of GSH may not be attributed to the increased activity of the enzymes involved in its metabolic disposal, but due to increased oxidation.

This investigation has indicated that this type of condition is similar to oxidation damage as observed in UV radiation. The change in the GSH content might be a possible cause in the impairment of the defence system by UV radiation. The results obtained in this study indicates that there is highly significant reduction in the GSH content. Normal lenses maintain a steady-state concentration of GSH; however, this begins to drop in lenses undergoing cataract formation. It is possible that the -SH groups of the lens crystallins remained reduced at the expense of the reducing system of the lens.
4.5 Ascorbic acid

Ascorbic acid is one of the important antioxidant present in aqueous humor as well as lens. It is well known that it cannot be synthesized by human beings and can be supplemented through diets. Ascorbic acid is believed to play an important role in tissue metabolism and probably essential for collagen synthesis (Heath, 1962). In any discussion seeking to link the changes in the level of a constituent with cataract formation, it should be remembered that, for a substance like ascorbic acid which probably functions as a link in a hydrogen carrier system, it is the turnover rate that is of importance. It is conceivable that the function of ascorbic acid might be so important that its level is maintained as far as possible and when it finally falls an increased turnover rate could maintain the necessary function.

The loss of ascorbic acid content in the lenses as associated with the development of cataract, seems to take place by a series of reactions. The two forms in which vitamin C exists namely ascorbic acid and dehydro ascorbic acid, are readily interconvertible. The ciliary body secretes it as a mixture of ascorbic acid and dehydro ascorbic acid, and the latter is being reduced by some factors in the aqueous humor (Kuch, 1970). Many of the metabolic functions of vitamin C can be traced back to the reversible oxidation-reduction system to which, both forms of vitamins are subjected. This explains its role in intracellular respiration in biological oxidation-reduction, linked with glutathione and nucleotide coenzymes. According to one hypothesis (Kinoshita, 1964), GSH reacts non-
enzymatically with dehydro-ascorbic acid to form ascorbic acid. The 
GSSG is reduced by glutathione reductase in the presence of NADPH 
produced by hexose monophosphate shunt (Reddy,1971).The oxidation 
of ascorbic acid in the selenite induced cataracts, could be by the 
formation of dehydro-ascorbic acid and hydrogen peroxide.

The participation of ascorbic acid in the oxidation reduction of 
glutathione in the lens has been clarified by the work of Pirie (1955), who 
suggested that the respiratory link between ascorbic acid and glutathione 
may be hydrogen peroxide rather than oxygen. In the development of 
some experimental cataracts, the level of ascorbic acid falls with the 
glutathione concentration (Merola and Kinoshita,1960).Glutathione 
peroxidase which catalyses the oxidation of glutathione hydrogen 
peroxide has been demonstrated in the lenses of several species. A 
highly significant loss of ascorbic acid was found in the advanced stages 
of selenite induced cataract. Since ascorbic acid cannot be synthesized in 
the lens, it is in the form of dehydro-ascorbic acid. The dehydro-ascorbic 
acid in the lens is usually reduced to ascorbic acid by oxidation of GSH in 
the presence of GSH peroxidase.

By the same reaction hydrogen peroxide formed in the lens may be 
converted to water (Pirie,1965).High ascorbic acid content in the lens is 
known to protect the tissue against the membrane damage to cation 
pump and free radical (Varma,1980).

From the experimental results it is clearly seen that with an 
increase in age the amount of ascorbic acid increases. The lowest value 
of ascorbic acid was seen in the age group of 31-40 years and the highest
value was in the age group of 81-90 years and hence it was evident that age has a role to play in case of ascorbic acid. In different types of cataract the value of ascorbic acid showed variations and in brown cataract it was 0.22 ± 0.02 mg/g and the value of total protein was 609.23 ± 12μg/mg respectively. In case of nuclear cataract the value was 0.82 ± 0.01mg/g and the total protein was found to be 412.12 ± 18 μg/mg respectively. The values of total protein showed variations in different types of cataractous lenses.

4.6 Electrophoresis

Thus it can be concluded that with an increase in age, the amount of insoluble protein decreases and no protein was found beyond the range of 29 kDa mol wt. protein. A detailed conclusion could not be drawn in absence of Gel-Scanner.