UNIT 18 BIOCHEMISTRY OF THE EYE

Structure

18.0 OBJECTIVES

After completing this unit, you should be able to understand:

- dynamics of vascular supply of eye;
- the metabolism of cornea;
- the reasons for transparency of cornea;
- the mechanism of catractogenesis; and
- the physio-chemical properties of vitreous.

18.1 INTRODUCTION

Every tissue in the body requires basic components which are either derived from the body itself or we acquire them from external source. These components are metabolised in our body to generate energy and for developinent of essential tissue components. The major factory for production of these essential components is the liver. Human eye also requires similar components for growth for its generation of energy and its tissue growth. Various biochemical reactions take place in the eye, so as to provide energy, perform regular maintenance and repair of the structure of the eye and perform the routine functions pertaining to vision. In this process metabolic by-products and waste material is also produced, which if accumulates may be toxic to the normal ocular tissue.

18.2 VASCULAR CIRCULATION OF EYE

Retinal vessels are derived from central retinal artery and are distributed within the inner two-third of retina. Outer one-third is supplied by choroidal circulation. The macula is entirely devoid of blood vessels. The retinal artery divides into arterioles, these arterioles are surrounded by capillary free zone.

Blood Ocular Barrier

All vascular beds of the eye are highly permeable to lipoid soluble substances (oxygen, carbon dioxide) and to water. The transport of material occurs through endothelial pores but some transport across endothelium occurs by the process of pinocytosis. Theblood ocular barriers primarily are:

- Blood retinal barrier
- Blood aqueous barrier

The permeability of these barriers can be measured by ocular flurophotometry for which several instruments have been developed. The most commonly used method employ fluorescein and indocyanine.

Blood Retinal Barrier

Blood retinal barrier is present in retina. In retina there are two tight junctions, outer blood retinal barrier is present between retinal pigment epithelial cells and inner blood retinal barrier is present between endothelial cells of retinal capillaries. Normally a defect of blood retinal barrier exists at the level of optic disc where the water soluble substance may enter the optic disc by diffusion from extravascular space of thechoroid.

Blood Aqueous Barrier

Blood aqueous barrier is present in anterior segment of the eye. There are two tight junctions one between the endothelial cells of iris capillaries and the other at the level of non-pigment layer of ciliary epithelium.

Ocular Haemodynamics

The pressure in the ocular arteries can be measured by ophthalmodynamometry. The intraocular pressure is raised to the level that stops the diastolic blood flow and then the level which stops the systolic pressure. The values are recorded with the help of direct visualization of retinal vasculature. The human eye pressure is lower then the rest of the body's blood pressure. The mean arterial pressure of ophthalmic artery is 77 mmHg whereas the mean arterial pressure of brachial artery is 100 mmHg. The pressure inside the intraocular veins is equal to the intraocular pressure at the site where these veins pierce the sclera. So the pulsation of retinal veins at optic disc indicate that the pressure inside them is above the intraocular pressure.

18.3 METABOLISM OF CORNEA

The corneal epithelium plays several roles in the process of image formation Its apical cell border interacts with the tear matrix to obtain an optically smooth surface and extremely regular thickness. Corneal smoothness is evaluated with a Placido's disc. Tight packing of individual epithelial cells is by the many desmosomes. The scattering of light by the epithelial cells decreases the clarity of image. The process that lead to variation of refractive index within the epithelial cells decreases the clarity of image, e.g., corneal edema either due to intraepithelial cell edema or intcrcellular edema. The common cause of intraepithelial cell edema is epithelial hypoxia resulting from contact lens over wear or intercellular edema may result from high IOP, as is seen in acute angle closure glaucoma.

Physiological roles of barrier function, refractive function (with interaction of tear film), response to wound and smoothing tendency is discussed to evaluate the transparency and functions of cornea.

Barrier Function

It is the first line of barrier between the external environment and the corneal stroma. The superficial epithelial cells are encircled by tight junctions (zonula occludens).

These superficial epithelial cells serve as semi-pernieable high resistance (12-16kΩ) membrane. It prevents niovement of fluid from tear to stroma. It is useful for maintenance of epithelial barrier. The maintenance is done by mitosis of basal cells and migration of new basal cells from the limbal stern cells. So the corneal epithelium is maintained by a balance among the process of centripetal cell migration (120μm/wk), mitosis, and shedding of superficial cells. The XYZ hypothesis of corneal epithelial maintenance by Thoft and Friend is X+Y=Z . The limbal epithelium has the highest mitotic rate followed by peripheral cells and central cells has the lowest mitotic mte.

Refractive Function

Cornea, through its interaction with the tear film, forms a smooth refractive function and uniform thickness.

Response to Wound

Response to wound initiates process of migration centripetally across the basement membrane to cover the abraded area and trigger a mechanism which is not known. The migration starts about 5 hrs after injury at the rate of 60-80 mm/hr until wound closes. There is mitosis and migration of cells. Hemidesmosomes disappear from basal cells during lag phase. Increase in vinculin synthesis occur during cell migration. Along with this there is synthesis of cell surface glycoproteins and glycolipids during wound healing.

Smoothing Tendency

In health and in disease condition the epithelium has a strong tendency to smoothen the underlying irregularities, e.g., epithelial facet by epithelial plug filling. The focal defects on bowman's membrane occurs as if we are repairing defects in the road. This smoothing tendency is also active in corneas after excimer photoablation therapy. There is a tendency of the epithelium to bring the underlying area back to mitigate the refractive effect of the ablation.

Electrophysiology and Ion Transport of Cornea

The trans-epithelial potential is 25-35 mV. It is almost 50 per cent of short circuit current and is due to C1-ion efflux in tear film, Na-K ATPase at the basolateral membrane with a chloride co-transport and Na-H exchanger and lactate-proton exchanger. These regulate intracellular pH by extrusion of lactate and H ions. Process is regulated by sympathetic corneal nerves. The in vivo epithelial ion transport has a minimal role, if any in corneal deturgescence, as compared to the endothelium.

Metabolism and Respiration of Cornea

The cornea requires energy for the maintenance of its transparency. Energy in the form of ATPs are provided as a result of glucose metabolism within the layers of the cornea. Glucose reach the cell by diffusion from aqueous humor. The corneal epithelial cell store high levels of glycogen. The glucose metabolism is mainly by EMP pathway. The remaining 30 per cent is by HMP pathway.

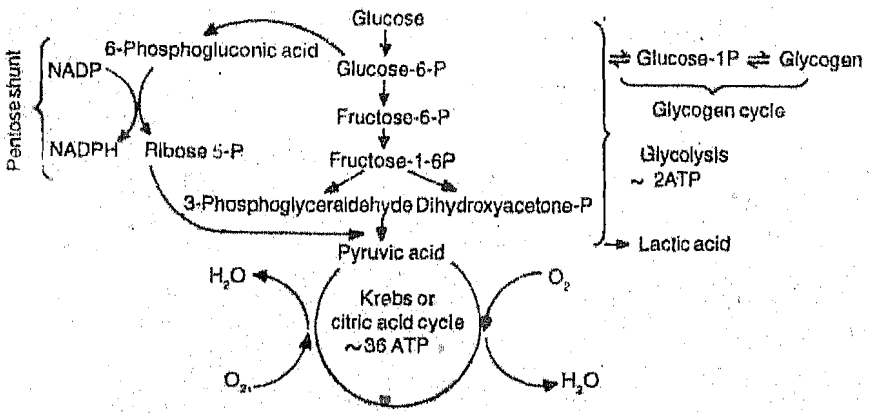

Fig. 18.1: Glucose metabolism by EMP pathway

Main Cycle: Glycolysis is represented by the central chain and involves the anaerobic conversion of glucose into pyruvic acid. Lower part of the cycle, the Kreb's Cycle (TCA cycle) is the aerobic utilistation of pyruvic acid for energy production. Left section represents the HMP (pentose shunt) which is a major substrate for protein synthesis.

In the process of the cycle providing the major part of the energy (Kreb's cycle) oxygen is required. The oxygen enters from atmosphere directly under open eye conditions when it is exposed to tear film pO_2 of 155 inm Hg. The pO_2 drops to 55 mm Hg in sleep. The cornea consumes about 3.5 ml/cm2/hr of oxygen. The corneal stroma is morphologically and physiologically divided into anterior and posterior lamellar stroma. It consists of bundles of collagen (type I and type V) fibres arranged as lamellae. In Bowman's layer, the interstices of the fibrils are filled with type VI collagen, proteogylcans, keratan sulphate, decorin, and fibromodulin.

The endothelium and epithelium are the most actively metabolizing of all layers.

Metabolic Pathways

1) 88 per cent of glucose is utilized through glycolysis.
2) Only 12 per cent of glucose utilized by TCA cycle (Kreb's Cycle).
3) HMP shunt also important for production of NADPH and ribose.

Sources of Nutrients of Cornea

1) Oxygen for the endothelium comes from aqueous humour and for epithelium from atmosphere through tear film. A small amount of oxygen is also provided to the peripheral parts of the cornea by the limbal capillaries and capillaries of the palpebral conjunctiva, especially during phases of eye closure.
2) The main source (90%) of glucose is aqueous humour. The remaining 10 per cent is provided by the lirnbal blood vessels and the tear film.
3) Amino acids come through aqueous humour by passive diffusion.

Metabolic Waste Products

During the metabolism various waste products including lactic acid, protein and fat breakdown products. A major metabolic waste product is carbon dioxide (CO_2). These metabolic waste products are removed primarily through the tear film, aqueous humor and limbal circulation.

Contact lenses act as a physical barrier to the transmission of oxygen and carbon dioxide between the atmosphere and cornea. Prolonged wear of contact lens of low water content and/or low permeability lead to a state of hypoxia.

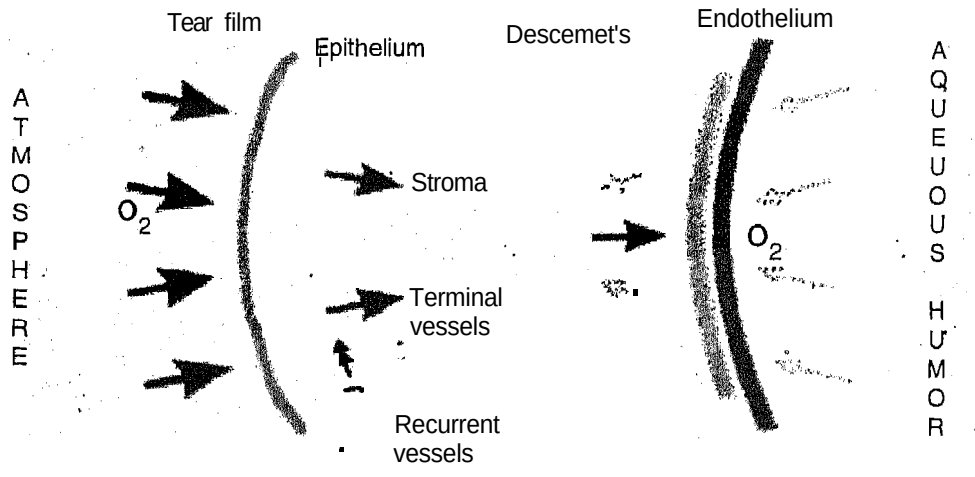

Fig. 18.2: Oxygen supply to the cornea

During prolonged hypoxic conditions, the ATP consumption remains the same but the production decreases (due to stoppage of the Kreb's cycle). The EMP pathway continues causing an accumulation of lactic acid. This lactic acid is not metabolised by the cornea and gets accumulated in the layers of the cornea, which imbibes water and causes epithelial and stromal edema.

Check Your Progress 1

What are the nutrient sources of cornea?

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18.4 CORNEAL TRANSPARENCY

For a good vision a clear optical pathway is essential, i.e., a light ray arising from ally object should be able to reach the retina un-interrupted or unhampered. However in the course of the ray of light, it has to pass through the tear film, the cornea, the aquous humour, the lens and the vitreous. It is obvious that if there is any opacity or obstruction to this path, vision would be adversely affected. A corneal opacity or opacity in the lens (called a cataract) would cause such problems.

Fortunately, our body's natural mechanisms ensure the transparency (or optical clarify) of the lens and the cornea. Various factors, both anatomical and physiological are responsible for maintenance of the transparency of the cornea. In this section we will try to understand the physiological factors maintaining the tranparency of the cornea. The normal composition of the cornea is 78 per cent water, 15 per cent collagen, 5 per cent other proteins, 1 per cent glycosaminoglycans and 1 per cent. salts.

The factors affecting the transparency include special properties of the corneal epithelium and tear film, anatomical arrangement of stromal lamellae of the cornea, corneal avascularity, and an immaculate balance of the corneal hydration.

- **Corneal Epithelium and Tear Film:** Normally the epithelium is transparent because of homogeneity of its refractive index. Other then this, the precorneal tear film plays an important role in transparency of cornea.
- **Arrangement of Stromal Lamellae:** The stromal lamellae of cornea is anatomically arranged in such a way that when light passes through, it do not get distorted in any way. Thus, stroma is responsible for corneal transparency. There are two main theories to explain the mechanism of corneal transparency.

1) **Maurice Theory**

The uniform collagen fibrils are arranged in regular lattice pattern separated by less than a wave length of light. The scattered light is destroyed by mutual interference.

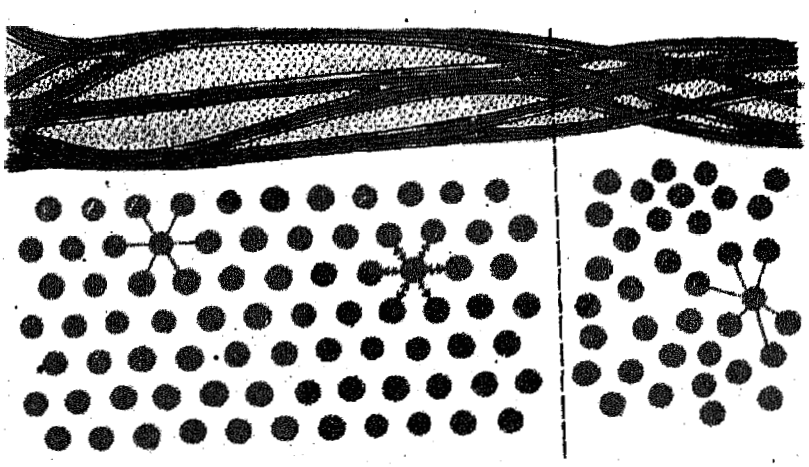

Fig. 18.3: Arrangement of stromal lamellae in cornea

2) **Goldman's Theory**

The fibrils are small in relation to light and do not interfere with light transinission unless larger than half a wave length of light.

Corneal Avascularity

Cornea is an avascular structure, except for its peripheral 1 mm, where small loops of vessels encroach. Progressive vascularization can interfere with transparency. There are various mechanisms responsible for its avascularity.

a) *Chemical Theory*

1) **Role of VIF:** thought to be sulfate ester of hyaluronic acid. Presence of this substance has been postulated, as a reason for corneal avascularity.

2) **Role** of VSF: thought to be an LMW amine. This substance has been demonstrated to he present at the site of lesion.

b) *Mechanical Theory*

The blood vessel is said to be stopped from growing into the cornea because of its compact nature.

c) Combination of mechanical and chemical theories has been thought as the cause of transparency lately.

d) *Role of Leukocytes*

These inflammatory substances has essential role in stimulating corneal vascular growth.

*Corneal **Hydration***

The normal water content of cornea is 78 per cent. The state of relative dehydration is necessary for corneal transparency. The factors responsible for corneal hydration are following:

a) Stromal swelling pressure

b) Barrier function of epithelium and endothelium

c) Hydration control by active pump mechanisms

d) Evaporation from corneal surface

e) Intraocular pressure

1) *Stromal Swelling Pressure*

Stromal Swelling Pressure (SSP) is the pressure exerted by GAG (Glycosaminoglycans) of the stroma which act like a sponge. Normally about 60 mm of Hg of pressure is exerted.

2) *Barrier Function of Epithelium and Endothelium*

Epithelium offers twice the resistance as endothelium. Barrier function of epithelium is maintained by desmosomes, mitotic activity of basal cells and migration of limbal stem cells into basal cell layer. Corneal endothelial barrier is leaky compared to epithelium. The permeability results from low resistance intercellular junctions at apical basement membrane. The tight junctions do not form complete zona occludens around the cells. The endothelial barrier function is calcium dependent.

3) *Hydration Control by Active Pump Mechanisms*

a) Na/K ATPase pump, which is more active in endothelium than epithelium

b) Bicarbonate dependent ATPase in endothelial cells

c) Carbonic anhydrase enzyme exclusively in endothelium

d) Na/H ATPase at lateral plasma membrane surface

Drug permeability across the cornea depends on:

1) Lipid and water solubility of the drug
2) Molecular size, weight and concentration
3) Ionic form of the drug
4) pH of the solution
5) Tonicity of the solution
6) Surface active agents
7) Pro drug form.

Check Your Progress 2

1) What is the total 'water content of cornea?

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2) Why should cornea be transparent?

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18.5 METABOLISM OF LENS

Water constitutes about 65 per cent of lens weight. Of the solid constituents highest concentration is of protein which is about 34 per cent of the total weight of an adult lens.

Proteins

Physical slate of protein is important for transparency of lens. Morner first time classified protein of lens into insoluble and soluble fraction. The insoluble fraction is albuminoid, it constitutes about 12.5 per cent of the lens protein. The soluble fraction is crystallins, which is of three types alpha, beta and gamma. They constitute 31.7 per cent, 53.4 and 1.5 per cent respectively.

Soluble Protein

Its synthesis takes place in the equatorial part on the surface of lens.

1) **Alpha crystallins:** It has the highest molecular weight, a positive charge and has got two chains A and B. Chain **A** has got thiol group and chain B has got no thiol group.
2) **Beta crystallins:** Has **got** three fractions and have disulphide linkages.
3) **Gamma crystallins:** Has got flour chains with similar molecular weight but differ from each other in chromatological properties.

Insoluble Proteins

When alpha crystallins combine with disulphide linkage or C-S bond, the insoluble fractions are formed. Insoluble fractions are urea or alkali soluble fractions,

Other Lens proteins: These are glycoproteins, nucleoproteins, phosphoproteins, lipoproteins and fluorescent protein.

Lens Transparency

1) Transparency depends on avoidance of large transitions of refractory index. This is in other words explained as low number of scattering areas.

2) Transparency was explained with more regular, uniform and lamellar configuration of lens fibres.

Changes in the Ageing Lens

1) **Physical changes:** Lens weight increases, light transmission decreases, light scattering increases, fluorescein property increases and refractive index increases with age.

2) **Metabolic changes:** Proliferative capacity of lens decreases, many enzymes decreases, insoluble albuminoids increases, glutathione decreases along with ascorbic acid, superoxide dismutase and glucose 6 phosphate dehydrogenase decreases.

3) **Changes in the crystallins:** Alpha and gamma crystallins decreases. Along with this gamma crystallins has a disulphide bond and increase in beta and non-tryotophan fluorescence.

4) Changes in plasma membrane and cytoskeleton.

5) There is loss of hexagonal cross linkage of fibres. Membrane protein and lipids are decreased. All the large membrane polypeptides are reduced along with changes in membrane rigidity.

Risk Factors of Cataractogenesis

1) Hereditary

2) Exposure to ultra violet radiation: The ultra violet radiation of 290-320 nm could induce cataract formation by photo oxidative damage involving free or plasma protein tryptophan residues in presence of molecular oxygen.

3) Dietary factors

4) Severe diarrhoea

5) Diabetes

6) Renal failure

7) Hypertension and diuretics

8) Myopia

9) Miscellaneous: smoking, alcohol, glaucoma and steroids.

Diabetic Cataract

1) Sorbitol Aldose Pathway: In patients with diabetes, there is increase in sugar alcohol which results in disturbance of NaK ATPase and concomitant lens swelling.

2) Auto-oxidation of protein

3) Theory of Non-enzymatic Glycosylation: According to this theory, the open chain of sugar attacks the amino acid of lens epithelium, resulting in S-S and thiol grouping and ultimately cataract formation.

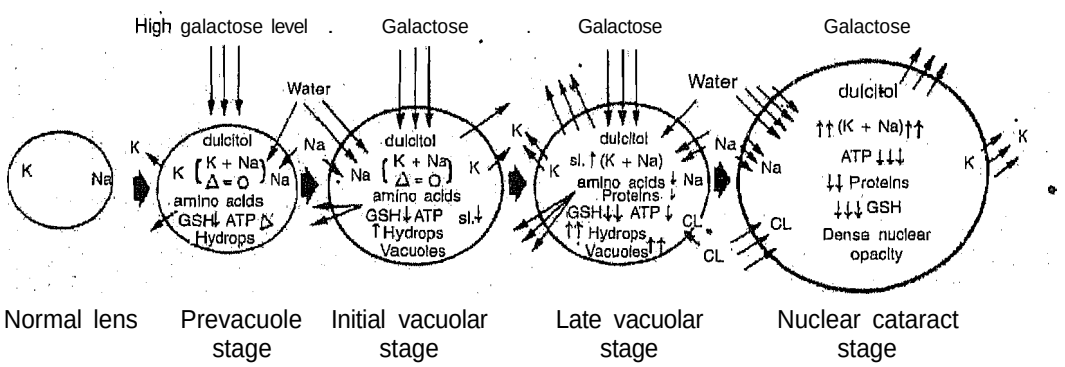

Fig. 18.4: Stages of cataractogeneris

Radiation Cataract

Exposure to radiation causes discoid posterior sub-capsular opacities. Mechanism of formation of cataract includes decrease in GSH, decrease in enzyme levels, decrease in K, increase in Na, S-S bond and decrease in protein concentration.

Steroid Induced Cataract

Posterior sub-capsular cataract is the type of cataract associated with use of steroid. Mechanism of cataract formation is leukocyte inhibition, increased proteolysis, decrease in glycosaminoglycans and MYOC gene.

Check Your Progress 3

1) Name soluble proteins of lens?

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2) Name three cataractogeneris factors?

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18.6 PHYSIO-CHEMICAL PROPERTIES OF VITREOUS

Vitreous is a clear gel like optically empty structure. Its physio-chemical properties are as under:

Weight and Volume

Body weight of vitreous is 4 grams, voluine is 4 cc and is approximately two-third the volume of entire globe.

Optical Properties

There is a fine random arrangement of vitreous fibres and a high water content. This leads to a gel like formation having a high transparency. It is optically empty.

Plasticity

Plasticity of vitreous is due to it's three dimensional network of randomly oriented road like collagen fibers. These collagen Fibers are electro-statically neutral and are not cross linked. This property allows vitreous to expand and give it its plastic property,

Viscoelasticity

This property is provided by the presence of hyaluronic acid molecular chains entangled around the collagen fibers. The numerous negatively charged group of hyaluronic acid molecules precipitates extreme volume changes, thus contributing to viscoelasticty.

Gel Stability

The combination of collagen fibers and hyaluronic acid furnishes the stability of gel system. The manner in which the collage and hyaluronic acid interact to stabilize the system is termed as frictional interaction.

Vitreous Expansion and Contraction

This property is a function of ionic charge of the vitreous structure. The positive charge in Na ions, NaCl molecule and protein neutralize the negative charge of hyaluronic acid. Washing the vitreous or neutralisation of the residual hyaluronic acid results in expansion of the vitreous gel. On the contrast if positively charged protamine sulphate is added there is contraction of the vitreous gel. Photocoagulation causes shrinkage of vitreous gel.

Blood Vitreous Barrier

It is a functional term with the inability of the vitreous to equilibrate with the blood and the surrounding fluid. The blood vitreous barrier consists of three components:

Tight junctional complexes at the level of vascular endotheliuin, pigment epithelium of retina and non pigment epithelium of ciliary body.

Basal lamina of vitreo retinal junctions: It blocks the passage of large molecules.

Vitreous Cortex: Cortical vitreous effectively blocks the movement of cells, macromolecules and cations.

Other then the factors given above the active transport pumping mechanism and small surface area are responsible for vitreo retinal barrier.

Check Your Progress 4

Give weight and volume of vitreous.

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18.7 LET US SUM UP

In this unit you have learnt that the blood ocular barriers primarily are blood retinal barrier and blood aqueous barrier. The human eye pressure is lower then the rest of the body's blood pressure. The mean arterial pressure of ophthalmic artery is 77 mmHg. The factors affecting the transparency include special properties of the corneal epithelium and tear film, anatomical arrangement of stromal lamellae of the cornea, corneal avascularity, and an immaculate balance of the corned hydration. Transparency of lens depends on avoidance of large transitions of refractoiy index. Also this.was explained with more regular, uniform and lamellar configuration of lens fibres. Visco-elasticity—This properly is provided by the presence of hyaluronic acid molecular chains entangled around the collagen fibers. The numerous negatively charged group of hyaluronic acid molecules precipitates extreme volume changes, thus contributing to viscoelasticty. In next block you will learn about ocular pathology and microbiology.

18.8 ANSWERS TO CHECK YOUR PROGRESS

Check Your Progress 1

Oxygen from: Aqueous humour, Air, and limbal blood vessels

Glucose from: Aqueous humour

Amino acids from: Aqueous humour

Check Your Progress 2

1) 78 per cent

2) For a clear optical pathway

Check Your Progress 3

1) Alpha, beta and gamma crystallins

2) Diabetes, radiation and hereditary

Check' Your Pı-ogress 4

Weight = 4 grams

Volume = 4 cc.

18.9 FURTHER READINGS

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