INTRODUCTION

CHAPTER 1
1.1 THE AQUEOUS HUMOUR (AQH)

Lens is located between anterior aqueous and posterior vitreous humour. Being avascular in nature, the lens contain no blood vessels, lymphatics, nerves or connective tissues. Owning to its avascular status, the lens is fully dependent upon the aqueous humour and partly dependent upon the vitreous humour for its nourishment and also to excrete waste products. Thus, the nutrition of the lens, the most important organ for cataract research, is different, most selective and highly specialized in several respects. Hence, it should be disturbed as little as possible during all operative procedure and should be studied as much as possible during cataract development and ageing.

1.1.1 Formation of AQH

The aqueous humour is a term which has been anatomical and a physiological cannotation. In its real term aqueous humour is the fluid found in the anterior chamber of the eye. The viscosity of the normal aqueous humour is little greater than water but less than blood. The pH value for plasma, aqueous humour and vitreous humour are as 7.4, 7.6 and 7.57 respectively (Kinsey V.E., 1953).

The formation of aqueous humour is a complex phenomenon involving movement of ions between blood vessels of the eyes and intra ocular fluids across a number of different barriers, each having individual physical and chemical
properties. Seidal (1921) must be given the credit for establishing beyond reasonable doubt that the aqueous humour is continuously formed and drained away.

1.1.2 Movement of molecules across blood - aqueous barrier
Two types of movements of the fluid as a whole, occur in the eye. The first is the thermal circulation which is a localized movement and relatively unimportant. The second, the movement of aqueous humour from the ciliary processes where it separates from its parent fluid, the blood to the epidermal veins where it rejoins the blood stream.

Since none of the membranes, which covers the anterior and posterior chamber, is completely impermeable to naturally occurring substances, it is clear that these substances could enter the aqueous humour from any of the surrounding tissues the ciliary processes, the lens, the iris, the cornea and the vitreous. Similarly any substance could leave the aqueous humour and pass into any of these tissues.

The AQH serves as a sole nutrient medium and also determine the intra ocular pressure. The intra-ocular pressure depends primarily on the rate of secretion of AQH, so the factors influencing these are of vital importance in understanding the physiology of the intra ocular pressure. Because of this reason, the chemistry of the fluid and the dynamics of exchange between it and blood should be considered primarily. The intra-ocular pressure also depends on drainage of AQH.
Corneal epithelium
Corneal stroma
Schwalbe's line
Schlemm's canal
Scleral spur
Zonular fibers
Ciliary muscle
Conjunctiva
Ciliary epithelium
Sclera
Corneal endothelium
Anterior lens
Iris pigment epithelium
Capsule
Anterior cortex
Lens nucleus
Posterior cortex
Descemet's membrane
Argon laser meshwork
Anterior lens
Iris pigment epithelium
Capsule
Anterior cortex
Lens nucleus
Posterior cortex
Descemet's membrane
Argon laser meshwork
The figure 1 shows site of AQH formation and drainage angle in greater detail.

1.1.3 Chemistry of AQH

The AQH is called secretion. It implies essentially that it should be formed from the plasma (blood), something more than a mechanical filtration process that "skims" off the proteins. The cells of the ciliary epithelium must perform work - what is called osmotic work - in order to produce a fluid like aqueous humour, which is not only different from plasma but also from a filtrate of plasma or dialysate. In making the selection of secretion certain ions and molecules may be transferred across the cells to give higher concentrations in the secretion than in the original plasma filtrate, e.g. bicarbonate and ascorbate, whilst others transfer at lower concentrations, e.g. Calcium, urea etc.

The most obvious chemical difference between AQH and blood plasma is in the protein contents of the two fluids. In the plasma it is of the order of 6-7 g/100 ml, whereas in the AQH it is only few mg/100 ml in man and 50mg/100 ml in the rabbit. Electrophoretic studies have shown that all proteins are derived from the plasma rather than synthesised during the secretory process (Jeanine et al., 1984). Lens is not in touch with blood directly hence it derives its oxygen requirement from the AQH which itself has very little amount of it.
The concentration of urea in aqueous humour parallels that in blood (Moore, 1942) and uraemic patient tend to develop cataracts (Hollwich, 1975, Orth, 1978) probably due to the acute osmotic change. And also lens opacities have been seen before dialysis, possibly caused by uraemia itself (Laqua, 1972). Increased urea level in the lens could have several effects: osmotic effects in both directions as aqueous urea concentration rise and fall, a direct effect in perturbing proteins and perhaps more importantly the formation of cyanate, which can carbomylate proteins (Carreras, 1976). Cyanate formation and carbomylation of lens proteins were found in urea solution used for electrophoresis (Hagel, 1971).

The other nitrogenous constituents such as uric acids, creatinine, amino acids etc. of the aqueous humour was estimated by Duke-Elder (1927) to be slightly less than that of plasma. Various electrolytes like sodium, potassium, calcium etc. are found to be present in AQH. Of the many constituent sodium ion has given the most important information regarding the mode of formation of this fluid. Sodium probably accounts for more than 95% of the total cation content of the AQH, making this ion of particular importance from the osmotic point of view.

The ascorbic acid content of the intra ocular fluid has excited great interest because its concentration is uniquely high in many species. The high ascorbate concentration in the AQH was shown by Berardinis et al., in 1965. The hypothesis
that the ciliary epithelial cells actively transport ascorbate into the eye is supported by several consistent pieces of evidence. A similar increase in the concentration of ascorbic acid in the aqueous can be induced by increasing the rate of blood flow through the ciliary body.

1.2 THE LENS

1.2.1 Location and Nature of lens

Lens is located between anterior aqueous and posterior vitreous humour. It is held by ciliary bodies and iris muscles. The lens is a semi-solid, elastic, elliptical, refractive biconvex, avascular highly organised cellular organ with smooth and shiny surface (Tripathi, 1983).

Structurally, it consists of a thickened resilient collagenous basal lamina called the capsule, a layer of epithelial cells existing on the anterior side, and a collection of radially, oriented, elongated lens cells or fibres. The figure -2 shows cross section of human eye.

The refractive power and transparency of the normal lens depends on a smooth refractive index gradient for visible light. This occurs due to regular arrangement of fibre cells and the presence of stable intracellular lens specific proteins, the various types of crystallins.

1.2.2 Lens Content

Content wise, lens has a higher protein concentration than
any other tissue and the concentration in the nucleus is greater than that in the cortex. The lens protein contribute 35% of the wet weight of the lens, and crystallins account for 80-90% of the soluble proteins of the lens.

The crystallins are generally referred to as structural proteins of the lens indicating that they are possibly responsible for the structural properties, consistency and transparency of the lens (De Jong, 1981). Three major structural lens proteins are α, β & γ crystallins.

A normal lens has exceptionally high concentration of reduced glutathione (GSH) which has been implicated in the maintenance of transparency and protection of the thiol groups of the lens. It has been suggested that this tripeptide may participate in the oxidation-reduction reactions which may be crucial in maintaining the transparency of the lens. An important function of GSH may be either to directly act as a protective agent by scavenging the free radicals that cause damage or to protect other reactive thiols of crystallins, enzymes or membrane sulfhydryls.

Ascorbic acid is an important constituent of lens as well as of aqueous humour. Ascorbic acid is a free radical scavenger and may act in this way in the lens. AQH gets ascorbic acid from the plasma which is then supplied to lens as required. The concentration of ascorbic acid seems to be higher than
that of plasma. It is suggested by the observation that, at physiological concentration, ascorbic acid inhibits (about 25%) the darkening of the lens from exposure to near UV light (Zigman et al., 1973). Ascorbic acids can effectively scavenge $\cdot O_2^-$ radical (Verma et al., 1977), since dehydro ascorbic acid can be reduced by glutathione. It seems possible that ascorbic acid could form part of a protective system such as that outlined below.

$$
\begin{align*}
2O_2^- + 2H^+ & \rightarrow \text{ascorbic acid} \\
H_2O & \rightarrow \text{dehydro ascorbic acid} \\
\text{G-S-S-G} & \rightarrow \text{NAD(P)H}_2 \\
\text{GSH} & \rightarrow \text{NAD(P)}
\end{align*}
$$

Evidence for the existence of such a system must await further experimental studies.

Earlier studies have indicated that the lens possesses the enzymatic mechanism to produce urea (Dardenne et al., 1962). Although the production of urea in the lens may not be of great significance, the production of ornithine by the action of arginase on arginine may have both metabolic and functional significance. The ornithine produced in the tissue may either be converted to putrescine in the biosynthetic pathway to polyamines, namely spermine and spermidine or it may be converted to glutamic acid by ornithine amino transferase. Another fate of ornithine may be to diffuse into aqueous and vitreous humours.
The polyamines have a regulatory role in nucleic acid and protein biosynthesis and this has a control over the production of lens proteins. Glutamic acid so produced may serve as the source for glutathione which is present in high amounts in lens and may also be used of the production of lens proteins which are known to contain high amounts of glutamic acid (Kuch, 1970).

1.2.3 Glutathione Metabolism in lens

Quantitatively, at least, the most significant protective system in the lens is that involving the reversible oxidation of glutathione. Like other tissue, the lens contains high concentrations of glutathione. The highest GSH level appear to be in the epithelium, in the rabbit lens for example GSH concentration in epithelium is 6 times higher than the whole lens concentration (Giblin et al., 1976).

Normal human lenses contain 3-4 mM reduced glutathione in the cortex and 1-2 mM in the nucleus. There is apparently a slow turnover of GSH. Reddy et al., (1973) reported a rate of 1.4%/hr. GSH turnover. Essentially the synthetic activity requires approximately 11% of ATP generated form glycolysis (Reddy et al., 1973).

Normal lenses maintain a steady state of concentration of GSH, however this begins to drops in lenses undergoing cataract formation. This has been found to be true in almost
all experimental cataracts, and also in human senile cataracts.

The disappearance of GSH may be due to its diffusion through 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. This can be illustrated by examination of the multiple role of GSH in the lens.

Although some oxidative changes are seen in the protein of the X-irradiated lens, it seems unlikely that these are the causes of the opacification. The gradual loss of GSH suggests that the primary damage may be at the membrane level or in the glutathione regenerating system. Thus, glutathione has been exhausted, either by loss from the lens or by oxidation as rapid oxidation of both soluble and membrane bound proteins takes place. The resultant inactivation of the sodium pump would be followed by the accumulation of sodium and water in the lens, damage to the capsule, loss of protein through this damaged capsule and insolubilization of some proteins due to alterations in their environment. Inactivation of sodium pump results into chloride influx and potassium efflux.

One of the functions of this high level of reduced Glutathione is probably to maintain protein sulfhydryls in the reduced form (Kinoshita et al., 1964). It appears that sulfhydryl groups in both cytoplasmic and membrane bound proteins need to be in the reduced form for the proper
functioning of these proteins. Reduced glutathione probably acts in two ways in the maintenance of protein sulphydryl groups - by preventing their oxidation which has taken place.

Being small and mobile molecules, glutathione (GSH) reacts with potential oxidants before they could interact with the lens proteins. Thus, it would react with oxygen and also act as a scavenger for any free radicals generated by ionizing radiation, UV and visible light, or univalent reduction of oxygen. In the process it would be oxidized to the disulphide.

\[
\text{G-SH} + \text{R.} \quad \rightarrow \quad \text{G-S} + \text{RH}
\]

\[
\text{G-S} + \text{GS.} \quad \rightarrow \quad \text{G-S-S-G}
\]

Any disulphide formed in the proteins can be reduced back to the sulfhydryls by glutathione. This takes place through thiol exchange reactions with the intermediate formation of mixed disulphide of protein and glutathione (Augusteyn, 1979).

1.2.4 Glutathione protective system

Oxidised Glutathione produced by any of these mechanisms is reduced under the action of glutathione reductase; i.e.

\[
\text{G-S-S-G} + \text{NADH (P)} \quad \rightarrow \quad 2 \text{GSH + NAD (P)}
\]

\[
\text{GLUTATHIONE REDUCTASE}
\]
The equilibrium in this reaction lies far towards the right thus ensuring that glutathione is in the reduced form at all times. Lens glutathione reductase is also capable of clearing mixed disulphide of glutathione and lens proteins. This provides the lens with a possible additional route for the regeneration of protein sulfhydryls. However, cataractous lenses contain substantial amount of mixed disulphide despite the presence of NAD (P) H and active glutathione reductase. Therefore it seems unlikely that glutathione reductase clears the mixed disulphide under physiological conditions. The reactions involving glutathione are summarized in figure-3.

![Figure-3](image)

**Figure -3** The glutathione protective system.

All the constituents of the protecting glutathione system including glutathione redox cycle and its enzymes, glutathione reductase, glutathione-s-transferase, g-glutamyl transpeptidase, GSH, GSSG, NADPH, etc. have been reported in the lens. Thus, GSH metabolism can be expected to be a significant factor in the defense of lens against
cataractogenesis. Altered activity of the enzymes associated with the synthesis, catabolism and utilization of glutathione in the lens have been reported with the progression of cataract (Rathbun et al., 1983).

Thus, a study of GSH metabolism appear to be the most relevant parameter in understanding the mechanism of cataractogenesis. It is quite obvious that if any of the oxidative processes described above were to take place unchecked, then a tissue would rapidly cease to function. The lens would be particularly susceptible if any disruption of its structure, and especially protein insolubilization would result in the scatter of light instead of its transmission.

The extensive tissue damage does not normally take place due to the presence of a number of protective mechanisms. These act by detoxifying reactive intermediates or by reversing any damage done by them. The lens contain several different mechanisms which seem to be primarily concerned with protecting it from the effects of oxidation. Some affect a general protection against a number of oxidants whilst others are aimed at specific oxidants. The activities of the various protective systems are generally higher in the cortex than in the nucleus with the epithelial layer being particularly active. These distributions probably reflect the general metabolic status of these tissues.
1.3 THE CATARACT

Cataract is a major cause of blindness. The word cataract is derived from the Latin word "Cataracta" meaning, cloudiness of the water fall' refers to the presence of an opacity in the normally clear and transparent ocular lens.

1.3.1 Blindness due to Cataract

A WHO report (1966) on blindness in different countries show widely varying percentage of blindness due to cataract as shown below.

<table>
<thead>
<tr>
<th>Countries</th>
<th>% of blindness due to cataract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceylon</td>
<td>28</td>
</tr>
<tr>
<td>China</td>
<td>07</td>
</tr>
<tr>
<td>England</td>
<td>22</td>
</tr>
<tr>
<td>India</td>
<td>39</td>
</tr>
<tr>
<td>Israel</td>
<td>28</td>
</tr>
<tr>
<td>Kenya</td>
<td>46</td>
</tr>
<tr>
<td>U.S.A.</td>
<td>22</td>
</tr>
</tbody>
</table>

Senile cataract is responsible for significant visual impairment with human etiologies. The magnitude of this overburden of cataract in the developing countries is indicated by a survey in Punjab in India (Chatterjee et al., 1982).

1.3.2 Senile cataract

The most common form of cataract is the "Senile cataract" occurring in the aged population, which has great socio-medical prevalence for many third world countries, where annually 2-3 million people become blind due to cataract.
which cannot be treated by surgical removal because of the lack of facilities, surgeons and shortage of funds. However, these cataracts are the result of age-related alterations in lens metabolism and a combination of risk factors.

Prior to 1976, so little research was being done on the mechanisms of human cataract formation that a useful system of classifying the features of a senile cataract was unavailable. Most of the 4,00,000 cataracts extracted each year were discarded after a gross pathological examination, this was possible only because there was so little demand for human lenses for study.

A single primary cause of cataract most likely does not exist. Epidemiological literature indicates that the prevalence of cataract is related to geographical location, climate and sun hours (Hiller et al., 1977, Zigman et al., 1979).

Number of other risk factors also contribute to the incidence of cataract. In the third world countries like India these includes: more than one attack of severe diarrhoea, malnutrition, diabetes, low education, residence in slums etc. Whereas in western countries with better living conditions the risk factors includes diabetes, renal failure, rampant use of tranquilizer, corticosteroids, alcohol, cigarettes etc. (Harding, 1991).
1.3.3 Risk factors

Several risk factors listed in an elegant summary of the epidemiology of cataract are as follows.

1. Ionizing radiation
2. Radio frequency and micro radiation
3. Toxic drugs and Chemicals
4. UV-A (Ultra-violet-light long wavelength UV)
5. Diabetes, Blood pressure
6. Family history

The aim to study human was the ultimate goal of any kind of research, to get maximum benefits for human welfare.

This work embodies the following aspects:

I. Normal and Cataractous human lens study.
   A) Biochemical analysis of the above mentioned lenses for:
      (i) Determination of - Total protein,
          Soluble protein,
          Insoluble protein
      (ii) Determination of Sulfhydryl Groups
          TSH : Total Sulfhydryl Groups
          PSH : Protein bound sulfhydryl groups
          GSH : Glutathione (Reduced)
      (iii) Fractionation of lens proteins
      (iv) Determination of ascorbic acid

17
(B) Enzymatic Assays

(i) Glutathione Reductase (GR) activity
(ii) Glutathione S-Transferase (GST) activity
(iii) \( \gamma \)-Glutamyl Transpeptidase (GTP) activity

II. AQH of Normal and Cataractous human eye were used for following biochemical analysis.

(i) Determination of sulfhydryl groups
   TSH : Total Sulfhydryl Groups
   PSH : Protein bound sulfhydryl groups
   GSH : Glutathione (Reduced)

(ii) Total Proteins

(iii) Ascorbic acid

1.4 AIM OF THE STUDY

The aim of this study was to investigate various biochemical changes occurring in different types of cataract (both) in human lenses and aqueous humour. Also to investigate the enzyme patterns in normal and cataractous human lenses.

It was determined to establish the relationship between various parameters with ageing and with cataract. The aim to study both lenses and AQH of human eye was to establish the relationship between them.