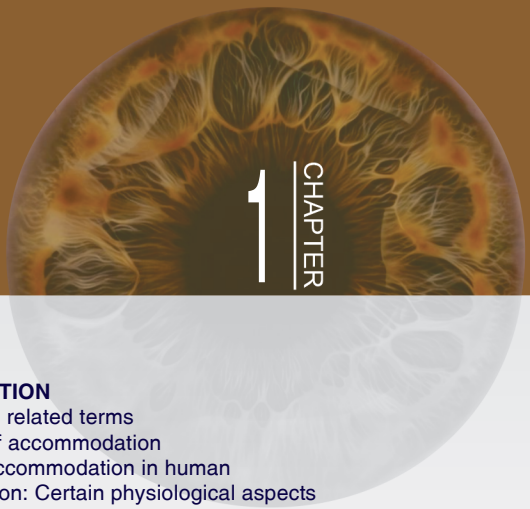
ANATOMY AND PHYSIOLOGY OF LENS



ANATOMY OF THE LENS

The lens is a transparent, biconvex, crystalline structure placed between iris and the vitreous in a saucer-shaped depression, the *patellar fossa.* The posterior surface of the lens capsule is in intimate contact with the vitreous in this fossa and is attached to it in a circular area with ligamentum hyaloidocapsulare (Weigert's ligament). Inside this circle, between hyaloid face and the lens capsule is a small cavity or potential space called retrolental or *Berger's space*. The *equatorial diameter* of the lens, about 6.5 mm at birth, increases to 9–10 mm in the second decade and then remains almost constant. Its *thickness* (axial or anteroposterior diameter) varies with age between 3.5 mm (at birth) and 5 mm (at extreme of age). Its *weight* varies from 135 mg (0–9 years) to 255 mg (40–50 years of age). It has two surfaces. The *anterior surface,* less convex than the posterior, is the segment of a sphere whose radius averages 10 mm (8–14 mm). The *posterior surface,* more curved than the anterior, presents a radius of about 6 mm (4.5–7.5 mm). These two surfaces meet at the *equator,* which is almost circular and has a rippled or undulated appearance. The centres of the anterior and posterior surfaces are called the anterior pole and posterior pole, respectively. The anterior pole is about 3 mm from the back of cornea.

The *refractive index* of the lens is 1.39 (nucleus 1.42, cortex 1.38). Its refractive power is about 16–17 dioptres. Its accommodative power varies with age, being 14–16 D at birth; 7–8 D at 25 years of age and 1–2 D at 50 years of age.

The *colour* of the lens also changes with age. A transparent lens in infants and young adults is colourless, acquires a definite yellow tinge after about 30 years of age and appears amber-coloured in old age. The *consistency* of the lens cortex differs from the nucleus; the former being softer than the latter.

STRUCTURE OF THE LENS

1. LENS CAPSULE

The lens capsule is a thin, transparent, hyaline collagenous membrane which surrounds the

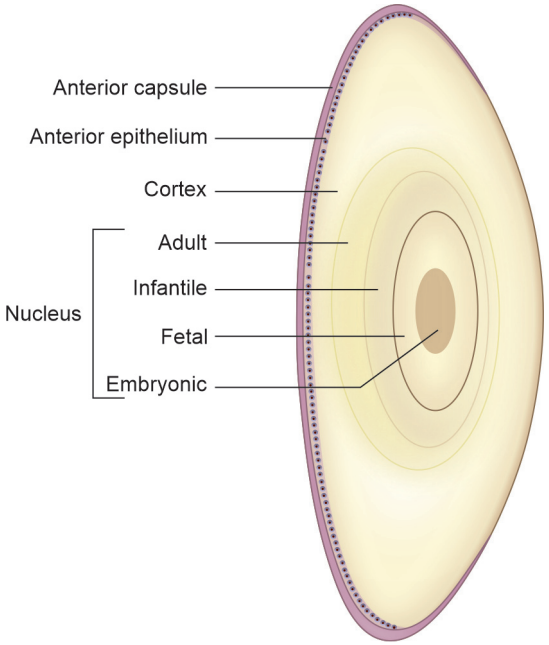


Fig. 1.1. *Structure of crystalline lens.*

lens completely (Fig. 1.1). The lens capsule is highly elastic but does not contain any elastic tissue. It is secreted by the basal cell area of the lens epithelium anteriorly and by the basal area of the elongating fibres posteriorly. Produced continuously throughout life, the lens capsule is the thickest base in membrane in the body. Capsule thickness varies according to the age and is not consistent through its extent.

It is thicker anteriorly than posteriorly and at the equator than at the poles, being thinnest at posterior pole (Fig. 1.2). The Salzman's data for capsular thickness are shown in Table 1.1.

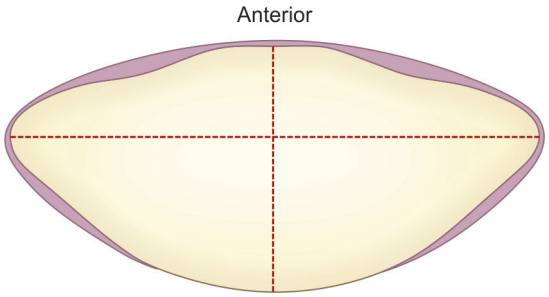


Fig. 1.2. *Variable thickness of lens capsule.*

Table 1.1 *Salzman's data for capsule thickness*

Age (yrs)	*Capsule thickness (mm)*		
	Anterior pole	*Posterior pole*	*Equator*
25	8	2	7
35	14	4	17
71	14	23	9

On light microscopy, the capsule appears as a homogenous structure and stains with PAS. However, on ultramicroscopic examination, it shows a lamellar appearance.

Each lamella contains fine filaments. In true exfoliation of the lens capsule, superficial zonular lamella of the capsule splits off from the deeper layer. The lens capsule is composed principally of type IV collagen and 10% glycosaminoglycans. It contains enzyme, ATP and glycolytic intermediates but cannot be considered to have an independent metabolism. There are chemical and antigenic similarities between the lens capsule and basement membrane of kidney and glomeruli, blood vessels, spleen and lungs.

2. ANTERIOR LENS EPITHELIUM

It is a single layer of cuboidal nucleated epithelial cells which lies deep to the anterior capsule (Fig. 1.1). These cells contain all the organelles found in a typical epithelial cell. Almost all the metabolic, synthetic and transport processes of the lens occur in this layer. In the equatorial region, these cells become columnar, are actively dividing and elongating to form new lens fibres throughout life. There is no posterior epithelium, as these cells are used up in filling the central cavity of the lens vesicle during development of the lens.

Zones of lens epithelium. The anterior lens epithelium can be divided into three zones:

a. *Central zone.* It consists of cuboidal cells which are polygonal in flat section. Their nuclei are round and located slightly apically. These cells are stable and their number, like those of corneal endothelium, slowly reduces with age. Under normal circumstances, these cells do not mitose, but can do so in response to a wide variety of injurious insults including uveitis. During injury repair, epithelial cells are elongated; resembling

fibroblasts and can pile up to 10 layers thick under the capsule. Metaplasia of these central zone lens epithelial cells into spindle-shaped myofibroblast-like cells can lead to anterior subcapsular cataract like the shield cataract in atopic dermatitis and glaukomflecken seen after an attack of acute congestive close angle glaucoma.

b. *Intermediate zone.* It consists of comparatively smaller and more cylindrical cells located peripheral to the central zone. Their nuclei are round and central. These cells mitose occasionally.

c. *Germinative zone.* It consists of columnar cells which are most peripheral and located just pre-equatorial. Nuclei of these cells are flattened and lie in the plane of cell axis. Cells of the germinative zone are actively dividing to form new cells which migrate posteriorly to become lens fibres. This process continues throughout life. These cells are extremely susceptible to irradiation. Dysplasia of these transitional zone cells can lead on to posterior subcapsular cataracts (PSCs) as seen in radiation cataract, myotonic dystrophy and neurofibromatosis-II.

Features of lens epithelium

- The anterior lens epithelium has the highest metabolic rate (content of ATP and enzymes is highest in this area of lens).
- The lens epithelial cells are remarkable in that they have a prominent, well-characterized cytoskeletal network consisting of actin, vimentin, spectrin, microtubules, alpha actinin and myosin. The cytoskeletal network is in the form of a polygonal array of geodomes located subjacent and attached to their apical membrane.
- The lateral membrane of the lens epithelial cells is markedly inflamed and has small number of gap junctions—hydrophilic passage between neighbouring cells. Uncommon feature of the lens epithelial cells' lateral membrane is that it lacks tight/occluding junctions.
- The apical membrane of the lens epithelial cells is planar and interfaces with the apical membrane of elongating fibre cells as they migrate to their sutural location. The unique apico-apical interface is known as epithelial fibre cell interface (EFI). The EFI is characterized by transcytotic events and plays a key role in the lens physiology.
- Na-K ATPases and acid phosphatases are localized on the apicolateral membrane.

3. LENS FIBRES

Formation

The epithelial cells elongate to form the lens fibres. At first, the lens fibres are formed from the posterior epithelium which runs from posterior to anterior to fill the lens vesicle. But later on, the lens fibres are derived from the cells of the equatorial region of the anterior epithelium. These cells divide, elongate and differentiate to produce long, thin, regularly arranged lens fibres that constitute the bulk of the lens. Successively, the new lens fibres are laid on the older deeper fibres. The superficial (new) fibres are nucleated with elongation of the cell; the nuclei assume a relatively more anterior position. As the new fibres are laid down, the anterior shifted nucleus forms a line convex forward at the equator, known as *lens* or *nuclear bow* (Fig. 1.3).

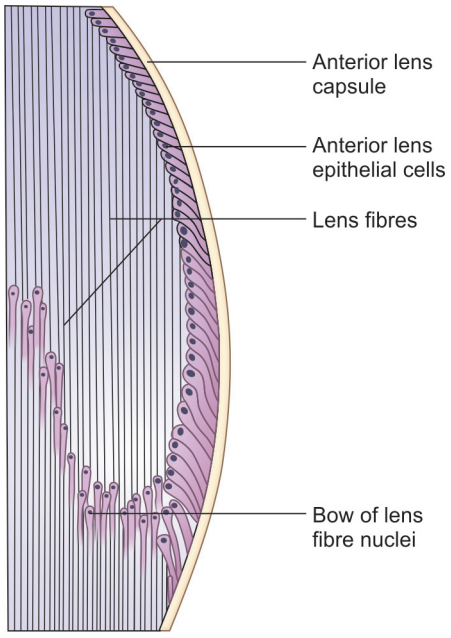


Fig. 1.3. *Nuclear bow.*

Structure of the lens fibres

On cross-section, the lens fibres are almost hexagonal in shape and are bound together by the ground substance (Fig. 1.4A). The cytoplasm of the cells of the superficial bow region and the newly formed lens fibres contain a nucleus, mitochondria, Golgi apparatus, rough endoplasmic reticulum, and polysomes. The ribosomal content of the newly formed lens fibres is more than the epithelial cells indicating an elevated protein synthesis. The nuclei of the lens fibres are present temporarily and disappear later on. Thus the cytoplasm of the older lens fibres is devoid of nuclei, is homogenous and granular with very few organelles. There are interlocking processes between cells (ball-and-socket and tongue-and-groove interdigitations) with zonulae occludentes present (Fig. 1.4B). It is interesting to note that the interdigitations are less complicated in the superficial zone of the lens; and this may permit moulding of the lens shape during accommodation.

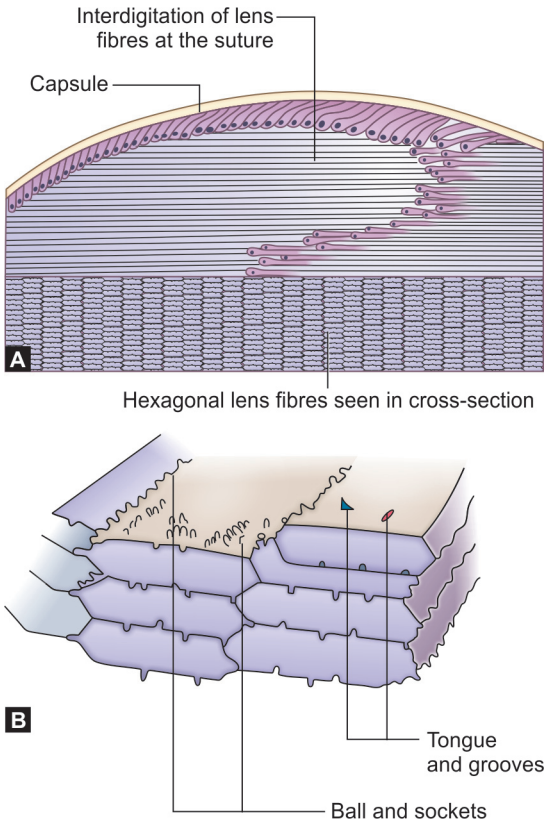


Fig. 1.4. *Structure of lens fibres:* ***(A)*** *Hexagonal arrangement of lens fibres in cross-section;* ***(B)*** *Interlocking processes in between the lens fibres.*

Structural arrangement of the lens fibres

The initial fibres forming the fetal nucleus just surrounding the embryonic nucleus are arranged in such a way that they terminate with two Y-shaped sutures on the anterior (upright Y) and the posterior (inverted Y) surfaces of the lens (Fig. 1.5). Later in gestation and following birth, the growth of the lens sutures is much more irregular. Instead of simple Y-sutures, more complicated dendritic patterns (Fig. 1.6) are observed due to asymmetrical fibre growth.

Zonal arrangement of the lens fibres

The lens fibres are formed throughout life and are arranged in zones that delineate the various periods of development of the lens (Fig. 1.1). This stratification is due to optical differences between the older, more sclerotic regions of the central lens and the newer, more transparent peripheral areas. In an adult, the lens fibres are arranged compactly as nucleus and cortex of the lens.

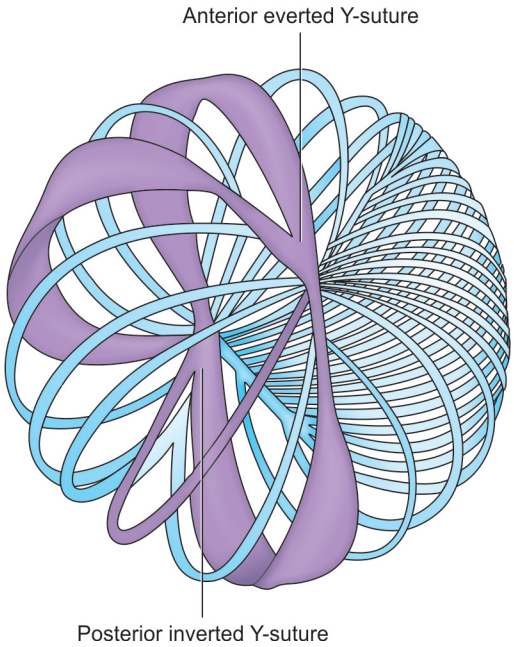


Fig. 1.5. *Y-shaped arrangement of lens fibres around embryonic nucleus.*

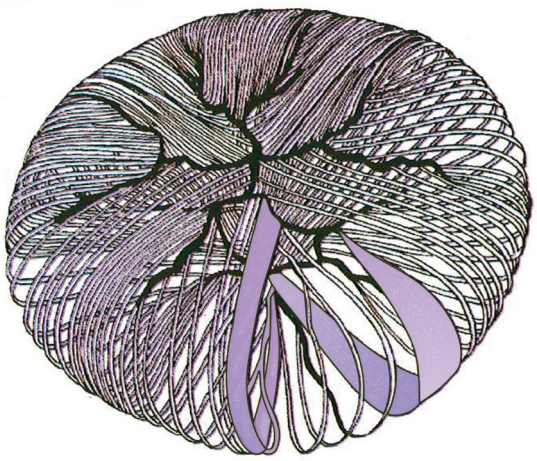


Fig. 1.6. *Dendritic pattern of lens fibres.*

a. ***Nucleus.*** It is the central part containing the oldest fibres. It consists of different zones, *embryonic nucleus* is its innermost part (formed at 1–3 months of gestation). Outside the embryonic nucleus, successive nuclear zones are laid down as the development proceeds and depending upon the period of formation, are called *fetal nucleus* (corresponding to lens from 3 months of gestation till birth), the *infantile nucleus* (corresponding to lens from birth to puberty) and the *adult nucleus* (corresponding to lens in adult life). The size of the embryonic and fetal nuclei remains constant while that of adult nucleus is always increasing.

b. ***Cortex.*** It is the peripheral part of the lens which lies just outside the adult nucleus. It comprises the youngest (most recently formed) lens fibres.

APPLIED ANATOMY OF THE LENS

BIOMICROSCOPIC STRATIFICATION OF THE LENS

Biomicroscopic examination of the lens with the pupil dilated reveals stratification of the lens into concentric layers from front to backwards which are as follows (Fig. 1.7A):

1. ***Capsule*** **(Ca).** The outermost layer, formed by capsule (Ca), is seen as a fine stratum.

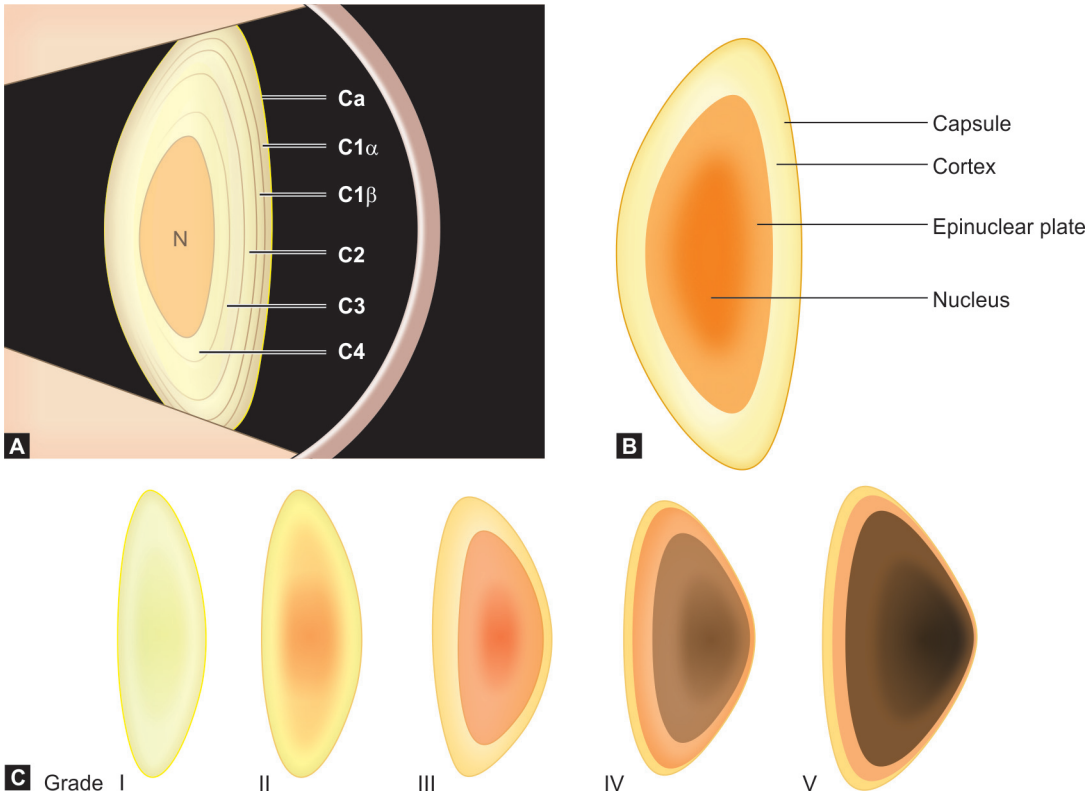


Fig. 1.7. *Applied anatomy of the crystalline lens:* ***(A)*** *Biomicroscopic stratification;* ***(B)*** *Surgical anatomy depicting capsule, cortex, epinuclear plate and nucleus;* ***(C)*** *Grading of the nucleus hardness in the cataractous lens.*

2. *Superficial cortex.* It is further stratified into three layers in the beam of slit-lamp:
- C1a. First cortical clear zone or the subcapsular clear zone.
- Cl b. First zone of disjunction, seen as a bright narrow, scattering zone of discontinuity.
- C2. Second cortical clear zone or the subclear zone of cortex.

3. *Deep cortex* is stratified into the following two perinuclear zones which autofluoresce a brilliant green under blue exciting light:
- C3. It is the bright light scattering zone of deep cortex.
- C4. It is the relatively clear zone of deep cortex.

4. *Nucleus* (N) represents the prenatal part of the lens. It shows following further stratifications:
- Central part of nucleus, which lacks scattering of light, represents the embryonic nucleus.
- Anterior and posterior peripheral light scattering zones of nucleus.

SURGICAL ANATOMY OF THE LENS

From the surgical viewpoint, the lens can be divided into four parts (Fig. 1.7B):
- A *central hard nucleus* surrounded by
- An *epinuclear plate* (EN) of varying thickness surrounded by
- A *layer of cortex*, and the outermost
- *Capsule*

GRADING OF NUCLEUS HARDNESS

Grading of nucleus hardness (sclerosis) in cataractous lens is important for setting the parameters of the machine for effective phacoemulsification. The sclerosis (hardness) of nucleus, depending upon its colour, can be graded as below (Fig. 1.7C):
- Grade I: Whitish/green yellow
- Grade II: Yellow
- Grade III: Amber
- Grade IV: Brown
- Grade V: Black.

The hardness of the lens nucleus can also be classified into:
- Ultra-soft (grade I)
- Soft (grade I+)
- Soft-medium (grade II)
- Medium-hard (grade III)
- Hard (grade IV)
- Ultra-hard (grade V).

CILIARY ZONULES

The ciliary zonules (zonules of Zinn or suspensory ligaments of lens) consist essentially of a series of fibres which run from the ciliary body and fuse into the outer layer of the lens capsule around the equatorial zone. Thus, they hold the lens in position and enable the ciliary muscle to act on it.

STRUCTURE

The zonular fibres are transparent, stiff and not elastic. Each zonular fibre has a diameter of about 0.35–1.0 μ. It is composed of microfibrils with a diameter varying from 8–40 nm. Zonular fibres are composed of glycoproteins and mucopolysaccharides and are similar in structure to the microfibrils of the elastic fibres. Their susceptibility to hydrolysis by α-chymotrypsin has been *used to advantage in intracapsular cataract surgery.* Structurally, three different types of zonular fibres have been described.
- *First type fibres.* These are thick, about 1 μ in diameter, wavy and usually lie near the vitreous.
- *Second type fibres.* These are thin and flat.
- *Third type fibres.* These are very fine and run a circular course.

Gross Appearance

Grossly, the ciliary zonules form a complete ring of fibres, which extend from ciliary body to the lens equator circumferentially (Fig. 1.8). On cut section, the ciliary zonules appear to be arranged in a triangular form. The base of the triangle is towards the equator of the lens and apex towards the ciliary body. The space between the triangle is filled with the zonular fibres except for a circumferential space around the equator of the lens between anterior and posterior zonular fibres—the canal of Hannover.

ARRANGEMENT OF ZONULAR FIBRES

A. Classical concept

I. *Main fibres of the ciliary zonules*

The main fibres of the ciliary armies which bind the lens with the ciliary body, depending upon their arrangement can be classified into the following four groups (Fig. 1.9A):

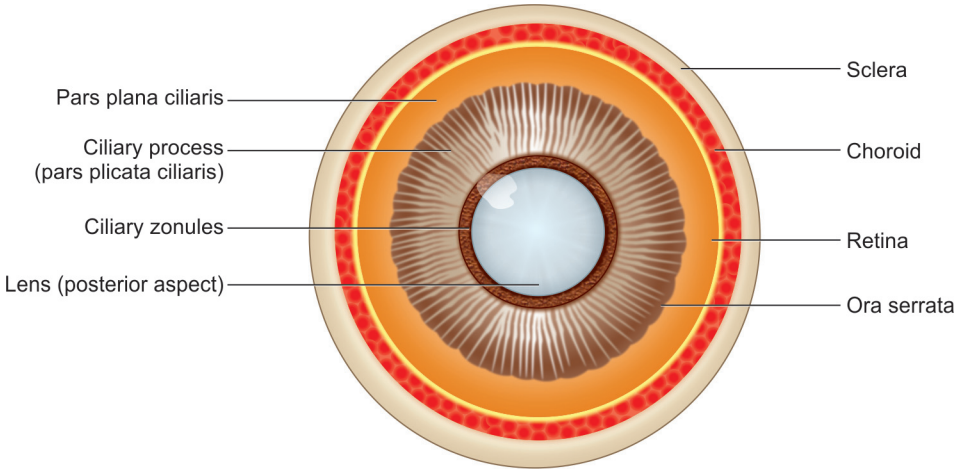


Fig. 1.8. *Gross appearance of ciliary zonules as seen from interior of the eyeball in relation to posterior surface of the lens.*

1. ***Orbiculoposterior capsular fibres.*** These are the most posterior and innermost zonular fibres. These take origin from the ora serrata, pass anteriorly in close contact with the anterior limiting layer of the vitreous and are inserted together with *hyaloidocapsular ligament* in the posterior capsule of the lens. Structurally, they are secona type fibres.

2. ***Orbiculoanterior capsular fibres.*** These are the thickest and strongest (structurally of first type) zonular fibres. They arise from the pars plana of ciliary body (orbicularis ciliaris), pass anteriorly to get inserted anterior to the equator. By the supporting (auxiliary) fibres, they are attached to the valleys and sides of the ciliary processes.

3. ***Cilioposterior capsular fibres.*** These are the most numerous zonular fibres. They arise mainly from the valleys and a few from the sides of the ciliary processes, pass posteriorly and get inserted on the posterior capsule anterior to the insertion of the orbiculoposterior capsular fibres.

4. ***Cilioequatorial fibres.*** These fibres arise from the valleys of the ciliary processes and pass almost directly inward to be inserted at the equator. They occupy the whole of the interval between the anterior and posterior group of fibres. These are third type of fibres. These are present in abundance in youthful eyes and tend to disappear and become sparse with advancing age.

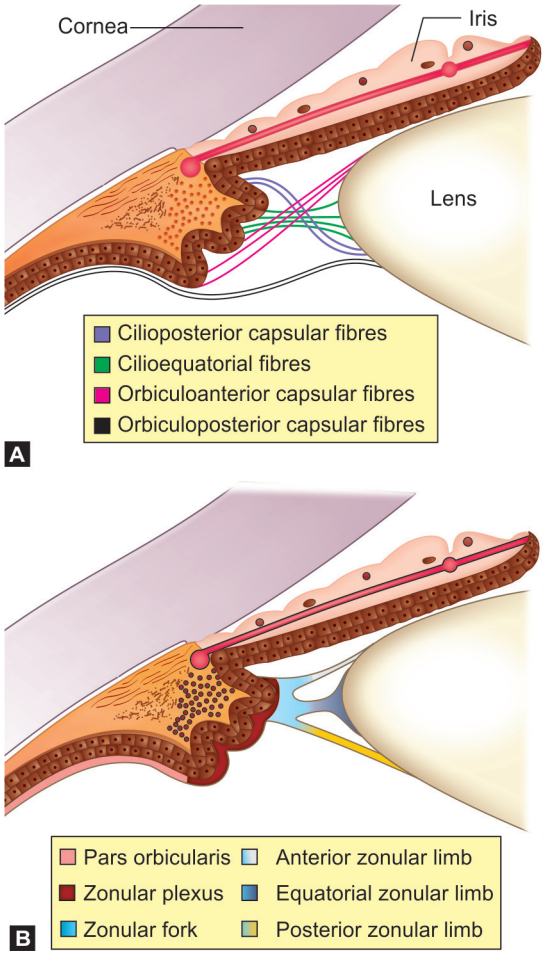


Fig. 1.9. *Arrangement of main zonular fibres:* ***(A)*** *Old concept;* ***(B)*** *New concept.*

II. *Auxiliary fibres*

The auxiliary or supporting fibres provide strength to main fibres by anchoring the individual portions of zonules. These also help to hold the various portions of the ciliary body together.

B. Recent concepts about the zonular fibres

I. *Main zonular fibres*

Recent scanning electron microscopy studies do not approve the above classical description of the arrangement of the zonular fibres. It is reported that vast majority of the zonules arise from the posterior end of the pars plana up to 1.5 mm from the ora serrata. They run a more or less complex but continuous course from ora serrata to the edge of lens. However, for description purposes, the suspensory zonular complex can be divided into four zones (Fig. 1.9B):

1. *Pars orbicularis.* After arising from the posterior end of the pars plana, the zonular fibres pass forward over the pars plana as a feltwork. This portion of the zonules forms the pars orbicularis.

2. *Zonular plexuses.* After reaching the posterior margin of the pars plicata, the zonular fibres segment into zonular plexuses, which pass through the valleys between the ciliary processes. Thus the zonular plexuses are that part of the zonules which lie between the ciliary processes in the region of the pars plicata. Each zonular plexus consists of fibres which cross and join each other in a regular pattern. The zonular plexuses are firmly attached to the bases of the ciliary valleys by fine and coarse fibrils known as *tension fibres.*

3. *Zonular fork.* After reaching towards the anterior margin of pars plicata, the zonular plexuses consolidate into zonular bundles which bend almost at right angle to proceed towards the lens. This point of angulation of the zonules at the mid-zone of the ciliary valleys has been referred to as the zonular fork.

4. *Zonular limbs.* At the level of the zonular fork, the zonular fibres divide into three zonular limbs—the anterior, equatorial and posterior—running to the anterior, equatorial and posterior lens capsule, respectively.

i. *Anterior zonular limb.* It is analogous to the *orbiculoanterior capsular fibres* of old classical description. The anterior zonular fibres are relatively dense and all insert at approximately the same distance from the equator (about 1.5 mm), as an irregular double row of bundles. It has been reported that anterior zonules decrease in number with age and that the site of the anterior insertion becomes displaced more centrally.

ii. *Equatorial zonular limb.* It replaces the term *cilioequatorial fibres* of old classical description. The equatorial fibres are sparse and poorly developed but fan out in a brush-like manner to get inserted into the capsule of the equatorial region.

iii. *Posterior zonular limb.* It can be considered a substitute for the orbiculoposterior capsular fibres and cilioposterior capsular fibres of old classical description. Posterior fibres fan out more and show less interconnections than the anterior zonules. They are inserted into the posterior capsule in two or three layers in a zone starting from the posterior edge of the equator to about 1.25 mm.

II. *Hyaloid zonule*

The hyaloid zonule comprises a single layer of zonules connecting the anterior hyaloid at the border of the patellar fossa with the pars plana and pars plicata. This layer is indistinguished in the untouched eyes because its fibres are closely apposed to those of the posterior zonules. The space between the hyaloid zonule and the posterior zonule is the *canal of Petit.*

III. *Hyalocapsular zonule*

It is a circular band of zonular fibres which is at the site where the anterior hyaloid membrane is attached to the posterior lens capsule at the rim patellar fossa. It probably corresponds to the ligament of Wiegert.

IV. *Circumferential zonular girdles*

Anterior ciliary girdle. It is a circular band of fibres which binds the ciliary processes with the anterior hyaloid membrane of the vitreous.

This band resists the pull of coronary vitreous tract, which is inserted in this area.

Posterior ciliary girdle. It is a circular zonular girdle present on the middle of pars plana at the internal surface of the main zonules. It binds the pars plana, 1–2 mm anterior to the ora serrata, with the anterior hyaloid membrane at a site into which the median vitreous tract inserts.

PHYSIOLOGY AND BIOCHEMISTRY

BIOCHEMICAL COMPOSITION OF LENS

Main constituents of the lens are water and proteins. Water constitutes about 65% of the lens wet weight. Of the solids, the highest is protein which constitutes about 34% of the total weight of an adult lens. The other constituents present in the lens are lipids, inorganic ions, carbohydrates particularly glucose and its derivatives, ascorbic acid, glutathione and amino acids.

LENS WATER

Lens is a relatively dehydrated organ, cortex being more hydrated than nucleus. Lens dehydration is maintained by an active sodium pump that resides within the membrane of the cell, in the lens epithelium and in each lens fibre.

The water content of the lens is about 65%. Fischer suggested that out of this about 80% is free while remaining is bound water. A small portion of the lens water is located in the extracellular space. Low amount of water in the lens is a natural consequence of the need for having a refractive index quite different from that of the watery fluids at the two optical interfaces of the lens. The normal human lens does not show significant alteration in hydration with age.

LENS PROTEINS

Protein content of the lens is higher than that of any other organ in the body. The physical state of proteins seems to be an important factor for the maintenance of transparency of the crystalline lens and has been studied in detail. Morner for the first time classically divided the proteins of crystalline lens of cattle into an insoluble fraction at physiological pH, called *albuminoids* and the soluble fraction called *crystallins.* The soluble fraction has three components, namely alpha-, beta- and gamma-crystallins. The three crystallins can be separated by precipitation at different pH, by salting out, by electrophoresis or by running through cellulose column. Clark *et al.* confirmed that the protein components of the human lens correspond to those of bovine lens.

Krause studied the various protein fractions in the lens as follows:

1. Insoluble albuminoids—12.5%
2. Alpha-crystallins—31.7%
3. Beta-crystallins—53.4%
4. Gamma-crystallins or albumin—1.5%
5. Mucoproteins—0.8%
6. Nucleoproteins—0.07%.

Besides these major proteins, a few minor proteins reported in the lens are glycoprotein, phosphoprotein, lipoprotein and fluorescent proteins.

In general, cortex contains more soluble proteins than nucleus which contains more insoluble proteins. The cortex of the young lens practically contains no albuminoid, whereas the nucleus of old lens is composed of almost entirely of this protein fraction. Since the concentration of albuminoid and alpha-crystallin is nearly inversely proportional, a close chemical relationship probably exists between these two. As the lens ages, the soluble alpha-crystallin is gradually converted into insoluble albuminoid. The close relationship of alpha-crystallin and albuminoid has further been shown by the fact that they are immunologically similar.

SOLUBLE PROTEINS

The lens crystallins make up the bulk of refractive fibres of the lens and are, therefore, considered structural proteins. The synthesis of soluble proteins takes place, to a large extent, in the equatorial part and on the surface of lens. The newly formed fibres contain very little or

no albuminoid. A part of the soluble lens proteins may be formed in deeper lens fibres, at least in those which contain nuclei.

Alpha-crystallins. Alpha-crystallin fraction has the highest molecular weight and at alkaline pH, it has the greatest positive charge. In a calf lens, the alpha-crystallin macromolecule has an average molecular weight of about 10 and is composed of several polypeptides held together by non-covalent forces. The molecular weight of the A chains is 19,500 and of the B chains 22,500. The A chains contain one thiol group per chain while B chains appear to contain no thiol group. It is believed that alpha-crystallins are polymers and consist approximately of 50 monomers.

Beta-crystallins. This fraction of lens proteins is a heterogenous group of proteins, not well defined, with molecular weights from 5×10^4 to 2×10^5. The beta-crystallins contain many polypeptide chains, some of which appear to be present in aggregates. Shapiro has reported that this protein group contains three different polypeptide chains of the molecular weights of 21,000, 23,000 and 29,000. They also have a relatively high thiol content and might have disulphide linkages.

Gamma-crystallins. These are composed of monomers only. This fraction constitutes about 60% of soluble lens proteins in young rat and dog fish. As the lens ages, there is a progressive and significant decrease in the relative concentration, until it reaches a level of 10% in the lens of two years old rat and in adult dog fish. Gamma-crystallin level is high in nucleus and low in cortex, especially in young cortex. Four proteins belonging to gamma-crystallin group have been purified, crystallized and characterized. A close similarity between all the four fractions has been demonstrated by free electrophoretic analysis. These four fractions have similar molecular weights but differ from each other in their chromatographic properties, amino acid composition, and number of their sulphhydryl groups. However, they are immunologically identical, with exception of fraction II which shows only partial identity with the other fractions.

INSOLUBLE PROTEINS

The chief insoluble protein of the lens is *albuminoid* which makes up to 12.5% of total proteins. Its molecular weight is 3,70,000. The albuminoid is obviously a mixture since it is only partly digested by urea and can be extracted by soidic aqueous solution. The amino acid composition of this protein is similar to alpha-crystallin. Waley described albuminoid as the urea and alkali soluble fractions of total insoluble material remaining in a decapsulated lens extract with water at pH 7. The relative amount of urea soluble and insoluble albuminoid varies with species. In bovine and human lens, most of the albuminoid is urea-soluble and appears to be derived from the alpha-crystallins, although there are small but significant amounts of beta- and gamma-crystallins present. The process involved in the formation of albuminoid fraction from the previous soluble crystallin may involve S–S and C–S bond formation. There is a quantitative difference in the distribution among the cortical and nuclear albuminoids, the latter contains more of beta and gamma antigens.

OTHER LENS PROTEINS

Glycoproteins are a group of proteins to which sugars are covalently bound. They are primarily associated with the lens cell membrane and are, therefore, of considerable importance. They also contribute to the intercellular ground substance. The lens cortex contains considerably more glycoproteins than the nucleus.

The other rare lens proteins include nucleoproteins, phosphoproteins, lipoproteins and fluorescent proteins.

IMMUNOCHEMISTRY OF LENS PROTEINS

Lens proteins are organ-specific and not species-specific. Since it has been seen that a rabbit sensitized to bovine lens proteins will develop antibodies will react with antigens in lens extract from almost all other species. It has also been seen that reaction does not occur with non-lens proteins. Thus, from this, it can be inferred that lens proteins from all are very similar. Further, since the lens proteins are organ-specific, an individual can become sensitized to one's own lens proteins.

AMINO ACIDS

Two groups of amino acids are present in the lens: proteogenic and non-proteogenic. *Proteogenic* group includes alanine, leucine, glutamic acids, aspartic acid, glycine, valine, phenylalanine, tyrosine, serine, isoleucine, lysine, histidine, methionine, proline, threonine and arginine. *Non-proteogenic* amino acids are taurine, alpha-amino butyric acid, ornithine, 1-methyl-histidine, 3-methyl-histidine and homo-carnosine. Hence the lens contains all the amino acids present in any other tissue except tryptophan, cysteine and possibly hydroxy proline.

Concentration of each amino acid is higher in the lens as compared to aqueous humour or vitreous humour leading to a conclusion that they are actively transported into the lens. Such a process is necessary to ensure that protein synthesis is not limited by the availability of amino acids. The ratio of concentration in the lens to that in aqueous humour is highest for acidic, lowest for basic and intermediary for the neutral amino acids. It has been shown that the free amino acid pool is quite characteristic and constant for similar animals. Barbar demonstrated that the amino acid concentration of lens is not appreciably affected by ageing, fasting or feeding a protein-free diet. The unaltered level of amino acids in lens may be a result of balance of protein synthesis and catabolism on one hand and amino acid excretion, uptake by lens and the synthesis and breakdown of amino acids on the other hand.

In the cellular membrane of lens exist special sites with which amino acids may attach during their transport. The extent of attachment of an amino acid with a particular class of site depends on the structure of amino acid. The smaller amino acids prefer 'A' site and have a relatively low affinity for combination with it. The amino acids with bulky side chains prefer the 'L' site for which they have a relatively high affinity. A third site 'X' appears to be selective for small amino acids.

CARBOHYDRATES

Carbohydrate metabolism of the lens is highly active and complex. Kuck stated that free carbohydrates of the normal crystalline lens are glucose, fructose, and glycogen. Derivatives of sugar found in lens are sorbitol, inositol, ascorbic acid, gluconic acid and glucosemine.

Glucose. Tbe glucose level of normal lens has been found to vary from 20 to 120 mg%. However, several workers have reported still lower concentration of glucose in lens in various species. Paulus and colleagues found 1–2 mg% in the bovine lens, while in the rabbit, Kuck obtained the figure of 7.2 mg/100 g of fresh lens.

The lenticular glucose has its source in aqueous humour. The level of glucose in lens is 1/10th of aqueous, where glucose concentration has been found to be 100 mg%.

Fructose. It is produced from glucose in the crystalline lens. Concentration of fructose varies considerably in many species, e.g. 1.4 mg/100 mg in leopard frog to 34 mg/100 mg in adult rat. Its concentration also varies with age, value being 6 mg at 14th day to 50 mg/100 mg at 550th day in rats.

Glycogen. Although in certain birds, the amount of glycogen is quite high, only traces of glycogen have been found in mammalian lenses. Its concentration varies with age and the region of lens examined. Lenticular glycogen is localised principally in the nucleus where it appears to replace gamma-crystallin normally present there. Rebaey has suggested an interesting hypothesis that glycogen replaces gamma-crystallin, functionally to increase refractive index. An earlier report concerning lenticular glycogen by histological evidence demonstrated that it was located as a thin layer of discrete intra-cellular granules, surrounding the nuclei of epithelial cells.

Sorbitol. The presence of sorbitol has been demonstrated in normal lens of several species, 17 mg/100 g fresh lens of rabbit (Kuck, 1965).

Inositol. It has also been demonstrated in the lens of several species. Its function is unknown although it may possibly be involved in the metabolism of phospholipids.

LIPIDS

Lipids were first identified in the lens by Berzelius. The total lipids of human lens amount

to about 2.5% of wet weight. The main substances concerned are cholesterol, various phospholipids such as cephalin, isolecithin, sphingomyelin and glycerides in addition to lipoproteins. Feldman and Feldman reported that human lens had lipids in two forms, a free form and a bound form as lipoproteins. The proteolipids constitute 2% of the wet weight of lens and that 65% of lenticular lipids are bound to proteins. Vass and Tapaszto found that lipids are most abundant in epithelial cells in children and in the cortex in adults.

Lipid material has been demonstrated between Jens fibres suggesting that it may function as a lubricating cement substance. The lipid content, particularly cholesterol, increases with age especially in nucleus while the glycerides decrease. Similar changes occur in cataract where lecithin is abundant and cholesterol is frequently evident macroscopically as crystals. Feldman and Feldman have demonstrated that in cataracts the concentration of free lipids increases, however, lipoprotein decreases.

ELECTROLYTES

As in any other tissue, sodium, potassium, calcium and magnesium are present in the lens in relatively large quantities, some being present principally in the extracellular fluid of the lens while others predominate within the cell.

Potassium. It is the predominant cation in lens. Its concentration in fresh human lens has been reported to vary between 114 and 130 mEq/kg lens water. The levels are higher than in any other eye tissue. This high level is probably a result of the unusually large proportion of intracellular space in lens.

Sodium. Its concentration in lens is about 10 to 50% of the potassium depending on species, age and state of the lens. In human lens, the sodium concentration is about 14–25 mEq/kg lens water. There is some variation in these levels between species and a marked regional variation in the concentration of sodium, which is more than twice in the superficial cortex as compared to central nucleus.

Calcium. The normal young lens has one of the lowest of all tissue calcium levels. A mean value of 0.14 mg/mg dry weight is reported for human lenses.

Anions. The main anions of the lens are chloride, bicarbonate, phosphate and sulphates. Phosphate is the predominant anion in the lens, comprising nearly half the ash. The level reported for the total phosphate is 240 mg/100 g and for inorganic phosphate 25 mg/100 g in young calf lens.

ORGANIC PHOSPHATES

The organic phosphates also form a significant group of lens constituents. These include nucleotides of both adenosine and pyridine. Adenosine triphosphate (ATP) is responsible for phosphorylation of glucose. Besides adenosine triphosphate, the presence of various other nucleotides such as the mono-, di- and triphosphates of adenosine has also been reported. Quantitative the phosphates of adenosine, particularly the triphosphates, are the most important constituents, making up half of the total nucleotide content.

Pyridine nucleotides act as coenzymes to the dehydrogenases, assisting with the transfer hydrogen in oxidation reduction processes. The pyridine nucleotides include diphosphopyridine nucleotide (DPN) also known as 'coenzyme 1' or NAD, and triphosphopyridine nucleotide (TPN), known as 'coenzyme 2' or NADP. These coenzymes exist either in oxidised forms (DPN, TPN) or in reduced forms (DPNH, TPNH) as they take part in the transfer of hydrogen in various stages of carbohydrate metabolism. Most of the remainder of the organic phosphates consist of glycerophosphates and related esters. Most of these substances in the lens decline with age and with the development of cataract.

GLUTATHIONE

The content of glutathione in the lens depending on the species and the method used for its estimation has been reported to vary from 3.5 to 5.5 mm/g wet weight of the lens. The level of glutathione in the lens is also known to be altered with the age of the individual. Its concentration falls with advancing age. This decrease in the level of glutathione with

advancing age is relative and not absolute. It is because of the increase in the wet weight of the lens with age.

Glutathione being a tripeptide consists of three amino acids: Glycine, cysteine and glutamic acid. It is also known as γ-glutamyl cysteinyl glycine. The cysteine fraction of glutathione by virtue of the presence of a sulph-hydryl group (–SH) is the most reactive constituent and enables glutathione to exist in two forms, i.e. oxidised glutathione (GSSG) and reduced glutathione (GSH). Reduced glutathione contains cysteine whereas oxidised glutathione contains cystine. In most tissues and so in the lens, a rough correlation exists between the concentration of glutathione and the activity of the tissue.

The glutathione contributes the so-called redox systems in the lens microenvironment. The lens is constantly exposed to attack by oxidative agents; indeed there is a high level of hydrogen peroxide in normal aqueous and peroxidase activity is also present in the lens itself. Several enzyme systems are available to minimize or buffer the effects of oxidants, including catalase, superoxide dismutase, glutathione peroxidase, and glutathione-S-transferase. The lens contains high levels of glutathione with the highest concentration in the epithelium, and detoxification via the mercapturic pathway is an important pathway in the lens. Glutathione is produced from the interaction between glutamate and cysteine in lens cells. Catalase and low levels of superoxide dismutase have also been identified in lens epithelium concluding that these systems are also probably important.

Glutathione is also important in protecting thiol groups in proteins, especially cation-transporting membrane proteins in the lens, which additionally accounts for its unusually high concentration in this tissue. More than 95% of glutathione is in the reduced state.

ASCORBIC ACID

A wide variation for ascorbic acid levels in human lenses (5–48 mg/100 g wet weight) have been reported. Adler reported that mean value of ascorbic acid in an adult man is 30 mg/100 g of the wet weight of the lens. In the aqueous humour, ascorbic acid is actively transported to a concentration some 15 times greater than that in plasma. Though the ascorbic acid content of the lens is even greater than that of aqueous humour, it is neither synthesized nor actively transported into the lens. Its accumulation within the lens might be explained by assuming that a portion of ascorbic acid is protein bound. Though the precise role of ascorbic acid in lens metabolism is not established, the conversion between ascorbic acid and the oxidized form, dehydroascorbic acid, might be coupled with other oxidation reduction systems in the lens.

METABOLIC ACTIVITIES OF THE LENS

GLUCOSE METABOLISM

Glucose metabolism is the main source of energy. The lens requires a continuous supply of ATP (energy) for active transport of ions, amino acids, maintenance of lens dehydration, and lens transparency, and for a continuous protein and GSH synthesis. Most of the energy produced is utilized in the epithelium which is the major site of all active transport processes. Only about 10–20% of the ATP generated is used for protein synthesis. On an average, about 3–4 mg glucose/day (or 1 mmol/hr) is utilised by an incubated rabbit lens *(in vitro).*

Glucose is very essential for the normal working of the lens. This has been proved by the fact that the lens can survive under incubation conditions in the absence of oxygen, so long as an adequate supply of glucose is available (but not if provided with oxygen only). When deprived of glucose, the lens rapidly uses up endogenous energy reserves (ATP, glucose, sorbitol and fructose) and begins to gain water and lose transparency. Cataracts can develop in infantile hypoglycaemia, a group of diseases in which low plasma glucose levels are present.

Glucose from the aqueous (mainly) and vitreous diffuses into the lens and is rapidly metabolized through four main pathways:

1. Anaerobic glycolysis
2. Krebs (oxidative) cycle
3. Hexose monophosphate shunt
4. Sorbitol pathway.

Various metabolic pathways are depicted in Table 1.2 and Fig. 1.10. Salient features of these processes are described below in brief.

1. ANAEROBIC GLYCOLYSIS

Anaerobic glycolysis, although not as efficient as the aerobic process, obviates the problem of oxygen starvation in a tissue totally dependent upon the aqueous humour, which has a rather low oxygen tension. About 80% of the lens glucose is metabolized through anaerobic glycolysis. The enzymes hexokinase and phosphofructokinase regulate the rate of glucose metabolism of the lens. The end product of glucose metabolism is lactic acid, some of which is metabolized further by the Krebs cycle, but the majority simply diffuses out into the aqueous humour to be eliminated from the eye. Metabolism of one molecule of glucose by anaerobic glycolysis yields only 2 molecules of ATP.

2. KREBS CYCLE

Krebs cycle requires oxygen and is very inactive in the lens, as there is a paucity of mitochondria and oxidative enzymes. Thus, ATP production in the Krebs cycle is effectively limited to the lens epithelium which possesses the necessary enzymes and has adequate oxygen supply. It has been estimated that only 3% of lens glucose is metabolized via the cycle; but because of the efficiency of the pathway (1 mol of glucose produces 36 mol of ATP), generates about 20% of the total ATP production from glucose in the lens. On an average, 8 ml/g/hr of oxygen is utilized in the rabbit lens. Carbon dioxide produced by the Krebs cycle diffuses out of the lens into the aqueous humour.

3. HEXOSE MONOPHOSPHATE (HMP) SHUNT

The hexose monophosphate (HMP) shunt uses glucose-6-phosphate as its initial substrate and does not generate ATP. However, it forms pentose and reduced nicotinamide adenine dinucleotide phosphate (NADPH). The pentose is utilized for ribonucleic acid synthesis. NADPH is an essential cofactor in many biochemical reactions. It is utilized to maintain lens glutathione in reduced state and is also a necessary factor in the sorbitol pathway. Further, some of the pentose produced is recycled to re-enter the glycolytic pathway.

It has been observed that about 14% of the total glucose utilization in the rabbit lens is through this pathway. Carbon dioxide produced in an HMP shunt diffuses into the aqueous humour.

4. SORBITOL PATHWAY

Under normal conditions, only about 5% of the glucose used by the lens is metabolized by the sorbitol pathway. This pathway does not generate any ATP and its purpose in the normal lens is not yet understood. However, this pathway has received wide attention mainly because of its pivotal role in the development of sugar cataract.

Table 1.2 *Pathways of glucose metabolism in the crystalline lens*

Sr. no.	*Pathway*	*Main intermediates*	*End products*	*Glucose through pathway (%)*	*Mol ATP gained/mol glucose metabolize*
1.	Glycolytic	Glucose-6-phosphate; fructose 1,6-diphosphate; pyruvic acid	Lactic acid	80	2
2.	Krebs cycle (oxidative + O_2)	Tricarboxylic acid	CO_2, H_2O	5	36
3.	Pentose shunt	Pentoses	CO_2, NADPH	15	—
4.	Sorbitol pathway	Sorbitol; fructose	Lactic acid*	Unknown	2

*Some lactic acid attributed to glucose metabolism by the glycolytic pathway may actually be formed through the sorbitol pathway.

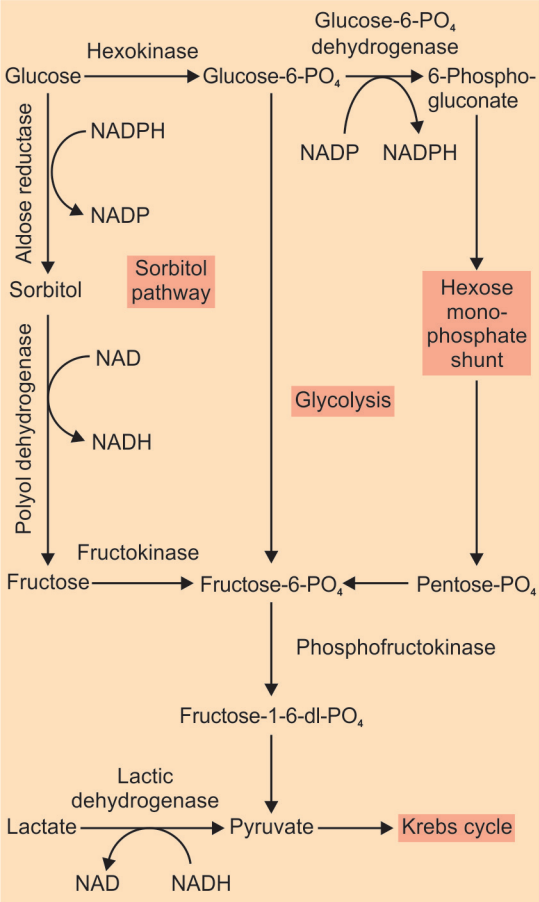


Fig. 1.10. *Pathways of glucose metabolism in the crystalline lens.*

In this pathway, glucose is converted into sorbitol (by the enzyme aldose reductase) which in turn is converted into fructose by the enzyme polyol dehydrogenase. The fructose is converted into fructose-6-phosphate (by the enzyme fructokinase) which enters into the glycolytic pathway (Fig. 1.10).

PROTEIN METABOLISM

Protein synthesis. Mechanisms occurring in the lens are similar to those occurring in all other tissues of the body. Proteins are synthesized from free amino acids which are actively transported into the lens from the aqueous. The formation of peptides from amino acids requires ATP and the appropriate RNA template. The ATP is acquired from glucose metabolism. Incorporation of amino acids into the RNA to form lens proteins occurs at a rather slow rate. It has been reported that glycine and serine are incorporated at no more than 5% per day. Further, rate of protein synthesis varies in different parts of the lens, nucleus being the slowest.

Protein breakdown in the lens is catalyzed by the enzyme peptidases and proteases. *In vitro* under sterile conditions, the lens undergoes autolysis. However, normally *in vivo,* the process of autolysis is inhibited.

PERMEABILITY AND TRANSPORT MECHANISMS OF THE LENS

Active and passive (permeability dependent) transport mechanisms of the lens are essential to provide nutrients for metabolism, to dispose of waste products of metabolism and to regulate water and cation balance in the lens. The salient features of biochemical composition of the lens vis-a-vis aqueous humour and the chemical exchange between the two is depicted in Fig. 1.11.

Active transport mechanisms are concerned with the transport of amino acid, potassium, taurine, inositol and extrusion of sodium. As discussed earlier, about 90% of the energy in

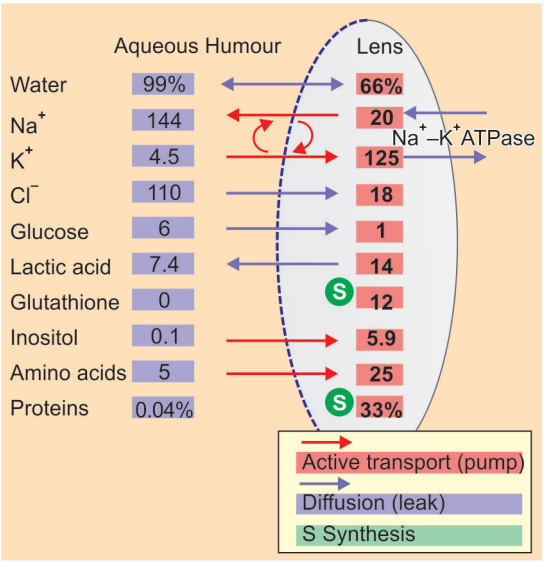


Fig. 1.11. *Chemical composition of the lens vis-à-vis aqueous humour and chemical exchange (pump-leak mechanism) between them. Values are in mmol/kg of lens water unless otherwise stated.*

the form of ATP generated from the glucose metabolism in the lens is utilized for these active transport mechanisms. ***Passive exchange (transport)*** across the lens capsule occurs for water, ions and waste products of metabolism, such as lactic acid and carbon dioxide. Exchange of these substances between the lens and aqueous humour meets with a little resistance from the capsule. It has been confirmed in laboratory studies that the isolated capsule is permeable to all low molecular weight compounds but restricts the movement of the larger colloidal material.

Following transport mechanisms of the lens need special description:

- Water and electrolyte transport
- Transport of amino acid and inositol
- Glucose transport

WATER AND ELECTROLYTE TRANSPORT

The electrolyte and water content of the lens resemble that of an intact cell, whereas Na^+, Cl^-, and K^+ ions and water content of the aqueous and vitreous are similar to that in plasma or extracellular fluids. The lens maintains its electrolyte and water gradient against the surrounding fluid by following mechanisms:

1. ***An energy-dependent cation pump.*** It is functioning at the level of anterior lens epithelium and plays an important role in cation balance in the lens. This pump mechanism involves the active extrusion of sodium (Na^+) coupled with uptake of potassium (K^+). This process is thought to be linked and mediated by the membrane bound enzyme Na-K-ATPase which degrades the ATP into ADP, inorganic phosphate and energy (used by cation pump). Oubain, a specific inhibitor of Na-K-ATPase, causes the lens to lose K^+ and gain Na^+ (confirming presence of the enzyme-mediated cation pump). Although there is some disagreement about whether individual lens fibres participate in cation transport, some workers have reported that another Na^+ extrusion 'pump' depending on the physio-chemical integrity of lens fibres also exists.

As a result of the active extrusion of Na^+ and uptake of K^+ at the anterior surface of the lens generates a chemical gradient which stimulates diffusion of Na^+ into the lens and K^+ out of the lens, primarily through the posterior surface and also to some extent from the anterior surface (Fig. 1.12). This process of active transport (cation pump) stimulating passive diffusion (leak) has been termed the *"pump-and-leak"* theory of cation transport.

2. ***The lens as an osmometer.*** One can consider the lens either as one giant cell whose ion distribution is regulated by the single layer of epithelium or as many individual cells functioning within a giant cell. The capsule confers on the lens properties of a giant in cell which swells up in hypotonic media and dehydrates in hypertonic media *in vitro.* The cations (Na^+ and K^+) which roughly equal 145 mEq/l, and anions (Cl^-, HCO_3^-, sulphate, ascorbate and glutathione) which equal 50 to 60 mEq/l, contribute to lens osmolarity. An anionic deficit of about 90 mEq/l is probably made by acidic groups of lens proteins and glycoproteins. The water equilibrium between the lens and the surrounding fluid is disrupted, if the concentration of osmotically active compounds (Na^+, K^+, others) increases inside the lens. For example, exposure of the lens to surface active detergent antibiotics disrupts the physiochemical integrity of the membrane and

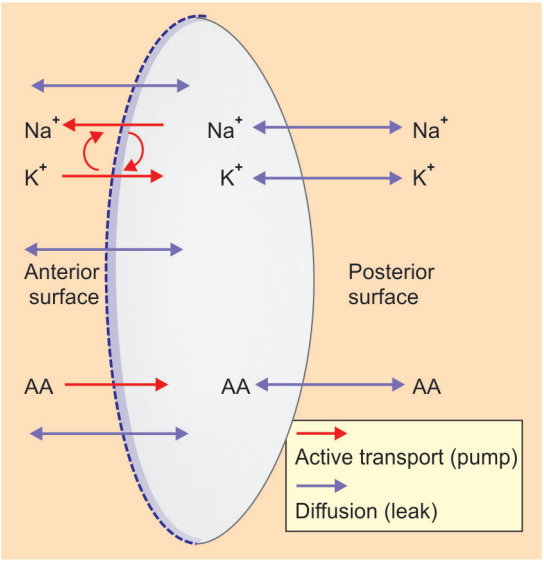


Fig. 1.12. *The 'pump-and-leak' mechanism of cation balance in the lens.*

the Na^+ extrusion pump which subsequent gain of Na^+ ions and water by the lens. Lens swelling, and eventually, complete loss of lens transparency follows.

TRANSPORT OF AMINO ACIDS AND INOSITOL

Amino acids are actively transported into the lens at the anterior epithelial surface and are, therefore, included in the "pump-and-leak" concept (Fig. 1.12). Three different pumps—one each for acidic, basic and neutral amino acids—have been reported. The transport of amino acids is in some way linked to the transport of cations. Inside the lens, amino acids are incorporated into the proteins, metabolized and used for energy or diffuse back into the aqueous by "leak" mechanism (Fig. 1.13).

Inositol is also actively transported into the lens, but its exact mechanism is not known.

GLUCOSE TRANSPORT

Simple diffusion and facilitated diffusion (mediated transfer) are the two mechanisms involved in the transport of sugars across the lens. Unlike the active transport of cations and amino acids which occur only at the anterior epithelial surface, the transfer of glucose occurs across both anterior and posterior surfaces of the lens.

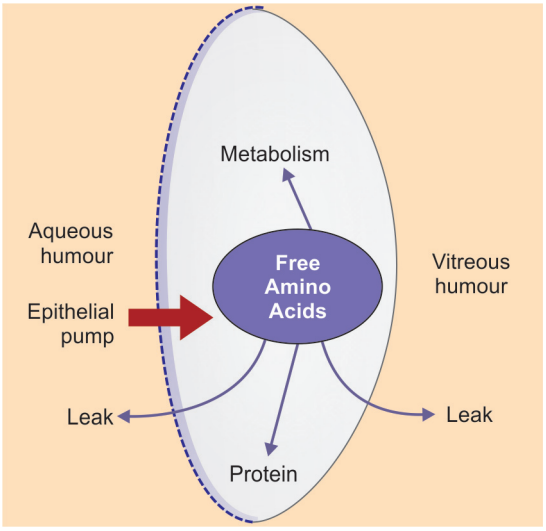


Fig. 1.13. *Fate of amino acids in the lens.*

ELECTRICAL PHENOMENA OF THE LENS

As a result of distribution of the ions inside the lens and its bathing medium (aqueous and vitreous), there exists a resting potential or an electrical potential difference in the lens. The inside of the lens is electronegative with respect to the bathing medium. Potential of about –70 mV is recorded across the intact lens capsule and of –23 mV in the lens fibres. Further, there exists a difference of –23 mV between the anterior and posterior surfaces of the lens. Thus, the flow of electrolytes into the lens is directed by an electrical gradient.

It is hoped that elucidation of electrophysiologic finding in the lens might eventually provide an ideal tool for detecting early membrane changes during cataractogenesis.

LENS CULTURE

The lens is an excellent tissue for *in vitro* incubation owing to its avascularity and simple structure. Thus, most of the physiologic and biochemical investigations on the lens are carried out *in vitro.* Various methods have been employed to incubate the lens in artificial culture media.

The most commonly employed method is *'closed* system' in which the carefully excised animal lens is placed in a culture flask containing an isotonic oxygenated balanced solution with glucose such as Tyrode's or Krebs-Ringer's solution. Under such conditions, a lens can be readily maintained for at least 24 hours. TC 199, a tissue culture medium containing salts, glucose, amino acids, and vitamins is used for long-term lens culture for days or weeks. The *in vitro* studies which are frequently carried out while the lens is in culture include: Glucose utilization, cation and amino acid transport and lens transparency. Chemicals or drugs harmful or beneficial to the lens in culture can be tested by comparing the treated lens with the control contralateral lens of the same animal. Several criteria for the viability of a cultured lens have been used, including mitotic activity and cation balance.

LENS TRANSPARENCY

Normal lens is a transparent structure transmitting almost 80% of light energy. It

consists of a composite system of fibre-cells with their own individual membranes separating their contents from the interstitial fluid or cement substance which is itself separated by the lens capsule and epithelium from the surrounding aqueous and vitreous humours. The transparency of this system must presumably depend upon the avoidance of large transitions of refractive index between cells and surrounding cement substance. In other words, the transparency of lens must simply be a consequence of the low number of scattering centres. Moreover, the lens cell is composed largely of protein molecules about 10 nm in diameter which are present in a non-opalescent colloidal solution. The particle size is sufficiently small and the concentration is sufficiently low so much that scattering, although present, reduces the transmission of white light only by a few percentage units. Torkel has proposed that the lens transparency is due to the regular arrangement of lens fibres and the uniform distribution and paracrystalline state of proteins within the cell. Jones and Lerman reported that the lamellar conformation of lens proteins rather than helical structure may also contribute to transparency. Benedek, however, has suggested that if the protein molecules are not too large, then their precise arrangement is of lesser importance.

In general, the factors that play significant role in maintaining the outstanding clarity of the normal lens are:

- Single layer of epithelial cells which is not thick.
- Semipermeable character of the lens capsule.
- Sparsity and highly packed nature of lens cells. The lens extracapsular space is less than 5% of its total volume, so the zones of discontinuity are very small compared to wavelength of light.
- Characteristic arrangement of lens proteins.
- Pump mechanism of the lens fibre, which regulates the electrolyte and water balance in the lens and thus maintains relative dehydration of the lens.
- Avascularity of the lens
- *Auto-oxidation.* High concentration of reduced glutathione in the lens maintains the lens proteins in a reduced state and ensures the integrity of the cell membrane pump.

Thus, appearance of vacuoles (both intracellular and extracellular), local precipitation of proteins resulting in larger aggregates and distortion of lens structure, probably all lead to increased light scatter and clinically the changes are described as cataract. Changes in transparency are certainly associated with changes in electrolyte and water content.

The layers of normal lens have different refractive indices. This change is not gradual, there is stepwise increase towards the interior. The refractive index of the nucleus is higher (1.40) than the cortex (1.38) owing to the comparative hardness of the nucleus.

CHANGES IN AGEING LENS

There are three stages of age-related changes in the crystalline lens: Development, growth and ageing. Many changes occur to the clear lens with increasing age. These are not to be confused with the changes occurring in the cataractous lens, though many of these changes are seen in a more extreme form in cataract and some are the forerunners of the cataractous changes. Changes in the ageing lens can be grouped as: Physical changes, metabolic changes, changes to crystallins and changes to plasma membranes and cytoskeleton.

1. *PHYSICAL CHANGES*

- *Lens weight and thickness* increase steadily with age. This results due to continued growth of the crystalline lens throughout life building up layers of new cells from the equator.
- *Light transmission* by the lens especially at lower wavelengths decreases with the increasing age indicating that light absorbance increases with the age.
- *Light scattering* is increased with the age. It has been reported to be caused by aggregation and formation of a gel-like state. Some workers have pursued the idea that the increased light scattering with age could be attributed to synerism, a process in which conformational changes to the protein release bound water enhancing the difference in refractive index between the 'drier' protein region and its surroundings.

- *Fluorescence* property of lens has been confirmed to increase with the age.
- *Refractive index* in the nucleus of bovine lens is reported to increase with age. However, no such change was found in human lens.

2. METABOLIC CHANGES

Most of the metabolic activities of the lens decrease with age. A few important ones are as follows:

- The proliferative capacity of human lens epithelial cells declines during adult life.
- Many enzyme activities decline in the whole lens with age.
- There occurs an increase in the urea-soluble proteins, at the expense of soluble proteins, on going from cortex to nucleus.
- Three enzymes of glutathione metabolism, viz. glutathione peroxidase, glutathione reductase and glutathione S-transferase do not significantly decline with age. However, both glutathione and ascorbate levels decrease in lens with age.
- Both superoxide dismutase and glucose-6-phosphate dehydrogenase activity are lost with age but the denatured enzyme protein remains.

3. CHANGES IN CRYSTALLINS

Various studies have been carried out to study the changes in crystallins with age. The reported changes are as below:

- The earlier claim that high-molecular weight aggregates accumulate in bovine and human lens nucleus with age has not been supported by recent experiments using fast high performance gel chromatography. However, α-crystallins have been reported to almost disappear from soluble extracts of the nucleus and β-crystallins become more polydisperse.
- There occurs an age-related loss of γ-crystallins.
- The γ-crystallin fraction in particular shows an increase in disulphide bonds with age.
- There occurs a limited unfolding of bovine crystallins with age.
- It has been reported that all crystallin fractions of human lens contain fluorophor other than tryptophan. The non-tryptophan fluorescence increases with age (greater in nucleus than the cortex).

4. CHANGES OF PLASMA MEMBRANE AND CYTOSKELETON

- The loss of hexagonal cross-section of fibre cells and of their interlocking devices, and the lack of cytoskeleton in the lens nucleus have been reported to occur as age changes.
- There occur age-related losses of membrane proteins and lipids and of cytoskeletal proteins.
- A loss of membrane potential and an increase in lens sodium and calcium occur with age.
- All the large membrane polypeptides are reported to decrease in parallel with age.
- Main junctional polypeptide (MP-26) is converted into smaller variants with age.
- Changes in membrane rigidity also occur with ageing.

ACCOMMODATION

DEFINITION AND RELATED TERMS

As we know that in an emmetropic eye, parallel rays of light coming from infinity are brought to focus on the retina, with accommodation at rest. Our eyes have been provided with a unique mechanism by which we can even focus the diverging rays coming from a near object on the retina in a bid to see clearly (Fig. 1.14). This mechanism is called accommodation. In it, there occurs increase in the power of the crystalline lens.

Far point, near point, range and amplitude of accommodation

The nearest point at which small objects can be seen clearly is called near point or *punctum proximum* and the distant (farthest) point is

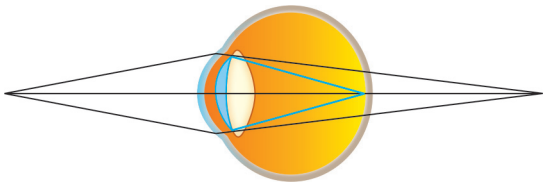


Fig. 1.14. *Effect of accommodation on divergent ray entering the eye.*

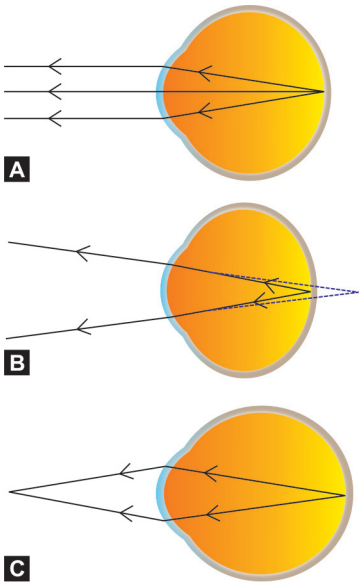


Fig. 1.15. *Showing far point in:* ***(A)*** *emmetropic eye;* ***(B)*** *hypermetropic eye;* ***(C)*** *myopic eye.*

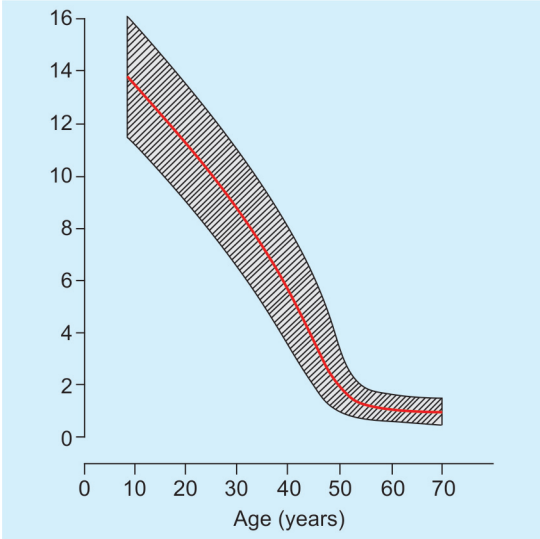


Fig. 1.16. *Showing decrease in the amplitude of accommodation with age in human (From Duane, A. Arch Ophthalmol 54; 568, 1925).*

called far point or *punctum remotum*. The distance between the near point and the far point is called *range of accommodation*. The difference between the dioptric power needed to focus at near point (P) and to focus at far point (R) is called *amplitude of accommodation* (A). Thus, A = P–R.

Far point and near point of the eye vary with the static refraction of the eye. In hypermetropic eye, far point is virtual and lies behind the eye, while in myopic eye, it is real and lies in front of the eye (Fig. 1.15). In an emmetropic eye, far point is at infinity and near point varies with age; being about 7 cm at age of 10 years, 25 cm at the age of 40 years and 33 cm at the age of 45 years. Thus the amount that the eye can alter its refraction is greatest in childhood and slowly decreases until it is lost in middle age. Amplitude of accommodation in dioptres as function of the age, as studied by Duane, is depicted in Fig. 1.16.

Depth of field and depth of focus

When an object is accurately focused monocularly, often the objects somewhat near and somewhat farther away are also seen clearly without any change in accommodation. This range of distance from the eye in which an object appears clear without change of accommodation is termed depth of field. Depth of field reduces the necessity for precise accommodation.

The range at the retina in which an optical image may move without impairment of clarity is termed depth of focus. The depth of field and depth of focus are markedly influenced by the diameter of the pupil (Fig. 1.17). Depth of field is inversely proportional to pupil size. Size of the blur circle produced on retina is proportional to pupil size.

Depth of field should not be mistaken for accommodation. The apparent range of accommodation also includes depth of field and tolerance of blur (i.e. depth of focus).

MECHANISM OF ACCOMMODATION

Accommodation is the result of a change in the form of the lens brought about by contraction of the ciliary muscle. Therefore, a review of the anatomy of ciliary body and ciliary muscle and lens with its capsule and the zonules will be useful to understand the mechanism of accommodation.

As we know, accommodation is a process by which one can focus the objects at different distances in a bid to have a clear vision. Its

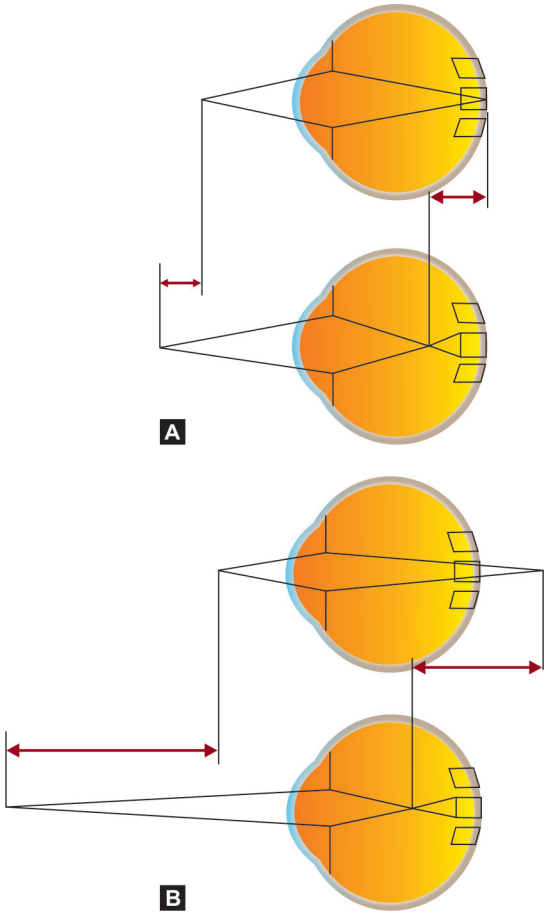


Fig. 1.17. *Effect of pupil diameter on depth of field and field of focus:* ***(A)*** *Large pupil;* ***(B)*** *Small pupil.*

mechanism varies from species to species. Just for interest, examples of a few species are given below.

- Some fish retract their lenses to focus on distant objects.
- Snakes and frogs have a mechanism to move the lens forward for near vision.
- Horses, by moving their heads, tilt the retina so that different regions lie at appropriate distances behind the lens.
- In man, the process of accommodation is achieved by a change in the shape of the lens.

THEORIES OF ACCOMMODATION IN HUMAN

The mechanism of human accommodation and disaccommodation, i.e. the ability of the focus from far to near and near to far, respectively, has been debated with surprising passion, since 1801 when Young reported that lens is responsible for accommodative properties of the human eye. In a bid to account for the changes taking place in the lens during accommodation, numerous theories have been proposed, a few of which warrant serious consideration are discussed below.

1. The relaxation theory (Helmholtz theory)

The relaxation theory also known as *capsular theory* is probably, most widely accepted, although it is not necessarily supported by experimental evidence. This theory was first proposed by Thomas Young and elaborated by Helmholtz in 1885, by whose name it is known generally. The importance given to the lens capsule was emphasized by Fincham in 1937. The main points of the relaxation theory are as follows:

- When the eye is at rest (unaccommodated) the malleable substance of young lens is compressed in its capsule (which is an elastic structure) by tension of the zonules. The surfaces of the compressed lens are less curved and these change the dioptric power in lens.
- Zonules are kept under tension by a pull executed on them by the elastic choroid (Helmholtz original assumption). However, recently it is being assumed by many workers that the zonules are kept under tension by the relaxation of fibres of the ciliary muscle.
- Contraction of the ciliary muscle causes the ciliary to shorten and move forward the equator of the lens. It also pulls the choroid forward. As a result, the zonules are relaxed *(basic mechanism of relaxation theory)*, the tension on the capsule is relieved and the lens attains a more spherical shape. As the refractive index of lens (1.39) is more than refractive index of aqueous and vitreous, increase in convexity of the lens increases its dioptic power and thus allows the near objects to be focused clearly on the retina.

Points in favour of relaxation theory

Glasser and Kaufman developed experiments to confirm the classical description of the mechanism of accommodation in primates. Many latest techniques also support this theory.

The imaging techniques show that the apex of the ciliary muscle moves antero-inward and the equatorial edge of the lens moves away from the sclera during accommodation (about 250 µm for 10 D of accommodation). Goniovideography shows that the zonular fibres extending from the ciliary processes to the lens equator are relaxed during accommodation. UBM imaging shows the posterior zonular fibre extending between the posterior attachment of the ciliary muscle, and the ciliary processes are stretched during accommodation by the forward and axial movement of the apex of ciliary muscle.

Points against relaxation theory

There are some points against this theory. According to Helmholtz hypothesis, since the equatorial diameter increases with age (i.e. since the crystalline lens equator is getting closer to the ciliary muscle), the zonules should relax. As one ages, the power of the crystalline lens should increase while viewing distant objects in the accommodated state. One should become more myopic and the crystalline lens should become unstable, but in fact, one becomes slightly hyperopic and the crystalline lens remains stable. Helmholtz theory also is not consistent with the decrease in spherical aberration that occurs during accommodation.

Helmholtz attributes the universal linear decrease in the amplitude of accommodation with age to hardening of the crystalline lens. No tissue in the body hardens in a linear fashion with age.

Role of lens capsule

Although, at first, Helmholtz regarded the lens to be an elastic body as a whole which would assume the spherical shape of its own when made free from the tension of zonules. However, he soon realized his fallacy that being a semisolid mass the lens may be deformed by the external force but being inelastic cannot return to its original shape when the deforming force is removed. Helmholtz found it necessary, therefore, to attribute elastic properties to the lens capsule to account for the change in the shape of lens when it was free from the tension of zonules. It was seen that the lens surfaces were not perfectly spherical in contour; the anterior surface, in particular, is more convex centrally during accommodation. Fincham suggested that variations in thickness of lens capsule (Fig. 1.2) account for the local variations in curvature. He proposed that during accommodation the thicker ring of anterior capsule surrounding the central region contracts under the lessened zonular traction, while the thinner central capsule bulges forward in a more pronounced fashion. The physiological anterior lenticonus thus formed has a short radius of curvature and high refraction.

Gullstrand mechanical model of accommodation

Based on the Helmholtz hypothesis, Gullstrand devised a mechanical model to explain the mechanism of accommodation. Description of Gullstrand's model (as shown in Fig. 1.18) is as below: The cord between the two springs represents the zonules. The upper spring represents the lens, and its contraction represents change of the shape of the lens during accommodation. The lower spring represents the elasticity of choroid. In the eye, at rest this spring is sufficiently strong to overcome the pull of the upper string, which must always be slightly on the stretch and, therefore, must be the weaker of the two (presently now the lower spring is thought to be represented by relaxation of the fibres of the ciliary muscle and not the choroid). A cord passed over the pulley supporting the weight represents the pull of the circular fibres of the ciliary muscle. In the unaccommodated eye (Fig. 1.18A), the weight is at rest and exerts no pull. When accommodation at place (Fig. 1.18B) due to pull exerted by the contraction of ciliary muscle, the lower spring pulled, and the zonules are slackened; this allows the upper spring to contract (i.e. change in the shape of lens).

2. Theory of increased tension (Tscherning theory)

This theory attributes to the increased curvature of the capsule increasing tension on the zonules. It states that contraction of the ciliary muscle pulls on the zonules directly and increases the

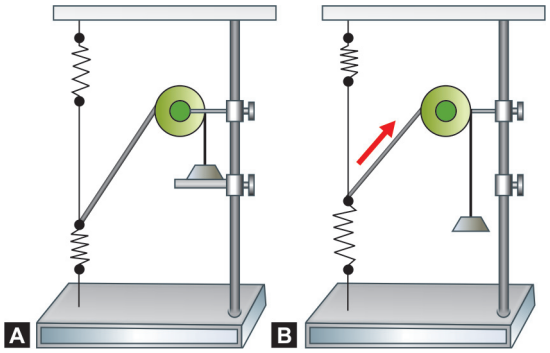


Fig. 1.18. *Gullstrand's mechanical model showing the forces which produce accommodation according to the Young-Helmholtz theory:* ***(A)*** *Unaccommodated stage;* ***(B)*** *Accommodated stage.*

tension on the capsule. This results in a compression of the capsule at the equator of the lens so that the poles bulge. Accommodation is brought about, therefore, by increasing the tension of the zonules.

However, now all the evidences (anatomical and physiological) are against this view and so this theory is no longer accepted.

3. Schachar's theory

Recently, media attention focused on a new theory of accommodation and presbyopia and its resulting surgical treatment, which Schachar suggested, may restore accommodation. This theory seems to be a modification of Tscherning's theory of increased tension and states that accommodation occurs when ciliary muscle contraction tenses rather than relaxes, the equatorial zonules. The lens would then be stretched equatorially or coronally (i.e. the lens edge moves toward the sclera) but the biomechanical properties of the lens are such that the central part of the lens rounds up and moves anteriorly, increasing its refracting power.

In Schachar's theory, the anterior and posterior zonules act like supportive ligaments of skeletal joints and are stabilizing components, which are tense during distance vision and relax during accommodation. The equatorial displacement of the crystalline lens occurs as a result of increased tension on the equatorial zonules produced by contraction of the anterior radial muscle fibres of the ciliary muscle. Since an active force is involved in accommodation, the amount of force that the ciliary muscle can apply is dependent on how much the ciliary muscle is stretched.

The Schachar's theory thus contradicts the classical Helmholtz mechanism, in which ciliary muscle contraction is thought to relax the zonules, allowing centripetal elasticity of the lens capsule to spherisize the lens (i.e. the equatorial or coronal diameter of the lens decreases, the equatorial edge of the lens recedes from the sclera, and the lens as a whole round up).

According to Schachar's theory, presbyopia results from growth in the equatorial diameter of the lens, such that the perilenticular space is reduced and ciliary muscle contraction can no longer tense the zonules and expand the lens coronally. Based on his theory, Schachar introduced a new surgery for presbyopia, i.e. the use of scleral expansion bands (SEB). The aim of these segments is to increase the working distance between the ciliary muscle and the lens equator, which should theoretically, as suggested by Schachar, allows the muscle to work again. However, recently conflicting reports regarding the effectiveness of SEB surgery have appeared in the literature and have challenged the validity of the Schachar's theory.

4. Cotenary (hydraulic suspension) theory of accommodation

Recently, Coleman et al. have stated that many observations during accommodation can neither be explained by 'capsular theory' of Helmholtz nor by Schachar's theory. The most difficult objection to these theories is to overcome the precise, rapid and anatomical reproducible shape of the lens in accommodated state. In addition, the ciliary muscle does not have the necessary anatomic rigidity or the attachments to support an equatorial fraction force to flatten the lens as proposed by Schachar theory. The capsule itself does not have the elastic properties to round up the lens mass reproducibly and rapidly. Coleman et al have demonstrated the observable feature of accommodation by a mechanical model of Cotenary theory of accommodation. The Cotenary theory of accommodation was proposed by Coleman in 1970 and demonstrated by a simple Cotenary

model to support his concept in 1986. The Cotenary (hydraulic suspension) theory propounds that the lens zonules and anterior vitreous comprise a diaphragm between the anterior and vitreous chambers of the eye. It has been proposed that contraction of ciliary muscle generates a pressure gradient between the aqueous and vitreous, causing anterior movement of the lens zonule diaphragm and steepening of anterior central lens curvature and slight flattening of the anterior peripheral lens curvature. Coleman and Fish in their mechanical model of Cotenary theory have demonstrated that the anterior capsule and zonules form a trampoline shape or hammock-shaped surface that is totally reproducible depending on the circular dimensions, i.e. the circumference of the ciliary body. Thus, he stated that the ciliary body directs the shape like the pylons of a suspension bridge, but does not need to support an equatorial fraction force to flatten the lens as required by a capsular theory.

According to the cotenary system of Coleman and Fish, presbyopia occurs due to increasing lens volume with age that results in a reduced response of anterior radius of curvature to the vitreous pressure gradient created by ciliary body contraction.

Conclusion

Despite a wealth of contradictory observations, the von Helmholtz capsular theory is probably most widely accepted. In light of the abundant evidence provided by experimental physiology throughout the last century, it is remarkable that fundamental elements of the mechanism of accommodation remain contentious. Nevertheless, using ultrasound biomicroscopy and goniovideography, Glasser and Kaufman have provided strong support for the classical Helmholtz theory, namely that upon accommodative effort, contraction of ciliary muscle releases zonular tension on the equatorial crystalline lens, allowing the lens to assume more spherical geometry. However, still there are elements of the mechanism that await further elucidation; for example, the role of the iris as suggested by Crawford et al. and role of the vitreous pressure as suggested by Cramer in 19th century and Coleman and Fish recently in 2001.

ACCOMMODATION: CERTAIN PHYSIOLOGICAL ASPECTS

STIMULUS FOR ACCOMMODATION

There is not only a single important stimulus to accommodation. All of the following factors must be responsible in eliciting the appropriate accommodative response:

- Image blur
- Apparent size and distance of object
- Chromatic aberrations
- Oscillation of accommodation
- Scanning movements of the eye

It has been reported that the initial accommodative adjustment is based on 'trial and error'.

REACTION TIME

Reaction time refers to the time lapse between the presentation of an accommodative stimulus and occurrence of the accommodative response. Some of the observations made regarding reaction time are as follows:

- Average reaction time for 'far-to-near' ac accommodation is 0.64 seconds.
- Average reaction time for 'near-to-far' accommodation is 0.56 seconds.
- Reaction time for accommodation is considerably larger than that for the contraction of the pupil to light (0.26–0.30 seconds). Reaction time of convergence response is about 0.20 seconds.

OCULAR CHANGES IN ACCOMMODATION

The changes which take place in the eye during accommodation can be summarized as below:

1. ***Slackening of the zonules.*** Zonules are normally tense and keep the lens flat. They slacken during accommodation due to contraction of ciliary muscle.

2. ***Changes in the curvature of lens surface.*** The principal change in the lens during accommodation is seen in the anterior surface of the lens. At rest, the radius of curvature of the anterior surface of the lens is 11 mm and that of posterior surface is 6 mm. In accommodation, the curvature of posterior surface remains almost the same, but the anterior surface changes, so

that in strong accommodation its radius of curvature becomes about 6 mm in the periphery and 3 mm in the central part which bulges more. The central part of the anterior surface bulges more because the anterior capsule is thinner here (Fig. 1.19) as compared to the peripheral part. The posterior capsule is the thinnest region and so the posterior surface has a greater curvature even in the unaccommodated lens.

3. ***Anterior pole*** of the lens moves forward carrying the iris with it, resulting in shallowing of the anterior chamber in the centre.

4. ***Axial thickness*** of the lens is increased owing to forward movement of the anterior pole (posterior pole remaining fixed).

5. ***Changes in the tension of lens capsule*** have also been studied. During accommodation, the anterior capsule becomes slack.

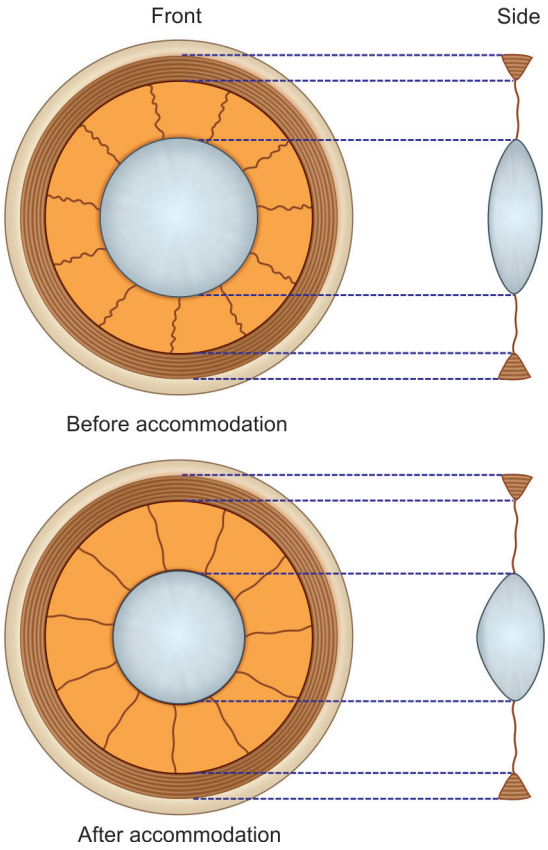


Fig. 1.19. *Changes in the ciliary body ring, zonules and shape of lens during accommodation.*

6. ***Lens sinks down*** because the accommodated lens is held less firmly by its zonular attachment, it is influenced by the force of gravity and tends to sink within the globe.

7. ***Changes within the lens substance.*** In addition to the changes in curvature of the lens, the changes in the lens substance also create a change in the refractive power of the lens. The internal changes are brought about by changes in curvature of the various portions of lens having different indices of refraction.

8. ***Pupillary constriction and convergence of eyes.*** In addition to the changes in the lens and zonular system, the pupil constricts and the eyes converge, almost simultaneously. These changes occur in a bid to achieve clear vision for near objects. The pupillary constriction is a synkinesis and not a true reflex, it does not depend on either accommodation or convergence alone for its appearance.

9. ***The choroid*** is stretched forward by the ciliary muscle contraction.

10. ***The ora serrata*** moves forward about 0.05 mm with each dioptre of accommodation.

AGE-RELATED CHANGES IN ACCOMMODATION

As discussed earlier, in an emmetropic eye, far point is infinity and near point changes with age, being about 17 cm at the age of 10 years, 25 cm at the age of 40 years, 33 cm at the age of 45 years and about 50 cm at the age of 50 years. Therefore, at the age of 10 years, amplitude of accommodation (A) = 100/7 (dioptric power needed to see clearly at near point)—I/a (dioptric power needed to see clearly at far point); i.e. A (at age 10) = 14 dioptres. Similarly 'A' at age 40 years = (100/25 – I/a) = 4 dioptres; at age 45 years A = 3 dioptres and at 50 years = 2 dioptres. Since, we usually keep the book at about 25 cm, so we can read comfortably up to the age of 40 years and after that the near point recedes beyond the normal reading or working range. This condition of failing near vision due to related decrease in the amplitude of accommodation or increase in the near point (punctum proximum) is called presbyopia.

Pathophysiology of presbyopia

Theories proposed to explain the development of presbyopia include:

1. Changes in the elastic properties of lens capsule
2. Hardness or sclerosis of the lens, and
3. Weakening of the ciliary muscle.

Changes in the elastic property of lens capsule

Lens capsule has a definite role in the occurrence of presbyopia. But many workers have reported that the elasticity of the lens capsule does not change markedly with age.

Sclerosis or hardening of the lens

It is generally believed that the principal cause of presbyopia is the result of sclerosis or hardening of the nucleus of the lens so that the forces (capsule's elasticity) which normally deform the soft lens during youth, are now no longer effective. For many years, it was thought that the hardening of the ageing lens correlated with a decrease in water content, particularly in the nuclear region. However, now it is clear that there is no significant loss of water in the aging human lens, although this is a frequent observation in other mammalian species. The age-related hardening of the lens must, therefore, be due to an alteration in the structural proteins of the lens or to increased adhesions between lens fibres.

Weakening of the ciliary muscle

Although, age-related morphologic changes are known to occur in the ciliary muscle, however, there is little evidence to support the concept of a weakened ciliary muscle. In presbyopes also, the ciliary muscle contracts vigorously under the influence of pilocarpine. Thus, from the above, it can be concluded that the principal factor for causing presbyopia is age-related hardening of the lens nucleus. But change in the elasticity of the lens and weakening of the ciliary muscle also might be playing some role.

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