CHAPTER – 1: INTRODUCTION

1.1 The Lens

1.1.1 The lens capsule
1.1.2 The lens epithelium
1.1.3 The lens fibers

1.2 The Cataract

1.2.1 Risk factors
1.2.2 Role of lens epithelia in cataract development

1.3 The significance of work

1.4 Experimental cataract models

1.4.1 Sodium selenite (Na₂SeO₃) induced cataract model
1.4.2 UV radiation induced cataract model
1.4.3 Hydrogen peroxide (H₂O₂) induced cataract model
The eye is an organ to sense the miraculous gift of vision. The fundamentals of the functional architecture of the human organ of sight may be attained as a spherical eye ball, housing an optical apparatus i.e., the ocular lens, that produces an inverted and reduced images of the outside world on a layer of nerve cells constituting the sensory apparatus. Science must search to keep this portal clear for the flow of light, as the flow of neurotransmitters in the brain.

1.1 THE LENS:

The ocular lens (Latin: Lentil or Lentil shaped) was recognised by ancient Greeks in first century and its location was established by Alexandrian school (Duke-Elder & Wyber, 1961). In recent years, the rapid advances in microscopical technology, molecular biology, immunochemistry, analytical biophysics and biochemistry have contributed immensely to our understanding of the ocular lens system.

The ocular lens referred variously as an organ or suborgan or a tissue which is formed by a sequestered group of modified ectodermal cells; that give rise to a highly organised, transparent, refractive, biconvex, elliptical, semisolid, cellular organ with smooth, shiny surface. The lens is a unique structure, which has no blood vessels, lymphatics, nerves or connective tissues and it has remarkably little extracellular space in its interior (Thoft and Kinoshita, 1965, Paterson, 1970). In addition, the lens can be regarded as syncytium (Rae, 1974). In postnatal life; the lens is nourished entirely by the freshly formed aqueous humor. Since the lens is enveloped completely by thickened basement membrane or capsule, and since new cells are continuously formed, the older fibers can not desquamate or be discarded; instead they are preserved in the interior of the lens, accounting for its continuous
PLATE 1. A Schematic diagram of rat eye.
AC, Anterior Chamber; Ca, Capsule; Co, Cornea; CP, Ciliary Process; I, Iris;
N, Lens Nucleus; ON, Optic Nerve; PC, Posterior Chamber; R, Retina and 
VH, Vitreous Humor.
increase in size throughout the life. Thus lens is characterized by growth rather than turnover.

The lens is located between the vitreous and the iris; the pupillary portion of the iris glides over its anterior surface. The anterior central region of the lens exposed by the pupil, forms part of the posterior boundary of the anterior chamber. Posteriorly, the lens is supported by the vitreous and lies in its anterior hollow, the patellar fossa. The lens is suspended in a fairly stable position in the visual axis by the zonule or the suspensory ligaments, which extends across the posterior chamber from the ciliary epithelium to circular zone on either side of the equatorial lens capsule. The lens has a sole purpose of refracting incident light on the retina.

Structurally, the lens consists of three components: the capsule, the epithelium and the lens cells or fibers.

1.1.1 The Lens Capsule:

The lens capsule is transparent, homogeneous, resistant and highly elastic envelope that encloses the lens substance and anchors the zonular insertions. It is one of thickest basement membrane in the body. The capsule appears to be formed from materials secreted by the epithelium. Histochemical and X-ray diffraction studies of the capsule indicate that it contains some form of collagen and immunochemical studies indicate that it is chemically similar to the basement membrane of other epithelial tissues. The thickness of the capsule varies in different regions of the lens. The capsule is thicker anteriorly than posteriorly and it is thickest peripherally on either side of equator in the region corresponding to the attachment zone of the suspensory ligaments (Tripathi and Tripathi, 1983).
The capsule is non-selectively permeable to electrolytes, some dyes and smaller molecules of plasma proteins but does not allow passage of large molecules of albumin or globulin and generally forms barrier to the passage of bacteria and inflammatory cells (Tripathi and Tripathi, 1983). The major role of the capsule is that of maintaining the proper cytoarchitecture of the lens during fibrogenesis, rather than in lens molding.

1.1.2 The Lens Epithelium:

The lenticular epithelium consists of single layer of polygonal, more or less cuboidal epithelial cells sandwiched between outer lens capsule and inner lens fiber cells, extending from anterior capsule to equator of the lens. It is derived from the original cells of the lens vesicle that did not differentiate into primary fibers. The term basal refers to the portion of the cells near the lens capsule and the term apical refers to the portion away from the lens capsule and in close proximity to the lens fibers. The terms adcapsular and anticapsular corresponding to the basal and apical regions of the cells is also in use. Because the epithelial cells adhere closely to the capsule, it is possible to make a flat preparation of the lens epithelium. Such preparations are most useful for qualitative and quantitative histocytologic and experimental studies (Harding et al., 1971; Howard, 1952). According to its proliferative kinetics, morphology and location, the lens epithelium has been divided into three regions (Duke-Elder and Wyber, 1961, Worgul, 1982, Harding et al., 1971):

1. The Central Region (CR)
2. The Preequatorial Region (PR) or Germinative Region (GR)
3. The Equatorial Region (ER)
The cells of the Central Region (CR) are polyhedral with oval to round nuclei when viewed on a whole mount and squamous with elliptical shape. Their basal surface is smooth, but firmly attached to capsule at various points, so that during accommodation, the epithelium and the capsule move together and do not slide on each other (Fransworth et al., 1986). The apical surface contains many folds and interdigitations with the underlying fibers, the two being joined by numerous gap junctions and occluding zonules. The lateral intercellular spaces are narrow but parallel. Juxtaposed cell borders are joined by a few gap junctions and desmosomes (Benedetti et al., 1974; Rafferty and Esson, 1974). Gap junctions play very important role in cell-cell communications and passage of intercellular materials in the lens. The CR of rat lens epithelium extending halfway from the center to the periphery of a whole mount preparations normally has less than 1% cells in cell cycle and normally devoid of proliferative activity (Maisel et al., 1981) and are blocked in the G1 phase of cell cycle (G0 phase) (Harding and Srinivasan, 1961). Thus they represents a true collection of aged ectodermal cells (Muggleton-Harris, 1970) of the body.

In the preequatorial region, the cells are smaller and more or less cylindrical with spherical nuclei located in the center. The cells were interdigitated in a more complex pattern, and the cytoplasm contains a large number of organelles. In the embryo the entire epithelium is mitotically active, giving rise to lens fibers but in adult, the cells in the peripheral region continues to proliferate and forms lens fiber cells at diminishing rate throughout the life (Harding et al., 1971). Hence, these region of the lens epithelium is responsible for all growth and differentiation in the lens. In the young rat, the rate of cells entering mitosis is approximately 100 cells/hour.
The Lens epithelium located beneath the anterior capsule is divided into Central Region (CR), Pre-equatorial Region (PR) and Equatorial Region (ER). Cell division is restricted to the epithelium where different regions adjacent to the Ciliary Process (CP), Iris Diaphragm (ID) and Pupil have distinct Mitotic Index (MI). The ER is the region where epithelial cells differentiate into fibers as the cells internalize.

C, Cornea and R, Retina.
In the equatorial region, cells gradually becomes columnar and assumes a pyramidal shape, the basal portion being wider than apex (Karim et al., 1987). The equatorial region of the lens is an important site where lens epithelium starts differentiating into lens fiber; this process is associated with the expression of several genes including those encoding crystallins (Wistow and Piatigorsky, 1988) and other unique gene product (Berthoud et al., 1999). Lens epithelial cells enter their final round of DNA synthesis before reaching the cortex (Worgul and Rothstein, 1977). Subsequent to this the cells typically divide, so that the terminal cell cycle coincides with the fiber differentiation phase. Following last division and prior to entering the lens bow, the cells line up and form the radiating columns. The proper alignment of the cells in the equatorial region is an absolute requisite for the transparency of the organ to be achieved (Worgul and Rothstein, 1977). Because of the pivotal nature of this process in maintaining the proper cytoarchitecture of the lens, the ER have become the subject of intensive investigations (Worgul and Rothstein, 1977). Approximately 1200 meridional rows are present in the rat lens (Rabl, 1900) and the cell advances from progenitor (p-cells) into nonproliferating compartments of ER at a velocity of 21.8 μm/day (Peer et al., 1977).

Ultrastructural study of both CR and PR exhibits monolayer arrangement and relatively uniform thickness. The prominent features of these sections include numerous intercellular spaces and interdigitating cellular processes. An intercellular spaces, interdigitations and pinocytotic vesicles are principally confined to the basal region of the cell. 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, smooth as well as rough endoplasmic reticulum, golgi complex, centriole and small vesicles.
Mitochondria are exceptionally smaller in size, have indistinct cristae and twisted in appearance. These organelles are concentrated more towards the apex of the cell. Epithelial cells are rich in various cytoskeletal elements such as actin, myosin, vimentin, microtubules, spectrum and α-actinin (Ramaekers and Blomendal, 1981) 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 PR where they take part in cell division (Hogan et al., 1971; Kuwabara, 1968). Lens epithelial cells have also shown to possess a network of polygonal arrays of actin combining microfilaments which may be important to stabilize the lens epithelial cells during the process of accommodation (Rafferty and Scholz, 1984, 1989, Rafferty et al., 1990).

The lens epithelium is responsible for the following important functions:

1. The growth of lens by division and differentiation of PR cells to form the lens fiber cells.
2. The transport of metabolites and ions across the lens capsule.
3. Control and repair of lens damage caused by photochemical reactions, oxidation, radiation, trauma and other injuries.

1.1.3 The Lens Fibers:

'Lens fibers', a misnomer for lens cells is a popular term because of the morphologic appearance of the cells. Young lens fibers originate from the cells of the equatorial region of lens epithelia. Here, the epithelial cells elongate, they orient themselves obliquely and come to lie beneath the epithelium of the anterior capsule. Thus, an elongated cell or fiber rotates through 90° so that its basal (capsular) aspect moves medially along the posterior capsule, while its apical aspect extends anteriorly to reach the anterior epithelium (Rabl, 1900). Since the oldest fibres are
entombed within the youngest and are never reabsorbed, the lens is characterized by growth rather than turnover (Young and Fulhorst, 1966; Jose, 1978). Lens fibers demonstrate a terminal differentiation process with loss of their organelles including nuclei. Chromatin disappearance is characterized by the same changes as most epithelial cells do in apoptosis (Counis et al., 1998).

In rats, approximately five new fiber layers are added each day, in the one year old animal, the rate slows down to one layer per day. It probably takes nearly three months for a cell of PR to differentiate into mature fibers. Along with nuclear disintegration, the fiber detaches from the epithelium anteriorly and from the capsule posteriorly; the detached ends of the contralateral fibers now interdigitate at the suture planes. Such a lens cell is now called the mature fiber (Kuwabara, 1975). The nuclear region of the lens represents internalization of the oldest fibers at any given age (Tripathi and Tripathi, 1983). In comparison to the cortex, the lens fibers in the nucleus are shorter, irregular in shape and densely packed with poorly defined intercellular organelles and only with a homogeneous granulomorphous protein material (Worgul, 1982; Maisel et al., 1981; Fransworth et al., 1980).

1.2 THE CATARACT:

The cataract is many different diseases of the lens, all of which causes increased light scattering. Most researchers consider the biophysical basis for this increased light scattering to be fluctuations of sudden changes "jumps" in the refractive index. An important molecular mechanism for such light scattering is the condensation of proteins into aggregates. Such aggregated states are also the basic pathogenic factor for a wide class of molecular condensation diseases, including sickle cell anaemia and Alzheimer's disease, etc. (Benedek, 1997).
The cataractogenesis is characterised by the increase in turbidity and light scattering of lens. With age on average, each lens shows a continual increase in turbidity until a threshold for 'cataract' is crossed. Healthy persons differ simply by virtue of variations in the rate of progression of turbidity. Thus, the common meaning of the term 'disease' is really not relevant to the process of cataractogenesis (Benedek, 1997). Indeed, a wide variety of post-translational chemical modifications of the lens proteins are believed to be associated with protein aggregation. Such modifications includes intermolecular disulphide crosslinks, methionine oxidation, carbamylation, glycosylation of amino groups and racemization. In these way multiple biochemical factors contribute to cataractogenesis (Harding, 1991). Nevertheless, these multiple factors have the net effect of producing 'clumps' or aggregates of protein. These spatial fluctuations in protein density produces increased light scattering and ultimately can result in opacification of the lens (Benedek, 1997).

The cytoplasmic solution of proteins within each lens cells is in fact not stable. Microscopic protein aggregation is one manifestation of this instability. A second instability and a source of spatial inhomogenity of the dense cytoplasmic protein solution is the spontaneous separation or the solution into coexisting protein rich and protein poor domains. Tanaka & Banedock (1975) were first to show that reversible opacification of lens is result of a phase separation of proteins (Benedek, 1997).

Cataract is the major cause of blindness and avascular morbidity throughout the world; and any means of delaying or preventing its onset would certainly have enormous social and economical benefits. In India, 3.8 million people becomes blind due to cataract every year. Besides that there is a backlog of 22 million cataract
cases (Minassian and Mehra, 1990). According to World Health Organization (WHO), there are about 42 million blind people in the world of which 17 million (40%) are blind due to cataract (WHO, 1979; 1987; Hyman, 1987). According to one study cataract causes 55% of visual impairment and blindness in India. If the development of cataract could be delayed by 10 years the numbers of cataract operations would decrease by 45% of the world's 40 million blind people, cataract affects about 17 million and about a third of these are in India (Kupfer et al., 1994; Balasubramanian, 1993). The economical loss from cataract in India is approximately US$ 2000 million annually. Economic and other losses associated with this disease are too huge for a common man; and these must be tackled in a suitable way. Thus, cataract is an important disease both in terms of the number addicted and the economical demand placed on budget which is more than 12% of total medicare (Stark et al., 1989).

1.2.1 Risk Factors:

A single cause of cataract in human probably does not exist. Several risk factors working concurrently lead to the loss of lens transparency. Some of the risk factors are listed below (Mathew et al., 1996b):

1. Age
2. Ultraviolet radiation – long wavelength (UV-L)
   (a) Sunlight
   (b) Occupational exposure
3. Nutritional status
4. Dehydrational crisis (Diarrhoea and heat stroke)
5. Toxic drugs and chemicals (i.e. corticosteroids, phenothiazines, mitotic cholinergies, metal ions and others)
6. Diabetes (High blood glucose-sorbitol and enzyme aldose reductase)
7. Ionizing radiation (Therapeutics and diagnostics, CT-scan, X-ray).
8. Blood pressure (elevated blood pressure is closely associated with the presence of cataract).
9. Family history (families with cataract prevalence).
10. Smoke (household fuel and cigarette smoke)

1.2.2 Role of Lens Epithelia in Cataract Development:

Lens epithelia is reported to be associated with various cataracts like senile cataract (Tseng et al., 1994), sugar cataract (Robison et al., 1990), UVR cataract (Hightower et al., 1994; Kleiman et al., 1990), oxidative stress induced cataract (Spector, 1995; Carper et al., 1999), steroid cataract and selenite cataract (Anderson et al., 1986; Hightower and McCready, 1994). A number of studies suggests 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).

UV radiation, X-ray, selenite, oxidative stress, galactose are associated with alterations in protein structure and its synthesis (Andley et al., 1990; Hoenders and Bloemendal, 1981; Shang et al., 1997; Padgaonkar et al., 1999). UV radiation, selenite and other experimental and human cataract leads to increase in sodium and calcium ion concentration (Duncan and Bushell, 1975; Salit et al., 1942, Shearer and David, 1982/1983; Delamere et al., 1983; Bando and Obazawa, 1989). The increase in calcium concentration leads to activation of proteolytic enzymes, the proteosome and calpain, which could result in proteolysis of crystallins, causing abnormal interaction of the crystallins, insolubilization of the proteins and further light scattering (Karlson et al., 1999; Anderson et al., 1999). Cation pump system in
a cell is critical for maintaining osmotic stress. Osmotic swelling contributes to cataract formation possibly through physical forces exerted on stretched membranes (Kinoshita, 1974). However, biochemical changes responsible for osmotic imbalance in human senile cataract is not a defect in the cation pump mechanism but rather an increase in lens membrane permeability (Pasino and Maraini, 1982).

The development of cataract is also associated with increased DNA damage and altered DNA synthesis (Andley et al., 1990). Selenite may cause a block in the S-phase of the cell cycle in the lens epithelial cells (Huang et al., 1990). Similarly in various cataract models, the mitotic figures of lens epithelium is found to increase first and then subsequently decreases. Multilayering of epithelial cells and formation of epithelial plaques are also reported (Gona, 1984; Gona and Gorelli, 1985).

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

1.3 THE SIGNIFICANCE OF WORK:

The anteriorly placed single layer of lens epithelium is essential for the continuous growth, differentiation and homeostasis of the lens. It contains highest activities of various enzymes and transport systems in the lens. It also acts as a barrier between aqueous humor and lens proper structure that is lenticular fibers. The epithelium being the most anterior portion in mammals, it is the first site of
ocular lens exposed to any sort of insult. Therefore, epithelium is first target site for damage which leads to lenticular opacification as found to be associated with the formation of various cataracts (Tseng et al., 1994; Robison et al., 1990; Spector, 1995; Hightower and McCready, 1994).

Significant evidences point to metabolic communication between the epithelium and the underlying terminally differentiated lens fiber cells. The mature fibers cannot replace the damaged proteins, as the lens epithelium is the major site for protein synthesis. Hence, the lens fiber cells have very limited capacity to repair damaged macromolecules and have a low level of defense against external insult.

Since all lens fibers are differentiated from the epithelium, it is reasonable to consider that changes to the lens epithelium, can give rise to defect or alterations in lens fibers which ultimately leads to cataract formation. There are so many laboratory evidences in several cataract models which has shown that during lens opacification, the first morphological changes appears in the lens epithelium.

Another important aspect of the lens epithelium is that the cells of central region are the true original cells which are formed during the embryogenesis and can be considered as oldest cells in the body (Mggleton-Harris, 1970). Therefore, central region of the lens epithelium is an interesting site for knowing about the aging process in the cells.

It is very well known that UV and oxidative stress are the major causes of cataract formation in tropical countries. The majority of them are senile cataract. The cataract induced by selenite, resembles the development of cataract in human. Several reports have explained that the first target for cataractogenic insult is lens epithelia (LEp), therefore, it has found to be advisable to know the effects of UV
radiation, oxidative stress and effect of selenium on lens epithelium and its probable and sequential role in the cataractogenesis. Although much more attention has been paid to the biochemistry and physiology of the lens epithelium but there are very few efforts in the direction of cellular integrity of such lens epithelia concomitant with the change in important biochemical parameters and its further influence on lens proper.

The investigation envisaged, is expressed in following chapters:

1. Introduction
2. Materials and Methods
3. Results
4. Discussion
5. Summary and Conclusions

1.4 EXPERIMENTAL CATARACT MODELS:

Owing to the non-availability of enough human sequential cataractous lens samples for conducting extensive morphological and biochemical studies, various experimental cataract models are being used to elucidate the underlying mechanism of cataract formation.

1.4.1 Sodium selenite (Na2SeO3) induced cataract model:

The salts of certain metals are capable of producing cataract. These includes selenium, cobalt and thallium. Selenium is present in nearly all materials of the earth crust, fundamentally in magnetic and sedimentary rocks. Selenium is usually concentrated in sulphur and sulphide minerals (Aller, 1996). It was first discovered by Berzelius and Gahn and occurs in different oxidation states like Se⁺, Se-organic complexes, selenides (Se²⁻), selenites (Se⁴⁻) and selenates (Se⁶⁶⁻).
Selenium, a component of glutathione peroxidase is known today to be an essential trace element in the mammalian diet (Schinogoethe et al., 1982; Lavender et al., 1983; Kien and Ganther, 1983) and their dietary requirement is well established (Hilton et al., 1980; Kukreja and Khan, 1994; Imai et al., 1997). Equally important are its toxic effects, both in experimental animals and in man (Hilton et al., 1980; Julius et al., 1983). Selenium compounds have been shown to cause cellular dysfunction or cytotoxicity in a number of tissues including the blood cells (Young et al., 1981; Zia, 1993), lungs (Bell et al., 1997), hepatocytes (Anundi et al., 1982), skeletal musculature (Lin-Shiau et al., 1989), ocular tissues (Rosenthal and Adler, 1962) and proptosis in fishes (Moxon and Rhian, 1943).

The cataractogenic potential of selenium was first reported by Algana and D'Aquino (1957). Since then, cataract was reported in experimental animals associated with selenium deficiency (Springler et al., 1971; Lägle et al., 1997) and/or selenium excessive intake as in young suckling rat pups given a single subcutaneous dose of 20 μmol sodium selenite per kilogram body weight on the 10th day of post-partum (Ostadalova, 1978). It is also reported in rabbit and guinea pig (Bhuyan et al., 1980). Advanced stages of cataracts have been produced within three to six weeks by injecting twice a week with sodium selenite (Vaezy et al., 1995; Hess et al., 1996).

The concurrent updated working hypothesis for selinite nuclear cataract formation is that selenite oxidizes critical sulphydryl groups in the lens epithelium (Shearer et al., 1992). This oxidation leads to impaired calcium homeostasis, elevation of calcium in nucleus and activation of the calcium dependent proteolytic enzyme calpain. Limited proteolysis of crystallins, especially β-crystallins polypeptides leads to abnormal interaction of crystallins, insolubilization of proteins.
and further light scattering. In addition, abnormal fiberogenesis also seems to contribute to selenite cortical cataract. The above findings are the results of experiments in cultured lenses, others in the vitro system and by the use of enzymes as well as cataract inhibitors.

Selenium model is useful and cataract develops quickly, easily and reproducibly involves many of the abnormalities found in human aging cataract (Matsushima et al., 1997). Rats of different species and from different origin, display variable susceptibilities to formation of selenium cataract. The selenite cataract model in Wistar rat is an appropriate specimen for the study of cataractogenesis, incorporating many of the characteristics observed in the morphology of human senile cataract.

1.4.2 UV Radiation induced cataract model:

Solar radiation, a major part of environmental radiation has wide influences on many non-ionizing biological and physical systems on earth. We are constantly exposed to a portion of the environmental radiation, mainly UV, visible and IR regions.

The ocular tissues are well protected from radiation below 290 nm because most of it is absorbed in the cornea and aqueous humor (Ringvold, 1996). The energy level that reach epithelium is also depend on the depth of anterior chamber which is 0.58 nm in rat than in primates (Reddy et al., 1998). Generally UV B, less than 290 nm is strongly absorbed by the cornea and produces photokeratins (Kinsey, 1948; Ringvold, 1996). UVR above 290 nm penetrates the cornea and is nearly to totally absorbed by the lens. UV B in 290 nm to 310 nm wavelength is thought to be more deleterious (Pitts et al., 1986; Bochow et al., 1989).
Since the early part of the 20th century, ophthalmologists have been suggesting an association between sunlight and cataract (Verehoeff et al., 1916; Duke-Elder, 1926). However, only in the past twenty years epidemiological studies documented this association. Besides skin, the only organ or tissue in the body that is particularly susceptible to UVR is the eye.

During the last decade or so, there has been increasing evidence that sunlight and particularly long wavelength ultraviolet UV-L (300-400 nm) and UV B (290-320 nm) are associated with cataract (Jose and Pitts, 1985; Bochow et al., 1989; Hiller et al., 1983). Van Heyningen (1973) has first time relate the cataract development with geographical location by the study of cataracts removed in Oxford and Pakistan. An excellent perspective epidemiological study of cataracts related to the environment has been described by Taylor (1980). This work related to the number of cataracts observed to several factors influencing Australian aborigen populations. A positive relationship was also found between number of sunlight hours and the prevalece of cataract. Hollows and Moran (1981) confirmed the incidence of cataracts observed among Australian aborigins was greater in areas of more intense UV radiation than in those areas with lower level radiation. Latitude and sunlight are also associated. A large increase in the fraction of brunescent cataract in relation to decreasing latitude was reported (Pirie, 1972; Zigman et al., 1979). The prevalence of senile cataract increases both with decreasing latitude or increasing altitude of place of residence. Residents of Zedang (China) of the Tibetean Plateau are exposed to twice the UV radiation in those living at sea level. They tend to develop cataract at younger ages (Mao and Hu, 1982). However, Chatterjee (1973) reported a negative correlation of cataract prevalence and altitude.
Cataract is found more common in cloudier areas. The reason would be that the pupil opens more in cloudier condition than in bright light to admit more radiation into the lens. Factually water vapour in the clouds filters visible and IR but not UV (Boetner and Wolter, 1962).

The increasing levels of UV B radiation reaching earth's surface as a consequence of depletion of stratospheric ozone layer creates much of academic interest. The most important study to date has been the Chesapeake Bay study -- a cross-sectional study on Wateman (Taylor et al., 1988) which revealed a significant association between total cumulative UV B exposure and cortical opacities. It is the only study to have examined the dose response relation between the UV B and cortical cataract. Most of all above studies inspite of different approaches derives at same conclusion with certain exceptions. All these studies support the hypothesis that senile cataract is associated with higher exposure to sunlight.

It is believed that the production of coloured pigments (fluorophores) in the lens is involved in the transfer of energy from light to receptor substances resulting in a free radicle formation (Andley and Clark, 1989). UV radiation can also lead to production of various reactive oxygen species (ROS) (Chalupecky, 1987; Zigman, 1985; Hightower and McCready, 1992a). The chromatophores like aromatic amino acids, phenyl alanine, tyrosine and tryptophan absorbs significant amount of UV radiation longer than 250 nm. The UV wavelength longer than 280 nm pose a potential threat of their presence in our environment. Two other amino acids cysteine and cystine are also important chromatophores since they absorb at wavelength longer than 250 nm at neutral and alkaline pH. Asides from chromatophores in protein, the peptide bonds can also be broken at wavelength
longer than 250 nm, although its absorptive maxima is 180 to 190 nm (Lerman, 1980).

N-formyl Kynurenine (NFK) is the photoproduct of tryptophan which has significant absorption at 300 nm and can thus, react via its triplet state with O₂ to generate various ROS. In vitro studies have shown that ROS including O₂⁻, H₂O₂, ¹O₂ and OH⁺ can be generated by light catalysed reaction. The photoproducts of tryptophan can be generated photochemically by near UVR to human or rat lenses in culture, their fluorescence property is identical to chromatophores which accumulates in normally aging lenses (Andley and Clark, 1989). The ROS formed during the UV exposure are strong oxidizers and together with H₂O₂ and O₂⁻ may lead to cataractous changes.

Sunlight cause various effects on lens which includes biochemical and histological changes. Pirie (1971) introduced the concept of lens protein alterations by sunlight and reported that exposure of human lens proteins to sunlight in vitro would cause their browning. If human lenses are exposed to sunlight in the presence of tryptophan, they turn yellow (Welter and Subramanian, 1978). Dilley and Pirie (1974) suggested that the photooxidation of tryptophan residues, probably with the formation of N-formyl Kynurenine (NKF) is a primary step in cataractogenesis.

Experimentally UV cataract can be produced in vitro in cultured rabbit lenses by exposing them to either near UV for 15 to 60 minutes (1700 – 6900 J/m²) (Hightower et al., 1994) by using a spectrolite lamp system; by using xenon lamps, 150W for about 30 minutes (Rao, 1990); by using 18", 15W fluorescent bulb (Kleimann et al., 1990) or by UV lamp or by using two black tube light 40 W each.
(Schmidt et al., 1992; Jain et al., 1999), experimental cataracts were performed both in vivo and in vitro in various animals like mice, rat, rabbit and primate lens (Zigman et al., 1974; Lerman, 1980).

1.4.3 Hydrogen peroxide (H₂O₂) induced cataract model:

The cataract appearing late in life, frequently referred as maturity onset cataract, is probably not associated with congenital conditions or other diseases such as diabetes. The difference between the constituents of young and older lenses reflects some light on the evidence that oxidative stress is associated with cataract. First, the post translational changes including racemization (Garner and Spector, 1978; Masters et al., 1977; Geiger and Clark, 1987), glycation (Chiou et al., 1980; Patrick et al., 1990), COOH terminal degradation (Van Kleef et al., 1975; 1976), deamidation (deJong et al., 1988), and non-covalent aggregation (Platigorsky, 1989) in the proteins of inner region of the lens where protein synthesis is very less (Halliwell and Gutteridge, 1990). Secondly, the numerous genes coding for major structural proteins, the crystallins is not necessarily active throughout life (Coring et al., 1992; Yu et al., 1990). Even its activity is different in the different regions of lens. Finally, a constant decrease in the concentration of various protective molecules along with increasing age is found (Hockwin and Ohrloff, 1984; Rathbun and Bovis, 1986). In cataract, all thiols are exposed to surface which were burried in normal and young lenses. So protein oxidation at the membrane proceeds cataract formation. The production of large aggregates of protein linked by disulphides are large enough to scatter light (Spector, 1984). Such findings suggests that oxidation participates in the development of cataract and proceeds the actual loss of transparency (Spector, 1995).
Further, there are several reports which suggest that major factor involved in the development of cataract is oxidative insult (Spector, 1995; Giblin et al., 1990; Ortworth, 1998; Spector et al., 1998). Both intra and intercellular oxidative stress affects the lens (Andley and Clark, 1989).

Oxygen being the most electronegative element with the exception of fluorine and also being one of the most abundant element in so far as the planet earth is concerned, it is one of the primary element for biological as well as nonbiological oxidation reactions. O₂ tension in the vicinity of the lens is low, less than 30 to 60 mm Hg. Yet this is sufficient to support some aerobic lens metabolism and is sufficient to act as a source of ROS (Kwan et al., 1972; Hanling and Crabbe, 1984). The major ROS arising from O₂ by consecutive one electron reduction reactions are the superoxide radicle (O₂⁻⁻), peroxide and hydroxyl radicle (OH*) (Fecundo and Augusteyn, 1983; Krishna et al., 1991). Singlet O₂ resulting from absorption of photochemical energy by O₂ is also a major ROS (Ortworth, 1998). O₂⁻⁻ is produced in all aerobic cells during mitochondrial oxidation-reduction reactions or during the oxidation of certain molecules and as a result of photochemical reactions. H₂O₂ is not a radical and is less reactive than O₂⁻⁻ or •OH. However, its reactive stability allows it to move from its origin to other locations, passing rapidly through cell membranes (Spector et al., 1993). H₂O₂ is produced due to detoxification of O₂⁻⁻ by an enzyme superoxide dismutase (SOD) (Halliwell and Gutteridge, 1990). A number of enzymes produces H₂O₂ and it also can be generated by photochemical reaction. OH* is the most reactive free radicle (Imlay et al., 1988) whose formation plays a key role in the damage suffered by tissues exposed to high energy radiation. It's reaction leaves behind a legacy in the cell, in a form of propagating chain reactions. But OH* has short half life reacting at or near
its site of formation and is rapidly formed from H$_2$O$_2$ through Fenton's reaction (Spector, 1995).

Aqueous humor of normal human eye contains approximately 25 to 30 μM of H$_2$O$_2$ (Spector and Garner, 1981). In rat, this value is 6 to 9 μM (Ramachandran et al., 1991). In aqueous humor of cataract patients, the value was found 2 to 7 fold higher than that of normal. Similarly, the lens possesses as much as 30 fold higher H$_2$O$_2$ than the normal lens (Spector, 1995). Thus, in a significant proportion of this cataract formation, elevated H$_2$O$_2$ levels accompanied the presence of an opaque lens. The high level of H$_2$O$_2$ may be due to decrease in lens systems capable of metabolizing H$_2$O$_2$, due to inflammation in older people and the presence of components present in the aqueous capable of photochemical reactions that may elevate H$_2$O$_2$ concentrations (Gasterland et al., 1979). H$_2$O$_2$ is also produced metabolically in the single layer of lens epithelial cells and the newly formed fiber cells (Fecundo and Augusteyn, 1983). There are also reports that aqueous humor and vitreous may be major component to the increase in the H$_2$O$_2$ level (Spector et al., 1998). Although ascorbate and GSH present in high concentrations can be involved in reactions producing H$_2$O$_2$ and other noxious ROS, however, the level found in cataract patients are not likely to be produced by ascorbate and GSH alone. Further analysis of human lens with respect to protein, lipid, DNA, epithelial cell death and transport show characteristics similar to those obtained when lenses were subjected to H$_2$O$_2$ treatment (Giblin et al., 1984; Spector, 1995).

A number of model systems have been used to induce cataract that involve oxidative stress,
1. Hyperbaric O₂ concentration (Padgaonkar et al., 1989; Giblin et al., 1988).

2. Adding certain toxins (such as alloxan paraqudt or doxorubicin (adriamycin) that increases intracellular formation of ROS.


5. Organ culture of normal lenses in H₂O₂ concentration bound in cataract patients (Giblin et al., 1985).

The last cataract model system is used in these experiments. However, the extent to which this model is applicable to human cataract is not clear. O₂* and OH* can be produced as aqueous humor contains Fe²⁺ or Cu²⁺ ions. However, in human, only one part of the lens is stressed but here both the parts are affected.