CHAPTER I: Cataract - An Overview
1.0. INTRODUCTION

The eye is an organ of visual transduction. Light entering the eye is focussed onto the retina, forming a reduced, inverted image of the external world. Consequently the media through which light passes must be sufficiently refractive and transparent. These are the cornea, the aqueous humor, the lens and the vitreous humor. The cornea, the anterior chamber of the aqueous humor and the iris are the anterior parts of the eye. Behind the iris is the posterior chamber of the aqueous humor and the transparent biconvex lens. The elastic lens is surrounded by a lens capsule. Fanning out from the lens capsule are the zonal fibers which are connected to the ciliary muscles and the outer layer of the retina (the choroid layer). Blood vessels, lymphatics and nerves are not found in the lens. The pupil is the space formed by the iris. The action of the iris muscles constricts or dilates the pupil to allow sufficient amount of light to fall on the lens, thus aiding in vision at varying ambient levels of light. Similarly, the contraction or relaxation of the ciliary muscles alters the shape of the lens thus aiding in seeing objects which are nearer or farther off; this is the process called accommodation.

1.1. Blindness and cataract

Since the eye is an organ of vision, the most frequent symptom of eye disease is the impairment of visual acuity to varying degrees. Falling eye sight may be due to an abnormality of the refractive media of the eye or due to a
lesion in the retina or optic nerve or the parts of the brain with which they are connected. The impairment of the eye sight may be unilateral or bilateral, sudden or gradual, episodic or enduring. The common cause for visual impairment varies with age. Nearsightedness or myopia is the usual cause in late childhood and adolescence. Presbyopia or farsightedness requiring refractive correction and wearing of eye glasses is common in the middle years. In later life cataract, glaucoma, retinal hemorrhages and detachments are frequent.

Alterations in refractive media that affect vision have certain medical implications. Degeneration of the outer receptor layer and the subjacent pigment epithelium occurs in retinitis pigmentosa and senile macular degeneration. In acromegaly and hyperphosphatemia, degeneration occurs of Bruch's membrane which supports the layer of pigment epithelium next to the rods and cones. Phenothiozone derivatives (used as drugs) conjugate with the melanin of the pigment layer resulting in degeneration of outer retinal layers. The commonest vitreous opacities are benign "floaters" which appear to patients as gray or black spots when they move their eyes. Hemorrhage may occur from rupture of the ciliary or the retinal vessel due to trauma which may not heal in disorders such as diabetes mellitus. The vitreous humor can also be affected by the deposition of calcium. Changes in the iridocorneal angle leading to impaired vision occurs during glaucoma or high pressure in the aqueous humor. Visual loss involving cornea may be due to band keratopathy where calcium carbonate and calcium phosphate deposits are seen below the corneal epithelium due to hyperparathyroidism and vitamin D intoxication. Other depositions under corneal
epithelium include cystine crystals in cystinosis, cholesterol in hypercholesterolemia, copper in hepatolenticular degeneration. Keratitis or corneal opacification may be due to bacterial or viral infections.

The commonest abnormality in case of the lens is cataract formation. The condition in which the transparent eye lens becomes opaque is termed cataract. Lens opacities may form in *diabetes mellitus* due to accumulation of sorbitol in the lens leading to high osmotic pressure. In galactosemia, the accumulation of dulcitol disrupts lens fiber cells, leading to cataract. In hypoparathyroidism, the lowering of calcium concentration in the aqueous humor opacifies the newly formed lens fibers. Lens opacity varies in extent and may be localized to the cortical or nuclear areas, or it might involve the whole lens. In immature cataracts the opacity does not obscure all anatomical regions of the lens, whereas in mature cataracts the whole lens becomes opaque without anterio-posterior swelling and in hypermature cataracts, the lens becomes totally opaque with marked anterio-posterior swelling.

It is believed that a proper classification of cataract helps in defining the sequence and progression of events that occur during cataractogenesis. Experimental cataracts in animals are classified according to the physico-chemical nature of the agent, dose and period of administration. However, classification of human cataracts based on lens appearance *in vivo* or *in vitro* is still controversial (Harding, 1981). There are two major classification systems for cataracts. One system is based on the location, shape and colour of the lens nucleus (Pirie, 1968), and includes a small number of totally opaque lenses (van
Heyningen, 1972). The other classification system is based on a strict description of each lens and a photographic reproduction, which has been adopted by the Cooperative Cataract Research Group in the United States (Chylack, 1978). The anatomical regions of the lens used in classification of cataracts are the cortical and the nuclear regions. These regions are subdivided into anterior subcapsular, equatorial and posterior subcapsular zones of the cortex; supranuclear and nuclear zones of the nucleus.

Epidemiological studies of cataracts are necessary to identify possible etiological factors and in order to understand cataractogenesis. Available data in the past decade indicate that cataract is the major cause of visual impairment in the world. In India alone, during the next decade, about 3.8 million people are projected to become blind due to cataract each year (Minassian and Mehra, 1990). In a case control study of age related cataracts, the association between nuclear, cortical, posterior subcapsular and mixed cataracts and a number of environmental, behavioral, physiological and biochemical variables were studied (Mohan et al., 1989). It was found that the high risk factors for cataract formation are associated with decreased cloud cover, lower level of nutrients in the diet, hypertension, lower body mass index, use of cheaper cooking fuel and lower levels of antioxidants in the blood.

Since the eye is constantly exposed to ambient solar radiation, part of the age related changes in the lens proteins may be attributed to sunlight or artificial light sources. The cornea absorbs all optical radiation below 293 nm (Bachem, 1956). Hence all radiation of longer wavelengths is transmitted through the
aqueous humor to the lens. One-third of the 300-400 nm radiation is absorbed by the cornea and about 2% by the aqueous humor (Zigman et al., 1977). The lens absorbs most of the radiation between 300-400 nm, transmitting light of longer wavelength to the retina. The filtering characteristics of the lens vary with age (Dayhaw-Barker and Barker, 1986). The young lens is clear and almost colourless but contains absorbing compounds in the range of 295-400 nm. During aging the human lens becomes yellow, with increased absorption between 300 and 400 nm. In the wavelength range 440 to 700 nm the optical density of intact 30 to 80 years old normal human lenses increases with age. The absorption at 440 nm after corrections for scattering represents then a measure of the colour of the lens. It was found that this "colour" increases slightly with normal lens until 50 years of age which from then onwards increases very rapidly till 70 years of age (Zigman et al., 1976).

1.2. Physical basis of lens transparency

Any material, in order to be transparent, should not contain chromophores that absorb light in the regions of interest. The material must also be free from static scattering centers, like particles, or dynamic fluctuations in the refractive index. The normal young lens possesses all these features to make it transparent in the visible region of the electromagnetic spectrum. But with aging and disease there is a gradual loss of lens transparency.

The major physical phenomena that limit lens transparency are the absorption and scattering of the visible radiation. From a quantitative standpoint,
the intensity of the transmitted radiation $I_t$ for an incident intensity $I_0$, passing through a sample of thickness $t$ and extinction coefficient $\varepsilon$ of the system are related as $I_t = I_0 e^{-\varepsilon t}$. Extinction of light occurs due to two physical phenomena, absorption and elastic scattering both of which remove energy from the incident radiation. It is thus necessary to consider absorption and scattering to analyze lens transparency. Absorption depends on the details of the chemical structure and the energy of the incident radiation. The turbidity of the sample is a measure of transparency caused by features other than absorption of light. For unpolarized light turbidity can be expressed as

$$\tau = 24\pi^3 \xi^2 N_a V_a / \lambda^4$$

where $\xi = (n_a^2 - n_t^2) / (n_a^2 + 2n_t^2)$

$n_a$ and $n_t$ are refractive indices of the solution and the solvent respectively, $N_a$ is the number of aggregate molecules per unit volume, $V_a$ is the volume of each aggregate. $V_a$ and $N_a$ can be expressed in terms of the molecular weight of the aggregate as

$$N_a = (c N_0 / M_a) f$$

$$V_a = v M_a / N_0$$

where $c$ is protein concentration in g/ml, $N_0$ is the Avogadro number, $M_a$ is mol. wt. of the aggregate, $f$ is fraction of the aggregated protein and $v$ is partial specific volume. From the above three expressions it is clear that turbidity

$$\tau = (24\pi^3 / \lambda^4) (c v^2 / N_0) \xi^2 M_a f$$

Thus there is a direct proportionality between the turbidity and the molecular weight of the aggregate.
Similarly, the equations for elastic scattering of the particle can be derived. Elastic scattering arises from the polarizability changes in the medium. When a particle in vacuo is subjected to an electric field $E$, an oscillating dipole moment $P$ is induced in the particle. The induced dipole moment is proportional to the applied electric field and the proportionality constant $\alpha$ is known as the polarizability. Thus we have

$$P = \alpha E$$

where $\alpha$ is the polarizability of the particle. An oscillating dipole emits radiation of the same frequency as that of the oscillation and thus becomes a secondary source of radiation of the same wavelength as that of the incident light. Lord Rayleigh has shown for polarized light of wavelength $\lambda$, the ratio of scattered intensity $I_s$ to the incident intensity measured at a distance $r$ from the particle is

$$r^2 I_s / I_0 = 16\pi^4 \alpha^2 / \lambda^4$$

However with unpolarized radiation the polarization factor $(1 + \cos^2 2\theta / 2)$ is needed. When the particle is immersed in a medium then particle polarizability is replaced by polarizability fluctuation $d\alpha$ (Debye, 1944). When the particle size is of the same order as the wavelength of light or larger, then scattering is not isotropic and exhibits an angular dependency (AD). Hence the total intensity of the scattered radiation is the sum of intensities scattered by $N$ identical particles modulated by an interference (IT) and angular dependency (AD) terms. Thus

$$r^2 I_s/I_0 = (16\pi^4 / \lambda^4) [(1 + \cos^2 2\theta) / 2] (N) (d\alpha^2) (AD) (IT)$$

The above equation suggests that large aggregates or domains are responsible for turbidity in the medium or opacity. Equations can be derived from the above
expression where scattering can be shown as a function of the scatterer concentration (c or $\phi$), size ($M_a$ or $V_a$), contrast ($\xi_a$) and particle distribution $S(c,0)$

$$r^2 I_s / I_0 = (32\pi^3 / 3 \lambda^4) \left[ (\xi^2_a / \lambda^4) \nu^2 \right] \left( M_a / N_a \right) S(c,0)$$

$$= (32\pi^3 / 3 \lambda^4) \left( \xi^2_a / \lambda^4 \right) (V_a \phi) S(c,0)$$

where $\xi_a = n_a^2 - n_i^2$ and $\phi = c \nu$

From the above discussion, it is clear that the lens must have specific structural features that avoid the formation of scattering centers. The constituents of the lens must have a proper spatial distribution and supramolecular organisation which avoids large dynamic changes in refractive index. In addition, the normal lens should be devoid of chromophores that can attenuate visible light due to absorption. In order to understand how the lens achieves these characteristics it is necessary to know the development of the lens and changes in the properties of the constituents during cataractogenesis.

1.3. Lens development and its constituents

The adult vertebrate lens is surrounded by a noncellular capsule. There is a temporal sequence of events that occur during differentiation of the lens. The lens placode is formed by the elongation of the cells constituting the presumptive lens ectoderm, the induction of which seems to be the direct contact of the optic vesicle as no natural inducing substance was found. The interfacial matrix between the basement membrane of the optic cup and the presumptive lens ectoderm becomes dense and contains glycoproteins, collagen and glycosaminoglycans (Hendrix and Zwaan, 1975). In vitro, the optic cup could condition
an agar slice to induce the presumptive lens ectoderm within 96 hours after the development of the chicken lenses (Karkinen-Jaaskelainen, 1978). The posterior cells of the lens vesicle form the primary lens fiber cells as they elongate. The central zone of the outer epithelial cells has a low rate of mitosis, whereas the cells in the equatorial plane are somewhat elongated and form the germination zone with high mitotic rate (Ramaekers and Bloemendal, 1981). The secondary (cortical) lens fiber cells form posteriorly from the equatorial epithelial cells and are added circumferentially by growth and differentiation throughout the life of the organism. The fiber cell elongation could be due to alignment of the microtubules (Piatigorsky et al., 1972a, 1972b), increase in cell density or crowding due to cell proliferation (Hendrix and Zwaan, 1974a, 1974b) and increase in cell volume (Beebe et al., 1979). As a result, the temporally formed fibers are progressively internalized. As all the cells are retained in the lens, the older fibers are always found towards the central region (nucleus) and newly formed fibers are found towards the periphery (cortex). There is a gradual loss of organelles in the cells from the periphery of the lens to the nucleus. Thus at any stage of development, the concentric layers of cells reflect the cell differentiation, maturation and aging of the lens fiber cells. The lens is also a very low metabolizing tissue and hence any changes in the cell constituents are cumulative. The lens is thus an excellent system for the study of aging processes.

Lens transparency appears to require a very high protein concentration (Delaye and Tardieu, 1983). The lens contains on an average 35% by weight of structural proteins. During lens differentiation, proteins called crystallins are
synthesized and reach high cytoplasmic concentrations varying between 20% in the soft lenses of birds and 50% in the very hard nuclei of fish lenses. The water content in the lens varies in different species and may or may not alter as a function of age of the species. Despite the general resemblance in morphology and function of all vertebrate lenses, there is large variation in the protein composition of the lens. The various crystallins found in the vertebrate lens are classified according to molecular weight, charge, subunit types and immunological properties. As the metabolism of the lens is low, there is very low turn over of these proteins. Apart from crystallins, the lens also contains membrane and cytoskeleton proteins and also some important enzymes. However, the crystallins constitute about 95% of the total lens proteins.

The crystallins are tentatively described as lens-specific water-soluble proteins. They are designated generally as "structural proteins" since their main function seems to be able to pack tightly and stably in the fiber cells, so as to reach high concentrations required for the necessary optical properties of the lens. The α-, β-, γ- and δ-crystallins are the major proteins of the vertebrate lens. α-, β- and γ-crystallins are present in mammals whereas birds and reptiles contain α-, β-, some γ- and in addition δ-crystallins. Several other types of crystallins are also found in vertebrates, albeit in low amounts. There is heterogeneity in the spatial distribution of these crystallins in the successive layers of the lens fiber cells. The differences in the protein composition is due to the differential temporal expression of the crystallin genes. α-crystallin is the first to appear in the rats (Marbaix et al., 1977) and mice (van de Kamp and Zwaan,
1973) β-crystallin is the first to be expressed in newts (McDevitt et al., 1969) and δ-crystallin in chickens (Rabey, 1962, Zwaan, 1968). The appearance of β- and γ-crystallins follows α- in rats, in chicken α-crystallin is expressed in the last while β- is expressed after δ-crystallin and in newts γ- appears after β- and α-crystallin appears in the last. In man, γ-crystallin appears first.

It is not known if the variation in temporal expression and spatial disposition of these crystallins in different species is directed towards achieving any specific distribution of crystallins in the lens. This variation might be necessary in order to attain the necessary inter-protein interaction and tight packing at low water levels; moreover the magnitude of the refractive index along the optical axis is determined by the concentration of crystallins along the axis. The dense packing of proteins ensures the absence of sharp discontinuities in the refractive index. However there is a gradual change in the refractive index from the cortex towards the nucleus of the lens, which increases the dioptic power and reduces spherical aberrations. In case of spherical lenses with uniform refractive index, the optical quality is poor since rays entering at different distances from the optic axis are focussed at different distances from the lens (Fernald and Wright, 1983), as such sharp images cannot form on the screen or the retina. The lens fiber cells and crystallins must have a certain uniqueness in order and packing that enable the whole lens to be transparent. Any physical or chemical event(s) that can disrupt the regular array of the lens fiber cells, such as fiber sliding, would lead to sharp changes in the refractive index and prevent proper focussing. In addition, the formation of large vacuoles in the lens fiber cell
or between fiber cells can also lead to refractive errors and transmission faults, as happens in the initial stages of sugar induced cataract, in animals.

1.4. Oxidative damage

1.4.1. Direct photolysis: The continued incidence of UV and visible light of the ambient environment may participate in the age dependent development of cataracts by initiating photochemical oxidation of susceptible moieties in the lens components. As the protein turnover and metabolism of the lens is low or absent, an increasing proportion of oxidatively modified components accumulate in time leading to the development of cataract. *In vivo* formation of oxyradicals, either photochemically or non-photochemically, plays a major role in altering the physicochemical properties and interactions of crystallins. These lead to changes in packing in the lens fiber cells thereby hastening cataractogenesis. Effect of irradiation by near UV-light and photosensitization seems to be dependent upon the spatial distribution and microenvironment of oxidation sensitive amino acids (cysteine, tyrosine, tryptophan, histidine and methionine), within the overall structure of the crystallins.

The suggestion of UV radiation as a possible factor in cataractogenesis (Duke-Elder, 1926) was first used in experiments on guinea-pigs and rabbits (Rohrschneider, 1936; Bachem, 1956). In these experiments wavelengths greater than 300 nm were used, since wavelengths below 295 nm are absorbed by the cornea and do not reach the lens. Sunlight and near UV light cause yellowing of human lens and of various proteins in the presence of added tryptophan.
(Zigman, 1971; Grover and Zigman, 1972). However, purified lens crystallins apparently do not show yellowing in the absence of tryptophan under the same conditions (Grover and Zigman, 1972). Thus the hypothesis for nuclear pigmentation seems to be either direct photooxidation of tryptophan residues in proteins leading to yellowing, increased fluorescence and alternatively, small molecules were photooxidized and then bind to proteins causing brunescence. It was also reported that in proteins lacking tryptophan, irradiation with simulated sunlight did not cause change in fluorescence or lead to formation of covalent crosslinks (Dilley, 1973). Other studies on lens and its proteins exposed to UV light, indicated that UV radiation in the region 290-310 nm is strongly absorbed by lens proteins and results in photochemical reactions which modify the proteins and produce photoproducts (Pirie, 1971; Grover and Zigman 1972; Kurzel et al., 1973). These properties were also compared with that of the aging human lens and cataracts (Spector et al., 1975; Dillon et al., 1976). Table 1.1 along with the epidemiological evidence (Hiller et al., 1977a; Zigman et al., 1979) suggests UV radiation as a possible factor for cataractogenesis. UV laser induced photolysis of lens has shown loss of tryptophan residues in proteins resulting in loss of tryptophan fluorescence, formation of photoproducts of different absorption and emission characteristics and formation of cross-linked proteins (Borkman, 1984).

Tryptophan is a major amino acid present in proteins that can absorb wavelength around 300 nm, and hence is the major photochemical target during protein irradiation in the UV-B range. Thus the photochemical behavior of tryptophan has been a subject of extensive studies. Near UV irradiation of Trp
Table 1.1 Changes common to UV-irradiated lenses and human cataracts.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UV-exposed lenses</th>
<th>Human cataracts</th>
</tr>
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<tbody>
<tr>
<td>Increased UV-visible absorption</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Protein cross-linking</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Increased non-tryptophan fluorescence</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Increased disulfide content</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Kynurenine derivatives</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>High molecular weight protein aggregates</td>
<td>yes</td>
<td>yes</td>
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in aqueous solution produces several photoproducts (Sun and Zigman, 1979) along with the generation of radicals (McCronick and Thomason, 1978; Inoue et al., 1982). Conventional stationary and laser flash photolysis studies of Trp in the 280 nm region have shown that the initial photoproducts are: the triplet state $^3$Trp, the radical cation Trp$^+$ and the neutral radical Trp$^-$ (Grossweiner and Usui, 1971; Baugher and Grossweiner, 1977). It is not known if a photolonization threshold exists (Amouyal et al., 1979) or not (Hoebcke et al., 1986).

The primary product of tryptophan photolysis is N'-formylkynurenine (NFK) which is also formed enzymatically in the human lens from tryptophan and subsequently converted to kynurenine, 3-hydroxykynurenine and hydroxykynurenine glucoside (van Heyningen, 1971). Recently it has been shown by diffuse reflectance spectroscopy that pale yellow cataractous lenses show an absorption band at 365 nm coincident with 3-hydroxykynurenine and that brunescent cataracts with high pigmentation showed an absorption peak over 400 nm possibly a dimerized product of 3-hydroxykynurenine, called xanthommatin (Tomoda et al., 1990). Similar results have been obtained in the extensive analysis of human lenses, carried out in our laboratory.

1.4.2. Photosensitization: Some of the metabolic or photooxidation products of tryptophan might act as photosensitizers in the lens. In the presence of oxygen they are capable of generating active species of oxygen like $^1$O$_2$, O$_2^-$, H$_2$O$_2$ and OH$^-$ (Grossweiner, 1984; Creed, 1984; Walrant et al., 1975; Balasubramanian et al., 1990). In the photosensitization phenomenon, the sensitizer molecule is promoted to its excited singlet state by the absorption of light; the
process of intersystem crossing then takes the system to a longer lived triplet state. From the triplet state of the sensitizer there are two major mechanisms which can lead to oxidation of substrate molecules. The type I or direct reaction pathway is a redox reaction of the triplet sensitizer involving either electron transfer or proton abstraction from the substrate by the sensitizer, with the production of active oxygen species such as superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and the hydroxyl radical (OH$^\cdot$). These radicals can rapidly react with the substrate and generate modifications including intermolecular crosslinking (Andley and Clark, 1989, 1989a; Varma and Mooney, 1986). In the type II reaction, the triplet sensitizer transfers its excited state energy not to the substrate but to ground state molecular oxygen, producing a highly reactive singlet oxygen ($^1$O$_2$), which can react with and oxidize the substrate (Goosey et al., 1980; Zigler and Goosey, 1984).

Photolysis in the presence of riboflavin has been shown to modify both the secondary and tertiary structure of crystallins in solution. After photolysis, both in aerobic and anaerobic conditions, the $\gamma$-crystallin becomes more acidic and also forms larger oligomers by non-disulfide covalent crosslinks (Andley and Clark, 1988a). Changes in the microenvironment of thiol groups is seen in riboflavin sensitized photooxidation of $\alpha$-crystallin (Andley, 1988) in both aerobic and anaerobic conditions although the SH-group environment remains unaltered in the anaerobic condition (Bose et al., 1986). These changes can be inhibited in the presence of radical scavengers (Andley and Clark, 1988b). Different classes of crystallins vary in their reactivity to active oxygen (Balasubramanian
It is however not known if this is due to differential accessibility of reactive residues in these proteins, to $^1\text{O}_2$. Oxidation of histidine has been shown to be necessary for formation of high molecular weight aggregates (Balasubramanian et al., 1990). The photophysical properties of endogenous sensitizers 3-hydroxykynurenine (3HK) and 3-hydroxykynurenine glucoside (3HKG) in the human lens have been studied (Dillon and Atherton, 1990; Dillon et al., 1990). More relevantly, there are variations in the photochemical quantum yields of generation of singlet oxygen and of hydroxyl radical by 3HK, N-formylkynurenine (NFK), Kynurenic acid (KUA) and kynurenine (Ky), various flavins and other pigments endogenous to the lens (Murall Krishna et al., 1991).

1.4.3. Non-photochemical damage: A nonenzymatic mixed-function oxidation system consisting of ascorbate and metal ions, like copper(II) or iron (III), in aerobic conditions has been shown to generate radicals that can oxidize lens crystallins (Garland et al., 1988). In the presence of such a transition metal ion, ascorbate is oxidized to dehydroascorbate generating active oxygen species via the Fenton reaction (McCord and Day, 1978; Halliwell, 1978). The superoxide drives the reaction by reducing the metal ions, thereby keeping the redox cycle (called the metal catalyzed Haber-Weiss reaction) going.

Thus the formation of nondisulfide covalent crosslinks, partial degradation, loss of more basic protein and increase in more acidic species, formation of non-tryptophan fluorescence products, browning and insolubilization of proteins can all occur via the non-photochemical pathway as well (Garland et al., 1988).
1.4.4. Autooxidation of sugars: In diabetic hyperglycemia there is an increase in intracellular sugar levels. Sugars undergo autooxidation to form dicarbonyl compounds and hydrogen peroxide via reactive intermediates of dioxygen and carbon-centered free radicals (Thornalley et al., 1984a,b). Increased non-enzymatic glycation can be seen in crystallins incubated in various sugars (Liang and Rossi, 1990), along with the formation of products of non-tryptophan fluorescence. Studies on the mechanisms of glycation indicate the participation of lysine residues which can form Schiff-base stabilized by Amadori rearrangement. Recently an yellow advanced glycosylation end product was isolated and characterized (Pognor et al., 1984). This was later shown to be the product of the acid hydrolyzed Amadori product (Njorge et al., 1989). The products of Amadori product degradation and subsequent reactions (advanced Maillard products) are usually brown fluorescent pigments and crosslinks which are thought to accumulate in long lived proteins such as the lens crystallins (Monnier and Cerami, 1981). Several glycated products that were isolated include N-α-formyl-ε-fructoselysine (fFL), N-ε-carboxymethyllysine (CML) and hexitol-lysine (Ahmed et al., 1986; Patrick et al., 1990).

In hyperglycemia, the excess glucose is converted via the aldose reductase pathway to sorbitol and other polyols. Accumulation of these polyols results in an increase in intracellular sodium concentration and a decrease in potassium concentration. The lens swells due to increase in the water content, brought about by the osmotic changes. Intercellular water pools are formed in the lens, resulting in phase separation and thus causing scattering of light. It was
suggested that opacity during hyperglycemia is caused due to osmotic imbalance (Kador and Kinoshita, 1984). In the case of galactose mediated cataracts marked spatial fluctuation in the protein density has been demonstrated by micro-radiography (Phillipson, 1969). Experimentally induced sugar cataracts can be reverted by use of drugs that can inhibit aldose reductase system. The extent to which the opacities can be cleared depends on the severity of cataract, dose and period of drug administered (Table 1.2).

Thus it appears that sugar cataracts can result in glycation of crystallins and subsequent browning. Glycated crystallins appear to differ in tertiary structure, with the loss of reactive sulfhydryls, and formation of high molecular weight aggregates (Liang and Chylack, 1987; Perry et al., 1987; Liang and Rossi, 1990).

1.5. Calcium – effects

The possible role of calcium in the physiology of cataract has been known for a long time (Burge, 1909, Adams, 1929). The calcium ion levels in cataractous human lenses are often quite different from those in normal lenses (Duncan and Bushell, 1975; Duncan and van Heyningen, 1977; Hightower and Reddy, 1982a). Increased calcium in the lens has been correlated with cataract formation in both human and animal model systems (Lohmann et al., 1986). Cortical opacities can be observed in lenses cultured in the presence of calcium (Clark et al., 1980; Hightower, 1985; Duncan and Jacob, 1984). Changes in cell fiber calcium content is also observed in cataractous lenses (Duncan and Jacob, 1984). These changes have been related to loss of membrane integrity (Patmore
Table 1.2 Effects of aldose reductase inhibitors on rats *in vivo*.†

<table>
<thead>
<tr>
<th>Inhibitor name</th>
<th>Type of cataract</th>
<th>Effect</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>AY 20,263</td>
<td>Galactose (50% diet)</td>
<td>18-day delay of nuclear opacity</td>
<td>Kinoshita (1974)</td>
</tr>
<tr>
<td>AY 22,284 (Alrestatin)</td>
<td>Galactose (30% diet)</td>
<td>80% delay of cataract after 29 days</td>
<td>Dvornik <em>et al.</em> (1973)</td>
</tr>
<tr>
<td></td>
<td>Galactose (50% diet)</td>
<td>7-day delay of nuclear opacity</td>
<td>Varma and Kinoshita (1976)</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Galactose (50% diet)</td>
<td>After 12 days, lens fibre integrity and growth preserved</td>
<td>Beyer-Mears and Farnsworth (1979)</td>
</tr>
<tr>
<td>Gossypin</td>
<td>Galactose (30% diet)</td>
<td>60% delay of cataract after 28 days</td>
<td>Parmar and Ghosh (1973)</td>
</tr>
<tr>
<td>CP 45,634 (Sorbinil)</td>
<td>Diabetic</td>
<td>No lens change after 6 months</td>
<td>Datiles <em>et al.</em> (1982)</td>
</tr>
<tr>
<td></td>
<td>Galactose (50% diet)</td>
<td>No lens change after 8 months</td>
<td>Fukushi <em>et al.</em> (1980)</td>
</tr>
<tr>
<td>M 79175</td>
<td>Galactose (30% diet)</td>
<td>No opacity after 33 days</td>
<td>Ono <em>et al.</em> (1982)</td>
</tr>
<tr>
<td>ICI 105552</td>
<td>Diabetic</td>
<td>170 day delay for 50% of lenses with greater than punctate opacities</td>
<td>Poulsom <em>et al.</em> (1983)</td>
</tr>
<tr>
<td>ONO 2235</td>
<td>Diabetic</td>
<td>Significant decrease in lens sorbitol after 5 months</td>
<td>Hotta <em>et al.</em> (1983)</td>
</tr>
</tbody>
</table>

† adapted from Kador and Kinoshita (1984)
The transparent structure of the lens is thus maintained, in part, by a careful control of the physiological concentration of calcium (Duncan and Jacob, 1984). Electron microscopy of calcium induced opacities showed domains of about $< 1000 \, \text{Å}$ containing heterogeneous aggregates of all crystallins (Tardieu and Delaye, 1988).

1.6. Temperature – effects

In the normal lens light is scattered by protein molecules, and correlation in position of protein molecules is thought to reduce scattering. The fluctuation in the number of protein molecules over the dimension comparable to the wavelength of light is small because of dense packing. Modification leading to the formation of aggregates can scatter light. Reversible opacification is seen in the nuclei of the lenses of young mammals upon cooling. This seems to be the intrinsic property of the membrane-free cytoplasmic extracts (Clark et al., 1982). Freeze fracture electron microscopy of the opaque regions of calf lens nuclear cytoplasm, undergoing cold cataract opacification, shows the presence of low molecular weight enriched domains, which act as scattering elements (Gulik-Krzywick et al., 1984). The phase separation behaviour of nuclear cytoplasm is dependent on the critical concentration taken. The coexistence temperature increases with concentration of the nuclear cytoplasm to a maximum value at fractional dry weight concentration (dry wt. / total wet wt.) of 37% and falls as the concentration increases to 42%. Dilution of nuclear cytoplasm with cortical cytoplasm lowers the coexistence temperature (Delaye et al., 1981).
Such reversible segregation of crystallins could be followed by crosslinking inside the domains, leading to high molecular weight aggregates or insoluble fractions. This might corroborate with the decrease in the low molecular weight proteins usually observed in the soluble fraction of human cataractous lenses (Horowitz et al., 1983).

It was shown by electron microscopy and immunogold electron microscopy that large domains of 1500 Å in cold cataracts contain low molecular weight proteins comparable to γ-crystallin (Gulik-Krzywicki et al., 1984; Lo, 1989). The contrast (\( E_a \)) between additional scatterers and bulk was different as the protein concentration in the bulk varied from 0.2 - 0.4 g/ml for cortical and 0.4 - 0.45 g/ml for nuclear extracts (Tardieu and Delaye, 1988).

### 1.7. Aging

Increasing amount of D-aspartic acid are found in the proteins during aging, which is brought about by the racemization of the L-residues. It is not known if racemization is the cause or effect of conformational change in the proteins (Harding, 1972; Masters et al., 1978; Fujii et al., 1989).

As the lens is a tissue with low metabolism, changes in the physicochemical properties of the proteins due to photo- or non-photochemical interactions become cumulative. These changes can result in the formation of several chromophores, alter the interprotein interactions and covalent modifications leading to aggregation and precipitation of proteins. The proportion of insoluble proteins increases during aging as well as in cataractous lenses (Clark
et al., 1969; Zigman et al., 1969; Garner and Spector, 1975), although the amounts isolated by various authors vary widely. Hence not only colour filtering occurs due to normal lens aging but significant amount of scattering by high molecular weight aggregates reduces transparency of the lens.

1.8. Scope of present study

From the above discussions it is clear that a combination of biophysical and biochemical studies of the crystallins in solution and the lens in situ are necessary to understand the process of cataractogenesis. Although cataracts are fairly of common occurrence in mammals, there is very little documentation of senile cataracts in birds. Cataracts are seen in birds which survive encephalomyelitis, toxoplasmosis and salmonellosis (Critchley and Tham, 1983). However cataracts are seen in the progeny of birds that are fed diets deficient in vitamin E. Transient cataracts can however be induced in embryonic chicken by administering glucocorticoid (Nishigori et al., 1983). It is not clear if the low incidence of senile cataracts in birds is related to the presence of δ-crystallin in the soft bird lenses. Accordingly chapter II presents a detailed spectroscopic and biochemical characteristics of δ-crystallin isolated from young and aged birds. The role of aging in determining protein stability and cataractogenesis are discussed along with the differential susceptibility of δ- and β-crystallins to photodamage in the presence and absence of calcium.

Since photodamage to lens proteins is one of the factors associated with cataractogenesis, the role of protein conformation in determining the vulnerability
of Trp residues to undergo aerobic photolysis is presented in chapter III. Particular attention is drawn to the differential vulnerability of the peptides or proteins that can undergo transconformational changes by changing the solvent system. Hence the two aspects of sequence dependent and secondary structure dependent photo-vulnerabilities could be delineated. This seems important in the light of changes in native structural organisation the crystallins during senile cataracts which are similar to changes inflicted by UV-light (Krivandin et al., 1989).

The above studies are limited to the molecular properties of isolated crystallins in solution at concentrations far below that present in the lens. Hence it is necessary to consider how these features can be related to the lens per se, with its very high concentration of crystallins and a definite variation in the different crystallins that are spatially distributed. It is not clear if these characteristics of the lens are needed to maintain proper intermolecular interactions so as to form the correct supramolecular ensemble with subtle plasticity in the spatial organisation for the lens to be transparent. Hence studies are necessary on the intact lens in situ. Chapter IV presents a novel way of looking at inter–crystallin interactions and protein organisation in the lens per se. The parameter called Red Edge Excitation Shift (REES) of fluorescence, which is sensitive to fragmental mobility of proteins, can be seen either in crystallins in solution or in the intact lens in situ. The importance of this method as a tool to monitor mobility changes and state of the lens are discussed.