CHAPTER I

HISTOCHEMICAL AND BIOCHEMICAL ANALYSIS

OF ACID MUCOPOLYSACCHARIDES IN THE EYES
INTRODUCTION

The term “mucopolysaccharides” was coined by Meyer (1938) for those substances isolated from connective tissues and having similar physico-chemical properties. With increased understanding of their biochemistry in recent times, terms like “glycoproteins”, “mucoproteins”, “glucosaminoglycans” were also used, but they failed to distinguish between bacterial polysaccharides and amino sugars containing antibiotics.

Glycosaminoglycans (GAGs) are long, unbranched polysaccharide chains composed of repeating disaccharide units. The repeating unit consists of a hexose (six-carbon sugar) or a hexuronic acid, linked to a hexosamine (six-carbon sugar containing nitrogen). When most glycosaminoglycan chains are synthesized, they are covalently linked at their reducing end to core proteins, thus forming proteoglycans (PGS)- the exception is the GAG hyaluronan, which is uniquely synthesized without a protein core and is spun out by enzymes at the end surface directly into the extracellular space (Spicer et. al. 2002; Toole, 2001, 2004). They may have specific biological functions conferred upon them by specific sequences within the carbohydrate chain (Carney, 1994).

The term “mucopolysaccharides” was introduced to describe 2-amino-2-deoxyhexose containing polysaccharide of animal origin and occurring either as free polysaccharide or as their protein derivatives (Kennedy and White, 1983). Mucopolysaccharides are of two main classes i.e. (i) those that are neutral and (ii) those that contain uronic acid. Acid mucopolysaccharide (AMPs) comes under the second class. Carney (1994) had indicated that glycosaminoglycans may have specific biological functions conferred upon
them because of specific sequences within the carbohydrate chain. “Glycosaminoglycan” is the systematic name for the carbohydrate residues which form linear chains of alternating acidic and basic monosaccharides. The basic units are usually N-acetylated and sometimes N-sulfated, while the acidic units are sometimes O-sulfated (Kennedy and White, 1983).

Acid mucopolysaccharides (AMPs) may be further sulfated or non-sulfated e.g. chondroitin sulfate and hyaluronic acid respectively. These terms i.e. AMPs and SMPs (Sulfated mucopolysaccharides) appear to provide an adequate description and also have the added advantage of continuous uses (Jaques, 1977). The different types of AMPS are as follows

1. Non-sulfated mucopolysaccharides e.g. Hyaluronic acid or Hyaluronan

2. Sulfated mucopolysaccharides

i. Chondroitins

a. Chondroitin sulfate A (ChS A) or Chondroitin 4-sulfate.

b. Chondroitin sulfate B (ChS B) or Dermatan sulfate.

c. Chondroitin sulfate C (ChS C) or chondroitin 6-sulfate.

ii. Heparitins

Heparitin sulfate A

Heparitin sulfate B

Heparitin sulfate C

iii. Keratins

Keratan sulfate/Kerato sulfate

iv. Heparins
It is to be noted here that although heparins belong to the group of mucopolysaccharides, they are usually absent in connective tissues. However, in some biological situations the function performed by heparins in some cells or species seem to be performed by chondroitins in other cells or species as in basophilic leucocytes and mast cells (Jaques, 1977).

Members of acid mucopolysaccharide types vary in the type of hexosamine, hexose or hexuronic acid they contain *e.g.* glucuronic acid, iduronic acid, galactose, galactosamine, glucosamine etc. They also vary in the geometry of the glycosidic linkage, molecular weight, binding energy of the components and in functional properties.

It is to be noted that glycosaminoglycan always comes within the mucopolysaccharides category irrespective of the ways in which the term has been used, and it is now known that glycosaminoglycans are attached covalently to proteins. Therefore, AMPs actually refer to glycosaminoglycans of a proteoglycan plus, sometimes a few amino acid unit.

Glycoproteins or proteoglycans also have a protein backbone, but the carbohydrate (which are glycosaminoglycans) are in the forms of liner chains with regularly alternating basic monosaccharides (2-amino-2-deoxy-D-galactose, 2-amino-2-glucose) and acidic monosaccharides (D-glucoronic acid, L-iduronic acid). The basic units are usually N-acetylated and sometimes N-sulfated, while the acidic units are sometimes O-sulfated. These compounds are the only source of hexuronic acids in animals, and occur in nearly all parts of mammalian bodies and to a lesser extend in fish and bacteria. According to Kennedy and White (1983), they are amongst the essential building blocks of the macromolecular frame work of connective and other tissues.

Among the various tissues of vertebrate, where mucopolysaccharides have been detected, the organic matrix of bone are reported to be the richest source (Herring, 1972). In addition to these, brain of some mammals like the rat are found to contain rich amount of some mucopolysaccharides. (DiBenedetta et al. 1969; Margolis and Margolis, 1970).

Compared to extensive amount of work on the AMPs of various vertebrate tissues, works on invertebrate AMPs is scanty. Hunt and co-workers (1970) have detected a glucan sulphate peptide component in mucin of marine snail, Buccinum undatum; Matthews (1975) has detected a chondroitin sulphate like substance from cranial cartilage of Locusta opalescens; Raghuvarman et al. (1998) from the body cuticle of the Peripatus Typhloperipatus wildoni; Vadgama and Kamat (1971), on various glands like salivary glands; Baldwin and Salthouse (1959) on dermal glands,
Mustafa and Kamat, (1970) in the connective tissue of brain, ganglia, and imaginal disc of the housefly, *Musca domestica*; Ashhurst and Costin, (1971a,b) have reported sulfated mucopolysaccharides in the connective tissue surrounding the ejaculatory duct and in the ganglia of the locust, *Locusta migratoria*. Ashhurst and Richards, (1964) have also detected mucopolysaccharides in the connective tissue, surrounding the nerve cord of pupa of the wax moth, *Galleria mellonella*.

Their presence in the visual system of vertebrates are also well documented. For example, they have been reported in the bovine cornea (Coster *et al.* 1987; Funderburgh *et al.* 1996; Corpuz *et al.* 1996; Plaas *et al.* 2001; Achur *et al.* 2004; and Conrad *et al.* 2010), in the eye of rabbit (Yue *et al.* 1984; Lutjen Drecoll, 1990; Fitzsimmons *et al.* 1992; Takahashi *et al.* 1993; Goes *et al.* 1999; Kato *et al.* 1999), in chick cornea (Conrad *et al.* 1977; Li *et al.* 1992; Mc Adams and McLoon 1995), human and rabbit cornea (Freund *et al.* 1995; Tai *et al.* 1997), in calf lens capsule (Mohan and Spiro 1991), and in the corneal stroma of squid (Anseth, 1961 and Moozar and Moozar, 1973).

Other visual apparatus where AMPs have been reported are in the cornea of elasmobranchs (Balazs, 1965), vitreous body of the eye of squids (Balazs *et al.* 1965), in aqueous and ciliary body (Cole, 1970; Schachtschabel *et al.* 1977), interstitial matrix surrounding the photoreceptor cell of the cattle (Berman and Bach, 1968; Berman, 1969), inter photoreceptor matrix of vertebrate (Rolich, 1970), sclera of ox (Robert and Robert, 1967) etc.

In the case of insects, AMPs have also been reported in the compounds eyes of *Periplaneta americana, Belostoma sp* (Dey, 1976),
Paelemon sp, Limunus polyphorus and Macrobrachium birmanicum (Dey et al. 1978) Musca domestica, Apis cerena indica (Dey, 1980)

The present study thus is an attempt to analyze the compound eyes of the two insects concerned with regards to the occurrence of AMPs and their possible roles in vision.

MATERIALS AND METHODS

Eyes were separated from the live insects and fixed in 10% buffered formalin until they were used.

Histochemical study: The tissues were embedded in paraffin and 8 µ thick sections were cut by microtome. The section were stained with Toluidine blue and Alcian blue (Humason, 1971) for detection of mucopolysaccharides.

Biochemical study according to Dietrich et al. (1977).

Extraction: Fresh eyes (1gm) were defatted in cold acetone for 3 hours and dried. Tissues were then homogenized and suspended in 20 ml of 0.05M Tris-Hcl buffer (pH 8). To the mixture, 10 mg of trypsin was added and then a few drops of toluene were added forming a layer at the surface, and incubated at 37°C for 24 hours. After incubation, pH of the mixture was brought to 11 with Conc. NaOH and maintained for 6 hours at room temperature. Then the pH was brought to 6 by the addition of Hcl and then mixture was centrifuged for 15 minutes at 3000rpm. To the supernatant, 0.1 ml of 2M NaCl and 2 volume of ethanol were added and kept overnight at 5°C. The mixture was centrifuged for 15 minutes at 3000 rpm and the precipitate collected and dried. The
resultant powder was re-suspended in 1 ml of 0.05M sodium acetate (pH 6.5) along with 1 mg of DNAase and RNAase. The solution was again incubated for 24 hours at 37°C with a layer of toluene. After incubation, 0.1 ml of 2M NaCl and 2 volumes of ethanol were added to the solution and kept overnight at 5°C. It was then centrifuged for 15 minutes at 3000 rpm and precipitate was collected and dried. The resultant powder was dissolved in 0.5 ml of water, heated at 100°C for 2 minutes and analyzed by paper chromatography and electrophoresis.

*Chromatography:* The extracted acid mucopolysaccharides were hydrolyzed with 6N HCl at 100°C for 12 hours. The acid hydrolysate was then evaporated to dryness. The dried residue was then dissolved in 0.5 ml of distilled water and spotted in whatman No 1 filter paper and ascending paper chromatograms run using butanol, acetic acid and water in the ratio of 4:1:1 (v/v) as solvent (Giri and Nigam, 1954).

The chromatogram was developed with silver-nitrate (0.1 ml of saturated solution in 20 ml of acetone) and sodium hydroxide (0.5 gm of NaOH in 25 ml of rectified spirit) as suggested by Trevelyan *et al.* (1950). The chromatogram was then washed in 6N ammonia hydroxide for 10 minutes and then washed in running water and dried at room temperature.

*Electrophoresis:* Electrophoresis of the acid mucopolysaccharides was carried out by applying streaks of the samples on Whatman No.1 paper strips using 0.1M phosphate buffer (pH 6.6) at 4v/cm for 8 hours. After removal from the electrophoretic apparatus, the paper strips were dried at room
temperature and stained with Toluidine blue (0.04% in 80% acetone). The staining of the strips was followed by 2-3 rinsing in 0.1% acetic acid and then 2-3 times in H₂O. The strips were then dried at room temperature.

**OBSERVATIONS**

*Histochemical:* Compound eyes of insects include the lens system, a retina and underlying optic ganglia. Lens is a modified cuticle and is composed of the cornea and underlying crystalline cone. Immediately behind the crystalline cone are the longitudinal sensory elements or the retinula cells. The inner sides of the retinula cells collectively secrete an internal light trapping rod like structure known as rhabdom.

**Lens cuticle of the butterfly, *Pieris brassicae:*** The histological preparations of the lens cuticle reacted positively when stained with Toluidine blue and Alcian blue. When the sections of the eyes are stained with Toluidine blue, cornea and the crystalline cone became purple in color showing metachromasia (Photoplate 1). This reaction indicates the presence of acid mucopolysaccharides. The region of the rhabdom was orthochromatic *i.e.* blue in colour. Rhabdom region is devoid of acid mucopolysaccharides. When the eyes were stained with Alcian blue, the lens and crystalline cone gave purple colour (Photoplate 2) which indicates the presence of acid mucopolysaccharides.

**Lens cuticle of moth, *Philosamia ricini:*** When the sections are stained with Toluidine blue, cornea as well as the crystalline cone became purple in colour
(Photoplate 3) showing the presence of mucopolysaccharides. The more intense reactions were observed towards the corneal lens. The rhabdom region however gave a blue colour reaction in the presence of toluidine blue *i.e.* the region is orthochromatic (Photoplate 4). When the eyes were stained with alcain blue the corneal lens and crystalline cone became purple in colour and the rhabdom became blue in colour.

**Biochemical:**

Chromatographic analysis of the acid mucopolysaccharide extract showed the presence of three sugars *viz.* lactose, galactose and xylose in case of *Pieris brassicae* and presence of three sugars *viz.* galactose, xylose and rhamnose in case of *Philosamia ricini* (Figure 1&2; Table 1).

Electrophorectic movement pattern of the crude extracts of the acid mucopolysaccharides from the eyes of *Pieris brassicae* and *Philosamia ricini*, when compared with several standard acid mucopolysaccharides showed that the mucopolysaccharides extracted resembles more of chondroitin 4-sulfate (Figure 3 & 4; Table 2).
PHOTOPLATES 1 & 2

**Photo plate 1:** T. S. of the eye of *Pieris brassicae* showing metachromasia with toluidine blue stain (400X).

- Co (Crystalline cone): Purple colour: mucopolysaccharides present;
- Rh (Rhabdom): Blue colour: mucopolysaccharides absent.

**Photo plate 2:** T. S. of the eye of *Pieris brassicae* showing metachromasia with alcian blue stain (400X).

Other details as in photo plate 1.
PHOTOPLATES 3 & 4

**Photo plate 3:** T. S. of the eye of *Philosamia ricini* showing metachromasia with toluidine blue stain (400X).

- **Co** (Crystalline cone): Purple colour: mucopolysaccharides present;

**Photo plate 4:** T. S. of the eye of *Philosamia ricini* showing metachromasia with alcian blue stain (400X).

Other details as in photo plate 3.
SUGAR COMPONENTS (BUTTERFLY)

- XYLOSE
- GALACTOSE
- LACTOSE
SUGAR COMPONENTS (MOTH)

RHAMNOSE

XYLOSE

GALACTOSE
CHONDROITIN-4-SULFATE

ACID MYCOPOLY SACCHARIDES (BUTTERFLY)
**Table 1:** Ascending paper chromatogram of sugar components of the butterfly, *Pieris brassicae* and the moth, *Philosamia ricini*.

Solvent: Butanol: Acetic acid: water (4: 1: 1 v/v)
<table>
<thead>
<tr>
<th>Insect</th>
<th>Rf value</th>
<th>Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Butterfly, <em>Pieris brassicae</em></strong></td>
<td>0.05</td>
<td>Lactose</td>
</tr>
<tr>
<td></td>
<td>0.18</td>
<td>Galactose</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>Xylose</td>
</tr>
<tr>
<td><strong>Moth, <em>Philosamia ricini</em></strong></td>
<td>0.16</td>
<td>Galactose</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>Xylose</td>
</tr>
<tr>
<td></td>
<td>0.43</td>
<td>Rhamnose</td>
</tr>
</tbody>
</table>

Ascending Paper chromatogram of some standard sugar components
Solvent: Butanol: Acetic acid : water (4: 1: 1 v/v)

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raffinose</td>
<td>0.03</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.05</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.10</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.13</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.18</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.25</td>
</tr>
<tr>
<td>Fructose</td>
<td>0.28</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.34</td>
</tr>
<tr>
<td>Ribose</td>
<td>0.38</td>
</tr>
</tbody>
</table>

**Table 2:** Paper electrophoretic movement patterns of the crude mucopolysaccharides from the eyes of butterfly, *Pieris brassicae* and moth, *Philosamia ricini*.
Solvent: Phosphate buffer (pH 6.5)
Paper electrophoretic movement patterns of some standard mucopolysaccharides. Solvent: Phosphate buffer (pH 6.5)

<table>
<thead>
<tr>
<th>Insect</th>
<th>Distance travelled (cms)</th>
<th>Acid mucopolysaccharide type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butterfly, <em>Pieris brassicae</em></td>
<td>6.4</td>
<td>Chondroitin 4-sulfate</td>
</tr>
<tr>
<td>Moth, <em>Philosamia ricini</em></td>
<td>6.8</td>
<td>Chondroitin 4-sulfate</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Standard mucopolysaccharides</th>
<th>Distance travelled (cms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin</td>
<td>5.5</td>
</tr>
<tr>
<td>Chondroitin 4-sulfate</td>
<td>6.6</td>
</tr>
<tr>
<td>Heparan sulfate</td>
<td>7.2</td>
</tr>
<tr>
<td>Chondroitin 6-sulfate</td>
<td>7.6</td>
</tr>
<tr>
<td>Keratan sulfate</td>
<td>8.7</td>
</tr>
<tr>
<td>Dermatan sulfate</td>
<td>10</td>
</tr>
</tbody>
</table>
DISCUSSION

Acid mucopolysaccharides play several important physiological roles owing to their capacity to bind and hold water. They serve as natural lubricants in the joints, impart elasticity to connective tissue, and as a component of cartilage and ligaments, are involved in support and motor functions, and also have bactericidal properties. Dysfunction in the mucopolysaccharide metabolism leads to a change in the composition of connective tissue and of the body fluids, resulting in diseases such as collagenosis, mucopolysaccharidosis, and rheumatism. Their dysfunction is also correlated with aging.

Vertebrates produce multiple chondroitin sulphate proteoglycans that play important roles in development and tissues mechanics. In the nematode, Caenorhabditis elegans, the chondroitin chains lack sulphate but nevertheless play essential roles in embryonic development and vulval morphogenesis (Olson et al. 2006). Corneal stromal glycosaminoglycans bind together and thus may influence keratocytes and nerve growth in cornea (Cornard et al. 2010). Glycosaminoglycans also play a central role in the physiological maintenance of trabecular meshwork as seen in the eyes of rabbits and humans (Yue et al. 1984 and Cavallotti et al. 2004).

Glycosaminoglycans and their core proteins have important physiological and homeostatic roles e.g. during inflammation and the immune response (Park et al. 2001; Li et al. 2002 and Wang et al. 2005). Bulow and Hobert (2006) also suggested that glycosaminoglycans may provide a specific code that contributes to the correct development of a multicellular
organism. They influence many biological functions, including cell division, differentiation, signal transduction, adhesion, migration, peripheral nerve extension or regeneration, and responses to growth factors (Miao et al. 1996; Groves et al. 2005; Manton et al. 2007; Fthenou et al. 2006, 2008). Chondroitin and dermatan proteoglycans have attracted much attention as inhibitors of axon growth and have been shown to be important components of the glial scar that prevents axon regeneration (Rhodes & Fawcett 2004).

The role of mucopolysaccharides in pathogenesis has been widely reviewed. They are responsible for dermal thickening in acromegalic patients (Matsuoka et al. 1982), involved in inborn errors of metabolism and/or storage disorders (Matalon et al. 1974a; Hall et al. 1978; Neufeld and Fratantoni 1970; Mc Kusick et al. 1978), maintenance of retinal structure and neural tube closure Knobloch syndrome (Sertie et al. 2000), treatment of diabetic nephropathy (Gombaro and Van DerWoude, 2000), calcification of bones (Rubin and Howard, 1950).

Matthews (1959) and Oosawa (1971) have suggested that one of the characteristic property of mucopolysaccharides is the selective association or binding with small inorganic cations, especially H⁺, Na⁺, and Ca²⁺, and also with cationic groups of macromolecules. In these regard, Farber and Schubert (1957) have shown that the percentage of chondroitin-sulphate binding of Ca²⁺ is greater than that of Na⁺. Urist et al. (1968) have also found a small preference for binding Ca²⁺ over Na⁺ in chondroitin sulphate. Matthews (1975) thus suggested that these substance act as a store for Ca²⁺ in cartilage tissue and that is why have specific roles have in tissue-calcification.
Mucopolysaccharides have been reported to play important roles in “water binding” and maintenance of tissue osmotic pressure (Ogston and Wells, 1972; Wells 1973b). According to Ogston (1970) the role of AMPs on tissue osmotic pressure is not only by influencing the water balance but also by introducing excess swelling pressure which is balanced by an internal structural resistance. Ogston and Wells (1972) and Wells (1973b) have also suggested the role of AMPs in maintaining mechanical flexibility and elasticity of tissues. AMPs may also have some functions in controlling metabolism of cells, and movement of metabolites on the basis of their rather specific chemical structure (Jeanloz, 1970). Kobayashi and Pedrini (1973) have suggested that AMPs have a major role in structural organization of intracellular matrix. According to them, AMPs may be involved in electrostatic and steric interactions with other macromolecules of the matrix, such as collagen and elastin.

Some roles of AMPs, especially in arthropodan cuticle have been reported by Meenakshi and Scheer (1959) and Sundara Rajulu (1969) in terms of calcification of the cuticle of Hemigrapsus mudus and Cingalabolus bungnioni respectively. Krishnan (1965) has suggested that AMPs may be associated with –S-S- bonding of the cuticle in the scorpion, Palaemoneus swammerdami. Sannasi (1969) reported that extreme flexibility of the intersegmental cuticles of the queen of Odontotermes obesus to be due to the occurrence of acid mucopolysaccharides. He based his conclusion on the fact that acid mucopolysaccharides are said to possess high water binding capacities (Ogston, 1966a and Katchalsky, 1964).
Thus it can be observed that there certainly exist some roles of AMPs in the visual process of insects, since their occurrence and their visual significance have been reported in the ocular tissues of various vertebrates and some invertebrates by various workers. Since the occurrence of acid mucopolysaccharides is not a general feature of the arthropod cuticle and it occurs in some special types of cuticle where it performs some special functions (Meenakshi and Scheer, 1959; Sundara Rajulu, 1969; Krishnan, 1965; Sannasi, 1969 and Raghuvaram et al. 1998), it is reasonable to presume that the specific occurrence of mucopolysaccharides in the lens cuticle and the crystalline cone may have a bearing on the visual system of the insects, examined in present study. Keeping the above account in view it is possible to assume a role of AMPs in the lens-cuticle of insects.

The lens-cuticle as already stated, besides playing a general defensive role, performs a special optical function of conducting light rays to the inner rhabdomere. It is possible to presume that the transparency of the lens-cuticle, which is more than that of other types of cuticle (e.g. body cuticle), may be affected by the occurrence of mucopolysaccharides. The basis of this presumption is that the transparency of the cornea of the vertebrates has been reported to be affected by mucopolysaccharides (Anseth and Fransson, 1970). It is known that the bulk of cornea of vertebrate eye is the stroma, which functions as a supporting structure and is adapted for the transmission of a high percentage of incident light of visible-wave length (Maurice, 1969). Anseth and Fransson (1970) have found that during chick corneal development, the occurrence of a highly sulfated keratan sulfate is associated with the rise in transparency of stroma. They have also suggested that stromal
transparency is correlated with the presence of normal proportions of keratan sulfate and chondroitin 4-sulfate. Funderburgh et al. (1996) have reported that keratan proteoglycans are the major proteoglycans of the bovine cornea and secreted by keratocytes in the corneal stroma and they are thought to play an important role in corneal structure and physiology, particularly in the maintenance of corneal transparency. Takahashi et al. (1993) have also reported that keratan sulfate and dermatan sulfate proteoglycans are associated with collagen in fetal rabbit cornea. Freund et al. (1995) also reported that the presence of AMPs in human and rabbit cornea is related to transparency. Blochberger, et al. (1992), has reported that corneal keratan sulfate proteoglycans contribute to corneal transparency in chick.

Transparency of the corneal stroma depends partially on the degree of spatial order of its collagen fibrils which are narrow in diameter and closely packed in a regular array (Maurice, 1957; Cox et al. 1970; Benedek, 1971; McCally and Farrell 1990 and Bron, 2001). Mc Adams and McLoon (1995) have shown that retinal axons grow in the presence of chondroitin sulphate and keratan sulfate proteoglycans and that these proteoglycans helps in developing chick visual pathway.

Payrau et al. (1967) observed that the transparency of the cornea is based on the stake of hydration of tissue. They based this on the fact that the corneal stroma of most vertebrates, including mammals, birds and teleosts absorb water wherever free water is accessible. In contrast, according to Maurice and Riley (1970) odema of the cornea leads to disorganization of its structure and less transparency, but dehydration does not appear to have serious optical affects. Maurice (1972) suggested that the presence of AMPs in
the cornea is mainly responsible for the dehydration properties of the tissue and hence transparency. This has been supported by workers like Hedbys (1961, 1963); Kikkawa and Hirayama (1970); Bettelheim and Plessy (1975); Lee and Wilson (1981) and Castoro et. al. (1988).

Many studies that focused on corneal swelling behavior noticed a gradual decrease in swelling from posterior to anterior side (Van Horn et. al. 1975; Bettelheim and Plessy 1975; Castoro et. al. 1988 and Cristol et. al. 1992). This process was thought to be related to the organization of the collagen lamellae and to the presence of different types of proteoglycans. In the posterior part, keratan sulfate, a more hydrophilic proteoglycan is prevalent, whereas in the anterior part dermatan sulfate, a much less hydrophylic proteoglycan, is present (Bettelheim and Plessy 1975; Castoro et.al. 1988). Muller et. al. (2001) studied the differential behavior of the anterior and posterior stroma during corneal swelling and drew an interesting conclusion that it is the high negative charge of the glycosaminoglycan components of the proteoglycans that is responsible for the corneal swelling. They also suggested that the structural stability of the anterior stroma under condition of extreme hydration imply an important role for this zone in the maintenance of corneal curvature and that this stability is determined by the tight interweave of the stromal lamellae.

The non- swelling properties of elasmobranchs cornea is supposed to be due to the high mannose content in their structural proteins (Moozar et. al. 1969b; Moozar and Moozar, 1972). On the other hand, the mechanism of corneal swelling is due to electrostatic repulsion between acidic groups of the macromolecules i.e mucopolysaccharide.
It is now known that the pH value is a decisive factor for the taking of water by the cornea (Cejkova and Brettschneider, 1969). The protein polysaccharides complex provides a more stable and specific configuration within the molecules than electro-static linkage could (Maurice, 1970). For the cornea to remain transparent, it is essential that an active mechanism counter the natural tendency of the stroma to increase its hydration, swelling and opacity. It is well-established that one of the corneal limiting cell layers i.e., the corneal endothelium, transports fluid at a substantial rate and that this transport is essential to maintain normal stromal hydration (Maurice, 1972; Candia, 1976; Candia and Zamudio, 1995; Narula et. al. 1992; Bonanno et. al. 1989 and Yang et. al. 2000). Anseth and Fransson (1969) had demonstrated the synthesis of AMPs by corneal epithelial and stromal cells, and that they are important in maintaining the corneal structure in relation to its environment. Deb and Raghuvarman (1994) have also observed that glucosaminoglycans are essential for the maintenance of corneal structure and function.

Acid mucopolysaccharides thus detected in the compound eyes of the butterfly, *pieris brassicae* and the moth, *Philosamia ricini* may play an important role in visual excitation, when light rays pass through the outer epicuticle to the inner endocuticular region (crystalline cone) - the sites of AMPs due to the fact that they act as a selective ion barrier (Jeanloz, 1970). It may also be noted that they are present not only in the corneal lens but also in the crystalline cone, which are in close connection with the inner rhabdomeres (the actual sites of photochemical reactions), the products of which may depolarize the membrane of the retinula cells and initiate impulse