Section

I

Anatomy and Physiology of Cornea and Ocular Surface

Chapter

1

Anatomy and Physiology of Conjunctiva and Ocular Surface

Chapter Outline

OCULAR SURFACE

COMPONENTS OF OCULAR SURFACE

Ocular surface, the term introduced by Thoft in 1978, refers to an integrated functional unit consisting of following structures (Fig. 1.1):

- *Epithelial lining* of the lid margins, conjunctiva, and cornea;
- *Eyelid glands* such as meibomian glands, glands of Moll and Zeis;
- *Ocular mucosal adnexa* (i.e. lacrimal gland, and the lacrimal drainage system); and
- *The tear film*.

These structures are not only interconnected through a continuous epithelium but share the same vascular, nervous, immune and hormonal control systems for their proper functioning. If one of these structures is not functioning properly, the functioning of other components is also affected and gradually the whole ocular surface is compromised.

Anatomy and physiology of ocular surface thus can be described under following heads:

- *Anatomy and physiology of conjunctiva,* described in the following text in this chapter.
- *Anatomy and physiology of corneal epithelium,* which is described in Chapter 2.
- *Anatomy and physiology of tear film,* which is described in Chapter 14: Tear Film and Dry Eye Disease.
- *Anatomy and physiology of meibomian glands,* which is described in Chapter 18: Blepharitis and Meibomian Gland Dysfunction.

CONJUNCTIVA

GROSS ANATOMY

The conjunctiva is a translucent mucous membrane which lines the posterior surface of the *eyelids* and *anterior aspect* of the eyeball. The name conjunctiva (conjoin: to join) has been given to this mucous membrane owing to the fact that it joins the eyeball to the lids. It stretches from the lid margin to the limbus and encloses a complex space called conjunctival sac which is open in front at the palpebral fissure.

Parts of conjunctiva

Conjunctiva can be divided into following parts (Fig. 1.1):

- *Palpebral conjunctiva:* Marginal, tarsal and orbital
- *Bulbar conjunctiva:* Scleral and limbal
- *Conjunctival fornix:* Superior, inferior, lateral and medial.

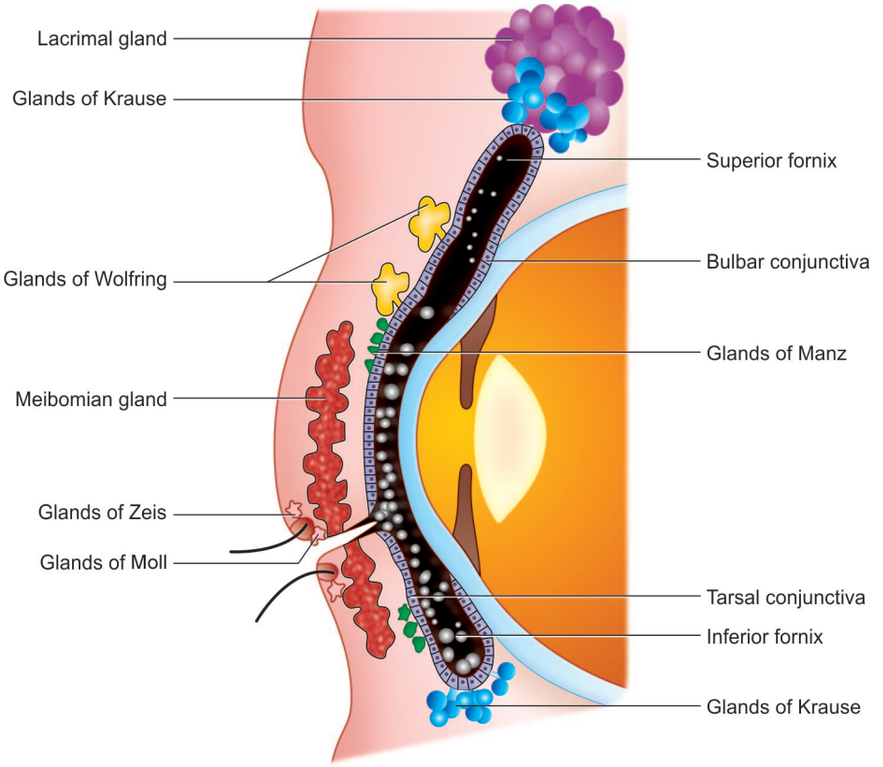

Fig. 1.1: *Ocular surface, parts of conjunctiva and conjunctival glands*

1. *Palpebral conjunctiva*

Palpebral conjunctiva is stratified squamous epithelium. It lines the lids and can be subdivided into marginal, tarsal and orbital conjunctivae.

i. ***Marginal conjunctiva*** extends from the lid margin to about 2 mm on the back of the lid up to a shallow groove—the *sulcus subtarsalis.* It is actually a transitional zone between skin and the conjunctiva proper. At the sulcus subtarsalis, the perforating vessels pass through the tarsus to supply the conjunctiva. This sulcus is a common site for lodgement of a conjunctival foreign body.

ii. ***Tarsal conjunctiva*** is thin, transparent and highly vascular. It is firmly adherent to the whole tarsal plate in the upper lid. In the lower lid, it is adherent only to half width of the tarsus. The tarsal glands are seen through it as yellow streaks. Tarsal conjunctiva is a common site for the follicular and papillary reactions.

iii. ***Orbital part*** of palpebral conjunctiva lies loose between the tarsal plate and fornix. Orbital conjunctiva of the upper lid is loose and lies over the Müller's muscle.

2. *Bulbar conjunctiva*

Bulbar conjunctiva is stratified columnar epithelium. It is thin, transparent and lies loose over the underlying structures and thus can be moved easily. It attaches firmly 1 mm posterior to sclerocorneal limbus. It is separated from the anterior sclera by episcleral tissue and Tenon's capsule. Subconjunctival vessels and the anterior ciliary arteries forming the pericorneal plexus can be seen in the loose tissue under the bulbar conjunctiva. A 3 mm ridge of bulbar conjunctiva around the cornea is called *limbal conjunctiva*. In the area of limbus, the conjunctiva, Tenon's capsule and the episcleral tissue are fused into a dense tissue which is strongly adherent to the underlying corneoscleral junction. It is the perferred site for obtaining a

firm hold (fixation) of the eyeball with the forceps during ocular surgery.

3. *Conjunctival fornix*

Conjunctival fornix is a continuous circular cul-de-sac, which is broken only on the medial side by caruncle and the plica semilunaris (Fig. 1.2). Conjunctival fornix joins the bulbar conjunctiva with the palpebral conjunctiva. It can be subdivided into superior, inferior, medial and lateral fornices.

i. ***Superior fornix.*** It extends from slightly above the upper border of the tarsal plate to a distance about 10 mm from the upper limbus and is thus located at the level of superior orbital margin. The extension of the fascial sheath of the levator and superior rectus muscles is attached to the conjunctiva in the upper part of the superior fornix. It helps in maintaining the recess of the superior fornix in the movements of the upper lid. In the subconjunctival tissue of the superior fornix are present glands of Krause and Müller's muscle. A knife passed through the superior fornix, enters the fibrous tissue between the levator and superior rectus muscles. A foreign body lodged in the superior fornix can be seen after double eversion of the upper lid.

ii. ***Inferior fornix.*** It extends from slightly below the lower border of the lower tarsal plate to a distance about 8 mm from the lower limbus and is located near the inferior orbital margin. The extension of the fascial sheath of the inferior rectus and inferior oblique muscles is attached to the conjunctival fold in the lower fornix. It helps in maintaining the recess of the inferior fornix during movements of the lower lid. Glands of Krause are lodged in the sub-conjunctival tissue of the lower fornix. A knife passed through the lower fornix will enter the fibrous tissue between the inferior rectus and inferior palpebral muscle and on further push it hits the aponeurotic expansion from the inferior rectus and inferior oblique muscles.

iii. ***Lateral fornix.*** It is a small cul-de-sac which extends to just behind the equator of the eyeball and is about 14 mm from the lateral limbus and about 5 mm from the lateral canthus.

iv. ***Medial fornix.*** It is a shallow cul-de-sac in which lie the caruncle and plica semilunaris dipped in the pool of tears called the 'lacus lacrimalis' or 'tear-lake'.

MICROSCOPIC STRUCTURE OF CONJUNCTIVA

Histologically, conjunctiva consists of three layers, namely (1) epithelium, (2) adenoid layer, and (3) fibrous layer (Fig. 1.3).

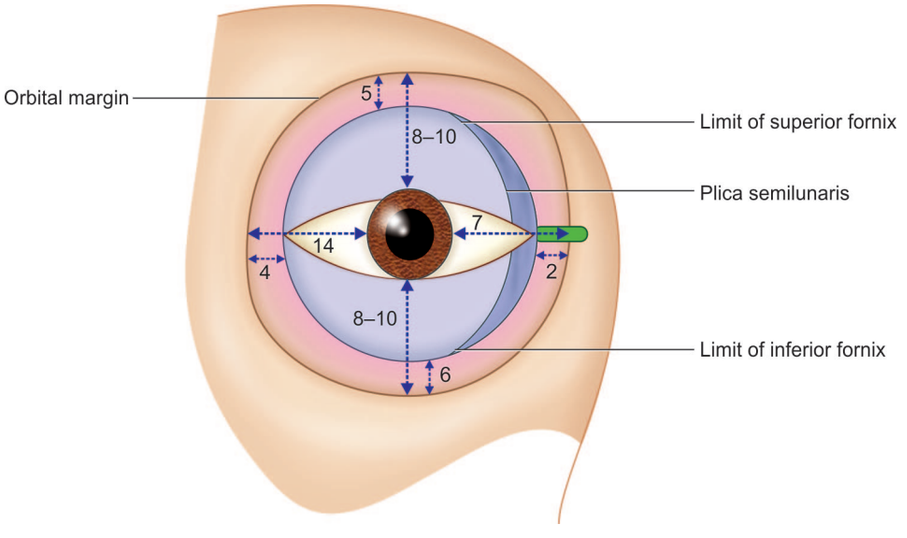

Fig. 1.2: *Conjunctival fornices*

1. Epithelium

■ ***Layers of epithelial cells*** in conjunctiva vary from region-to-region and its different parts are as follows (Fig. 1.3B):

- *Marginal conjunctiva is* 5-layered non-keratinised stratified squamous type of epithelium. The most superficial layer is of squamous cells, intermediate 3 layers of polyhedral cells and deepest layer of cylindrical cells. Goblet cells, absent at mucocutaneous junction, begin to appear in this part of conjunctival epithelium.
- *Tarsal conjunctiva* has two-layered epithelium, superficial layer of cylindrical cells and a deep layer of cubical cells in the upper lid. While the lower tarsal conjunctiva is composed of 3–4 layers of cells which from deep to superficial are layers of cubical cells, polygonal cells, elongated wedge-shaped cells and the cone-shaped cells.
- *Fornix and bulbar conjunctiva* has three-layered epithelium, a superficial layer of cylindrical cells, middle layer of polyhedral cells and a deep layer of cuboidal cells.
- *Limbal conjunctiva* is again many-layered (8 to 10) stratified squamous epithelium. Most superficial layers are one to two layers of squamous cells. Intermediate several layers are of polygonal cells and basal layer is of small cylindrical or cubical cells. The limbal epithelium forms the papillae of the limbal palisades of Vogt. The epithelium of pallisade zone provides the germinative zone for the corneal epithelium.

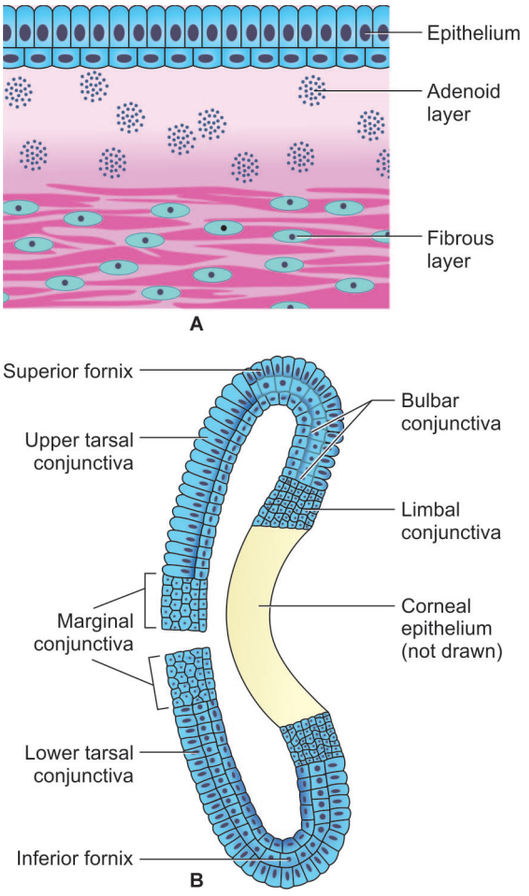

Fig. 1.3: *Microscopic structure of conjunctiva showing three layers (A); and arrangement of epithelial cells in different regions of conjunctiva (B)*

■ ***Goblet cells*** are present in between the epithelial cells in all the regions of conjunctiva, particularly aggregated in the tarsal conjunctival crypts (Henle's crypts) and on the bulbar conjunctiva nasal to the limbus (Maaz's glands).

■ ***Melanocytes*** are found in the conjunctiva at limbus, fornix, caruncle and at the site of entry of anterior ciliary vessels.

■ ***Langerhans cells*** were originally described in humans as dendritic cells in the basal corneal epithelium. Now it has been demonstrated that they are also present in almost all parts of the conjunctiva. In fact, Langerhans cells appear to represent a highly differentiated cell line from bone marrow related to the monocyte-macrophage-histiocyte series, which are present in the epidermis, mucous membranes, thymus and lymph nodes. These cells stain positively for ATPase and have no desmosomes. The Langerhans cells have surface receptors for the Fc component of IgG, the third component of compliment and surface HLA-DR (la) antigen. They are not phagocytic but function in antigenic presentation, lymphokine and prostaglandin production, and stimulation of T lymphocytes. They are reported to be involved in allograft rejection of the cornea, and in contact hypersensitivity of the skin.

Conjunctival stem cells

Conjunctival stem cells, thought to be present predominantly in forniceal conjunctiva, account for the self-renewing property of conjunctiva. These cells are intrinsically distinct from the limbal stem cells and have distinctly separate lineage than the limbal stem cell. Unlike limbal stem cells, the conjunctival stem cells are bipotent and produce both goblet and non-goblet epithelial cells.

Conjunctiva-associated lymphoid tissue

Conjunctiva-associated lymphoid tissue (CALT) is similar to mucosa-associated lymphoid tissue (MALT) present in the gut and bronchi, and plays a significant role in antigen uptake and processing. The specialized epithelial cells of MALT, called M-cells, engulf and deliver antigens to neighbouring antigen-processing cells (APCs). Thus, the CALT plays a key role in local active immunity and in developing immune tolerance. In addition to conjunctiva, MALT-like tissue is also present in lacrimal gland, tear film and cornea.

2. Adenoid layer

It is also called lymphoid layer and consists of fine connective tissue reticulum in the meshes of which lie lymphocytes. This layer is most developed in the fornices and ends at the subtarsal fold. It is not present since birth but develops after 2–3 months of life. For this reason, conjunctival inflammation in an infant does not produce follicular reaction.

3. Fibrous layer

It consists of a meshwork of collagenous and elastic fibres. It is thicker than the adenoid layer, except in the region of tarsal conjunctiva, where it is very thin. This layer contains vessels and nerves of conjunctiva. It blends with the underlying Tenon's capsule in the region of bulbar conjunctiva. The adenoid layer and the fibrous layer are collectively known as the *substantia propria* of the conjunctiva.

CONJUNCTIVAL GLANDS

The conjunctiva contains two types of glands: The mucin secretory glands (goblet cells, crypts of Henle, glands of Manz), and the accessory lacrimal glands (glands of Krause and glands of Wolfring) (Fig. 1.1).

The mucin glands

1. *Goblet cells.* These are unicellular mucous glands located abundantly within the epithelium of all the regions of the conjunctiva except the marginal mucocutaneous junction and limbal conjunctiva. The goblet cells are round or oval in shape with an eccentric flattened nucleus near the base of the cell. It contains a prominent Golgi apparatus with numerous mucus pockets in the cytoplasm. The goblet cells are formed from the deepest cells (basal layer) of the conjunctiva and migrate towards the surface. These cells are destroyed after discharging their content, the mucin. The density of goblet cells is high in children and young adults. They are more numerous on the nasal side, particularly in the bulbar conjunctiva and inferior fornix (Fig. 1.4).

The mucin secreted by goblet cells lubricates and protects the epithelial cells of the conjunctiva and the cornea and ensures the tear film stability by lowering the surface tension.

The absence of tear fluid has no effect on the desiccation, but destruction of the goblet cells as in epithelial xerosis (hypovitaminosis A) and parenchymatous xerosis, leads to desiccation of

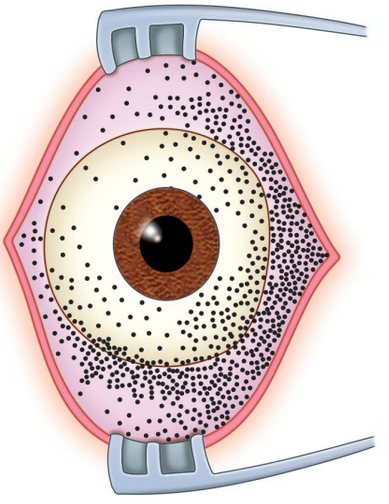

Fig. 1.4: *Goblet cell density (black dots) in different parts of conjunctiva*

the conjunctiva. The number of goblet cells is greatly increased in the inflammatory conditions.

2. *Henle's glands.* Crypts of Henle are not true glands but folds of the mucous membrane present in the palpebral conjunctiva between the tarsal plates and the fornices. These are tubular structures with lumina of 15–30 µm which contains a few goblet cells. These resemble Lieberkühn's crypts in the large intestine.

3. *Glands of Manz.* These are found in the limbal conjunctiva in animals like pig, calf or ox. Their existence in human beings is controversial.

Accessory lacrimal glands

These include:

1. Glands of Krause
2. Glands of Wolfring
3. Intraorbital glands
4. Glands in the caruncle and plica semilunaris

ACCESSORY CONJUNCTIVAL STRUCTURES

Plica semilunaris

It is a pinkish crescentric fold of the conjunctiva, present in the medial canthus. Its lateral free border is concave which becomes less prominent on abduction but forms a cul-de-sac about 2 mm in depth when the eyeball is adducted. It is a vestigial structure in human beings and represents the nictitating membrane (the third eyelid) of the lower animals.

- ***Microscopic structure.*** The epithelium of this part of the conjunctiva consists of 8 to 10 layers of cells with many goblet cells. The deepest layer is cylindrical instead of the normal cubical.

The substantia propria is composed of loose connective tissue containing numerous blood vessels, a lobule of fat, a few non-striated muscle fibres and melanophores.

Caruncle

The caruncle is a small (5 mm × 3 mm), soft, ovoid, pinkish mass situated in the inner canthus, just medial to the plica semilunaris. In reality, it is a piece of modified skin (a part of the margin of the lower lid which gets cut off due to development of the inferior canaliculi) and so is covered with stratified squamous epithelium and contains sweat glands, sebaceous glands and hair follicles. It differs from the skin by the presence of accessory lacrimal glands of Krause, presence of plenty of goblet cells and absence of keratinisation in the epithelium. The connective tissue underlying the caruncle is in contact with the septum orbitale and the medial check ligament.

- *Blood supply* is through the superior medial palpebral artery.
- *Lymphatics* drain into submandibular lymph glands.
- *Nerve supply* is from the inferior trochlear nerve.

VESSELS AND NERVES OF CONJUNCTIVA

Arterial supply of conjunctiva

Arteries supplying the conjunctiva (Fig. 1.5) are derived from three sources:

1. Marginal arterial arcade
2. Peripheral arterial arcade, and
3. Anterior ciliary arteries.

1. *Marginal arterial arcade*

It is formed by anastomosis of medial and lateral palpebral arteries and lies in the submuscular plane in front of the tarsal plate, 2 mm away from the lid margin, in each lid. The perforating branches from the marginal arterial arcade pierce the tarsus at the sulcus subtarsalis to reach the conjunctiva, where they divide into marginal and tarsal branches. The tarsal branches anastomose with the branches from the peripheral arcade.

2. *Peripheral arterial arcade*

It is situated at the upper border of the tarsus in the upper lid. Its perforating branches pierce the palpebral muscle to reach the conjunctiva and sends off descending and ascending branches.

The descending branches supply the tarsal conjunctiva and anastomose with the branches of the marginal arterial arcade at the level of sulcus subtarsalis.

The ascending branches pass upwards and then bend round the superior fornix to descend under the bulbar conjunctiva as *posterior conjunctival arteries*. At about 4 mm from the limbus, the posterior conjunctival arteries anastomose with the anterior conjunctival arteries (branches of

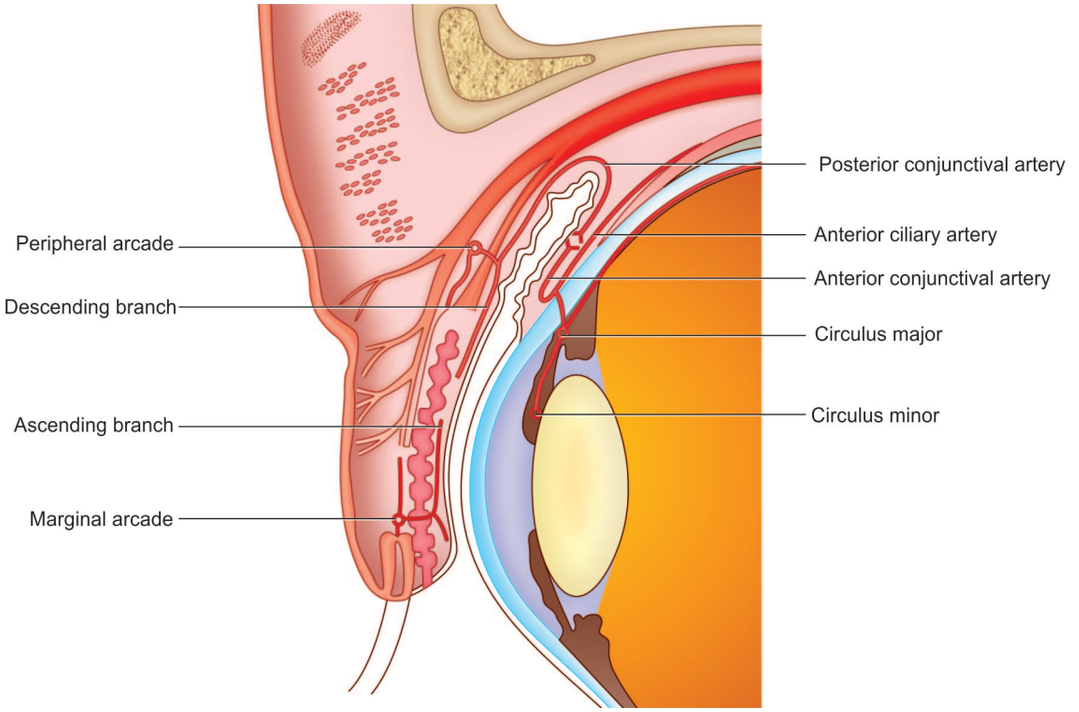

Fig. 1.5: *Blood supply of conjunctiva*

anterior ciliary arteries) forming the pericorneal plexus. The posterior conjunctival vessels move with the movement of the bulbar conjunctiva. In conjunctivitis, there is hyperaemia of the superficial conjunctival vessels derived from the posterior conjunctival vessels.

3. *Anterior ciliary arteries*

These are branches of muscular arteries (total 7—2 from each rectus muscle except 1 from the lateral rectus). These arteries give off *anterior conjunctival arteries* just before piercing the sclera at about 4 mm from the limbus.

The anterior conjunctival arteries move forward towards the limbus at a plane deeper than the posterior conjunctival arteries. These anastomose with each other forming a series of arcades parallel to the corneal margin and also form the pericorneal plexus.

To summarize

- *The palpebral conjunctiva and fornices* are supplied by branches from the marginal and peripheral arcades of the eyelids.
- *Bulbar conjunctiva* is supplied by posterior and anterior conjunctival arteries.

Venous drainage of conjunctiva

The veins from the conjunctiva drain into the venous plexus of eyelids which in turn drain into the superior or inferior ophthalmic veins.

A circumcorneal zone of veins about 5–6 mm from the limbus drain into the anterior ciliary veins.

Lymphatics of the conjunctiva

Conjunctival lymphatics are arranged in two layers: A superficial and a deep. Lymphatics from the lateral side drain into *preauricular lymph nodes* and those from the medial side into the *submandibular lymph nodes* (Fig. 1.6).

Nerve supply of conjunctiva

■ ***Distribution of nerve supply of conjunctiva*** is as below:

- *Bulbar conjunctiva, in its anterior two-thirds circumcorneal zone,* is supplied by the branches

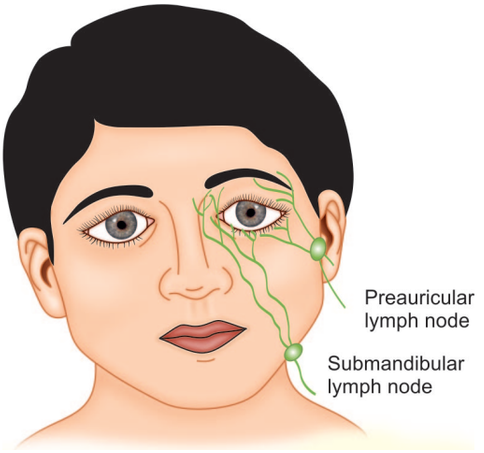

Fig. 1.6: *Lymphatic drainage of conjunctiva*

from *long ciliary nerves* (of V1) which also supply the cornea.

- *Superior plapebral conjunctiva, superior fornix and superior peripheral one-third of bulbar conjunctiva* are supplied by branches from the supratrochlear and supraorbital nerves (of infratrochlear nerve (branch of nasociliary nerve), frontal nerve and lacrimal nerves (of V1).
- *Lateral inferior palpebral conjunctiva, lateral fornix and lateral part of inferior peripheral one-third of bulbar conjunctiva* are supplied by branches from the lacrimal nerve (of V1).
- *Nasal inferior palpebral conjunctiva, nasal fornix and nasal half of peripheral one-third of bulbar conjunctiva* are supplied by branches of infraorbital nerve (of V2).

■ ***Pattern of nerve supply.*** The nerve branches supplying the conjunctiva form a subepithelial plexus in the superficial part of substantia propria. From this plexus, the fibres pass to form an intraepithelial plexus around the base of the epithelial cells and send free nerve fibrils between these epithelial cells. These nerves do not possess the myelin sheath.

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Chapter

2

Anatomy and Physiology of Cornea

Chapter Outline

ANATOMY OF CORNEA

The cornea is a transparent, avascular, watch-glass-like structure. It forms anterior one-sixth of the outer fibrous coat of the eyeball.

DIMENSIONS

- *Anterior surface* of cornea is elliptical with an average horizontal diameter of 11.75 mm and vertical diameter of 11 mm.
- *Posterior surface* of cornea is circular with an average diameter of 11.5 mm.
- *Thickness* of cornea in the centre is about 0.52 mm; while at the periphery, it is 0.67 mm.
- *Radius of curvature:* The central 5 mm area of the cornea forms the powerful refracting surface of the eye. The anterior and posterior radii of curvature of the central part of cornea are 7.8 mm and 6.5 mm, respectively.
- *Refractive power* of the anterior surface of cornea is about +48 D and that of its posterior surface is about –5 D. Thus, the net refractive power of cornea is about +43 D which is three-fourths of the total refractive power of the eye (60 dioptres).
- *Refractive index* of the cornea is 1.37.

HISTOLOGY

Histologically, originally the cornea was considered to consist of five distinct layers, from anterior to posterior, these are: Epithelium, Bowman's membrane, substantia propria (corneal stroma), Descemet's membrane and endothelium. However, recently a new layer between the corneal stroma and Descemet's membrane named Dua's layer (pre-Descemet's membrane) has been discovered, and thus now, the cornea is considered to consist of six layers (Fig. 2.1).

1. Epithelium

Corneal epithelium is of stratified squamous non-keratinized type and becomes continuous with epithelium of the bulbar conjunctiva at the limbus. It is about 50–90 µm thick, represents 10% of corneal thickness and consists of

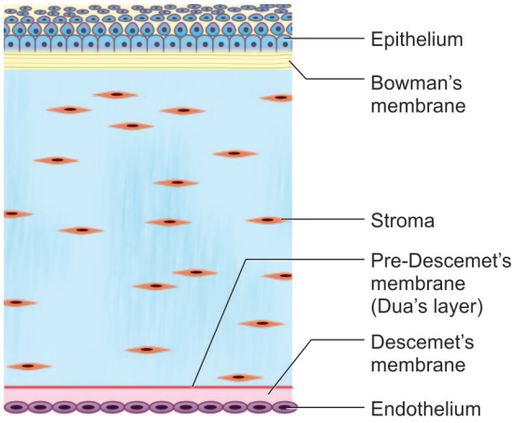

Fig. 2.1: *Microscopic structure of cornea*

5–7 layers of cells. The deepest (basal) layer is made up of columnar cells, next mid-epithelial 2–3 layers of wing or umbrella cells and the most superficial two layers are of flattened cells. The corneal epithelium sheds at a regular interval and is replaced by growth from its basal cells. It is estimated that the entire epithelium is replaced in a period of 6–8 days via mitotic activity of basal cells. Some important features of various layers of epithelium are described below.

Basal layer

Basal layer (Fig. 2.2) comprises tall columnar polygonal-shaped cells arranged in a palisade-like manner on a basement membrane. Basal cells have a width of 12 µm and density of approximately 6000 cells/mm^2. It forms the germinal layer of the epithelium and undergoes mitosis to produce daughter cells which continuously migrate anteriorly into the wing cell layer. The basal cell has an oval nucleus and its cytoplasm contains a few organelles. Some intermediate filaments, microtubules, free ribosomes, rough-surfaced endoplasmic reticulum and occasional Golgi apparatus are seen. The mitochondria are small and a few, suggesting low aerobic oxidation and more dependence on the pentose shunt for metabolism.

The basal cells are firmly joined laterally to the other basal cells and anteriorly to the wing

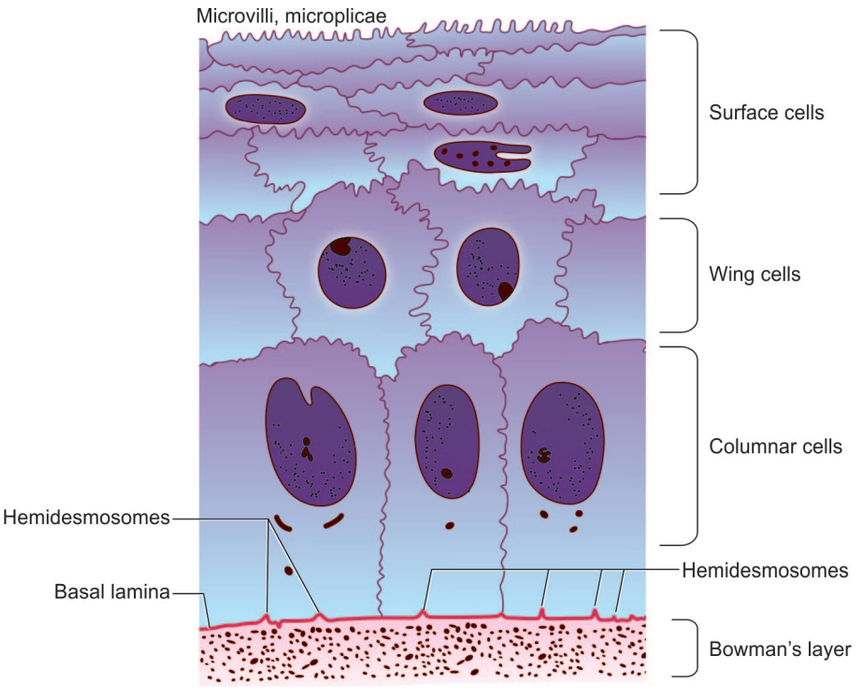

Fig. 2.2: *Microscopic structure of corneal epithelium*

cells by desmosomes and *zonula occludens*. These tight intercellular junctions account for the epithelium's transparency as well as its resistance to the flow of water, electrolytes and glucose, along with inhibiting pathological entrance, i.e. its barrier function.

■ ***Limbal stem cells.*** The basal cells of the limbal area constitute the so-called limbal stem cells. Epithelial stem cells are the undifferentiated pluripotent stem cells found in the limbal basal epithelium of palisades of Vogt. They are the source of new corneal epithelium. These slow-cycling stem cells divide and give rise to a progeny of daughter cells (*transient amplifying cells*) which amplify, proliferate continuously, migrate centripetally and serve to maintain the corneal epithelium.

Diffuse damage to the limbal stem cells (e.g. in chemical burns, Stevens-Johnson syndrome, pemphigoid, trachoma) leads to chronic epithelial surface defects and invasion of conjunctival epithelium onto the cornea.

■ ***Non-epithelial cells*** also appear within the corneal epithelial layer especially in the peripheral cornea. These cells include wandering histiocytes, macrophages, lymphocyte, and pigmented melanocyte. Antigen presenting Langerhans cells are formed peripherally and move centrally with age or in response to keratitis.

■ ***Basal lamina.*** The basement membrane of the basal cells is PAS positive structure. It is extracellular secretory product of basal epithelial cells. Posteriorly, it blends indistinctly into Bowman's membrane. Anteriorly, firm adhesions are formed between it and the basal cells by the filaments extending from the latter called *adhesion complex*, made up of hemidesmosomes and type VII collagen fibrils. In fact, the basement membrane is of utmost importance in epithelial cell adhesions. When abnormal, it is associated with recurrent erosions and epithelial defects.

Applied aspects of anchoring/adhesion complex

- Holds epithelium to basement membrane and its stroma, so its defect as seen in epidermolysis bullosa, can lead to bullae formation.
- Reduplication of basement membrane as seen with ageing or in diabetes mellitus leads to abnormal epithelial adhesions and increased predisposition to epithelial erosions.

Wing cells

Wing cells form 2–3 layers of the polyhedral-shaped cells. Their nuclei are flattened parallel to the surface and cytoplasmic organelles decrease as compared to the basal cells. They are attached with the basal cells posteriorly and other wing cells laterally and anteriorly, via tight junctions.

Flattened cells

Flattened cells constitute two most superficial cell layers. Superficial cells represent the highest level of differentiation and are chronologically the oldest epithelial cells. These cells are long (45 mm) and thin (4 mm) with flattened nuclei. The desmosomal attachment and maculae occludentes are more numerous in these cells. Further, zonulae occludentes seen in the lateral cell walls are also found in this layer. The anterior cell wall of the most superficial cells has many microvilli (each 0.5 mm in height) which play an important role in the tear film stability. The microvilli contain glycocalyx which is associated with tear film. An important feature of the superficial cell layer is the presence of junctional complexes formed with laterally adjacent cells which maintain the barrier function of the epithelium. A clinical test to determine whether the barrier is intact uses dyes such as fluorescein.

Functions of epithelium

- It is extraordinary regular in thickness with smooth wet apical surface for serving as major refractive surface of the eye.
- It serves as a major surface to respond to wound healing.
- It helps in providing barrier to fluid loss and pathological entrance to the organisms.

2. Bowman's membrane (layer)

This layer consists of acellular mass of condensed collagen fibrils. It is about 8–14 μm in thickness and binds the corneal stroma

anteriorly with basement membrane of the epithelium. It is not a true elastic membrane but simply a condensed superficial part of the stroma.

Bowman's layer is secreted during embryogenesis by the anterior stromal keratocytes and epithelium. It is composed of randomly packed type I and type V collagen fibres that are enmeshed in a matrix consisting of proteoglycans and glycoproteins.

It shows considerable resistance to infection and injury. Unlike Descemet's membrane once destroyed, it does not regenerate.

- ***Function of Bowman's membrane.*** It acts as a smooth base for epithelium uniformity thus helps in refraction.

3. Stroma (substantia propria)

This layer is about 0.5 mm in thickness and constitutes most of the cornea (90% of total thickness). It consists of collagen fibrils (lamellae) and cells embedded in hydrated matrix of proteoglycans (ground substance).

Corneal lamellae

The lamellae consist of fibrils with a macroperiodicity (640Å) typical of collagen. The stroma's collagen types are I, III, V and VI. Among the various subtypes, type I predominates. Type VII forms the anchoring fibril of the epithelium. The corneal lamellae are arranged in many layers (200–250). In each layer, they are not only parallel to each other but also to the corneal plane and become continuous with the scleral lamellae at the limbus. They vary in disposition according to the area of the cornea. They have oblique orientation in the anterior one-third of the stroma. In the posterior two-thirds of the stroma, the alternating layers of lamellae are at right angles to each other.

Studies, using X-ray diffraction techniques, have shown that the parallel arrangement of the central corneal fibrils extends to the periphery where the fibrils adopt a concentric configuration to form a 'weave' at the limbus. This imparts considerable strength to the peripheral cornea and permits it to maintain its curvature and thus its optical properties. Previous studies have shown that the corneal fibrils, running in two preferred orientations in the central cornea, bend as they approach the peripheral cornea to run circumferentially and form the peripheral collagen ring.

The parallel arrangement of lamellae in the cornea allows an easy intralamellar dissection during superficial keratectomy and lamellar keratoplasty. The peculiar arrangement of lamellae has also been implicated in the corneal transparency.

Stromal cells

The cells present among the lamellae are keratocytes, wandering macrophages, histiocytes and a few lymphocytes.

- ***Corneal keratocytes*** about 2.4 million in number constitute 2–4% of the volume of the stroma in humans. The keratocytes are fibroblasts which are found throughout the stroma, between, and occasionally extending into the lamellae. The keratocytes have a flattened cell body, a large eccentric nucleus and long branching processes which form contact with other cells in the same layer, but do not form a syncytium. It is believed that these cells produce ground substance and collagen fibrils during embryogenesis and after injury.
- ***Wandering cells*** *of* the stroma migrate from the marginal loops of the corneal blood vessels to the site of injury.

Ground substance of stroma

Ground substance of cornea consists of hydrated matrix of proteoglycans that run along and between the collagen fibrils. The primary glycosaminoglycans of stroma are keratin sulphate and chondroitin sulphate in the ratio of 3:1. Maximum concentration of keratin sulphate occurs in the centre and that of chondroitin sulphate in the periphery. The glycosaminoglycan components (e.g. keratin sulphate) of the ground substance are highly charged and account for the swelling property of the stroma. The keratocytes which lie between the corneal lamellae synthesize both the collagen and proteoglycans.

Functions of stroma

It acts as a window to the right passage and meshes with surrounding scleral connective tissue to form a rigid frame for maintaing IOP.

4. Pre-Descemet's membrane

Pre-Descemet's membrane, also known as *Dua's layer,* has been discovered in 2013 by Dr. Harminder Dua, an ophthalmologist of Indian origin working in Great Britain. Located anterior to Descemet membrane, it is about 15 mm thick acellular structure which is very strong and impervious to air. Dua's layer (DL) primarily composed of collagen type 1. Collagens 4 and 6 are also present (more in DL compared to corneal stroma). Collagen 5 is also present (weakly positive in both DL and stroma). Proteoglycans present are lumican, mimecan and decorin (intensity equal in DL and stroma). CD34 negative, which proves lack of keratocytes in DL. Unlike DM, DL does not extend to the periphery.

5. Descemet's membrane (posterior elastic lamina)

It is a strong homogenous layer which is separated from the stroma by pre-Descemet's membrane. It represents the basement membrane of the corneal endothelium from which it is produced. Though elasticity is one of its physical characteristics, it is made up of collagen and glycoprotein with no elastic fibres visible by electron microscopy. Its thickness varies with age, being 3 µm at birth and 10–12 µm in young adults. It is very resistant to chemical agents, trauma, infection and pathological processes. Even when whole of the stroma is sloughed off, the Descemet's membrane can maintain the integrity of the eyeball for long. Further, unlike Bowman's membrane, when destroyed it can regenerate. Normally, it remains in a state of tension and when torn it curls inwards on itself. In the periphery, it appears to end at the anterior limit of the trabecular meshwork as Schwalbe's line (ring).

On electron microscopy, Descemet's membrane can be divided into two distinct regions: An anterior one-third having a vertically banded pattern and the posterior two-thirds appearing amorphous and granular. The posterior surface of the Descemet's membrane, at the periphery, shows rounded wart-like excrescences called *Hassall-Henle bodies,* which increase with advancing age. Similar central excrescences, known as guttatae, are seen with advancing age in Fuchs' dystrophy.

6. Endothelium

- *Consists of a single layer of flat polygonal (mainly hexagonal) cells*, which on slit-lamp biomicroscopy appear as a mosaic on Descemet's membrane.
- *Cell density of endothelium* is around 6000 cells/mm^2 at birth. In the human adults, these cells have hardly any ability to divide. The cell count falls by about 26% in the first year and a further 26% is lost over the next 11 years. Therefore, with increasing age, the number of cells is reduced to about 2400–3000 cells/mm^2 in young adults. The defect left by the dying cells is filled by enlargement (polymegathism) of the remaining cells. Hence, these cells vary in diameter from 18 to 20 micron early in life to 40 micron or more in the aged.
- *There is a considerable functional reserve* for the endothelium. Therefore, corneal decompensation occurs only after more than 75% of the adult age cells are lost (i.e. when the endothelial cell count becomes less than 500 cells/mm^2).
- *Endothelial cells are best evaluated* by specular microscopy.
- *Endothelial cells are attached to the Descemet's membrane* by hemidesmosomes and laterally to each other by tight interdigitating junctional complexes. The desmosomal linkages and zonulae occludentes are continuous around the entire cell, and thus close the intercellular space from the anterior chamber. This linkage is calcium-dependent and plays an important role in maintaining the barrier function of endothelium.
- *Endothelium also contains an active pump mechanism* and is involved in active secretion and protein synthesis.
- *High metabolic activity and energy production* for *the above process* by the endothelial cells is

evidenced by the presence of abundant mitochondria, free ribosomes, rough- and smooth-surfaced endoplasmic reticulum and Golgi complexes in the cytoplasm of the cells. In the eye, next to photoreceptors, the endothelial cells contain the highest number of mitochondria.

BLOOD SUPPLY AND NERVE SUPPLY

Blood supply of the cornea

The cornea is an avascular structure. Small loops derived from the anterior ciliary vessels invade its periphery for about 1 mm and provide nourishment. Actually, these loops are not in the cornea but in the subconjunctival tissue which overlaps the cornea.

Nerve supply of the cornea

The cornea has a rich supply of sensory nerve endings derived mainly from the long ciliary nerves which are branches of the nasociliary nerve (a branch of ophthalmic division of the trigeminal nerve). The long ciliary nerves after arising from the nasociliary nerve enter the eyeball around the optic nerve along with the short ciliary nerves and run forward in the suprachoroidal space. A short distance from the limbus, these nerves pierce the sclera to leave the eyeball, divide dichotomously and connect with each other and the conjunctival nerves to form *a pericorneal plexus of the nerves.* About 60–80 myelinated trunks from the pericorneal plexus enter the cornea at various levels, viz. sclera (the principal regions), episclera and conjunctiva. After having gone for 1–2 mm in the stroma, the corneal nerves lose their myelin sheath, branch dichotomously and form a *stromal plexus.* Although, some nerves end in mid-stroma, most pass anteriorly and form a *subepithelial plexus.* The fibres from here penetrate the pores in Bowman's membrane, lose their Schwann's sheath, divide into filaments under the basal layer of epithelium which extend between the cells of all the layers of epithelium, and form *intraepithelial plexus.* The nerves end in the epithelium as fine-beaded filaments (Box 2.1).

Thus, the cornea has an extensive innervational density which is highest near the centre and gradually decreases towards the periphery. However, there are no nerves in the central posterior part of the cornea, Descemet's membrane and endothelium (Box 2.2).

Box 2.1 ***Pattern of corneal innervation***

Myelinated and non-myelinated axons distribute radially around periphery of cornea
↓
Enter substantia propria of stroma in radial manner and branch dichotomously (loose myelin sheath)
↓
Preterminal fibres form a plexus in mid-stroma
↓
Subepithelial plexus formed
↓
Intraepithelial plexus formed where the axons are devoid of Schwann cells

Box 2.2 ***Physiological variations in corneal sensation***

- Most sensitive at apex, least at superior limbus.
- Sensitivity lowest in morning and highest in evening.
- Sensitivity decreases with age

PHYSIOLOGY OF CORNEA

INTRODUCTION

Functions of cornea

The two primary physiologic functions of the cornea are:

1. *To act as a powerful refracting lens* of fixed focus that transmits light in an orderly fashion for proper image formation, and
2. *To protect the intraocular contents.*

In addition, the cornea also plays an important role in:

- Absorption of topically applied drugs, and
- Wound repair after anterior segment surgery or trauma.

Cornea performs these functions by maintaining its transparency and replacement of its tissues.

BIOCHEMICAL AND PHYSIOLOGICAL PROCESSES CONCERNED WITH THE FUNCTIONING OF THE CORNEA

- Biochemical composition of cornea
- Metabolism of cornea
- Corneal transparency
- Cell turnover and wound healing in the cornea

BIOCHEMICAL COMPOSITION OF CORNEA

Biochemical composition of cornea is heterogenous owing to differences in cellularity and morphology of its different layers, namely epithelium, Bowman's membrane, stroma, pre-Descemet's membrane, Descemet's membrane and endothelium.

Under normal conditions, biochemically cornea consists of approximately 80% water and 20% solids (19.8% organic matter and 0.2% inorganic salts). However, hydration of cornea is quite variable, since exposure of the cornea in the living animals allows sufficient evaporation to reduce corneal hydration.

EPITHELIUM

Corneal epithelium constitutes 10% of the total wet weight of the cornea. Its essential biochemical components are as follows:

- *Water* represents 70% of the wet weight.
- *Protein* synthesis in epithelium is five times higher than the stroma and about 2 times higher than the endothelium and Descemet's membrane.
- *Lipids* (phospholipids and cholesterol) are mainly present in the cell membranes and constitute about 5.4% of the dry weight of the epithelium. *Enzymes* necessary for glycolysis, Krebs cycle and Na^+, K^+ activated ATPase are present in high levels in the epithelium.
- The epithelium contains *ATP* (2000 mmol/kg wet weight), *glycogen* (10 mg/g), *glutathione* (75 to 180 mg/g) and *ascorbic acid* (47 to 94 mg/100 g).
- *Acetylcholine* (ACh) and cholinesterases are also present in high levels in epithelium. Perhaps these play a role in cation transport as well as in trophic nerve function.
- *Electrolytes.* Epithelium contains high concentration of K^+ (142 mEq/L H_2O) and low concentration of Na^+ (75 mEq/L H_2O) and Cl^- (30 mEq/L H_2O) as compared to the stroma (Table 2.1).

Table 2.1: *Electrolyte composition (in mEq/L H_2O) of rabbit cornea, plasma, aqueous humour and tears*

	Na^+	K^+	Cl^-
Cornea			
Whole cornea	156	28	97
Stroma	172	21	108
Epithelium	75	142	30
Aqueous humour	143.5	5.2	108
Plasma	151	5.2	109
Tears	149	12	131

STROMA

Stroma constitutes main bulk (90% of total thickness) of the cornea. It contains 75 to 80% water (wet weight). Remaining solids (20 to 25%) include mainly extracellular collagen, other soluble proteins, mucopolysaccharides (chondroitin, keratan, and ***dermatan*** sulphates) and salts (Table 2.2).

Differences in the biochemical composition of anterior and posterior stroma

- *Anterior stroma has less water* (3.04 gm H_2O dry weight) as compared to posterior stroma (3.85 gm H_2O dry weight). This is due to atmospheric drying and increased amount of dermatan sulphate which has less water sorptive capacity.
- *Less glucose,* 3.89 µm/gm H_2O in anterior stroma as compared to posterior stroma (4.93 µm/gm H_2O).
- *More dermatan sulphate and less keratan sulphate* in anterior stroma.

Table 2.2: *Biochemical composition of stroma*

Substance	%
Water	78
Collagen	15
Other proteins	5
Keratan sulphate	0.7
Chondroitin sulphate	0.3
Salts	1

Collagen

Collagen fibrils (lamellae), embedded in hydrated matrix of proteoglycans, essentially constitute the corneal stroma. These present the typical 64–66 nm periodicity of the collagen.

Collagen constitutes approximately 70% of dry weight of human cornea. Type I collagen is the predominant type, although collagen types III, V (10–20%), VI (15.1%), VII, XII and XIV have also been found in normal adult cornea. The diameter of corneal collagen fibrils (35 nm) and spacing between these fibrils (55 nm) is remarkably constant. There appears to be an inverse correlation between the number of carbohydrate units and the fibril diameter of collagen. The corneal collagen, like the collagen from other structures such as skin and tendons, has a high glycine, proline and hydroxyproline content. The mature collagen is a helix composed of two alpha chains (molecular weight 80,000) and one beta chain (molecular weight 160,000). The corneal collagen is dissolved by proteolytic enzymes such as collagenase, which has important implication in corneal ulceration.

In boiling water or acids, the corneal collagen is converted into gelatin, which accounts for the acid corneal burns being less serious than the alkali burns.

Soluble proteins

These constitute 5% of wet weight of stroma (and 25% of dry weight of the tissue) and include albumin, immunoglobulins, and glycoproteins. The high levels of antibody proteins (immunoglobulins G, A and M), present in the cornea have immunologic implications. The corneal immunoglobulins are probably derived from the serum and diffuse into the centre from the limbus.

Proteoglycans

Proteoglycans are a family of glycosylated proteins that contain at least one glycosaminoglycan chain covalently bonded to a protein core. Glycosaminoglycans (GAGs) or the so-called acid-mucopolysaccharides represent 4 to 4.5% of the dry weight of the cornea.

- ***Cornea contains three major GAG fractions,*** namely:
  - *Keratan sulphate* (50%),
  - *Chondroitin sulphate* A (25%), and
  - *Chondroitin* (25%), present exclusively in the cornea).
- ***GAGs are present in the interfibrillar space of the corneal stroma*** and account for the *'stromal swelling pressure'* (normal—60 mm Hg), i.e. its tendency to imbibe water and thus plays an important role in the maintenance of the corneal hydration level and transparency as they have water sorptive capacity.
- ***An abnormal accumulation of GAG occurs in the corneal stroma*** of the patients affected by the inborn errors of GAG metabolism known as *mucopolysaccharidosis*.

Enzymes

Glycolytic and Krebs cycle enzymes are present in the stromal keratocytes. However, the enzymatic activity of the corneal stroma is very low when compared with that of the epithelium, on weight basis. The adenosine triphosphate (ATP) content of the stroma is also low (10 to 15 mmol/kg wet tissue).

Matrix metalloproteinases

Matrix metalloproteinases (MMPs) are calcium-dependent zinc containing endoproteinase family of enzymes that breakdown components of extracellular matrix. In the cornea, they help maintain the normal framework and have a crucial role in remodelling after injury. MMPs are secreted as proenzymes by infiltrating inflammatory cells or by cells resident in the tissue. They are then activated by cleavage of a peptide from their N-terminal end. All MMPs require a metal cofactor. The MMPs of cornea have different substrates:

- MMP-1 (collagenase I) is active against collagen types I, II, and III.
- MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are active against collagen types IV, V, and VII as well as gelatins and fibronectin.
- MMP-3 (stromelysin I) breaks down proteoglycans and fibronectin.
- MMPs 7, 8, 9, 11 are other substrates.

Note. Only MMP-2 has been detected in normal cornea, the other MMPs mentioned above are found in the cornea only after injury. MMPs 1, 2 and 3 are products of stromal cells, whereas MMP-9 is produced by the corneal epithelium which is most importantly involved in the corneal inflammation.

Proteinase inhibitor of cornea

Proteinase inhibitors of cornea are mainly synthesized by resident cells of cornea; and some are derived from tears, aqueous humour, and limbal blood vessels. These include:

- α_1 proteinase inhibitor,
- α_1 antichymotrypsin,
- α_2 macroglobulin,
- Plasminogen activator inhibitors 1 and 2, and
- Tissue inhibitor of metalloproteinases

Functions Proteinase inhibitors of the cornea play a key role in corneal protection by restricting damage during corneal inflammation, ulceration and wound healing.

Electrolytes

Concentration of Na^+ is high and that of K^+ is low in the stroma as compared to the epithelium. Concentration of electrolytes in the cornea and three fluids surrounding it (viz. plasma, aqueous and tear) is shown in Table 2.1. In the stroma, the sum of diffusible cations, sodium and potassium (172 + 21 = 193 mEq/L H_2O) is in excess of the diffusible anion chloride (108 mEq/L H_2O). Part of the anions may be provided by bicarbonate ions (25 to 35 mEq/L H_2O) and remaining by the acidic GAG (acting as anions) and the corneal collagen molecules.

PRE-DESCEMET'S MEMBRANE AND DESCEMET'S MEMBRANE

These layers are made up of collagen (73%) and glycoproteins. The collagen differs from typical connective tissue collagen in that it lacks typical 640-A banded collagen fibrils and have a high content of hydroxyproline, glycine and hydroxyglycine. Unlike stroma, the Descemet's membrane does not contain GAG. The collagen of Descemet's membrane is insoluble (except in strong alkali or acids), and extremely resistant to chemical and enzymatic (collagenase) action than the corneal stromal collagen. This accounts for the resistance offered by Descemet's membrane to trauma, chemical agents, infection and a barrier to perforation in deep corneal ulcers.

ENDOTHELIUM

Owing to delicate nature of this single cell-layered structure, it has not been possible to analyse its biochemical composition without inducing artifactual changes. However, the histochemical examination of the endothelium has shown the presence of enzymes needed for glycolysis and Krebs cycle.

METABOLISM OF CORNEA

The cornea, among other activities, requires energy for maintenance of its transparency and dehydration. Energy in the form of ATP is generated by the breakdown of glucose. The most actively metabolising layers of the cornea are epithelium and endothelium, the former being ten times thicker than the latter, requires a proportionately larger supply of metabolic substrates. The sources of nutrients required for the corneal metabolism and the metabolic pathway involved are described below briefly.

SOURCES OF NUTRIENTS

1. Oxygen

- *Epithelium* derives oxygen mainly from atmosphere through the tear film (an active process) as well as through the limbal capillaries. A tight-fitting contact lens made of non-oxygen-permeable material such as polymethyl methacrylate (PMMA) interferes with oxygen uptake of epithelium and causes intracellular oedema, a decrease in epithelial glycogen and an increase in lactic acid. This confirms the fact that atmosphere is the main source of oxygen for the epithelium. Epithelium consumes oxygen ten times greater than stroma. The oxygen required by the epithelium is about one-tenth of that available from the atmosphere when the eyes are open and about one-fourth of that available from the palpebral conjunctiva when the eyes are closed.
- *Endothelium* derives most of its required oxygen from the aqueous humour, which has an oxygen tension of about 40 mm Hg.

- *Mean total corneal oxygen consumption* (QO_2) of the human cornea is approximately 9.5 ml O_2 cm^{-2} hr^{-1}. Mean QO_2 for epithelium and endothelium is 5 to 6 and that of stroma alone is 0.44.
- *Minimum oxygen tension for normal corneal hydration* ranges between 11 and 19 mm Hg. Below this critical range, the cornea will hydrate and swell.

2. Glucose

The respiratory quotient (RQ) for the cornea is 1.0, indicating that glucose metabolism is the prime energy source. It is now confirmed that the aqueous humour is the main source of glucose supply not only for the endothelium but also for the corneal stroma as well as the epithelium. A negligible amount of glucose also enters the cornea from the tear film and by diffusion from the perilimbal capillaries. It has been estimated that the minimal requirements of epithelial glucose consumption are 50–60 mg/cm^2/hr. In the absence of an exogenous supply of glucose or when the tissue needs additional energy (especially after injury or surgical wounds), the glycogen stored in the corneal epithelium is broken down to glucose at a rate of 25 mg/hr/cornea. It has been assumed that perhaps fatty acids might also be used by the epithelium and stroma after prolonged periods of glucose deprivation.

3. Amino acids

A continuous supply of the amino acids is required to allow synthesis of proteins needed for the constant shedding and replacement (by mitosis) of the epithelial cells of the cornea. It appears that like glucose, amino acids are also supplied from the aqueous humour, principally by passive diffusion. Amino acids in quantities sufficient for the synthesis of about 10 mg/hour of proteins by the epithelial cells can enter by diffusion. An additional active transport of amino acids has not been totally ruled out.

METABOLIC PATHWAYS IN CORNEA

Following pathways of glucose metabolism are present in the cornea (Fig. 2.3).

1. Glycolysis

In normal conditions, all the glucose consumed by the cornea can be accounted for by either respiratory or glycolytic activity. Histochemical studies have shown that many enzymes of the Embden-Meyerhof pathway of glycolysis and the tricarboxylic acid (Krebs) cycle are present in all of the cells of cornea.

Rate of glucose consumption by the whole cornea is approximately 100 mg/hr/cm^2 with 90% being consumed by the epithelium. Through glycolysis, glucose is broken down to lactic acid

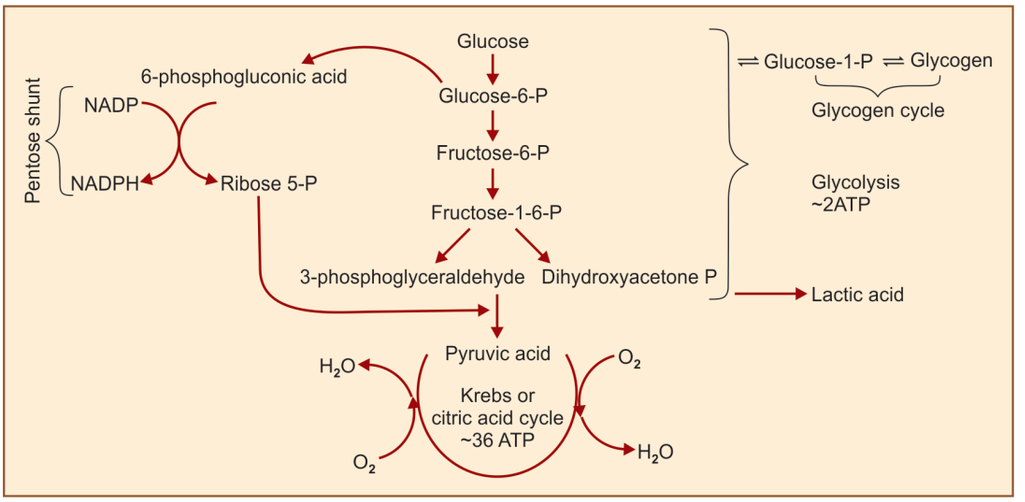

Fig. 2.3: *Pathways of glucose metabolism in cornea*

producing energy in the form of 2 molecules of ATP/molecule of glucose broken down. While, through Krebs or citric acid cycle, 36 ATP molecules are produced per molecule of glucose oxidized (Fig. 2.3).

Corneal glucose utilization studies indicate that 88% of the total glucose consumed is metabolized no further than lactic acid, leaving 12% to be oxidized by the cellular respiratory systems. This high activity of glycolysis in comparison to the limited activity of tricarboxyl acid cycle, leads to accumulation of lactate even in aerobic condition. It is likely that the lactate is eliminated from the cornea by diffusion through the epithelium.

2. Hexose monophosphate/pentose shunt

In the corneal epithelium and endothelium, glucose is also metabolised through the hexose monophosphate shunt (pentose shunt), but without a net gain in ATP. The purpose of glucose metabolism through the pentose shunt is the production of NADPH which is utilized in the biosynthesis of lipids by corneal epithelium. The ribose produced by the pentose shunt may be used to build the nucleic acids—DNA and RNA.

3. Krebs cycle

It is a complex cycle which yields H_2O, CO_2 and 36 molecules of ATP/cycle of oxidation of pyruvic acid (Fig. 2.3).

CORNEAL TRANSPARENCY

The main physiologic function of the cornea is to act as a major refracting medium, so that a clear retinal image is formed. Maintenance of corneal transparency of high degree is a prerequisite to perform this function.

Normal corneal transparency is the result of anatomical and physical factors:

- *Anatomical factors* include uniform and regular arrangement of the corneal epithelium, a peculiar arrangement of the corneal lamellae and corneal avascularity.
- *Physiological factor is* relative state of corneal dehydration.

Note. Therefore, almost any process which upsets the anatomy or physiology of the cornea will cause loss of transparency to some degree.

FACTORS AFFECTING CORNEAL TRANSPARENCY

1. Corneal epithelium and tear film

Normal epithelium is transparent due to the homogenicity of its refractive index. The basal cells are firmly joined laterally to the other basal cells and anteriorly to the wing cells by desmosomes and maculae occludentes. These tight intercellular junctions account for the epithelium's transparency as well as its resistance to the flow of water, electrolytes and glucose, i.e. its barrier function. Normal precorneal tear film plays an important role in maintaining the transparency of epithelium. Therefore, conditions associated with tear film abnormalities and/or corneal epithelial abnormalities may result in loss of corneal transparency.

2. Arrangement of stromal lamellae

Following theories have been put forward to explain the role of a peculiar arrangement of the stromal lamellae in corneal transparency. These are:

i. ***Maurice theory.*** Maurice in 1957 proposed that the cornea is transparent because the uniform collagen fibrils are arranged in a regular lattice so that scattered light is destroyed by the mutual interference. He stated that as long as the fibrils are regularly arranged in a lattice, having less diameter (275–300Å) and separated by less than a wavelength of light (4000 to 7000Å), the cornea will remain transparent (Fig. 2.4). Loss of transparency will result, if this regular arrangement is altered by stromal oedema or mechanical stress (Fig. 2.5). So, whenever the distance between these collagen fibrils is increased, it can lead to bullae formation as in pseudophakic bullous keratopathy (PBK).

Absence of lattice arrangement of fibrils on electron microscopy reported by some workers is a point against the Maurice theory.

ii. ***Theory of Goldman et al.*** Goldman *et al* in 1968 after applying diffraction theory to the problem concluded that the lattice arrangement is not a necessary condition for stromal

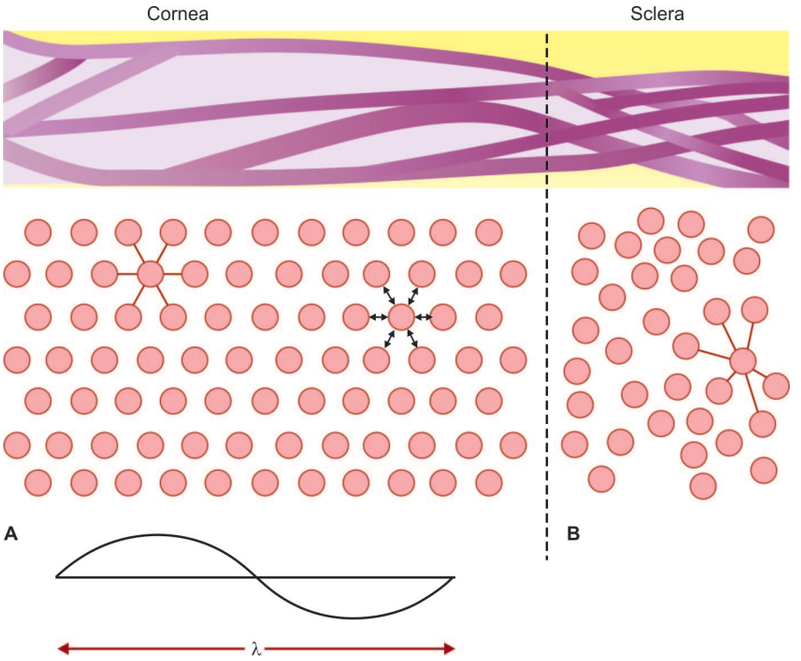

Fig. 2.4: *Cross-sectional view showing regular arrangement of the corneal fibrils as basis of corneal transparency (Maurice theory) (A); vis-à-vis irregular arrangement of sclera fibres (B)*

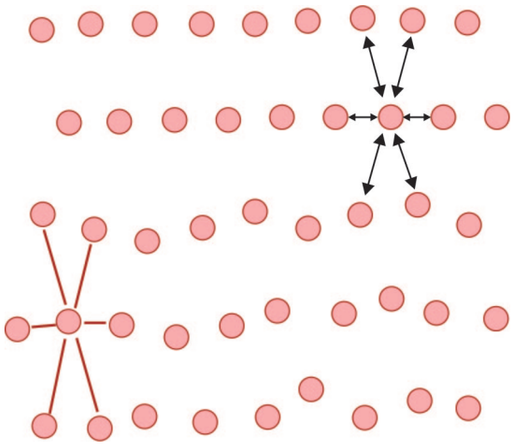

Fig. 2.5: *Cross-sectional view showing irregular arrangement of corneal fibrils as basis of loss of the corneal transparency (Maurice theory)*

transparency. Rather they postulated that the cornea is transparent because the fibrils are small in relationship to the light and do not interfere with light transmission unless they are larger than one-half a wavelength of light (2000Å). In confirmation of their theory, they found *'lakes'*—areas devoid of collagen with dimensions greater than 2000Å, in swollen non-transparent human corneas. Similar 'lakes' can be found surrounding keratocytes in oedematous human corneas.

iii. ***Expression of corneal crystallins in keratocytes*** is a further structural adaptation to minimize light scatter.

Note. However, theory of Maurice as well as that of Goldman *et al* fail to explain the occurrence of rapid clouding of cornea associated with acute rise in intraocular pressure and the rapid clearing of the cornea with reduction of intraocular pressure.

3. Corneal vascularization

The cornea is avascular except for small loops which invade the periphery for about 1 mm. However, various disease processes of the cornea may be associated with corneal vascularization. The purpose of this process is to bring the defence mechanisms into play against the noxious agents. It facilitates nutrition, transport of systemic antibiotics and

drugs. Progressive vascularization, however, is a harmful process—as it interferes with the functional properties of the cornea, especially its transparency.

Pathogenesis of corneal vascularization

The factors that keep the normal cornea avascular are unknown and so is the pathogenesis of corneal vascularization. However, various theories which have been proposed largely agree on the role of chemical and mechanical factors in producing corneal vascularization.

i. *Chemical theory.* There may be presence of vasostimulatory factor (VSF) or the breaking down (destruction) of previously existing vasoinhibitory factor (VIF).

- *Role of vasoinhibitory factor (VIF).* The idea that avascularity of the cornea might be due to the presence of a VIF preventing vascular invasion was put forward by Meyer and Chafre. They postulated that the sulphate ester of hyaluronic acid (stromal glycosaminoglycan) acts as VIF. However, other workers after experimental studies have discarded this view.
- *Role of vasostimulatory factor (VSF).* Campbell and Michaelson 1949, using experimental corneal burns, postulated the release of VSF at the site of lesion which diffuses through the stroma to the limbus and stimulates new vessel growth from the limbal plexus. The exact nature of VSF is unknown, but it is thought to be a low molecular weight amine. It has also been postulated that corneal hypoxia also induces neovascularization by activation of VSF.

ii. *Mechanical theory.* Cogan postulated that blood vessels cannot invade normal cornea because of its compact structural nature and that loosening of the compactness of corneal tissue due to oedema was mandatory for neovascularization. This mechanical theory was agreed to by many workers. However, Langham doubted the adequacy of oedema alone being responsible for the vascularization. Clinically also it has been seen that in Fuchs' dystrophy and aphakic bullous keratopathy, it is rare for the vascularization to occur even when the oedema extends to limbus.

iii. *Combined mechanical and chemical theory.* Maurice *et al* have demonstrated that both release of some vasostimulatory factor (VSF) and structural loosening of compact corneal stroma by oedema are necessary for the neovascularization to occur.

iv. *Role of leucocytes.* Some workers have postulated that corneal vascularization is a manifestation of inflammatory response and that leucocytes perform an essential role in stimulating corneal vascular growth. However, other investigators could not confirm this.

Types of corneal vascularization

Normal corneal vascularization according to depth of involvement may be:

- *Superficial vascularization*: Vessels originate from superficial limbal plexus. Superficial vessels are usually arranged in arborizing pattern, present below the epithelial layer and their continuity can be traced with the conjunctival vessels. They are dark red in colour and they branch dichotomously.
- *Deep vascularization*: Vessels are derived from anterior ciliary arteries. Deep vessels are usually straight, lie in the stroma, not anastomosing and their continuity cannot be traced beyond the limbus. They are pink in colour.
- *Retrocorneal pannus*: It is seen in syphilitic cause of interstitial keratitis.

4. Corneal hydration

The normal cornea maintains itself in a state of relative dehydration, which is essential for the corneal transparency. The water content of normal cornea is approximately 80%, which is the highest water content of any connective tissue in the body. It is kept constant by a balance of factors which draw water in the cornea (e.g. swelling pressure of the stromal matrix and intraocular pressure) and the factors which prevent the flow of water in the cornea (viz. the mechanical barrier action of epithelium which constitutes a relatively impermeable membrane) and those which draw water out of the cornea (e.g. active pumping action of the corneal endothelium). Disturbance of any of these factors leads to corneal oedema, wherein its hydration becomes above 80%, central thickness

increases and transparency reduces. Since the cornea swells only in the direction of thickness, therefore, corneal thickness and hydration are linearly related. Clinically, corneal thickness is measured using a corneal pachymeter and from it an idea of corneal hydration is made.

Factors affecting corneal hydration

i. *Stromal swelling pressure.* Swelling pressure (SP) is the 'keystone' of corneal biophysics. It is a pressure (60 mm Hg) exerted by the glycosaminoglycans (GAGs) of the corneal stroma which act like a sponge. The electrostatic repulsion of the anionic charges on the GAG molecule expands the tissue, sucking in the fluid with equal but negative pressure called imbibition pressure (IP). The values of imbibition pressure are equal to swelling pressure *in vitro* (i.e. in excised corneal tissue) but *in vivo* IP is reduced by the values equivalent to intraocular pressure (IOP), i.e. IP = IOP–SP or IP = 17–60 = –43 mm Hg. The cornea thus has a swelling pressure and a metabolic pump (the endothelium) designed to maintain it as described below. The swelling pressure generates a level of interfibrillar tension and may be the biophysical mechanism whereby the fibrils are maintained in their normal arrangement. In addition, the swelling pressure may reciprocally activate chloride channels.

ii. *Barrier function of epithelium and endothelium.* Both the epithelium and endothelium function as barriers to excessive flow of water and diffusion of electrolytes into the stroma due to their semipermeable nature. The corneal epithelium offers twice the resistance to water flow as does the endothelium and is thus practically a perfect semipermeable membrane for small solutes such as sodium chloride and urea when they are used to produce hypertonicity of the solution bathing the cornea. While, in the case of endothelium, these solutes diffuse across the layer, while water is extracted osmotically. The barrier function of the endothelium is calcium dependent. Corneal transparency is decreased and corneal thickness is increased when the corneal endothelium is damaged and to a lesser extent when the epithelium is damaged.

iii. *Hydration control by active pump mechanisms.* It is now established that the corneal endothelium plays a predominant role in controlling fluid transport due to several enzyme pump systems present in it. The pump mechanisms are active processes, requiring energy and are thus dependent upon the metabolic activity of cornea (cyclic AMP does not seem to be involved). The enzyme pump systems which collectively regulate fluid and ionic transport across the cell layer are as follows (Fig. 2.6).

- *Na^+/K^+–ATPase pump system* present in the endothelium is several folds more active than its counterpart in the epithelium. The enzyme Na^+/K^+ activated ATPase mediates the active extrusion of the Na^+ from the tissue. Oubain, a specific ATPase inhibitor, when applied topically to the eye or injected into the anterior chamber, blocks endothelial fluid transport and results in corneal overhydration.
- *A bicarbonate-dependent ATPase* has also been reported in endothelial cells; depletion of bicarbonate from incubation/perfusion medium induces swelling. The enzyme seems to be present in mitochondria and not on the plasma membrane. Enzyme inhibition by thiocyanate is paralleled by inhibition of fluid transport.
- *Carbonic anhydrase enzyme* has also been implicated in the regulation of fluid transport, since carbonic anhydrase inhibitors decrease the flow of fluid from stroma to aqueous humour. The enzyme has been localized almost exclusively in the corneal endothelium and, as in most tissues, produces bicarbonate (HCO_3) ions and hydrogen (H^+) ions.
- *Na^+/H^+ pump* has also been postulated at the lateral plasma membrane surface. From the above, it is quite clear that a complex series of metabolically dependent reactions occur in the endothelium and epithelium to maintain proper fluid/ionic balance and deturgescence in the cornea. Besides these systems, passive ion movement also occurs, in that K^+, Cl^- and HCO_3^- ions diffuse into the aqueous humour. In the contralateral direction, Na^+, Cl^- and HCO_3^- passively diffuse from the aqueous into the cornea.

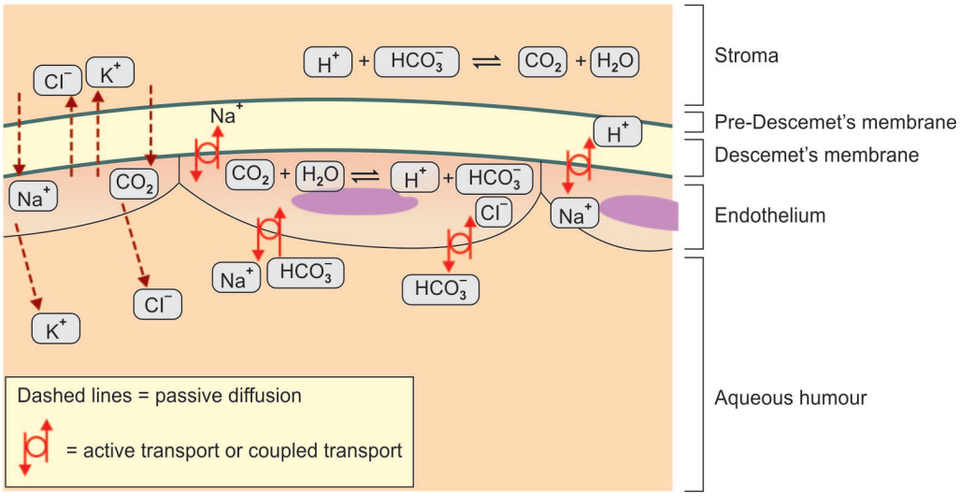

Fig. 2.6: *Endothelial active pump mechanisms controlling the corneal hydration*

iv. ***Evaporation of water from the corneal surface.*** The evaporation of water from the precorneal tear film would concentrate this fluid and increase its osmolarity relative to the cornea. The hypertonicity of the tear film could draw water from the cornea. However, this loss of fluid is readily replaced by aqueous and, therefore, figures little in corneal dehydration.

v. ***Intraocular pressure.*** It has been shown that when the intraocular pressure (IOP) exceeds the swelling pressure of the corneal stroma, epithelial oedema will occur. This correlates well with the occurrence of clinically detectable (on slit-lamp examination) corneal oedema when the IOP is raised above 50 mm Hg. However, when swelling pressure is low, corneal oedema can occur even with normal IOP as seen in endothelial dystrophy.

5. Cellular factors affecting transparency

Corneal fibroblasts (keratocytes) are important in maintaining transparency, as they are the source of stromal collagens and proteoglycans. Although most of the changes that occur in the assembly of the matrix are post-transitional, the enzymes that induce these changes are present in keratocytes in which these genes have been specifically induced. (Specific enzyme defects are associated with corneal opacification as in the mucopolysaccharidoses.)

- *Collagen turnover in early postnatal life* is about 24 to 50 hours but there is a little information on adult collagen metabolism. For both collagen and GAGs, studies on cultured keratocytes have not been very informative since these cells produce a range of GAGs not found *in vivo.* In contrast, organ cultures of cornea produce a panel of GAGs more akin to that found *in vivo.* The preferential production of KS-PG to CS/DS-PG in corneal cells from different species has been attributed to relatively hypoxic conditions and to anaerobic glycolysis, which favour the former in rabbit cornea, the development of transparency correlates with dramatic increase in concentration of KS-PG in the early postnatal period.
- *Corneal crystallins*, i.e. water-soluble protein of keratocytes (transketolase and aldehyde dehydrogenase class IA1), also contribute to corneal transparency at the cellular level.

CELL TURNOVER AND WOUND HEALING IN THE CORNEA

EPITHELIUM

Corneal epithelium heals by sequence of three processes: Migration, mitosis and differentiation. The epithelium is constantly being regenerated by mitotic activity in the basal layer of cells. However, after epithelial debridement, the

initial response of epithelium is to migrate as a flattened sheet of single cells across the stroma to close the defect. Hemidesmosomes and intercellular contacts then reform and gradually the single layer is restored to its six-layered architecture by mitotic activity in the peripheral basal cells.

- *Migration of the epithelial cells occurs in a predictable manner* as sheets of cells that produce geometric patterns as the advancing sheets meet in the centre. Migration of cells begins after 5–6 hours of injury and occurs at a rate of 60–80 μm/hr. Migration of epithelial cells is achieved by marked cytoskeletal and cell shape changes involving redistribution of actin–myosin fibrils. Changes in actin distribution in the cell are preceded by changes in actin-binding proteins such as fodrin and E-cadherin, which are under genetic regulation via growth factors. Vinoclin protein is also increased which promote interaction of contractile protein actin with adhesion proteins like talin and integrin.
- *Migration of the cells is also dependent on matrix-induced intracellular signaling* via components such as fibronectin/fibrin, laminin and collagen peptides through cell surface integrins. The role of fibronectin/fibrin in corneal epithelial resurfacing is unclear since these proteins do not appear to be essential for migration *in vitro.* However, it has been suggested that they facilitate healing where the normal basement membrane and, in particular, its laminin component has been lost, but are not essential for wound healing. The clinical application of fibronectin/fibrin to non-healing corneal ulcers has, however, been disappointing. It is also noticed that cell migration requires increased Ca^{2+}, cyclic AMP, cyclic GMP and increased acetylcholine levels.
- *Adhesion of epithelium to the basement membrane and Bowman's layer* is normally achieved via hemidesmosomes, the lamina densa and the anchoring type VII collagen fibrils. However, while hemidesmosomes form during the early stages of pre-epithelialization (18 hours), many days elapse before anchoring fibrils reappear, and many months pass before full ultrastructural integrity is restored. This may explain in part the phenomenon of recurrent erosion where there has been damage to the superficial stromal layers of the cornea.
- *Proteolytic activity in repairing epithelial defects is also important*—both urokinase type plasminogen activator and matrix metalloproteinases have been implicated.
- *Most of the mitotic activity in the epithelium takes place at the limbus* where stem cells undergo several rounds of division to repopulate the entire corneal surface. However, attempts to promote wound healing using growth-promoting agents such as epidermal growth factor and retinoic acid have not met with great success.
- *Conjunctival cells, limbal stem cells, and normal corneal epithelial cells* can be distinguished by their cytokeratin profile and by the types of protein present in their junctional complexes.

STROMA

Incisional wounds of the cornea that involve the stroma may be accidental or intentional. The immediate effect is to cause wound gape and imbibition of water from tears by the GAGs (*see* above). This causes localized opacification (light scatter) and initiates a series of events in the cornea directed to closing the wound. These include deposition of fibrin within the wound, rapid epithelialization of the wound incision, and activation of the keratocytes to divide and synthesize collagen and GAGs. During the early phase of corneal wound healing, there is loss of specialization in the keratocytes such that they revert to a fibroblast-like function and lay down collagen and GAGs found in any typical wound, e.g. hyaluronic acid, types I and III collagens, and matrix glycoproteins. In addition, the size and arrangement of the fibrils are not regular, further contributing to the corneal opacity. In extensive wounds, this opacification remains permanently, however, in smaller, well-defined wounds there is an attempt by the cornea to restore clarity by producing normal corneal matrix components.

Since the corneal curvature is a function of tension in its circumferential fibres, restoration of the normal curvature will not be achieved

unless the edges of the wound are apposed by surgical reconstruction. This is the basis of refractive corneal surgery where partial thickness wounds are intentionally left to heal in a gaping configuration; limited incisions in the periphery of the cornea alter its refractive power, the degree of which can be precisely determined by the number and depth of incisions. More recently, there has been an explosion of interest in the use argon-Fl and ultraviolet laser energy to produce precise customized incisions in the stroma by 'ablating' the tissues. Ablation is thought to be caused by photon-photon interactions derived from thermal reactions or directly by photoablation, whereby molecular disintegration is induced. Further developments in refractive surgery include laser *in situ* keratomileusis (LASIK) in which the surface of the cornea is reconfigured by raising a corneal flap, laser ablation of the exposed stromal bed and restoring the corneal flap without sutures. Both these and conventional surgical corneal incisions are fully epithelialized in the normal manner, with epithelial migration into the depths of the wound sometimes producing excessive layer of cells.

ENDOTHELIUM

The corneal endothelium does not normally undergo mitosis in humans even after direct injury as in a perforating corneal wound. Endothelial defects are repaired by migration and enlargement of surrounding cells. With age, there is a decline in the number of endothelial cells with an increase in their size and variable morphology. The response to direct wounding is to undergo 'cell slide', as occurs in the epithelium in the early stages of migration. If sufficient numbers of endothelial cells are lost, the cell layer cannot perform its pumping action and the cornea imbibes water (decompensates) and becomes opaque.

VASCULARIZATION

Vascularization of the cornea occurs when vessels from the conjunctiva or the deep episcleral plexus invade the periphery of the cornea during healing of the wound or corneal ulcers. When corneal epithelial or stromal defects fail to close promptly, often as a result of infection or during the severe inflammatory response of chemical injury such as acid and alkali corneal burns, the continued release of proteolytic enzymes causes degradation of the stroma and increases the risk of spontaneous perforation. Matrix metalloproteinases, such as matrilysin and stromelysin and MMP-9 as well as plasminogen activators (uPA and tPA) are released both by the incoming leucocytes and the resident epithelial and stromal cells. Cytokines, such as interleukin 1 (IL-1), IL-6, and IL-8, tumour necrosis factor α (TNF-α) and TGF-β, macrophage inflammatory proteins (MIP) la and b, and granulocytes-macrophage colony-stimulating factor (GM-CSF), liberated from the inflammatory and local cells stimulate further ingress of inflammatory cells and initiate a vascularization response. Vessels advance across the cornea to the site of injury or infection and contribute to the eventual opaque 'leucoma' of the healed cornea. Inhibitors of angiogenesis are also released during the process such as angiostatin and Kl-5, which are proteolytic fragments of plasminogen itself.

CORNEAL PHYSIOLOGY: APPLIED ASPECTS

Important applied aspects to be considered in relation to corneal physiology are:

- Drug permeability across cornea, and
- Effects of contact lens wear on corneal physiology.

DRUG PERMEABILITY ACROSS THE CORNEA

Topically instilled medications largely penetrate intraocularly through the cornea. Many factors which affect the drug penetration through the cornea are as follows.

1. Lipid and water solubility of the drug

The corneal epithelium and endothelium being lipophilic are crossed readily by the non-polar (lipid-soluble) drug. The stroma being hydrophilic is easily crossed by polar (water-soluble) compounds. Therefore, a drug should be amphipathic, that is, have both lipid and water solubility to readily penetrate across the cornea.

2. Molecular size, weight and concentration of the drug

As discussed above, the lipid-soluble molecules can cross the corneal epithelium easily irrespective of their molecular size, while water-soluble molecules with the molecular size less than 4 A only can filter through the pores which exist in the cell membrane.

It has been reported that the substances with molecular weights of less than 100 can pass readily through the cell membrane and those with more than 500 cannot.

It has also been reported that when the substances with large molecular size are used in high concentration, then a small amount of drug can cross the cornea following laws of mass action. The rate of penetration through the cornea of the drug such as pilocarpine, homatropine, atropine and steroids depends upon their concentration in the solution.

3. Ionic form of the drugs

The drugs intended for topical use in eye must have capacity to exist in both ionized and non-ionized form for a better penetration through the cornea since only non-ionized drugs can penetrate through the epithelium and the ionized drugs can pass through the stroma. True electrolytes or non-electrolytes cannot penetrate these various barriers. Therefore, fluorescein, a negatively charged ion, cannot penetrate the intact epithelium and this property forms the basis of fluorescein dye test.

A typical model of the drug existing both in non-ionized and ionized form for penetration through cornea has been proposed by Kinsey for homatropine. As shown in Fig. 2.7, in the tear film, ionized homatropine (R_3NH^+) converts into non-ionized free base (R_3N) which readily crosses cornea through the epithelium and gets ionized (R_3NH^+) in the stroma. Near the endothelium, it again becomes non-ionized (R_3N), crosses it and in the aqueous humour becomes ionized (R_3NH^+).

4. pH of the solution

pH may affect the penetration of the solutes by its effect on the electrical charges and stability of solutions. The pH of the solution may be varied from 4 to 10 without affecting the permeability of the epithelium, but solution outside this range increases the permeability.

5. Tonicity of the solution

Hypotonic solutions (those below 0.9% of sodium chloride) increase the permeability of the epithelium considerably.

6. Surface active agents

Agents that reduce surface tension, increase corneal wetting and, therefore, present more

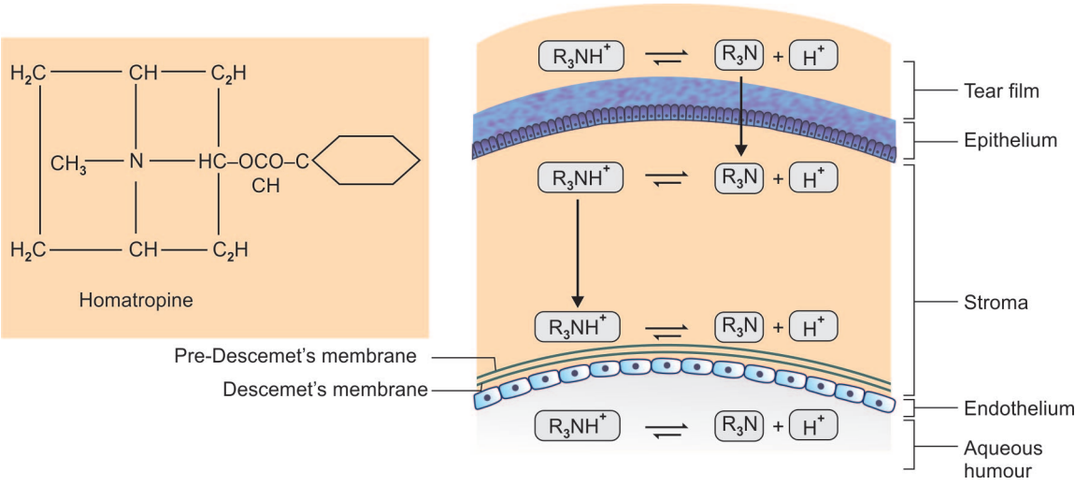

Fig. 2.7: *A model of drug (homatropine) existing in both ionized and non-ionized from for penetration through the cornea (after Kinsey)*

drug for absorption. Benzalkonium chloride used as preservative also acts as wetting agent and thus increases the drug absorption.

7. Pro-drug form

Pro-drug forms are lipophilic and after absorption through epithelium are converted into proper drug which can easily pass through stroma. For example, dipivefrin is pro-drug which is converted into epinephrine after its absorption into the eye. Dipivefrin is more lipophilic than epinephrine and thus its corneal penetration is increased 17 times.

EFFECTS OF CONTACT LENS WEAR ON CORNEAL PHYSIOLOGY

Contact lenses predominantly affect the function of the epithelium. This layer receives its oxygen from tears and its glucose from the circulation via the aqueous and the limbal vessels. Contact lenses reduce the direct availability of oxygen to the epithelium, thus shifting the balance from aerobic to anaerobic metabolism. Lactate levels in the cornea are doubled with contact lens wear and carbon dioxide production is increased. The induced acidosis has a direct effect on stromal hydration by impairing stromal deturgescence mechanism (see below).

Hard (rigid) contact lenses are usually made from polymethylmethacrylate (PMMA) and have the greatest effect on corneal function; in addition to restricting oxygen availability, hard lenses deplete glycogen stores, even though the level of glucose availability is not reduced. It has been suggested that hard lens-induced inhibition of aerobic enzymes such as hexokinase reduces direct glucose utilization by the cornea. Prolonged wear of hard contact lenses is, therefore, not possible owing to the damaging effect on corneal transparency induced by the disturbed metabolism.

Soft contact lenses are made from polymers of HEMA, poly-HEMA vinylpyrrolidones, silicone, or other similar materials, and permit extended wear of lens owing to their permeability to oxygen and carbon dioxide. However, there is still some degree of lactate accumulation with soft lenses and prolonged use appears to affect the function of the endothelium. Manufacturers of contact lenses are continually producing newer biomimetic type lenses with increased water content (up to 59%) in attempts to support normal corneal physiology (hydrogel lenses).

Gas permeable rigid lens, which combines the reduced toxicity of PMMA with high gas transfer capability, is a popular compromise in contact lens type.

DK value of contact lenses. The wide variety of lens types and materials have led to their being characterized on the basis of their oxygen flux defined as the DK value,

$$\text{Oxygen flux} = DK/L \times DP$$

where D is the diffusion coefficient, K is the solubility and L is the thickness of lens material. DP is the change in the partial pressure of oxygen across the material. HEMA and PMMA have a low oxygen flux, while hydrogels and silicones have a high flux.

Both the thickness of the lens and the DK value determine its suitability for use in term of its gas permeability. The actual amount of oxygen that reaches the cornea is the most important factor in the design of a contact lens and most practioners describe contact lenses in terms of their equivalent oxygen performance (EOP).

Contact lenses may have deleterious effects on the epithelium, causing:

- Thinning, reduction in the hemidesmosome density and the number of anchoring fibrils, and reduced adhesion of the epithelium to the basement membrane. This may be a direct effect of low oxygen transmitting lenses on basal epithelial cell proliferation. This is especially true of extended wear hydrogel lenses.
- In severe cases, excessive use of contact lenses produces epithelial oedema and keratopathy in the form of punctate epithelial erosions.
- Rigid contact lenses also produce tear film instability by causing damage to the epithelium in the mucin layer in particular.
- They also cause limbal redness, epithelial microcytes formation and endothelium polymegethism.

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