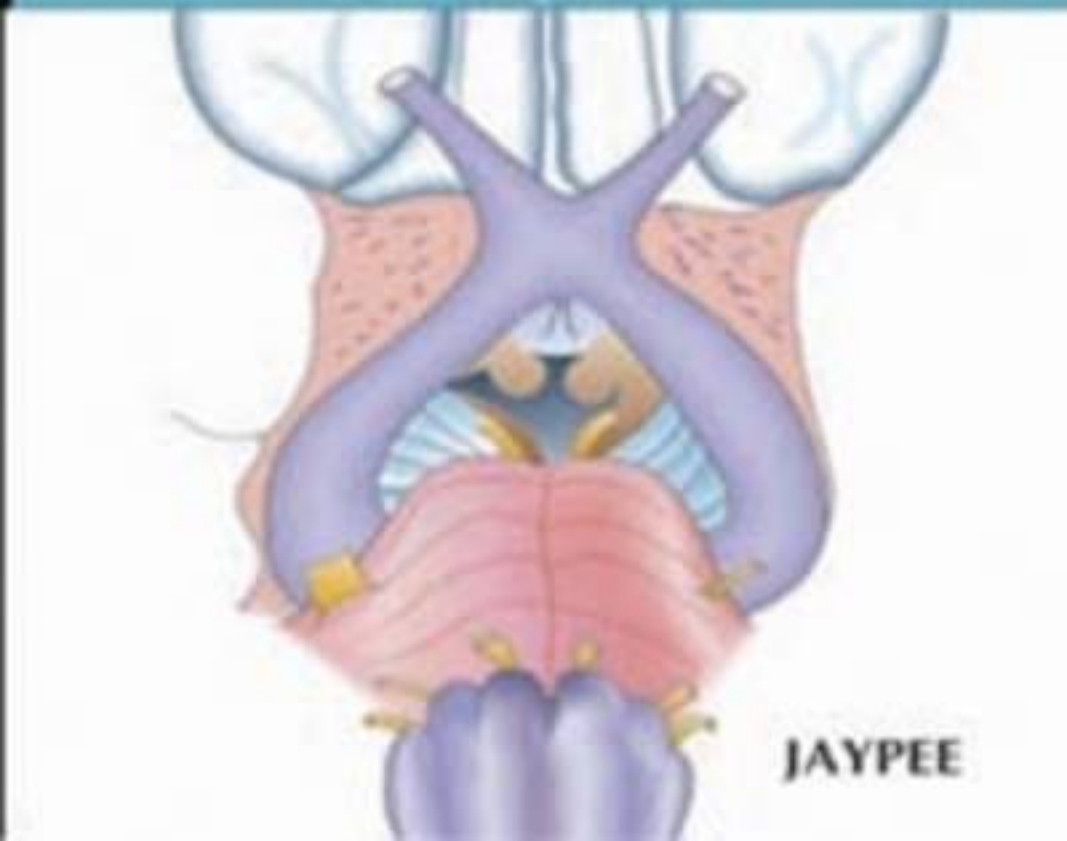
Basic Sciences in OPHTHALMOLOGY

V Velayutham



JAYPEE

Basic Sciences in OPHTHALMOLOGY

Basic Sciences in OPHTHALMOLOGY

Author

V Velayutham MS, DO

Director and Superintendent
Regional Institute of Ophthalmology
Madras Medical College, Chennai
Tamil Nadu, India

Co-Authors

K Vasantha MS FRCS
Head Cornea Department
Regional Institute of Ophthalmology
Madras Medical College, Chennai
Tamil Nadu, India

Mythili MD (Biochem)
Professor of Biochemistry (Rtd)
Madras Medical College, Chennai
Sri Balaji Medical College, Chennai
Tamil Nadu, India

G Leela MD DCP
Director Institute of Pathology
Madras Medical College, Chennai
Tamil Nadu, India

Sundar MD (Micro)
Assistant Professor
Regional Institute of Ophthalmology
Madras Medical College
Chennai
Tamil Nadu, India

JAYPEE BROTHERS MEDICAL PUBLISHERS (P) LTD

New Delhi • Ahmedabad • Bengaluru • Chennai • Hyderabad
Kochi • Kolkata • Lucknow • Mumbai • Nagpur • ST Louis (USA)

Published by
Jitendar P Vij

Jaypee Brothers Medical Publishers (P) Ltd

Corporate Office
4838/24 Ansari Road, Daryaganj, **New Delhi** - 110002, India, Phone: +91-11-43574357

Registered Office
B-3 EMCA House, 23/23B Ansari Road, Daryaganj, **New Delhi** - 110 002, India
Phones: +91-11-23272143, +91-11-23272703, +91-11-23282021
+91-11-23245672, Rel: +91-11-32558559, Fax: +91-11-23276490, +91-11-23245683
e-mail: jaypee@jaypeebrothers.com, Website: www.jaypeebrothers.com

Branches

- 2/B, Akruti Society, Jodhpur Gam Road Satellite
  Ahmedabad 380 015, Phones: +91-79-26926233, Rel: +91-79-32988717
  Fax: +91-79-26927094, e-mail: ahmedabad@jaypeebrothers.com
- 202 Batavia Chambers, 8 Kumara Krupa Road, Kumara Park East
  Bengaluru 560 001, Phones: +91-80-22285971, +91-80-22382956, 91-80-22372664
  Rel: +91-80-32714073, Fax: +91-80-22281761, e-mail: bangalore@jaypeebrothers.com
- 282 IIIrd Floor, Khaleel Shirazi Estate, Fountain Plaza, Pantheon Road
  Chennai 600 008, Phones: +91-44-28193265, +91-44-28194897, Rel: +91-44-32972089
  Fax: +91-44-28193231 e-mail: chennai@jaypeebrothers.com
- 4-2-1067/1-3, 1st Floor, Balaji Building, Ramkote Cross Road,
  Hyderabad 500 095, Phones: +91-40-66610020, +91-40-24758498
  Rel:+91-40-32940929, Fax:+91-40-24758499, e-mail: hyderabad@jaypeebrothers.com
- No. 41/3098, B & B1, Kuruvi Building, St. Vincent Road
  Kochi 682 018, Kerala, Phones: +91-484-4036109, +91-484-2395739
  +91-484-2395740 e-mail: kochi@jaypeebrothers.com
- 1-A Indian Mirror Street, Wellington Square
  Kolkata 700 013, Phones: +91-33-22651926, +91-33-22276404
  +91-33-22276415, Rel: +91-33-32901926, Fax: +91-33-22656075
  e-mail: kolkata@jaypeebrothers.com
- Lekhraj Market III, B-2, Sector-4, Faizabad Road, Indira Nagar
  Lucknow 226 016 Phones: +91-522-3040553, +91-522-3040554
  e-mail: lucknow@jaypeebrothers.com
- 106 Amit Industrial Estate, 61 Dr SS Rao Road, Near MGM Hospital, Parel
  Mumbai 400 012, Phones: +91-22-24124863, +91-22-24104532,
  Rel: +91-22-32926896, Fax: +91-22-24160828
  e-mail: mumbai@jaypeebrothers.com
- "KAMALPUSHPA" 38, Reshimbag, Opp. Mohota Science College, Umred Road
  Nagpur 440 009 (MS), Phone: Rel: +91-712-3245220, Fax: +91-712-2704275
  e-mail: nagpur@jaypeebrothers.com

USA Office

1745, Pheasant Run Drive, Maryland Heights (Missouri), MO 63043,
USA, Ph: 001-636-6279734

e-mail: jaypee@jaypeebrothers.com, anjulav@jaypeebrothers.com

Basic Sciences in Ophthalmology

First Edition: 2009

ISBN 978-81-8448-608-7

Typeset at JPBMP typesetting unit
Printed at Ajanta Offset & Packagings Ltd., New Delhi

To
Regional Institute of Ophthalmology
and
Government Ophthalmic Hospital–My Temple

Preface

It has been my long time wish to bring a comprehensive book on *Basic Sciences in Ophthalmology*. For a postgraduate in ophthalmology, a comprehensive book on Basic Sciences is an absolute need from examination point of view. This book does not claim to be a complete treatise in Basic Sciences, but the aim is to guide the postgraduates to prepare the Basic Sciences for the theory examination.

This attempt is a teamwork. I must acknowledge and thank my colleagues Prof (Dr) K Vasantha, Prof (Dr) Leela, Prof (Dr) Mythili and Dr Sundar for their hard and sincere efforts and also their contributions. A feedback from teachers and students is most welcome.

I thank my wife for supporting me in my academic activities.

Finally, I thank M/s Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, for having taken the sincere efforts to publish this book.

V Velayutham

Contents

Section 1

Anatomy

1 The Eyeball

The eyeballs, which are responsible for vision the most precious sensation, is well protected by the bony orbit. The eyeballs are situated in the anterior part of the orbit closer to the roof and lateral wall than the floor and the medial wall. When a line is drawn from the superior orbital margin down to the inferior margin it will just touch or will be very close to the cornea. But on the lateral side the eyes are exposed. Hence, any injury to the eye is more common from the lateral side causing rupture globe on the upper medial part of the eye. Beside the orbit, the lids also give protection to the eyeballs.

The eyeball is not a true sphere. The anterior part, which is formed by the cornea is more curved with a radius of curvature of 8 mm or even less. The posterior part formed by the sclera is a bigger sphere of 12 mm radius. Since the cornea is more curved the anteroposterior diameter is around 24 mm while the horizontal diameter is about 23.5 mm. The vertical diameter is still less (23 mm).

The eyeball has three layers: The outer tough sclera with the cornea anteriorly, the vascular choroid forms the middle layer and the neurosensory retina forms the inner most layer.

Now let us see the different parts of the eye individually (Fig. 1.1).

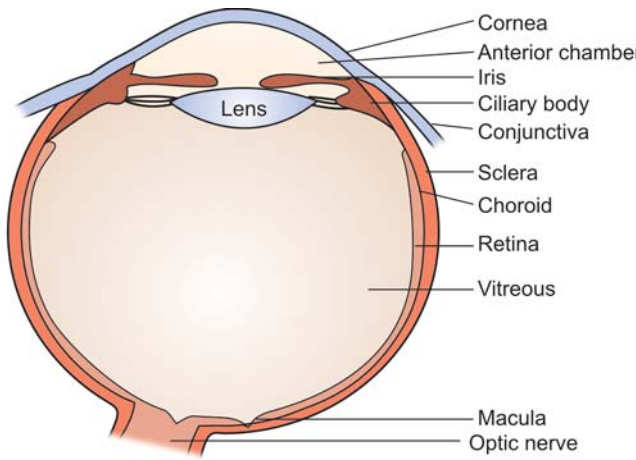


Fig. 1.1: Parts of an eyeball

Anatomy of the Eyelids and Adnexa

The eyelids are highly mobile folds of skin, which are very essential for the protection of eyes. The lids protect the eyes from injuries as well as control the amount of light entering the eyes. Only when the eyes are closed the visual cortex can take rest.

The lids spread the tear fluid uniformly over the eyeball by blinking there by keeping the surface of the eye moist. The excessive secretion of tears is also pumped out of the palpebral fissure by the lids. Correct eyelid position and function are needed for spreading the tear fluid uniformly over the conjunctiva and the cornea.

Embryology

The lids develop from the mesenchymal condensations above and below the optic cup (the frontonasal and maxillary processes). The skin develops from the surface ectoderm. Differentiation begins in the 4th to 5th week of gestation. It starts at the lateral canthus and then elongates medially. By the 8th week both the lids are seen. The palpebral fissure is initially round. The mesenchyme from the condensations forms the tarsus and the orbital septum while that from the second visceral arch form the nerve fibres and blood vessels. By the 10th week the lids begin to fuse from the edges. By the 12th week the lids are fused together protecting the developing cornea from the amniotic fluid. The cilia also develop at about this time while the hair follicles develop a little later. The meibomian glands and the glands of Zeis and Moll develop from the epithelial cells. Separation of eyelid margins start at the anterior margin around the fifth month. This is completed by 7th or 8th month. The palpebral muscles develop from the mesoderm.

Superficial Anatomy (Fig. 1.2)

The contour of the eyelids is different in different races but usually the lateral canthus is 2 mm higher than the medial. The highest point of the upper eyelid is just medial to the centre of the lid. The lid covers about 2 mm of the upper part of the cornea. The lower eyelid lies just below the cornea and rises just a little when the eyes are closed. The upper eyelid extends up to the eyebrow while there is no clear-cut demarcation from the lower lid. Medially the nasojugal sulcus and laterally the malar sulcus are present in the skin. At the sulci the skin is adherent to the periosteum thereby limiting any effusion.

The Canthi

The lateral canthus has an acute angle and there is a groove, which passes downward from the lateral canthus which lies 1 cm from the fronto-zygomatic suture.

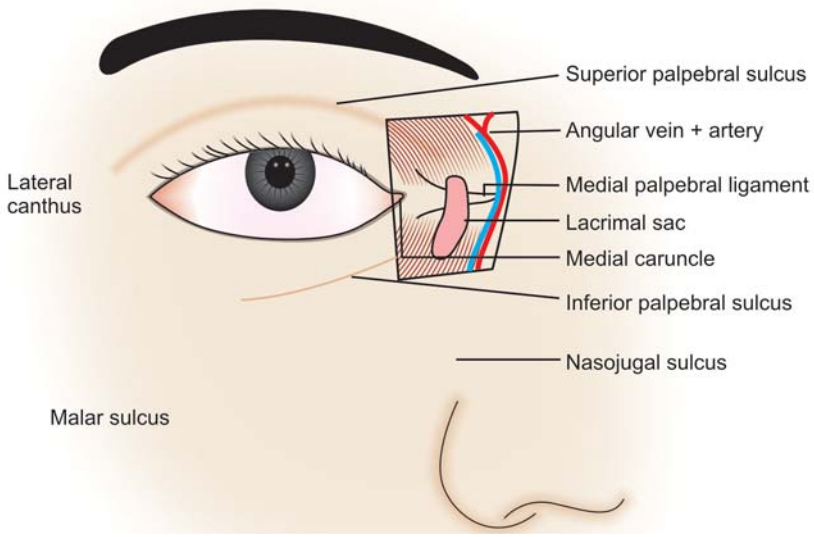


Fig. 1.2: Superficial anatomy

The lower margin of the medial canthus is horizontal while the upper margin is directed slightly upwards. A small area called the lacus lacrimalis separates the medial canthus from the globe. This gap is filled by a yellowish lacrimal caruncle and a reddish semilunar fold called the plica semilunaris. It is the rudimentary form of nictitating membrane, and it contains sympathetic muscle fibres. As the caruncle is modified skin, sebacious glands and hair follicles will be present in the caruncle. Closely opposed to the plica is an elevation called the lacrimal papilla near the medial end of the lids in which the lacrimal puncta are situated. This demarcates the ciliary portion of the lid from the lacrimal portion.

The palpebral fissure measures 30 mm horizontally and 15 mm vertically. When the eyes are shut the upper lid moves down and covers the eyeball but when the eyes are partially closed the part of the cornea just below the centre is exposed. This is the reason for exposure keratitis and injuries occurring in this area.

The Structure of the Lids (Fig. 1.3)

The margin of the lid is 2 mm broad. The anterior border is rounded and has two or three rows of eyelashes. The upper eyelid has more number of lashes, which turn upwards while lower lashes turn downwards. It takes about ten weeks for the lashes to grow and replace lost ones.

The posterior margin is sharp and is closely opposed to the eyeball. Between the orifices of the meibomian glands and the eyelashes is the grey line, which separates the anterior part of the lid from the posterior.

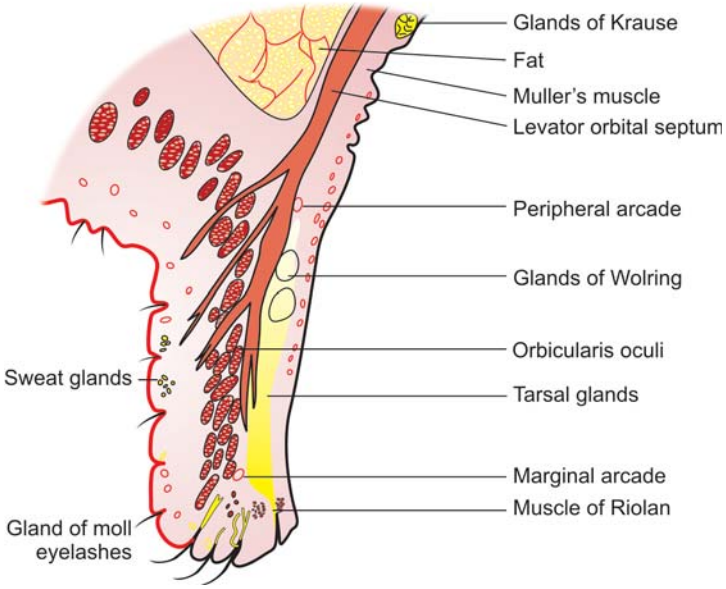


Fig. 1.3: Structure of the lids

The anterior most structure is the skin followed by a layer of subcutaneous areolar tissue, the orbicularis oculi, submuscular areolar tissue, the tarsal plate, a layer of nonstriated muscle and finally the conjunctiva.

The skin is very delicate and because of this during old age it develops many folds and even overhangs the lateral part of the upper lid. A furrow is formed in the upper lid by the attachment of the levator palpebrae superioris. The nasal portion of the lids is smoother and contains less number of hairs. The hairs on the lids are smoother and have small sebaceous glands. The sweat glands are also of small size. Besides this light pigmented cells are present in the eyelid skin which can wander and change the color of the skin in the same individual. The mucocutaneous junction lies behind the openings of the meibomian glands.

The subcutaneous areolar tissue has loose connective tissue and this can easily accommodate large amount of fluids and blood. At the medial and lateral ends the skin is adherent to the ligaments underneath.

Muscular Layer

The fibers of orbicularis palpebrarum supplied by the facial nerve are circularly arranged around the palpebral aperture. The fibers overlap as in a dove's tail. The ciliary part of the muscle occupies the whole of the lid margin and is called the muscle of Riolan.

The submuscular areolar tissue occupies the area between the orbicularis and the tarsal plate. The main nerves to the eyelids lie in this space. Hence,

when one wants to anesthetize the lid the anesthetic agent has to be injected under the orbicularis into this space. In the upper lid this space is divided into pretarsal and preseptal space by the levator. The peripheral arterial arcade is present in the pretarsal space. The preseptal space is bounded in front by the orbicularis and behind by the septum. Above this is a cushion of fat.

Fibrous layer: This forms the framework of the lids and foundation for attachment of muscles and orbital septum. The central thick portion is called the tarsal plates and the peripheral thin portion is formed by the septum orbitale.

The tarsal plates give shape and firmness to the lids. This consists of dense fibrous tissue in which the tarsal glands are situated. Even though there is no cartilage the tarsal plate appears like cartilage. The lateral ends are 7 mm from the Whitnall's tubercle. Medially they end at the level of the lacrimal puncta. The upper tarsus is larger; crescent shaped and is about 11 mm in size at the center. The lower tarsus is 5 mm in size and is oblong in shape. Both are 29 mm in size and 1mm thick. The orbicularis oculi is situated anteriorly while the palpebral conjunctiva, which is firmly attached to it, lines the posterior surface. The free border of the tarsus joins the septum orbitale.

The superior border of the tarsus gives attachment to the nonstriated muscle (Mullers) while the inferior palpebral muscle is attached to inferior border of the lower tarsus.

The medial palpebral ligament arises from the medial end and is attached to the frontal process of the maxilla. Its lower border is free but its upper border is continuous with the periosteum. At the anterior lacrimal crest the ligament divides into two. The posterior part covers the upper part of the lacrimal sac. The anterior part which contains the lacrimal canaliculi encloses the caruncle and pass over the lacrimal fossa. The lacrimal sac lies under the medial angle. If an incision is made 2 mm medial to the medial canthus the dissection must be made laterally to expose the sac (Fig. 1.2).

The lateral palpebral ligament is attached to the orbital tubercle in the zygomatic bone 11 mm below the frontozygomatic suture. This is not as strong as the medial palpebral ligament. A lobule of lacrimal gland is present between the lateral palpebral ligament and the lateral check ligament. The levator palpebrae superioris is attached to the upper border. The lower border is continuous with the expansion of the inferior oblique and the inferior rectus.

The palpebral fascia or septum orbitale: When the periorbita merges with the periosteum a thickening called arcus marginale is formed. The septum orbitale extends from the arcus to the tarsal plates. The membrane freely moves with the lid. It is thicker and stronger on the lateral side compared to the medial and in the upper lid than the lower lid. Through the weaker areas of the septum fat protrudes out in old age. On the lateral side the septum lies in front of the lateral palpebral ligament then it runs along the orbital margin, in front of the pulley of the superior oblique and gets attached to the posterior

lacrimal crest behind the Horner's muscle, lacrimal sac and medial palpebral ligament. It lies in front of the medial check ligament. It then gets attached to the anterior lacrimal crest at the level of the lacrimal tubercle. Inferiorly this the septum is attached to the orbital margin. The inferior medial palpebral artery runs in between the caruncle and the Horner's muscle.

The septum is pierced by the following structures:

a. the lacrimal vessels and nerves
b. the supraorbital vessels and nerves
c. the supratrochlear nerve and artery
d. the infratrochlear nerve
e. the anastomosis between the angular and ophthalmic veins
f. the superior and inferior palpebral arteries
g. The levator palpebrae superioris in the upper lid and in the lower by the prolongation of the inferior rectus.

The nonstriated muscle fibers lie deep to the septum orbitale in both the lids. The fibers of these muscles arise from the levator in the upper lid and prolongation of the inferior rectus in the lower.

THE GLANDS OF THE LIDS

The Meibomian Glands

These are otherwise called the tarsal glands. These are sebaceous glands without hair follicles. They are arranged vertically in a parallel row 25 in the upper lid and 20 in the lower lid. They secrete the oily layer of the tear film. It is an acinar gland arranged around a central tubule.

Ciliary glands of Moll: Spiral tubules of sweat glands lie close to the bulbs of the cilia. This is different from the glomerulus type present in the sweat glands. They open separately or into the glands of Zeis.

The sebaceous glands of Zeis: Two modified sebaceous glands are present for each eyelash. Unlike other hair follicles there is no erector pilorum muscle.

Blood supply: The medial and lateral branches of the ophthalmic artery and lacrimal arteries supply the lids. The two palpebral arteries join together to form the tarsal arches in the submuscular plane close to the lid margin. In the upper lid there is another arch formed by the medial palpebral artery in front of the upper margin of the tarsal plate.

The lids are supplied by blood from two sources. The external carotid through the facial, superficial temporal and infraorbital branches and the internal carotid through branches of ophthalmic artery such as dorsal, nasal, frontal, supraorbital and lacrimal arteries.

The veins form a dense plexus in the fornices and drain into the veins of the forehead and temple or into the ophthalmic vein.

Lymphatics

The lymphatics in front of the tarsus drain into the skin whereas those of the structures behind drain into the conjunctiva. The lateral lymphatics drain into the preauricular and parotid nodes whereas those from the medial drain into the submandibular lymph glands.

Nerve Supply

The medial part of the eyelid is supplied by the supra-trochlear and infra-trochlear nerves while the lateral side is supplied by the lacrimal branches of the ophthalmic division of the trigeminal nerve. The lower lid is supplied by the infraorbital nerve.

Development the cartilages, fat and connective tissue develop from the frontonasal and maxillary processes formed by the neural crest cells.

MUSCLES PRESENT IN THE LID

Orbicularis Oculi (Fig. 1.4)

This muscle forms an elliptical sheet surrounding the palpebral fissure and spreads a little over the cheek, forehead and also temporally.

Orbicularis is the sphincter muscle of the eyelids. The palpebral part closes the lids without effort and is used for blinking to prevent drying of the cornea. It also holds the lid in contact with the globe. The orbital part is used to close the lids tightly. The palpebral portion is opposed by the levator palpebrae while the orbital portion is opposed by the frontalis. Eyelid closure during sleep involves active tonus of orbicularis oculi and inhibition of levator palpebrae superioris.

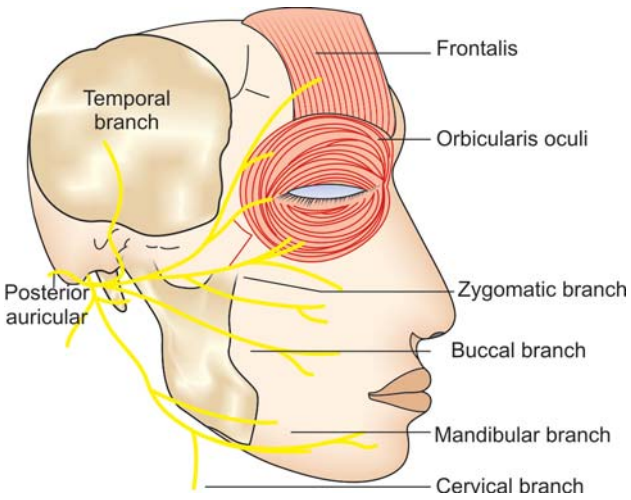


Fig. 1.4: Orbicularis oculi

The palpebral portion is divided into pretarsal and preseptal fibers. The junction between the two, forms furrows in the upper and lower lid. The fibers meet laterally in the lateral palpebral raphe. It is adherent to the dermis at the medial and lateral canthi.

The orbital portion originates from the medial side of the orbit medial to the supraorbital notch, from the maxillary process of the frontal bone, frontal process of the maxilla, medial palpebral ligament and lower orbital margin medial to the infraorbital foramen. The fibers sweep across the orbital margin some of which forms a complete ring.

The Horner's Muscle

Pars lacrimalis or tensor tarsi arises from the upper part of the posterior lacrimal crest and lacrimal fascia behind the lacrimal sac. It divides into two and surrounds the canaliculi and becomes continuous with the pretarsal fibers and the muscle of Riolan, which lies in the eyelid margin.

The upper zygomatic branch and the temporal branch of the facial nerve supply the upper part of the muscle. The lower zygomatic branch supplies the lower part.

The Corrugator Supercilii

Originates from the medial end of the superciliary ridge, passes laterally and inserts into the skin of the eyebrow about its middle. It pulls the eyebrows towards the nose producing a furrow in the forehead skin when a person frowns. It also forms a protection to the eye from bright sun light by forming a projection. The superior zygomatic branch of the facial nerve supplies it.

Occipitofrontalis

The occipital part of the muscle arises from the nuchal line of the occipital bone and its extension into the mastoid bone and passes into the epicranial aponeurosis. The frontalis part arises from the epicranial aponeurosis midway between the coronal suture and the orbital margin and is inserted into the skin of the eyebrow. It raises the eyebrows and draws the scalp forwards producing wrinkles on the forehead. It is the muscle of attention.

This muscle supplied by the posterior auricular and temporal branches of the facial nerve.

Musculus Procerus

It occupies the bridge of the nose and is attached to the nasal bones and lateral nasal cartilages and also to the skin. It pulls the skin down producing the transverse ridges giving it the name the muscle of aggression or menace. The superior buccal branch of the facial nerve supplies it.

Eyebrows

The eyebrows give protection to the eye from small particles along with the eyelashes, which stimulates reflex eyelid closure when something comes in contact.

The brows demarcate the junction of the upper eyelid with the scalp and are formed by a hairy elevation of skin. The skin is very thick but highly mobile. The hairs are also thick but silky and are arranged in a comma shape. The medial end lies under the orbital margin, the middle portion lies along the orbital margin and the lateral end lies above it. The subcutaneous tissue contains fat and fibrous tissue.

The frontalis, orbicularis oculi and corrugator supercilii are present in the eyebrows. The submuscular layer continues with the dangerous area of the scalp. Any infection or hemorrhage in the layer IV of the scalp extend to the eyebrow.

THE LACRIMAL APPARATUS

The lacrimal gland secretes tears, which drain through a series of ducts into the upper fornix of the conjunctival sac. These ducts pass through the palpebral part of the gland before opening into the palpebral fissure. Hence if this part is removed it is equal to removing the whole gland. Besides lubrication the tears are needed to keep the epithelial cells alive.

From the conjunctival sac the tear evaporates. The excess tears pass through the canaliculus into the lacrimal sac and then into the inferior meatus of the nose through the nasolacrimal duct.

Lacrimal Gland

The lacrimal gland is situated above and anterolateral to the eyeball. It is divided into two parts anteriorly by the levator palpebrae superioris. The large orbital portion, which is almond shaped, is situated in the fossa present in the roof of the orbit behind the septum orbitale. The superior convex surface is in contact with the bone while the inferior concave surface lies in contact with the levator palpebrae superioris, its tendon and the lateral rectus.

The palpebral portion is smaller and lies just above the lateral end of the upper fornix where it can be seen when the upper lid is pulled upwards and laterally.

The lacrimal gland is a tubulo racemose gland resembling the parotid. It consists of masses of lobules, which are pinhead sized with fat in between. The acini consist of two layers of cells surrounding a central canal. The basal cells are myoepithelial and can contract. The other cells secrete tears and are cylindrical in nature. They contain granules, which disappear after secretion of tears. There is very little connective tissue in the young.

Ligaments to the lacrimal fossa loosely connect the gland superiorly to the bone and to the zygomatic bone inferiorly. The lacrimal nerves and vessels enter the gland at the posterior border. The veins join the ophthalmic vein.

The lymphatics of the lacrimal gland drain into the preauricular node.

Nerve supply: The lacrimal nerve is the sensory nerve, and the greater superficial petrosal nerve is for tear secretion. Sympathetic supply is from the cervical sympathetic trunk innervate the lacrimal gland.

The greater superficial petrosal nerve: (nerve of tear secretion) The axons of start from the superior salivatory nucleus, continue as the nervus intermedius along with the seventh cranial nerve to the geniculate ganglion without synapsing and becomes the greater superficial petrosal nerve. It runs in front of the petrous temporal bone and then under the trigeminal ganglion to join the deep petrosal nerve (sympathetic) to form the nerve of the pterigoid canal (vidian nerve) in the foramen lacerum. Following this the parasympathetic fibers relay in the pterygo palatine (sphenopalatine or Meckel's) ganglion and pass through the zygomatic nerve to reach the lacrimal gland with the lacrimal nerve.

Post-ganglionic sympathetic fibers reach the gland along the lacrimal artery, deep petrosal nerve or the lacrimal nerve, which gives the sensory supply.

The glands of Krause, which are present in the upper fornix and Wolfring present in the orbital border of the tarsus, are accessory lacrimal glands.

Development

During the sixth or seventh week of development the mesenchyme present in the neural crest cells develop into acini. The tear production starts only about a month after birth.

Conjunctiva

The conjunctival sac is so called since it joins the eyeball with the lids and is formed by a transparent membrane, which is divided, into the bulbar conjunctiva, fornix and the palpebral conjunctiva. The conjunctival epithelium is continuous with the corneal epithelium.

The palpebral conjunctiva is closely adherent to the tarsal plate unlike the bulbar part, which is freely mobile except for a few millimeters close to the cornea where it is firmly attached to the Tenon's capsule.

The conjunctiva develops from the ectoderm lining the lids and the ectodermal cells in front of the globe.

The Fornix

The superior fornix extends up to the level of the orbital margin but the inferior stops a bit above the inferior margin. The levator and the expansion of the inferior rectus muscle deepen the fornices when they contract. On either side

of the center the conjunctiva is in contact with the orbital fat and it is in this region the infiltrations and hemorrhages from the base of the skull reach the subconjunctival space. The conjunctival epithelial cells and proliferating goblet cells are present in the fornix. Few cells are present in the bulbar conjunctiva and the limbus also. Since the stem cells are well protected the conjunctiva is replaced easily after injuries.

The loose episcleral tissue under the bulbar conjunctiva contains the anterior ciliary vessels and the tendons of the recti. The tissues blend at the limbus where a series of radiating ridges called the palisades of Vogt appear.

The epithelium at the margin of the lid is formed by keratinized stratified epithelium, which later becomes nonkeratinized as it reaches the tarsus. The stratified cuboidal cells over the tarsus become columnar in the fornices and squamous in the bulbar conjunctiva.

The goblet cells that form 10% of the basal cells are more numerous in the fornix, over the tarsal conjunctiva and inferonasal bulbar conjunctiva. These cells are unicellular glands secreting mucin which is very essential for keeping the eye moist.

The submucosal layer contains lymphocytes, which interact with the epithelial cells through growth factors, cytokines and neuropeptides. The lymphatics of the lateral part drain into the parotid and the medial into the submandibular lymph nodes.

The bulbar conjunctiva is supplied by the anterior ciliary vessels. The palpebral conjunctiva is supplied by the peripheral arterial arcade and the marginal arcade. The arterial plexus around the cornea is arranged in two layers superficial and deep. In superficial corneal lesions, the superficial layer is congested. In diseases of iris, ciliary body and deep portions of the cornea the deep plexus will be congested. The veins drain into the palpebral veins, ophthalmic vein and the muscular veins.

The sensory innervation is by lacrimal, supraorbital, supratrochlear and infraorbital branches of the fifth cranial nerve.

Lacrimal Drainage System (Fig. 1.5)

The Puncta

Each punctum is situated on an elevation called the papilla lacrimalis. They are small transversely oval or round and are situated at the medial end of the ciliary part of the lid. The area around the punctum is slightly avascular making it easier to locate the punctum when it is occluded. When the eyes are closed the upper punctum lies 0.5 mm medial to the lower punctum. The puncta normally face backwards and are kept patent by a ring of fibrous tissue. The orbicularis oculi press the punctum towards the lacus lacrimalis in which the puncta glide when the eye is open or shut.

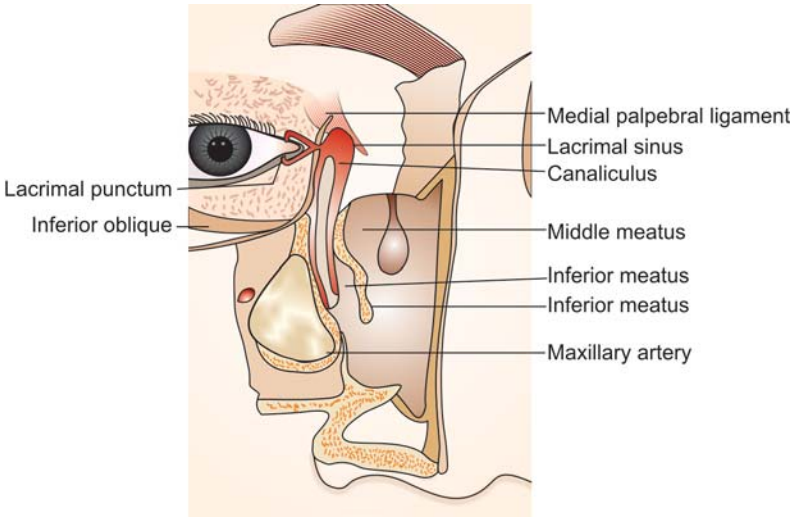


Fig. 1.5: Lacrimal drainage system

Lacrimal Canaliculi

The canaliculi at first run vertically downwards from the punctum for 2 mm and then bend medially to run for another 8 mm before entering the sac at the sinus of Maier a diverticulum situated 2.5 mm below the apex and behind the middle of the lateral surface of the sac. The lower canaliculus is longer and extends more laterally than the medial. The canaliculi enter the sac either separately or they form a common canaliculi and then enter the sac.

The canaliculi are lined by stratified squamous epithelium surrounded by elastic tissue. It can be dilated 3 times its normal size of 0.5 mm.

Lacrimal Sac

The lacrimal fossa in which the sac is located is in the lacrimal bone and frontal process of the maxilla in the anterior part of the medial wall of the orbit. Below it is continuous with the nasolacrimal duct. The sac slopes laterally and slightly backwards. The nasolacrimal duct slopes laterally forming an obtuse angle at its junction with the sac. The sac is enclosed by the periorbita (lacrimal fascia). The ethmoidal sinuses and the middle meatus are present medial to the sac. The skin and orbicularis oculi are present laterally and the origin of inferior oblique is from the floor of the orbit just lateral to the lacrimal fossa.

The lacrimal sac is formed from the cells derived from, surface ectoderm that sinks into the space between the lateral nasal and maxillary prominences. The canaliculi develop from the upper part of this solid column of cells. The cells grow downwards to form the nasolacrimal duct. This solid column must undergo degeneration and shedding of the central cells to form the nasolacrimal

duct. This occurs first in the sac region. The canalization of nasolacrimal duct is complete by about the sixth month. If the debris inside is not shed properly congenital dacryocystitis occurs.

The angular vein crosses the medial palpebral ligament 8 mm from the medial canthus. Sometimes a tributary may be present medial to this vein. Hence, incisions for sac surgeries must be made close to the medial canthus.

Since the upper part of the medial palpebral ligament is closely attached to the sac any injury can tear the sac wall causing swelling in the sac region during blowing of the nose. Any swelling of the sac also occurs below this attachment.

The lacrimal fascia, which surrounds the sac and the septum orbitale and the check ligament of the medial rectus are present behind the sac.

Nasolacrimal Duct

The lacrimal sac continues downwards as the nasolacrimal duct. It opens into the inferior meatus of the nose and is about 15 mm long. When the duct is narrow as in females it is more prone for obstruction leading on to chronic dacryocystitis. The canal as such is formed by a groove in the maxilla and by the lacrimal bone and the lacrimal process of the inferior concha or the so-called turbinate bone. The duct descends downwards, laterally and slightly posteriorly. A line drawn from the medial canthus to the first upper molar will correspond to the direction of the duct. If the duct stops at the level of the bony canal itself the orifice is round and can be easily seen. Sometimes it has a membranous tube and ends in slit like opening, which is difficult to find. There is a valve called the valve of Hasner at the orifice. This will prevent air from entering into the sac when one blows the nose.

From the figure one can see that the duct is very close to the maxillary antrum. Any lesion in the antrum will easily affect the duct also.

The duct and the sac are lined by two layers of epithelium. The inner layer is columnar and outer layer is formed by flattened cells. They also contain goblet cells and mucus glands. The duct is surrounded by a rich plexus of veins, which when engorged as in inflammation of the nasal mucous membrane can obstruct the duct.

The duct is supplied by the superior and inferior palpebral branches of ophthalmic artery and angular artery and infraorbital artery.

The lymphatics drain into the submandibular and the deep cervical glands.

Nerve supply: Is by infratrochlear and anterior superior alveolar nerves.

Excision of the sac often causes reduction in tear secretion. So it is thought that there is some reflex relation between the nerve supply of the sac and the lacrimal gland.

Distribution of the tears: The tears secreted by the lacrimal gland enter the conjunctival sac through the ducts and are spread over the anterior surface of

the eyeball by the blinking of the lids. The tears then form a small strip along the lid margin which can be seen very clearly when seen with a slit lamp. This is prevented from overflowing by the meibomian secretion. There is a collection of tears close to the medial canthus in front of the plica and medial to the caruncle called the lacus lacrimalis. The tears then enter the canaliculi through capillarity and by expansion of the canaliculi by the action of orbicularis. When the orbicularis contracts it also opens the sac due to its attachment to the sac and the medial palpebral ligament. The tear fluid is then sucked into the sac. The tears are then pushed into the nasolacrimal duct by the elastic sac. When the elasticity of the sac wall is lost it is called an atonic sac. In this condition there will be a swelling in the region of the sac, but when it is pressed the tears will enter the nose, as the duct is not obstructed.

This matter on conjunctiva can be shifted after the lacrimal drainage system at point marked with place conjunctiva here.

2 The Cornea

The cornea is the most important structure needed for convergence of the parallel rays falling onto the eye. The curvature of the cornea is greater than that of the rest of the globe. Hence, a slight furrow is formed at the junction of the cornea and sclera.

When the cornea is observed from the front it appears elliptical as it is partly covered above by the sclera with a horizontal diameter of 12 mm and vertical diameter of 11 mm. From behind it forms a circle of 11.5 mm diameter. As the cornea is usually more curved in the vertical meridian astigmatism with rule is found.

The cornea is 0.52 mm thick in the center and about 0.67 mm thick in the periphery. The radius of curvature is 7.8 mm anteriorly and 6.5 mm posteriorly.

The cornea is made of five layers (Fig. 2.1).

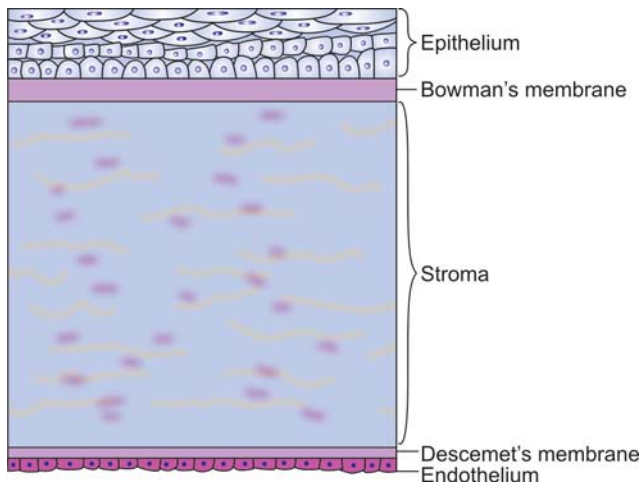


Fig. 2.1: Corneal layers

EPITHELIUM

This is the most superficial layer with 5 or 6 layers of cells and about 50 microns thick. The basal cells are columnar with flat bases and rounded heads. The nuclei are oval and placed near the head of the cell. The basal cells form

the germinal layer and are continuous with the same type of cells in the conjunctiva. Above the basal cells there is a layer of wing cells, which sit over the basal cells. The next two layers are polyhedral. The uppermost cells are flattened but are not keratinized. The flattened nuclei project backwards so that an extremely smooth surface is created. The cells are also tightly arranged so that the tear fluid cannot enter into the cornea. The cells have microvilli, which project into the tear film. Adhesions between the cells are maintained by desmosomes and cell bridges called prickles. The basement membrane merges with the Bowman's membrane.

Bowman's membrane is a thin sheet of homogenous tissue, which is parallel with the corneal surface. It does not regenerate when it is destroyed. It is composed of type IV collagen, laminin and other proteins.

The stroma or the substantia propria forms the major part of cornea and is made of multiple lamellae of collagenous tissue. The bands of each lamella are parallel to each other while alternate layer make a right angle. Each lamella covers the whole cornea and it is difficult to separate the lamellae in live corneae. The posterior lamellae have thick fibers and minimal interlamellar connections whereas the anterior lamellae have thinner fibers. A few oblique fibers are also seen. The lattice arrangement of the collagen fibrils is partly responsible for the corneal transparency. This pattern acts like a diffraction grating to reduce light scattering by destructive interface. The size of the lattice elements is smaller than the wavelength of visible light.

Corneal transparency also depends on keeping the water content of the cornea at 78%. This is controlled by both the epithelium and endothelium. The endothelial pump is by ion transport between the aqueous and endothelium, maintained by sodium and potassium ATPase. The negatively charged stromal glycosaminoglycans tend to repel each other producing a swelling pressure (SP). The intraocular pressure tends to compress the cornea. The imbibition pressure of corneal stroma is IOP—SP.

A few flattened cells called keratocytes are present in between the collagen fibrils. The keratocytes continuously digest and manufacture stromal molecules. The extracellular matrix of the stroma contains collagens and proteoglycans. The collagen fibrils are of type IV and type V types with type VI collagen in between. Corneal proteoglycons are decorin, dermatan sulphate and lumican along with keratan sulphate.

The Descemet's membrane is a strong homogenous membrane that is very resistant. The membrane is sharply defined from the stroma and is very resistant to chemical agents and toxins. When cut it curls up into the anterior chamber, but it does not contain any elastic tissue. In the periphery a few well-defined wart like elevations are seen on the posterior surface called Hassal Henle bodies. The Descemet's membrane contains two layers the lamina densa on the stromal side and the lamina lucida on the endothelial side. The lamina lucida is composed of a homogenous, fibrillogranular material and is called the nonbanded zone.

Folds in Descemet's membrane begin to occur when corneal thickness is increased by 10% or more. Epithelial edema occurs when the thickness is more than 0.7 mm. A central corneal thickness greater than 0.62 mm suggests a higher risk for symptomatic corneal edema after intraocular surgery.

The endothelium consists of flattened epithelium like cells continuous with fetal epithelium, which may persist in front of the iris. These are immature cells and hence they cannot multiply.

Blood supply: Small loops of anterior ciliary vessels invade the periphery for about 1 mm in the subconjunctival connective tissue. Otherwise the cornea is avascular and it receives nutrition from the aqueous. Oxygen also diffuses through the tear film.

Nerve supply: The ciliary nerves branch of the ophthalmic division of the trigeminal nerve supply the cornea. After entering the cornea the nerve fibers loose their myelin sheath and divide into anterior and posterior branches. The anterior fibers form a plexus under the Bowman's membrane. Besides this a subepithelial and intraepithelial plexus are also seen. Neurotransmitters in the cornea include acetylcholine, catecholamines, substance P and calcitonin gene related peptide.

Limbus (Fig. 2.2)

This is the junction between the cornea and sclera. At the limbus the stratified columnar conjunctival epithelium of the conjunctiva transforms into the stratified squamous epithelium of the cornea. Anterior limbus is the transition from the clear cornea to gray–posterior limbus is where the gray cornea becomes the white sclera. There is a gap of 1-1.5 mm between the two limbi. The anterior limbus corresponds to the end of Bowman's membrane and the Descemet's membrane. The structures present at the limbus are the conjunctiva, Tenon's capsule, episclera, corneoscleral stroma and the trabecular meshwork.

The surgical limbus is 2 mm wide. The anterior bluish zone overlies the clear cornea and extends from the Bowman's layer to the Schwalbe's line where the Descemet's membrane ends (the Descemet's ends slightly more towards the sclera). The posterior white zone overlies the trabecular meshwork and extends from the Schwalbe's line to the scleral spur.

The limbus also contains the epithelial stem cells of the cornea and the limbal plexus of vessels, which provides nutrition to the limbal stem cells. The limbal stem cells are present in the basal limbus. The limbal stem cells move towards the cornea and become transient amplifying cells. These cells form the basal layer of the cornea and differentiate to form the other epithelial layers of the cornea, i.e. the wing cells and then the superficial cells. The stem cells also form a barrier between the conjunctival epithelium and the corneal epithelium and prevent the former from growing over the cornea.

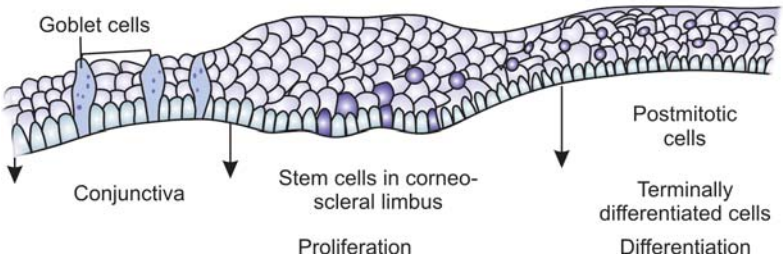


Fig. 2.2: Corneoscleral junction—Limbus (stem cells)

Development (Fig. 2.3)

Once the lens vesicle separates from the surface ectoderm the cornea starts developing. By the fifth week the ectoderm contains two layers of cells. The basal layer secretes collagen fibrils and glycosaminoglycan, which will become the stroma. Later the cells derived from the neural crest cells form the first wave of ingrowth of the endothelial cells. By 20-24 weeks differentiation of the epithelial cells would have occurred with stroma and a double layered endothelial cells.

A second wave of ingrowth of mesenchymal cells from the rim of the cup occurs with two layers of cells. The cells, which grow in between the developing lens and the cornea becomes the pupillary membrane. The other group of cells enters the stroma to become the keratocytes. The keratocytes secrete the stroma.

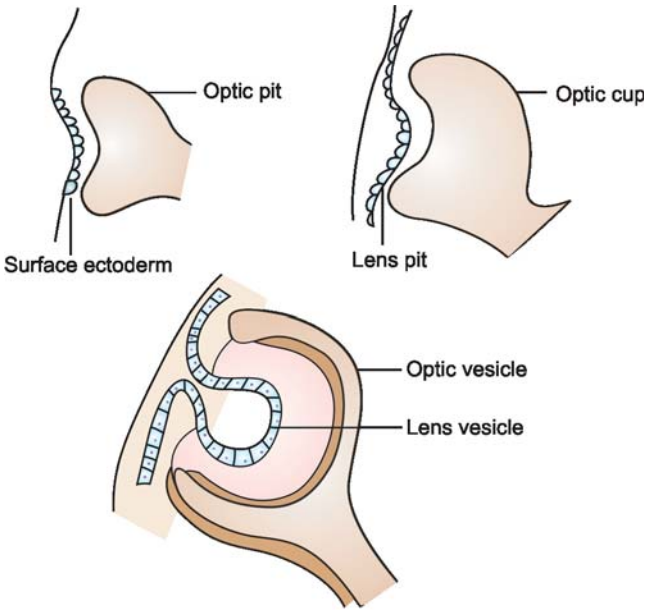


Fig. 2.3: Development of the eye

Initially the stroma is thick. It then undergoes dehydration of hyaluronic acid and compression of the stromal tissue to attain the normal thickness.

By the 12th week the two-layered endothelial cells become a single layer and secrete the Descemet's membrane. The Bowman's membrane develops by the 16th week.

The cornea measures 2 mm by the 12th week and is 9.3 mm by the 35th week.

ANGLE OF THE ANTERIOR CHAMBER (FIG. 2.4)

The angle of the anterior chamber lies at the junction of cornea and the iris and it consists of:

a. The Schwalbe's line, which is the most prominent portion and is called the anterior annular line.
b. The Schlemm's canal a venous channel, which resembles a lymphatic. It is lined by non-fenestrated endothelium and has a thin connective tissue wall. The cells have tight junctions. Large vesicles are seen in the cells. When this canal is empty it is invisible but when filled with blood it can be seen clearly just in front of the scleral spur.
c. Trabecular meshwork (ligamentum pectinatum iridis or cribriform ligament of Henderson) this is triangular in shape with apex at the Schwalbe's line. The base is formed by the scleral spur and the ciliary body. It is present all around between the sinus venosus sclerae and the anterior chamber. It has three parts the uveal portion, corneoscleral meshwork and the juxta canalicular portion. The part in front of an imaginary line drawn from the Schwalbe's line to the scleral spur is the uveal portion. Pigmentation of the uveal part of trabecular meshwork is more in people with dark irises. The

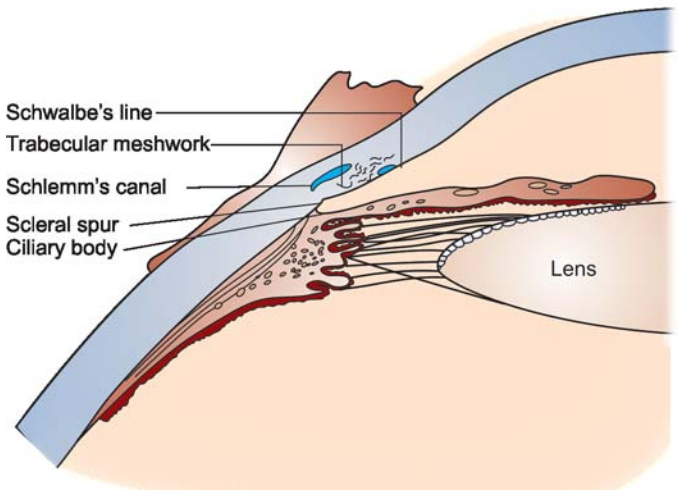


Fig. 2.4: Angle of anterior chamber

uveal meshwork is larger than the corneoscleral meshwork. The pores in the meshwork are called spaces of Fontana.

The corneoscleral meshwork consists of thin sheets of connective tissue arranged in a laminar pattern. Each fibril is formed by a central core of collagen and elastic fibrils surrounded by a basal lamina and then a single layer of endothelial cells. These cells have many vesicles for pinocytosis.

d. The scleral spur. This is seen as a white line behind the scleral trabeculae and is called the posterior annular line.
e. The iris processes are the true ligamentum pectinatum. They bridge over the angle and are seen as yellowish vertical lines.
f. Anterior border of the ciliary body. The anterior surface of the ciliary body forms a concave recess or sinus. It can be recognized by the fact it is darker in color compared to the iris, which lies next.
g. Iris.

Development

After the in growth of the first wave of endothelial cells and the posterior extension of the second wave which forms the pupillary membrane there is a slit like space in between the two layers of cells. This becomes the angle of the anterior chamber. During the seventh week the angle is occupied by mesenchymal cells derived from the neural crest cells. The trabecular meshwork develops from these cells. Around the 15th week the endothelial cells extend up to the angle of the anterior chamber and meets the anterior surface of the developing iris. Full development of angle develops only after the age of four. As the cornea develops the angle recess moves posteriorly. Around the 14th week the trabecular meshwork becomes perforated.

The Schlemm's canal develops during the 12th week of gestation from the venous plexus. It is derived from the mesoderm. The circulation of aqueous humor begins at this time and the Schlemm's canal acts as a conduit for aqueous drainage and does not become a blood vessel.

SCLERA

Sclera is composed of type I collagen and proteoglycans (decorin, biglycan and aggrecan), elastin and glycoproteins such as fibronectin. Fibroblasts lie along the collagen bundles. Long posterior ciliary artery nerves supply the anterior sclera. The intrascleral loop of these nerves sometimes form a nodule over the ciliary body. Deposits of calcium phosphate anterior to the insertion of medial and lateral rectus will cause dehydration of sclera. This will produce thinning of sclera, which will appear blue in color due to the underlying uvea.

Development

The sclera is formed by the mesenchymal cells derived from the neural crest. Just like the choroid also develops from the anterior end. This occurs during the seventh week.

3 The Lens

The human lens is a transparent biconvex structure with a refractive index of 1.39 suspended between the iris and the vitreous by the zonules. The lens is able to refract light as its refractive index is different from the refractive index of the aqueous and vitreous, which surround the lens. The refractive power of the lens is about 15-20D. The diameter of the lens is 9-10 mm at the equator from right to left. Anteroposteriorly it measures about 4 mm in the young but it continuously increases in size as new lens fibers are laid throughout life.

The anterior surface of the lens is less convex (10 mm radius) than the posterior surface (6 mm). Anterior to the lens are the pupil, the anterior chamber, iris, the posterior chamber and the ciliary processes. The anterior pole of the lens lies 3 mm from the cornea.

The posterior surface is separated from the vitreous by a space (Berger's) which is filled by the primary vitreous. The equator of the lens lies 0.5 mm within the ciliary processes. The indentations formed by the zonules disappear during accommodation.

Parts of the Lens (Fig. 3.1)

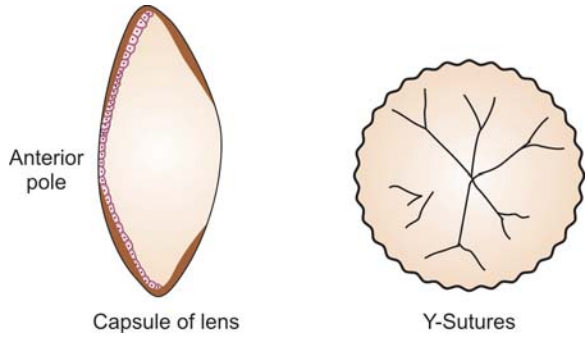


Fig. 3.1: Sutures of the lens

Lenticular capsule is not a true capsule but only a thickening of the basement membrane of the epithelial cells. Though it does not have any elastic tissue the fine fibrils of collagen present in the capsule gives it elasticity. It is thicker in

front than behind. The part of the capsule just in front and behind the equator is also slightly thicker than other areas. The posterior capsule is very thin just 2 to 4 micrometers in the center. The capsule is made of type IV collagen.

Lenticular epithelium is formed by a single row of cubical cells present under the capsule, which becomes columnar as they reach the equator. Mitosis is seen more near the equator. These fibers elongate to form the lens fibers.

The lens fibers are 15 micro m/6 micro m in size. The fibers remain parallel except at the sutures where they converge. The central fibers loose their nuclei and other organelles so that light will not get scattered, and become part of the lens nucleus. As newer fibers are laid externally closer to the capsule a laminated structure is formed. An anteroposterior section will show concentric layers. The nuclei lie closer to the equator. The lens fibers start and finish in such a way that anterior and posterior y sutures are formed.

The older fibers lack nuclei have serrated edges and are compactly arranged to form the nucleus. There are around 2000 fibers in an adult lens. The inter digitations between the lens fibers help in movement of the lens fibers during accommodation.

The lens epithelium is metabolically very active. As the cells elongate into lens fibers they loose all the cell organelles so that light is not scattered.

The nucleus is harder than the cortex. Though the lens becomes flatter as age advances the refractive power remains the same due to increase in the refractive index. The color of the lens also becomes more yellow as years pass by and on oblique illumination appears gray. The oldest fibers form the embryonic or fetal nucleus, then the infantile and finally the adult nucleus.

Ciliary zonule (zonule of Zinn or suspensory ligament of the lens). The lens is suspended from the ciliary body in between the iris and the vitreous by a series of fine fibers called zonules. Thus, it divides the eye into a small anterior and a large posterior portion.

The zonules are attached to the equator and parts of the anterior and posterior lens capsule on one side and the ciliary body on the other up to the ora serrata.

The posterior chamber is situated in front and the vitreous behind. The space between the layers of zonule is called the canal of Petit.

The zonules are smooth and inextensible. The ends spread in a fan shaped manner to become continuous with the lens capsule. The zonules have many auxilliary fibers also for strengthening the main fibers.

The main fibers are subdivided into:

Orbiculoposterior capsular fibers which originate from the ora and are attached to the posterior capsule,

Orbiculoanterior capsular fibers which are the thickest, arising from orbiculus ciliaris and attaching itself to the anterior capsule.

Cilioposterior capsular fibers which are the most numerous, arise from the valleys of the ciliary body and inserted into the posterior capsule,

And the cilioequatorial fibers present only in young eyes.

The zonules can be seen in the living through a slit lamp when there is coloboma of iris.

The lens being avascular is nourished by the aqueous humor.

Development of the Lens (Fig. 3.2)

The lens develops from the surface ectoderm in front of the primary optic vesicle. There are four stages, lens placode or plate, lens pit or recess, lens pouch or sac and lens vesicle.

a. Between the 3rd and 4th week of development the surface ectoderm becomes thicker as the cells become columnar. This thickened single layer of cells is called the lens placode.
b. A groove appears in this layer towards the end of fourth week. This is called the lens pit.
c. The groove closes to form a pouch.
d. This is converted into a vesicle between the 4th and 5th week.

Once the lens vesicle forms it moves inwards and gets separated from the surface ectoderm. At this time the optic vesicle invaginates to form the optic cup.

When the lens vesicle forms, the cells are arranged in such a way that the basement membrane of the anteriorly situated cells face anteriorly. The posterior epithelial cells grow forward towards the cavity of the vesicle. The cells

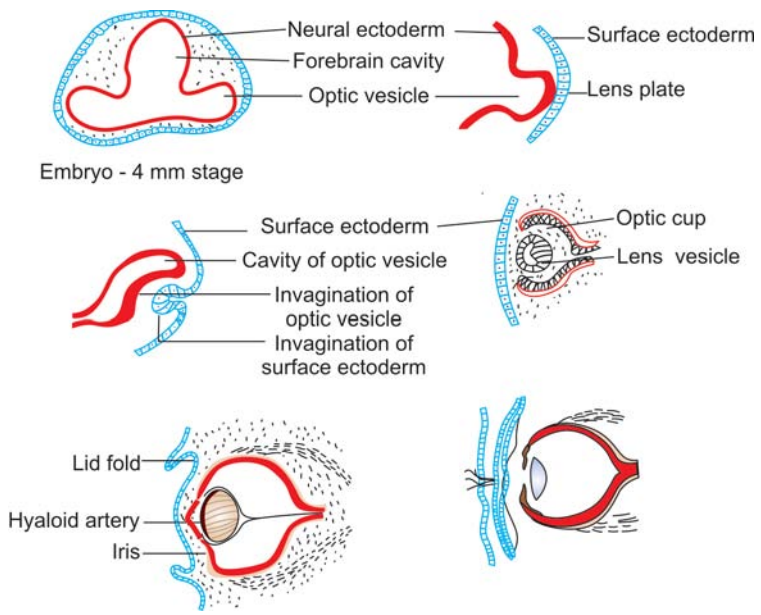


Fig. 3.2: Development of lens

become columnar. The central fibers elongate first followed by the fibers on either side. These fibers fill the lens vesicle by the later part of 6th week. These fibers extend from the posterior surface to the anterior surface. Once the cells elongate they do not multiply. As these fibers grow forward the nuclei also move forward and meet the nuclei of the anterior epithelium to form the nuclear bow. The cells of the anterior layer of the lens vesicle continue to form lens fibers throughout life. The new fibers are laid concentrically around the fibers formed by the posterior epithelium. This gives a laminated appearance to the lens. The fibers at the equator are arranged radially. The fibers do not make a complete circle but stop short at various levels depending on where they start. The fibers are of equal length. This forms the sutures of the lens (Fig. 3.1).

The lens is almost spherical at birth. It then becomes flattened anteroposteriorly. The capsule is secreted by the epithelium around the sixth week. It is only the basement membrane of the epithelium.

4 The Vitreous

This is a gelatinous mass, which is firmer than egg white containing 98.5% water and traces of albumin. It is transparent and colorless. There is a depression anteriorly called the patellar fossa just behind the lens. The vitreous supports the ciliary body and the retina. The vestige of the hyaloid artery called hyaloid canal is present in the center.

The vitreous is adherent to—

a. The edge of the optic disc
b. To the ciliary epithelium just anterior to the ora and 4 mm onto the retina. This part is called the base of the vitreous and here it is firmly adherent.
c. To the lens as a circle – this is a weak attachment, which disappears as age advances.
d. To the macula.

Microscopically

A limiting layer is formed by the condensation of the vitreous. The posterior part is in contact with the retina and is attached to the internal limiting membrane at the optic disc.

The cortex of the vitreous is formed by the condensation of the collagen fibrils, cells, proteins and mucopolysaccharides. The main mass is formed by a reticulum of extremely fine fibrils. The intervening space is filled up by hyaluronic acid and a protein called vitrein.

The vitreous cells or hyalocytes are PAS positive and are perhaps needed for production of mucopolysaccharides.

Development

During the 4th week of gestation the space behind the lens vesicle is filled with mesenchymal tissue called the primary vitreous. The hyaloid artery and veins also develop at this time. The fibrils in the vitreous develop from the ectoderm. By the eighth week the primary vitreous is vascular and well developed. The secondary vitreous is avascular and is made of type II collagen and hyalocytes. The primary vitreous starts retracting during the eighth week and the hyaloid

system regresses. The vitreous increases in volume. The Cloquet's canal represents the remnants of the primary vitreous and hyaloid vessels. The anterior most end of the hyaloid vessel is slightly nasal and below the posterior pole of the lens. Sometimes the degenerated vessel remains attached to the posterior capsule. It can even form an opacity at this position. This is called Mittendorf's dot. Condensation of the fibrils extends from the rim of the optic cup to the lens equator. This is called the tertiary vitreous. The zonules develop anterior to this condensation from the neurectoderm.

5 The Uveal Tract

The uveal tract or the vascular tunic of the eye is formed by the iris, ciliary body and the choroid from before backwards. The three layers are continuous. Since it resembles a grape fruit when the outer coat that is the sclera is removed it is called the uvea.

IRIS

The iris, which is the anterior most part of the uveal tract, controls the amount of light entering the eye (Fig. 5.1). During accommodation the pupil constricts so that peripheral rays of light are prevented from entering the eye. This reduces spherical aberration and increases visual acuity. The anterior part of the iris is developed from the mesoderm and the posterior part is from the neuroectoderm.

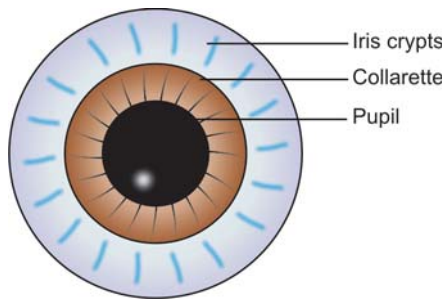


Fig. 5.1: Iris

The iris has the following structures.

a. Anterior endothelium
b. Stroma
c. Smooth muscles—sphincter pupillae
   dilator pupillae
d. Two layered pigmentary epithelium

Development

a. The stroma and blood vessels of the iris develop from the vascular mesoderm present anterior to the optic cup.

b. Sphincter and dilator pupillae muscles are derived from the neuroectoderm.
c. Both layers of the posterior epithelium are derived from the marginal region of the optic cup, i.e. the neuroectoderm. Though this corresponds to the neurosensory retina it is pigmented. The pigmentation starts from the pupillary margin and goes up to the root of iris.
d. The anterior layers are formed by the neural crest cells. They are designated as mesenchymal cells and are divided into superficial and deep layers.

During the sixth week the annular vessel near the rim of the optic cup gives rise to vessels which grow into the mesenchymal cells in front of the lens. The anterior part of the tunica vasculosa lentis forms the pupillary membrane. At the end of the 12th week both walls of the optic cup grow forward. The sphincter pupillae forms from the anterior epithelial layer during the 12th week. The dilator starts developing during the 24th week and continues to develop even after birth. Due to this it is difficult to dilate an infants eye. The tunica vasculosa lentis is reabsorbed during the 24th week. Parts of the arteriovenous anastomosis remains at the level of the collarette, which is closer to the pupil in the newborn.

Gross Appearance

The iris separates the anterior chamber from the posterior chamber and is situated between the cornea and the lens. It is the anterior most part of the uveal tract. It is bathed on both sides by the aqueous. The diameter is approximately 12 mm. The collarette is the thickest part of the iris while the root of the iris is the thinnest. Hence, during injuries the root of the iris gets torn causing iridodialysis.

The iris is a contractile diaphragm with a central aperture called the pupil.

The pupil is situated slightly nasal to the center but still lies in the optical axis. The anterior surface of the iris is slightly convex as it lies on the lens, which is convex. When the lens is removed the iris is flat and often tremulous. Contact between the posterior surface of the iris and the lens causes a relative pupillary block to the flow of aqueous humor, which is most marked during mid dilatation. This, further causes forward bowing of the iris (physiological iris bombe), which in persons with narrow angles may precipitate angle closure glaucoma.

The anterior surface of the iris is divided into two zones, the central pupillary zone and the peripheral ciliary zone. At the junction of these two zones is a circular ridge called the ‘collarette’. The collarette marks the embryonic site of minor vascular circle of iris from which the embryonic pupillary membrane originates. The ciliary zone has many ridges and crypts, which are unique to each individual just like fingerprints. The pupillary zone is relatively flat.

The superficial mesenchymal layer is shorter than the deeper layer. It extends from the ciliary border to the collarette and gives the ciliary portion of the iris its color. It contains the crypts and trabeculae of the collarette.

The deeper mesenchymal layer is transparent and extends from the ciliary border to the edge of the pupil. The superficial layer glides over it. This is why when the pupil dilates the edge of the pupil seems to be nearer to the collarette and the pupillary ruff disappears.

The pupil regulates the entry of light into the eye. It is pinpoint in bright sunlight and widely dilated in the dark. The diameter of the pupil can vary from 1.5 mm to 8 mm. In old people the size of the pupil becomes smaller due to fibrotic changes in the sphincter and atrophy of the dilator muscle. When mydriatics are used the pupil can dilate up to 9 mm.

The color of the iris is determined by melanocytes in the stroma and the anterior mesenchymal layer. Blue irises are thinly pigmented.

Microscopically the line of demarcation between the pupillary zone and the peripheral ciliary zone is marked by the collarette. This lies about two millimeters from the edge of the pupil. The iris is thickest at this region. An incomplete vascular circle called the circulus vasculosus iridis minor lies here. Remnants of the vascular connection to the tunica vasculosa lentis is sometimes present. It usually appears as thin fibrils attached to the collarette. Rarely even a thick sheet may be seen obscuring vision.

The anterior surface of the iris has a trabecular structure with pit like depressions. These are called Fuch's crypts. The extension of the posterior pigmented epithelial layer onto the front surface is called the pupillary ruff. This is the anterior edge of the optic cup. If the extension is prominent it is called ectropion uveae. This often occurs due to abnormal traction by tumors or any other pathological conditions.

The posterior surface of the iris is smooth with faint radial and concentric folds. The crenated appearance of the pupillary edge is due to the Schwalbe's contraction folds. The Schwalbe's furrows start as narrow depressions 1.5 mm from the pupil and become broad and shallow towards the periphery. The circular furrows are formed by the variations in the thickness of the pigment epithelium and due to the arrangement of the stroma. The epithelial cells here have a high rate of proliferation and hence cysts are more common here. Besides furrows few pits are also seen.

Layers of Iris

a. Anterior border (limiting) layer.
b. Stroma and sphincter pupillae muscle.
c. Anterior epithelium and dilator muscle.
d. Posterior pigment epithelium.

Anterior Border Layer

This is a condensation of connective tissue and pigment cells. Fibroblasts form a continuous sheet of flat stellate cells and interlacing processes throughout

the anterior surface. The melanocytes lie deep to the fibroblasts and parallel to the surface. Rarely iris processes an extension of the anterior border layer may extend up to the Schwalbe's line.

Stroma: Contains highly vascular connective tissue, collagen fibers, fibroblasts, melanocytes and matrix. Nerve fibers, sphincter pupillae and myoepithelial cells of the dilator muscle are also present. The spaces between the collagen fibers are filled with mucopolysaccharides. There are no elastic fibers. Mast cells, macrophages and lymphocytes are also present in the stroma.

The sphincter pupillae muscle is situated in the pupillary zone of the iris. It forms a ring of smooth muscle fibers around the pupil. It is one millimeter wide. The muscle fiber bundles are separated by nerve fibers, blood vessels and connective tissue. When this muscle contracts the pupil will constrict. It is supplied by the postganglionic, parasympathetic fibers, which travel through the oculomotor nerve, branch to the inferior oblique and then the short ciliary nerves. Sympathetic innervation is also seen which probably has an inhibitory role to relax the sphincter in the dark. As the muscle fibers are attached to the surrounding tissues even after a sector iridectomy the remaining fibers can contract.

The blood vessels run radially and are sinuous to allow for the dilatation and contraction of the iris. The vessels are from the long ciliary arteries and the anterior ciliary arteries.

The dilator pupillae muscle is a thin layer of myoepithelium. It extends from the root of iris to the sphincter pupillae. It is derived from the anterior layer of the iris epithelium and is supplied by the postganglionic fibers of the superior cervical sympathetic ganglion via the long ciliary nerves. Here also parasympathetic innervation is also seen for inhibition. Both these muscles are derived from the external layers of the optic cup. The dilator and constrictor muscles fuse together near the edge of the pupil. In the periphery the dilator continues into the ciliary body from where the muscle originates. Thus when the muscle is stimulated it draws the pupillary edge towards its origin thereby dilating the pupil.

Epithelial layer: The two layers of epithelium the anterior and posterior are derived from the neuroectoderm of the optic vesicle. The cells are opposed apex to apex. This polarity of cells is maintained from embryogenesis. Between the two layers lies a potential space. When fluid gets into this space an iris cyst is formed. The anterior epithelial layer is closely associated with the muscular processes of dilator pupillae and is continuous with the outer pigmented layer of the ciliary epithelium.

The posterior epithelium is in contact with the aqueous. The cells in this layer are larger than those in the anterior layer and are cuboidal in shape. They are full of melanin granules. This is continuous with the inner pigmented layer of the ciliary epithelium. The pigmentary layer is sometimes seen prominently

projecting around the pupil. This is called ectropion uveae. But this is a misnomer as the posterior layers are derived from neuroectoderm.

Blood supply: The major arterial circle, which lies in the stroma of the ciliary body, is formed by the long posterior ciliary arteries and the anterior ciliary arteries. Radial arteries arise from the major arterial circle and converge towards the pupil in a spiral pattern. These form the radial ridges formed on the surface of the iris. The spiral pattern is needed as the pupil has to dilate and constrict. On reaching the collarette the arteries and veins anastomose with the minor arterial circle.

The veins follow the arteries and form the minor venous circle and then drain into the vortex veins. The vessels are non-fenestrated and the endothelial cells have tight junctions. So they are less permeable. There are few smooth muscle fibers but no elastic lamina.

Nerve supply: The iris has both sensory and autonomic nerve supply.

The long ciliary nerves, branches of the nasociliary division of the ophthalmic branch of the trigeminal nerve contains sensory fibers that ascend through the trigeminal nerve. The long ciliary nerves also contain postganglionic sympathetic fibers from the superior cervical ganglion. These fibers innervate the dilator pupillae and the blood vessels.

The short ciliary nerves arise from the ciliary ganglion and contain the postganglionic parasympathetic fibers that originate from the Edinger Westpal nucleus of the oculomotor nerve. It supplies the sphincter pupillae.

THE CILIARY BODY

The ciliary body is divided into two parts the pars plana and the pars plicata (Fig. 5.2).

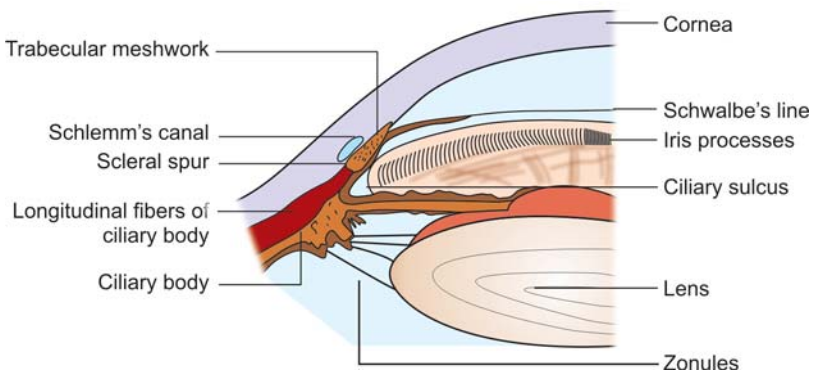


Fig. 5.2: Ciliary body

Pars plicata has seventy radiating ridges called the ciliary processes with darker valleys in between them. Its width is two millimeters. The width of the whole ciliary body is 5.9 mm on the nasal side and 6.7 mm on the temporal

side. The pars plana is 4 mm wide and is situated 3-4 mm from the limbus. During surgeries like vitrectomy this is the safest region to make the incision.

The ciliary body is triangular in shape the anterior face being the shortest. Part of this surface forms the angle of the anterior chamber. The iris arises from the middle of this surface. On the scleral side of the triangle lies the ciliary muscle. Suprachoroidal tissue lies in between the ciliary body and the sclera. The inner side of the triangle is formed by the ciliary processes and the suspensory ligaments. The equator of the lens is situated 0.5 mm from the ciliary body

Parts of the Ciliary Body

Suprachoroidal lamina consists of collagen fibers.
The next layer the *ciliary muscle* is triangular in shape. It has bundles of non-striated fibers. The external fibers are longitudinal or meridional, the intermediate ones are oblique or radial and the internal fibers are circular. The internal circular fibers form the sphincter.

The longitudinal fibers are also called Brucke's muscle. It takes origin from the scleral spur and the trabecular meshwork. This attachment is the main attachment to the sclera. The muscle fibers pass backwards into the suprachoroidal lamina to even beyond the equator. The fibers are slender spindles. Some of these fibers incline obliquely towards the inner side of the eye to form the oblique layer of the muscle. As the longitudinal muscle fibers are inserted into the scleral spur it has a pumping action on the canal of Schlemm. The pull of the muscle opens the canal. Thus, it facilitates drainage of aqueous.

The circular fibers are called the Muller's muscle. This is situated more internally, close to the lens. Some of the radial and circular fibers also are attached to the scleral trabeculae. The tendinous fibers of origin form a ring. The long posterior ciliary and the anterior ciliary arteries pass through the muscle and supply the same. The anterior ciliary veins drain the blood into the choroidal vessels.

The three layers of ciliary muscle act as one component. Miotics do not have much effect in infants as the muscle tendon attachment is not well developed in them.

The stroma is continuous with the suprachoroidal lamina. In the radial portion of the ciliary body the stroma consists of the blood vessels, nerves and melanocytes. As age advances the stroma becomes sclerosed and hyaline degeneration also sets in.

The function of the ciliary body is to slacken the zonules. This results in decreased tension to the capsule of the lens so that it can bulge forwards and become more convex. When the circular fibers constrict its circumference becomes less and the zonules are relaxed. The longitudinal muscle also may play a part by drawing the choroid forward but this is very doubtful.

According to Fincham when the eye is at rest the lens capsule is stretched by the zonule and the lens is in its normal form. When the ciliary muscle contracts the zonules are relaxed and the capsule compresses the lens matter at the equator. This makes the lens to bulge forward and the lens reaches its accommodative state. So normally the lens is in its passive state.

Nerve supply: The short ciliary nerves from the ciliary ganglion form a rich plexus in the muscle. Proprioceptive and sympathetic nerve endings are not demonstrated clearly in this tissue.

The ciliary processes are the most vascular part of the eyeball as they are formed mostly by blood vessels. This is a continuation of the choroid without the choriocapillaries. Each ciliary process is a ridge 2 mm long and 0.5 mm high. Anterior end is broader and is called the head of the process. The ridges are white in color whereas the valleys are pigmented and dark. The vessels are mostly veins that run parallel to each other. They run backwards to become continuous with the choroid.

The basal lamina is a continuation of the same lamina of the choroid. But at ora serrata the lamina splits into two and has connective tissue between the two layers. The inner layer is a continuation of the basement membrane of the pigment epithelium and forms the basement membrane of the deeper layer of pigmented epithelial cells.

The stroma has vessels and some elastic fibers. The vessels are mostly capillaries and veins. Loose connective tissue separates the ciliary muscle from the anterior chamber. The major arterial circle of the iris is situated in the stroma of the ciliary body.

The Epithelium

The basal lamina has two layers of cells. The outer layer consists of pigment cells and is the forward continuation of the pigment layer of the retina. These cells are smaller in size and do not have pigment processes. But the cells are more pigmented and hence the ciliary body appears darker. The inner layer is equivalent to all the layers of retina except the pigment layer. It is a single layer of nonpigmented cells. The cells are adherent to the pigment epithelium.

The capillaries in the ciliary body are thin walled and fenestrated. The junctional zone between the epithelia may get opened up. The mitochondriae in the ciliary epithelium are more in the apical region. These cells seem to be secretory in nature.

The internal limiting membrane is on the inner side of the non-pigmented epithelium and is the continuation of the internal limiting membrane of the retina.

The ciliary epithelium develops from the neurectoderm during the 12th week. The ciliary muscles develop from the mesoderm. The meridional fibers develop first followed by the circular and radial fibers. The circular muscle continues to develop up to one year after birth.

The Choroid

The choroid is the most posterior part of the uveal tract. It is homologous to the pia-arachnoid. It supplies blood to the outer part of the retina. It extends from the optic nervse (to which it is firmly attached) to the ora serrata. It is thicker posteriorly especially in the macular region. The choroid is also attached at the points where the vessels and nerves enter it. The choroid is formed by three layers, the layer of vessels in between two laminae.

The suprachoroidal lamina has a grill pattern. This is more evident when fluid gets collected in between choroid and the sclera, i.e. the suprachoroidal space. Since the two laminae are more adherent in the posterior region and the suprachoroidal lamina is attached to the sclera, choroidal detachment occurs only anteriorly. Un-striped muscle fibers are also seen in this layer. This gives the rough appearance to this layer when it is stripped off from the sclera.

The long and short posterior ciliary arteries and nerves pass through this space.

The blood vessels in the choroid are arranged in three layers. The external layer of larger vessels (Haller's), internal medium sized (Sattler's) layer and the inner layer of choriocapillaries. The arteries are deep posteriorly. But in the anterior region they lie superficial to the veins. Near the macula and where the veins join the vortex veins the vessels are larger in size. No valves are seen in these veins.

The stroma of the choroid contains loose collagenous tissue with elastin and reticulin fibers. The melanocytes are present in this layer. The pigment granules in these cells are of the same size in all the cells in a particular individual. It is also smaller and lighter in color compared to the granules in the retinal pigmentary epithelium. Pigmentation of the choroid and its density are of importance when photocoagulation is considered.

Macrophages, lymphocytes, mast cells and plasma cells are also present in the stroma.

The layer of choriocapillaries end at ora serrata whereas the larger vessels continue in to the ciliary body. This layer is not pigmented. The capillaries in the macular region form a dense network and have a wider bore. The chorio-capillaries in general are thin walled with fenestration. The pericytes in the vessels are present only on the outer wall. The capillaries have a lobular pattern. This is better seen with fluorescein angiogram. Each lobule is supplied by a central arteriole. Blood flow in the choroid is so high that the oxygen level in the venous blood is only 2-3% less than that of the arteries.

Bruch's membrane: This membrane is two microns thick and is closely adherent to the pigment epithelium. Near the optic disc the fibrils become circular and merges with the connective tissue of the optic nerve. Though it has been called a membrane this PAS positive tissue has five parts.

a. basal lamina of the retinal pigment epithelium
b. inner collagenous zone
c. band of elastic fibers
d. outer collagenous zone
e. basal lamina of the outer layer of choriocapillaries,

The basal lamina is highly permeable to small molecules like fluorescein. Defects can develop in myopia, pseudoxanthoma elasticum, trauma, inflammations etc. These defects cause neovascularization.

Development: The choroid starts developing from the anterior part of the optic cup and then proceeds posteriorly. The cells of choroidal stroma and melanocytes develop from the neural crest cells. The blood vessels develop from the mesenchymal cells. The choroidal capillaries develop after the fourth week. The junctional complexes develop before the fenestrations in the endothelium. The vessels are initially supplied by the internal carotid and then by the ophthalmic artery. The superior and inferior orbital venous plexuses drain into the developing cavernous sinus. The three layers of the choroidal vasculature are seen by the 12th week. The anterior ciliary and the long posterior ciliary vessels develop by the 16th week.

6 Lymphatic Drainage of the Eye

The eyelids have true lymphatics, which drain into the parotid lymph node and the submandibular lymph nodes. Otherwise the aqueous is considered the lymph of the eye.

Anteriorly the aqueous goes into the posterior chamber then through the pupil into the anterior chamber. From there it passes through the trabecular meshwork into the sinus venosus sclerae and then the aqueous veins. The crypts of Fuchs on the surface of the iris also help in drainage. The aqueous passes through the ciliary veins and suprachoroidal lymph space and perivascular lymphatics to the Tenon's space.

Posteriorly the lymph passes backward through the slit like spaces of the suspensory ligament into the canal of Petit around the equator of the lens. From here it reaches the Berger's space and through the hyaloid space into the optic disc.

Anterior chamber associated immune deviation: This reaction is supposed to be the basis for the immunological privilege of the eye. When antigens like viruses, allogenic spleen cells or tumor cells are introduced into the anterior chamber the delayed type of hypersensitivity, which should occur, is downregulated, while antibody and cytotoxic T cell responses are enhanced. Down regulation of hypersensitivity is caused by the presence of immuno-suppressive cytokines in the anterior chamber. These cells suppress the reaction of T cells. These antigen-presenting cells do not induce hypersensitivity but induce cytotoxic T cell response. The down-regulation of hypersensitivity prevents tissue damage caused by cell mediated immune responses. Transforming growth factor beta is the most important inhibitory factor found in the aqueous.

7 Retina

The retina is part of the central nervous system and is made of 10 layers of tissues. There are three types of tissues namely neural, glial and vascular (Fig. 7.1).

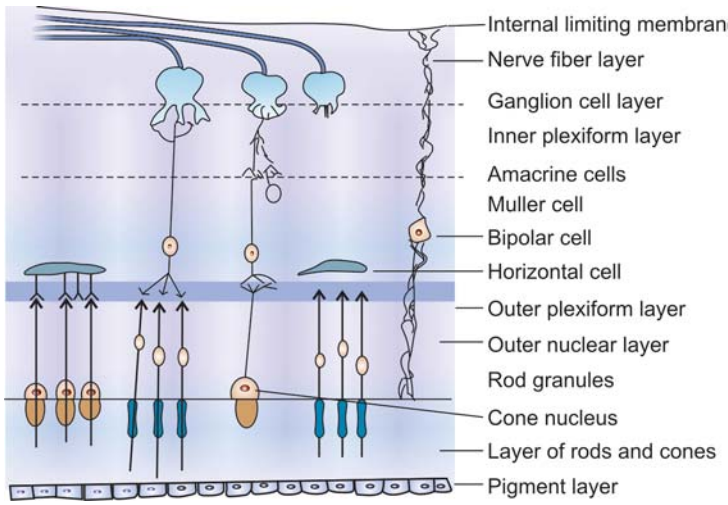


Fig. 7.1: Layers of retina

The pigment epithelium, which is close to the choroid, is made of a single layer of approximately 5 million cells extending from the ora serrata to the optic disc. The cells are hexagonal in shape. They are uniformly tall and narrow in the posterior pole, and more pigmented in the region of the macula but wider and shorter in the retinal periphery. Near the ora they assume a cuboidal configuration and continue as the pigment epithelium of the ciliary body. At the optic nerve the pigment epithelium falls short of basal lamina of choroid and the terminal depigmented cells heap up to form a ring at the edge of the optic disc.

The fine mottling of the cells is due to irregular distribution of pigments and this gives the fundus a granular appearance. Absence of specialized adhesion molecules (laminin and fibronectin) in the interphotoreceptor matrix and lack

of junctional complexes between apical microvilli of RPE cells and outer segments of photoreceptors makes the sensory retina prone to detachment in pathological conditions. The size of melanin granules varies between uveal and retinal pigment epithelium.

The pigment epithelium is needed for

a. vitamin A metabolism
b. maintenance of outer blood retinal barrier
c. phagocytosis of outer segments of the photoreceptors
d. absorption of light
e. heat exchange
f. formation of basal lamina
g. production of mucopolysaccharide matrix in the outer segment
h. Active transport of materials in and out of the cells.

Disruption of interaction between the RPE and photoreceptors causes retinal degeneration. Drugs like hydroxy chloroquine binds with the melanin granules in the retinal pigment epithelium and interferes with maintenance of photoreceptors (Fig. 7.2).

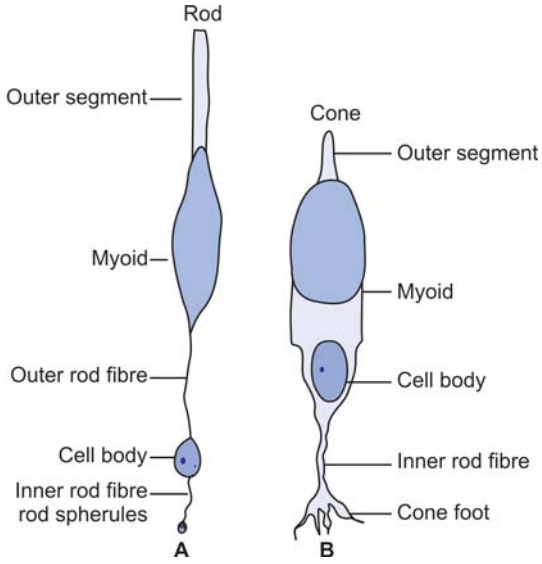


Fig. 7.2: Rods and cones

RODS AND CONES LAYER

The rods and cones that are the photoreceptor cells are highly specialized cells. They constitute the neuroepithelium. These cells absorb light by the visual pigments. Light alters the molecules of these pigments thereby producing nerve impulses. Thus, they transform physical energy into nerve impulses. The rods and cones have inner and outer segments. The transition between

the two segments is marked by a constriction, which lies exactly between the external limiting membrane and RPE. The plasma membrane of inner and outer segments is continuous. Extensions of Muller's cells called the fiber baskets of Schultz separate adjacent inner segments.

The rods are 40-60 microns in length. The outer segment is cylindrical and thin. It is refractile and shows striations on the surface, which run both transversely and longitudinally. The rhodopsin in rods is called the visual purple. The inner segment is slightly thicker and has transparent protoplasm. From the inner end of this segment a thin sinuous fiber exits and crosses the external limiting membrane. The nucleus of the cell which, is situated in the outer fiber is located in the outer nuclear layer. This nucleus is called the rod granule. The inner fiber extends further and ends in the outer molecular layer. The terminal end is bulbous and is called rod spherule. It transmits impulses to the dendrites of the bipolar cells.

The cones are larger in the foveal region, i.e. about 85 microns. In the periphery they are about 40 microns in size. The outer segments of the cones are conical and shorter than that of rods. The inner portion of the cones is in close contact with the nucleus, which lies in the outer nuclear layer. The cone fibers which are thicker than the rod fibers end in a pedicle. The lateral processes of the cone pedicle meet the dendrites of the bipolar cells in the outer molecular layer.

The rod and cone outer segments contain a series of lamellae, which appear discoid. These discs have larger diameter close to the inner segment. The inner segment has a segment called ellipsoid and a basal myoid.

The cell bodies of the cones are arranged in a single layer where, as that of the rods are in multiple rows (Fig. 7.3).

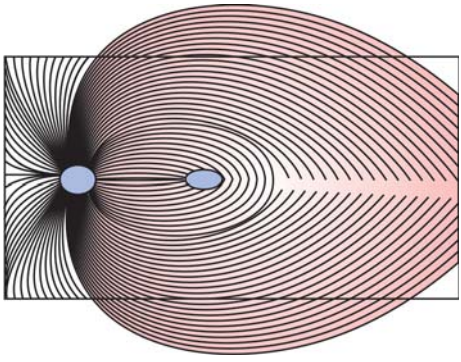


Fig. 7.3: Arrangement of nerve fibers

The outer segments of rods and cones are shed everyday. Rods shed the discs one-hour after exposure to light in the morning. The outer segments of the rods are entirely replaced in 10 days.

At the fovea only cones are present. 50% of the cones are present in the macula. Highest number of rods is present in the elliptical ring that passes through the optic nerve. Rods degenerate as age advances.

EXTERNAL LIMITING MEMBRANE

It extends from the ora serrata to the optic disc. It is pierced by the processes of the rods and cones and hence it is fenestrated. It is actually formed by the terminal expansion of the fibers of the Muller's cells and junction between the rod and cone processes. It separates the photoreceptor layer from the outer nuclear layer.

OUTER NUCLEAR LAYER

This lies internal to external limiting membrane and it contains the nuclei of the rods and cones. The nuclei of the cones are close to the external limiting membrane. The axons of these cells synapse with horizontal and bipolar cells in outer plexiform layer. There are 4 rows of nuclei on the temporal side of the disc, 8 or 9 on the nasal side and 10 rows of cone nuclei in the foveal area. The rest of the retina has one row of cone and four rows of rod nuclei.

OUTER PLEXIFORM LAYER

This is the junctional zone between photoreceptors and first order neuron, i.e. the bipolar cells. It is thickest around the fovea. A middle limiting membrane exists in this layer and it helps in demarcating the outermost extent of normal retinal vasculature.

The following synaptic units are present in this layer:

a. Presynaptic elements – spherules and pedicles,
b. Synaptic elements – bipolar and horizontal cells are in contact with the spherules and pedicles. Except for the fovea in other places many photoreceptors connect with one bipolar cell.
c. Postsynaptic elements – bipolar dendrites and horizontal cell processes.

INNER NUCLEAR LAYER

This layer has four layers of cell bodies from within outward. These are:

a. The nuclei of amacrine cells,(so called as they lack axons)
b. Muller's cells,
c. Bipolar cells and
d. Horizontal cells

Bipolar cells represent the 1st order neuron and transmit nerve impulse from photoreceptors to ganglion cells. Horizontal and amacrine cells have long branches, which extend horizontally and act as integrating circuits. The Muller's cells are supportive and also supplies nutrition. They also help in transmission and modification of nerve impulses. The capillaries of central retinal vessels are present in this layer.

The bipolar cells are neurons of the first order. Their dendrites are in contact with the rod and cones in the outer plexiform layer and on the retinal side they synapse with the ganglion cells. These cells have large nuclei and minimal protoplasm. This layer is thinner than the outer nuclear layer, which it resembles. It is thickest at the macular region and in the fovea it is almost absent.

The horizontal cells spread horizontally parallel to the surface of the retina. There are two types of horizontal cells. Type one synapse with the cones and type two with rods.

Amacrine cells have a single process, which ends in the inner plexiform layer. While the horizontal cells are placed close to the outer plexiform layer the amacrine cells are placed close to inner plexiform layer. They are connected to the axons of the bipolar cells and the dendrites of the ganglion cells.

The Muller's cells provide support to the retina. These cells occupy the space between the structures in the retina.

INNER PLEXIFORM LAYER

This layer is situated between inner nuclear layer and ganglion cell layer. Synapses between the first order neuron (bipolar cells) and the second order neuron (ganglion cells) occur in this layer. It contains processes of bipolar, amacrine, ganglion and Muller's cells.

GANGLION CELL LAYER

This layer consists of cell bodies of second order neurons, i.e. the ganglion cells. They are similar to nucleus gracilis and cuneatus. They are either bipolar or multipolar. Nasal to the optic nerve it consists of single row of nuclei, temporal to the disc a double row and in the macular region eight to ten rows of nuclei. In the foveola the nuclei entirely disappear. Temporal to the macula again a single row of nuclei is seen. The density of the cells decrease as the periphery is approached. The cells are of two types, the midget ganglion cells synapse with the midget bipolar cells and are present in the central retina. The other type that is the polysynaptic ganglion cells is more in the peripheral retina and synapse with many bipolar cells.

This layer contains fibers of Muller's cells, neuroglial cells and branches of the retinal vessels.

NERVE FIBER LAYER

This layer is composed of the axons of ganglion cells that are surrounded by processes of glial cells. The fibers that are nasal to the disc course like the spokes of a wheel towards the disc, whereas the fibers that are temporal form an arcuate pattern around the macula to enter the disc at its upper and lower

poles. Fibers from the macula and fovea go directly forward to the temporal side of the disc forming the papillomacular bundle. Besides the nerve fibers there are:

a. centrifugal fibers
b. fibers of Muller cells
c. neuroglial cells and
d. retinal vessels in this layer.

The nerve fiber is thinnest on the temporal side of the disc. The upper lateral and lower lateral come next followed by the medial part. The thickest parts are the upper medial and lower medial quadrant. When papilloedema occurs the thickest parts are affected first, so the upper medial and lower medial quadrants develop edema first. In the foveal region just like other layers it is almost absent.

Normally fibers in the nerve fiber layer are nonmyelinated because during development, myelinization of optic nerve stops at lamina cribrosa. Sometimes myelinated fibers are seen clinically in otherwise normal fundi as patch of white fibers with a feathery edge usually radiating out of the disc or rarely as isolated patch anywhere in the retina.

This layer contains some centrifugal fibers, fibers of Muller cells, neuroglial cells and retinal vessels.

INTERNAL LIMITING MEMBRANE

In between the retina and vitreous there is a membrane called the internal limiting membrane of the retina or the hyaloid membrane of the vitreous.

This is a PAS positive filamentous basement membrane of the Muller's cells and stains with Mallory's triple stain. The inner surface of the membrane is even but the retinal side is irregular conforming to the irregularity of basal plasma membranes (foot-plates) of Muller's cells. But over a vessel it is smooth on both sides.

GLIAL SYSTEM

Muller's Cells

These are the largest retinal cells. They span the entire retina inner limiting membrane to even beyond external limiting membrane. They synthesize and store glycogen and provide glucose to the surrounding neurons. Its cell body is in the inner portion of the inner nuclear layer.

Accessory Glia

Cells like astrocytes, oligodendrocytes and microglia are also present in the retina but only in the nerve fiber layer, ganglion cell layer and inner plexiform layer. Microglia are phagocytic, while the star shaped astrocytes send processes

around the nerve cells and blood vessels giving support to them. The oligodendrocytes in the retina do not produce myelin.

BLOOD SUPPLY

The retina internal to middle limiting membrane receives its blood supply from branches of the central retinal artery. The central retinal artery and vein divide into 4 main branches, the superior and inferior nasal and temporal vessels. Subsequent branching tends to be at right angles in the posterior part of the eye and dichotomous or γ shaped in the periphery. In 20% of normal eyes a cilio retinal vessel arises from the choroidal circulation and supplies the foveal region. The retina external to the middle limiting membrane is nourished by choriocapillaries.

Beyond the first bifurcation, vessels become arterioles, i.e. they loose their continuous muscular coat and internal elastic lamina. The venules, which course in the nerve fiber layer ultimately break up into an extensive capillary network and lie at all levels of retina internal to the middle limiting membrane present in the outer plexiform layer.

Arterioles differ from venules. They are more circular in cross-section and are thick walled. Arterioles lie internal to venules where they cross and have a broader surrounding avascular zone. Capillaries have a single layer of continuous endothelial cell lining surrounded by an interrupted layer of pericytes. A basement membrane derived from the endothelial cells and pericytes completely surrounds the outer surface of endothelial cells and cell surface of pericytes.

Around the disc is a distinct capillary network called the radial peripapillary network. This layer of capillaries follows the nerve fiber layer and supplies the axons arising temporal to the nerve head. The pattern of distribution of these capillaries is responsible for the arcuate scotoma seen in glaucoma (Fig. 7.4).

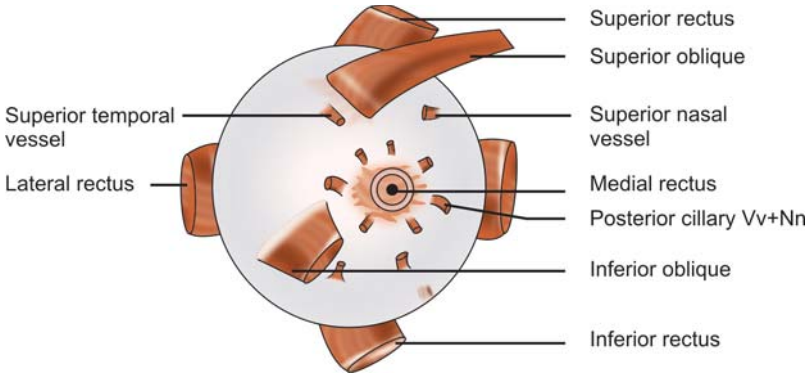


Fig. 7.4: Arrangement of posterior ciliary vessels and nerves

REGIONAL ANATOMIC VARIATIONS

Central Retina

Contains the macula and the optic disc.

'The optic disc is a circular to oval structure 1.5-mm in diameter.

The macula is clinically seen as an ill-defined yellow zone. This is free of capillaries and located temporal and slightly below the center of the disc. In the middle of this yellow region is a depression called the fovea. The sidewall of this depression is called the clivus. The edge of this slope shines bright when light is shone on the retina with an ophthalmoscope. A tiny bright reflex is seen in the center of this annular reflex. That is the region of the foveola. The macula around the fovea is divided into the inner parafoveal and outer perifoveal area.

The macula contains 2 or more layers of ganglion cells with an obliquely oriented outer plexiform layer. There is a high concentration of cones and the internal limiting membrane here is very thick.

The fovea is depression in the center of the macula. Its size is equal to the optic disc. Along the slope of the fovea called the clivus there is a transition from the thick internal limiting membrane to a thinner membrane. The retina is also avascular here. The ganglion cells are also smaller compared to other areas. The ganglion cells are 5 to 7 layers in thickness at the periphery and disappear gradually as the foveola is approached. The outer nuclear layer is also 8 layers thick in the periphery and only 2 or 3 cell layers in the center and are displaced towards the inner limiting membrane. The outer plexiform layer consists exclusively of cones and a few internally displaced photoreceptor nuclei. There is no nerve fiber layer, ganglion cell layer, inner plexiform layer or inner nuclear layer. As the inner photoreceptor fibers travel horizontally away from the center to reach the displaced bipolar neurons they form a plexiform lamina known as the Henle's layer. Because of this any edema or deposition of exudates take a radial pattern here.

The masking of choroidal fluorescence during FFA is due to the presence of xanthophyll pigments as well as the increased pigmentation of the retinal pigment epithelium at the fovea.

Rods are absent in the fovea. This is the reason for physiological central scotoma in dim light.

Peripheral Retina

Neural retina ends anteriorly at the ora serrata. It is a serrated zone approximately 2 mm wide temporally and 1mm wide nasally. The dentate processes, which produce serrations, are prominent nasally. They extend into the pars plana and point towards the valleys between ciliary processes. It is 8.5 mm from the limbus, 6 mm from the equator and 25 mm from the optic nerve.The peripheral

retina is thin and the distinctive anatomical layers are not seen. There is loss of nerve fiber layer and ganglion cell layer. The inner and outer nuclear layers merge. The internal limiting membrane becomes thinner and multilaminar finally interweaning with the collagenous filaments of the vitreous base. The outer limiting membrane continues forward into the ciliary body as junctional complexes between pigmented and nonpigmented epithelia of ciliary body. The rods disappear approximately 1mm posterior to the ora and are replaced by primitive or malformed cones. The retinal pigment epithelium is continuous with the pigment epithelium of the ciliary body. The ora is firmly attached to the choroid. Hence, retinal detachment ends here. The vitreous is also firmly adherent to the retina in this site. The retina continues beyond this point as the outer cubical and pigmented and inner columnar and nonpigmented layers of the ciliary body.

The fundus appears red when seen with an ophthalmoscope due to the blood in the choroid. It will appear darker in darkly pigmented individuals due to increase in the pigmentary level in the RPE. A tessellated fundus is seen when the choroid is more pigmented while that of RPE is less. In this type of fundus the choroidal vessels are seen. These vessels are seen more clearly in albinos. Unlike retinal vessels the choroidal vessels anastomose, are deeper and do not have a bright reflex.

The optic disc appears light colored due to the presence of lamina cribrosa and absence of any pigments. Though the optic disc is called a papilla it is in level with the retina and not raised. Hence, the vessels have a continuous bright reflex as they cross the edge of the disc. When there is edema of the disc this reflex will be broken.

Development

The development of retina is divided into three stages.

a. Epithelial stage
b. Stage of differentiation
c. Stage of growth.

The nervous portion of the retina develops from the distal part of the optic vesicle which after invagination forms the inner layer of the optic cup. It has a single layer of cylindrical epithelium. These cells divide and form a multiple layer of cells within four weeks after fertilization. The oldest cells lie close to the vitreous. The retina is then divided into the outer two thirds of cells called the primitive or the nuclear zone and the zone without nuclei called the marginal layer of His. The marginal zone contains the processes of the retinal cells and the neuroglial cells and becomes the nerve fiber layer. The retina develops further after the closure of the embryonic fissure.

The ganglion cells develop from the innermost cells of the nuclear zone during the 5th week. Once these cells invade the marginal zone in the region of

the future macula the nerve fibers grow out of them and reach the optic stalk. By the fifth week, two layers of neuroblastic cells the inner and outer are seen with the cell processes in between. The dendritic processes from the ganglion cells create a non-nuclear zone, which forms the future inner plexiform layer. The ganglion cells are the first cells of retina to become clearly differentiated. Initially there are a large number of ganglion cells. These later undergo apoptosis and reduce in number but increase in size.

The outermost cells of the nuclear zone give rise to the rods and cones. High mitotic activity is seen between the 4th and 12th week. The outer plexiform layer develops by the 5th month. Differentiation of cones begins at 5th month. Rods develop later around the 7th month. No new retinal cells are formed after the 6th month. The development of the fovea starts at the end of 6th month by thinning of ganglion cells. This thinning causes a depression in the center. Later the inner nuclear layer also becomes thinner. The cones appear later in the macular region and they are longer and thinner than the cones in other areas. Only when the child is several months old the cones become mature and they continue to differentiate until the fourth year of life. The location of the fovea is same at birth as in an adult.

Amacrine cells develop by 14th week and bipolar cells by 23rd week.

The pigment epithelium develops from the outer wall of the optic cup. These cells which are at first cylindrical gets pigmented at about three weeks. These are the earliest cells to become pigmented in the body. The anterior portion is pigmented first. The cylindrical cells then become cubical and are arranged in a single row. The pigment epithelial cells induce growth of choroid, sclera and neurosensory retina. So if growth of this layer is affected other layers also will be affected.

8 The Orbit

The bony cavities in the skull on either side of the nasal cavity in which the eyeballs are situated are called the orbits. Each orbit is roughly shaped like a quadrilateral pyramid with the base formed by the orbital margin. At the apex the optic foramen and the superior orbital fissure are situated. The orbit has not got a perfect geometrical shape, as there are many depressions and ridges. Hence, it was likened to a pear with the optic nerve as its stalk. The medial walls are parallel while the lateral forms an angle of 45 degrees with the median plane. The orbit runs forwards, laterally and slightly downwards (Fig. 8.1A).

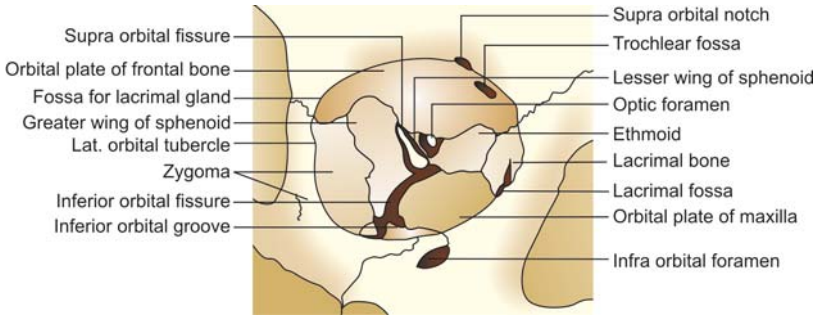


Fig. 8.1A: The right orbit

The orbit is surrounded by—

- Nasal cavity and ethmoid on the medial side,
- Frontal sinus and anterior cranial fossa above,
- Middle cranial fossa, temporal and pterygopalatine fossa on the lateral side and
- The maxillary and palatine air cells below.

Its volume is 30 cc. It is 35 mm high, 45 mm deep and 45 mm wide. 1cm behind the orbital margin the orbit is wider than the other areas and corresponds to the equator of the eyeball.

The following bones are part of the orbit:

Frontal bone, zygoma, maxilla, ethmoid, sphenoid, lacrimal bone and the palatine bone.

The orbital margin that is quadrilateral is formed by—

- Superiorly the frontal bone

- Medially the frontal bone, maxillary bone
- Inferiorly maxillary and zygomatic bones and
- laterally by the zygoma and frontal bones.

The margin is like a spiral, as the superior margin is continuous with the posterior lacrimal crest while the inferior is continuous with the anterior lacrimal crest. Each side is approximately 40 mm in size, but the width is slightly more than the height. The ratio between the two varies in different races and is called the orbital index.

The supraorbital notch or foramen transmits the supraorbital nerve and vessels. This can be felt through the skin. Local anesthetic agents for ptosis surgeries or suturing cuts near the brow can be injected here. Medial to this groove is another one for the supratrochlear nerve and vessels.

The inferior margin is above the floor of the orbit. The suture between the zygoma and maxilla forming this margin is marked by a tubercle. This is situated about the middle of the lower margin. The infraorbital foramen transmitting the infraorbital nerves is situated just below the tubercle. Hence this serves as a landmark when the infraorbital nerve has to be anesthetized.

The four walls of the orbit (Fig. 8.1B).

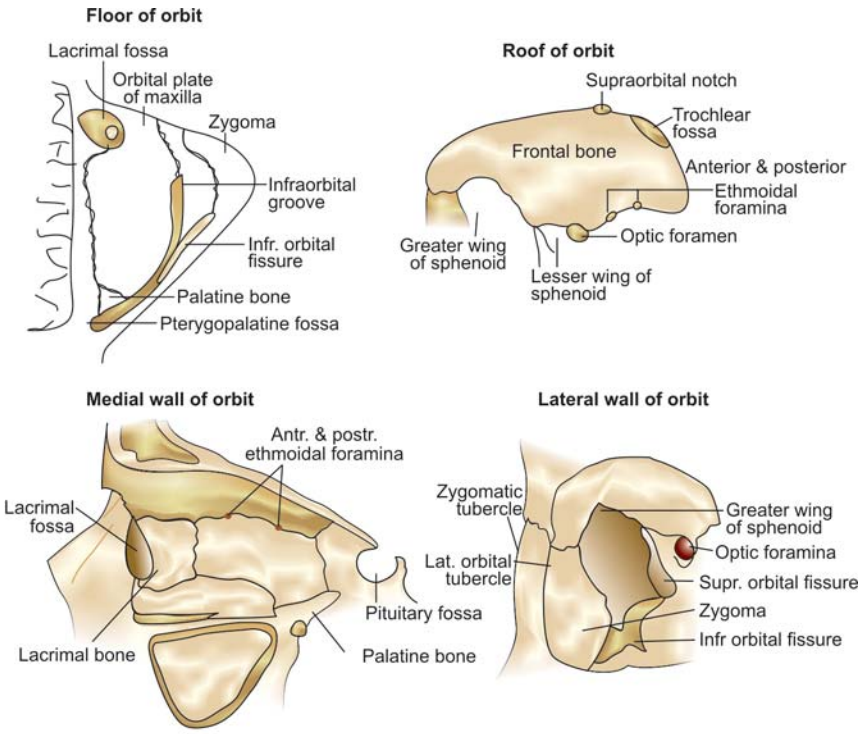


Fig. 8.1B: The four walls of orbit

Orbital Roof

This is triangular in shape, formed by the orbital plate of the frontal bone and the lesser wing of the sphenoid bone. There is a smooth depression on the anterolateral portion of the roof, which accommodates the lacrimal gland. This is called the fossa for the lacrimal gland. The roof is more concave about 1.5 cm from the orbital margin. This area corresponds to the equatorial region of the eye. Medially about 4 mm from the orbital margin there is a depression in the roof called the fovea trochlearis. A curved piece of hyaline cartilage the trochlea which forms the pulley for the tendon of the superior oblique muscle is attached to the trochlea. This may sometimes be ossified. Above the fronto-ethmoidal suture the anterior and posterior ethmoidal canals are situated. Through these openings the anterior and posterior ethmoidal nerves and vessels pass. Besides this numerous small pores are seen in the roof through which many vessels pass from the anterior cranial fossa to the orbit.

Medial Wall

This is formed by the frontal process of the maxilla in front, followed by the lacrimal bone, the orbital plate of the ethmoid and the lesser wing of sphenoid. Ethmoid occupies the largest portion.

The lacrimal fossa is situated between the anterior lacrimal crest in the frontal process of the maxilla and the posterior lacrimal crest in the lacrimal bone. This fossa in which the lacrimal sac is situated is continuous with the inferior meatus of the nose through the nasolacrimal duct. The lacrimal bone separates the fossa from the ethmoidal sinuses above and the middle meatus of the nose below. Hence, when an opening is made in the bone for doing a dacryo-cysto-rhinostomy the bone must be broken lower down. Otherwise the entry will be made into the ethmoidal sinus. The medial wall is very thin and is called lamina papyraceae. Hence, infection of ethmoidal sinus can easily affect the orbit.

Floor of the Orbit

The floor is slightly shorter than the other walls and slopes slightly downwards by about 20 degrees. It does not reach the apex. Hence at the apex the orbit is triangular and not quadrilateral. The roof of the maxillary sinus, which is situated in the maxilla, the orbital plate of the zygoma and the palatine bone form the floor of the orbit.

In the posterior part of the floor there is a groove which is transformed into a canal anteriorly. The infraorbital nerve and vessels pass through it and reach the anterior face of the maxilla through the infraorbital foramen. The foramen is situated 4 mm below the lower orbital margin. The floor of the orbit just lateral to the nasolacrimal duct gives origin to the inferior oblique muscle.

Blunt trauma can cause dehiscence of the orbital floor. It should be remembered that the inferior rectus is in close contact with the floor posteriorly while in the anterior part the inferior oblique muscle comes in between the two.

Lateral Wall

The lateral wall is the thickest of all. It protects the eyeball from any blow from the side. The posterior part that separates the orbit from the middle cranial fossa is thin. It is formed by the zygoma and the greater wing of sphenoid. The lateral orbital tubercle called the Whitnall's tubercle is an important landmark. It gives attachment to the check ligament of the lateral rectus muscle, suspensory ligament of the eyeball, lateral palpebral ligament and aponeurosis of the levator muscle. This is situated 11mm below the frontozygomatic suture. Above and below the sphenoidal portion of the lateral wall is the superior and inferior orbital fissure.

Superior Orbital Fissure

This is also called as the sphenoidal fissure. It is gap between the roof and the lateral wall of the orbit. It lies between the greater and lesser wing of sphenoid. The lateral end is closed by the frontal bone. It is retort or comma shaped with the broader end on the medial side below the optic foramen. At the junction of the broad medial and narrow lateral end there is an elevation called the spine for the lateral rectus.

The fissure is 22 mm long. It is the largest communication between the middle cranial fossa and the orbit. It is separated from the optic foramen by the posterior root of the lesser wing of sphenoid. The infraoptic tubercle is situated on this root. Anastomosis between the middle meningeal and lacrimal arteries occur near the superior orbital fissure through small frontosphenoidal foramina.

The common tendinous ring divides the fissure into three compartments. Trochlear, frontal and lacrimal nerves (LFT), and the vessels like the superior ophthalmic vein and the recurrent lacrimal artery pass through the lateral most part. The two divisions of the oculomotor nerve, the nasociliary branch of the trigeminal, the sympathetic root of the ciliary ganglion and the abducent nerve pass in between the two limbs of the annulus or the two roots of the lateral rectus. The abducent nerve initially lies lateral to the two divisions of the third nerve. Later it lies in between the two divisions. Sometimes the ophthalmic veins also pass through this opening. The medial compartment is usually empty. Rarely inferior ophthalmic vein passes through it.

The Inferior Orbital Fissure

Through this fissure the orbit is connected to the pterygopalatine and infra-temporal fossae. It starts close to the medial end of the superior orbital fissure

and lies between the lateral wall and the floor of the orbit, i.e. in between the maxilla and the greater wing of the sphenoid. It is about 20 mm in length. As the maxillary sinus develops the fissure will become narrow. It transmits the infraorbital nerve, zygomatic nerve, branches to the orbital periosteum from the pterygopalatine ganglion and a communication between the inferior ophthalmic vein and the pterygoid plexus. The fissure is closed by the periorbita and the muscle of Muller.

The Optic Foramen (Fig. 8.2)

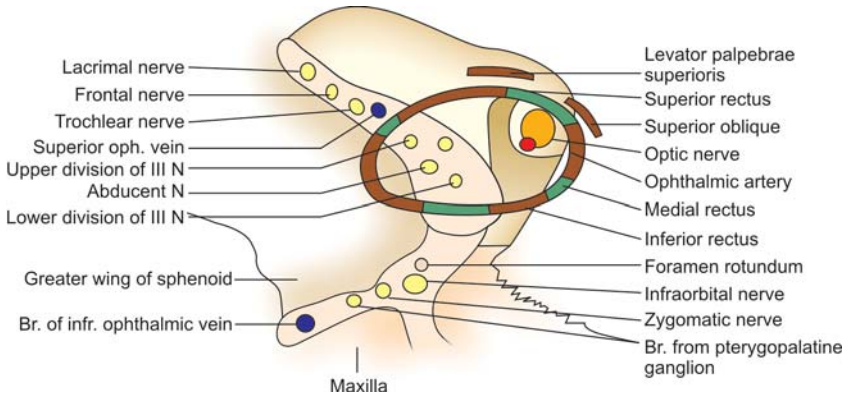


Fig. 8.2: Orbital fissures and optic foramen origin of extraocular muscles

The optic foramen connects the orbit to the middle cranial fossa. It is situated at the apex of the orbit, in between the two roots of the lesser wing of sphenoid. It transmits the optic nerve, the ophthalmic artery and the sympathetic plexus around the vessel. This is rather a canal than just a foramen. It descends laterally and forwards making an angle of 36 degrees with the median sagittal plane. Thus, the distance between the intracranial optic foramina on the cranial side is 25 mm whereas on the orbital side it is 30 mm. If a line is drawn backward from both the foramina they will meet at the dorsum sellae at an angle of 30 degrees. The anterior opening is slightly larger than the posterior and vertically oval in shape. The posterior opening is horizontally oval. The lateral wall of the foramen is well defined. The four recti muscles jointly form the muscle cone in the posterior part of the orbit. In the oval opening created by the origin of muscles lie the optic nerve, ophthalmic artery and the third and sixth cranial nerves.

The optic strut is bony ridge that joins the lesser wing of sphenoid to the body of sphenoid. This part that separates the optic canal from the superior orbital fissure is an important landmark. When X-rays are taken for intracranial lesions any erosion or enlargement of the strut is looked for.

Measurements

The roof and medial walls are longer than the other walls. Roof measures 10-12 mm, lateral wall 5-7 mm. The orbital opening is 5/6 mm in size.

The tubercle for the tendinous ring is situated lateral to the optic foramen. The sphenoidal air sinus and sometimes the posterior ethmoidal sinus are in close contact with the optic canal. Since the bone separating the sinuses from the canal is very thin any infections occurring in the sinus can easily affect the optic nerve. Above the optic canal is the gyrus rectus of the olfactory tract.

Development

The bones of the orbit are membranous except the sphenoid. Because of this the ostium we make during DCR do close. Ossification of the membranes start by the 12th week.

9 Extraocular Muscles (Extrinsic Muscles)

The eye is the only sense organ capable of independent movement. This is needed for greater field of vision, foveal vision for a large portion of visual field and binocular vision for both distance and near.

There are four recti and two oblique muscles for moving the eyeball in various directions. The four recti and the superior oblique muscle arise from the apex of the orbit. The recti are inserted into the anterior part of the sclera in a spiral fashion called the spiral of Tillaux. The spiral is formed thus. The medial rectus is inserted close to the sclera, the inferior rectus next and the superior rectus furthest back. The four recti pull the eyeball back against the orbital pad of fat. The obliques pull the muscle forward. The relation ship of ora serrata to the insertions must be remembered as during squint surgeries and while taking the superior rectus suture one may injure the retina.

Origin of extra ocular muscles: The muscles take origin from the annulus of Zinn and the sphenoid bone around it. The annulus has a superior ligament called the upper tendon of Lockwood and the inferior part is called the lower tendon of Zinn. The medial rectus takes origin from the upper part of the ligament, the inferior rectus from the lower and the lateral from both the upper and the lower. The superior oblique muscle originates from the periostium of the body of sphenoid above and medial to the optic foramen. The inferior oblique arises from the anterior part of the floor of the orbit just lateral to the lowermost part of the lacrimal fossa. The two oblique muscles approach the globe from the medial side of the orbit anteriorly and cross the globe in an oblique fashion to reach the lateral aspect. Both the obliques get inserted into the sclera posterior to the equator on the temporal part of the globe (Figs 9.1 to 9.4).

The origins of superior and medial recti are slightly anterior and they are more closely attached to the dural sheath of the optic nerve. Because of this patients with retrobulbar neuritis often complain of pain during elevation and adduction.

Insertion: The recti are flat narrow bands but the tendons are broader than the muscles. The muscles are inserted into the sclera by glistening tendons. The fibers of the tendons run parallel to the long axis of the muscle and are made of collagen. Some of the fibers may be attached further back from the main

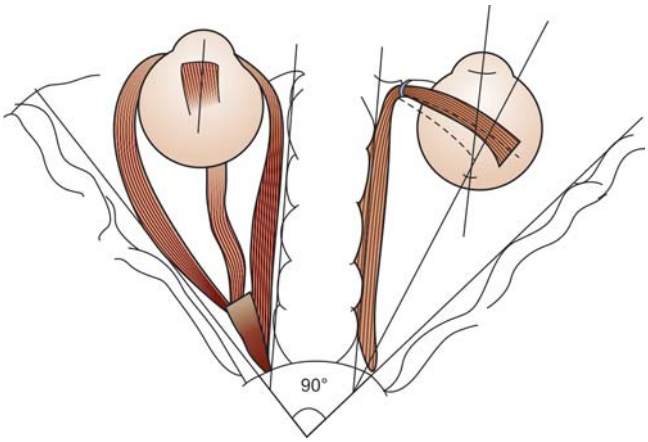
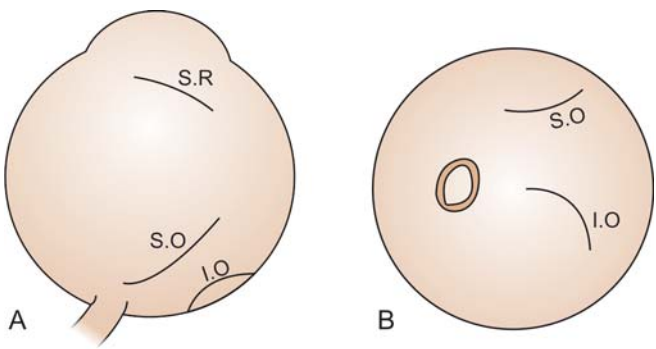


Fig. 9.1: Location of muscles in the orbit



Figs 9.2A and B: **A.** Superior view, **B.** Posterior view

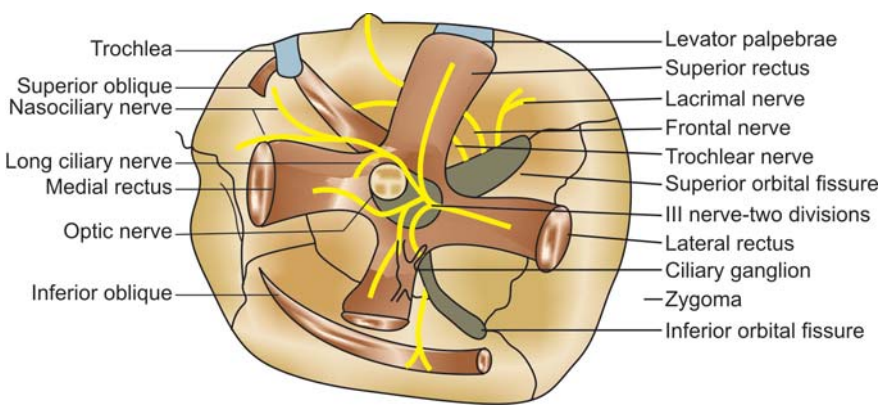


Fig. 9.3: Origin of extraocular muscles

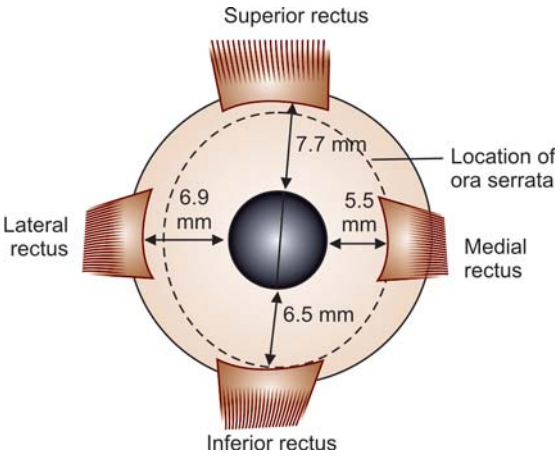


Fig. 9.4: Insertion of muscles

tendon. During squint surgery these fibers must be taken care of.

Blood supply: The muscles are supplied by the muscular branches of the ophthalmic artery, lacrimal artery and infra orbital artery. Each rectus muscle receives two anterior ciliary arteries. The lateral rectus is supplied by only one branch from the lacrimal artery.

Structure of Extraocular Muscles

The extraocular muscles are very highly differentiated. They are striated muscles but the diameter is smaller than that of other striated muscle fibers. The fibers are loosely united unlike other muscles where the fibers are bundled tightly together. The number of nerve fibers supplying the muscle is also numerous. There is a separate cranial nerve for a small muscle like the lateral rectus. Each muscle fiber is a multinucleated structure containing sarcoplasm surrounded by sarcolemma.

The muscle fibers are two types. The type with slow fibers have grape like motor nerve endings, are poorly defined, thinly innervated, with central nuclei, and sparse cytoplasm and have a good response to acetyl choline. The twitch fibers with plaque like nerve endings have abundant cytoplasm, are well defined, with thick innervation, peripheral nuclei and poor response to acetyl choline.

The large number of elastic fibers and the rich nerve supply present in these muscle help them to have a very smooth action. The muscles are enclosed in a sheath.

The extraocular muscles contain very small muscle spindles. Each muscle spindle has a group of thin muscle fibers with rich nerve supply. The fibers are enclosed in a thin capsule. The intrafusal fibers are thinner and their nuclei are placed in the center unlike the extrafusal fibers.

Actions of Extraocular Muscles

When the eyes look in different directions the eyeballs are not displaced. The movements occur around the center of the eye.

The three primary axes around which the eye moves pass through the center of the movement. The three axes are:

a. Vertical axis (Z axis of Fick)—Around which the eye moves medially and laterally
b. Transverse axis (X axis of Fick)—Around which the eye moves up and down
c. Sagittal axis (Y axis of Fick)—Corresponds to the line of vision. Round this occurs the wheel rotation of the eye. When the twelve O' clock region of the cornea rotates nasally it is called intortion and when it turns outward it is called extortion.

Each extraocular muscle has a primary action and two subsidiary actions. When the primary action is taking place the subsidiary actions will not come into play. For each action a group of muscles have to function together; they are called the synergists. The muscle, which opposes that action, is called the antagonist.

Details about Individual Muscles

Muscle	*Length*	*Length of tendon*	*Width*	*Insertion from cornea*
Superior	40 mm	5.8 mm	10.8 mm	7.7 mm
Inferior	42 mm	5.5 mm	9.8 mm	6.5 mm
Medial	40 mm	3.7 mm	10.3 mm	5.5 mm
Lateral	40 mm	8.8 mm	9.2 mm	7 mm

Superior Rectus

Relationships—At the apex of the orbit the levator palpebrae superioris is above and it is continuous with the medial and lateral recti on either side. The muscle passes forwards and slightly laterally below the levator at an angle of 23-25 degrees with the visual line. The frontal nerve is also above the superior rectus. Orbital fat, ophthalmic artery, nasociliary nerve and optic nerve are below the muscle. The lacrimal artery and nerve are situated laterally and the ophthalmic artery and nasociliary nerve lie on the medial side.

Anteriorly the tendon of the superior oblique passes below the superior rectus close to the globe.

Medial Rectus

This muscle is bulkier than the other muscles. It is also stronger than the lateral rectus, which is its antagonist.

Relations the muscle passes along the medial wall of the orbit. The superior oblique muscle is situated above with the ophthalmic artery and its anterior and posterior ethmoidal branches and the anterior and posterior ethmoidal nerves and infra-trochlear nerve in between. Below the muscle is the floor of the orbit. On the medial side is the orbital plate of the ethmoid with the ethmoidal sinuses. The central orbital fat is present on the lateral side.

Inferior Rectus

The inferior rectus is the shortest. It passes forwards and somewhat laterally along the floor of the orbit, at an angle of 23–25 degrees with the visual line. The inferior rectus is attached to the lower lid by means of the fascial expansion of its sheath.

Relations: Above the muscle is the optic nerve, inferior division of the oculomotor nerve and the globe with orbital fat in between. The nerve to the inferior oblique is on the lateral side. The floor of the orbit with the maxillary sinus is below the muscle. The inferior oblique muscle passes below the inferior rectus. A sheath binds the muscles together.

Lateral Rectus

The origin of this muscle is strengthened by its attachment to the spina recti lateralis of Merkel on the greater wing of sphenoid. The muscle passes along the lateral wall of the orbit.

Relations: At the apex of the orbit the space in between the two heads of origin of the lateral rectus muscle is called the oculomotor foramen. Part of the lesser wing of sphenoid separates this area of the superior orbital fissure from the optic nerve. The upper division of oculomotor nerve, the nasociliary nerve, a branch of the sympathetic and then the lower division of the oculomotor nerve pass through this opening. Sometimes ophthalmic veins also may pass through it. The abducent nerve passes lateral to the oculomotor and supplies the lateral rectus.

Above the lateral rectus muscle the trochlear, frontal and lacrimal nerves and the recurrent lacrimal artery are present along with the superior ophthalmic vein.

As we come forwards from the apex the lacrimal artery and nerve are above, the floor of the orbit is below with the inferior oblique below and then medial. The ciliary ganglion and ophthalmic artery are situated medially. Laterally besides the orbital fat the lacrimal gland separates the muscle from the bone.

Action of the Recti Muscles

Muscle	*Nerve*	*Blood*	*Primary action*	*Subsidiary action*
Superior	Upper div. of III nerve	Muscular Br. Ophthalmic A	Elevation	Adduction, intorsion
Inferior	Inferior div. of III nerve	Muscular Br. Ophthalmic A	Depression	Adduction, extorsion
Medial	Inferior div. of III nerve	Muscular Br. Ophthalmic A	Adduction	
Lateral	Abducent N.	Lacrimal A. Muscular Br. Ophthalmic A.	Abduction	

The nerves enter the recti muscles at the junction of the posterior 1/3rd and anterior 2/3rd.

Superior Oblique

The site of origin of this muscle is above and medial to the optic foramen. The muscle then passes forwards along the upper part of the medial wall. It then reaches the trochlea at the angle between the superior and medial wall. The trochlea is like a tube 4–6 mm long called the trochlear fossa of the frontal bone. The muscle then turns laterally and backwards forming an angle of 54 degrees with the pretrochlear part of the muscle.

The fibers of the tendon have few inter fibrillar connections. Each fiber moves in a sliding and telescoping fashion. The central fibers move for a longer distance than the peripheral, i.e. about 8 mm in either direction.

The tendon of the muscle starts 10 mm before the trochlea. It then passes under the superior rectus, fans out and merges with the sclera.

Insertion: The anterior end of the insertion lies 3–4.5 mm behind the lateral end of the insertion of superior rectus and 13.8 mm behind the limbus. The posterior end of the insertion lies 13.6 mm behind the insertion of superior rectus. The width of the tendon varies but on an average it is 11mm. The medial end of the insertion lies 8 mm from the posterior pole and is close to the superior vortex vein.

The muscle is 40 mm long. The tendon is 19 mm long. Physiologically the trochlea is the origin of the muscle.

Action

The primary action is depression and this increases as the eye is adducted. When the eye is adducted superior oblique is the only muscle that can depress the eyeball. The muscle can also abduct and intort the eye which happens when the eye is abducted. So when the superior oblique and the inferior rectus act together the eye is depressed directly down.

Nerve supply: The superior oblique is supplied by the trochlear nerve, i.e. the fourth cranial nerve.

The superior muscular branch of the ophthalmic artery supplies the muscle.

Inferior Oblique

This is the shortest extraocular muscle. It is 37 mm long. If a line is drawn perpendicularly down from the supraorbital notch it will pass through the origin of this muscle which is just lateral to the upper most part of the nasolacrimal duct. From its origin the muscle goes backwards, upwards and laterally passing below the inferior rectus. Its tendon, which is just 1-2 mm long gets inserted into the posterior and temporal aspect of the eyeball. The width of the tendon is 9 mm. The anterior end of the insertion is 10 mm behind the lateral rectus. The posterior end is 1mm below and in front of the macula. It is close to the inferior vortex vein. The muscle forms an angle of 51 mm with the vertical plane of the globe.

Action

The inferior oblique is the elevator of the eye on adduction, opposite to that of superior oblique. It also abducts and extorts the eyeball. Along with superior rectus this muscle elevates the eyeball upwards.

The muscle is supplied by the inferior division of the oculomotor nerve and by the infraorbital artery and the medial muscular branch of the ophthalmic artery.

Fascias

Tenon's capsule: The Tenon's capsule is a condensation of fibrous tissue that covers the eyeball from the optic nerve to the cornea. At the limbus it is fused with the conjunctiva. In other areas there is a space between the two called the subconjunctival space. The space between the Tenon's and the sclera is called the episcleral space. The capsule fuses with the orbital reticular tissue and the orbital fat. It is pierced by the extraocular muscles before their insertion where the Tenon's capsule reflects over the muscle.

Development: The extraocular muscle develop from the myotonic cells of the mesoderm around the optic vesicle during the 5th week. The tendons fuse with the sclera by the 12th week.

Muscle sheaths and their extensions: Each muscle has an extracapsular portion and an intracapsular portion. The extracapsular portion is enveloped by a sheath. It is a reflection of the Tenon's capsule. The sheaths of the four recti are connected by the intermuscular membrane. These sheaths form fibrous attachments to the orbit. They also support the globe and check ocular movements.

The sheath of the superior rectus is attached to the levator.

Sheath of the inferior rectus has two layers. The upper one is attached to the Tenon's. The lower one passes between the tarsus and orbicularis forming part of the Lockwood ligament.

Sheath of the superior oblique is attached to the levator, superior rectus and the Tenon's.

The sheath of the inferior oblique fuses with that of the inferior rectus, lateral rectus and the optic nerve.

Suspensary ligament of lockwood: The sheaths of the inferior oblique and inferior rectus blend and join with the medial rectus and lateral rectus to form a hammock, which supports the eyeball. This tissue is called the ligament of Lockwood. Extensions from the ligament are to the:

- Tarsal plate
- Orbital septum
- Periosteum
- The floor of the orbit

Check ligaments: Connections are present from medial rectus and lateral rectus to the orbital walls. These are called the check ligaments. By this ligament the lateral rectus is attached to the zygomatic tubercle, to the lateral palpebral ligament and the lateral conjunctival fornix.

The check ligament of the medial rectus is attached to the lacrimal bone behind the posterior lacrimal crest and to the orbital septum. It is also connected to the sheath of the levator and superior rectus. Its inferior border fuses with extensions of inferior rectus and inferior oblique. The check ligaments of other muscles are not so well defined.

The episcleral tissue covers the intracapsular portion of the muscles. At the insertion this covering thickens to form the falciform fold of Guerin.

Role of fascial sheaths:

- To support the globe
- To protect the globe
- To form a smooth cavity with in which the eyeball can easily move
- To prevent retraction of globe
- To prevent movement of the globe in the direction of action of the muscle. (e.g. when the medial rectus contracts the visual axis must turn inward without the whole eyeball getting pulled towards the apex of the orbit)
- To keep the centre of rotation constant
- To limit the action of the contracting muscle and reduce the effect of relaxation of the opposing muscle
- To lessen the shaking up of the contents of the eyeball during sudden movements of the eye.

Levator Palpebrae Superioris

The surface of lesser wing of sphenoid above and in front of the optic foramen gives origin to the levator. At its origin the tendon fuses with the superior rectus. The belly of the muscle is flat. It passes forward between the roof and the superior rectus to reach 1cm behind the septum orbitale. This position corresponds to the upper fornix and a few millimeters in front of the equator. The muscle now gives off a membranous expansion or aponeurosis which spreads like a fan and covers a wide area of the upper surface of the eye ball. The muscular part is horizontal whereas the tendinous part is vertical. Along with the upper lid the tendon moulds itself around the eyeball.

Insertion

a. Main insertion of the levator is to the skin of the upper lid at and below the upper palpebral sulcus. To reach the skin the muscle fibers naturally have to pierce the fibers of the orbicularis oculi.
b. Part of the aponeurosis is attached to the front and lower part of the tarsal plate. One must remember that the superior palpebral (Muller's muscle) arising from the levator is attached to the upper border of the tarsus.
c. The facial sheathe of the muscle is attached to the upper fornix.
d. The ends of the aponeurosis are called the horns of the muscle. The lateral horn divides the lacrimal gland into its orbital and palpebral part and supports it. The aponeurosis is then attached to the orbital tubercle and to the upper aspect of the lateral palpebral ligament. The weaker medial horn is attached the bone below the fronto-lacrimal suture and to the medial palpebral ligament.

10 Cranial Nerves Connected with the Eye

The optic nerve consists of:

a. Intraocular part
b. Intraorbital part
c. Intracanalicular part
d. Intracranial part (Figs 10.1 and 10.2)

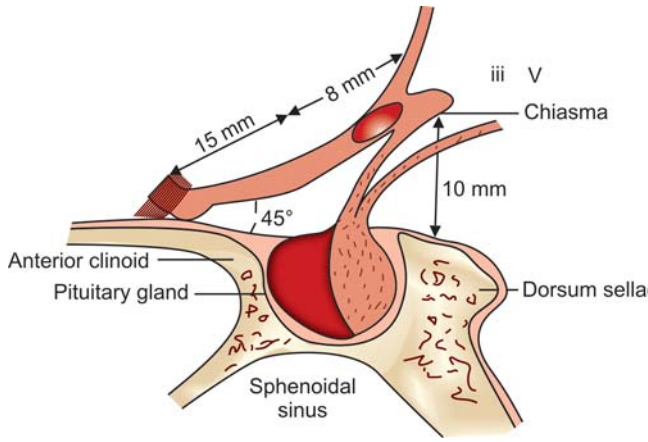


Fig. 10.1: Optic chiasma

Intraocular part: The optic disc is the exit site of the axons of the ganglion cells of the retina. The portion that is visible ophthalmoscopically is the prelaminar portion of the optic nerve. It is situated 3 to 4 mm nasal to the fovea or the posterior pole. It is slightly vertically oval and is 1.5 mm/2 mm in size. The optic nerve head contains four types of cells, ganglion cell axons, astrocytes, capillary associated cells and fibroblasts. As there are no other layers of retina other than the nerve fiber layer it produces an absolute scotoma in the visual field called the blind spot of Mariotte. The central depression that is circular is called the optic cup. The larger the scleral canal larger will be the physiological cup. If the canal is small the cup also will be small. This type of disc is called 'disc at risk' because when the axons are affected due to any reason the resultant swelling will cause ischemia to a larger number of fibers. This is because whatever may be the size of the disc, the number of fibers

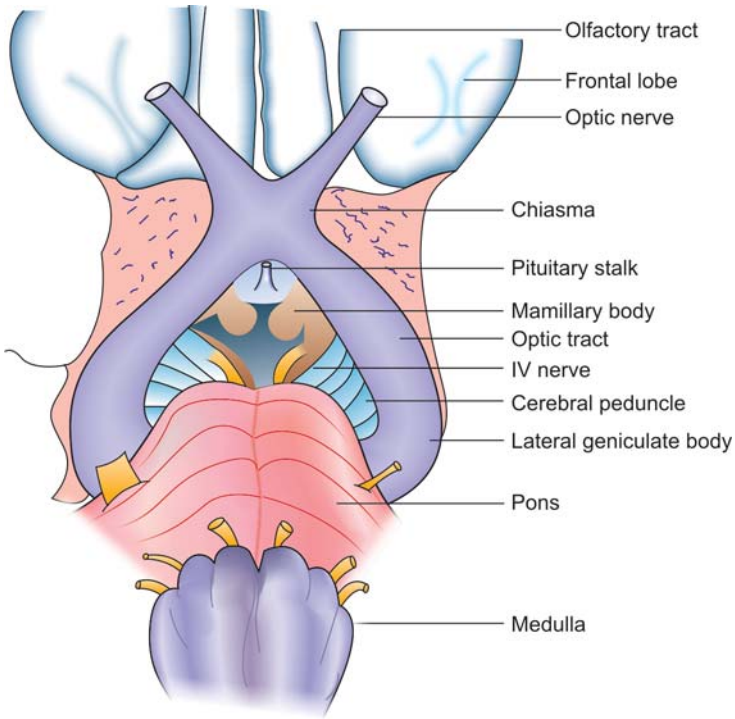


Fig. 10.2: Relationship of optic tract

passing through are almost the same. The deepest axons, i.e. those close to the sclera occupy the peripheral optic nerve while the axons close to the vitreous occupy the center of the nerve.

The fibers of the optic nerve, which are one million in number, are grouped together into small bundles called the fascicles. These are axons of secondary neurons, i.e. part of the central nervous system. The axons are mostly concerned with vision. Few fibers are responsible for eye movements and pupillary reactions. A few fibers supply motor fibers to the blood vessels. They are supported by astrocytes. The Muller's cells are replaced by astrocytes. The nerve fibers perforate the sclera making it sieve like. Hence, this part of the sclera is called lamina cribrosa. The nerve fibers become myelinated posterior to the lamina by the oligodendrocytes. This is similar to the Schwann cells of the peripheral nerves. But as the optic nerve is an extension of the white matter of the brain it is supported by oligodendrocytes. Sometimes the myelination of the optic nerve can extend on to the optic nerve head and rarely on to the retina also. This is more likely in premature births as light will help in myelination.

The laminar portion contains astrocytes along with elastic fibers and the collagenous connective tissue, which forms the scleral lamina. The lamina

cribrosa present in this layer is a scaffold for the nerve fibers and a point of fixation for the retinal vessels. It also reinforces the posterior segment.

Blood Supply

The prelaminar portion and the lamina cribrosa are supplied by the branches of posterior ciliary arteries which are branches of ophthalmic artery. The optic nerve head is supplied by the retinal arterioles which are branches of the central retinal artery. The circle of Zinn-Haller which supplies blood to the intraocular portion of the optic nerve receives blood from choroidal feeder vessels, from short posterior ciliary arteries and a small contribution from the pial vessels. The radial branches from the circle of Zinn-Haller, that supply the laminar portion of the optic disc are autoregulated like the retinal blood vessels. Venous drainage is through the central retinal vein. Fluid can pass from within the eye to the subarachnoid space due to hydrostatic pressure. This is due to the fact the intraocular pressure is more than the optic nerve tissue pressure.

The disc vessels do not leak during fluorescein angiography. The staining of the disc seen during late phases of FFA is due to the choroidal capillaries, which are permeable to fluorescein and the scleral collagen, which will take up the leaked dye.

The posterior ciliary arteries are terminal branches. Hence, the ends of the capillaries do not anastomose with other capillaries. This creates a watershed zone between two capillary beds. When blood supply is affected this area is affected first. In cases like acute anterior segment ischemia of the optic nerve head portions of the nerve head which falls in this watershed area are affected.

The retrolaminar portion extends from the lamina cribrosa to the apex of the orbit.

Intraorbital Portion

This portion lies within the muscle cone. At the apex it is surrounded by the annulus of Zinn with the origin of the recti muscles. The length of the nerve in the orbit is about 3 cm, which is longer than the distance between the lamina cribrosa and the apex of the orbit. This is to facilitate free movement of the eyeball in various directions.

The orbital portion is supplied by the pial plexus and the intraneural branches of the central retinal artery. Ophthalmic artery lies below and lateral to the optic nerve. At the middle of the orbit it crosses under the optic nerve to lie medial to it.

Intracanalicular Portion

At the apex of the orbit the optic nerve enters the optic canal which is 5-10 mm long and 5-7 mm wide. Its bony wall is thinnest on the medial side. Here, the nerve is separated from the ethmoid and sphenoidal sinuses by this

thin bone. The canal transmits the ophthalmic artery, which is on the lateral side, the filaments of the sympathetic plexus and the intracranial meninges around the optic nerve. The ophthalmic artery supplies the nerve. Small lesions arising within the canal will grossly affect the optic nerve. These lesions may not be picked up by even CT scans.

The dura and arachnoid around the optic nerve are attached to the periosteum making the nerve immobile. If any blunt injury occurs or, when there is edema of the optic nerve at this region the blood supply to the nerve is affected as this is a confined space.

Intracranial Portion

Posteriorly the nerve passes through an unyielding falciform fold and ascends at an angle of 45 degrees to join the optic chiasma.

The two optic nerves pass posteriorly over the cavernous sinus. Both nerves lie above the ophthalmic arteries and above and medial to internal carotid arteries. The anterior cerebral arteries, inferior surface of frontal lobe and olfactory tract lie above them. The two anterior cerebral arteries are joined together by the anterior communicating artery. The wall of the sphenoidal sinus, which is close to the inferomedial aspect of the nerve, is very weak. Hence, any inflammation of the sinus will affect the nerve, as the dura mater is absent here around the nerve.

This part of the optic nerve is supplied by the internal carotid and ophthalmic artery.

Meningeal sheathes: The pia mater covers the nerve from the retrolaminar portion, and ends in the intracranial part. It gives support to the nerve. The small arteriorles and venules present in the pia mater supply the nerve. The arachnoid mater continues with that of the brain. The dura mater is 0.3-0.5 mm thick. It has dense bundles of collagen and elastic tissue that fuse anteriorly with the outer layers of the sclera. The meninges have sensory nerve supply. Hence, optic neuritis can cause pain.

Development (Fig. 10.3)

The optic nerve develops from the optic stalk or the pedicle, which connects the optic vesicle and the forebrain. Its has a cavity which is connected to the cavity of the diencephalon posteriorly and the cavity of the optic vesicle anteriorly. The stalk contains neuroectodermal cells in the center and neural crest cells in the periphery. The stalk, which is initially short lengthens as the brain develops. As the fetal fissure closes the optic stalk becomes a rounded cord. The cavity is filled by the nerve fibers, which develop from the ganglion cells. Cells that were already in the stalk die to give room to these fibers. The nerve fibers first occupy the ventral and lateral portions. The glial tissue forms

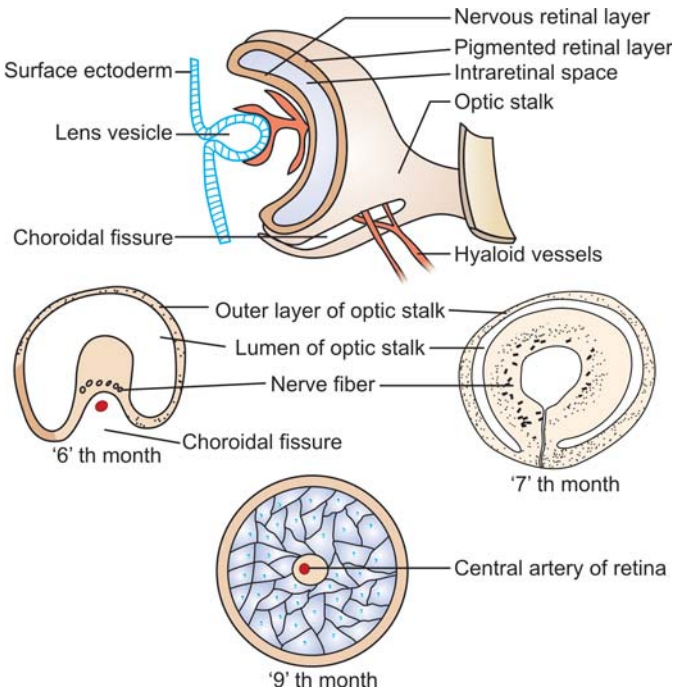


Fig. 10.3: Development of optic nerve head

around the 12th week. As the nerve fibers pass towards the optic stalk they displace some glial cells in the center of the disc. This is known as the Bergmeister's primitive epithelial papilla. This is vascularized by the hyaloid artery. When this vessel atrophies the papilla also disappears. Remains of the papilla are seen very often.

Myelination of the nerve fibers start by the 28th week on the chiasmal side and proceeds towards the eye. It ceases at the lamina cribrosa at about 4 weeks after birth.

Chiasma: is situated just below the anterior part of the third ventricle, above the diaphragma sellae posterosuperior to the optic groove on the sphenoid. It is a flat oblong band 8 mm long, 12 mm wide and 4 mm thick. The posterior border of the chiasma is slightly higher than the anterior. In 80% of cases part of the hypophysial fossa is anterior to the chiasma. In 12% the fossa is behind the chiasma. When the intracranial portion of the optic nerve is shorter than 12 mm the chiasma will sit over the tuberculum sellae. Then it is called a prefixed chiasma. If the optic nerve is longer than 18 mm the chiasma will be over the dorsum sellae. Then it will be called postfixed chiasma.

The chiasma is about 10 mm above the sella. Hence, any tumor of the pituitary gland must be more than 10 mm in size to affect the visual fields.

The anterior cerebral arteries lie anterior to the chiasma. The internal carotids lie on either side and the infundibulum (the stalk of the hypophysis) lies posterior. The third ventricle and the lamina terminalis lie above while the olfactory tract lies above and laterally. The hypophysis lies directly below and the cavernous sinus lies below and laterally. The chiasma is covered by the pia mater and the arachnoid is spread like an apron between the optic nerves.

55% of the fibers forming the chiasma decussate.

Optic Tract

It contains the uncrossed temporal and crossed nasal fibers from the optic nerves and the pupillary afferent fibers. The fibers from the upper part of the retina both crossed and uncrossed lie in the medial part of the tract. The macular fibers lie dorsolaterally. The tracts run backwards laterally and slightly upwards around the interpeduncular space. When the tracts go around the cerebral peduncles they turn slightly so that the part that was facing up faces upwards and medially. It is close to the anterior perforated substance and tuber cinereum. The tracts are separated from each other by the pituitary stalk inferiorly and the third ventricle superiorly. The tract then becomes part of the lateral surface of the cerebral peduncle. Here it lies between the internal capsule and basis pedunculi. The tract is attached to the brain by glial fibers. The anterior choroidal and posterior cerebral arteries lie below. The anterior choroidal artery crosses the tract twice. The optic tract crosses the third nerve and the pyramidal tract. Hence if there is a lesion in this region hemianopia is associated with motor and sensory loss also. As optic radiations are also close to the sensory and motor tracts in the posterior part of the internal capsule similar symptoms can occur. But the resultant hemianopia will be more congruous. Posteriorly the tract lies close to the roof of the inferior horn of the lateral ventricle. Here it develops a sulcus, which divides the tract into medial and lateral roots. The axons of the lateral root of the optic tracts terminate in the ipsilateral lateral geniculate ganglion. A small fascicle ends in the paraventricular nucleus of the hypothalamus. It is thought to send visual input to control diurnal rhythm. A slightly larger fascicle enters the medial geniculate nucleus and then terminates in the pretectal nucleus of the rostral midbrain and mediates the pupillomotor light reflex. The medial root enters the superior colliculus. This may be responsible for general reflex responses to light.

The optic tracts are supplied by the anterior choroidal arteries, branches of internal carotid arteries (Fig. 10.4).

Lateral Geniculate Nucleus

This is the thalamic visual center linking the retina and the striate cortex. It is situated in the lateral geniculate body. It is an oval or cap like structure resembling a closed fist situated in the posterior aspect of the thalamus, hidden by

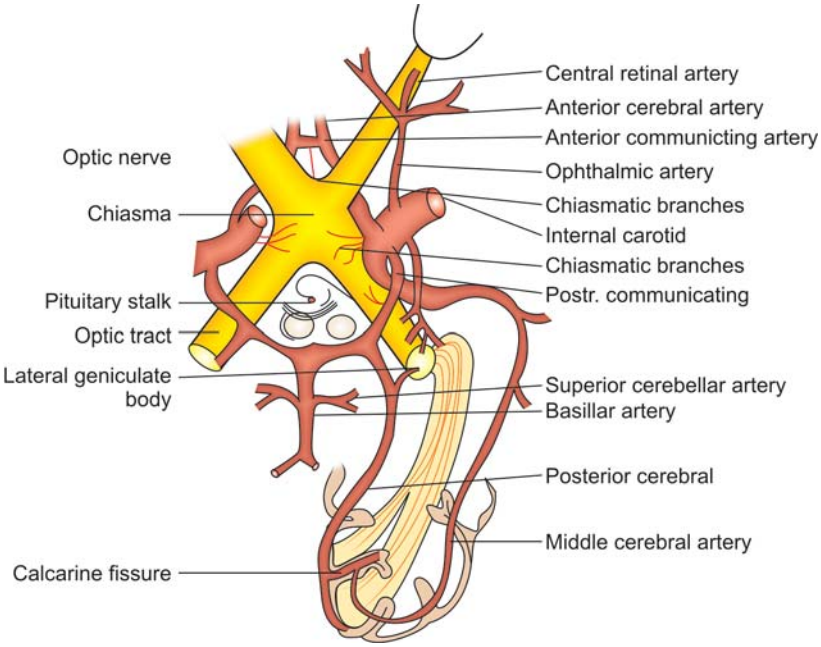


Fig. 10.4: Blood supply to visual pathway

the pulvinar. It is below and lateral to the pulvinar and above the lateral recess of the ambient cistern. Because of its location it is difficult to visualize LGN by neuroimaging. The optic radiations are situated dorsolaterally with the internal capsule on the lateral side. The acoustic radiations are present dorsomedially. The hippocampal gyrus and the inferior horn of the lateral ventricle are present posteriorly (Fig. 10.5).

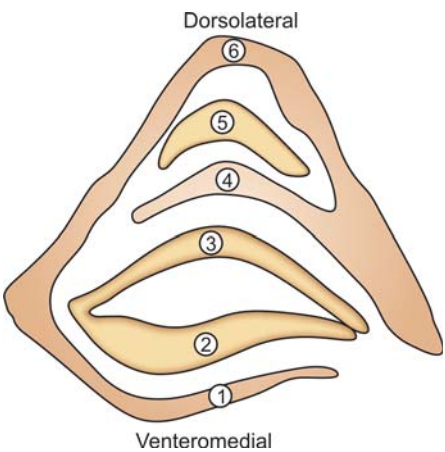


Fig. 10.5: Lateral geniculate body

LGN plays a major role in the appreciation of contrast. The number of neurons in the LGN are exactly same as the number of ganglion cells.

The fibers of the optic tract enter the nucleus anteriorly. Inferiorly the nucleus is hollow. This is called the hilum. The superior brachium connects the LGN to the superior colliculus.

Arrangement: The fibers of the tract divide into two layers. When seen after a sagittal section the inferior layer enters the hilum while the superior layer enters the dorsal part. It receives fibers from the retinal ganglion cells and project it to the cerebral cortex through the optic radiation. The nucleus contains 6 layers. The laminae are connected to each other by the inter neurons.

Afferent pathways to lateral geniculate nucleus:

a. Retinal ganglion cells: this is the main afferent pathway. There are three major types of retinal ganglion cells.
   I. The medium sized cells have small receptive fields, high spatial resolution, low contrast sensitivity and temporal resolution. The axons project to parvocellular layer of LGN. They are important for color perception and spatial contrast under photopic conditions.
   II. Large sized ganglion cells have larger receptor fields, low spatial resolution, higher contrast sensitivity and temporal resolution. They transmit information in mesopic and scotopic conditions.
   III. The smallest ganglion cells
b. Occipital cortex
c. Sensory fibers from the spinal cord and medulla.

Efferent fibers cross and connect with the oculomotor nuclei and the tectospinal tracts.

Optic Radiations of Gratiolet (Geniculo-calcarine Pathway)

The visual impulses from the lateral geniculate nucleus is carried to the occipital lobe by the optic radiations. The fibers pass forwards from the LGN and travel close to the retrolenticular part of the internal capsule behind the sensory fibers. They spread out to form the optic lamina. The fibers travel lateral to the temporal and occipital horns of the lateral ventricle in the lateral sagittal stratum.

The ventral part pass forward into the temporal pole and then passes backwards as the fasciculus of Meyer. Any lesion in this part will cause superior homonymous quadrantic hemianopia (pie in the sky). The optic radiations then pass backwards deep to the middle temporal gyrus. Hence, tumors of the temporal lobe can affect the optic radiations. The radiation ends in the striate cortex (Fig. 10.6).

The striate cortex is just 1.4 mm thick. It has a distinctive striae called the striae of Gennari. The striate cortex is situated around the calcarine sulcus. The calcarine sulcus is a deep sulcus extending from the occipital pole, and situated on the medial side of the cerebral hemisphere. It begins at the lunate

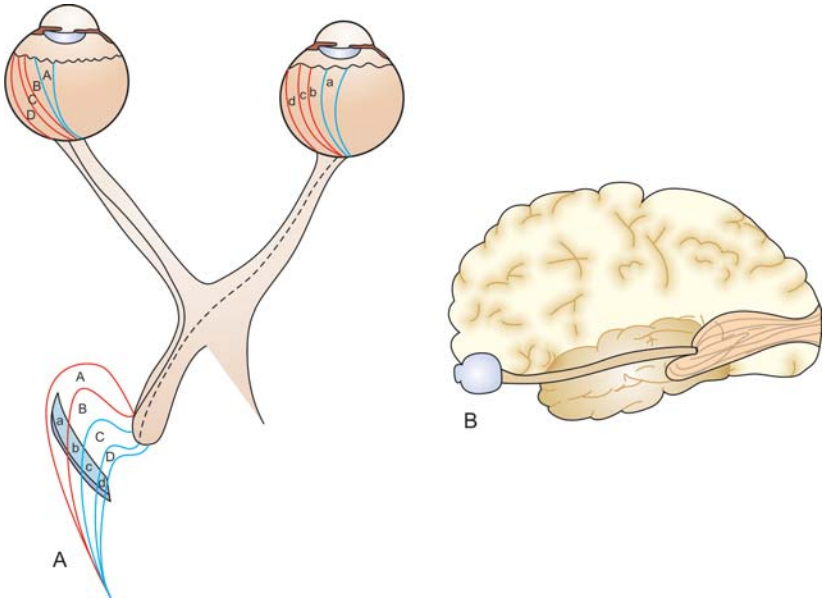


Fig. 10.6: **A.** Representation of inferior retinal quadrant in the temporal lobe. **B.** Optic radiation

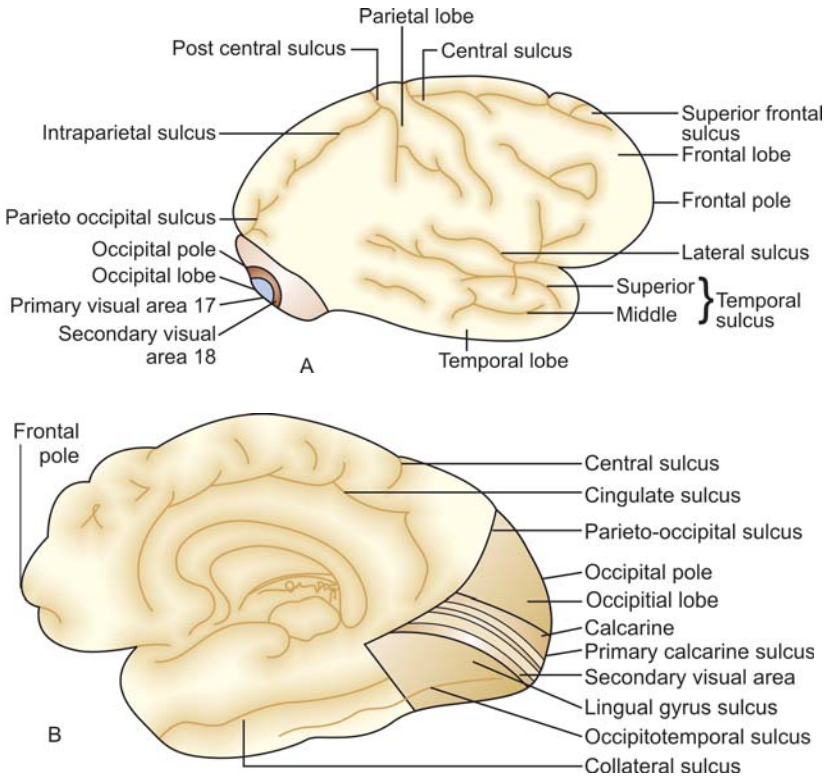
sulcus and passes forwards to end below the splenium of the corpus callosum. The posterior end often extends to the lateral surface of the cerebrum. The parietooccipital sulcus divides the calcarine sulcus into and an anterior and posterior part. The cuneate gyrus is found in between the two sulci.

The lunate sulcus separates the striate area from the peristriate area of the cortex. The parastriate area is buried within the walls of the sulcus. From the upper and lower end of the lunate sulcus arise the superior and inferior polar sulci which enclose the cortex representing the macular area. The lingual gyrus lies between the calcarine and collateral sulci.

The visual cortex or the striate cortex (Brodmann's area 17) is situated around the calcarine sulcus. The cortex is present on either side of the sulcus in the posterior portion but only on the inferior side anteriorly. The cortex extends from the parietooccipital sulcus above to the collateral sulcus below. Anteriorly it extends up to the splenium of the corpus callosum. The striate appearance is due to the interconnecting fibers in the fourth layer of the cortex (Figs 10.7A and B).

The cortex has six layers.

1. The plexiform lamina is the outermost with axons and dendrites. The processes of stellate cells, dendrites of the pyramidal cells and afferent fibers from other parts of the central nervous system are found here.
2. The next layer is the external granular lamina. This layer has a granular appearance due to the presence of many nuclei. Here also some cells are pyramidal while others are stellate.



Figs 10.7A and B: Cerebral hemisphere (A. lateral view, B. medial view)

3. The pyramidal lamina contains the pyramidal neurons and stellate interneurons with vertical or horizontal processes.
4. The next layer is called the internal granular lamina. It has stellate interneurons and a few pyramidal cells. There are both vertical and horizontal processes, which form the external band of Baillarger.
5. The ganglionic lamina also contains stellate and large pyramidal cells along with axons and dendrites of cells from the other layers.
6. This layer called the multiform lamina is the innermost layer. It is close to the white matter and contains small neurons, which are more stellate than pyramidal.

The axons project to the basal ganglia, thalamus, hippocampus, brainstem nuclei and the spinal cord.

Besides the main visual cortex the peristriate area (area 18) and parastriate area (area 19) also receive fibers from the optic radiation. The visual cortex is also connected to the opposite side, the frontal visual fields, parietal association areas, superior colliculus and oculomotor and other cranial nerve nuclei.

Arrangement of the Nerve Fibers in the Visual Pathway

When the light falls on the retina the rods and cones are stimulated and the impulses pass through the bipolar cells and the ganglion cells and reach the lateral geniculate ganglion. From here the optic radiations take the impulses to the cortex. Throughout this pathway the nerve fibers are arranged in such a way the visual impulses falling on the right side of retina (that is the image of objects on the left side), go to the cortex on the right side. The fibers are arranged in a very regular and precise manner (Fig. 10.8).

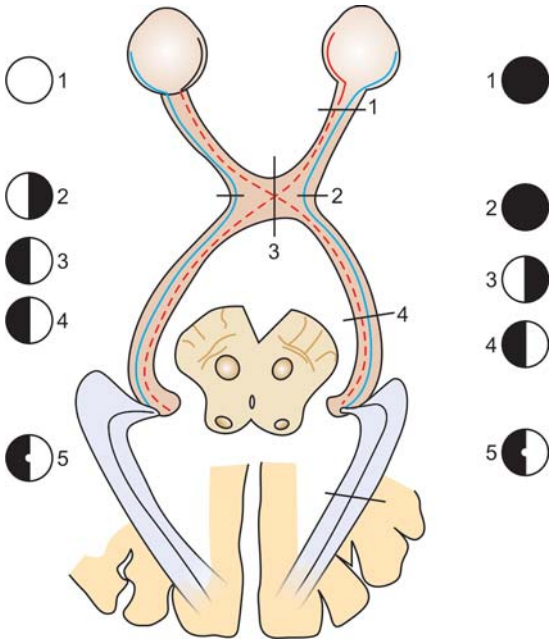


Fig. 10.8: Arrangement of nerve fibers

Retina: In the retina the line dividing the nasal from the temporal fibers pass through the fovea. Those that are temporal to the line go uncrossed, and the others cross over to the other side. The upper temporal fibers are separated from the lower by the macular fibers. The fibers from the periphery of the retina lie deep in the optic disc.

Optic nerve: Just behind the disc the fibers are as in the retina. The macular fibers occupy a large space and are located temporally. The peripheral temporal fibers lie above and below these fibers. The nasal fibers occupy the nasal side of the disc. As we go closer to the chiasma the macular fibers lie in the center.

Chiasma: In the chiasma the nasal fibers cross to the opposite side. Fibers from the lower and medial quadrant bend to reach the anterior portion of the chiasma and then descend to enter the optic tract of the opposite side. The most anterior fibers enter the terminal part of the opposite optic nerve before

crossing. These fibers are called the anterior loop of Willebrand. Hence, a lesion affecting the optic nerve close to the chiasma will cause an upper temporal field defect in the opposite eye besides affecting vision on the same side. These fibers are also close to the pituitary and hence affected early in tumors of this gland.

The upper nasal fibers go along with the uncrossed fibers and form a loop in the tract before crossing to the opposite side. The uncrossed temporal fibers run directly backwards and enter the tract of the same side.

The macular fibers cross in the posterior aspect of the chiasma. These fibers are above the peripheral crossing fibers.

Optic tract: The fibers, both crossed and uncrossed are arranged in such a way that the macular fibers are present in the dorsolateral region. The fibers from the lower retinal quadrants are (both crossed and uncrossed) are placed laterally. The fibers from the upper quadrants are placed medially.

Lateral geniculate nucleus as in the tract the lower fibers are lateral and the upper fibers are medial. The macular fibers occupy all the laminae on the posterior two thirds of the nucleus. The laminae in the lateral geniculate ganglion represent fibers alternately from the retina of the same side and opposite side. Of the six laminae present in the nucleus the first two layers close to the hilum contains large cells compared to the other four layers. The crossed fibers end in 1, 4 and 6 layers. The uncrossed fibers end in 2, 3 and 5 layers. There is a point to point localization of the retina. This is projected in the same way to the cerebral cortex. The crossed and uncrossed fibers lie in such a way in the laminae that the fibers from corresponding areas of two retinae end in adjacent areas. Each fiber in the optic tract divide into five or six branches and synapses with the dendrites of a single neuron in the lateral geniculate nucleus. There are inter-neurons connecting the cells to each other.

Optic radiation: The fibers representing the upper portion of the retina (which is present in the medial part of the lateral geniculate nucleus) occupy the upper portion of the radiation and end in the upper lip of the calcarine fissure. The lower part of the radiations represent the lower part of the retina and end in the lower part of the calcarine fissure.

Visual cortex: while the fibers from the corresponding visual areas are in adjacent laminae in the lateral geniculate body they are located side by side close to each other in the cortex. The localization of the retina in the cortex is very precise and sharply represented (see Fig. 10.6).

As the nasal fibers cross in the chiasma all the visual impulses falling on the right side of each retina will reach the right lateral geniculate body and the cortex on the right side. As already mentioned the upper fibers end above the calcarine sulcus. The macula is represented in the posterior most part of the visual cortex occupying a very large area when compared to the size of the

macula in the retina. It may even extend on to the lateral side of the cerebral hemisphere.

The nasal periphery of the retina is represented in the anterior most part of the striate area. This area represents the temporal crescent in the visual field. The vision is monocular in this area.

Though the macular area is supplied by the posterior cerebral arteries when this vessel develops thrombosis the middle cerebral artery supplies the area for central vision (see Fig. 10.8).

OCULOMOTOR NERVE

The oculomotor nerve or the third cranial nerve contains 15000 axons. It innervates the levator palpebrae superioris, superior rectus medial rectus, inferior rectus, and the inferior oblique.

Besides this the parasympathetic fibers to the sphincter pupillae and the ciliary muscle also pass through this nerve.

The nucleus of the third nerve lies in the midbrain at the level of the superior colliculus, ventral to the gray matter around the aqueduct of Sylvius. It is just above the nucleus of the fourth nerve. The medial longitudinal bundle lies inferolaterally. The internuclear neurons project to the nucleus of the VI nerve on either side.

The nucleus is divided into subdivisions each supplying one extraocular muscle. The central caudal nucleus supplying the levator palpebrae superioris is single whereas the other nuclei are paired. Fibers for the superior rectus cross the nucleus and supply the contralateral superior rectus.

The Edinger Westphal nucleus is situated above and dorsomedially. This supplies parasympathetic preganglionic efferent innervation to the pupillary sphincter and ciliary muscle.

Abnormalities of oculomotor nerve may be caused by absent or incomplete development of the nucleus, nerve or both, causing congenital third nerve palsy.

Since both LPS are supplied by the caudal end of oculomotor nerve nucleus complex lesions of this part causes bilateral ptosis. Conversely this nucleus may be spared while the other subnuclei are involved.

Since the pupillary area is represented in the rostral part lesions in the caudal region will not affect the papillary reactions (Figs 10.9-10.11).

The fascicular portion of the third nerve (the portion of the nerve within the brain) passes ventrally through the red nucleus and corticospinal fibers and enters the interpeduncular space. If any lesion occurs at this level involving the red nucleus and third nerve ipsilateral third nerve palsy and contralateral involuntary movements will occur (Benedict's syndrome). If the lesion occurs at the level of the cerebral peduncle contralateral hemiplegia will occur along with involvement of the lower face and tongue (Weber's syndrome).

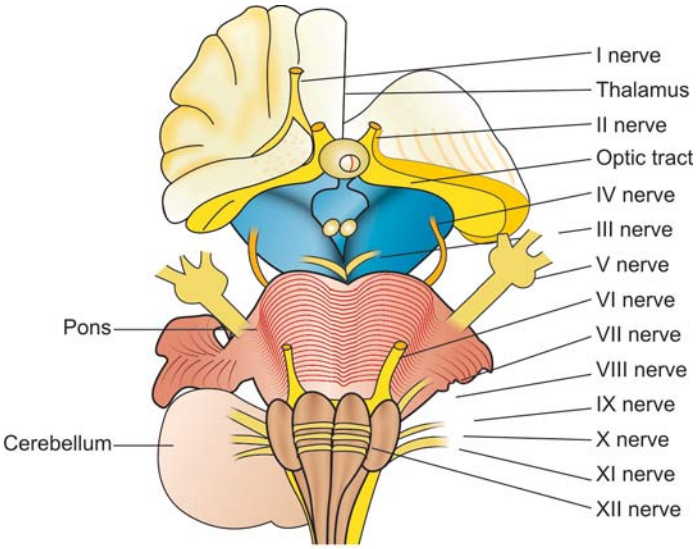


Fig. 10.9: Ventral aspect of brain

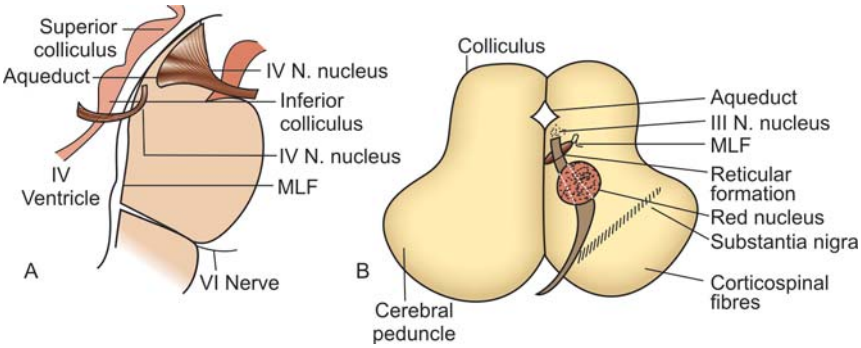


Fig. 10.10: A. Nuclei of III, IV, VI nerves and B. Exit of III nerve

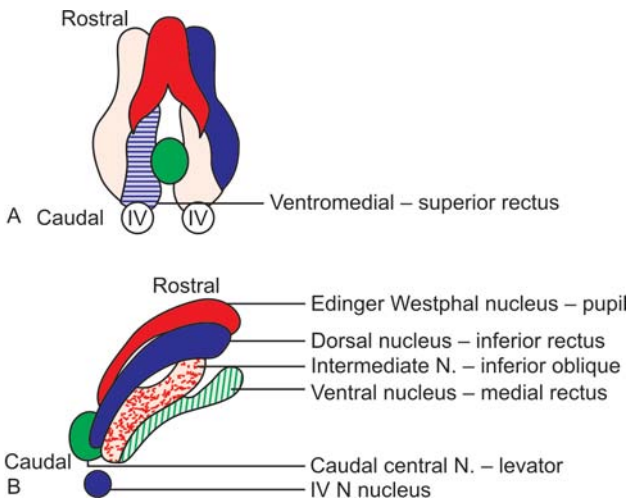


Fig. 10.11: A. Oculomotor nucleus complex and B. Right lateral aspect

The differentiation into the superior and inferior division occurs at the level of the fascicular portion itself. Hence, partial third nerve palsy can occur due to lesions here also.

Lesions involving the fascicle can cause both, complete and incomplete nerve palsy. The pupil also may or may not be involved. Lesions at the level of the brachium conjunctivum will cause cerebellar ataxia (Nothnagel's syndrome).

In the inter-peduncular fossa the nerve passes below the posterior cerebral artery and above the superior cerebellar artery. Aneurysms, at the junction of the basilar artery and the superior cerebellar artery or the internal carotid and posterior communicating artery will affect the third nerve at this level. Meningitis also can affect the third nerve when it is crossing the inter peduncular fossa. These two are the major branches of the basilar artery. The terminal arteries supply the red nucleus and the cerebral peduncles. In the event of any obstruction to this vessel exclusive third nerve palsy or lesions involving the red nucleus and cerebral peduncles occur. Here, the nerve lies lateral to the posterior communicating artery. The nerve then lies close to the free and attached borders of the tentorium cerebelli. Herniation of the hippocampal gyrus compresses the oculomotor nerve where it passes over the ridge of the dura associated with the attachment of the free edge of the tentorium to the clivus. The nerve is compressed between the free and attached borders of the tentorium. The herniating mass also displaces the pons and stretches the posterior cerebral arteries. This will compress the nerve still further.

The third nerve pierces the dura in the lateral side of the posterior clinoid process to enter the roof of the cavernous sinus. Where it pierces the dura it is firmly attached and is vulnerable to stretch and contusion injuries like to trauma to the frontal area. It then changes course to lie in the lateral wall of the sinus initially above the fourth nerve later below the same. When the nerve is traveling in the lateral wall of the cavernous sinus any lesion here may affect the sympathetic fibers also causing a miotic pupil along with third nerve palsy. Any suprasellar lesion may affect the third nerve alone in this region. Close to the superior orbital fissure it divides into two branches. It enters the orbit within the annulus of Zinn, i.e. the middle portion of the superior orbital fissure. The upper division supplies the levator and the superior rectus. The fibers for the LPS runs along the lateral aspect of the superior rectus and penetrate the muscle from the under surface. The lower division supplies the medial and inferior rectus and the inferior oblique. Absence of the superior division of the nerve, which occurs in autosomal dominant mutations of cause congenital bilateral ptosis, with eyes, fixed at down gaze and external ophthalmoplegia.

Since the pupillary fibers are situated superficially they are affected due to compression at an earlier stage.

Blood supply: from the nucleus to the orbit, the nerve is supplied by the posterior cerebral, superior cerebellar artery, meningeal branches of the internal carotid and the ophthalmic artery.

TROCHLEAR NERVE

The trochlear or the fourth cranial nerve is the thinnest cranial nerve with very few fibers (2100) supplying just one muscle the superior oblique. It has a long intracranial course 75 mm. Its nucleus is located just below (caudal) to the third nerve nucleus with which it is continuous. The nucleus lies at the level of the inferior colliculus ventral to the aqueduct. The medial longitudinal bundle is ventro lateral to the nucleus (Fig. 10.12).

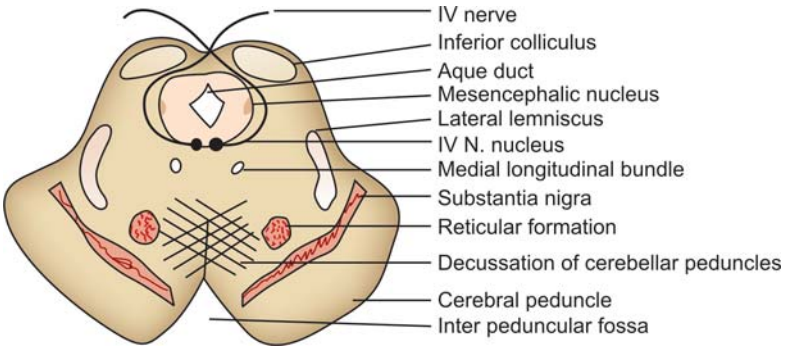


Fig. 10.12: IV nucleus and exit

Unlike other motor nerves the fibers of the IV nerve almost completely decussates at the level of the anterior medullary velum, over the roof of the aqueduct of Sylvius. It then exits on the dorsal aspect curving around the periaqueductal grey matter. Since it exits dorsally it does not cross any important structures. Hence isolated lesions can occur anywhere along its course. Rarely contralateral Horner's syndrome and relative afferent pupillary defect can occur without involvement of the optic nerve. At this level the sympathetic fibers are affected as they run through the dorso lateral tegmentum close to the fascicular part of the IV nerve. The pupillary fibers are affected due to involvement of the brachium of the superior colliculus.

After exiting the midbrain just below the inferior colliculus it lies in the ambient cistern. As it emerges from the dorsal surface it is susceptible to injury or compression. An injury can avulse or stretch the rootlets of the nerve as it is very thin. Both nerves can be affected simultaneously as they are close together. From below the edge of the tentorium the nerve runs between the posterior cerebral and superior cerebellar arteries lateral to the third nerve. It then pierces the dura to enter the cavernous sinus. In the lateral wall of the cavernous sinus it runs below the third nerve initially. Then it crosses the third nerve and enters the orbit through the lateral most part of the superior orbital

fissure. Since it is situated outside the muscle cone any lesions in the cone or retrobulbar anesthesia will not affect this nerve.

ABDUCENS NERVE

The nucleus of the sixth cranial nerve is situated in the floor of the IV ventricle, beneath the facial colliculus in the lower part of pons. The facial colliculus is formed by the fibers of the VII nerve, which loop around the nucleus of the sixth nerve. The medial longitudinal bundle is medial to the nerve. There are 6500 cells in the nucleus. Some of these cells travel upwards along the medial longitudinal bundle on the contra lateral side. This connection is involved in horizontal gaze. As the abducens nucleus contains neurons for the ipsilateral lateral rectus as well as the interneurons that supply the opposite medial rectus lesions of the nucleus will cause horizontal gaze palsy to the ipsilateral side. As the facial colliculus may also be involved a VII nerve palsy may co exist. If the lesion affects the MLF on the same side one and a half syndrome results. The basilar artery supplies blood to the nucleus (Fig. 10.13).

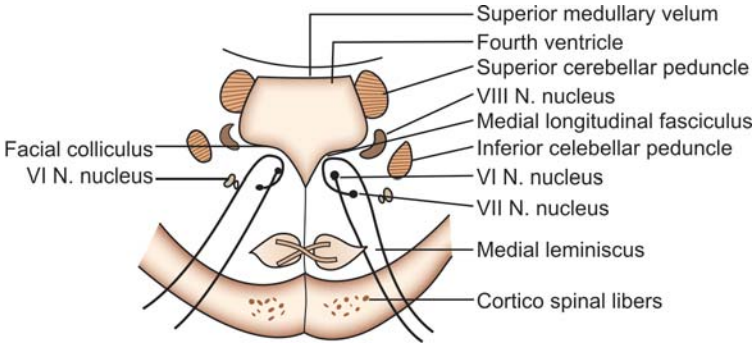


Fig. 10.13: Caudal part of pons VI and VII N. nuclei

The fascicular portion of the nerve runs downwards, laterally and ventrally through the paramedian pontine reticular formation and the pyramidal tract. The nuclei of the fifth nerve are also very close.

As the fascicular portion of the abducens nerve passes through many structures complex neurological findings will be present when this part of the sixth nerve is affected. As fascicles of both VI and VII nerve are affected paralysis of abducens and facial nerve will be present together. If nucleus of tractus solitarius is affected loss of taste in the anterior two thirds of the tongue will be seen. Besides this the central trigeminal tract (ipsilateral Horner's syndrome), spinal tract of the trigeminal nerve (ipsilateral analgesia of the face) and superior olivary nucleus (ipsilateral peripheral deafness) may also be affected. This is called the syndrome of anterior inferior cerebellar artery.

In Millard Gubler syndrome VI and VII are affected along with contra lateral hemiplegia.

In Raymond Cestan syndrome VI nerve is affected with hemiplegia.

Lesions in the ventral paramedian pons affects the cortico spinal tract along with VI and VII nerves causing ipsilateral lateral rectus palsy with contra lateral hemiplegia.

It exits the brain at the junction of the pons and medulla. The nerve travels upwards on the ventral surface of the pons lateral to the basilar artery. The anterior inferior cerebellar artery crosses the nerve. On the surface of the clivus, the nerve is surrounded by Batson's venous plexus. As the nerve has got a long course it is frequently involved in many conditions. As the nerve is bound to the ventral surface of the pons by the anterior inferior cerebellar artery, it may be compressed by the vessel. Atherosclerotic, posterior inferior cerebellar artery and basilar artery also can affect the nerve.

As the nerve ascends vertically in the subarachnoid space it is vulnerable. Any descent of the brain stem following injury to the head, space-occupying lesions above the tentorium will cause sixth nerve palsy. The nerve gets stretched where the nerve exits from the pons and at the clivus. Meningitis also can affect the sixth nerve in this region along with other cranial nerves.

False localising sign follow increased intracranial tension due to the nerve being compressed between the pons and the basilar artery or the clivus or due stretching at the sharp edge of the petrous temporal bone.

About 2 cm below the posterior clinoid process and below the crest of the petrous part of the temporal bone it is closely connected with the inferior petrosal sinus. Thrombophlebitis of the lateral sinus can extend to the inferior petrosal sinus and affect the sixth nerve.

The nerve runs either through the sinus or around it and then under the petro clinoid (Gruber's) ligament. Here it is close to the mastoid air cells and hence can be affected by mastoiditis and otitis media. The inflammation may cause meningitis at the level of the tip of the petrous part of the temporal bone causing Gradenigo's syndrome. In this syndrome the Gasserian ganglion and the facial nerve are also affected. This area under the ligament is called the Dorello's canal.

The nerve then enters the cavernous sinus and runs below and lateral to the internal carotid artery. The sympathetic fibers from the paracarotid plexus may run along with this nerve. As it is located close to the internal carotid vascular lesions affect the sixth nerve first. The sympathetic plexus around the carotid will also be affected. When lesions occur close to the spheno palatine fossa VI nerve, spheno palatine ganglion (loss of tears) and maxillary division of the fifth cranial nerve will be affected together.

It enters the orbit through the middle portion of the superior orbital fissure. After a short course in the orbit it supplies the lateral rectus. Due to the short course isolated lesions do not usually occur at this level. All the fibers ramify in the orbit and enter the muscle in a diffuse fashion.

Supra Nuclear Connections of the Ocular Motor System

The normal smooth movement of the eyeballs involves the neurons of the third, fourth and sixth nerves on either side or the internuclear pathways in the brain stem. Conjugate gaze involves both visual and auditory systems.

The posterior part of the right middle frontal gyrus is responsible for conjugate deviation of the eyes to the left side. Stimulation of the area above the central part will result in downward movement and the area below will result in upward movement.

The occipital center is responsible for moving the eye to focus on the object of interest. Stimulation of this area will also result in conjugate deviation.

Inter Nuclear System

This is called the medial longitudinal fasciculus. This tract extends from the oculomotor nucleus to the spinal cord. It lies close to the median line beneath the aqueduct and the fourth ventricle. It also lies close to the oculomotor nuclei lateral to the third and fourth but medial to the sixth.

The fibers in the tract are:

a. the vestibular nuclei
b. ipsilateral sixth nerve connecting to the contra lateral subnucleus of the medial rectus.

All three oculomotor nuclei will be affected if the medial longitudinal bundle is affected.

The fibers of MLF synapses with nucleus of Darkschewitsch, interstitial nucleus of Cajal and rostral interstitial nucleus of MLF. Lesions of interstitial nucleus of Cajal will affect the vertical pursuit and vertical gaze holding.

Immediate Premotor Structures of the Brain Stem

Consists of the abducens nuclei and the paramedian pontine reticular formation for horizontal gaze and rostral mesencephalic reticular formation for vertical gaze.

Paramedian Pontine Reticular Formation

Is located near the abducens nucleus within the pons. Lesions of PPRF cause paralysis of conjugate gaze to the ipsilateral side. If the sixth nerve alone is affected abduction to the ipsilateral side only is affected. Interconnections, both afferents and efferents exist between PPRf and vestibular nuclei, reticular formation, spinal cord and cerebellum. The main projections are to ipsilateral abducens and to rostral intestitial nucleus of MLF, which is concerned with vertical gaze.

The premotor commands for horizontal gaze are sent from the abducens nuclei to ipsilateral lateral rectus and contralateral medial rectus through the internuclear neurons ascending in the contralateral MLF.

Rostral Interstitial Nucleus of the MLF

Is a group of cells situated on either side of midline in the rostral mesencephalon. It has projections to oculomotor and trochlear nerve nuclei for vertical recti and oblique muscles. It causes vertical saccadic movements.

Nucleus of the Posterior Commissure

Lesions of this nucleus, which is situated in the caudal end of diencephalon, on its dorsal side will affect upward movements.

Cerebral Cortex

Movements both voluntary and involuntary are represented in the cerebral cortex. The cerebral regions involved are:

a. Frontal eye fields in the frontal cortex — posterior part of the second frontal convolution area 8 of Brodman. Stimulation of this area results in conjugate ocular movements to the opposite side. The fibers from the cortex pass through the posterior part of the anterior limb of the internal capsule near the genu to the medial part of the cerebral peduncle.
b. Internal sagittal stratum in the parieto occipital region.

Fibers from area 8 also project to layer IV of superior colliculus and pretectal region, contra lateral pontine tegmentum, thalamic nuclei, medial pulvinar and PPRF and the oculomotor nuclei.

Parieto Occipital Eye Fields

Pursuit movements originate here. The eyes deviate to the opposite side when this area is stimulated.

Superior colliculus and thalamus: The superior colliculi project from the upper part of the mesencephalon. It contains alternating layers of cells. The superficial layers are involved in visual sensory function. The deep layers are involved with ocular movements. It receives projection from the other supranuclear structures associated with eye movements. The deepest layer consists of efferent fibers.

Vestibular System

The vestibular impulses that affect the vertical gaze fibers from ipsilateral vestibular nuclei pass to the brain stem on the opposite side. It then ascends in the medial longitudinal fasciculus to the oculomotor subnuclei involved in vertical movements.

For horizontal vestibular ocular impulses there is a projection to the contralateral abducens nucleus and ipsilateral medial rectus. This is through the ascending tract of Dieter's and the contra lateral MLF.

TRIGEMINAL NERVE

The trigeminal or the fifth cranial nerve has both sensory and motor fibers. It carries sensory nerve supply to the scalp, forehead, face, lids, eye, lacrimal gland, extraocular muscles, ear, dura mater and the tongue. The mandibular division supplies the muscles of mastication.

The nuclear complex is very long extending from midbrain to the upper cervical segments up to C4.

The mesencephalic nucleus: mediates proprioceptive and deep sensation from masticatory, facial and extraocular muscles.

Main Sensory Nucleus

Is situated in the pons lateral to the motor nucleus. It is continuous above with mesencephalic nucleus and below with the spinal nucleus. It serves light touch from skin and mucus membrane. The sensory root of the fifth cranial nerve divides into ascending and descending tracts at the level of the pons. Ascending root ends in the main sensory nucleus and the descending root ends in the spinal nucleus.

Spinal nucleus and tract: extends from the medulla to C4. It receives pain and temperature afferents. It also carries cutaneous components of VII, IX and X from the ear. The sensory fibers of ophthalmic division enter the ventral portion of the nucleus. Fibers from perioral and perinasal areas end rostrally.

Peripheral face and scalp end caudally.

Midface enters the central portion of the nucleus.

So if the sensory loss is bilateral and affects one of the concentric circles it must due to a lesion in the brain stem. If distribution of a particular division is affected the lesion must be after the exit of the nerve from the brain stem.

The axons from the nuclei terminate at the ventral postero medial nucleus of the thalamus after crossing at the level of pons. The axons from the nucleus then reach the postcentral gyrus of the cerebral cortex through the internal capsule.

Motor Nucleus

Lies medial to the sensory nucleus. It receives fibers from both the cerebral hemispheres, the reticular formation, red nucleus, tectum, medial longitudinal bundle and mesencephalic nucleus.

The fifth nerve emerges from the upper lateral portion of the ventral pons, passes over the petrous apex, forms the Gasserian ganglion and then divides in to three branches—ophthalmic, maxillary and the mandibular.

The Gasserian ganglion is situated in the Meckel's cave, a recess in the duramater at the apex of the petrous part of the temporal bone, in the middle cranial fossa. The cavernous sinus lies anteromedial to the ganglion. The internal

capsule lies close to the ganglion on its medial aspect. The ganglion contains the cells of origin of all the sensory axons of the fifth cranial nerve.

Ophthalmic Division

Travels along the lateral wall of the cavernous sinus lateral to internal carotid artery. At this position it lies beneath the third and fourth nerve. It supplies sensory nerve supply to the cerebral vessels, duramater of the anterior cranial fossa, cavernous sinus, sphenoidal wing, petrous apex, Meckel's cave, tentorium cerebelli, falx cerebri and dural venous sinuses. It divides into three branches the frontal, lacrimal and nasociliary. The frontal and lacrimal enters the orbit through the lateral portion of the superior orbital fissure while the nasociliary enters through the central portion (Fig. 10.14).

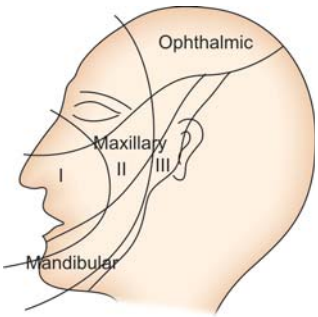


Fig. 10.14A: Sensory distribution of supranuclear V nerve

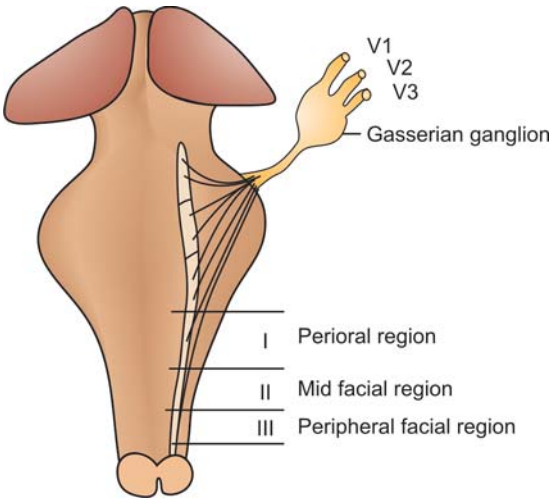


Fig. 10.14B: Nucleus of V nerve

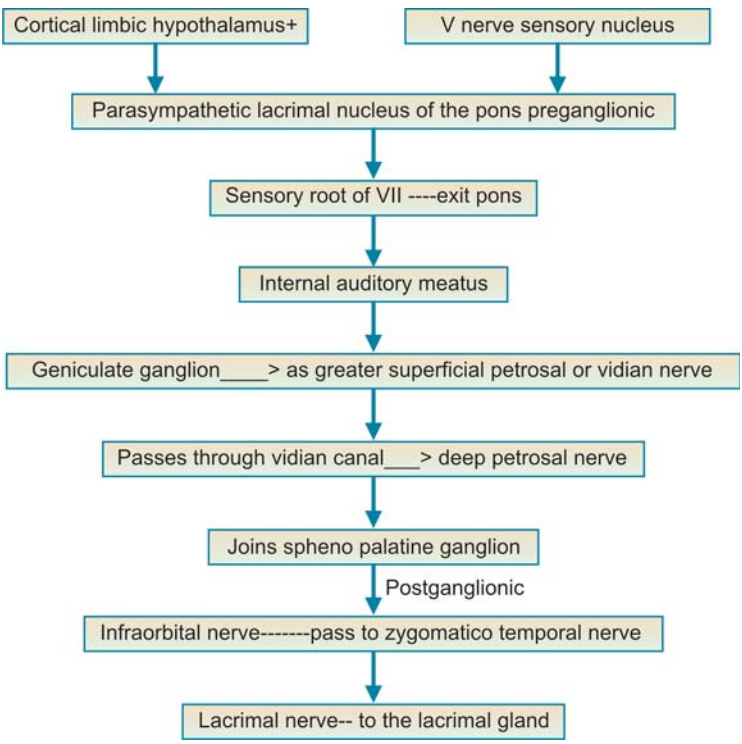
Frontal Nerve

Divides into supraorbital and trochlear nerve and provides sensation for the medial portion of the upper lid and conjunctiva, forehead, scalp, frontal sinus and the side of the nose.

Lacrimal Branch

Supplies the lateral portion of the upper lid and conjunctiva, lacrimal gland and carries postganglionic parasympathetic fibers for reflex lacrimation.

Pathway for Reflex Tearing



The Naso Ciliary Nerve

Supplies sensation through nasal branches to the middle and inferior turbinates, septum, lateral nasal wall and tip of the nose.

Its supratrochlear branch supplies the conjunctiva and skin on the medial side besides the lacrimal sac and the nasolacrimal duct.

The long ciliary nerves carry sensory fibers from the ciliary body, iris, cornea and sympathetic innervation to the dilator muscle.

The short ciliary nerves carry sensation from the globe. The ciliary nerves also carry postganglionic parasympathetic fibers from the ganglion to the pupillary sphincter and the ciliary muscle.

Hutchinson's sign: If the tip of the nose is involved in Herpes zoster the eye is likely to be involved as the naso ciliary supplies the tip of the nose and the eye.

Maxillary Division

Exits the skull through the foramen rotundum. It reaches the infraorbital fissure after crossing the pterygo palatine fossa and runs as the infraorbital nerve along the floor of the orbit. It occupies the infraorbital canal and exits the orbit through the infraorbital foramen.

This branch supplies the lower lid, side of the nose, upper lip, the teeth, maxillary sinus, roof of the mouth and soft palate. Lesions in any of these areas can cause referred pain in the eye.

Mandibular Division

Exits through the foramen ovale and supplies muscle of mastication and skin of the mandible, buccal mucosa, lower lip, tongue, external ear and tympanum.

FACIAL NERVE

The motor area for the facial nerve is situated in the precentral gyrus of the frontal cerebral cortex. The fascicles from here pass through the genu of the internal capsule and cerebral peduncles to the pons. The facial nucleus that supplies the frontalis, orbicularis and corrugator receive fibers from both right and left side but the lower face receives only crossed fibers. Hence supra-nuclear lesions will involve only the contralateral lower face.

The nucleus of the facial nerve contains 7000 nuclei and is located in the lower pontine tegmentum in the ventrolateral angle. It has four groups of nuclei

a. auricular and occipital areas receive fibers from the dorso medial nucleus
b. frontalis, corrugator and orbicularis oculi receive fibers from intermediate nucleus
c. platysma from ventromedial
d. buccinator and buccolabial from the lateral group of nuclei.

The axons exit from the nucleus dorsally and the fibers loop around the nucleus of the sixth nerve to form the facial colliculus. It then emerges from the lateral side of the pons. The parasympathetic fibers for the sublingual, submandibular and lacrimal glands arising from the superior salivatory nucleus forms the nervus intermedius. These two nerves along with the VIII cranial nerve exit from the ventrolateral aspect of the pons. This occurs at the cerebello pontine angle. Near the internal auditory meatus the nervus intermedius joins

the facial nerve. The eighth cranial nerve is closely situated and will be affected in any lesions affecting the facial nerve at this level. The secretion of saliva and tears also will be affected. A tumour at this position can also affect the V, 9th, 10th and 11th nerve. Vascular compression here will cause hyperkinesia of the facial muscles.

The facial nerve enters the temporal bone through the internal auditory meatus and passes through the fallopian (facial) canal, which is 28-30 mm long. There are three segments here the labrynthine, tympanic and mastoid segments. The geniculate ganglion belongs to the labrynthine part. Here the facial nerve gives off its first branch the greater superficial petrosal nerve. This branch travels along the floor of the middle cranial fossa to synapse in the spheno palatine ganglion. The postganglionic fibers travel with the zygomatico temporal branch of the fifth cranial nerve (maxillary) and join the lacrimal nerve (nasociliary) to supply secretory fibers to the lacrimal gland (Fig. 10.15).

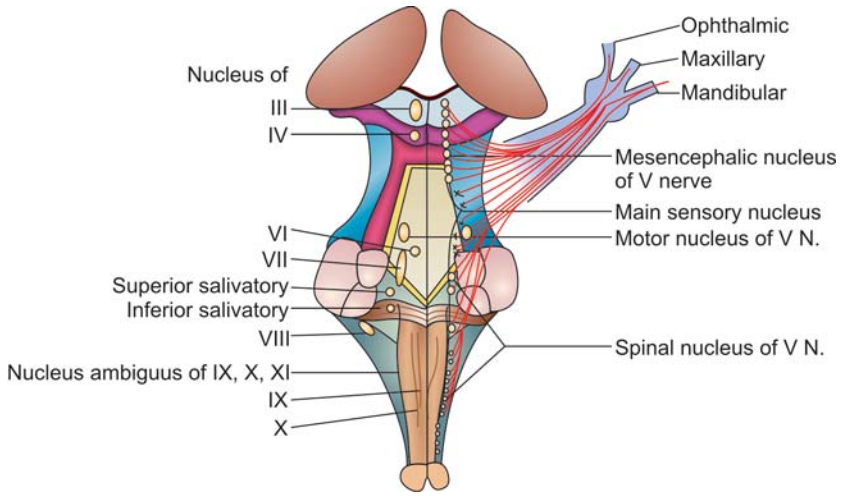


Fig. 10.15: Nuclei of cranial nerves

At the geniculate ganglion the facial nerve turns posteriorly to form a genu to enter the tympanic portion of the facial canal. It then emerges from the middle ear below the incus between the auditory and horizontal semicircular canals. Here the facial nerve turns downwards. The terminal branch of the nervus intermedius called the chorda tympani arises here. This nerve supplies the submandibular and sublingual salivary glands and sensory fibers for the external auditory meatus and taste from the anterior 2/3rds of the tongue. The nerve then exits through the stylo mastoid foramen behind the mandibular angle. It gives motor branches to the posterior belly of the digastric, stylo hyoid and posterior auricular muscles. The main trunk enters the parotid gland and divides in to upper and lower branches, which again divide into temporal, zygomatic, buccal, mandibular and cervical branches.

11 Pituitary Gland and the Hypophysis Cerebri

INTRODUCTION

The pituitary gland is a small oval structure 8 to 12 mm in size. It is well-protected in the sella tursica (fossa for the pituitary gland) present in the body of the sphenoid. If a slanting line drawn from the root of the nose to the posterior margin of the foramen magnum the pituitary gland can be located in the middle of this line. The sella is situated in between the tuberculum sellae and optic groove in front and the dorsum sellae behind (Fig. 11.1).

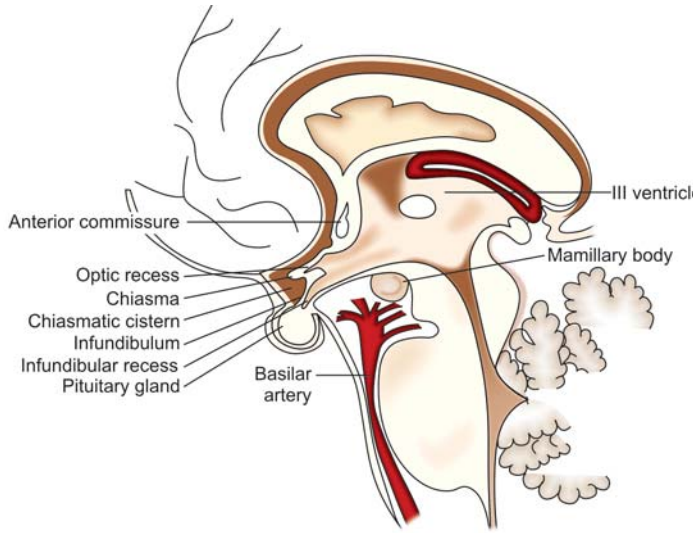


Fig. 11.1: Pituitary gland

The pituitary fossa is covered by the dura which is called the diaphragma sellae. The pituitary stalk or the infundibulum pierces this. The cavernous sinus is present on either side with the dura in between. Hence tumors of the pituitary gland will affect the 3rd and 4th nerve present in the wall of the sinus but the sixth nerve is protected by the internal carotid.

The intercavernous sinuses are present in front and behind the pituitary gland while the sphenoidal air sinuses are present below. The circle of Willis is present above.

The gland is supplied by the hypophysial branches of the internal carotid and is drained by the veins into the intercavernous plexus.

BLOOD SUPPLY TO THE EYE AND OCULAR ADNEXA

The ophthalmic artery a branch of the internal carotid supplies the eye and the other structures like scalp up to the vertex, orbit and the lateral wall of the nose. The vessel arises just after the internal carotid makes the fifth bend and pierces the roof of the cavernous sinus. The artery travels upwards for a few millimeters and then forwards to lie on the medial side of the optic nerve (intra-cranial portion). Then as the vessel passes strait ahead it comes to lie on the lateral side of the nerve.

It enters the orbit through the optic canal within the dural sheath of the nerve piercing the sheath only after it enters the orbit. Thus the vessel is attached to the nerve. In the orbit, it lies within the muscle cone between the optic nerve and the lateral rectus muscle. The ciliary ganglion is also situated lateral to the artery. It then ascends and crosses the optic nerve under the superior rectus muscle to reach the medial side. The vessel travels forwards above the medial rectus up to the maxillary process of the frontal bone. Here it divides in to the dorsal nasal and supra trachlear branches (Fig. 11.2).

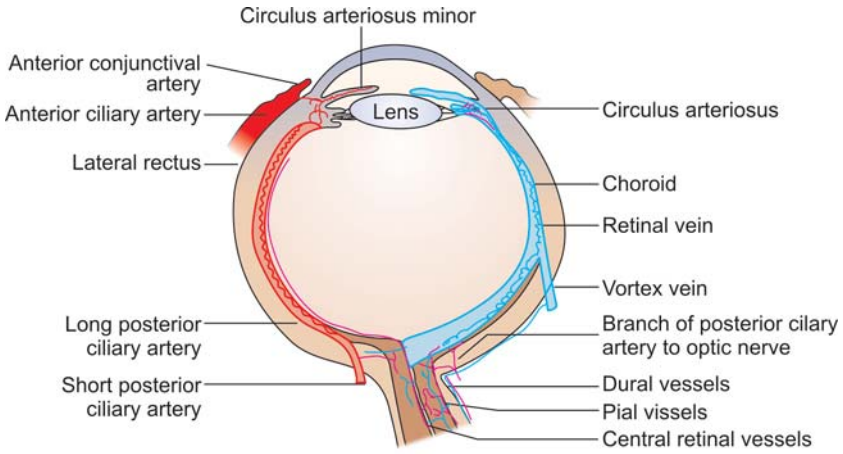


Fig. 11.2: Blood supply to the eyeball

BRANCHES OF THE OPHTHALMIC ARTERY

1. *Central retinal artery*: This is an end artery with minimal anastomosis at the level of the circle of Zinn. This vessel is very thin about 0.28 mm in diameter. The central retinal artery, is given off by the ophthalmic artery near the optic foramen, along with the medial ciliary trunk. It travels forward very close to and below the optic nerve. It pierces the optic nerve from the inferomedial aspect about 15 mm from the optic foramen. It is covered both by the dura and arachnoid. It then runs forward and pierces the pia, 12 mm behind the optic disc, to enter the nerve. It carries with it the pia mater, pial blood vessels and sympathetic nerve plexus called the nerve of Tiedemann.

The central retinal artery then bends forwards, and with the vein on its lateral side pierces the sclera at the lamina cribrosa to reach the retina. It then divides in to superior and inferior retinal vessels to supply the retina.

Branches

a. When the vessel is within the optic nerve branches are given off to pial plexus.
b. A collateral branch, which joins the pial network, gives a branch, which runs backwards and supplies the macular fibers in the optic nerve.
c. The two terminal branches superior and inferior seen in the retina divide in to a smaller nasal and a bigger temporal vessel. This happens either at the margin of the disc or in the nerve itself. The nasal vessels run radially while the temporal vessels take an arcuate course. The retinal vessels divide dichotomously and do not have any anastomosis. They supply the retina up to the inner nuclear layer. The retina behind this is avascular and is supplied by the choroidal vessels. The capillaries are present in the inner nuclear and ganglion cell layers. The capillaries are two-layered in most part of the retina. Close to the disc they are four layered, as the retina is thick at this level. The endothelium of retinal capillaries is non-fenestrated.

   The circle of Zinn-Haller is situated close to the optic nerve. It is formed by the anastomosis between the short ciliary arteries after they have pierced the sclera. From this plexus vessels pass to the choroid, optic nerve at the level of lamina cribrosa and to the pial plexus. It also supplies the retina around the optic nerve head.

   The cilio retinal artery a branch of this anastomosis enters the eye on the temporal side of the disc close to its edge and supplies the macula.

   The retinal veins follow the pattern of the arteries and the central retinal vein is formed at the level of the lamina cribrosa posterior to the division of the artery. It lies temporal to the artery and drains either directly in to the cavernous sinus or in to the superior ophthalmic vein.

2. *Posterior ciliary arteries:* When the ophthalmic artery is still below the optic nerve it gives off two posterior arteries. It divides into 10-20 branches. Of these two large branches called the long posterior ciliary arteries pierce the sclera on the medial and lateral side of the nerve. They run forwards in the supra choroidal space and supply the ciliary body and forms the major arterial circle of the iris by anastomosing with the anterior ciliary arteries. The other branches called short ciliary arteries pierce the eyeball around the optic nerve and supply the choroid.
3. *Lacrimal artery:* Runs along the upper border of the lateral rectus and supplies the lacrimal gland.
4. *Lateral palpebral:* Branches arise from the lacrimal artery and supply the conjunctiva and eyelids on the lateral side.

5. *Recurrent meningeal artery:* Anastomoses with the middle meningeal artery, which belongs to the external carotid system.
6. *Muscular branches:* Are two in number. The lateral branch supplies the lateral rectus, superior rectus, levator and the superior oblique. The medial branch supplies the inferior and medial recti and the inferior oblique.
7. *Anterior ciliary arteries:* Arise from the muscular branches. They form a subconjunctival network around the cornea and enter the suprachoroidal space and anastomose with the posterior ciliary arteries.
8. *Supra orbital artery:* Branches off the ophthalmic artery above the optic nerve and along with the supraorbital nerve reaches the space under the frontalis muscle by passing through the supraorbital foramen. This vessel supplies the upper lid, scalp, levator, periorbita and the diploe of the frontal bone.
9. *Medial palpebral arteries:* The palpebral arteries of the upper and lower lid anastomose with the lateral palpebral vessels and form arcades in the submuscular layer of the lids. They supply the structures in the lid and the conjunctiva.
10. *Posterior ethmoidal artery:* Supplies the posterior ethmoidal air sinus.
11. *Anterior ethmoidal artery:* Supplies the anterior ethmoidal air sinus, dura mater of the anterior cranial fossa and enter the face between the nasal cartilage and the nasal bone to supply the skin of the nose.
12. *Dorsalis nasal artery:* Is situated above the medial palpebral ligament and supplies the skin over the root of the nose and lacrimal sac. It anastomoses with the facial artery.
13. *Supratrochlear artery:* It reaches the forehead by curving around the medial end of the superior orbital margin and supplies the medial part of the forehead.
14. *Episcleral and conjunctival arteries* and other small branches arise from the larger branches.

CEREBRAL VESSELS SUPPLYING THE VISUAL PATHWAY

Branches of Internal Carotid

1. *The anterior cerebral artery* supplies the intracranial portion of the optic nerve and the upper surface of the chiasma.
2. *Middle cerebral artery* supplies the infero lateral aspect of the chiasma and the anterior part of the optic tract. The deep optic branch supplies the optic radiation close to the internal capsule. Its terminal branches supply the cerebral cortex representing the macular area.
3. *Posterior communicating artery* runs backwards from its origin and joins the posterior cerebral artery a branch of the basilar artery (anastomosis between the internal carotid and vertebral system). It supplies the under surface of the chiasma and anterior 1/3rd of the optic tract.

4. *Anterior choroidal artery* supplies the internal capsule, chiasma, posterior 2/3rd of optic tract, anterior and lateral aspect of lateral geniculate body and optic radiation at its origin.

Circle of Willis is formed by the anastomosis of the two internal carotids with the basilar. It lies in the subarachnoid space around the structures in the interpeduncular cistern.

Basilar artery is formed by the union of the two vertebral arteries. It gives off pontine branches supplying the cranial nerve nuclei present in the pons and the posterior cerebral arteries formed by the bifurcation of the basilar artery. These vessels supply the lateral geniculate ganglion, visual cortex and the posterior part of the optic radiation.

Venous Drainage

The blood from the orbit and its contents is drained into the cavernous sinus through the superior and inferior ophthalmic veins.

Superior ophthalmic vein: Starts at the root of the nose. After the supra-orbital vein joins the angular vein, it gives a communication which joins the superior ophthalmic vein. Its tributaries are

a. Inferior ophthalmic vein
b. Anterior and posterior ethmoidal veins
c. Muscular veins
d. Lacrimal veins
e. Central retinal veins (may drain into the cavernous sinus)
f. Anterior ciliary veins
g. Venae vorticosae

The inferior ophthalmic vein starts in the floor of the orbit in its anterior part. It runs on the lateral rectus and joins the cavernous sinus either on its own or after joining with the superior ophthalmic vein. It receives blood from the inferior vortex veins, lacrimal sac and the inferior and lateral rectus. It communicates with the pterygoid plexus through the inferior orbital fissure.

Vortex veins: The four venae vorticosae drain blood from the choroid. There is no corresponding veins to posterior ciliary arteries. The small veins from the optic nerve head also join the choroidal veins. The veins that join together to form the vortex veins have radial and curved branches, which give it a whorled (vortex) appearance. These veins drain blood from the choroid, optic nerve head, iris and ciliary body. When they enter the sclera the veins develop an ampulliform dilatation. Then they pierce the sclera on either side of superior and inferior rectus. They are 6mm or more behind the equator. The superior branches are more behind the equator. The lateral branches are closer to the mid-vertical plane compared to the medial. The superior lateral vein is 8 mm behind the equator and is the most posterior. It is close to the tendon of the superior oblique. The inferior lateral vein is the most anterior and is 5.5 mm

behind the equator. As they pierce the sclera obliquely, they form a canal which is 4 mm long. The canal runs posteriorly towards the midvertical plane of the eye. The veins can be seen through the sclera as dark lines. The superior branches drain into the superior ophthalmic vein and the inferior vessels drain into the inferior ophthalmic vein.

Angular Vein

This vein is situated about 8 mm medial to the medial canthus. It is close to the medial edge of the medial canthal ligament. It is frequently encountered during surgery for dacryocystitis. It continues below as the facial vein. It receives blood from the supraorbital vein, supra trochlear, the superficial veins from the skin of the nose and the superior and inferior palpebral veins. The superior palpebral vein crosses the medial palpebral ligament between the angular vein and medial canthus. The facial vein accompanies the artery and runs downwards and backwards across the face. It joins the retromandibular vein to form the common facial vein, which joins the internal jugular sinus.

Cavernous Sinus

The cavernous sinuses are situated on either side of the hypophysis and body of the sphenoid in the middle cranial fossa. They extend from the medial end of the superior orbital fissure to the petrous part of the temporal bone. It is formed by the splitting of the dura mater and is lined by endothelium. The vein is traversed by fibrous tissue giving it a spongy or cavernous appearance.

The internal carotid passes through the carotid canal and lies between the lingula and petrosal process of the sphenoid. It runs forwards in the groove on the body of the sphenoid and reaches the medial side of the anterior clinoid process. It then pierces the roof of the cavernous sinus. In the sinus, it is surrounded by the sympathetic plexus and is closely related to the sixth cranial nerve, which passes with in the sinus to reach the superior orbital fissure.

The 3rd and 4th cranial nerve and the ophthalmic branch of the fifth nerve travel in the lateral wall of the sinus to reach the superior orbital fissure. The maxillary branch is also present in the lateral wall but it exits the skull through the foramen rotundum. The temporal lobe is present lateral to the sinuses. The trigeminal ganglion is also present on the lateral side posteriorly.

Both sinuses are connected to each other by the intercavernous plexes, which are situated in front and behind the pituitary gland. Any infection in the face can spread to the cavernous sinus and produce thrombosis as the ophthalmic vein, which drains into the cavernous sinus is connected to the angular vein. Because of the intercavernous plexes the infection spreads to the other sinus also.

The ophthalmic veins, sphenoparietal sinus, inferior cerebral vein, central retinal vein and the emissary veins drain into the sinus.

The cavernous sinus drains into the superior petrosal sinus, which drains into the transverse sinus. The inferior petrosal sinus connects the cavernous sinus to the internal jugular vein below the base of skull. As this sinus drains blood from the inner ear, infection of this region can affect the cavernous sinus. As the mastoid emissary veins are affected edema is seen in the mastoid region.

The pterygoid plexus also drains blood from the cavernous sinus.

Development of the Vascular System

During the fourth week of gestation vascular channels develop in the mesenchyme around the optic vesicle. The vessels develop from the internal carotid. Initially, a dorsal and a ventral artery develop and join the capillaries developing around the optic vesicle. Capillary plexes drain into the developing carotid sinus. A transient vessel called stapedial artery develop from the carotid to supply the orbit. This joins the ophthalmic artery.

The hyaloid artery develop from the dorsal ophthalmic artery when the embryonic fissure starts closing. At the level of the rim of the optic cup both dorsal and ventral vessels give branches and develop an annular vessel. The hyaloid artery grows forward and around the lens to join the annular vessel and supply the different parts of the eye. The dorsal ophthalmic artery becomes the ophthalmic artery. During the sixth week the temporal long ciliary artery, short ciliary arteries and the central retinal artery develop.

An arterial circle forms in front of the annular vessel from the long ciliary artery. This forms the major arterial circle of the iris. Radial vessels develop from these two circles to form the pupillary membrane over the iris and lens. Central part of this membrane disappears. The peripheral part forms the minor arterial circle of the iris.

The retinal vessels develop from the hyaloid artery during the sixteenth week (fourth month) of gestation and grows centripetally. Vascularisation of the nasal retina is complete before the temporal as the nasal ora is nearer to the optic disc. The capillaries reach the ora by the eight month but maturation of vessels continues up to three months after birth. The hyaloid system and tunica vasculosa lentis disappear in the third trimester.

PATHWAYS CONNECTED TO THE VISUAL SYSTEM

Light Reflex

When light falls on the retina the outer portions of the rods and cones are stimulated. These are the receptor organs for the light reflex. The impulses are then sent to the bipolar cells and then to the ganglion cells of the retina. From ganglion cells the reflex passes through the optic nerve to the optic tract and then they reach the visual cortex through the optic radiations.

Pupillary reflex: The fibers for the pupillary reflex leave the posterior part of the tract and without entering the lateral geniculate body run superficially in the superior brachium and reach the pre tectal nucleus. The fibers for pupillary reflex terminate here. Some of the fibers emerging from the nucleus cross in the posterior commissure ventral to the aqueduct. The fibers run along with the medial longitudinal fasciculus to reach the Edinger Westpal nucleus which controls the sphincter pupillae muscle. From here the fibers for the pupillary reflex passes along with the third nerve. In the orbit the fibers travel with the branch to the inferior oblique. The preganglionic, para-sympathetic myelinated fibers separate from this branch and enter the ciliary ganglion. From this ganglion the fibers reach the eyeball through the short ciliary nerves to innervate the sphincter pupillae and ciliary muscle. These postganglionic fibers are myelinated. This helps in getting quick reactions.

Pathway for Dilatation

The dilator fibers leave the lateral column of the spinal cord at the junction of the thoracic and cervical regions through the four upper thoracic nerves. They reach the cervical sympathetic chain and travel upwards. The fibers synapse in the superior cervical sympathetic ganglion. The postganglionic fibers run upwards with the sympathetic plexus around the internal carotid artery. The fibers for the pupil leave the plexus to reach the trigeminal ganglion. They then run with the nasociliary nerve and enter via the long ciliary nerves to innervate the dilator muscle fibers.

Paralysis of sympathetic fibers will cause ptosis, miosis and enophthalmos. This is called Horner's syndrome. In this condition both the pupils will be equal in size in bright light. But in the dark the affected pupil will not dilate giving rise to anisocoria.

The sympathetic fibers control the blood circulation in the eye.

Accommodation Reflex

The power of the human lens has to change for us to see clearly at various distances. Besides this to see the near objects the eyes must converge to fixate on them. The pupils also must constrict. These three changes bring about accommodation.

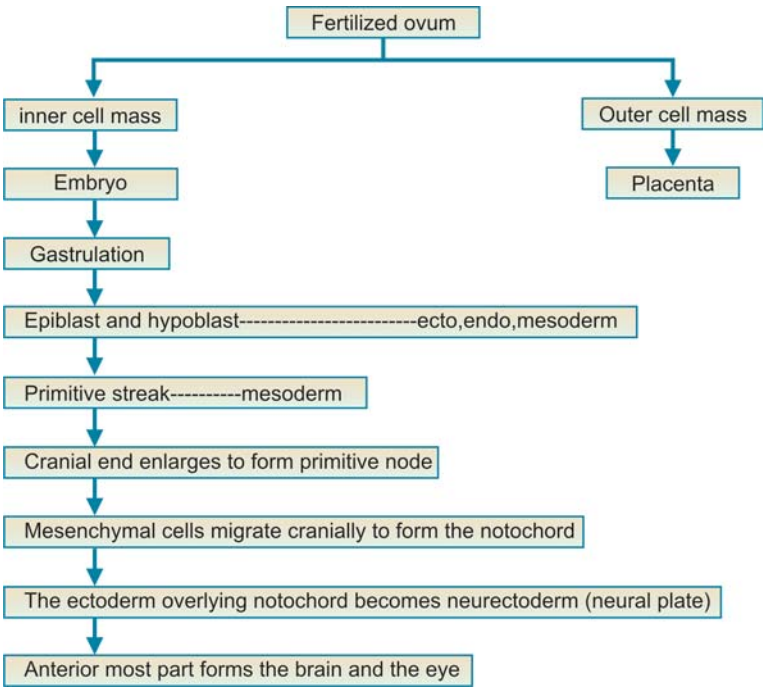
The afferent pathway for this is the visual reflex. The reflex for convergence, which may involve the visual cortex, stimulates the proprioceptive impulses in the medial recti muscle. Through the inferior division of the V nerve it reaches the mesencephalic nucleus of the trigeminal nerve. From here the fibers pass to the center for constriction of the pupil, i.e. the Edinger-Westpal nucleus. The fibers travel with the oculomotor nerve and then without entering the ciliary ganglion it reach the accessory ganglion and then the sphincter pupillae.

Clinical Aspects

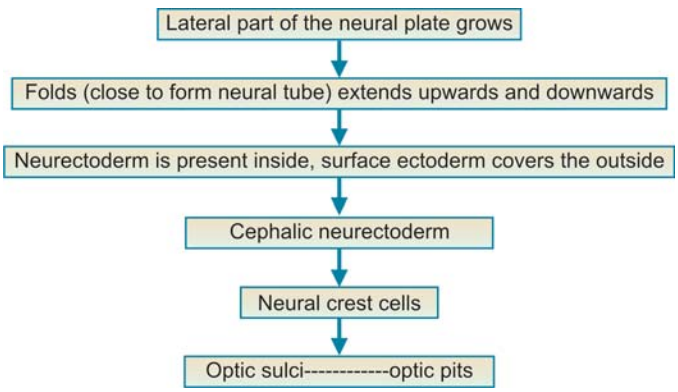
a. Lesions at the level of the superior brachium will result in loss of light reflex but accommodation reflex will be spared. (AR pupil)
b. AR pupil can result due to lesions affecting the pupillary fibers before they reach the III nerve nucleus.

Total pupillary paralysis can result from supranuclear lesion, nuclear or infranuclear lesion in the nerve, in the ciliary ganglion or short ciliary nerves.

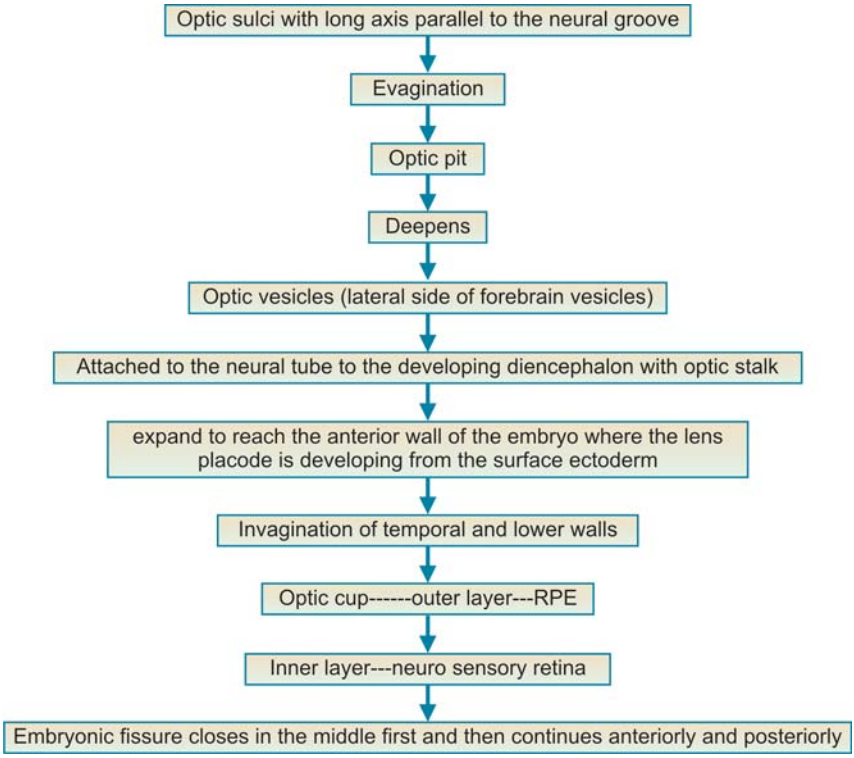
Embryogenesis



Development from the Neural Plate



Organo Genesis of the Eye



Neural Crest Cells

Neural crest cells make a major contribution to the connective tissue components of the eye and orbit. But the extraocular muscles and the blood vessels develop from the mesoderm only. Neural crest cells arise from the neurectoderm located at the crest of the neural folds just before the folds fuse to form the neural tube. They migrate to different parts of the embryo where differentiation will occur due to local factors.

Mesenchymal cells of the facial primordia are derived from the neural crest cells. The neural crest cells in the mesenchephalic region move forward to form the maxilla and mandible. The cells from the diencephalon form the frontonasal mass. These cells along with the cells from the anterior midbrain settle around the optic vesicles. The migration of neural crest cells are promoted by fibronectin and inhibited by proteoglycons.

Any abnormalities that involves the tissues that develop from the neural crest cells are called neurocristopathy. These congenital anomalies result either from faults in migration or differentiation. Anomalies in the anterior segment are found to be associated with dental malformation, middle ear deafness, malformations of the skull, shoulder girdle and upper spine. Malformations of

the median face cause hypertelorism due to impaired midline coalescence of the frontonasal process.

Initially, when the globe develops the axes between the two eyes are at an angle of 180°. By 12th week, it becomes 105. As the head develops, it reduces further to become 71° just before birth. By the age of three it is 68.

CONGENITAL ANOMALIES (Fig. 11.3)

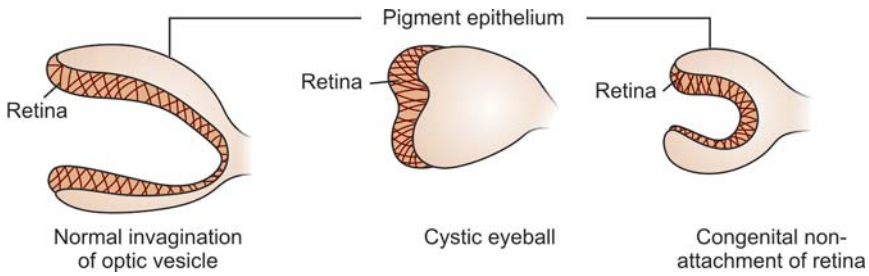


Fig. 11.3: Congenital anomalies

As in any organ teratogenic influence affecting the foetus at a very stage of development will cause severe damage to the eye. In these cases, the surrounding tissues also will be affected. It the damage occurs later the lesion will be more localized.

Developmental anomalies fall into three categories. Developmental arrest, abnormal differentiation or both.

If the development is affected very early the optic vesicle will not grow fully. This will cause gross anomalies.

E.g. Anophthalmos: though the term means total absence of the globe very often a rudimentary eyeball will be seen. This condition is bilateral.

Microphthalmos: This may be associated with other anomalies like persistence of primary vitreous or incomplete closure of the embryonic fissure. Leucoma, cataract and poorly developed retina, and optic nerve also will be seen.

Nanophthalmos: Here the size of the eye is less than normal. But the eyes will otherwise be normal except for hypermetropia, thick sclera and deep-set eyes. *Mode of inheritance:* Both autosomal dominant and recessive.

Cyclopia: Here, there is a single eye in the middle of the face. There are gross deformities and the child will not survive. In synophthalmia two incomplete globes are seen fused together.

Cystic eye: In true cystic eye, the optic vesicles remain as cysts without any further development. The skin covering the cyst does not show any eyelid formation.

In cystic coloboma, when there is incomplete closure of fetal fissure. Hence, there is a cyst with a small, deformed eyeball.

Orbital teratomas as in other teratomas contains all the three germinal layers. Single or multiple cysts are also found.

An encephalocoele also may occupy the orbit but this lesion will be pulsatile. Though the bony defect is present at birth the encephalocoele may develop later.

In cryptophthalmos, the eyelids do not develop properly. This defect may be complete or partial. The eyeballs are covered by skin. As the lids fail to form the exposed corneal and conjunctival epithelium undergo metaplasia into skin. There is absence of lashes, brows and sometimes even the lacrimal gland. The cornea is absent and the lens will be poorly developed. The retina and optic nerve are likely to be developed normally.

In an abortive form, the upper lid is replaced by a fold of skin that is adherent to the cornea. The lower lid will be normal in these cases.

Though the eyeball appears to move if an incision is made into the skin the eyeball will be opened up. This condition can occur unilaterally also.

Inheritance: Autosomal recessive.

In ankyloblepharon the upper and lower lids are formed but fused. This may be complete or partial. The cornea is normal in these cases. This can be differentiated from cryptophthalmos by the presence of lashes and brows. If the lids are incised the patient can get some useful vision but the lids may fuse together again.

Differentiation of anterior segment structures occurs mainly between sixth and sixteenth week. Any factors affecting growth during this period will cause anterior segment anomalies.

Congenital Anomalies of the Cornea

Any cornea that is smaller than 9 mm at birth in an otherwise normal eye, is called microcornea. Inheritance is autosomal dominant or recessive. Unlike sclerocornea and cornea plana a normal limbus is present here. If it is associated with a small eye, it is called microphthalmos.

If the cornea is larger than 12 mm it is called megalocornea. This condition is not associated with glaucoma and the cornea is histologically normal. 90% of the affected persons are males. This condition is usually X linked recessive but may be inherited as autosomal dominant or recessive manner. The gene is located in the X12-q 26 region. This anomaly is due to inadequate growth of the anterior tip of the optic cup. This leaves a large gap, which is filled by the cornea.

Sometimes, the peripheral part of the cornea appears like sclera with a variable size of clear cornea in the centre. This is called sclero cornea.

If the corneal curvature is very low, around 38 D it is called cornea plana. Here the curvature is similar to sclera. The anterior chamber will be shallow but glaucoma is not common. During the sixteenth week of gestation the

cornea curvature starts increasing. If this fails, cornea plana results. Inheritance is autosomal dominant or recessive.

Oval cornea is seen in Turner's syndrome, Rieger's anomaly and along with microphthalmos.

Keratectasia in which the cornea is ectatic and opaque is due to intrauterine infection or due to failure of mesenchyme to migrate and develop the cornea.

Posterior keratoconus is a condition where there is a localized diffuse thinning of the central or paracentral cornea on the posterior aspect with an opacity in that area. Unlike Peter's anomaly the Descemet's membrane and endothelium will be present in the defective area. Vision is reasonably good as the anterior surface is normal.

Congenital leucomas of any shape and size can occur but they are not so common. This may be associated with other anterior segment anomalies, which were called mesodermal dysgenesis. But these are called neurocristopathies as the structures, which develop from neural crest cells are affected in this condition. Congenital rubella and syphilis also can cause corneal opacities. Multilple macular opacities are sometimes seen bilaterally in some cases. It may also be part of congenital glaucoma and mucopolysaccharidosis.

In arcus juvenilis an opacity similar to arcus senilis is seen. This may be present at birth or develop a little later.

Dermoids: Can be found in the cornea, conjunctiva or orbit. These are choristomas and are due to epidermal and connective tissues being included in between the two sides of a cleft. The dermoid will have many types of tissues like hair follicle, sebaceous glands, nerve fibers, fat and or glandular tissue.

Dermolipomas consist of fat and fibrous tissue and are seen in the fornix at the superotemporal quadrant.

Chromosomal anomalies with corneal abnormalities:

Trisomy 21	—	Keratoconus
Trisomy 17,18	—	Anterior corneal opacities
Abnormal Ch 18	—	Posterior keratoconus, microcornea, opacities, Peter's anomaly, oval cornea, other neuro cristopathies
Trisomy13	—	Poorly defined cornea with leucoma and sclera-lization
Turner's XO	—	Blue sclera, oval cornea and corneal opacity.

Angle anomalies. These develop from failure of cell differentiation, incomplte migration of secondary mesenchyme, or due to defective regression of mesenchymal cells present in the space between the developing endothelium and iris. Depending up on the degree of defect it ranges from simple prominent Schwalbe's line which is present in 15% of normal people to gross anomalies in the angle. If insertion of iris processes anteriorly is included along with prominent Schwalbe's it is called Axenfeld's anomaly. If glaucoma is also present, it is called Axenfeld's syndrome.

If iris strands and hypoplasia of iris stroma are also present it is called Rieger's anomaly. In Rieger's syndrome non-ocular defects like macular hypoplasia with a broad flat root of nose, small teeth or even absence of teeth, deafness, mental retardation and osteogenesis imperfecta will be present. These signs may be seen in syndromes like Marfan's, Down's and Ehler Danlos.

If central corneal opacity is added to this it is called Peter's anomaly the inheritance pattern of which is autosomal dominant. In some cases of Peter's anomaly the lens may be cataractous. In such cases other ocular anomalies like aniridia, sclero cornea, microphthalmos with choroidal coloboma can be present. Systemically trisomy 13 and 15, congenital heart defects, genitourinary disorders, mental retardation, cleft lip and palate and other skeletal abnormalities may also be associated with this condition.

Congenital peripheral anterior synechiae and posterior keratoconus is also part of this type of anomaly. In posterior keatoconus as the anterior curvature is normal the vision is not affected.

Abnormal differentiation of mesenchyme could lead to congenital hereditary endothelial dystrophy, congenital hereditary stromal dystrophy, posterior polymorphous dystrophy, sclero cornea and cornea guttata.

Iris

Anisocoria of 0.5-2mm can occur in normal people. Polycoria (many pupils), ectopia (displaced pupil) and corectopia (deformed pupil) also are seen. Cysts can occur congenitally in the iris.

In aniridia though it appears as if the whole iris is absent some rudimentary tissue is present in the periphery. It can be inherited as an autosomal dominant or recessive trait. When it is associated with Wilm's tumor deletion of part of short arm is seen.

Colobomata

Coloboma literally means mutilation. When a part of a structure of the eye is missing it is called coloboma of that part. When this is due to defective closure of the embryonic fissure it is called typical coloboma. This anomaly will occur when the development of the fetus is affected during the fourth and fifth week of gestation. This is the time when the optic vesicle invaginates and fusion of the fissure occurs. Normally the cleft starts closing in the center and then proceeds both anteriorly and posteriorly. Depending on at what stage the defect starts the coloboma will be partial or complete.

Colobomata may be due to primary defect in the ectodermal development.

Inheritance: Autosomal dominant.

Coloboma of retina and choroid: A complete coloboma involves the iris, lens, choroid and optic disc. It is bilateral in 60% of cases. Instead of a total coloboma

multiple defects may be seen along the embryonic fissure. In these cases the sclera will be thin and sometimes ectatic. The choroid is absent except for a few large vessels. Though the macula is normal vision will be affected and nystagmus may be present. When coloboma occurs in the ciliary body the zonules will be defective causing coloboma of the lens.

Coloboma of the Iris: is usually a triangular or tear drop-shaped defect in the lower nasal quadrant of the iris. The broad base is usually towards the pupil but the shape may vary. This coloboma is either due to defective closure of the embryonic fissure in the anterior part or persistence of the vessels in the tunica vasculosa lentis. In the later case, the coloboma can occur in any clock hour. The vessel anastomosing with the hyaloid artery at the level of the embryonic fissure is larger. Hence colobomas are more common at this site. If many vessels persist many colobomas result and if all the vessels persist aniridia ensues.

Sclera: Translucent sclera makes the uveal tissue more visible giving it a bluish appearance. Hence it is called blue sclera. Staphyloma can occur congenitally in the peripapillary region.

Uveal Tract

Iris: Hypoplasia of iris is very common. This may be limited to one quadrant or either the mesodermal or ectodermal parts of the iris. In pseudopolycoria multiple holes are seen in the iris. In iridodiastasis the defect is seen in the ciliary margin. This resembles an iridodialysis but the pupil will be round in shape.

Optic Nerve

Bilateral hypoplasia of the optic nerve is usually associated with other anomalies like microphthalmos or absent septum pellucidum. EG de Morsier syndrome. Unilateral hypoplasia is sometimes seen in otherwise normal persons. Colobomas may be confined to optic nerve head alone.

Optic nerve pit is a deep circular depression in the nerve head located more commonly in the inferior temporal quadrant. The cause for the formation of the pit is the presence of a small pouch of retinal tissue between the nerve and the sclera. There will be a defect in the lamina cribrosa. It is thought that there is a continuity between the subretinal space and the subdural space. Through this space cerebrospinal fluid can enter the subretinal space causing a condition resembling central serous retinopathy. An arcuate scotoma will be present in these cases.

Hyaloid vessels may be present in the vitreous. Depending on when the regression has stopped the extent of remaining vessel will vary. If the posterior end is persistent it forms a elevation called Bergmeister's papilla over the disk.

If the anterior end persists, it will be attached to the back of the lens slightly nasal to the center and is called Mittendorf's dot.

Very rarely the whole empty vessel will be hanging between the disc and the lens.

In persistant hyperplastic primary vitreous or persistant fetal vasculature there is a fibrovascular sheath behind the lens. This contracts and pulls the ciliary body causing elongation of the ciliary processes which is pathognomonic for this condition. The vessels may enter the lens and even cause hemorrhage in the lens. As the development of the eye is affected there is microphthalmos, retinal dysplasia and amblyopia. The anterior chamber will be shallow with a clear or cataractous lens. Fortunately, this condition is uniocular.

Defects in the Eyelids

Coloboma of the eyelids where there is a defect in the eyelid margin is due to defective fusion of the facial processes.

The skin over the eyelids and at times the sclera and uveal tract are heavily pigmented on one side alone. It is called nevus of Ota. As primary open angle glaucoma is more common in these cases they must be followed up for the same.

Nevi may be present as isolated lesions on the eyelids, conjunctiva, caruncle, iris and choroid.

In the orbit dermoids which are choristomas can occur. It is usually found in the superotemporal quadrant. Though they are present from birth they become evident in the second or third decade. These tumors produce a defect in the orbital wall. Sclerosis of the bone at the tumor margin will be present.

At birth the eye is only about 12.5 to 16 mm in size. This makes the eye hypermetropic. The cornea is 10 mm in diameter, which makes it very large compared to the anteroposterior diameter of the eye. It is also more curved in the periphery the reverse of what is seen in an adults eye. The medial rectus is closer to the cornea.

Pigmentation of the iris continues after birth. In infants the pupil will be constricted and will not dilate properly as the dilator muscle fibers are not well developed. In addition to this the anterior chamber will also be very narrow. This makes it difficult to do surgeries in congenital cataract. The ciliary processes also will not be fully developed at birth. The ciliary muscle will be very short which makes the retina lie very close to it. Hence any incision through the parsplana must be very carefully made.

During the first three years of life the eye grows rapidly. It continues at a slower rate till puberty following which, there is another spurt of growth. Myelination of the optic nerve is completed by the third week after birth.

As age advances the cornea is flattened in the vertical meridian, giving rise to astigmatism against the rule. Deposition of lipids in the cornea causes arcus

senilis. Lipids in the sclera causes thickening and yellowish discoloration. The pupil becomes miotic and the sphincter pupillae becomes rigid. The pigment epithelium atrophies especially around the disc.

CHRONOLOGY OF DEVELOPMENT

Period	*Organogenesis*
3rd week	Formation of optic vesicle from optic pit. Lens plate forms in the surface ectoderm
4th week	Invagination of optic vesicle to form optic cup formation of lens pit and towards the end of 4th week of lens vesicle.
5th week	Fetal fissure starts closing
6th week	Closure of fetal fissure Separation of lens from the surface. Formation of primary lens fibers Differentiation of various layers of retina Appearance of tunica vasculosa lentis
Neofetal period:	
12th week	Formation of secondary lens fibers, lid folds and ectodermal layers of iris.
Fetal period:	
16th week	Development of central retinal vessel, ciliary body, ciliary, sphincter and dilator muscles Sclera and outer layer of choroid. Regression of vascular capsule of lens
28th week	Regression of pupillary membrane myelination of the optic nerve starts
9 months	Hyaloid artery disappears and medullation reaches lamina cribrosa.
After birth:	
3 weeks	Myelination is complete, lens becomes flatter but continues to grow throughout life. Hypermetropia
1 month	Fovea is fully-developed. Location same as adults.
6 months	macula is fully developed
2-4 years	Angle of anterior chamber reaches adult size. The anterior part of the orbit grows more than the posterior
Adult life	Eyeball is three times that found at birth.

Section 2

Biochemistry

12 General Biochemistry of the Eye

The various types of tissues that form the eye are specifically or specially designed for transmission of light and conversion of light energy into cellular signals that are transmitted through second messengers like ion channels, G protein, tyrosine kinases etc. Like the cells in any other organ, the ocular cells also have basic structural organelles such as plasma membrane, nucleus, cytoplasm with endoplasmic reticulum, golgi apparatus, mitochondria and intracellular matrix. Intracellular matrix has filaments of 3 types as microfilaments, intermediate filaments and cytoskeletal fibres. The cells lie in the extracellular matrix that form the structural framework with proteins such as collagen and proteoglycans (glycosaminoglycans bound to core proteins). All these structures are designed for the specific function of light transmission. The metabolism in these ocular cells also occur similar to the other cells.

STRUCTURAL PROTEINS

Collagen is a protein of great structural importance to the eye. About 80-90% of the bulk of the eye contains collagen. It is an extracellular, insoluble molecular complex. It acts as a supporting medium to hold cells and to maintain the tissue structure. It may be fibrous or non-fibrous. So far, 19 types of collagen have been identified. All types of collagen are protein complexes of 3 polypeptides wound around each other in a helical structure like a rope. Each polypeptide chain has á helical structure and so collagen is a triple helix (Fig. 12.1). The polypeptides are rich in proline and lysine. These amino acids are post translationally hydroxylated by hydroxylase enzymes to form hydroxyproline and hydroxylysine which are necessary for cross linking and hydrogen bonding (Fig. 12.2). In the polypeptide chain every third amino acid is glycine. The small size glycine is useful to maintain the helical structure of the collagen. The 3 chains associate themselves by hydrophobic interactions and are linked by disulfide bonds at their 'C' terminal region enabling them to twist and form a stable triple helix of procollagen (Fig. 12.3). The stability is enhanced by interchain hydrogen bonding at the hydroxyl groups. The amino acid sequence of these polypeptide chains may be similar and it is called homopolymeric or dissimilar known as hetero - polymeric, e.g.–Type I collagen is heteropolymeric and Type II is heteropolymeric. The procollagen undergoes hydrolysis of the

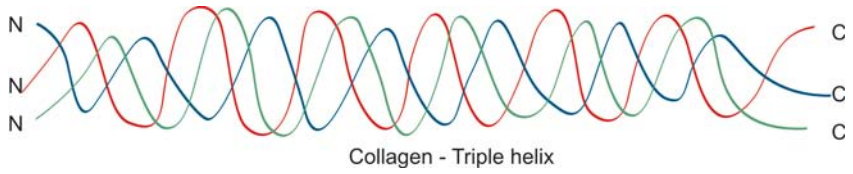


Fig. 12.1

non helical N and C terminal portions (extension peptides) to form the tropocollagen (Fig. 12.4). The tropocollagen comes out of the cell and assembles close to the cell surface. The tropocollagen units associate laterally and staggered lengthwise by hydrophobic interactions and cross linking of lysine and hydroxylysine. 5 such rows of cross linked tropocolllagen units is called microfibril (diameter 10 - 300 nm (Fig. 12.5)). Many such microfibrils make a fibril (Fig. 12.6). Association of several fibrils together is termed fiber (Fig. 12.7). Alternate arrangement of procollagen into nets or spider web structures and polygonal surfaces give rise to the sheet forming, non fibrous collagen.

CELLULAR SYNTHESIS OF COLLAGEN (Figs 12.2 to 12.7)

Collagen types: Those having structural role and non structural role

Structural

Fibrillar – Type I, II, III, Vand VII

Non fibrilar – Type IV, VI, VIII and X

Non structural

Types IX, XI, XII, XIII, XIV

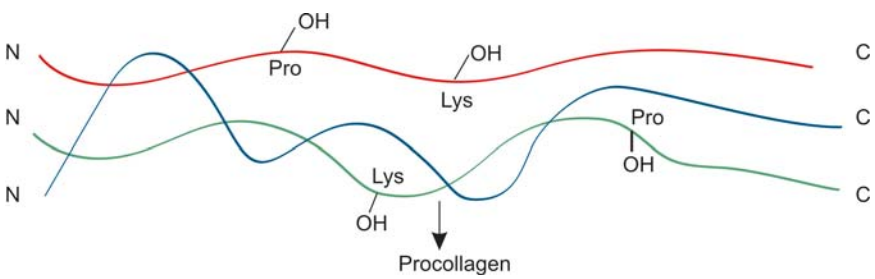


Fig. 12.2

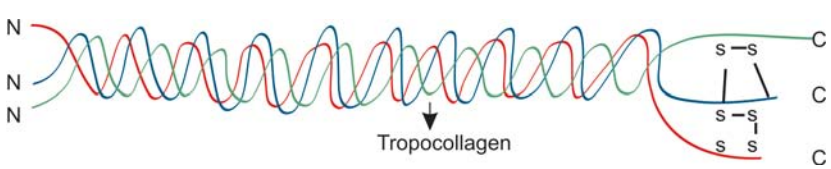


Fig. 12.3

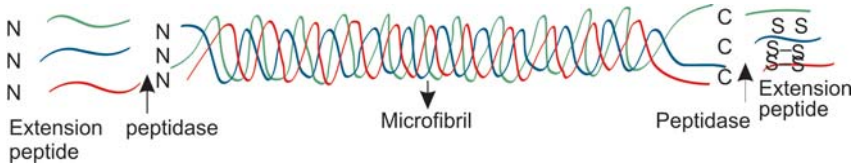


Fig. 12.4

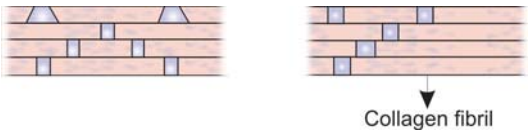


Fig. 12.5

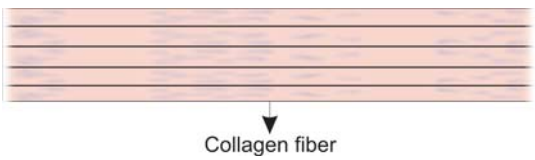


Fig. 12.6

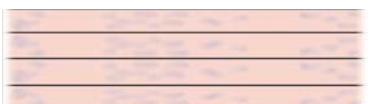


Fig. 12.7

Fig. 12.2 to 12.7: Cellular synthesis of collagen

STRUCTURAL CARBOHYDRATES

They are sugar oligomers and polymers.

OLIGOMERS

Oligomers are oligosaccharides: They are a class of carbohydrates in which ten or fewer sugars are held together by oxygen bridges. Oligosaccharides are commonly found as covalent appendages to many proteins known as glycoproteins. They are bound to protein by either oxygen bridges (O-linked through serine/ threonine) or nitrogen bridges (N- linked through asparagine). Some oligosaccharides are also bound to lipids in cell surface membranes.

POLYMERS: (GLYCOSAMINO GLYCANS) - GAG

They are found throughout all bodily tissues including the eye. They exist predominantly in the extracellular matrix that maintains the structure of the organism. They are involved in cushioning, lubricating and attaching the matrix to various kinds of cells. The polymer is made of repeating units of sugar bound to an amino sugar by oxygen bridge. They are also called muco-polysaccharides (MPS) as they were first found in mucous tissues and are

abundant there. Most GAGs occur linked to core proteins, the entire assembly is known as proteoglycan. Proteoglycans and GAGs are often associated with collagen in extracellular matrices. The common GAGs found in ocular tissues are:

Hyaluronic Acid

Composed of Glucuronic acid and N- acetyl glucosamine linked together by ß 1-3 glycosidic linkage within the unit and β 1-4 glycosidic linkage in between units.

Chondroitin Sulfate

Glucuronic acid and N- acetylgalactosamine sulfate in ß 1-3 glycosidic linkage.

Keratan Sulfate

Galactose and N–acetyl glucosamine sulfate in 1-4 glycosidic linkage.

Dermatan Sulfate

Iduronic acid and N acetyl galactosamine sulfate in β 1-3 glycosidic linkage.

Sulfation of the right hand unit adds considerable acidity and charge density to the entire unit. The negative charge density is an important characterstic of GAGs that attract counterions (principally sodium) and water.

The major function of structural carbohydrates is to:

- Increase the stability of protein (hydrophilic).
- Stabilise protein conformation.
- Aid in proper orientation of protein in membrane.
- Act as recognition marker for cell sorting prior to protein transport.
- Acts as immunological identifiers for immune reaction.

Proteoglycans

The core proteins to which GAG is bound to form the proteoglycans are:- Keratocan, mimican, lumican—bound to keratan sulfate and decorin – bound to dermatan sulfate.

One to 3 GAGs are bound to core protein through oligosaccharide chain to form the proteoglaycan. The link is N glycosidic bond between N-acetyl glucosamine of keratan sulfate and asparagine in core protein (Fig. 12.8).

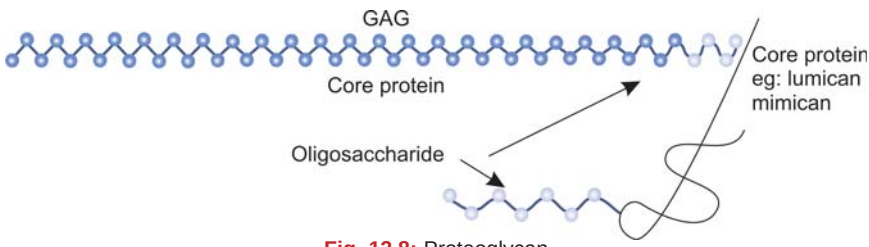


Fig. 12.8: Proteoglycan

LIPIDS

Are the structural basis of cell membranes. Membrane is composed of phospholipids, glycolipids, cholestrol etc.

Phospholipids

Are the most important lipids for the formation of maintainance of cell membrane. In this, glycerol is used as the frame on which 2 fatty acid esters are attached to the 1st and 2nd carbon and a phosphate ester to the 3rd carbon to which any of the 4 polar groups - ethanolamine, choline, serine or inoistol is attached, e.g. Phophotidyl choline (Fig. 12.9).

In an aqueous medium, lipids arrange themselves in such a way that the polar (hydrophilic) groups face the aqueous phase, whereas the hydrophobic groups face each other to form a miscelle (Fig. 12.10). But owing to the bulky nature of 2 chains of fatty acids, membrane lipids do not readily from the miscelles,but group together to form a lipid bilayer (Fig. 12.11).

Phospholipid bilayers have the fatty acid composition designed by the cell as per its functional need. For, e.g: RBC must have somewhat a rigid membrane to assume its biconcave disc shape. So, it tends to have a shorter chain with unsaturated fatty acids and a lower percentage of long chain, highly unsaturated fatty acids. But *rod* **outer segment discs** require a high degree of membrane fluidity to carry out the process of visual transduction. So, higher percentage of long chain unsaturated fatty acid is required. Cervonic acid – (a 26 carbon fatty acid with 6 double bonds) is one such fatty acid.

Cholesterol is also a component of membranes. It is hydrophobic predominantly except for the hydroxyl group on 3rd carbon. It gives rigidity to the membrane so, the rod outer segment has lower percentage of cholesterol ie only 8%.

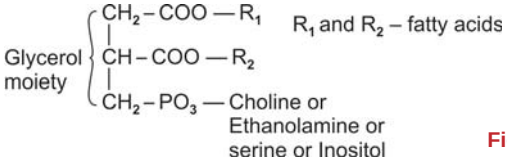


Fig. 12.9

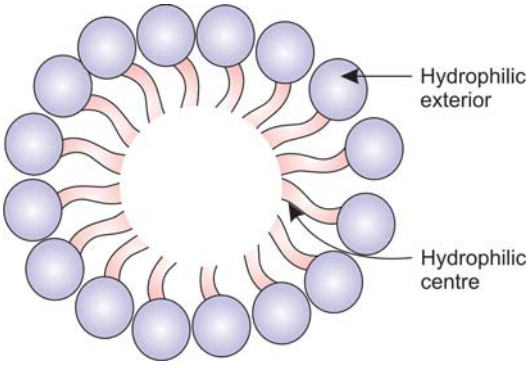


Fig. 12.10: Miscelle

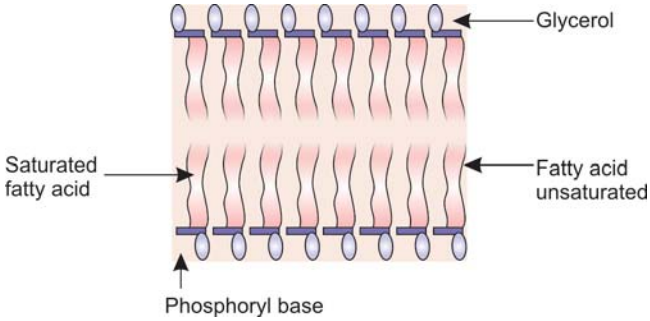


Fig. 12.11: Lipid bilayer

Glycolipids

Glycolipids are another important membrane components found in ocular tissues. They contain carbohydrates like galactose, N- acetyl galactose and N-acetyl neuraminic acid (sialic acid). The basic structure that binds the glycolipids is not glycerol, but a long chain amino alcohol known as sphingosene.

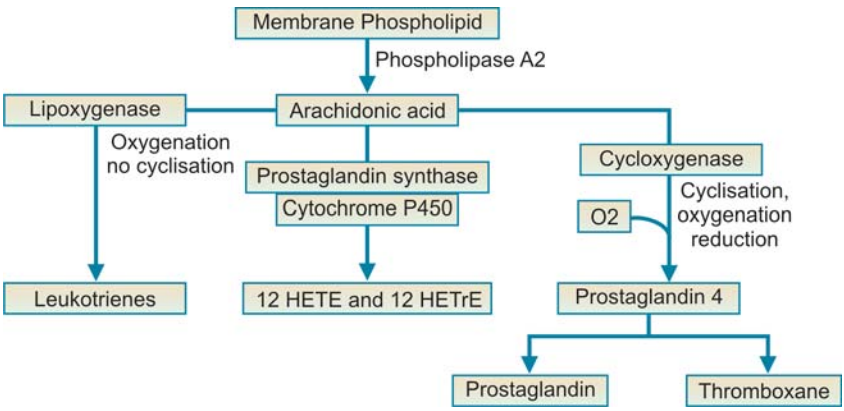
Sphingosene + fatty acid → ceramide.

Ceramide + phosphatidylcholine → sphingomyelin.

If phosphocholine is replaced by carbohydrate, it is glycolipid or glycosphingolipid. They are cerebrosides and gangliosides. Apart from these above mentioned lipids, there are some derived lipids called eicosanoids. They are cyclic lipids derived from eicosonic acid such as arachidonic acid. They include prostaglandins, thromboxanes and leukotrienes which are short acting local hormones. They are formed from membrane phospholipids by the hydrolytic action of the enzyme phospholipase A2.

Synthesis of Eicosanic Acids

Prostaglandins



Flowchart 12.1

Prostaglandins on release to neighbouring cells, bind to their receptor protein and affect the production of second messenger leading to stimulatory or inhibitory effects. They have profound effect on inflammation in the eye, aqueous humour dynamics and blood ocular barrier functions (prostaglandins – PG)

PGF2α- contract smooth muscles, miosis, reduce the intraocular pressure (A chemical analogue of PGF2α is effective in glaucoma).

PGE2α- relaxation of iris and ciliary muscles,

Control of cell division

Opens the tight junction between vascular endothelial cells leading to breakdown of blood retinal barrier resulting in macular oedema and blurring of vision. Here, trauma to the retina is the cause for the release of prostaglandins.

PGI2 (Prostacycline) synthesized mainly in endothelial cells of vascular tissues.

Potent vasodilator, platelet separator (prevents aggregation), stimulator of adenlyate cycalse

Thromboxane A2 (TXA2)- synthesized mainly by platelets

Potent vasoconstrictor, platelet aggregator.

TXB2- contracts smooth muscle.

12HETE (12 hydroxy eicosa tetraenoic acid) - inhibit Na+ / K+ ATPase in corneal cells leading to corneal swelling.

12 HETrE (12 hydroxy eicosa trienoic acid) - induce chemotaxis as part of inflammatory response.

Initiate capillary proliferation in cornea.

Leukotriene E4 – exudation of plasma.

Steroids are also lipids; steroid hormones are glucocorticoids and mineralocorticoids.

Aldorsterone – induces gene expression to increase the synthesis of Na / K ATPase resulting in sodium retention and potassium excretion.

Dexamethasone – inhibit the synthesis of Na / K ATP ase in lens protein.

Topically applied steroids increase the corneal thickness. Prolonged use of steroids for some systemic disease leads to—

Development of posterior subcapsular cataract. This is due to the inhibition of Na/K ATPase resulting in retention of Na+ and hence osmotic inclusion of water, increased intraocular pressure.

LIPID PEROXIDATION

Lipid peroxidation is the non enzymatic autoxidation of lipids exposed to oxygen especially the polyunsaturated fatty acids (PUFA). This reaction is initiated by light or metal ions. It damages the cell membrane directly, then the cells by the interaction of breakdown products (free radicals) with cellular proteins. This lipid peroxidation proceeds as a chain reaction and occurs in 3 steps of initiation, propagation and termination.

Intiation – production of free radicals or reactive oxygen species as correctly named viz $R^{\cdot}$ and $ROO^{\cdot}$.

R° is called the carbon centered radical. $ROO^{\cdot}$ is the lipid peroxide radical. Interaction of PUFA with free radicals generated by other means

(such as univalent reduction of oxygen in some enzymatic reactions and by metal ions) leads to the formation of $R^{\cdot}$ and $ROO^{\cdot}$. Free radicals are molecules or atoms that have an unpaired electron which makes them highly reactive.

$$O_2 \rightarrow O_2^{\cdot} \xrightarrow{e^- + 2H} H_2O_2 \xrightarrow{e^- + H^+} OH^{\cdot} \xrightarrow{e^- + H^+} H_2O$$

H_2O

E.g. superoxide ($O_2^{\cdot}$) hydroxyl radical ($OH^{\cdot}$) hydrogen peroxide (H_2O_2).

$R^{\cdot}$ and $ROO^{\cdot}$ in turn are degraded to injurious products.

$ROOH + metal \longrightarrow ROO^{\cdot} + H^+$

$RH\ (Fatty\ acid) + OH^{\cdot} \rightarrow R^{\cdot} + H_2O.$

Propagation Phase

R° rapidly react with molecular oxygen to form peroxyl radical (ROO°) which can attack another polyunsaturated lipid molecule and the reactions continue to produce peroxyl radical from PUFA of membrane destroying the integrity of the membrane.

$R^{\cdot} + O_2 \longrightarrow ROO^{\cdot}$

$ROO^{\cdot} + RH\ (Fatty\ acid) \rightarrow ROOH + R^{\cdot}$

Termination Phase

When 2 peroxyl radicals react with each other inactive products form, thus terminating the damaging chain reaction.

$ROO^{\cdot} + ROO^{\cdot} \rightarrow RO - OR + O2$

$R^{\cdot} + R^{\cdot} \longrightarrow R–R$

$ROO^{\cdot} + R^{\cdot} \rightarrow RO - OR$

The oxidation of membrane phospholipids is thought to increase the membrane permeability of cell and inhibit membrane ion pumps. This loss in barrier function leads to edema, disturbances in electrolyte balance, elevation of intracellular calcium, all resulting in malfunctioning of cell.

The damage produced by reactive oxygen species may be prevented by antioxidants which are of 2 types –preventive and chain breaking.

Preventive antioxidants inhibit the initial production of free radicals, they are catalase, glutathione perioxidase trdiamine diethyl penta acetate (DTPA) and ethylene diamine tetra acetate (EDTA). Once the peroxyl radicals are generated, the chain breaking antioxidants inhibit the propagative phase. They include superoxide dismutase, Vit E and uric acid. Vit E (alpha tocoferol T-OH) would intercept the peroxyl free radical and inactivate it before a PUFA can be attacked.

$T.OH + ROO^{\cdot} \longrightarrow ROOH + TO^{\cdot}$

$TO^{\cdot} + ROO^{\cdot} \longrightarrow$ Inactive product

Vit E is the most effective, naturally occurring chain breaking antioxidant in tissues and only traces are required (1 tocoferol for 1000 lipid molecules). Ascorbic acid acts as antioxidant in the aqueous phase.

Intracellular Signalling Mechanism

Cells respond to external stimuli (1st messenger or ligand) by means of cell surface receptors, which convert the stimuli into intracellular signals (second messenger). These second messengers transmit and magnify the signals that effect the cellular function through a cascade of reactions involving a set of coupled proteins.

Steroid hormones have intracellular receptors. On binding to ligand hormones, these intracellular receptors cause the effect at gene level. Cell surface receptor signal transduction may occur through the second messengers, Tyrosine kinases, cyclic nucleotides, inositol triphosphates, calcium or nitric oxide (unorthodox second messenger recently described) .

1. Tyrosine kinase is part of the insulin receptor. On binding with the ligand, the activity of the enyzyme is altered to cause intracellular effects.
2. Ligand gated ion channels: Ligand like neurotransmitter binds to the receptor that function as ion channel and causes it to open and allow the cations into the cell.
3. G protein coupled reactions: This involves the release of cyclic nucleotides– c AMP and c GMP from ATP and GTP by the action of Adenyl cyclase and Guaylate cyclase which are activated by a G protein (an intermediate in the process) coupled receptor on ligand binding.

G protein may also activate phospholipase C which will hydrolyse the membrane bound phosphotidyl inosiotol into 1,2 diacylglycerol (DAG) and Inositol 1,4,5 triphosphates both of them being second messengers.

IP3 causes release of Ca^{++} into cytosol. Both DAG and Ca++ stimulate protein kinase C to initiate another cascade of reactions

Ca^{++} can also bind to calmodulin and activate several protein kinases and c AMP phosphodiesterase.

G protein activates phospholipase A2 to cause release of arachidonic acid from membrane phospholipids leading to the formation of prostaglndins.

Nitric oxide stimulates guanylate cyclase to form c GMP.

METABOLISM IN OCULAR TISSUES

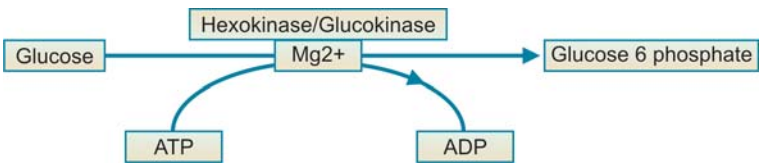
Carbohydrate Metabolism

Carbohydrates are the energy source to the tissues. Complex carbohydrates are broken down to glucose which enters cells by facilitated diffusion through

glucose transporters (GLUT). GLUT allows the diffusion of glucose into the cells down their concentration gradient. GLUT are proteins found in cell membranes with 12 transmembrane domains. They are of 5 types — GLUT 1- GLUT 5. They are all facilitative bidirectional transporters. SGLUT1 alone is sodium dependant unidirectional transporter, which is an active mechanism against concentration gradient. Some are insulin dependant or regulated by insulin. E.g: GLUT 4 others are not regulated by insulin. The GLUT in ocular tissue is mainly GLUT1 andGLUT 3.

Glucose on entering the cells through GLUT undergoes many types of reactions for varied uses in the cells.

The initial reaction is the conversion of glucose to glucose 6 phosphate, utilizing energy in the form of hydrolysis of a high energy compound, ATP. This is to prevent the escape of glucose from the cell. The negative charge on the phosphate group will not allow the glucose to pass through the hydrophobic interior of the plasma membrane of the cell.



The enzyme, hexokinase catalyzing this reaction has high affinity for glucose and other enzyme glucokinase, catalyzing the same reaction has low affinity for glucose. So only when the glucose concentration is high, glucokinase will act.

Glucose 6 phosphate is the metabolic junction for many pathways-viz

Glycolysis – Aerobic for production of enrgy in the form of ATP
– Anaerobic

Pentose phosphate pathway – for production of NADPH and pentoses on

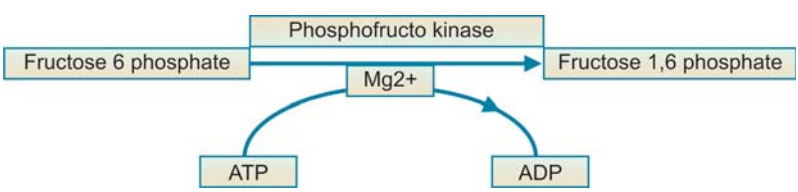
Glycogenesis for storage of energy.

Glycolysis

Glucose 6 phosphate is prepared for fractionation into 3 carbon units,split apart then further rearranged to generate 4 molecules of ATP.

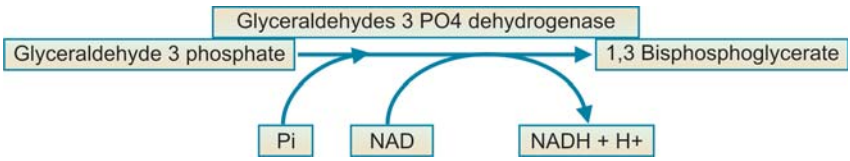
Glucose 6 phosphate —(Phosphoglucoisomerase)— fructose 6 Phosphate

The next reaction energetically primes the molecule for splitting

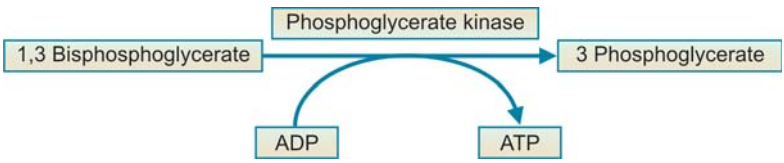


Fructose 1,6 bisphosphate —(Aldolase)→ Glyeraldehyde 3 PO4 + Dihydroxy acetone phosphate

Presence of phosphate group in both fractions assume that they will remain within the cell cytoplasm. These two are isomers and can be interconverted by isomerase. Actually, dihydroxyacetone is converted to glyceraldehydes 3 phosphate which only undergoes further reactions to produce ATP. In the subsequent reactions, there is shuffling of phosphate groups which are ultimately transferred onto ADP to form ATP.

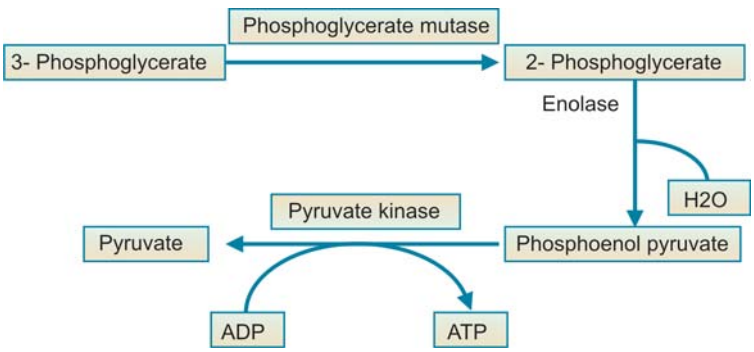


Inorganic phosphate in the cytoplasm is added in this reaction with the help of co-enzyme NAD which is reduced to NADH + H [this NADH enters the respiratory chain in the mitochondria where ATP is formed by electron flow].

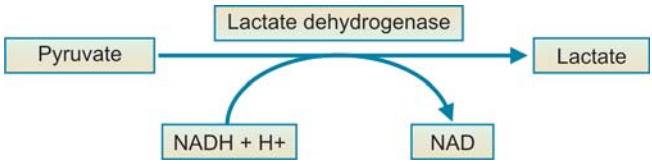


The high energy PO_4 group on carbon 1 is transferred to ADP to form ATP and this is called substrate level phosphorylation.

Then, the remaining PO_4 group is prepared for transfer to ADP by isomerisation and dehydration of the glycerate molecule. These reactions increase the potential energy for transfer of phosphate group by four fold so that ATP is formed from ADP easily.



Upto this reaction, 2 molecules of ATP have been consumed and 4 molecules of ATP have been formed with the net gain of 2 ATP per glucose molecules. Pyruvate is at the junction point between aerobic and anaerobic metabolism. In anaerobic metabolism, there is only a single reaction that forms lactate from pyruvate and with that the pathway is terminated. The NADH formed in the earlier reaction is utilized here, instead of entering, etc.



This is a quick and relatively uncomplicated means for cells to obtain ATP in the absence of oxygen, though the yield is small (2 ATP/ glucose molecule). If the cell obtains its glucose from the breakdown of stored glycogen, it usually realizes a netgain of 3 ATPs anaerobically because no ATP is required for formation of glucose 6 phosphate from glycogen.

The cell obtain high energy supply in a short period. So, a relatively significant percentage of glucose is utilized via this pathway in ocular tissues. Moreover, sufficient amount of NAD must be regenerated from NADH for use in the earlier reaction, i.e. formation of 1,3 bisphosphoglycerate from glyceraldehyde 3 phosphate for that purpose also, this anaerobic glycolytic pathway is necessary.

Aerobic Glycolysis

Pyruvate, if not converted to lactate, will diffuse into cellular mitochondria, to begin the aerobic phases of ATP production (the mitochondria has a double membrane, the inner membrane has a large surface area with infoldings known as 'crista' and it is impermeable to most of the molecules and ions without a transporter. It contains a number of insoluble electron transfer proteins and an enzyme, ATP synthase. The inner most compartment called "matrix" contains many soluble enzymes not found in cytoplasm).

Pyruvate is converted to acetyl CoA in the matrix and the complete oxidation occurs in matrix. The reducing equivalents that are produced during oxidation, enter the respiratory chain or electron transport chain in the inner membrane to produce ATP and CO_2 which is used for formation of oxaloacetate from pyruvate.

TCA cycle (Fig. 12.12)

Respiratory chain or Electron transport (ETC) is the site of ATP production during oxidation of the reducing equivalents.

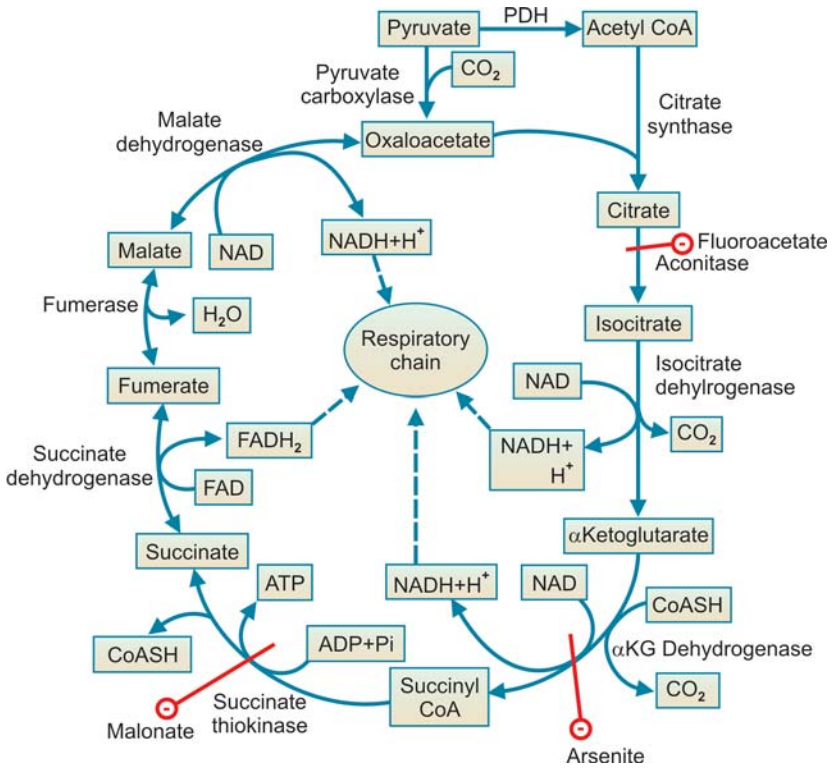
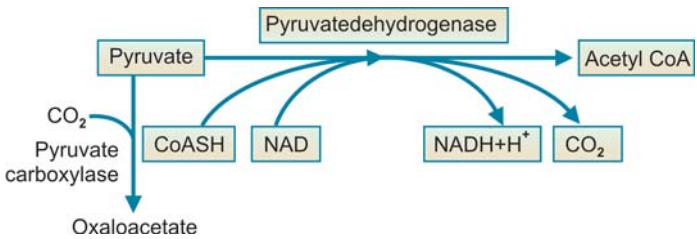


Fig. 12.12

$$NADH + H+ \xrightarrow[O_2]{ETC} NAD + H2O + 3\,ATP$$



$$FADH_2 \xrightarrow[O_2]{ETC} FAD + H_2O + ATP$$

Substrate level phosphorylation (ATP from ADP) during conversion of succinyl CoA to succinate. Totally 12 ATPs are produced per citric acid cycle (TCA cycle). Thus, on complete oxidation of one molecule of glucose 36/38 ATPs are produced aerobically.

Glycogen Formation and Degradation

It is limited and minimal in ocular tissues (corneal epithelial, muller cells in retina).

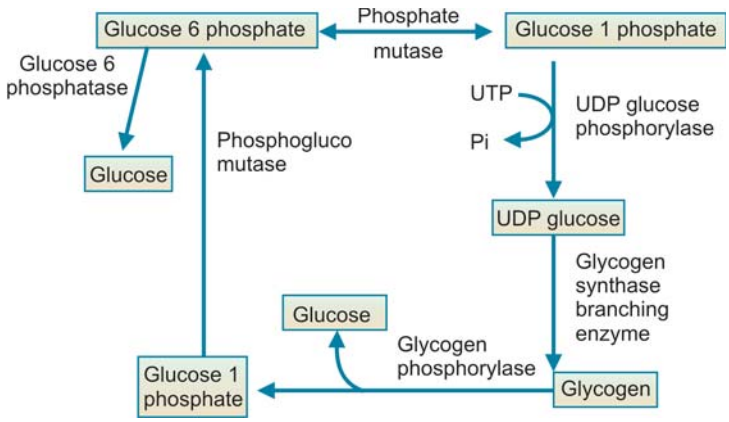


Fig. 12.13: Debranching enzymes

Pentose shunt or HMP (Hexose Monophosphate) shunt (Fig. 12.14)

It serves 3 principal functions:

1. Generation of pentoses – for synthesis of nucleic acids
2. Generation of NADPH necessary for – synthesis of fatty acids, cholesterol etc, cell detoxification – by removal of destructive form of oxygen or reactive oxygen species like H_2O_2.
3. Recovery of certain metabolic intermediates such as fructose, glucose etc.

Two other pathways of significance in ocular tissues are

1. **Gluconeogenesis** in retina where there is formation of glucose from non carbohydrate sources like lacatate, due to the high demand for glucose. Mostly reversal of glycolysis with 3 exceptions.
2. **Polyol pathway** which is dormant normally. It is stimulated when there is excess glucose as in Diabetes mellitus, galactosemia, etc.

HMP Shunt

Aldose reductase has a very high Km for glucose – 700 times that of hexokinase ie very low affinity. Polyol dehydrogenase also has a very high Km for sorbitol. So, there is chance for accumulation of sorbitol in the cell, before it is further metabolized to fructose.

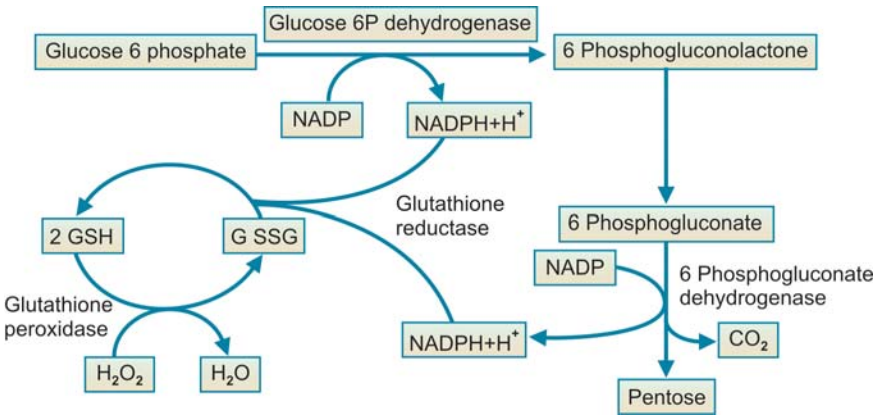
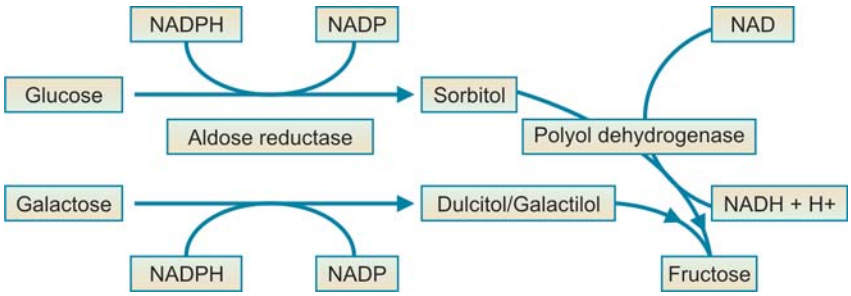


Fig. 12.14



Glycation of proteins: occurs when there is an excessive level of glucose as in diabetes mellitus or non-enzymatic glycation as a part of aging.

PROBLEMS OF CARBOHYDRATE TRANSPORT AND METABOLISM

Diabetes and Galactosemia

Diabetes is a metabolic disorder of cellular carbohydrate uptake that affects not only the carbohydrate metabolism, but also lipid and protein metabolism. Primary effect occurs to blood vessels of brain, eyes, kidney and external limbs. In the eyes , retina, cornea and lens may be affected pathologically resulting in visual debilitation and blindness.

Diabetes occurs in 2 forms. Type I or Juvenile onset diabetes and Type II or maturity onset diabetes ~ 10% of diabetes are Type I and it is more severe. In both forms, there is inability of glucose to enter certain classes of cells in the body that are dependent upon insulin activated, transport protein systems.

In Type I, there is lack of insulin due to autoimmune destruction of β cells of pancreas and in Type II, there is problem with the insulin receptor protein. It may be less in number or less responsive receptors in normal numbers, e.g.

obesity is one of the causes of Type II diabetes. The enlarged adipocytes secrete a protein known as tumor necrosis factor α– that inhibits insulin receptor autophosphorylation necessary for insulin response. So, the cells that depend on insulin for transport of glucose become starved for nourishment while other cells, not dependent on insulin become exposed to higher than normal cytoplasmic levels of glucose ie toxic levels.

Insulin dependent cells of our interest are cells of blood vessel walls.

Cells not dependent on insulin—lens fibre cell

To compensate for the lack of glucose, the insulin dependent cells alter their metabolism in pathological ways (abnormal ways) to produce energy precursor in the form of acetyl CoA from the breakdown of lipids and protein. This leads to formation of ketone bodies in excess resulting in ketosis and consequent acidosis.

In the cells that are not dependent on insulin the glucose is taken up by cells in excess and is bound to proteins both extra and intracellularly. This is known as glycation and it is a permanent change. This reaction continues and produces more complex forms that result in the denaturing of protein. Glucose in higher concentration can cause DNA damage.

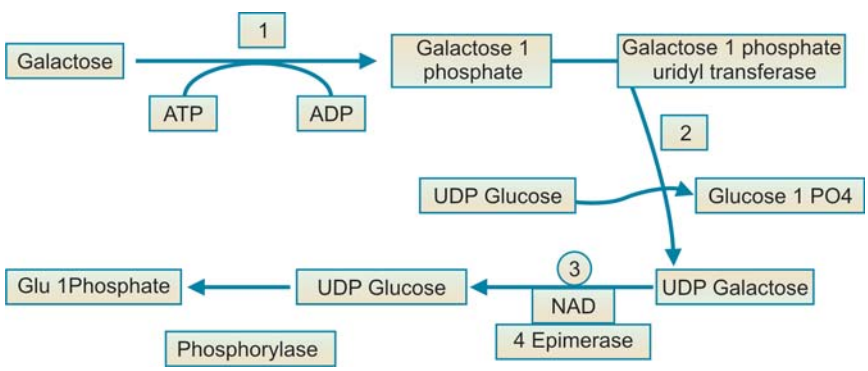
Galactosemia

Galactose is incorporated into glycolytic pathway via glucose 1 phosphate by the activity of 3 enzymes.

1. Galactokinase.
2. Galatose 1 phosphate uridyl transferase.
3. UDP galactose epimerase.

Glucose 1 phosphate is converted to Glucose 6 phosphate by isomerase as in glycogen breakdown.

A deficiency in any of these three enzymes numbered in the pathway results in accumulation of either galactose or galactose 1 phosphate in tissues.



The resulting condition is known as galactosemia. The general systemic symptoms and signs are, liver failure and mental deterioration from depletion of inorganic phosphate by the uridyl transferase deficiency, accumulating Galactose 1 phosphate. Epimerase deficiency is symptom free. Galactokinase deficiency results in accumulation of Galactose.

The ocular effects of galactosemia are as follows

Zonular or nuclear cataract development in 30% of galactosemic patients due to rapid accumulation of galactitol in the polyol pathway. The accumulated galactose is converted to galactitol due to the stimulation of aldose reductase. This galactitol is an extremely poor substrate for polyol dehydrogenase. So, the accumulation of osmotically active galactitol causes swelling of the lens fibre cell, leading to bursting of the cell resulting in cataract formation.

13 Biochemistry of Individual Ocular Tissue

Biochemistry of ocular tissue can be discussed under the following sections:
1. Ocular surface - lid, tear film, conjunctiva, cornea, sclera.
2. Uveal tract.
3. Aqueous humor.
4. Lens.
5. Vitreous.
6. Retina.
7. Photo transduction.

THE LIDS

Eyelids contain specialized mucus secreting glands that secrete a different type of mucus gamma MUC4 and oil secreting glands that contribute to the makeup of the tear film. Lashes and their hair follicles are important, in protecting the eye from foreign particles.

Abnormalities: E.g. Trachoma.

Defect in lid apposition due to scarring and deformation of lids leading to corneal exposure; ulceration and blindness.

CONJUNCTIVA

Specialized cells like immune cells (T and B cells, mast cells, dendrite cells) and mucus secreting cells are present in conjunctiva. The epithelium is more than a simple covering layer for the conjunctiva. The conjunctival epithelium is an intermediate type between keratinized squamous epithelium and mucosal epithelium with intraepithelial lymphocytes and dendrite cells organized as a part of mucosa associated lymphoid tissue.

Functions of Epithelium
1. Acts as a barrier to external organisms.
2. It is the source of tear mucins derived from the intraepithelial goblet cells which are under neuro endocrine control.

Clinical application

Abnormality in eptithelial and goblet cells result from Vit A deficiency – leading to severe dry syndromes.

Conjunctival stroma: is highly vascular structure and contains aqueous veins.

It has a superficial lymphoid layer and a deep vascular layer. Through this stroma, waste materials from anterior chamber of the eye are transported to periauricular draining lymphnodes and venous drainage systems in the neck.

SCLERA

This is the outermost coat of the eye. It is a tough and non-compressible layer of connective tissue with minimal amount of fibroblasts. Few are found to be myofibroblasts, i.e. contractile fibroblasts conferring refractive properties to sclera.

Connective tissue is made up of Type I and III collagen fibres. The fibres have variable diameter and are irregularly distributed. Hence, they cannot transmit light and sclera is non-transparent.

The Glycosaminoglycans present in sclera are dermatan sulfate and chondroitin sulfate. They are bound to core protein to form the proteoglycans of small non aggregating type and are localized to the collagen fibres. Sclera has some large, aggregating proteoglycans also viz. versican, neurocan and brevican combined with hyaluronic acid. As the amount of proteoglycans in sclera is less, it is less hydrated (70%). Uveoscleral outflow of aqueous is a bulkflow at constant rate and a portion of it drains directly transclerally. Fluid flowing across the sclera is absorbed by the matrix proteoglycans with low water binding capacity to maintain normal 70% hydration.

BIOMEDICAL IMPORTANCE OR CLINICAL APPLICATION

In Uveal effusion syndrome and Nanophthalmia – the sclera contains high levels of abnormal proteoglycans especially dermatan sulfate proteoglycans. These bind and trap large volumes of water leading to thickening of sclera. This may cause obstruction of choroidal venous drainage further increasing the swelling and water retention.

In myopia or near sightedness, the ocular globe is lengthened along its anteroposterior axis. This condition is associated with remodelling or reformation of the extracellular matrix proteins in the posterior sclera. The enzyme gelatinase A digests the scleral proteins (collagen) partially so that new scleral proteins can be formed to establish a new scleral length resulting in myopia. The inhibitor of this enzyme is less (TIMP - Tissue Inhibitor of Metallo Proteinase) and so, activity of the enzyme gelatinase A proceeds uninhibited resulting in digestion of scleral proteins. Gelatinase A is a type of Matrix Metallo Proteinase (MMP).

PRECORNEAL TEARS

Precorneal tears bathes the ocular surface compised of conjunctival and corneal non-keratinised epithelium. Precorneal tears form a film between the inside of

the lids and the cornea; when the eyes are closed and remain so for 15-45 seconds after the eyes are opened and then rupture stimulating the next blink. This is called tear break up time.

Functions of Precorneal tears

1. It is the lubricating fluid for the lid cornea interface.
2. It is the protective covering for cornea and forms a smooth optical surface at the air – eye interface.
3. Antibacterial medium to protect the eye.
4. Perfusion fluid washing away debris from corneal surface.
5. Temporary depository for instilling topical drugs therapeutically.
6. Supplies oxygen to corneal epithelium.

Precorneal tears, as a film are a complex mixture of 3 µm thick, composed of 3 layers viz.

1. The anterior or superficial lipid layer or surface oily layer.
2. Central aqueous layer, and
3. Posterior mucous layer.

Lipid layer is derived from meibomian gland secretion.

Composition of Human Meibomian gland lipids

Lipid component	*% composition*
Cholesteryl esters	29.5
Waxes	35
Triacylglycerol	4
Cholesterol	1.8
Fatty acids	2.2
Diesters	8.4
Unidentified	rest of it

Waxes are esters of long chain (14–36 carbons) fatty acids and long chain alcohol (16–20 carbons) derived from fatty acids.

Cholesteryl esters are esters of unsaturated and hydroxy fatty acids.

Diesters are double esters, i.e. hydroxyl fatty acids esterified to (a) other other fatty acids, or (b) one fatty acid and an alcohol, or (c) a fatty acid and cholesterol.

Fatty acids are long chain fatty acids (14–36 carbon) saturated and unsaturated (with 0-3 double bonds) inclusive of odd chain and hydroxy fatty acids.

Functions of lipid layer

1. Allows free flow of tears from their ducts to the eyelid edges.
2. Forms a film over aqueous layer.
3. Prevents speedy evaporation of tears.
4. Prevents spill over of tears at the lid margin by adhering to the eyelid skin.
5. Forms a water tight seal when the lids are closed.
6. Prevents migration of skin lipid onto the ocular surface.
7. Provides a clear optical medium.

Abnormalities of lipid layer may result from meibomian gland dysfunction where excessive production of keratin occurs in the ductal epithelium (Keratin is a protein characteristic of skin hard coverings such as animal horns) leading on to reduction in the amount of steryl esters, increase in cholesterol and appearance of ceramides, a further complication of this process is that the epithelial cells may detach from the gland and block the flow of new lipids to the tear film. Bacterial infection in the area of blocked lipids can also occur. Staphylococcus aureus and other bacteria produce the enzyme cholesterol esterase and fatty wax esterase capable of hydrolyzing the meibomian lipids.

Central aqueous layer

The tear film contains 98 % H_2O, pH 7.5, volume 6–9 µl, osmolality 310–334 m osm.

Lacrimal gland secretion provides the aqueous component of tears. The dilute aqueous solution from the exocrine acinar glands contain proteins, small molecular weight components and electrolytes as shown in table on comparison to blood.

Composition of aqueous layer in comperison to blood

Component	*Blood concentration*	*Aqueous concentration*
Ascorbate	1.3 mg%	0.4 mg%
Bicarbonate	27 mmol /L	23 mmol/L
Glucose	90 mg%	6 mg%
Chloride	96–106 mmol/L	118–135 µmol/L
Potassium	4.3 mmol/L	30 mmol/L
Sodium	150 mmol/L	138 mmol/L
Protein	7 gm%	0.7 mg%
Magnesium	0.75–1.25 mmol/L	0.7–0.9 µmol/L

Functions of aqueous layer

The principal proteins present in aqueous are:

1. Lysozyme – antibacterial.
2. Tear specific pre-albumin – induces lipid spreading by interacting with lipid layer, removes harmful lipophilic molecules.
3. G protein – signal protein.
4. Lactoferrin.
5. Immunoglobulin A, etc.

Na^+, K^+, Cl^-: Regulate the osmotic flow of fluids from cornea to the tear film and maintain the osmolality at 310–334 mosm.

HCO_3^- regulate the pH of tear film maintains at 7.5, other electrolytes are enzyme co-factors.

Regulation of aqueous layer

Psycho-neuroendocrine control:

1. Neural control mediated by autonomic nervous system (instillation of drugs that modulate this system, e.g. Pilocarpine, atropine affect tear secretion).

Direct effect on acinar cells through intracellular second messengers. Parasympathetic system increases the tear flow via its effects on the myoepithelial cells surrounding acinar cells.

2. Hormonal control

   Testosterone stimulates secretion of certain tear components such as Ig A.

   Reduction in tear flow in women after menopause.

3. Psychological factors also contribute for controlling tear flow.

Posterior Mucus Layer:

Mucins are secreted by conjunctival goblet cells e.g., MUC5AC which is the principal tear mucin and it is mucus glycoprotein. Mucins have larger amounts of carbohydrates with short chain sugars. The carbohydrates are attached in numerous short chains along the length of polypeptide chain. This layer is composed of glycocalyx of the epithelial cell surface e.g., MUC1, MUC4, sialomucin complex and an additional layer of tear specific nucleoproteins.

Functions: mucus imparts viscosity to the tear film, maintains the stability of tear film. Mucins support the stability of tear film by increasing tear film viscosity and by trapping, lipids within their structure, so that they can be, reused after blinking.

Clinical applications:

Abnormalities in mucus layer: Reduction or absence of mucin in tear film occur due to the following :

1. Vit A deficiency.
2. Ocular pemiphigoid (conjunctival ulceration).
3. Stevens-Johnson syndrome (an attack of the mucous membranes and skin).
4. Alkali burns.

All these conditions lead to destruction of goblet cells with consequent loss of mucin production. This results in rapid breakup of tear film even when there is adequate volume of aqueous layer of tears (Fig. 13.1).

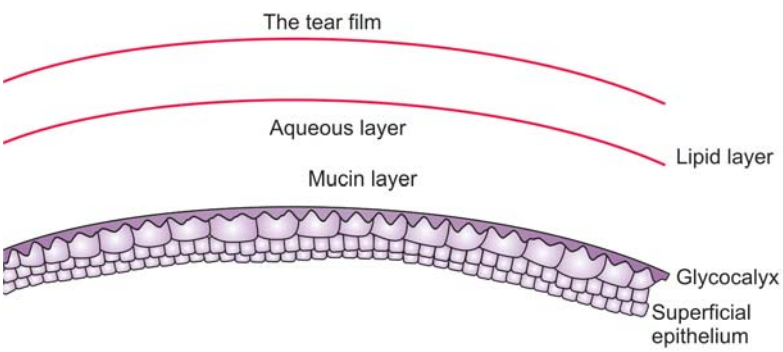


Fig 13.1: The tear film

CORNEA

Cornea is designed for light transmission as well as light refraction. Cornea is made of cellular and extracellular matrix of collagen and glycosaminoglycans. They are organized in such a way to reduce the disparity in their refractive indices and this enables the function of cornea.

Cornea consists of epithelium with basal lamina, Bowman's membrane, stroma, Descemet's membrane and endothelium. All the layers of cornea contain collagen.

Corneal Epithelium

It is six cell thick stratified layer on a basal lamina. It is the first refracting interface for transmitted light. Short wave length light are absorbed by the epithelium and majority of the light of the visible spectrum is transmitted through this epithelium. Epithelial cells express keratin, the intermediate filament that gives mechanical strength to the junctional structure between cell and basement membrane such as hemidesmosomes.

They also contain receptors for basement membrane components such as fibronectin, laminin and collagen. Hemidesmosomes are bound to corneal stroma through a band of anchoring fibrils. These fibrils are made of type VII collagen. In addition, type XVI collagen also support firm adhesion between the basal cells.

Function

1. Effective barrier to fluid transport through ion channels.
2. The arrangement of collagen fibers with proteoglycans of glycosaminoglycans such as keratin sulphate, dermatan sulphate and chondroitin sulphate provides elasticity and deformability to the cornea while maintaining high levels of transmission.
3. Synthesis of cell surface glycoproteins and the intracellular matrix intermediate filament keratin.

Clinical application

Vitamin A or retinol is required for the control of epithelial keratin expression, cell surface glycoprotein synthesis and for normal corneal wound healing. Retinol also promotes the synthesis of α-1 proteinase inhibitor which inhibits a wide range of proteolytic enzyme. So, vitamin A deficiency leads to a form of keratinisation of corneal epithelium, impaired epithelial function, loss of luster, bitot's spots, punctuate erosions. Different types of collagen are present in the cornea. These are the matrix proteins in the cornea as shown in the Figure 13.2.

Corneal Stroma

Collagen fibres form sheets termed lamellae and have uniform diameter of 30 nm. 70% are type I collagen, 15% type V and 15% type VI collagen. Type I

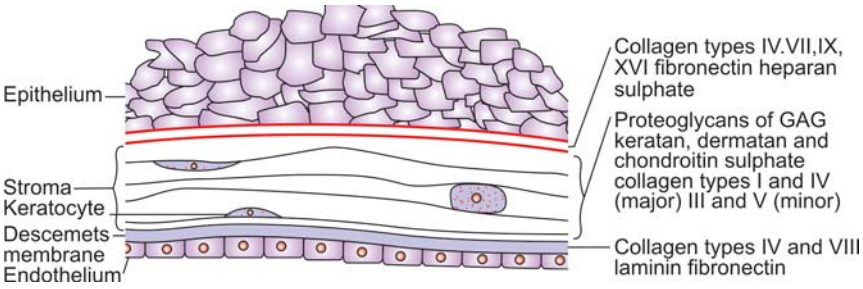


Fig 13.2: Cornea—Matrix proteins

collagen forms oblique running lamellae in the stroma. Type V assumes anterior conformation that prevents the attachment of additional fibres and this limits the diameter of type I. This tight control of the diameter prevents light scattering in the corneal stroma. Type V aminoterminal domains project onto the fibril surface and when sufficient numbers have accumulated, they block further accretion of collagen monomers and there by limit growth in diameter. Type VI collagen stabilizes the proteoglycans and keratocytes located between the lamellae.

The inter fibrillar distance is maintained at around 55 nm. Only when the distance between the regions of different refractive indices becomes greater than 200 nm, the light scattering occurs. So the critical factor is the inter-fibrillar distance for maintaining the corneal transparency.

The parallel arrangement of the central corneal fibrils extends to the periphery to form a concentric configuration and curvature of cornea. The curvature of cornea is important in refracting and focusing light to produce an image on the retina. In the corneal stroma, 2 types of glycosaminoglycans viz, keratan sulphate and dermatan sulphate are found linked to core protein lumican and decorin respectively. These proteoglycans bind to collagen at specific binding sites.

Function

Maintenance of corneal clarity and curvature. The proteoglycans serve as spacer molecules between the collagen fibres of the stromal lamellae. Glycosaminoglycans carry negative charge and hence, attract sodium and water. This is important to maintain the corneal clarity as it generates a level of interfibrillar tension that maintains the interfibrillar distance necessary for light transmission.

Clinical Application

In certain diseases of the cornea, for example; corneal dystrophies-water enters the space occupied by the proteoglycans and increases the distance between the collagen fibers. So, light scattering occurs resulting in corneal opacity.

Mucopolysaccharidoses are diseases associated with degradation of glycosaminoglycans due to degradative enzyme deficiency. The resultant partially degraded glycosaminoglycans deposit in the cornea leading to corneal

opacity, e.g; Hurler's syndrome: Deficiency of α iduronidase, GAGs containing iduronic acid viz heparan sulfate and Dermatan sulfate are not broken down after the initial removal of sulfate. These GAGs accumulate in cell lysozymes, spill over and get deposited in cornea causing opacity.

Abnormalities in curvature can distort image even with clear cornea, e.g. astigmatism, keratoconus, wound scarring. Keratocytes are corneal fibroblast and are the site for post-translational modification of collagen and also proteoglycans synthesis as they contain the necessary enzymes and hence important for corneal transparency.

Bowman's Layer

Bowman's layer is immediately posterior to epithelial basal lamina and is secreted by keratocytes (anterior stromal). It is usually of Type IV collagen. Here, one type IV collagen joins the other type IV collagen by association of its non-collagenous peptide extensions to form a spider web like structure or open mesh and not a fiber (Fig. 13.3). This layer is acellular and anterior keratocytes do not repair, damage to this layer after initial formation during embryogenesis.

Function

It separates epithelial cells from adjoining stroma and supports epithelial cells in their location.

It acts as a sieve or barrier for molecules that may approach its associated cells.

Descemet Membrane

Descemet membrane is secreted by endothelial cells and is made of type VIII collagen which forms geometric patterns resembling a box spring mattress or lattice structure. The non-helical regions of this type VIII collagen can form bonds with type IV collagen (Figs 13.4 and 13.5).

Function

1. Provides elasticity and deformability to cornea.
2. Imparts strength and resilience to corneal stroma.
3. Maintains light transmission.

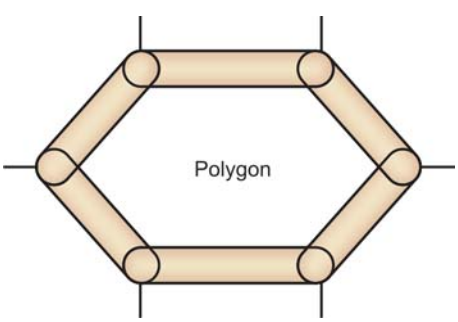


Fig 13.3

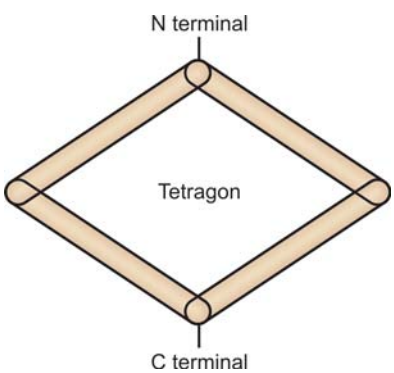


Fig 13.4

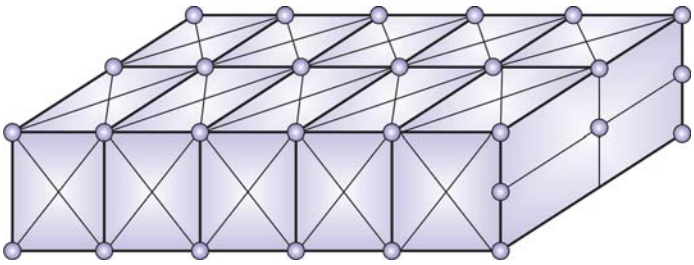


Fig 13.5: Partial lattice structure

4. It is the main resistance to intraocular pressure.
5. It is resistant to proteolytic enzymes.

Anchoring fibrils extend between the basal epithelial cells and the outer most lamella of the corneal stroma, extending through the Bowman's membrane. It is of type VII collagen. It is attached from the hemidesmosomes of the epithelial basal cells to stromal type I collagen fibers.

Function

It serves to attach the epithelium to the stroma strongly.

Biomedical Importance/Clinical Application

In diabetes mellitus, the synthesis of anchoring fibrils is reduced resulting in loose adhesion of the epithelium to its underlying stroma.

Endothelium

Functions as a fluid pump to keep the cornea in a clear, deturgesced state. ~ 10 µl/hr of fluid is pumped back into the anterior aqueous by the corneal endothelium. Endothelium is a non-vascular, highly metabolic, single cell layer of hexagonal cells of uniform size and shape, bathed by aqueous humor of the anterior chamber. No mitosis occurs in adult corneal endothelium and so cells decrease in number throughout life.

It is termed "metabolic pump" instead of "fluid pump" because it allows the water to move osmotically down the gradient setup by active transport of ions. pH and osmotic requirements of endothelium are: 6.8–8.2 pH and 200–400 mosm / kg of osmotic tolerance with 304 mosm/kg as optimum.

Transport of ions in cornea:

1. Epithelial ion transport.
2. Endothelial ion transport.

Endothelial ion transport:

Several ion transport systems exist in the corneal endothelium.

1. Sodium Potassium Pump (Na^+ /K^+ ATPase).
2. Sodium / Hydrogen exchanger.
3. Bicarbonate / Sodium cotransporter.

1. Sodium Potassium Pump (Na^+ /K^+ ATPase) (Fig. 13.6)

It has two special functions in ocular tissue.

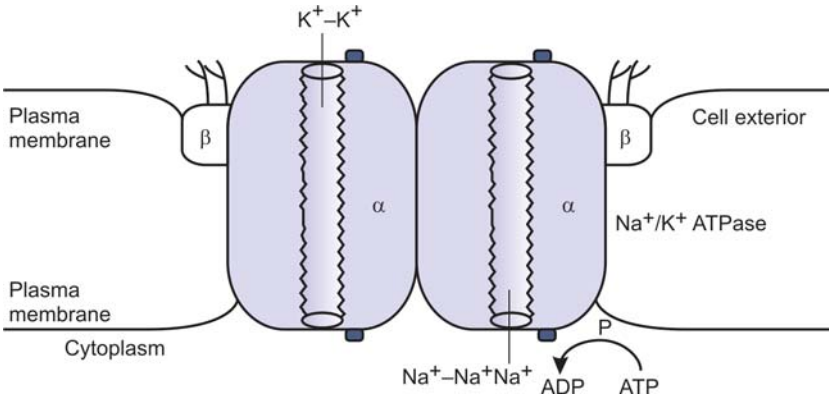


Fig 13.6: Sodium potassium pump

1. Control of corneal hydration.
2. Production of aqueous fluid.

It is located in basolateral membrane of the endothelial cells about 1.5 × 1000000 pump sites / cell. It is an enzyme – sodium, potassium stimulated adenosine triphosphatase, in short Na^+ / K^+ ATPase. It is membrane bound or an integral protein, spanning the width of the cell plasma membrane. It consists of 4 polypeptide chains 2 α and 2β chains. β chains with small chains of carbohydrate are necessary for membrane insertion, stabilization and orientation of α subunit.

Alpha chains are the actual catalytic molecules for which the substrate is the high energy compound ATP.

$$ATP \xrightarrow{Na+/K+\ ATPase} ADP + Pi$$

This reaction is energetically coupled to an ion transport process. Pi becomes bound to one of the α subunit and in the process supplies energy necessary to transport 3 Na^+ out of the cell and $2K^+$ inward. The actual transport of ions is assumed to take place either by a conformational shuttle within the α subunit or ions pumped through the pores present in the subunits. The pumping out of Na^+ into the narrow channel between the adjacent endothelial cells generates a counter osmotic pressure drawing water also out of the stroma. The other transporters support this enzyme in their action.

2. **Sodium / Hydrogen exchanger:** It is present in the basolateral membrane moving Na^+ into the cell and H^+ outward. This is necessary to maintain the intracellular Na^+ that is pumped out by Na^+/ K^+ ATPase. The hydrogen ions acidify the extracellular fluid, increasing the level of CO_2 which diffuses into the cell.
3. **Bicarbonate / Na^+ cotransporter:** It is present on the apical membrane

of the cell. The CO_2 inside the cell combines with water in the presence of the enzyme, carbonic anhydrase to form carbonic acid which then dissociates into $H^+$and HCO_3^-. Both are transported out, H^+ from this reaction exchanges with Na^+, so that this can provide the ions necessary for the action of Na^+/ K^+ ATPase. The HCO_3^- from this reaction goes out through the bicarbonate transporter which carries one Na^+ also for 2 HCO_3^- transported. All these transport systems are interlinked or dependent on each other.

Na^+ from the lateral space goes into aqueous humor which has negative potential and along the path of least resistance into the stroma. Water follows sodium through osmotic gradient.

Chloride transport also has a role in this. Thus, the deturgescence or corneal hydration is maintained by these endothelial pumps (Fig. 13.7).

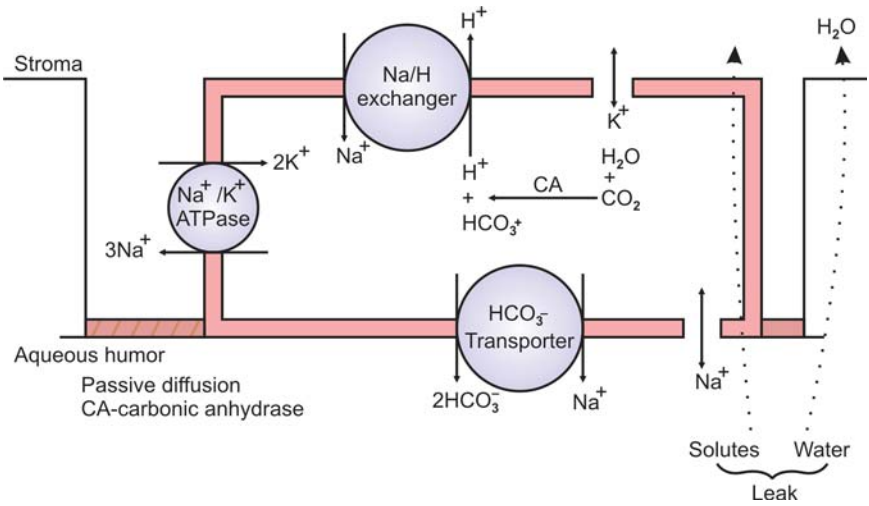


Fig 13.7: Endothelial pumps

Epithelial Transport

Sodium potassium pump.

Sodium/chloride co-transport pump.

Model of ion transport, ion channels and sympathetic neural control of chloride channels in the corneal epithelium (Fig. 13.8).

The Na^+/ K^+ ATPase ion pump in the basolateral membrane maintains the Na^+ gradient for Na^+/ Cl^- co-transport. Chloride diffuses down its chemical gradient through apical channels which are opened by cAMP. Corneal epithelium is a tight epithelium with low ionic conductance for Na+ through its apical cell membrane (sodium is pumped from tears to stroma while chloride is transported into tears).

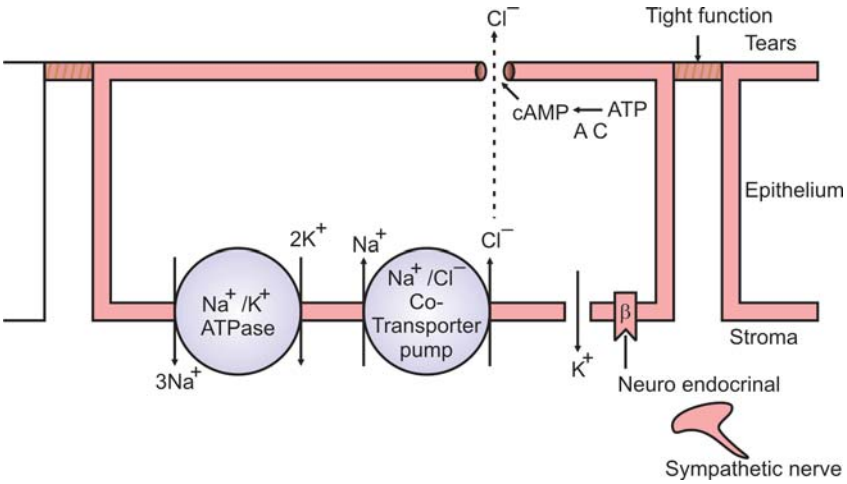


Fig 13.8: Epithelial transport system

Sodium from tears enters the corneal epithelial cell through low conductance channels in the apical membrane of the superficial cells. It is then extruded into the stroma by the Na^+/K^+ ATPase located in the basolateral membrane of the cells, i.e. 3 Na^+ out in exchange for $2K^+$ into the cell. As there develops a Na^+ gradient in the stroma, it moves down the gradient into the epithelial cell again of while moving it carries the Cl^- also along with it. Cl^- movement against the concentration gradient is possible because of this Na^+ co-transport pump. There is a high conductance K^+ channel in the basolateral membrane transporting K^+ into the stroma. Once inside the cell, Cl^- diffuses into the tears through channels in the apical cell membrane. The transport processes in the corneal epithelium result in osmotic transport of water out of the cornea. Epithelial chloride is stimulated by catecholamines through ß adrenergic receptors and second messenger cAMP.

The epithelial transport of water and electrolytes also helps in maintaining the corneal transparency.

Biomedical importance

Chemical burns of cornea: caused by mineral acids and alkali, requires immediate medical attention e.g., NaOH is the alkali, exposure to which causes instantaneous cloudiness of cornea.

Early chemical damage results in cell destruction and disruption of collagen of proteoglycans. With sufficently concentrated alkali, the cell membrane is damaged by saponification or lysis of lipid ester bonds in the membrane resulting in cell death. The OH^- of the alkali binds to the basic amino acid of collagen and through Vanderwalls forces and hydrogen bonding gets sorbed to the collagen molecules, allowing water molecules to enter the interfibrillar spaces. This causes swelling and distortion of collagen fibres. The peptide bonds of

the exposed and distorted fibres are hydrolysed by the alkali. Also, the proteoglycans are cleaved at their linkage with GAGs, all these result in obliteration of the regular interfibrilar distance and hence the loss of transparency.

Immunochemical damage

When the alkali burns is severe (>0.1 M alkali exposure) the initial chemical damage is followed by destructive inflammatory response as the reparative process cannot commence due to cell destruction. The response is by infiltration of polymorphonuclear lymphocytes within the first 2 days of the burns, ulceration of tissues, release of matrix metallo proteinase, which will further lyse and damage the corneal collagen and proteoglycan proteins. This continual degradation of corneal collagen can lead onto perforation of cornea.

Cell turnover and wound healing in Cornea

Epithelium is constantly being regenerated by mitotic activity in the basal layer of cells. The initial response following epithelial debridement is migration of cells as a flattened sheet of single layer across the stroma to close the defect. Migration is achieved by the redistribution of cytoskeletal actin – myosin fibrils. Adhesion of epithelium to basement membrane and Bowman's layer is through hemidesmosomes that form within 18 hours. The lamina densa and the anchoring fibrils of type VII collagen take many days to form. Thus, single cell layer is restored to its six layered architecture.

Stroma: Immediate effect is to cause wound gaping and imbibing of water from tears by GAGs causing local opacity. Then, keratocytes are activated to synthesize GAGs and collagen. In early stages of healing, there is loss of specialization in keratocytes and loss of regularity in the arrangement and size of fibrils leading to further opacity in the cornea. Only after (many months) a long time, cornea restores clarity by producing normal corneal matrix component in small, well defined wound and not in extensive wounds.

Endothelium: does not have mitotic activity, but undergoes cell slide i.e. migration and loss of cells. If considerable number of endothelial cells are lost, the pumping cannot be performed; cornea will imbibe water and become opaque.

Vascularisation occurs during healing, if the defect does not close promptly.

Metabolism of Cornea

The primary metabolic substrate for energy in cornea is glucose. Glucose is provided to the stroma, primarily from aqueous humor by carrier mediated transport through the endothelium to the epithelium by passive diffusion through stroma. Oxygen is supplied largely by the atmosphere. The percentage of glucose utilization through various pathways is given below.

Metabolic pathway	*Epithelium*	*Stroma*	*Endothelium*
Anaerobic glycolysis	57%	57%	70%
Aerobic glycolysis	8%	8%	23%
Pentose pathway	35%	35%	7%
Glycogenesis	+	-	-
Polyol pathway may be present in diabetes mellitus.			

Though the percentage of glucose entering the anerobic glycolysis is more, the aerobic glycolysis gives much more energy with less glucose.

Anerobic glycolysis 57 %	aerobic glycolysis
ATP production 57 × 2 = 114	8 × 36 = 254 ATP

Total yield is 368 ATP from 100 glucose molecules in epithelial and stromal cells.

Since immediate supply of energy by quick release of ATP without O_2 is needed of the %, anerobic glycolysis is more in epithelial cells to sustain its transport function and cell division.

The relatively high % of pentose shunt in the corneal epithelial and stromal (keratocytes) cells may be related to their physiological role. Epithelial cells are in constant cell division requiring consistent production of proteins and lipids. The pentose i.e. ribose produced in pentose shunt is useful for synthesizing nucleic acid in protein formation and the NADPH obtained from this pathway is utilized for synthesis of fatty acids required to build up cell membrane.

The role of keratocytes is one of maintenance and repair of the structure that constitute the stroma. Although, these cells occupy only 5-10% of stromal volume, they are involved in the production of protein (collagen, proteoglycans) and structural carbohydrates. Thus, they also have to maintain an adequate supply of pentose for nucleic acid formation.

The endothelial cells of cornea, have a higher demand for ATP to maintain the deturgescence (corneal clarity) through the Na^+/ K^+ ATPase that is dependant on ATP. So, it requires both anaerobic and aerobic glycolysis at a higher level than other cells of cornea. Here, it is 140 ATP from an aerobic and 838 ATP from aerobic, totalling 968 ATP, 26 times more than other corneal cell types.

Biomedical importance

Corneal epithelia have a reduced amount of available O_2 during contact lens wear and this causes them to increase the percentage of anaerobic glycolysis. This was once a major problem in the use of hard contact lenses as nearly 80 % of the glycogen would be used in just 8 hours of lens wear compared to soft lenses. The resultant metabolic strain on the epithelial cell caused significant swelling of both epithelium and anterior stromal tissue. This is due to the high amount of lactate formed in epithelial cells from anaerobic glycolysis, causing an osmotic strain and consequent swelling. The increase in total corneal swelling could be about 20% of tissue volume especially when the partial

pressure of O_2 falls below 54 mmHg. The recent use of rigid gas permeable lenses has largely eliminated this problem as these lenses allow the passage of O_2 more efficiently than even soft contact lenses. Actual O_2 consumption rate is only 2 µl/mg of corneal tissue / hour.

Diabetic cornea

Diabetes mellitus produces 3 pathological effects on the cornea:

1. Reduced epithelial adhesion to the corneal stroma.
2. Loss of corneal neural sensitivity.
3. Possible increase in corneal thickness principally in the stroma.

1. Reduced epithelial adhesion is due to the glycation of the protein laminin that forms the extra cellular matrix needed for spreading and attachment of epithelial cells. This impairs the ability of corneal epithelium to repair itself especially after corneal surgery, the collagen anchoring fibrils are defective leading on to tissue erosions.
2. Loss of corneal neural sensitivity is due to protein AGEs (Advanced Glycation End products) in the basal lamina of Schwann cells at the anterior cornea. It impedes the ability to sense corneal contact such that patient is unaware of bacterial infections and so undetected corneal ulceration can occur.
3. Increase in corneal thickness results in corneal swelling from the osmotic effect due to stimulated polyol pathway.

UVEAL TRACT

Uveal tract is the vascular, middle compartment of the eye consisting of three parts viz. Iris, ciliary body, anteriorly and choroid in the posterior uvea.

The major functions of uvea are:-

1. To regulate the pupil size for optimal vision through the muscles of the iris viz. sphincter iridis and dilator pupillae muscles.
2. To regulate the production and composition of aqueous humor.
3. To influence the ionic environment and metabolism of lens, cornea and trabecular meshwork.

Blood vessels of iris have tight junction and lack fenestrations rendering them relatively impermeable to large molecules, thus forming a second component of blood – aqueous barrier.

Stroma of the iris is composed of pigmented melanocytes and non-pigmented epithelial cells and matrix of collagen Type III fibrils with hyaluronic acid as GAG. Anterior border is avasucular and allows the passage of aqueous humor through the loose stroma.

Ciliary body bridges the anterior and posterior segments of the eye and forms the blood aqueous barrier. It forms the aqueous humor, maintains the intra-ocular pressure, and uveoscleral outflow of aqueous humor apart from its role in accommodation of lens through ciliary muscle.

Ciliary muscle forms the major part of ciliary body with 3 bundles of outer longitudinal, middle radial and inner circular fibres. The cells contain multiple myofibrils, with mitochondria and nucleus. A basal lamina surrounds the smooth muscle cells.

Contraction of ciliary muscle relaxes the zonules allowing the lens to adapt a more spherical shape owing to the elasticity of the lens capsule. This accommodation is mediated by parasympathetic stimulation of longitudinal fibres followed by the action of circular fibres. It produces greater ability to focus on near objects owing to the increased refractive (diopteric) power of the lens.

Biomedical importance

Myopia may be induced by intense near work for prolonged periods and in children sleeping in dimly lit rooms due to persistence of poorly focused images through thin eyelid skin.

Ciliary processes are inward projections of ciliary body; zonular fibres of lens are attached in between these ciliary processes. These processes consist of a central core of highly vascularised connective tissue stroma and specialized double layer of epithelium covering the stromal core. The connective tissue consists of fibroblasts and Type III collagen fibrils. The vessels are highly fenestrated leaking most of the plasma components into the stroma.

Double layer epithelium

The inner layer is non-pigmented epithelium (NPE) and is in direct contact with the aqueous humor.

The outer layer is pigmented (PE) and lies in between NPE and stroma. The basement membrane of PE is the Bruch's membrane. The PE cells are cuboidal and contain numerous melanosomes, but poor in other intracellular organelles. The internal limiting membrane of the ciliary body is the basement membrane of NPE (Fig. 13.9).

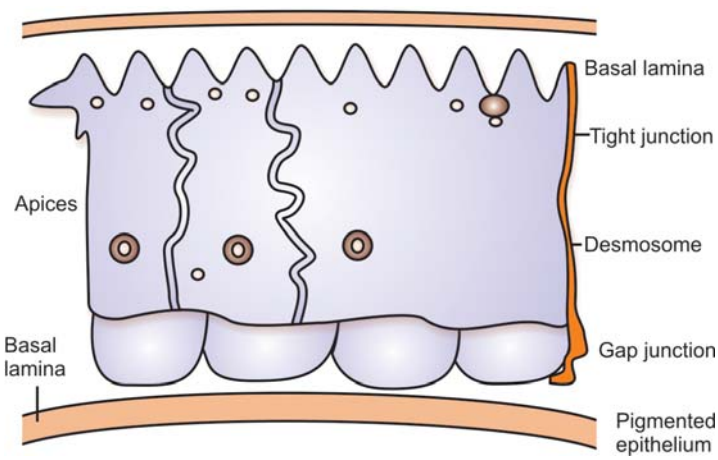


Fig 13.9: Posterior Chamber

The NPE cells are columnar and contain numerous mitochondria, smooth and rough endoplasmic reticulum. They are highly active forming aqueous humor. The cell membrane of NPE has numerous basal infolding s and multiple convoluted lateral interdigitations forming the ciliary channels. Various types of intercellular junctions join the PE and NPE cells like desmosomes, gap junctions, puncta adherentia, and tight junctions all useful for the fluid transport of aqueous humor. The ciliary epithelium is rich in antioxidant activity with high concentration of catalase, superoxide dismutase and glutathione peroxidase. This is to reduce H_2O_2 present in normal aqueous humor derived from the non-enzymatic interaction between reduced ascorbate and molecular O_2.

The iris ciliary body contain adrengeric, cholinergic, peptidergic, prostaglandins, and serotonin receptors. Cytochrome P450 is present in NPE of ciliary body to detoxify many compounds, first by hydroxylation and then conjugation with glucuronide or glutathione.

Blood Aqueous Barrier

The tight junctions between the NPE cells together with non-fenestrated iris vessels (tight junctions between the vascular endothelial cells) containing the protein occludin and cingulin contribute to the blood aqueous barrier. It does not allow large molecules like proteins to pass through. It maintains IOP by allowing the surrounding tissues to remove waste products of metabolism.
Breakdown of blood aqueous barrier can occur due to various causes like mechanical trauma, inflammation, paracentesis, vascular disease, administration of hyperosmotic agents, after cyclodestructive procedures for glaucoma, or after argon laser trabeculoplasty etc.

Mechanism and Effects

In these conditions tight junction is fragmented, leading to leakage of plasma proteins into the aqueous seen as "flare" and so IOP increases.

In addition, cell membrane disruption releases arachidonic acid from the phospholipids of the membrane. Arachidonic acid gives rise to prostaglandins through cycloxygenase pathway. Prostaglandins have irritative effects on the eye like miosis, vasodilatation, release of protein into aqueous and increased IOP.

Prevention of these prostaglandins effects may be achieved through pretreatment with inhibitors of PG synthesis such as aspirin, indomethacin, and other NSAIDs.

Mechanism of action of inhibitors: These NSAIDs bind irreversibly to cycloxygenase enzyme and inhibit the pathway synthesizing PG e.g., topical NSAIDs used in the treatment of anterior segment inflammation, aphakic and pseudophakic cystoid macular edema, allergic conjunctivitis (Ketorolac tromethamine 0.05%).

Pain after refractive surgery - Diclofenac 0.1% (voveran).

Preoperative use of flurbiprofen and suprofen drops (1%) - prevent PG mediated papillary miosis during ocular surgery.

Leukotrienes formed from arachidonic acid by lipoxygenase pathway is not inhibited by NSAIDs. Only nordihydroguaiaretic acid (NDGA) inhibits lipoxygenase.

AQUEOUS HUMOR

It is an ultra filtrate of blood produced by the ciliary body. It enters the posterior chamber from the ciliary processes through various mechanisms such as diffusion, ultra filtration and active transport and carbonic anhydrase II activity. The rate of formation is 2 µl/ minute. It exerts a hydrostatic pressure (IOP) of 10 -20 mmHg which maintains the shape of the ocular globe and protects it from physical shock to some extent. IOP is maintained by steady formation and drainage of aqueous.

Aqueous nourishes the cells of posterior cornea (corneal endothelium, stromal keratocytes, most of the corneal epithelial cells), lens and iris. (Fig. 13.10)

Aqueous humor is the source of antoxidants (ascorbate) for lens and corneal endothelium and removes their metabolic waste products. It does not contain RBC, still it carries released O_2 and nutrients to the cells, it serves. Due to the lack of cellular components, proteins and K^+ content are low in aqueous. This

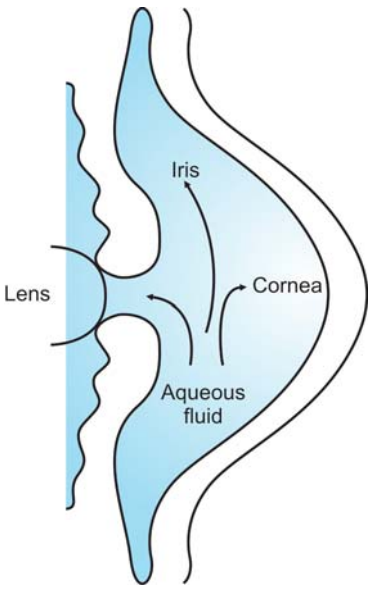


Fig 13.10

enhances the capacity of aqueous to transmit light but reduces the buffering capacity. Still, pH is maintained by HCO_3^- and PO_4^- retention in sufficient quantity.

Composition of Aqueous humor in comparison with plasma.

Components	*Aqueous humor*	*Plasma*	*Units*
Glucose	2.7-3.9 (47 mg%)	5.6- 6.4 (98mg%)	Mmol/litre
Lactate	4.5	0.5-0.8	Mmol/litre
Ascorbate	1.1 (19 mg %)	0.04 (1.3 mg %)	Mmol/litre
Albumin	5.5-6.5	3400	Mg/dl
Transferrin	1.3-1.7	-	Mg/dl
Fibronectin	0.25	29	Mg/dl
IgG	3	1270	Mg/dl
Phosphate	2.1	3.8	Mg/dl
Globulin	5	2900	Mg/dl
Na+ (sodium)	142	130 -145	Meq/litre
Potassium (K+)	4	3.5- 5	Meq/litre
$HCO3^-$ (bicarbonate)	20	24- 30	Meq/litre
Chloride	131	92 - 125	Meq/litre
Calcium	1.2 (0.01mg%)	2 -2.6 (4.8 mg %)	Meq/litre
Magnesium	1	0.7-1.1	Meq/litre
pH	7.5	7.4	

Constituents of aqueous humor:

Glucose: passes across the ciliary epithelium by facilitated diffusion through transporters, not dependent on insulin. In diabetes mellitus, concentration of glucose increases.

Concentration of inositol, important for phospholipids synthesis is 10 times higher that of plasma.

Lacatate concentration is higher than that of plasma due to the increased anaerobic glycolysis in intraocular tissues.

Glutathione concentration is high for the antioxidant activity.

Urea enters by passive diffusion.

Enzymes: Hyaluronidase, carbonic anhydrase and lysozyme.

Growth modulatory factors such as fibroblast growth factor, ß transforming growth factor, insulin like growth factor (IGF _1), insulin like growth factor binding proteins, vascular endothelial growth factor.

Formation of Aqueous humor: from the ciliary processes.

1. Diffusion: Movement of ions like Na+ across the membrane towards the side with most negative potential and down a concentration gradient.
2. Ultrafiltration: is the non-enzymatic component of aqueous formation that is dependent on IOP, BP and blood osmotic pressure in ciliary body. It is the "bulk flow" of material across epithelium and increases by augmenting its hydrostatic driving force.

3. Carbonic anhydrase II activity (CA II): CA II is present in the pigmented and non-pigmented epithelium. The inhibitors cause a reduction in the rate of entry of Na^+ and HCO_3^- in the posterior aqueous leading to reduction in the aqueous flow.
4. Active transport: This requires cellular energy (ATP) to secrete solute against a concentration gradient. This is the major mechanism of aqueous humor formation by the inner non-pigmented epithelium involving membrane associated Na^+/K^+ ATPase found in highest concentration along the lateral cellular interdigitations. A surplus of Na+ is pumped into the posterior aqueous chamber by Na^+/K^+ ATPase causing water flow into the chamber osmotically (Fig 13.11). The enzyme thus generates an IOP indirectly. HCO_3^- also contributes to this mechanism. Thus, aqueous humor is formed by the transport of water and electrolytes from the leaky fenestrated capillaries of the ciliary process to the epithelial syncytium and hence, across the plasma membrane of the non-pigmented epithelium as shown in figures 13.12 and 13.13.

Regulations of aqueous flow

1. Adrenergic receptors present in the ciliary epithelium regulate the IOP through adenylate cyclase system.
2. Cholinergic receptors are linked to phosphotadyl inositol second messenger system.

Adrenergic receptors (α and ß receptors stimulation):

Stimulation of α 2 receptors - inhibition of adenylate cyclase through G protein - decreased production of aqueous --- reduced IOP.

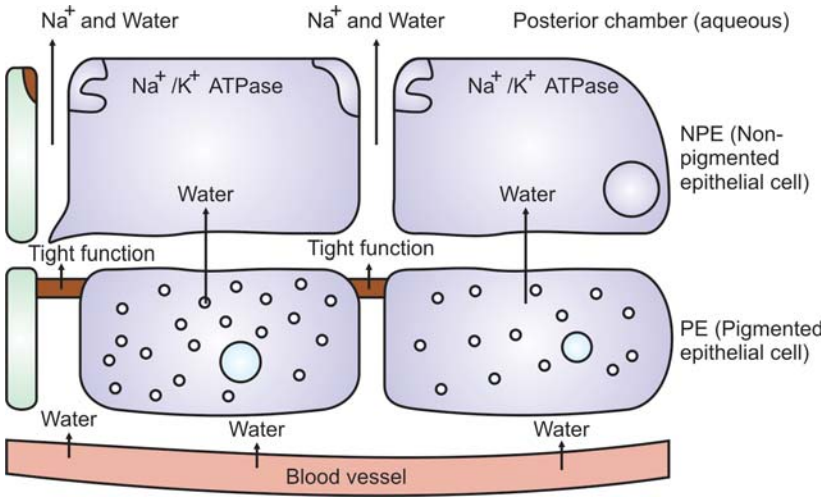


Fig 13.11

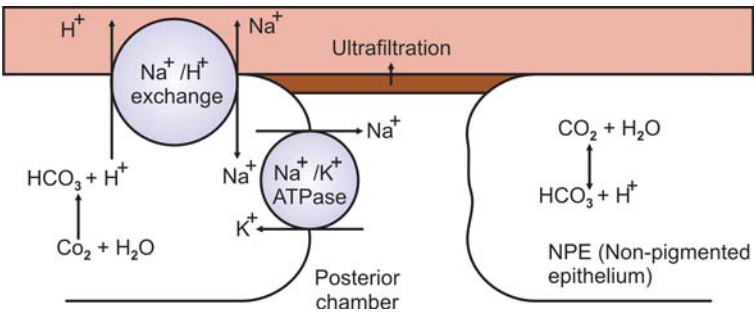


Fig 13.12

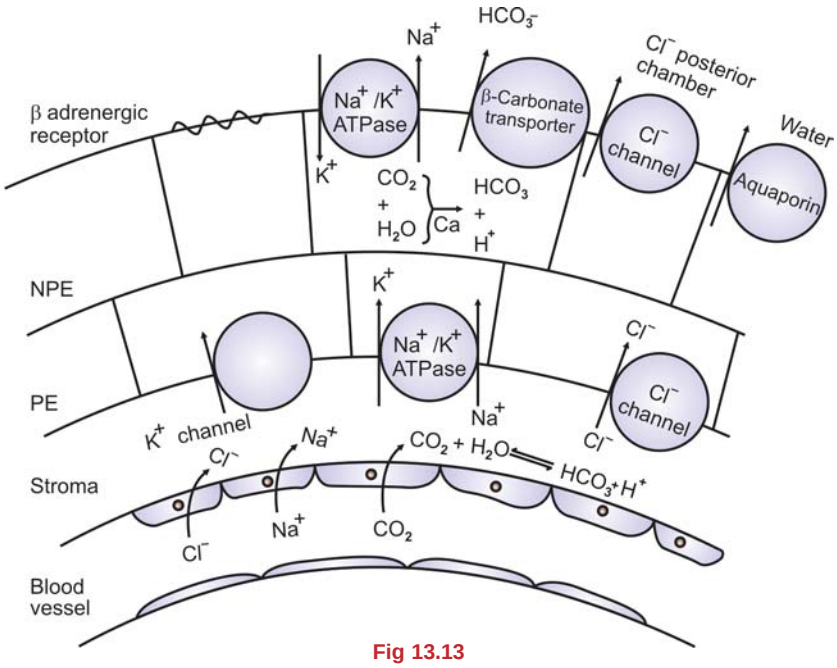


Fig 13.13

Epinephrine, an α adrenergic agonist, stimulates prostaglandin synthesis, PGE2 and PGF2 which decrease IOP.

Stimulation of β receptors - activation of adenylate cyclase through G protein - increase the production of aqueous and secretion.

Outflow of aqueous from the eye is regulated at different levels like trabecular meshwork, uveoscleral system and episcleral vessels.

Trabecular Meshwork

a) Trabecular meshwork - The juxtacanalicular cribriform meshwork contributes significant resistance to the outflow. The trabecular meshwork is formed by Type I, III, IV collagen and other matrix proteins such as

laminin, fibronectin and elastin separated by GAG filled spaces, particularly hyaluronic acid that retards the flow of fluid by its hydrophilic properties and large hydrodynamic volume. Varying amounts of chondroitin sulfate, heparan sulfate, dermatan sulfate and keratin sulfate with some unidentified proteoglycans and trace amounts of type V and VII collagen are also found.

b) The endothelial cells of trabecular meshwork are specialized for endocytic transport of water and solutes and also for contractiliy. This is possible due to the presence of cytoskeletal actin microtubules, vimentin and desmin in these cells, similar to smooth muscle cells. They derive energy from anaerobic glycolysis. Actin mobilsation is mediated by β2 adrenergic receptors, highly responsive to epinephrine. This stimulates adenylate cyclase through G protein increasing the formation and secretion of aqueous.
c) They also synthesise and degrade the trabecular meshwork matrix components like GAG that retard the outflow.
d) They have high level of TPA (tissue plasminogen activator) to maintain potency of outflow passages and to reduce the resistance to the outflow.
e) Trabecular meshwork cells also have significant amount of PG and leukotriene synthesis which reduce the production and secretion of aqueous humor and hence decrease IOP.
f) The cells have free radical scavenging enzymes like catalase and glutathione peroxidase.

Uveoscleral Drainage

3 - 20 % of the aqueous drains into the anterior uvea at the ciliary body, immediately posterior to cornea and then into the suprachoroidal space.

Uveoscleral drainage is possible owing to the lower pressure in the suprachoroid by 2 to 4 mm Hg than the anterior chamber. But, this can be reversed after trabeculectomy and can lead to choroidal effusions. The risk of choroidal effusion is greater with advancing age in such patients as the pressure difference lessens with age.

Prostaglandins may increase the uveoscleral outflow and thus reduce the IOP.

Episcleral Circulation

When the IOP is < 15mmHg, aqueous fluid will not drain into the episcleral veins (normally aqueous humor passes through large transcellular channels and giant vacuoles on the meshwork side of the canal of Schlem into aqueous veins and then through the communicating vessels on the outer wall of canal into scleral veins).

CHOROID

Choroid is rich in immune cells viz. mast cells, macrophages and dendritic cells responding massively to intraocular inflammation. It acts as the lympho-

vascular supply to posterior segment of the eye. The choroid is almost entirely composed of vessels embedded in a loose connective tissue matrix with a high content of type III collagen necessary for an expansile or spongy tissue. Blood vessels are highly fenestrated and leaky like ciliary vessels. 85 % of total ocular blood flow passes through the choroid. The vessels are sensitive to changes in partial pressure of O_2 and CO_2 and also to vasoconstrictors.

The Normal Constituents of the Lens

Water	66% of wet weight.
Protein	33% of wet weight.
Sodium	17 meq/kg lens water.
Potassium	125 meq/kg lens water.
Chloride	30 meq/kg lens water.
Calcium	0.4 meq/kg lens water.
Glucose	1mM
Lactic acid	14 mM
Glutathione	12 mM (decrease with age).
Ascorbic acid	1.6mM (functions as a link in H_2 carrier system).
Inoistol	5.9mM (inhibits the effect of UV radiation and it acts as shock absorber).
Lipids	28mg/ wet weight (cholesterol,phospholipids, glycosphingolipid).

An increase in Ca^{2+} is the hallmark of degenerative changes in the lens such as normal aging, sclerosis and cataract formation. The nucleus of the lens contains most of the calcium. It is involved in the maintenance of normal cell membrane permeability.

LENS

Next to cornea, the second refracting unit of the eye is the lens. The transparency of the lens is a function of the highly ordered state of its cells and extracellular matrix. It transmits long wavelength light but filters the majority of short wave length light< 360 nm and is an absolute barrier to light below 300 nm.

The extracellular matrix of lens is the capsule. It is a typical basement membrane surrounding the lens. The epithelial cells form a syncytium with interlocking cellular processes.

Anterior capsule is formed by the underlying epithelium and contains fibronectin.

Posterior capsule is formed by the cortical fibres and has tenacin. The capsule is non-cellular and composed of glycoprotein associated Type IV collagen. The GAG, heparan sulfate comprises < 1 % of the lens capsule and is important in maintaining the clarity of the capsule. The dense fibrillar outer layer of capsule is the zonular lamella and zonules running from ciliary processes fuse into the outer layer.

Function

- Capsule is the source of anti-angiogenesis factors.
- It is a barrier to vitreous, bacteria, chemicals and growth factors. Thus, it helps in the prevention of postsurgical secondary open angle glaucoma, reduction in the incidence of endophthalmitis following cataract surgery, if remained intact, reduction in the incidence of anterior segment neovasularisation.
- It is impermeable to small molecular weight proteins < 50,000 KDa including low molecular weight crystallins.

Epithelium

It is a single cell layer of cuboidal epithelium. It does not scatter or reflect light. It is a highly active, mitotic layer with a ring of germinative zone around the anterior lens. Anterior lens epithelium contains $\alpha 6\beta 1$ integrin and $\alpha 5\beta 1$ integrin receptor for laminin is present in the equatorial and lens fibre cells. Both are migratory. The newly formed epithelial cells migrate equatorially to form the lens fibre cells. As the epithelial cells progress to the 'bow region', they change in morphology and synthetic activity. There is increase in cell size, increase in mass of cellular proteins and in membrane of each cell. It elongates at both ends of the cell anteroposteriorly with decrease in and or disappearance of other cellular organelles. This is terminal differentiation into lens fibre cells.

The new fibre cell has its centre at the equator and the ends of each fibre meet the ends of other fibre at the anterior and posterior regions of lens giving rise to a pattern called "suture line". The new cell is layered over the old one. The oldest fibre lies in the centre of the lens as "nucleus" surrounding which are cortical fibres that are formed recently. The epithelium maintains the fluid and electrolyte balance of the lens syncytium via ion pump mechanisms. Fig 13.14.

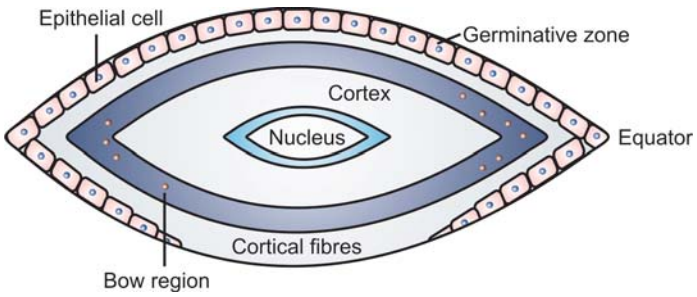


Fig 13.14

Transport Function in Lens: Controlled by Membrane Proteins

A specific internal ionic, osmotic environment is important is normal lens metabolism and this is maintained by the cellular communication between epithelium and fibres. The epithelial layer shows typical polarization potentials, but lack tight junctions at the lateral surface. But, they have gap junctions that permit rapid intercellular communications. They restrict the passage of high molecular weight solutes between the cells.

Na^+/K^+ ATPase pump in the epithelium actively exchanges Na^+ for K^+ to maintain the internal Na^+ at 20 meq and K^+ at 120 meq (where as in aqueous it is 150 and 5 meq respectively), from the back of the lens, Na^+ passively diffuses down a concentration gradient in the vitreous, across the posterior lens capsule into the lens body and rapidly diffuses to anterior epithelium and pumped out into the aqueous. K^+ moves passively in the reverse direction across the posterior capsule into the vitreous.

Lens Fibre Cell

Gap junctions between lens fibre cells allow rapid movement of metabolite by forming channels between the cells e.g., MIP26 (main intrinsic polypeptide of 26,000 KDa) which is also called Aquaporin for transport of water out of the lens to maintain transparency. There is abundance of Na^+/K^+ATPase in the lens fibre around the lens sutures, Ca++/Mg ++ ATPase in the lens cortex, specific transporter proteins for glucose, and amino acids in the plasma membrane of lens epithelium and fibre cells. Lens fibre cells are organized in a densely packed cellular arrangement with interdigitations, having the gap junction protein for intercellular communication.

During development of lens fibre cells, they become anucleate and specialized for the production of specific lens protein, the crystallins.

The plasma membrane of lens fibre is very stable and rigid due to the high content of saturated fatty acids, cholesterol and phospholilpid with high amount of sphingomyelin contributing to the tight packing and low fluidity of membrane. The structural frame work of lens cells formed by the cytoskeleton is a complex system of intracellular filaments, as detailed in the table below.

Cytoskeletal component	*Cytoskeletal protein*	*Importance*
Microfilaments	Actin	Maintenance of lens shape during accommodation.
Intermediate filaments	Vimentin	Found in epithelium and outer cortex.
Microtubules	Tubulin	Found in epithelium and outer cortex. involved in maintenance of lens shape and developmental events.
Beaded chain filaments	Back bone protein	Unique to lens. Found in cortex and nucleus. Associated with integrity of cell membrane.
Stress fibres	Myosin, α Actin, tropomyosin	Involved in wound repair in lens.
Membrane skeleton	spectrin	

The concentration of lens protein is 35% of its wet weight. The majority of the proteins are in lens fibres making up the bulk of the lens. These fibre proteins are of two types - water soluble and water insoluble. Crystallins are the water soluble proteins and membrane proteins are the water insoluble protein e.g., MIP specific to fibre cell and not epithelial cell. MIP first appears in the lens just as the fibres begin to elongate and concentrated in the gap junctions. It makes ~ 50% of the membrane protein and hence known as Main Intrinsic Polypeptide.

CRYSTALLINS

Crystallins are the lens proteins with structural role. They are responsible for the light refraction and light transmission as the orderly arrangement of the molecules make the protein transparent. They also serve to maintain the elongated shape of the lens. By their relative ability to migrate in an electric field, they are broadly classified into alpha (fast moving), beta and gamma crystallins (slow moving)and alpha is further divided into αA (acidic)and αB (basic) and ß into ßH (heavy) and ßL (light) and gamma has 6 subtypes gamma A to gamma F.

The primary structure of crystallin (i.e., the sequence of amino acids) has the N - terminal aminoacid acetylated for prevention of cellular degradation of protein. The sequence of amino acids varies in each type. The number of cysteine is 16, 25 and 41 per 1000 residues in alpha, beta and gamma respectively.

The secondary structure assumes a beta pleated sheet conformation (arrangement of the polypeptide folding itself into antiparallel design associated by hydrogen bonds between the amino acids) for all 3 types. Alpha type has alpha helical structure (winding of the primary structure onto itself stabilized by hydrogen bonds) also.

The tertiary structure assumes a globular shape with hydrophilic surface and hydrophobic interior stabilized by hydrophobic interactions.

Quaternary structure (association of polypeptide when present in more than one number among themselves with hydrogen bonds, hydrophobic interactions,Vanderwalls forces) of alpha and beta having 40 and 6 subunits respectively are called aggregates and gamma being monomer does not have quaternary structure.

Molecular weight of alpha crystalline is 750,000 dalton.

Beta crystallin is 50,000 (light) and 160,000 (heavy) dalton.

Gamma crystallin is 20,000 dalton.

The isoelectric pH (the point or pH at which precipitation of protein occurs) is ~4.9 - alpha, ~ 6.4 - beta, ~7.6 - gamma.

The lens protein crystalline totally contains 35% alpha, 55% beta and 10% gamma; gamma comprises 90 % of soluble protein of the lens. All the 3 crystallins occur in lens fibre cells, but alpha crystallin alone is found in lens epithelium. These crystallins undergo no replacement throughout life. There is no turnover of these proteins as the lens fibre cells have lost their ability to synthesize new protein.

Alpha crystallin function as molecular chaperones i.e., they help to maintain the normal molecular conformation of other lens crystallins by binding to them and prevent the crystallins aggregation that may lead to cataract formation. Light scattering is a phenomenon associated with beta crystallin aggregation and this is inhibited by alpha crystallin.

Molecular Biology of Crystallins

Crystallins are related to heat shock proteins (heat shock proteins are those made in response to an unusual temperature rise in organism to protect the organism by solubilising and refolding any heat denatuated proteins).
The genetic patterns of crystallins are given below:

Crystallin	*No. of genes*	*Chromosomal location of genes*	*No of exons per gene (sequence of gene is expressed as mRNA)*
α	2	αA - 21 αB- 11	3
β	6	βA1 βA3 17 βA2- 2 βB1 ┐ βB2 ├ 22 βA4 ┘	6
gamma	7	gamma A to gamma F-2 gamma S-3	3

Transcription of gene is initiated by binding of certain specific nuclear protein (alpha ACRYBP1) to the promoter region in the DNA. Another transcription factor PAX6, helps to control the hn RNA and hence, the mRNA formation. DNA damage by UV radiation may produce cataract of the lens.

Genetic Defects of Crystallin

Any chemical or physical process that produces a defective gene and causes it to be transmitted or causes any normal gene to be over / under transcribed will result in some pathological process or disease. So, there will be production of either abnormal protein or normal protein in abnormal amounts resulting in congenital cataract e.g., given in table.

Type of congenital cataract	*Location of chromosomal mutation*	*Type of mutation*	*Protein involved*
Anterior polar cataract	17p 12-13		Beta-crystallin
Zonular pulverulant	2q 33-35	Abnormal 5 base insertion	Gamma crystallin
Aniridia	Pax 6 gene 11p13	Deletion of a segment	PAX6 LDH- A
Microphtahlmia	Pax 6gene 11p13	Deletion of a segment	PAX6 LDH-A

Aging Process and Effects on Crystallin

Crystallins undergo several biochemical transformations as age advances, though there is no protein turnover. The cells loose cytoskeletal organization and develop vacuolation and electron dense bodies. The transmission of light decreases with age. The biochemical processes as age advances are as follows:

Phosphorylation, disulfide bond formation, deamidation, peptide bond disruption, and glycation.

The amount of both soluble and insoluble proteins change with age.

1. **Phosphorylation:** is the process of addition of phosphate groups, most often to serine amino acids. In mature lens fibre cells, phosphorylation of alpha A chain of alpha crystallin is maximum while that of alphaB chain is minimum. Thus, the total amount of phosphorylated chains of alpha crystallin increases as lens fibre cells age. This addition of phosphate amplifies the negative charges and potential energy of a protein. The cascade systems that are responsible for the phosphorylation are cAMP dependent protein kinase and a phospholipids dependent protein kinase C.
2. **Disulfide bond formation:** (cross linking) between different polypeptide is an oxidative process and increases with ageing. The cysteine content of each crystallin is important as it is the potential source of molecular bridging or aggregation via disulfide bonding. Gamma crystallin with more cysteine (41/1000) is the most likely candidate for cross linking.
3. **Deamidation:** is the loss of an amide group from aspargine and glutamine by oxidation to convert them to aspartate and glutamate (dicarboxylicamino acid) and this is part of aging process.
4. **Peptide bond disruption:** seen in beta and gamma crystallins with ageing due to the catalytic accessibility of an exopeptidase over time. Majority of peptide bond breakage occurs at N- terminus of beta and C-terminus of gamma crystallin.
5. **Glycation:** Non-enzymatic glycation of crystallins occurs at the epsilon amino group of lysine, aminoacid. This is likely to be due to interaction of crystallins with oxidized ascorbic acid rather than glucose depending on the intralens concentration. Glutathione inhibits this process by maintaining the ascorbic acid in the reduced state.

In the normal aging process all the 3 types of crystallins get incorporated into the insoluble fraction by disulfide bonding and cross linking so that the concentration of insoluble protein is larger with concomitant reduction in soluble protein after the age of 50.

Of the insoluble protein, only 1 % are normally insoluble proteins of the cell plasma membrane. With advancing age, this increases to 50% or more of all the lens proteins in an eighty year old person and form high molecular weight aggregates (HM) HM1, HM2, HM3, HM4.

- HM1 and HM2 are actually soluble crystallin complexes.
- HM3 and HM4 are found in cataractous lens.

- HM3 associated woth cortical cataracts and held by disulfide bonds.
- HM4 occurs exclusively in nuclear cataracts and composed of crosslinks that are not disulfide bonds.

These fractions cause light scatter and light absorption so that the images are blurred and glaring. MIP26 also undergoes modification with age, loosing a 5000 Da peptide to become MIP22. This leads to appearance of water clefts in lens disrupting fluid transport, ion channels and membrane potentials. Senile cataracts occur due to the growing HM fractions that form at the inner layer of lens cell plasma membrane and grow into the cell cytoplasm. The protein complexes continue to grow as the lens ages, by forming either disulfide bond or other covalent bonds. This is the stage to which nuclear cataract form but with non-disulfide bonds. An important difference with cortical cataract is that the strain produced on the cell membrane by the growing HM aggregates may lead to membrane rupture. The resultant cellular debris with membrane fragments get attached to the HM aggregates to form the cortical cataract as shown in figure (Figs 13.15A and B).

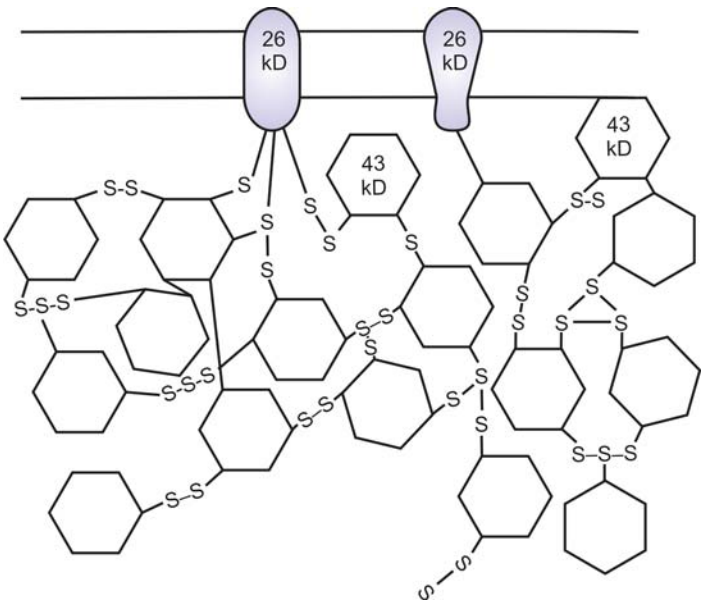


Fig 13.15A: Nuclear (similar) catract

Cause for Cataract Formation

Cortical Cataract

In the non-cataractous state, the sulfur containing amino acids are buried in normal conformation of crystallins and are prevented from getting oxidized to sulfoxide and disulfide bonds. Minimal oxidation of exposed groups may cause

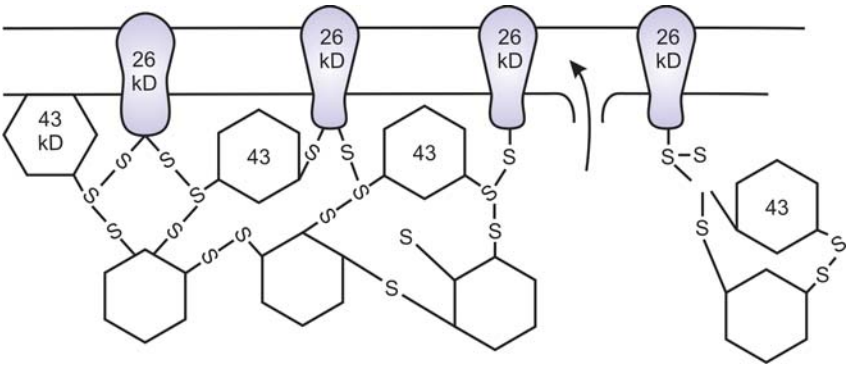


Fig 13.15B: Cotical cataract

conformational changes in the other oxidizable groups resulting in oxidation and aggregate formation.

H_2O_2 in the aqueous may induce progressive oxidation. Glutathione protects crystallins from cross linking by binding to such exposed groups. Function of glutathione may be overwhelmed in cataract formation.

Nuclear cataract

Radiation in the range of 300- 400 nm (including sunlight) has the potential of being cataractogenic since the lens absorbs these wavelengths.

The UV light oxidises tryptophan in the crystallin protein to N - formyl kynureniune (responsible for the dark colour of cataract), 3 hydroxy kynurenine, anthranilate, beta- carboline etc., leading to non-sulfide cross linking of HM4 aggregates. 3 hydroxykymurenine can form reactive intermediates that covalently bind to the lens protein.

Other inherited disorders resulting in impairment of lens function are as follows:

Autosomal Dominant disorders

1. Marfans - Dislocation of lens (upwards) due to defect in collagen cross linking.
2. Ehler's Danlos syndrome- Ectopia lentis due to defective Type II collagen from lysyl hydroxylase gene mutation.
3. Myotonic dystrophy - Cataract due to mutation in chromosome 19.

Autosomal recessive:

1. Homocystinuria - dislocation of lens due to the enzyme cystathionine synthase deficiency. Sulfite deficiency - ectopia lentis.
2. Hyperlysinemia - deficiency of the enzyme lysine dehydrogenase resulting in microspherophakia.
3. Alport's syndrome - anterior lenticonus, cataract due to mutation in COL4A5 gene.

Lens Metabolism

Carbohydrates

Energy demands in the lens are lower compared to cornea as there is slower production of lens fibre cells after birth. Glucose is the source of energy and it is derived from aqueous humor. Glucose enters the cells by facilitated transport independent of insulin. Due to the low oxygen tension in the lens; only about 3% of lens glucose passes through TCA cycle and also there is loss of sub-cellular organelles necessary for TCA cycle in the lens fibre cells. So, in the lens, the more active pathway is anaerobic glycolysis. This can maintain the ion pump and the amino acid pump activators of the lens and also the mitotic activity of the epithelial cells. Actual O_2 consumption rate is only 0.5 µl/ mg of lens tissue / hour. In the lens epithelial cells, 81% of glucose is metabolized through anaerobic glycolysis and only 4 % through aerobic glycolysis, 15% glucose enters pentose phosphate pathway to produce NADPH needed for detoxification and to nullify the oxidative stress created by the H_2O_2 present in aqueous.

In lens fibre cells, 83 % of glucose enters anaerobic glycolysis, 2 % aerobic and 15 % HMP shunt. So, the products of ATP in lens epithelial cells are 228 ATP totally from 81 x 2 =162 anaerobically and 2 x 36= 72 aerobic glycolysis. Normally < 5 % of glucose enters polyol pathway. The polyol pathway is induced only when there is excess glucose as in diabetes.

Diabetic Lens: Cataract

As the lens cells are not dependent on insulin for glucose uptake, toxic levels of glucose enter lens fibre cells in diabetes mellitus and activate aldose reductase which converts glucose to sorbitol. Due to the high Km of polyol dehydrogenase to sorbitol, and also due to the poor permeability of lens to sorbitol, there is retention of sorbitol in the lens. The accumulated sorbitol generates high intracellular osmotic pressure that is sufficient to burst the lens cells. The cellular debris generated by this damage becomes the manifestation of cataract. Apart from this, protein denaturing effects of glycation changing the 3 dimensional structure of protein and oxidative stress also lead to cataract formation. When the blood glucose level increases, lysine residues of the protein are glycated. This leads to increased susceptibility for sulfhydryl oxidation and consequent aggregation of protein resulting in opalescence and cataract. Protein aggregates with molecular weight > 50 million will produce light scatter.

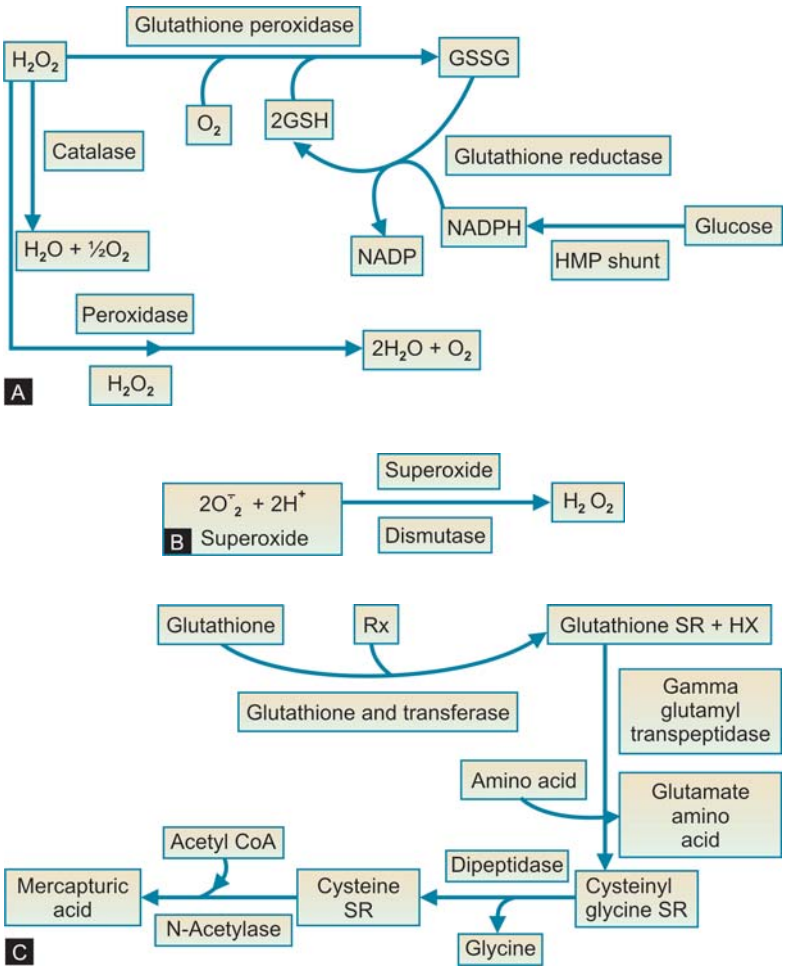
Protein metabolism: Synthesis of new protein causes with lens fibre cell formation and all changes that occur to lens protein, after this stage are post-translational modification only. Phosphorylation of many proteins occur including crystallins, cytoskeletal proteins and MIP26.

Lipid Metabolism

Cholesterol is present in the lens fibre membrane and it imparts rigidity to the lens membrane along with sphingomyelin. It makes up 50 - 60 % of lipids and its concentration increases with age.

Redox system in the lens microenvironment:

Lens is constantly exposed to attack by oxidative agents. There is high level of H_2O_2 in normal aqueous and peroxidase activity is also present in lens. The other enzymes to neutralize the effect of oxidants in the lens epithelium are catalase, superoxide dismutase, glutathione peroxidase and glutathione S transferase. The concentration of glutathione in lens is high with highest concentration in lens epithelium for detoxification via mercapturic pathway and for the protection of thiol groups in protein especially cation - transport membrane protein (Figs 13.16A, B, C).



Figs 13.16A,B,C

VITREOUS

The vitreous body is a specialized connective tissue containing collagen and glycosaminoglycans and cells. It is 98 % water, 1% macromolecules and the rest solutes and low molecular weight materials. Thus, it's a mixture of fluid and gel, the ratio of which varies with age. It begins as 80 % gel and 20% fluid and gradually changes to 40% gel and 60% fluid. Gel portion is stiff but a semirigid precipitate retaining the liquid.

The peculiar characteristics of vitreous are due to the presence of Type II collagen and other collagens, as well as proteoglycans. Collagen imparts the gel structure and hyaluronic acid contributes to the viscosity of vitreous. The gel like nature of vitreous gives it rheological (deformation) properties that it is able to cushion the eye from shock by absorbing exterior forces placed on it. This is known as viscoelasticity and it prevents mechanical shock being transmitted to the retina while at the same time exert continuous intraocular pressure to the retina preventing detachment of its delicate structure of functional neurons. Vitreous gel also has resilience (the ability to reform its original shape after deformation) and some flow (movement without stored energy)

Components of vitreous gel:

Matrix or the gel has interacting GAG and collagen. The collagen of vitreous is termed vitrosin and it consists of type II and IX. The proteoglycans are versican and agrin. The GAGs are chondroitin sulfate and predominantly hyaluronic acid and some proteins, opticin.

Type II collagen is the principal, structural collagen and accounts for 75% of the total vitreous collagen. It has more of galactosylglucose side chain and higher content of alanine. Its presence is essential for gel formation. Combined Type V, XI collagen represent ~ 10 % of total collagen.

Type IX collagen is a non-fibrillar collagen with 2 domains of importance. Collagenous and non-collagenous. Chondroitin sulfate is attached to the alpha2 chain of noncollagenous domain (NC3) (Type IX collagen is classified also as a proteoglycan due to the attachment of the GAG). The collagenous domain COL2 has sites for cross linking to Type II collagen (Fig 13.17).

Thus, type IX collagen serves as a linking molecule between Type II collagen and GAG chondroitin sulfate. Some completely, non-collagenous proteins are also present e.g., opticin (Fig. 13.18)

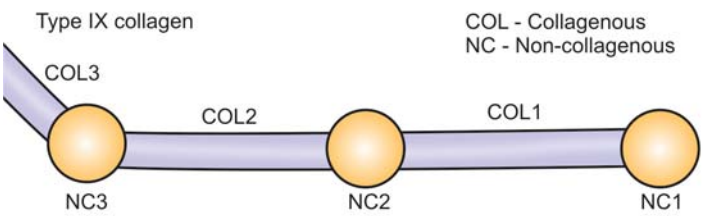


Fig 13.17

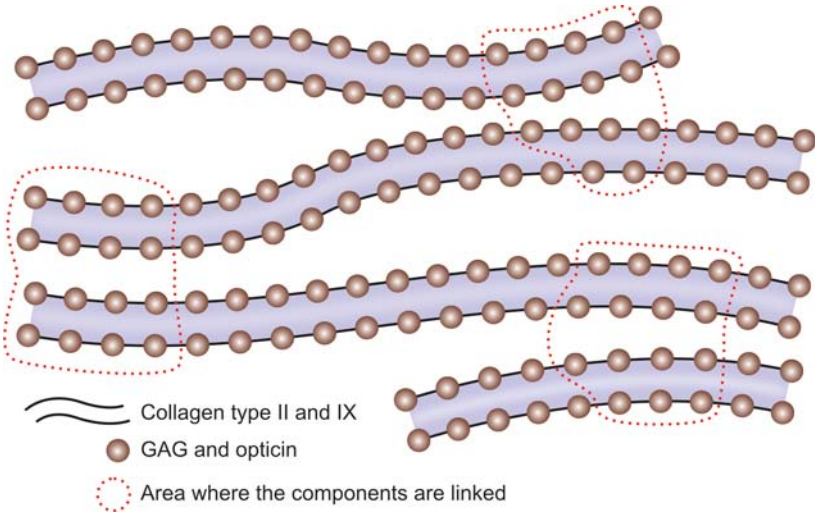


Fig 13.18

Opticin is an extracellular matrix protein with leucine rich repeats (ECM, LRR protein) that binds non-covalently to collagen and prevents the fusion or aggregation of collagen fibrils.

Versicon

It is the principal proteoglycan found in the vitreous. The protein component of this versicon consists of 3 functional domains:

1. A C-terminal domain for binding to non GAG sugars.
2. A central domain where GAGs - chondroitin sulfate bind.
3. A N-terminal domain that binds to hyaluron.

Hyaluron

It is hyaluronate, the negatively charged version of hyaluronic acid, not linked to a core protein in vitreous. It may form a twofold helix in solution and stabilized by non-collagenous protein there. It is a GAG with repeating unit of glucuronic acid and N - acetyl glucosamine linked by beta 1, 3 glycosidic bond. The repeating units are linked by beta 1, 4 glycosidic bonds. At physiological pH, it occurs as sodium hyaluronate, which is spheroidal and can hold an extremely large volume of water relative to its weight. This hyaluronin binds to versicon. It increases the mechanical stability of vitreous. The concentration varies between 100 - 400 µg/ml combination of these molecules represents the vitreous.

Vitreous Cells

Vitreous contains a monolayer of cells called hyalocytes that line the adult vitreous cortex. They are responsible for production of hyaluronic acid in the gel. These cells are two types 1) Fibrocyte like 2) Macrophage like.

Vitreous transmits light by the same mechanism as cornea i.e., its collagen fibrils (10 - 20 nm) are thinner than half the wavelength of light and the interfibrilllar space is filled sith GAGs (hyaluronic acid) at intervals that reduce the effect of diffraction in the system.

Vitreous retards the bulkflow of fluid and diffusion of small molecules. due to the presence of hyaluronic acid. Thus, flow of aqueous from posterior chamber towards the retina is slower in young eyes.

Comparison of some component of blood with vitreous

Component	*Concentration in blood*	*Concentration in vitreous*
Ascorbate	1.3 mg%	7.6 mg%
HCO_3^-	27 mmol/L	25 mmol/L
GAG (hyaluronin)	None	25 mg%
Protein	7.3 mg %	55 mg% (mainly as collagen)
Na+	150 mmol/l	137 mmol/ L
K+	105 mmol/l (in RBC)	3.8 mmol/ L
Glucose	98 mg%	50 mg%

This table reveals that ascorbate is more in vitreous probably due to the high level present in aqueous fluid which dissipates into the vitreous.

Molecular Effects of Aging and Liquefaction

As age advances, a process called syneresis occurs. In this process, hyaluronic acid molecules are degraded to smaller moieties and the collagen fibril conglomerate to form larger fibrils, becoming visible as floaters.

Liquefaction is already at 20 % around the age of 18 and progress to greater than 50 % by 80th age. As liquefaction increases GAGs become equally divided between the gel and liquid portion of the vitreous while collagen is only associated with gel portion. With aging, vitreous collagen fibres collapse or contract resulting in decreasing volume of gel and pocket of fluid form in the vitreous. These small, central pockets of water then coalesce and may cause posterior vitreal detachment (PVD) Fig. 13.19. In time, this could bring about a retinal detachment also. PVD occurs in 25 % population. Thus, age related

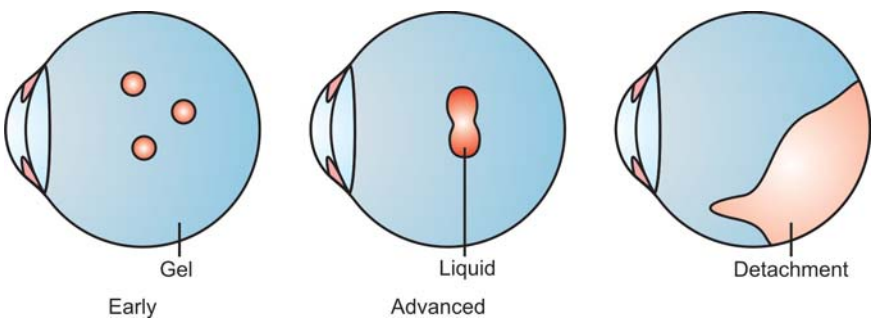


Fig 13.19

liquefaction of the vitreous is a result of the breakdown of spacing mechanism in vitreal collagen. Such a breakdown allows the formation of non-collagenous volume within the vitreous ie liquid areas. Apart from this, there may also be genetic abnormalities in opticin protein resulting in liquefaction. Opticin is the protein that prevents aggregation of collagen fibrils. 4 different amino acid sequence variations of this protein has been detected in certain cases of vitreous liquefaction.

Causes for decreased collagen and hyaluronic acid resulting in liquefaction;

1) syneresis -age related.
2) Myopia - Axial length of globe increases and so relative decrease in collagen and hyaluronic acid.
3) Aphakia - Intracapsular cataract extraction (ICCE) causes decrease in hyaluronic acid.
4) Diabetes mellitus - decreased collagen and increased hexosamine.
5) Injury with hemorrhage and inflammation- the catabolic products of RBC - hemosiderin and iron causes depolymeristion of hylauronic acid.

When the collagen and hyaluronic acid content of vitreous decrease, water collects in the space leading to liquefaction and PVD.

RETINA

Retina is a thin, transparent structure consisting of outer pigmented epithelial layer and an inner neurosensory retina. The inner neurosensory retina consists of 6 neuronal cell types viz. photorecepthors, bipolar cells, horizontal cells, amacrine cells, interplexiform cells and ganglion cells.

Photoreceptors are the light detecting cells in the outer nuclear layer (ONL) and contain the specialized protein rhodopsin and also transducin of the retina. The important lipids of the retina are glycolipids (gangliosides), phospholipids - phosphatidyl choline and ethanolamine mostly with serine and inoistol to some extent. Sphingomyelin is only 4 %. The predominant fatty acid is cervonic acid having 6 double bonds to maintain the fluidity of membrane and to facilitate the phototransduction.

Clinical Aspects

Absence of this essential fatty acid. Otherwise called DHA or docosahexaenoic acid (22 carbon atoms with 6 double bonds) reduces the amplitude of a and b waves of ERG, reduces visual acuity, prolongs the recovery time of the dark adapted ERG, measured after a saturating flash of light and raises the rod thresholds. This can be seen in preterm infants fed with standard formulas containing no DHA, instead of mother's milk containing DHA. The absence of DHA does not affect the cone function much, as the cone rich macular region contains less DHA than the rod rich peripheral region.

DHA is an important component of retinal membrane and it is presumed to function as a spring, helping to expand the membrane to facilitate the opening of the active state of rhodopsin. The polyunsaturated fatty acids of the retina are vulnerable to destruction by oxidative processes in the retina. But there is sufficient Vit E in the retina to prevent such destruction by absorbing the free radicals. Lipid soluble Vit A has an important dynamic role in visual excitation and perception described as Vit A cycle or wald's visual cycle.

Tay-Sachs Disease

Glycolipids storage disease due to deficiency of the enzyme hexosaminidase A that normally catalyze the breakdown of gangliosides as new ones are synthesized. So, accumulation of GM2 in retina, degeneration of ganglion cells and formation of cherry - red spot in macular region and blindness in early age. Gangliosides are glycolipids predominantly located in the membranes and have negatively charged sugar, sialic acid. Tay-Sachs ganglioside, GM2 is partially degraded ganglioside. The complete in situ, ganglioside, known as GM1 has a galactose attached to N- acetyl galactosamine which is detached by beta- galactosidase in neural and ocular cell lysosomes. In Tay-Sachs disease N- acetyl galactosamine (which would be normally be hydrolysed next) is not removed due to deficiency of the enzyme Hexosaminidase A.

Sandhoff disease (GM2 gangliosidosis type II)

Due to Hexosaminidase A and B deficiency is also a lipid storage disorder forming macular cherry red spot.

The proteins of retina are

1. Visual pigments of 4 classes one in rod i.e., Rhodopsin - visual transduction protein of rod photoreceptors functional at lower light levels. And 3 colour sensitive visual pigments i.e., cone photoreceptor proteins- for day light vision and colour vision of 3 types : blue, green, and red light absorbing or long wavelength sensitive (570 nm) (red sensitive) mid wavelength sensitive (540 nm) (green sensitive) short wavelength sensitive (440 nm) (blue sensitive).

   All 4 calsses of protein have the same prosthetic group or chromophore viz. 11-cis retinal dehyde and all are derived from a common ancestral gene. Only the interaction of chromophore with the aminoacids of protein decides the spectral characterstics of each pigment or the capacity to absorb different wavelength.

   Clinical aspect; colour blindness or colour deficiency and retinitis pigmentosa are caused by absence or mutation of visual pigments.
2. Transducin - mediates signal coupling for phototransduction.
3. Enzyme proteins - phosphodiesterase, rhodopsin kinase, guanylate cyclase, phospholipase C, protein kinase, phosphoprotein phosphatase, retinal dehydrogenase.

4. Channel proteins - cGMP regulated channel protein, Na^+/Ca^{++} - K^+ exchanger, glucose transporters.
5. Other proteins like arrestin, recoverin, tenascin C, peripherin, phosducin.

Visual pigments absorb light and initiate visual excitation. These protein molecules are embedded in a lipid bilayer. It is the rhodopsin an integral membrane protein of 348 amino acids found in the disc membrane. It is oriented in such a way that the N-terminus faces the intradiscal space or the interphotoreceptor matrix (extracellular space between photoreceptors). This orientation is necessary to maintain its function. The N-terminal region is located inside the disc and has 2 short chain oligosaccharide bound to asparagine. These sugars anchor the molecule and may stabilize its structure. The C ternminal region exposed to cytoplasm contains several hydroxyl amino acids (ser, Thr) which can be phosphorylated. Phosphorylation is a mechanism to " turn off" the sensitivity of activated protein to light. The portion of the molecule that traverses the membrane consists of 7 alpha helices in secondary structure with many hydrophobic amino acids. Of the alpha helices, 3 are cytoplasmic and 3 are intradiscal loops. Another cytoplasmic loop is generated by the insertion of palmitoyl residues of cysteine 322 and 323 into the lipid bilayer. The cytoplasmic loops are associated with binding of another protein, transducin (Fig 13.20).

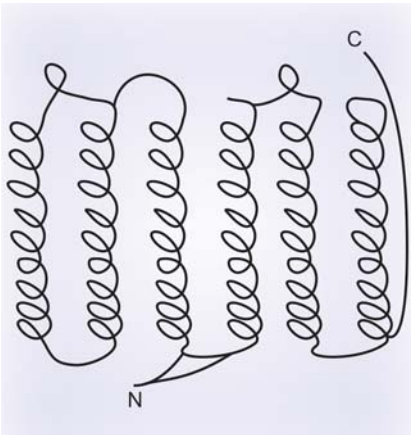


Fig 13.20

Clinical Aspect

Point mutation at codon 23 in opsin gene results in formation of histidine in place of proline giving rise to retinitis pigmentosa.

Rhodopsin is the holoprotein containing the apoprotein, opsin with the prosthetic group as VitA. Vit A is bound to the protein at its 296 Lys as 11 cis-retinaldehyde which is the energetically favourable configuration for confinement among the helices of the protein. It is a protonated (H^+ added)

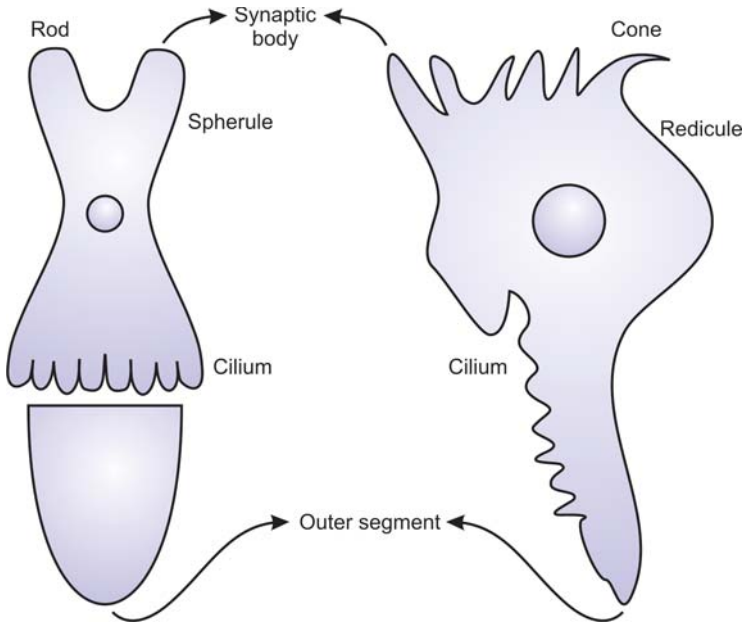


Fig 13.21

Schiff base linkage (i.e. Retinal – CH = NH^+ protein). A Schiff base has the characterstic of being less permanent or less stable than many covalent bonds. This is useful for easy detachment of Vit A upon light stimulation.

Rhodopsin is prevented from migrating around the ends of the discs by other proteins such as peripherin, rds- ROM -1 present at the rim of each disc.

Peripherin contributes to the integrity of the disc structure and controls the population of rhodopsin molecules, on each side of the disc.

Clinical aspect: mutation in peripherin genes gives rise to retinitis pigmentosa.

Synthesis and Turnover of Photoreceptor Outer Segments

Rods and cones visual cells in the retina do not undergo cell division. Instead, the old membrane are shed from the apical tips of both rods and cones, phagocytosed by the retinal pigment epithelium and new membrane material is added at the junction between inner and outer segments.

Renewal of the Outer Segment

Lipids and opsin are synthesized in the endoplasmic reticulum of the inner segment, and transported to the outer segment in vesicles or through exchange protein. Lipids and cone opsins diffuse throughout the entire outer segment. The phospholipids are hydrolysed by phoapholipase A2 and C. Rhodopsin is acylated with 2 moles of palmitic acid on adjacent cysteines near C terminus. There is no denovo synthesis of lipids in ROS (rod outer segment).

Shedding of Rod and Cone Outer Segments

These shed during the period of time when they are functionally less active. So, rods shed early in light cycle and cones shed in the early dark hours. This orderly shedding of photoreceptor tips provides them with a mechanism for renewing the components of their visual membrane. Photoreceptors are highly susceptible to damage at any stage from synthesis to phagocytosis. They degenerate, if separated from RPE as in retinal detachment or in subretinal collection of fluid. They are lost in inflammatory and metabolic retinal disease,retinitis pigmentosa, free radical damage etc.

Antioxidants Vit A and E are present in retina for free radical scavenging along with the enzyme, superoxide dismutase, glutathione peroxidase, glutathione S transferase and also melanin.

Clinical Aspects

Retinoblastoma is a tumor of primitive photoreceptor cells and is the commonest intraocular malignancy of childhood. Autosomal transmission defect : Deletion of 13q14 in 4 % cases. Normally present tumor suppressor gene is mutated or inhibited.

RPE (Retinal pigment epithelium): Is a single layer of cuboidal epithelial cells with basal infoldings and apical microvilli ensheathing and interdigitating between the photoreceptor outer segments. It has numerous gap junctions and tight junctions. It forms part of the blood ocular barrier and hence controls the exchange of metabolites. Many components of RPE are in constant state of turnover through autophagocytosis and synthesis.

Composition of RPE

Lipids - Mostly phospholipids and predominantly phosphotidylcholine and ethanolamine. Palmitic, stearic acids esterify retinol and also used for energy metabolism. The level of PUFA is lower in RPE except arachodinic acid. Phospholipids form 3 % of weight of RPE. RNA is continuously synthesized by the active nuclei of RPE to replace the portion of apical plasma membrane that is internalized along with the outer segment tips.

Proteins of RPE are cytoskeletal proteins mainly actin, receptors in plasma membrane and micro-peroxisomes with hydrolytic enzymes such as glutathione peroxidase, superoxide dismutase, catalase form 8% of the weight of RPE. Lysosomal enzymes are also present. RPE contains all the enzymes of 3 major biochemical pathways viz glycolysis, Kreb's cycle, and pentose phosphate pathway.

Pigment Granules

RPE contains a large number of ellipsoidal and spherical pigment granules having melanin that absorb the stray light. Melanin also provides binding site

for free radicals generated by photochemical events in RPE. A peroxidase is associated with the melano-lysosome complex to detoxify the peroxides produced in these reactions. Melanin is synthesized from tyrosine, through many enzyme catalysed reactions starting with tyrosinase, a copper dependent enzyme. There is a slow and steady renewal of melanin in RPE throughout life.

Clinical aspects: deficiency of tyrosinase results in albinism, poor visual acuity and nystagmus in children—Albinism is of 2 types - oculo-cutaneous and ocular.

Ocular is autosomal recessive inheritance and also X linked trait. There is mosaic pattern of pigment distribution.

Oculo-cutaneous albinism—is also of autosomal recessive inheritance. It is of 2 subtypes tyrosinase positive and negative.

In tyrosinase positive - some visible pigment is present.

In tyrosinase negative - there is lack of all visual pigments, profound visual loss, photophobia, nystagmus, absent fundal pigmentation and foveal reflex.

Autophagy and phagocytosis are two of the important functions of RPE.

1) **Autophagy:** Under physiologic conditions, RPE is a non-mitotic tissue. So, in order to replace and repair its various constituents and organelles, the post-mitotic RPE undergoes autophagy (autophagocytosis). The old organelles are destroyed by autophagocytosis and this stimulates the biosynthesis of new membrane, ribosomes, lysosomes, peroxisomes, mitochondria and melanosomes. Autophagic vacuoles form from the fusion of lysosomes with organelles. A small amount of RPE plasma membrane is lost, each time the tip of an outer segment of photoreceptor is internalized.
2) **Phagocytosis:** RPE phagocytose the tips shed from the constantly renewing photoreceptor outer segments. First, the pseudopodial processes of the RPE envelope the shed tips of rods and cones and internalize them into the cell. Then the lysosomes of the cell fuse with the ingested tips to form the phagolysosome. The degradative, digestive enzymes of the lysosome digest the phagolysosome and leave a residual body. This residual body accumulates throughout life and fuse to form lipofusin granules noticed along the basal margin in older eyes. Actin filaments and microtubules of RPE are involved in phagocytosis. Each photoreceptor completely renews its outer segments every 10 days and so phagocytic load for RPE is high.

   Clinical aspects: Defects in rds locus leads to lack of recognition of the material to be digested and hence retinal degeneration.
3) The RPE plays a role in maintaining the proper ionic and fluid environment by acting as part of blood- retinal barrier. The apical membrane of RPE has the transporters and channel proteins. They are bicarbonate transporter, carbonic anhydrase enzyme and Na^+/K^+ATPase. These help in the transport of Na^+ towards subretinal space and K+ in the opposite direction (Fig 13.22).

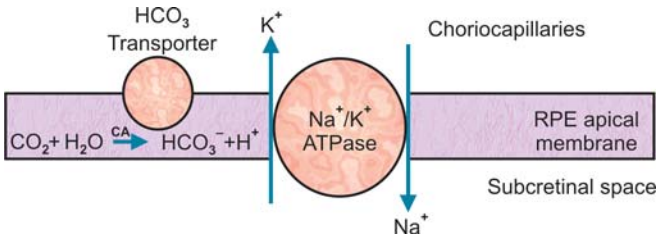


Fig 13.22

4) RPE along with photoreceptors helps in maintaining the subretinal space (interphotoreceptor matrix IPM) by synthesizing and secreting some of the proteoglycans and GAGs of IPM.

IPM - interphotoreceptor matrix or sub retinal space: Lies between RPE and photoreceptors. Proteins, GAG, glycoproteins and proteoglycans make up the major part of IPM. Interphotoreceptor retinoid binding protein is the glycoprotein and is soluble. But the predominant chondroitin sulfate proteoglycan is insoluble. The proteoglycan around the rod outer segment has sialyl conjugates also. The rods and cones associated IPM domains are tubular structures surrounding the outer segment and extend from the external limiting membrane of retina beyond the distal tip of outer segment to terminate at the apical surface of RPE.

Functions of IPM

1. Retinal attachment by promotion of adhesion between RPE and photoreceptors cells.
2. Visual pigment chromophore exchange.
3. Transport of metabolites between the RPE and rod outer segment. Fig 13.23.

Clinical aspect:

Defects in IPM manifests as retinal detachment, macular degeneration and retinitis pigmentosa.

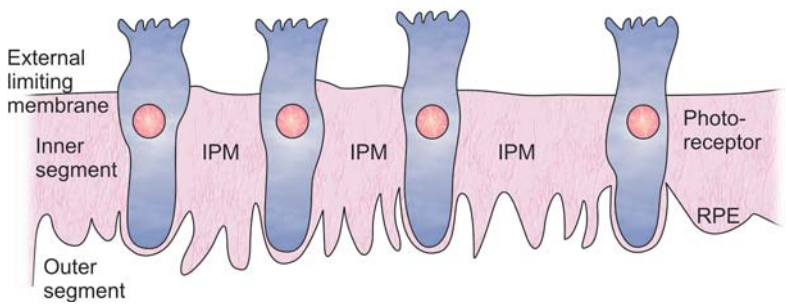


Fig 13.23

Wald's visual cycle and photo transduction

In 1958 George Wald demonstrated that light isomerizes 11 cis retinal, the chromopore in rhodopsin to 'All trans retinal'. Thus, absorption of a photon by a visual pigment molecule is the initial event in visual excitation. This is followed by many reactions that transmit the visual message to the brain.

The cis form of Vit A (11–cis retinal) is folded on itself to form a compact molecule (Fig 13.24). On isomerisation it unfolds and straightens (disrupting the conformation of protein which also unfolds) to form the trans form (i.e., the double bonds in trans position) occupying more space in membrane provided by changes in DHA Fig. 13.25. The isomerisation reaction creates an energitcally unstable molecule that rapidly proceeds through several intermediate forms with different spectral activities in milliseconds as shown below (Fig. 13.26).

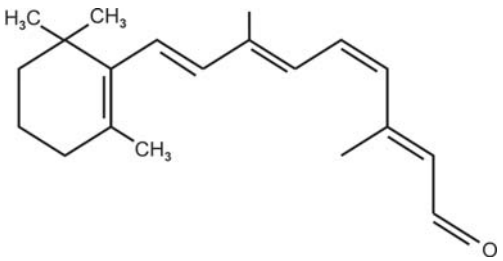


Fig 13.24: 11-cis-retinal

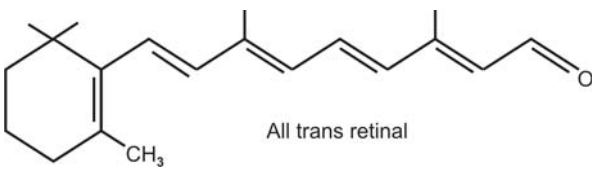


Fig 13.25: All trans retinal

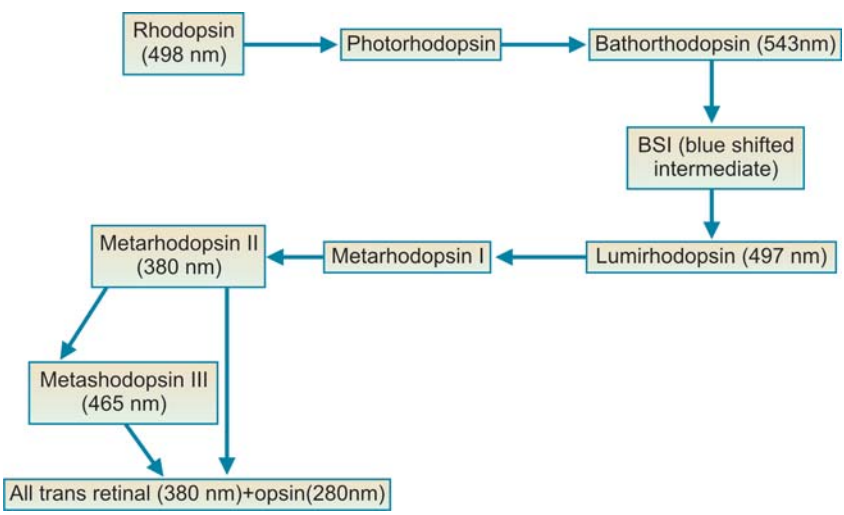


Fig 13.26

As the reaction proceeds to the formation of metarhodopsin the Schiff base linkage is deprontonated and finally broken. The completion of the reaction results in the release of Vit A from the protein as 'All transretinal'. The vitamin detached protein is then called opsin. The conversion of rhodopsin to opsin is only the beginning of visual transduction process. This process is called bleaching of rhodopsin. The light absorbing properties of rhodopsin change on bleaching. The curve shows the absorption spectrum before and after exposure to light or isomerisation, 3 peaks seen (Fig. 13.27).

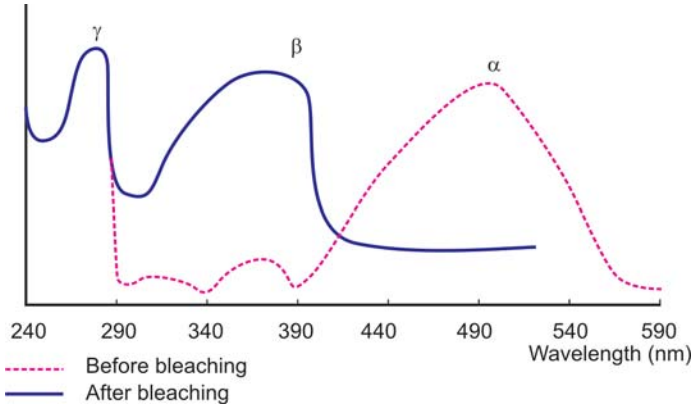


Fig 13.27

Alpha - absorption of 11 cis-retinal bound to opsin (~ 515 nm)- rhodopsin- Beta - absorption of all transretinal which is not bound (~ 375 nm), Gamma - protein absorption (~ 280 nm) peak common to both opsin and rhodopsin.

All trans retinal is rapidly reduced to all trans retinol by retinol dehydrogenase present in rod outer segment. NADPH is the cofactor for this reaction. This enters RPE, esterified and isomerised to 11 cis-retinol. Again, it enters rod outer segment and oxidized to 11 cis-retinal. As the opsin without chromophore is unresponsive to light, functional rhodopsin molecule has to be regenerated. By condensation of 11 cis-retinal with newly synthesized opsin that had been added to rod outer segment, rhodopsin is regenerated. During the normal course of light and dark adaptation, an efficient recycling of the liberated chromophore within the eye, between the RPE and ROS occurs and total amount of vit A compounds is maintained constant. This is referred to as visual cycle or Wald's visual cycle.

Processing and transport of Vit A in RPE and ROS (Fig. 13.28)

All transretinol enters RPE from ROS or from circulation. It should bind to retinol binding protein before transcellular transport to RPE. In RPE, it is esterified with palmitic, stearic or oleic acid to all trans retinyl ester. All trans-retinol in cone photoreceptors is reisomerised to the 11 cis form before transport to the RPE.

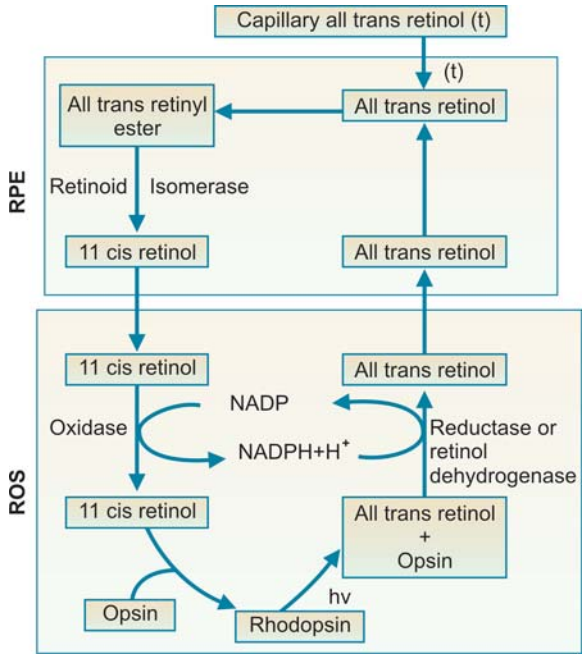


Fig 13.28: Processing of transport of Vit A in RPE and ROS

Processing and transport of vit A to its target cells (Fig. 13.29)

Vitamin A is stored in liver as retinyl ester and it may be hydrolysed to retinol and free fatty acids. The retinol combines noncovalently with serum retinol binding protein (SRBP) with a molecular mass of 21 KD. This small complex is bound to a larger protein, prealbumin in serum. As the choroid capillaries are fenestrated this RBP prealbumin complex (76KD) penetrates Bruch's membrane and interacts with specific receptors on the basal side of the RPE. The protein, RBP does not enter the cell but delivers the retinol (vit A) to the membrane for transport inside the cell. The receptor now shows reduced

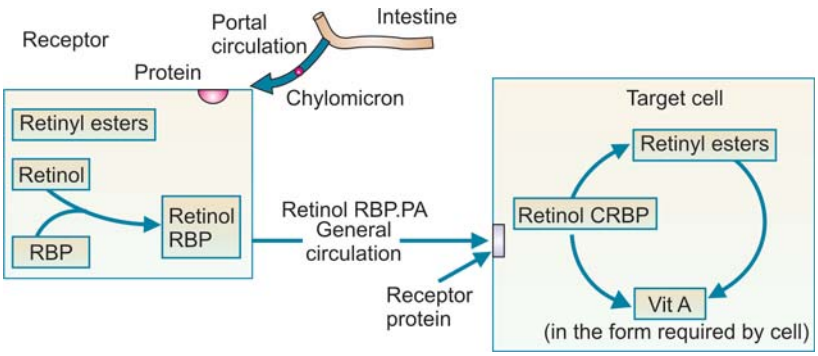


Fig 13.29: Processing of Transport Vit A to its target cells

affinity for RBP as the vitamin A is separated and can be replaced by another RBP with vitamin A.

Clinical aspect: An early effect of vitamin A deficiency is loss of night vision. Light transduction is a cGMP mechanism.

It is the coupling of absorption of a photon by rhodopsin in disc membrane with the closure of cation channels in the plasma membrane of outer segment.

It is similar to the mechanism of hormone action. Light replaces hormone. Rhodopsin is the receptor protein. 'G' protein is transducin. The activated enzyme here is phosphodiesterase. Transducin and phosphodiesterase are peripheral membrane proteins. Transducin has 3 subunits, alpha, beta, gamma. Presence of gamma keeps it inactive. It is bound to GDP in the dark.

Phosphodiesterase is a tetramer with 1 alpha, 1 beta and 2 gamma subunits. Here, also presence of gamma subunits keep the enzyme in inactive state. The action of phosphodiesterase is to hydrolyse cGMP into GMP.

GTP $\xrightarrow{\text{Guanylate cyclase}}$ cGMP $\xrightarrow{\text{Phosphodiesterase}}$ 5´GMP

On the cytoplasmic side of each disc, rhodopsin (receptor protein), transducin (G protein) and guanylate phosphodiesterase are located in close proximity on the disc membrane.

When light strikes a rhodopsin molecule, the molecular rearrangement of vitamin A (to an unprotonated Schiff base) and the protein conformation of opsin portion (to Metarhodopsin II) causes close contact with transducin.

The activated rhodopsin interacts with transducin causing the transducin to incorporate GTP with the removal of GDP. The alpha subunit of transducin gets detached and diffuses to the inactive phosphodiesterase. The alpha subunit of transducin, now binds to the gamma subunit (inhibitor) of phosphodiesterase causing it to be released from the enzyme. This release activates the enzyme, bringing about the catalytic hydrolysis of cGMP to GMP. So, the concentration of cGMP in the cytoplasm of photoreceptor outer segment is lowered. The above said events are shown in the figures 13.30 and 13.31.

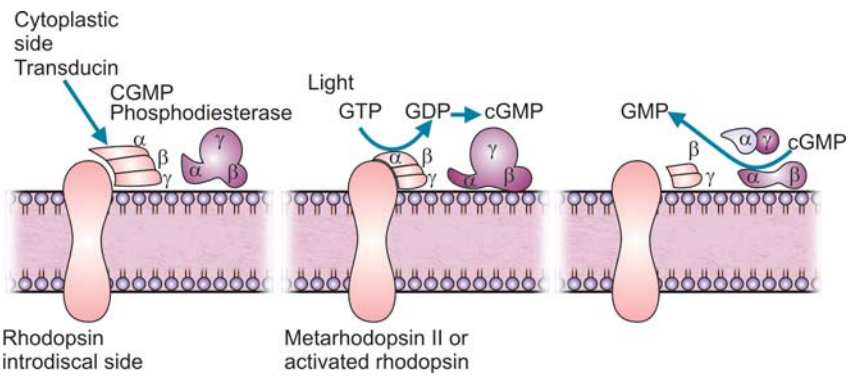


Fig 13.30

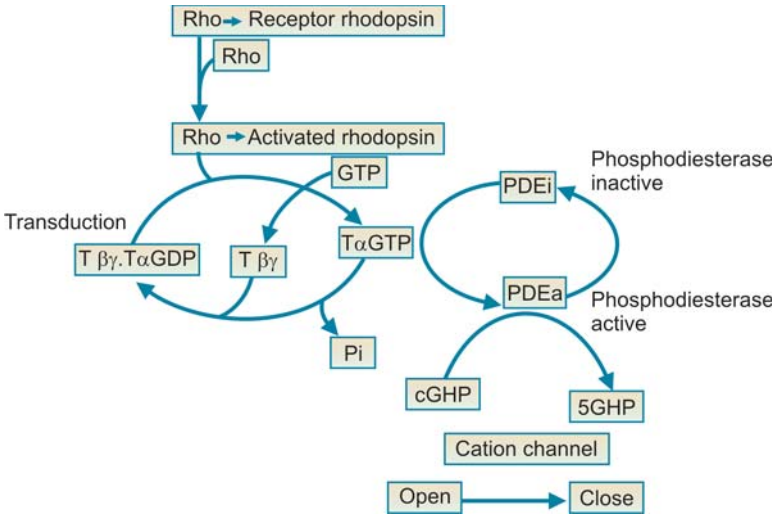


Fig 13.31

Cation channels require cGMP to be bound to the gate protein for it to remain opened. When the concentration of cGMP is lowered, it dissociates from the gate protein which close and interfere the ion flow. In the dark, there is a potential difference between the inner retina (+ve) and the tips of the outer segment (-ve). The resulting ionic flow is called dark current. In the outer segments of photoreceptors, 2 kinds of protein maintain a constant flow of Na+, Ca++ into the outer segment.

1. **Gate protein -** allows the passage of Na+, Ca++ into the outer segment. It is maintained in the open state by the cGMP and even Ca++ itself.
2. **Na/Ca- K exchange protein** - allows a controlled amount of Na+and Ca++ to exit the outer segment in exchange for the inward passage of K+. The function is to maintain the cytoplasmic Ca++ at ~ 400 nm in dark and at ~ 40 nm in strong light.

On the inner segment of photoreceptors, cation flow is controlled by the actions of Na/KATPase and voltage gated K+ channel protein.

1. **Na^+/K^+ ATPase:** maintains an excess of Na+ on the outside of the cell, and K+ inside the cell, the flow is at a slower, controlled rate.
2. **Voltage gated K^+ channel protein:** Selectively extrudes K^+ in a voltage dependant manner and maintains a steady flow of K^+ outward to contribute to the overall dark current flow of polarization potential ~ 40mv and the release of glutamate neurotransmitter to bipolar cells. When there is continuous interruption in the dark current the change in membrane potential alters the K+ outflow (this is why the protein is called a voltage gated K+ channel) to adopt the cell to continued presence of light. This is a form of light adaptation.

The departing positively charged ions cause the photoreceptor to acquire a net negative charge of ~ 65mv. The cells hyperpolarize. The hyperpolarisation stops the flow of current (i.e., the discharge of the neurotransmitter, glutamate is interrupted) to the ganglion cells of the retina causing the ganglion cells to discharge. This discharge is ultimately transmitted to area of the brain where it is perceived as light (Figs 13.32 and 13.33).

There is amplification of the response in cGMP cascade. One activated rhodopsin can lead to the hydrolysis of 100,000 molecules of c GMP. So, re-establishment of cGMP levels is necessary for further visual acuity of photoreceptor cell. Control of cGMP level is exerted at several points in the cascade.

1) T α (alpha subunit of transducin) is a GTPase. So, it hydrolyses GTP to GDP. Formation of GDP leads to reassociation of T α with Tβλ to form the complete molecule of transducin. Tαβ. GDP which cannot activate phosphodiesterase.
2) Phosphorylation of opsin by rhodopsin kinase and (C terminal serine and threonine are exposed on bleaching) binding with arrestin inhibits the ability of activated rhodopsin to bind transducin (arrestin combines with rhodopsin only after phosphorylation). Thus, changes in levels of illuminance alter the level of c GMP in rod outer segment.

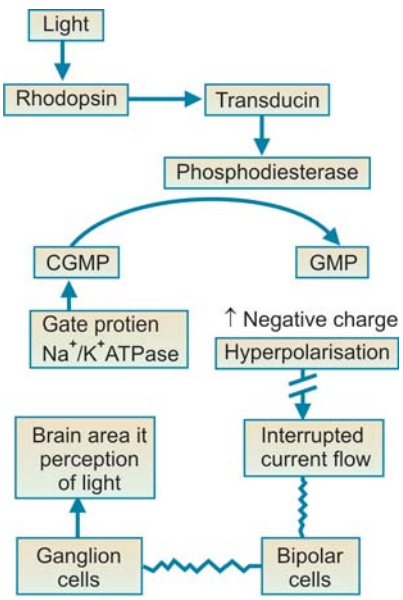


Fig 13.32: Visual transduction

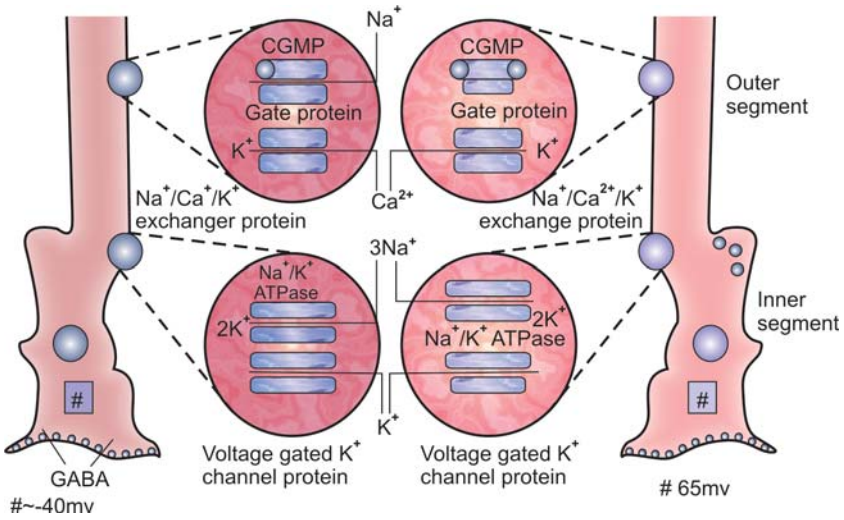


Fig 13.33

Calcium ions also play a significant role in phototransduction.

1. Calcium calmodulin complex binds to calcium channel protein and decreases its ion permeability. This mechanism acts together with the exchange protein to limit the upper concentration of calcium ions in the outer segment.
2. Calcium ions bind to guanylate cyclase binding protein (GCBP) and inhibit guanylate cyclase. So, when the intracellular Ca++ concentration decreases, guanylate cycalse is activated to produce more cGMP (the reaction catalysed by guanylate cyclase is production of cGMP from GTP). The cGMP will bind to the gate protein and allow more Ca++ to enter the cell through the opened channel.
3. Calcium ions have a direct / indirect influence over voltage gated K+ channel protein of inner segment also.
4. Ca^{++} bound to recover in the ROS, inhibits rhodopsin kinase. So, the life time of activated rhodopsin is extended (only the activated rhodopsin is phosphorylated by rhodopsin kinase as the serine, threonine amino acids at the C terminus of opsin are exposed on bleaching). Photoreceptors send electrochemical signals to the brain by both direct (cone) and indirect (coneand rod) synaptic mechanisms (Fig. 13.34).

The cone photoreceptors have many triad synapses on their pedicle (Fig. 13.35). Whereas the rod photoreceptors have a single triad synapse at the end of its presynaptic process (spherule). The constant release of glutamate neurotransmitter is necessary to maintain the synapse in the inactive state i.e., to prevent post synaptic fibres from depolarizing. This is facilitated by the synaptic ribbon apparatus with a RIBEYE protein (synaptic ribbon protein of the eye) as its essential part. It binds to the synaptic vesicles that hold the neurotransmitter and transpose the vesicles to the synaptic membrane at a

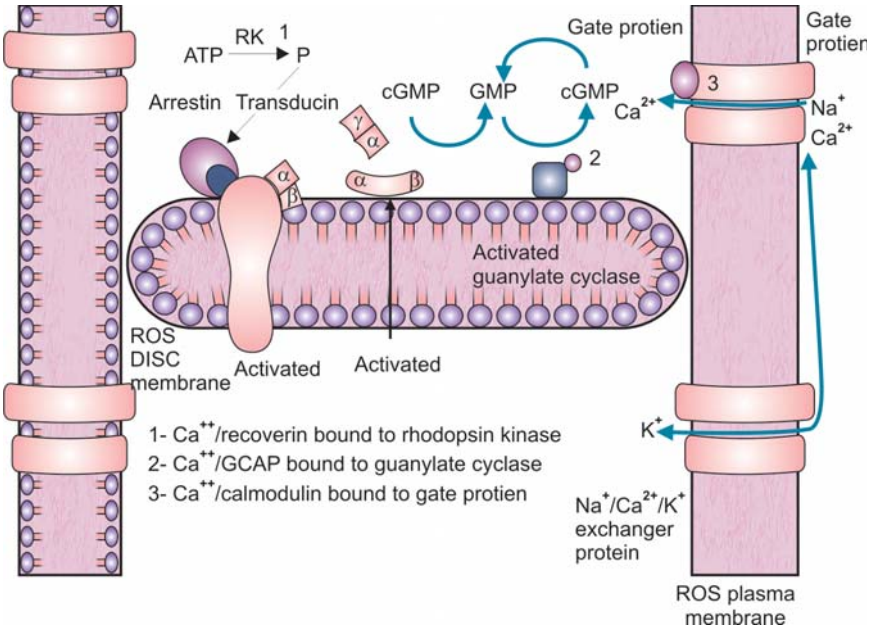


Fig 13.34

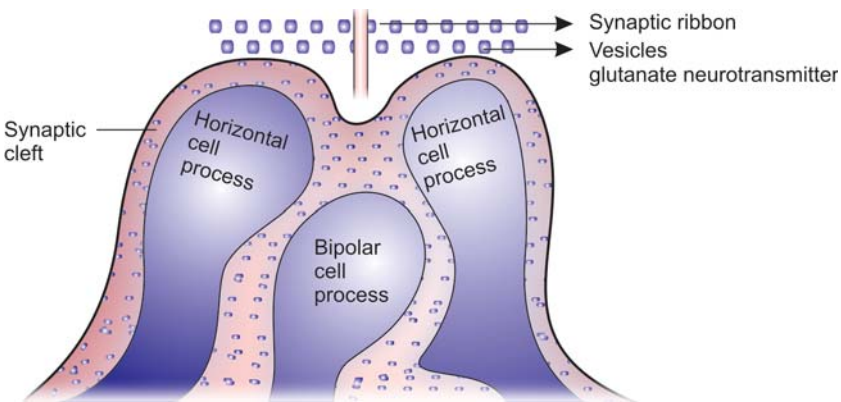


Fig 13.35: Cone photoreceptor-Triad synapse

rapid rate to facilitate their release. This protein consists of 4 domains with a molecular weight of 120 KD. 2 identical A domains are essential for the formation and stabilization of the ribbon structure and 2 identical B domains are responsible for actual binding to the presynaptic vesicles. Vesicle fusion with the membrane occurs in the active zone at the bottom of the ribbon adjacent to the cleft membrane.

Calcium channels also facilitate vesicle fusion and neurotransmitter release by increasing the intracellular calcium necessary for fusion and release and decreasing the glutamate release.

Some biomedical synaptic pathways of the retina:

Type	*Synapse (neurotransmitter)*	*Receptor mechanism*
Cone, center, on	Photoreceptor → bipolar (less Glu)	Opening of Na+ channels via cGMP
	Bipolar → Ganglion (Glu)	Depolarization
Cone, center, off	Photoreceptor → Bipolar (Glu)	Closing of Na+ channel via PDE
	Bipolar → Ganglion (Glu)	Depolarization
Cone surround on (neighbour cells)	Photoreceptor $\xrightarrow{1}$ Horizontal (less Glu)	Opening of Na+ channels protein
	Horizontal $\xrightarrow{1}$ photo receptor (GABA)	Maintenance of Glu release
	Photoreceptor $\xrightarrow{2}$ Bipolar (no Glu)	Opening of Na+ channels protein
	Bipolar $\xrightarrow{2}$ Ganglion (Glu)	Depolarization
Rod, low light	Photoreceptor $\xrightarrow{2}$ rod Bipolar (less Glu)	Closing of Na+ channel protein
	Rod bipolar → amacrine (Glu)	Opening of Na+ channels protein
	Amacrine → cone Bipolar (indoleamine)	Opening of Na+ channels protein
	Cone bipolar → Ganglion (Glu)	Depolarization

1. Indirect pathway to adjacent cone photoreceptor.
2. Direct pathway to ganglion cell equivalent to centre on type.

Glu- Glutamate neurotransmitter.
GABA- Gamma amino butyrate.

Clinical Aspects

- Retinitis pigmentosa (degenerative condition) → disruption of neurotransmission → loss of vision.
- Horner's syndrome -nerve lesions outside the eye → disruption of neurotransmission → loss of vision.
- Parkinson's disease → decreased levels of dopamine in retina (normal 1ng) → loss of contrast sensitivity ability → decreased ability to read. Dopamine is a NT found in some amacrine cells and interplexiform cells.

Intermediary Metabolism of the Retina

Visual excitation is the unique function of retina and requires energy in the form of ATP derived from metabolism of glucose mainly. Glucose from the retinal capillaries (inner layers) and choroidal (photoreceptor cells) circulation

diffuses into the retinal cells by facilitated transport through glucose transporters GLUT1and GLUT3. It does not depend on insulin for glucose uptake.
Glucose is rapidly metabolized through 3 main pathways viz.

3. Aerobic glycolysis - 25%.
4. Anerobic glycolysis- 60%.
5. HMP shunt - 15%.

Retina consumes oxygen at a higher rate than any other tissue. 70% of the oxygen uptake is for glucose utilization as glucose is the major fuel for retinal metabolism. It is actually 31µl oxygen / mg tissue dry weight/ hour and the blood flow supplying this O_2 and glucose to retina is 12ml/g tissue/ minute.

Even with this adequate amount of oxygen supply, there is accumulation of lactate (end product of glucose metabolized anaerobically). This is because pyruvate oxidation in the mitochondria does not keep pace with the pyruvate production from glucose. So excess pyruvate is reduced to lactate which then accumulates. Excess lactate is lost by diffusion.

900 molecules of ATP are produced aerobically and 120 molecules anaerobically (60x2=120) per 100 glucose molecules. The glucose entering the HMP shunt provides the pentose for nucleic acid synthesis and it is the major source of NADPH in the retinal cell for reduction of "all transretinal" in the photoreceptor outer segments emphasizing the importance of this pathway in retinal metabolism.

Retina cannot function anaerobically even when abundantly supplied with glucose. During anoxia as seen in diabetic retinopathy and central retinal vein occlusion, the b wave and oscillatory potentials of ERG do not survive. In the absence of glucose the amplitude of 'a' and 'b' wave of ERG decrease.

Thus, the electrical activity of retina is very much dependent on glucose and oxygen. Retinal glycogen is very low and is present in Muller cells (glial cells). Muller cells have glucose 6 phosphatase activity so that they can convert their glycogen to glucose for use by neighbouring neuronal cells.

The maintenance of ionic gradients across the cell membrane, requires a constant utilization of ATP (cation pump in visual cell associated with the maintenance of dark current). This may be reduced by light. Light induces turnover of cGMP in the ROS, hydrolysis of GTP, phosphorylation of rhodopsin etc..which all consume ATP.

Clinical Aspect

Diabetic retina: retina is vulnerable to diabetes due to deterioration of blood vessels in diabetic retinopathy. In the retina, pericytes are destroyed while the lumen of the vessel becomes blocked due to vessel swelling as the basement membrane thickens (Fig. 13.36). This is followed by hemorrhages and retinal detachment, resulting in loss of vision.

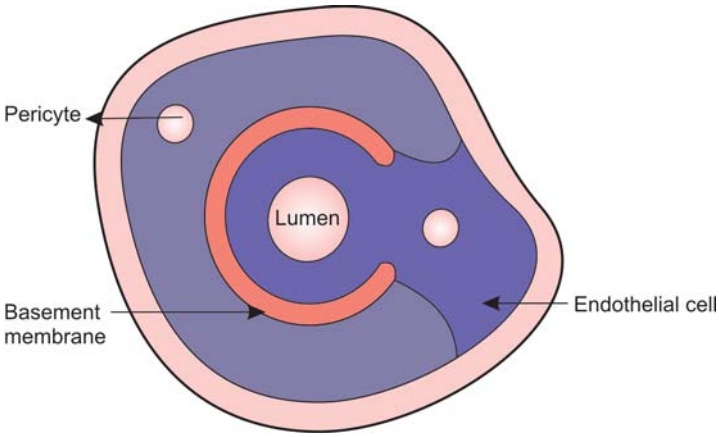


Fig 13.36

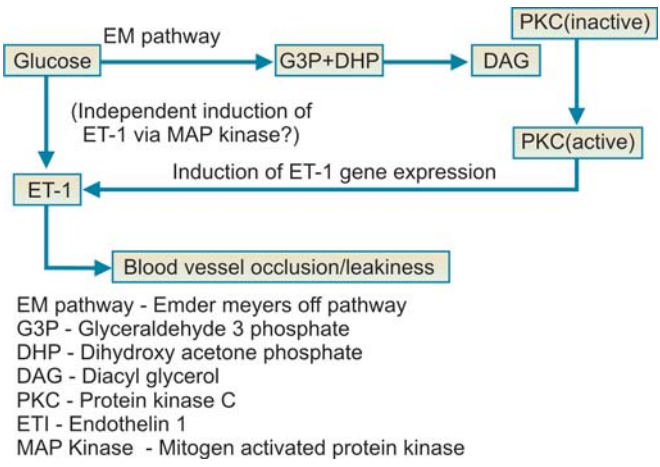


Fig 13.37

Biochemical mechanism behind this is

1. Reaction of aldose reductase ie polyol pathway. As there is no restriction of glucose uptake (retina being not dependent on insulin for glucose uptake) the toxic levels of glucose induce aldose reductase leading to polyol pathway. The end product, Sorbitol of this pathway is an osmotically active component causing the cells to swell and rupture.
2. Diacylglycerol (DAG) synthesized from glyceraldehyde 3 phosphate and dihydroxy acetone phosphate stimulates protein kinase C (PKC) which increases the blood vessels permeability and excessive synthesis of blood vessel membranes (basement membrane thickening). Also PKC induces synthesis of endothelin1 (ET1) which again increases the permeability and blood vessel thickening. Mitogen activated protein kinase (MAP) also have similar effects (Fig. 13.37).

3. Glycation of Proteins: protein, carbohydrates conjugates occur in Na+/K+ATPase, Hb etc. These with time form complex, permanent and undefined "Advanced glycation end products (AGE)". AGEs bind to receptors on vascular endothelial cells to produce blockage in the vessel, leakage of vessel, dilatation of vessel, thickening of vessel and cell death by apoptosis.
4. Oxidative mechanism also contributes.

Section 3

Ocular Pathology

14 Ocular Pathology

INFLAMMATION

The inflammatory response involves a variety of specialized effector cells and a complex interplay of cells, mediators and biochemical reactions that protect the body against microorganisms. The inflammatory process includes mechanisms to repair and restore tissues damaged by foreign invaders, trauma or chemical and physical agents. The intact immune system, which is the corner stone of the inflammatory process, is effective in protecting us from potential invaders. This is evidenced by the wide spectrum of opportunistic infections and tumors that afflict patients who have AIDS.

Definition

Inflammation is defined as a localized protective response elicited by injury to or destruction of tissues by physical, chemical and biologic stimuli. The process serves to destroy, dilute and sequester the injurious agents and to partially isolate the injured tissue.

It is an expression of the host's attempt to localize and eliminate metabolically altered cells, foreign particles, micro-organisms or antigens. It involves a complex series of events- dilatation of arterioles, capillaries and venules resulting in increased blood flow and vascular permeability, exudation of fluid and plasma proteins and migration of leukocytes into the inflammatory region.

The suffix *-itis* is used to designate an inflammatory process. If several tissues are simultaneously involved the one that is most prominently or initially affected is designated first in the terminology.

"True" intraocular inflammatory processes must be separated from pseudoinflammatory processes in which the inciting agent is ischemic or neoplasia e.g. Pseudohypopyon produced by leukemia, retinoblastoma or pseudouveitis produced by anterior segment ischemic syndrome.

Intraocular inflammation is subdivided into *acute*, *subacute* and *chronic*. *Acute inflammation* begins within minutes of injury and lasts for several days or weeks before resolving.

There are *five cardinal signs* of acute inflammation:

1) *Rubor* or redness.
2) *Calor* or heat.

Above two signs are caused by increased rate and volume of blood due to increased vascular permeability.

3) *Tumor* or swelling or edema is due to the exudation of the fluid and the cellular components of the blood into the extravascular space.
4) *Dolor* or pain is due to prostaglandin inflammatory mediators, which stimulates contraction of smooth muscle, e.g., Spasm of the ciliary muscle and sphincter muscles of the iris contributes to the pain of anterior uveitis.
5) *Functio laesa* or loss of function.

Effects of Increased Vascular Permeability

Aqueous humor normally is totally devoid of protein. When inflammation disrupts the blood ocular barrier, protein content rises. Inflammatory cells normally move with the convection currents in the aqueous. An absence of cellular convection currents may indicate clotting of fibrin rich aqueous in a patient with severe vascular permeability. *Adhesions* readily form in the fibrin rich milieu of ocular inflammation e.g., posterior synechiae and seclusio pupillae. Aggregates of inflammatory cells called keratic precipitates (KP) form on the posterior surface of cornea. KP may be small or large and lardaceous (mutton fat) as seen in chronic granulomatous inflammation.

Outline of Acute Inflammatory Response

Principal features are vasodilatation, increased blood flow, increased vascular permeability and exudation of blood proteins and leukocytes. *Emigration of leukocytes* into the inflammatory focus is divided into three stages:

1) *Margination*—leukocytes pass from the center of the vessel to contact the endothelial lining.
2) *Adherence* of white blood cells to the microvasculature.
3) *Diapedesis* or migration of leukocytes into the extra vascular space.

Initially *neutrophils* are the predominant inflammatory cells. *After 24 hours* they are replaced by *monocytes* and *macrophages*. The inflammatory cells are attracted to the region of injured tissues by specific chemical mediators or chemotactic agents within the tissues- *chemotaxis*. Chemotaxis is followed by the process of *phagocytosis*, which shows three phases:

1) *Attachment* of phagocytes to the foreign antigenic particles.
2) *Ingestion* and intracellular degradation of the particle.
3) Extracellular release of the leukocyte degradation product.

The various *chemical mediators* of inflammation released during the process of extracellular exudation have specific actions that enhance the acute inflammatory response. Mediators that *increase vascular permeability* are histamines, serotonin, kinins, prostaglandins and platelet activating factors.

Cytokines are synthesized and secreted by inflammatory cells and include interleukins, interferons and colony stimulating factors. The balance of synthesis and degradation of these mediators of inflammation helps to determine the nature, time course and severity of the inflammatory response.

Thus, acute inflammation may subside with complete resolution and the area of inflammation is healed by scarring or abscess formation. The inflammation may persist or may change and progress to subacute or chronic inflammation.

Subacute inflammation: Destructive force or inciting agent continues to elicit a modified inflammatory response over a period of weeks or months.

Chronic or recurrent intraocular inflammation: Usually follows an acute or subacute form. Some inflammatory process presents a chronic appearance throughout their entire course.

Histopathologically inflammation is categorised as acute or chronic, based on the type of inflammatory cells found in the tissue or exudates. *Acute inflammation* is characterized by presence of polymorphonuclear leukocytes and pus (exudates composed of numerous polymorphs and tissue destruction). Presence of pus is called suppurative inflammation. Hypopyon is seen in acute keratitis and endophthalmitis. Vitreous abscess is seen in acute purulent endophthalmitis.

Chronic inflammation may be nongranulomatous or granulomatous. *Chronic non-granulomatous* inflammation shows lymphocytes and plasma cells and denotes involvement of the immune system. Presence of numerous eosinophils is suggestive of an allergic reaction or parasitic infestation. Mast cells or tissue basophils can be seen in allergic disorders, e.g., vernal conjunctivitis and also in neurofibromas.

Chronic granulomatous inflammation is characterized by activated macrophages or epithelioid histiocytes and inflammatory giant cells. Epithelioid cells are derived from blood monocytes. It refers to inflammation in which granulomas are present and occurs in response to certain infectious agents. *Granuloma* is a mass or nodule less than 2 mm in diameter, composed of macrophages known as epithelioid cells and may contain multinucleated giant cells. *HPE*- the lesion is surrounded by a rim of lymphocytes. Actively growing fibroblasts and small capillaries may be seen within a granuloma.

Chronic granulomatous inflammation can be induced by—

1) *Infection*—Bacteria (tuberculosis, syphilis, leprosy), Mycoses (candida, toxoplasmosis, histoplasmosis, cryptococcosis), Parasites (toxocara, cysticercosis) (Fig. 14.1).
2) *Reaction to autologous intraocular tissue*—phacoanaphylactic endopthalmitis, resolving vitreous hemorrhage, cholesterol granuloma, sympathetic ophthalmia, chalazion (lipogranulomatous inflammation).
3) Immunological processes of unclear etiology- sarcoidosis, Still's disease, scleritis in rheumatoid arthritis, juvenile xanthogranuloma.
4) Intraocular foreign bodies.

Three histological patterns are seen in granulomatous inflammation:

- Nodular pattern seen in tuberculosis.
- Zonal pattern seen in phacoanaphylactic uveitis.
- Diffuse pattern seen in sympathetic ophthalmia (Fig 14.2).

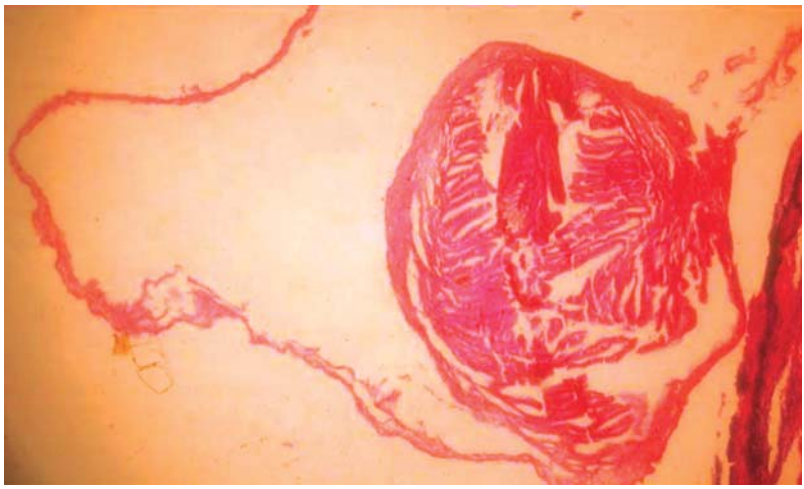


Fig. 14.1: Shows cystic structure with a scolex showing papillary infoldings and hooklets

During inflammation epithelioid cells aggregate forming giant cells. Inflammatory giant cells are of three types:

1) Langhan's giant cell seen in tuberculosis. Nuclei are arranged around the periphery (Fig. 14.3).
2) Foreign body giant cell- nuclei are randomly arranged throughout the syncytium. May contain foreign material.
3) Touton giant cell- shows a rim of cytoplasm that contains lipid peripheral to the ring of nuclei.

Granulation tissue forms during the reparative phase of inflammation and plays an important part in wound healing. Granulation tissue is composed of proliferating capillaries, activated fibroblasts with contractile properties called

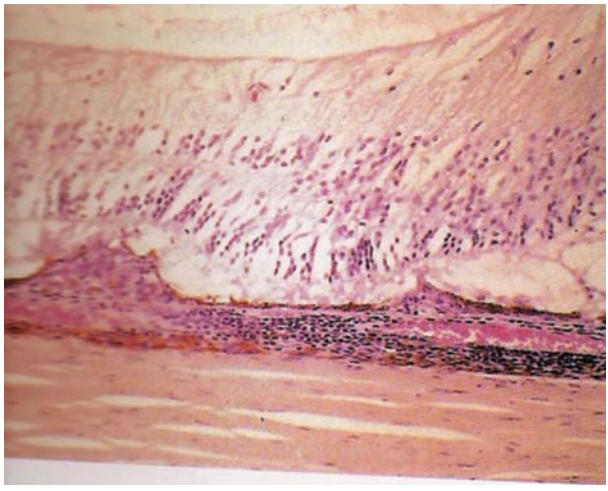


Fig. 14.2: Dalen Fuchs nodule showing collection of epithelioid histiocytes beneath the RPE

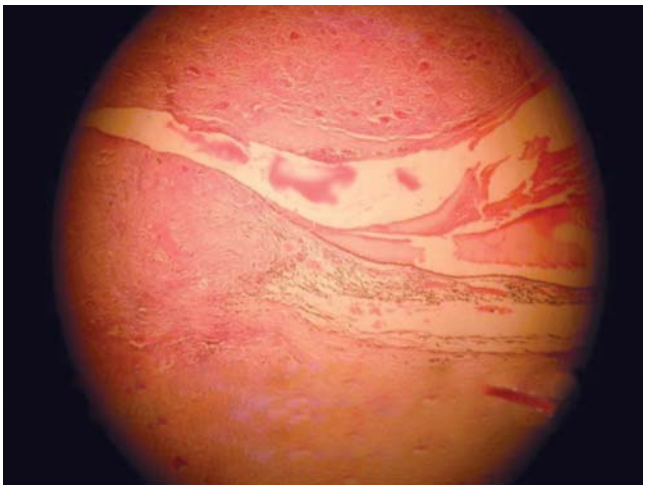


Fig. 14.3: Tuberculous endophthalmitis—shows numerous caseating epithelioid cell granulomas in vitreous and sclera

myofibroblasts and a mixture of polymorphs, lymphocytes, plasma cells, macrophages and eosinophils. Myofibroblasts are involved in scar contraction. *Pyogenic granuloma* is an exuberant proliferation of granulation tissue seen after surgery or minor trauma.

Sequelae of Ocular Inflammation

Intraocular fibrosis and membranes are caused by the organization of inflammatory debris. Contraction of cyclitic membrane causes tractional detachment of ciliary body and retina. Disorganization and destruction of intraocular structures results in ocular hypotony and shrinkage. Clinically the term "*phthisical*" refers to blind hypotonous eyes that are soft, partially collapsed and have vague cuboidal shape due to rectus muscle traction.

Pathologically, eyes that are markedly atrophic and disorganized and have thickened folded sclera are designated *phthisis bulbi*. Interior of such phthisical eyes usually are filled with scar tissue. Intraocular structures are unrecognizable.

The term "*atrophic bulbi with shrinkage*" is used if intraocular structures can be identified. Intraocular ossification (osseous metaplasia of the retinal pigment epithelium) may be seen in atrophic and phthisical eyes that have chronic retinal detachment.

Aggregates of inflammatory cells called Keratic precipitates form on the posterior surface of cornea. KP may be small or large and lardaceous. The latter are called mutton fat keratic precipitates and are seen in eyes with chronic granulomatous inflammation.

Phases of The Inflammatory Process

All inflammatory processes show the following phases

1) Exudative phase- cells and serum proteins migrate from intravascular space to extravascular space.

2) Acute adaptive phase—there is cytotoxicity and tissue necrosis. Varies in severity and duration. Ultimately progresses to sub acute or chronic inflammation.
3) Reactive phase of homeostasis restoration. There is reactive proliferation and restoration of tissue substance with newly formed parenchymal tissue, scarring, fibrosis or granulation tissue.

Exudation

In anterior uveitis, there is exudation of lymphocytes, plasma cells or polymorphs into the anterior chamber-Hypopyon.

Inflammatory exudate in the stroma of the iris inhibits free movement of the iris-causes miosis in iritis.

Inflammation of the ciliary body leads to decreased production of aqueous humor and ciliary muscle spasm.

Posterior uveitis results from exudation into the vitreous.

Bacterial infections cause diffuse infiltration of the vitreous with extensive liquefactive necrosis.

In contrast, mycotic infections cause multifocal microabscesses.

Exudation beneath the retina or choroid may result in retinal and choroidal detachments. Serous detachment of the sensory retina results in degeneration of photoreceptors and outer retinal layers, due to reduction of oxygen and nutritional supply from the choriocapillaris.

Edema also may be present within the retina. Cystoid macular edema results when fluid accumulates in the outer plexiform layer or Henle's nerve fiber layer. Retinal vasculitis and perivasculitis are common features associated with intraocular inflammatory processes.

Cytotoxicity and Tissue Necrosis

Cytotoxic immunologic mechanisms while destroying noxious agents often result in some destruction of normal host tissue.

Inflammatory exudates in the anterior chamber often become adherent to the corneal epithelium and can cause focal destruction of the corneal epithelium with swelling of the corneal stroma.

In granulomatous inflammation or viral induced inflammation, the stroma and pigment epithelium of the iris and ciliary body show sectoral or diffuse iris necrosis, but remain intact in nongranulomatous inflammatory processes.

Chronic recurrent inflammations of any type can lead to focal or diffuse atrophy of the iris and ciliary body.

Choroid shows severe changes in bacterial endophthalmitis, but is spared in viral retinopathies.

Toxoplasmic retinochoroiditis causes marked focal destruction about the retina and the choroid.

The pigment epithelium and outer layers of the retina are damaged secondarily in primary choroidal inflammatory diseases. The inner retinal layers remain intact due to the external limiting membrane.

In contrast, primary inflammations affecting the retina (retinits) are characterized by cewllular infiltrates throughout the entire retinal structure.

Viral induced retinitis (Herpes simplex and Herpes zoster retinitis and Toxoplasma induced retinitis) are characterized by necrosis of all retinal layers.

Reactive Proliferation and Chronic Inflammatory Changes

Third phase of inflammation is generally reparative in nature.It involves reactive proliferation of parenchymal tissue and replacement with fibrous tissue. The reparative process may result in restoration of normal function, but more often results in destructive sequelae.

Reactive proliferation of the retinal pigment epithelium can result in the formation of chorioretinal scars, cyclitic membranes and Dalen Fuchs nodules. The proliferating pigment epithelial cells undergo metaplasia, lose their melanin granules and assume a spindle shape. There is extensive deposition of collagen fibres due to fibrous metaplasia of the pigment epithelium. Basement membrane material is found within the fibrous bundles because pigment epithelium has the capability to produce basement membrane material. Osseous metaplasia of the RPE may occur especially in phthisical eyes.

Proliferation of iris pigment epithelium can cause posterior synechiae formation between the iris pigment epithelium and anterior lens capsule. Secondary proliferation and fibrous tissue metaplasia of the lens epithelium can produce anterior capsular cataract.

Cyclitic membranes are formed by proliferation of nonpigmented ciliary epithelium and may extend across the entire width of the eye. Proliferation of the pars plana epithelium anterior to the ora serrata is associated with long standing retinal detachment. The mounds of proliferated RPE beneath the detached retina is known as ringschwiele.

Perivascular pseudoretinitis pigmentosa occurs secondary to several types of disease entities including various inflammatory processes. Proliferating retinal pigment epithelium migrates into the retina in a perivascular fashion.

Dalen Fuchs nodules are focal cellular accumulations that often take the form of a pile of cannon balls that accumulate between Bruch's membrane and an elevated RPE. Typically seen in sympathetic ophthalmia. It consists of collection of epithelioid histiocytes beneath the RPE (Fig. 14.2).

Reactive proliferation of vascular connective tissue also occurs. Potential sites of neovascularisation and the proliferation of fibrovascular tissue are:

1) Anterior surface of iris stroma producing rubeosis iridis.
2) Inner surface of the ciliary body producing cyclitic membrane.
3) The surface of peripheral retina as in pars planitis.
4) Surface of the posterior retina and optic nerve head producing vasoproliferative retinopathy.
5) The choriocapillaries producing subretinal neovascular membrane.

Rubeosis Iridis

It consists of neovascular budding from the anterior surface of the iris stroma. Appears most often at the pupillary margin or on the surface of the far peripheral iris. This fibrovascular membrane on the anterior iris surface may undergo contraction, that results in tugging of the posterior iris pigment epithelium from the posterior surface of the iris around the pupillary margin onto the anterior surface resulting in *ectropion uvea*.

Chronic inflammatory process of the pars plana typically causes proliferation of the ciliary epithelium and fibrovascular tissue. These epithelial and fibrovascular elements migrate along the anterior hyaloid surface towards the equator of the lens eventually forming the complete cyclitic membrane in the retrolental space. Contraction of the myofibroblasts within the membrane may exert traction on the ciliary body and produce a ciliochoroidal detachment.

PROLIFERATIVE VITREO RETINOPATHY

Proliferative vitreoretinopathy is characterized by proliferation of fibrovascular tissue and neovascularisation and is due to -

1) Organization of intraocular hemorrhage, exudates, inflammatory infiltrates and other noxious substances with associated formation of granulation tissue.
2) Retinal neovascularisation resulting from intraocular hypoxia or ischemia.

Neovascularisation generally occurs as a response to relative lack of oxygen. The combined presence of both viable tissue in the affected region and partial hypoxia induces the formation of an angiogenic factor that initiates the neovascularisation process. Clinically and histopathologically proliferative vitreoretinopathy has two distinct characteristics:

1) An abnormal location in which new vessels are present, where they normally do not exist.
2) Increased vascular permeability.

In contrast normal retinal vessels have an intact lining with tight junctions formed by zonula occludens, which prevent the leakage of fluid into the surrounding retina. These tight junctions are absent in neovascular vessels and extravasation of fluid and lipid occurs. The vascular channels break through the internal limiting membrane of the retina and grow along the surface of the retina. In addition to this flat growth the buds often proliferate anteriorly onto the vitreous scaffolding forming the arborizing fibrovascular fronds, which are typical of proliferative retinopathy.

The preretinal membranes are composed of proliferating vascular elements, vitreous hyalocytes, retinal glial elements and macrophages derived from the blood. When simultaneous retinal defects are present, metaplastic proliferating retinal pigment epithelium may grow through these holes to form fibrovascular membrane.

Sub retinal neovascularisation: It is a common sequelae of inflammation of the retina and choroid. Almost all focal inflammatory processes of the choroid and retina can induce neovascularisation derived from the choroid. This is particularly true when basement membrane attachments to the RPE are compromised or there is interruption of Bruch's membrane. Sub-retinal neovascularisation can lead to subretinal hemorrhages.

Reactive gliosis: refers to reactive proliferation of glial tissue occurring in response to damage to the pigment epithelium. The cells of the RPE are strongly inclined towards reactive hyperplasia in contrast to the elements of sensory retina. Massive retinal gliosis of retina is a relatively unusual process. It involves a massive proliferation of retinal glial tissue often simulating a tumor. It may occur following hemorrhage or severe intraocular inflammation.

Fungal Granuloma

Fungal infection of retina, choroid and vitreous may be seen in post-operative patients, patients with systemic mycotic infections, IV drug users and patients with surgical or non-surgical ocular trauma. It can occur as chronic panuveitis or as fungal endophalmitis. There are fluffy deep retinal or choroidal yellow white lesions and the vitreous may contain fungal abscesses. Commonly isolated organisms are candida, fusarium, aspergillus and mucormycosis.

Candida endophthalmitis is most common cause of disseminated fungal infection. It begins as focal yellow white, deep retinal or choroidal infiltrative lesions. Usually multiple, often there are one or more associated vitreal abscesses or vitreous fluff balls. HPE reveals neutrophils, macrophages, lymphocytes and either yeast forms or pseudohyphae consistent with Candida. Non-purulent granulomatous intraocular inflammation can also be seen with lymphocytes, macrophages, multinucleated giant cells and fibrosis.

Mucor mycosis is a saprophytic fungus. It can complicate diabetes. Infection begins in the paranasal sinuses and then invades the orbital tissue secondarily. Large non-septate hyphae invade vessels producing thrombosis and necrosis. Other organisms causing endogenous fungal endophthalmitis include coccidiodomycosis, cryptococcus, histoplasmosis, and blastomycosis (Figs 14.4 and 14.5).

CORNEAL DYSTROPHIES

Dystrophy denotes an inherited, relatively symmetric bilateral disease that is unassociated with vascularisation or inflammation in its early stages. Pathology appears to be localized to an ocular tissue. It is not evident at birth but becomes clinically evident later in life.

Topographic Classification

1) Epithelial-
   a) Epithelial basement membrane dystrophy.
   b) Meesman's dystrophy.

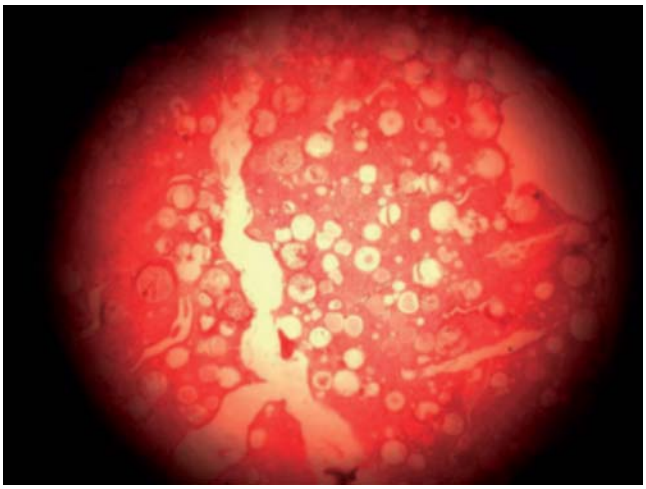


Fig. 14.4: Rhinosporidiosis showing multiple sporangia in varying stages of maturation—mature sporangia contain endospores

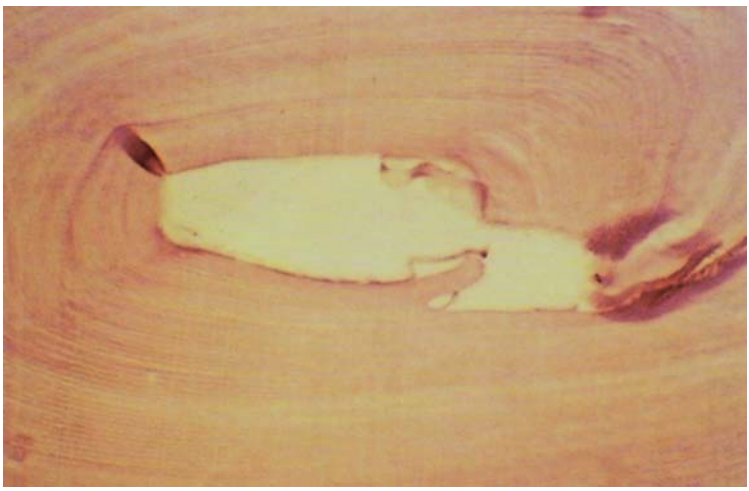


Fig. 14.5: Hydatid cyst shows outer non-nucleated layer composed of laminated hyaline layers

2) Bowman's layer / superficial stroma (subepithelial)
   c) Familial subepithelial amyloidosis.
   d) Reis Buckler's dystrophy.
3) Stroma
   e) Central crystalline dystrophy.
   f) Fleck dystrophy.
   g) Granular dystrophy.
   h) Lattice dystrophy- type1, type2, type3, type3a.
   i) Macular dystrophy- type1, type2.

4) Endothelium
   j) Congenital hereditary endothelial dystrophy.
   k) Fuchs dystrophy.
   l) Posterior polymorphous dystrophy.

Corneal epithelium is rich in genetic protein product keratoepithelin. Variations in the aggregation or precipitation of the several mutant forms of keratoepithelins in the cornea result in granular, lattice, avellino and Reis Buckler dystrophy.

Meesman's Dystrophy

It is characterized by presence of myriad small punctate vacuoles in the corneal epithelium. *HPE*- both corneal epithelium and its basement membrane are thickened. Epithelium has a disorderly appearance and contains cells that have a hyalinized appearance with small intra epithelial cystoid spaces.

Anterior Basement Membrane Dystrophy and Map-dot Finger Print Dystrophy

It shows spectrum of epithelial abnormalities like- reduplication, intraepithelial segments of corneal epithelial basement membrane, intraepithelial cystoid spaces filled with devitalized cellular debris and focal epithelial scarring. The *subtypes* are-

Cogan's microcystic dystrophy- shows multiple intraepithelial cysts filled with white putty like cellular debris. The devitalized cells that fill the cystoid spaces are trapped by duplication of the epithelium, which prevents their desquamation.

Finger print subtype shows parallel lines representing basement membrane material separating sheets of duplicated epithelium.

Map like changes are due to irregular, geographically shaped areas of sub-epithelial scarring. Corneal epithelial basement membrane is often thickened.

Reis Bucklers Dystrophy and Thiel-Behnke's Honey Comb Dystrophy

It affects epithelium, Bowman's layer and anterior stroma. Clinically recurrent erosion is seen. *HPE*- epithelium is irregular in caliber and shows saw toothed appearance. Thick multilaminated pannus composed of alternating layers of collagen and an abnormal eosinophilic material. More eosinophilic than normal collagen resembling colour of rock candy deposits of granular dystrophy. This elevates the epithelium anterior to the plane of Bowman’s layer and Bowman’s layer is usually destroyed.

Difference- Reis Buckler's dystrophy resembles superficial variant of granular corneal dystrophy- keratoepithelin forms small, sharply angulated crystalloids (Masson's trichrome positive) . Subepithelial deposits in honeycomb dystrophy are less pronounced, and show less intense positivity with Masson's trichrome.

Primary Gelatinous Drop-like Dystrophy (Familial Subepithelial Corneal Amyloidosis):

Focal mounds of amyloid seen in sub epithelium and superficial stroma (positive staining with Congo red). Overlying epithelium is atrophic. Bowman's layer is usually disrupted. Amyloid is composed of the antimicrobial protein lactoferrin produced by the lacrimal gland.

Dystrophies of Corneal Stroma

Granular dystrophy: (Bucklers Type I)

It is the most benign of dystrophies of cornea. Visual loss develops late in life. Multiple white crumbs or ring shaped opacities are seen in central cornea of both eyes. Most are superficial opacities separated by clear corneal stroma, hence excellent visual acuity. *HPE*- mutant keratoepithelin resembles hyaline 'rock candy'. More eosinophilic, less PAS positive than surrounding normal stroma. Intense red staining with Masson's trichrome. Granular dystrophy can recur in the graft after keratoplasty- then the granular material typically accumulates in the anterior cornea underneath the epithelium.

*Lattice Corneal Dystrophy Type1 (*Bucklers *Type II)*

It is a form of amyloidosis confined to the cornea. Amyloid is a mutant form of keratoepithelin clinically. It appears as a latticework of branching relucent lines in corneal stroma. HPE shows smudgy round or oval deposits of eosinophilic amyloid material in the stroma. Lattice dystrophy can recur in the corneal graft. Amyloid first accumulates superficially underneath the epithelium and in suture tracts.

Lattice Corneal Dystrophy Type II- Meretoja Syndrome

It is a form of familial amyloidosis not related to keratoepithelin.

Amyloid deposits are located in mid-peripheral cornea and in corneal nerves. They are composed of a protein called gelsolin, which is involved in actin metabolism. Patients have amyloid neuropathy with cranial nerve palsies, dry itchy skin and mask like ' hound dog ' facies with protruding lips.

Macular Corneal Dystrophy

It is the most severe of the classic corneal stromal dystrophies. Clinically, it appears as grayish opacities with indistinct borders seen in superficial stroma. The stroma between the macules is diffusely hazy. It is a type of localized corneal mucopolysaccharidosis. Patients lack keratan sulphate in their serum and cartilage. Keratan sulphate is a major constituent of corneal ground substance. Lack of this results in abnormal hydration of the corneal stroma. So, the cornea appears thinner than normal and is hazy and poorly hydrated in systemic mucopolysaccharidosis (Hurler's disease).

Cornea is cloudy and massively thickened

HPE- cytoplasm of keratocytes and corneal endothelial cells show frothy vacuolated appearance in hematoxylin and eosin due to accumulation of insoluble non-sulfated keratan. Also seen are large extracellular deposits in the subepithelial stroma, which appear as large vesicular granules that are mildly basophilic and PAS positive. Acid mucopolysaccharide can be demonstrated by colloidal iron and Alcian blue stain.

Schnyder's Crystalline Dystrophy

It shows needle shaped polychromatic crystals of cholesterol in the anterior corneal stroma, seen as subtle pattern of stromal vacuolization in routine sections, lipid being dissolved during processing. Some patients have elevated serum lipids and xanthelasma.

Francois Neetan's Fleck Dystrophy

It is an incidental clinical finding- unilateral. Visual acuity is not affected. Keratinocytes are swollen and contain mucin and lipid.

Congenital Hereditary Stromal Dystrophy

Shows bilateral corneal clouding. Cornea is of normal thickness but its collagen fibres are one half of normal diameter.

Pre-Descemet's dystrophy shows fine flour like opacities called cornea farinata. It is thought to be an age related degenerative change.

In deep filiform dystrophy, keratocytes are enlarged and contain fat and phospholipid inclusions.

Corneal Edema, Bullous Keratopathy and Endothelial Dystrophies

Corneal edema can occur in corneas with damaged or dysfunctional endothelium (endothelial decompensation) due to aphakia, pseudophakic bullous keratopathy, and corneal graft failure. Primary endothelial disease can also cause corneal edema. Endothelial dystrophies include Fuch's dystrophy (common), congenital hereditary endothelial dystrophy and posterior polymorphous dystrophy.

HPE of edematous cornea- Cornea is thickened with partial obliteration of artifactitious intralamellar stromal clefts. Lamellar margins are indistinct. edematous stroma is pale and has frothy appearance- "cotton candy". As secondary epithelial edema develops basal cells have pale edematous appearance. Fluid accumulates in intercellular spaces and beneath the epithelium, forming focal bullous areas of epithelial detachment called bullous keratopathy.

A degenerative pannus is found in many corneas with chronic edema and bullous keratopathy. It is seen as opaque layers of connective tissue between base of the epithelium and Bowman's layer, which is intact.

Fuch's Endothelial Dystrophy

It is the most common corneal dystrophy and presents during adult life with corneal edema and bullous keratopathy. It is due to primary defect in the corneal endothelium. Descemet's membrane is typically thickened and studded with anvil or mushroom shaped guttate excrescences of abnormal basement membrane material produced by dystrophic endothelial cells.

HPE- central cornea shows guttae that have been buried by a newly elaborated posterior layer of Descemet's membrane. Descemet's membrane shows thickening and multilamination. Rarely, Descemet's membrane lacks guttate excrescences and appears diffusely thickened. Endothelium is atrophic. Moderate number of corneal endothelial cells usually persist. Cytoplasm of the residual endothelium contain round granules of melanin pigment from iris pigment epithelium.

Pseudophakic Bullous Keratopathy: Latrogenic Disease

It is due to direct or delayed endothelial damage associated with cataract surgery and IOL implantation. It is the most common cause of corneal transplantation in recent years.

HPE- Descemet's membrane is not thickened and is regular in caliber. No guttate excrescences. Endothelium is severely atrophic or totally absent. Bullous keratopathy may be severe and some cases show total epithelial desquamation. Degenerative pannus formation is not so frequent as pseudophakic bullous keratopathy has a more acute course.

Identical picture of corneal edema is seen in failed corneal transplants. Eosinophilic retrocorneal fibrous membrane is found on the posterior surface of Descemet's membrane.

Congenital Hereditary Endothelial Dystrophy: (CHED)

It presents as massive corneal edema. Endothelium may be normal or atrophic. Massive thickening of Descemet's membrane seen in some cases.

Posterior Polymorphous Dystrophy

There is no corneal edema.Endothelium is hypercellular and shows multilayering. The abnormal endothelial cells express surface epithelial cytokeratins.

DEGENERATIONS OF CORNEA

Variety of secondary corneal degradations initiated by inflammatory, traumatic (accidental or surgical), metabolic or aging processes occur. Degenerations are classified as -

Epithelial and subepithelial degeneration.

Stromal degeneration.

Epithelial and subepithelial degeneration

1. Keratoconjunctivitis sicca and xerophthalmia.
2. Exposure keratopathy and Dellen.
3. Neurotrophic keratopathy.
4. Pterygium.
5. Band keratopathy.
6. Degenerative and inflammatory pannus.
7. Salzmann's nodular degeneration.
8. Marginal degeneration.

Stromal degeneration

1. Keratoconus.
2. Acute keratoconus (hydrops) .
3. Pellucid marginal corneal degeneration.
4. Polymorphic amyloid degeneration.
5. Secondary amyloid degeneration.
6. Deep or posterior crocodile shagreen.

Keratoconjunctivitis sicca (Sjogren's syndrome) and xerophthalmia (Severe vitamin A deficiency) - Due to deficient or abnormal tear production. There is corneal drying, superficial punctate keratopathy and filamentary keratitis composed of strands of detached corneal epithelium and mucous.

There is epithelial thickening and opacification, accompanied by secondary subepithelial pannus and stromal haze or scar, leading to thinning and perforation. In late cases, epithelium shows histologic changes characterized by irregular acanthosis and epidermalisation. May be associated with subepithelial vascularisation, fibrosis, loss of Bowman's layer and inflammatory cell infiltration. When secondary infection occurs, partial or full thickness ulceration may be seen.

Exposure Keratopathy and Dellen

It is due to incomplete lid closure due to facial palsy, proptosis, eyelid deformity or coma due to incomplete lid closure, exposed epithelium becomes dry and undergoes punctate degeneration in interpalpebral zone. In severe cases larger areas of epithelium breakdown, leading to secondary infection with or without ulceration.

Dellen - Epithelium overlying a thinned area of dehydrated stroma exhibits punctate irregularities and results in depression of the surface called dellen.

Neurotrophic Keratopathy (Trophic Ulcer)

It is due to lesion of the trigeminal nerve that interrupts sensation and trophic impulses to the epithelial cells. There is persistent epithelial edema, vesicle formation and erosion. Stroma is diffusely hazy. Late vascularisation may also develop. Following breakdown of epithelium, healing is slowed. If untreated, secondary bacterial ulceration can occur.

HPE- In late or secondarily infected cases there is stromal scarring, vascularisation and acanthotic epithelial changes.

Calcific Band Keratopathy

It is a superficial opacity that extends across the part of cornea exposed in interpalpebral fissure. It is caused by dystrophic calcification of Bowman's membrane and the anterior stroma. It is seen in iridocyclitis, long-standing glaucoma, phthisis bulbi, vitamin D excess and hypercalcemia.

In early cases Bowman's layer is of irregular thickness with stippled deposition of calcium salts in the basement membrane of overlying epithelium, Bowman's layer and subjacent superficial stroma. In advanced cases, there is calcification and fragmentation of Bowman's layer. Subepithelial fibrosis may be seen.

Chronic actinic keratopathy (spheroidal keratopathy, Labrador keratopathy, Climatic droplet keratopathy):

It shows multiple spherules of yellow hyaline material in the anterior cornea. HPE shows drop like deposits of varying sizes seen as amorphous hyaline material that stains light gray or amphophilic with hematoxylin and eosin stain. The deposits stain intensely with elastic tissue stains and are considered as a form of elastic degeneration of the collagen.

Degenerative and Inflammatory Pannus

Term pannus is applied to a flat superficial scar of anterior cornea. There are two types- inflammatory and degenerative.

Inflammatory pannus occurs in trachoma. Here, Bowman's layer is destroyed and replaced by vascularised connective tissue with appreciable round cell infiltration.

Degenerative pannus occurs in corneas with chronic edema and bullous keratopathy. There is subepithelial fibrovascular membrane with minimal inflammatory cells and preservation of Bowman's layer. The vascular connective tissue of the pannus arises from the conjunctiva.

Salzmann's Nodular Degeneration

It is a secondary degenerative process of unknown cause. Corneal epithelium shows elevated white mounds of dense collagenous connective tissue. HPE- resembles a massive focal pannus. There are mounds of relatively acellular hyaline connective tissue that elevate the corneal epithelium anterior to the plane of Bowman's membrane which may be destroyed.

Marginal Degeneration

It begins in upper cornea adjacent to accompanying arcus senilis. Degeneration originates in the stroma. Initially, Bowman's layer and the peripheral superficial lamellae undergo fibrillar degeneration followed by thinning and partial

replacement with vascularised connective tissue. Deep lamellae eventually degenerate and only a thin fibrovascular layer separates the outwardly bulging Descemet's membrane from the epithelium.

Lipid Keratopathy

Lipid deposition occurs in the peripheral corneal stroma in arcus senilis. If patient is below 40 years, it can signify a hyperlipemic state. It is a secondary phenomenon caused by lipid deposition in a heavily vascularised stroma.

Corneal epithelial keratinisation and epidermalisation

It is due to deficient tear production or mucous secretion. It occurs in severe vitamin A deficiency. Dry white spots are seen at the limbus (Bitot's spots) , which may contain colonies of Corynebacterium xerosis.

HPE- shows irregular acanthosis of the epithelium, hyperkeratosis and formation of discrete ridges. Bowman's layer is destroyed and replaced by subepithelial fibrovascular tissue with mild chronic inflammatory cell infiltration (Inflammatory pannus).

Keratoconus

It is a bilateral degenerative disorder. There is progressive thinning or ectasia of the central stroma that imparts a conical configuration to the cornea.

HPE- sectioned cornea assumes an irregular wavy configuration. There is a central or apical thinning of the stroma (less than 1/10 th of normal thickness). Characteristic dehiscences in Bowman's membrane are common and appear wavy in configuration. Corneal epithelium usually is intact and irregular in caliber, with areas of thinning and compensatory hyperplasia. Descemet's membrane is thin. Endothelium is well preserved.

Acute Keratoconus (hydrops)

In advanced cases, rupture of Descemet's membrane and the endothelium results in severe stromal and epithelial edema with rapid onset of clouding of the cornea.

HPE- shows elastic retraction of the free ends of Descemet's membrane, which coils on itself and is thrown into folds.

Pellucid Degeneration of Cornea

It resembles keratoconus histopathologically, but is located in the periphery of the cornea.

Polymorphic Amyloid Degeneration

It resembles lattice dystrophy clinically and microscopically. HPE- Corneal epithelium and Bowman's layer are normal. Small amyloid deposits are present in the stroma.

Degeneration of Conjunctiva

Chronic irritation due to exposure to ultra violet actinic exposure damages the stromal connective tissue of the bulbar conjunctiva, which is exposed in the interpalpebral fissure and results in pinguecula and pterygium.

Pinguecula- it is a localized yellowish grey elevated mass close to the limbus on either side of cornea in the interpalpebral portion of bulbar conjunctiva.

Pterygium is similar in appearance and seen more often nasally and involves cornea also.

HPE- both lesions show similar features. Overlying epithelium can be thin from atrophy or thickened by reactive secondary changes of hyperplasia, hyperkeratosis, acanthosis, dyskeratosis or even dysplasia. Subepithelium shows accumulation of amorphous, eosinophilic staining, hyalinized or granular appearing material resembling degenerated collagen interspersed with coiled or fragmented fibres resembling abnormal elastic tissue (elastoid or elastotic degeneration). Stromal fibrocytes are increased in number.

Older lesions also show aggregates of proteinaceous substance, acid mucopolysaccharide and calcific concretions.

Conjunctival Amyloidosis

It is a localized phenomenon seen in healthy adults without systemic amyloidosis. It can involve any part of conjunctiva. Subepithelial amyloid can form circumscribed, polypoidal, yellowish waxy nodules on the epibulbar surface or diffusely infiltrate the substantia propria.

HPE- shows relatively acellular amorphous deposits of eosinophilic hyaline material. It can be confirmed by special stains for amyloid.

RETINAL DEGENERATIONS

Peripheral Retinal Degenerations

Peripheral chorioretinal degeneration (cobble stone or paving stone degeneration):

It is seen in more than 25% of individuals above 20 years. It is more common in myopes. Lesion appears as yellow white patches of chorioretinal atrophy. Borders are sharply demarcated, scalloped and often pigmented. Pigmentation is due to hyperplasia of adjacent RPE. Patches appear white because the overlying sclera is bared by the severe atrophy of RPE and choriocapillaries.

HPE- Outer retina is firmly adherent and welded to the inner surface of Bruch's membrane, which is devoid of RPE. Rods and cones are absent in the area of chorioretinal adhesions and replaced by gliosis. Underlying choriocapillaris is atrophic.

Peripheral microcystoid degeneration:

It occurs in two forms- typical and reticular.

In *typical* form there is a stippled pattern that corresponds to an array of interconnecting channels or lacunae in the peripheral retina just posterior to

the ora serrata. HPE- Outer plexiform and inner nuclear layer contains multiple cystoid spaces called Blessig- Iwanoff cysts, which are filled with hyaluronic acid and are separated by residual pillars of Muller cells. With time the spaces may spread vertically to involve the retinal layers above and below.

Reticular cystoid degeneration is seen posterior to typical cystoid degeneration and principally involves the ganglion cell layer and the nerve fibre layer. It may be the cause of reticular retinoschisis, where the split in the retina occurs in the nerve fibre layer.

Retinoschisis

There is splitting of the layers of sensory retina. Cystoid degeneration is called *schisis*, when the spaces measure more than 1.5 mm linearly. Typical degenerative schisis forms from typical cystoid degeneration involving the outer plexiform and inner nuclear layers.

Reticular schisis forms from reticular degeneration. With time the inner layer consists of only the internal limiting membrane and retinal vessels. This inner layer may form a large bullous separation.

Age Related Macular Degeneration (ARMD)

It causes loss of central vision because it involves the fovea. Peripheral vision is retained. Manifestations may be atrophic (dry) or exudative (wet) , both are part of the clinical and pathologic spectrum and hence seen in the same patient.

Dry (atrophic) macular degeneration is characterized by atrophy and death of the subfoveal RPE. This leads to photoreceptor degeneration and outer retinal atrophy. Bruch's membrane thickens with age and become disorganized. HPE- there is increased basophilia of Bruch's membrane due to calcification and a progressive increase in lipid deposition. With increased age wide banded collagen is also deposited in Bruch’s membrane.

Drusen are the initial clinical findings in ARMD. They are clinical markers for sick RPE and consist of mounds or accretions of abnormal extracellular matrix material that form on the inner surface of Bruch's membrane. They are probably synthesized by the RPE.

There are two types of drusen—hard and soft. *Hard* or cuticular drusen are discrete, round or globular mounds of homogenous deeply PAS positive hyaline material with thinning of the overlying RPE. They are found beneath the basement membrane of the RPE and are formed due to apoptosis of the photoreceptors after a variety of injuries.

Soft drusen have less well defined boundaries. They are identical to small retinal pigment epithelial detachments. The involved area is larger and more irregular than that of a hard drusen and the material appears granular and less uniform. There is diffuse deposition of material beneath the pigment epithelium called basal laminar deposits, which adhere loosely to the Bruch's membrane predisposing to RPE detachments, tears, neovascularisation and scarring.

With time the retinal pigment epithelial cells overlying drusen atrophy and the drusen become fibrotic and sometimes calcify.

Window defects—correspond to the thinned pigment epithelium over the apex of hard drusen in ARMD. They may be a precursor to areolar atrophy.

Areolar Atrophy

It is a "dry" type of macular degeneration. Histologically, there is loss of photoreceptors and the RPE, with adhesions of the outer plexiform layer against Bruch's membrane.

Serous Retinal Pigment Epithelial Detachment

It is actually a separation between the split layers of Bruch's membrane and results from enlargement and coalescence of soft drusen.

Subretinal neovascularisation and disciform scarring can result from ARMD, myopia, angioid streaks and ocular histoplasmosis.

Disciform degeneration- There is loss of photoreceptors and only few nuclei remain in the outer nuclear layer. There is proliferation of pigment epithelium with retention of pigmentation. Bruch's membrane is split; the outer portion overlies intact choriocapillaris. Blood vessels are seen interior to this portion of bruch's membrane within the disciform scar. Other degenerative changes include cystoid degeneration, lamellar and full thickness macular holes.

Subretinal neovascular membrane characterizes the exudative type of ARMD. Untreated subretinal neovascularisation occasionally undergoes spontaneous involution. More often the vessels leak or bleed forming serous or hemorrhagic detachments of the RPE.

Fibrous disciform scar formation, the end stage of exudative ARMD, usually results from the organization of hemorrhagic RPE detachment. HPE shows mature scars composed of mounds of dense collagenous connective tissue on the inner surface of Bruch's membrane. The collagenous scar usually contains vessels and aggregates of RPE cells. Massive fibrous dysplasia of RPE is seen in eyes with chronic retinal detachment. Bone formation (osseous metaplasia of the RPE) is almost always the rule in phthisical eyes. Subretinal neovascular membrane also complicates other conditions such as angioid streaks.

Angioid Streaks

They are idiopathic in 50% of patients. It may be associated with Pseudoxanthoma elasticum, sickle cell anemia, other hemolytic anemias and Paget's disease. Angioid streaks resemble blood vessels. It appears as irregular reddish-brown crack like lesions radiating outward from the optic disc.

HPE- the streaks correspond to discontinuities in Bruch's membrane, which is thickened and calcified at the level of elastic layer. Calcification causes the increased brittleness of Bruch's membrane, which predisposes to subretinal neovascularisation.

Macular Holes

Can be lamellar or full thickness. Clinically, appear as discrete, small round areas of retinal discontinuity in the fovea. Epiretinal membrane and small drusen are often present. Hole formation is due to detachment caused by tangential vitreous traction in the macular regions.

HPE- Macular holes show rounded margins. Sometimes proliferated RPE binds the margins creating a chorioretinal adhesion. Epiretinal membranes may be present. Photoreceptor segments extend close to the hole margins.

Hereditary Retinal Dystrophies (Vitreoretinal Dystrophies)

1) *Lattice degeneration:* The term is derived from lattice like pattern of criss crossing sclerotic vessels seen in 12% of lesions. There are oval areas of retinal thinning, which are sharply demarcated, circumferentially oriented and located anterior to the equator.
   HPE- Focal areas of retinal thinning seen. Inner retinal layers are atrophic. Internal limiting membrane is absent. A pocket of liquefied vitreous overlies the discontinuity in internal limiting membrane. Thick walled vessels are present. RPE shows hypertrophy, hyperplasia and intraretinal migration. The firm vitreoretinal adhesions to the margins of atrophic retina predispose to tractional retinal breaks and rhegmatogenous retinal detachment.
2) *Familial exudative vitreoretinopathy:* It resembles Coat's disease or retinopathy of prematurity. Retina has preretinal acellular fibrous membranes peripherally with retinal traction and macular dragging. Later there is intraretinal exudation, leading to total detachment. Peripheral retina is avascular.
3) *Stickler syndrome:* Striking feature is vitreous degeneration with syneresis (optically empty vitreous cavity). Fundus shows perivascular pigmentation overlying atrophic RPE, lattice degeneration and retinal breaks.
   HPE- Advanced cases show complete retinal detachment. Preretinal membrane extends through the retinal holes into the retrolental space.
4) *Wagner disease:* It shows degenerative fundus changes resembling retinitis pigmentosa and gliotic membrane formation, but without retinal detachment.

Photoreceptor Dystrophies

Retinitis Pigmentosa

Early symptom is decreased night vision (nyctalopia). Fundus shows RPE atrophy and characteristic bone spicule pigmentation arranged along retinal vessels, which are markedly narrowed. Waxy pallor of optic nerve is due to decreased vascularity. Macular edema, pre retinal membrane formation and optic disk drusen may be seen. Posterior subcapsular cataract may develop.

HPE- shows varying degrees of photoreceptor degeneration that initially affects the rods and ultimately the cones. Earliest changes occur in the equatorial

zone and then extend peripherally and centrally. Nuclei of photoreceptor cells migrate outward with subsequent degeneration of photoreceptors and atrophy of outer nuclear layer. RPE proliferates and invades the atrophic retina and grows in the space around retinal vessels forming perivascular cuffs of intensely pigmented cells (evident clinically as bone spicule pigmentation) and also in small clusters.

The endothelial cells of the surrounding retinal vessels become thin and develop fenestrations. Vascular walls are thickened and gliotic. The remaining retina adheres to Bruch's membrane. Cystoid macular edema, hole formation and epiretinal membrane may be seen. Gliosis is seen overlying the optic disc with retinal gliosis in advanced cases. Inner retina remains relatively more intact, but there is some loss of ganglion cells. Overlying vitreous contains pigment epithelial cells, uveal melanocytes, macrophages, retinal astrocytes and free melanin pigment.

Stargardt's Disease And Fundus Flavimaculatus: (Yellow Spotted Fundus)

They are part of the same disease and are a type of inherited macular dystrophy.
Clinically- Retina shows poorly defined yellow white linear or fish tail opacities at the level of RPE.
HPE- Opacification of RPE is due to massive accumulation of an abnormal lipofuscin like lipopigment, which is yellow brown in colour in the cytoplasm of the RPE cells which become intensely PAS positive. RPE cells are taller than normal and their nuclei are displaced towards the apex of the cells. Groups of enlarged RPE cells surrounded by smaller, relatively normal cells may be responsible for the yellow-flecked appearance of the fundus.

All the RPE cells contain yellow brown lipopigment but the lipofuscin is hidden by a relatively normal complement of apical melanin in the smaller cells. The pisciform aggregates of larger cells appear yellow because these grossly abnormal cells are relatively amelanotic. Macrophages or detached lipopigment laden RPE in the subretinal space could also contribute to the flecked retinal appearance. The massive accumulation of pigment probably contributes to RPE dysfunction and death of subfoveal RPE cells leads to photoreceptor degeneration and atrophic macular degeneration.

Vitelliform Dystrophy (Best's Disease)

In the early stages of the disease, a striking yellow deposit, which is smooth and round, resembling the yolk of an egg, is seen at the macula or elsewhere in the posterior pole. Visual loss develops when the egg "scrambles" becoming irregular in appearance and chorioretinal scarring develops.

Lipofuscin accumulates within the apices of the RPE cells. In the macular region an acellular fibrillar material has been found beneath the RPE. This may represent disordered shedding of photoreceptor outer segment or diffusely

impaired metabolism of RPE cells. Secondary subretinal neovascularisation may occur. Likely cause is a gene defect for a protein expressed in the photoreceptor outer segment. Pale macular lesions are seen in the adult form of vitelliform dystrophy, which is a retinal pigment epithelial dystrophy.
HPE - RPE may show atrophy and hypertrophy with fibrosis or retinal pigment epithelial nodular proliferation.

Pattern Dystrophies

They are congenital abnormalities of the RPE. Depending on the arrangement of pigment they are-
1) Butterfly shaped dystrophy.
2) Maculoreticular dystrophy.

Choroidal Dystrophies

There is primary selective atrophy of the choriocapillaris. Loss of the choriocapillaris in turn causes loss of RPE and outer retina. Junction between involved and uninvolved regions is sharp and abrupt. Involved areas are pale with prominent sclerotic choroidal vessels.

Gyrate Atrophy

It is due to defect of the enzyme ornithine aminotransferase found in mitochondrial matrix. Fundus shows confluent, sharply demarcated areas of depigmentation in the mid periphery.
HPE- Retina shows abrupt photoreceptor and pigment epithelial loss in the atrophic area with focal pigment epithelial hyperplasia.

Parsplana Cysts

They are innocuous acquired degenerative lesions seen in 1/3 rd of normal people above 70 years of age. They are found incidentally. They are formed by detachment of the inner nonpigmented layer of ciliary epithelium and contain hyaluronic acid in normal individuals.

Multiple pars plana cysts occur in patients with multiple myeloma. The cysts contain Bence Jones protein and on fixation appear as milky white opacification due to precipitation of protein.

HYPERTENSIVE AND ARTERIOSCLEROTIC RETINOPATHY

Arteriolar sclerosis and hypertension are separate phenomena, but usually occur together. They are graded separately from grade I to IV. Hypertensive retinopathy is due to vascular incompetence and breakdown of the blood retinal barrier. Acute severe elevation of blood pressure causes retinal arteriolar narrowing and focal vasospasm which when persistent causes necrosis of the muscular and endothelial coats of the vessels.
HPE- Shows endothelial damage with resultant retinal edema and necrosis of smooth muscle and insudation of fibrin rich plasma in the vessel wall. There

may be exudation and even serous retinal detachment. Small exudates called edema residues may form a stellate pattern around the fovea (macular star figure). Retinal hemorrhages and papilledema are additional manifestations of hypertensive retinopathy.

Grade I - There is thickening of the arterioles so that the blood column appears narrower than normal.

Grade II - There is increased narrowing and focal spasm of the arterioles.

Grade III - In addition there are hemorrhages at any level of the retina, cotton wool spots (microinfarctions of the nerve fibre layer) and hard exudates that may form a macular star.

Grade IV - All the above changes along with papilledema.

Fibrinoid necrosis caused by the insudation and accumulation of plasma proteins in vessel walls may affect retinal and choroidal vessels. Microinfarctions of the nerve fibre layer (cotton wool spots) occur due to occlusion of small damaged vessels. Occasionally larger areas of retina show infarction. Focal choroidal infarction with patchy proliferation of RPE is evident clinically as Elschnig spots and Siegrist lines (increased pigmentation along a sclerotic vessel) – unknown lesions.

Arteriolar Sclerotic Changes

Grade I - Subintimal hyaline deposition and thickening of the vascular wall (increased light reflex).

Grade II - Arteriolar- venular crossing defects are seen in addition.

Grade III and IV - Above changes and increased thickening of the arteriole, so that the blood column is narrowed.

The increased light reflex gives a coppery appearance to the vessel, "copper wire" change in grade III and "silver wire" change in grade IV. Low grade chronic hypertension induces fibrosis in the walls of retinal arterioles.

HPE- the vessels are encompassed by a thick mantle of collagenous connective tissue referred to as "onionskin".

Like the surrounding neurosensory retina, the walls of the healthy retinal vessels normally are transparent. Retinal vessels are seen as columns of pigmented RBCs filling the lumina. Progressive accumulation of connective tissue in the vessel walls in retinal arteriolar sclerosis gradually obscures the blood column. The light reflex is widened and imparts an orange or coppery hue to the arterioles (copper wire in grade III lesions).

If the process is prolonged and severe, perivascular fibrosis may totally hide the blood column and vessels appear as white lines (silver wire change) in grade IV lesions. Arteriovenous crossing defects (A-V nicking) results when the opaque walls of thickened arterioles obscure part of the underlying venules. This is because the arterioles and venules normally share a common adventitial sheath where they cross. So, with thickening and increased rigidity of arteriolar wall the venular wall is compressed. Clinically, this appears as a gap in the course of the venules where the arteriole crosses it.

OPHTHALMIC PATHOLOGY OF DIABETES MELLITUS

Pathologic features of diabetes mellitus in the eye are due to two basic and partially interrelated mechanisms- thickening of the basement membranes and ischaemia.

Thickening of basement membrane is due to the activation of aldose reductase by the persistently elevated glucose levels. Ischemia may result from the pathologic thickening of the basement membrane of the retinal capillary endothelium. The most severely affected ocular tissues are the retina, vitreous, choroid and corneal epithelium.

Diabetic retinopathy is the retinal manifestation of the generalized microangiopathy that occurs throughout the body in diabetes mellitus. Three forms are recognized clinically:

1) Background retinopathy- initial stage of diabetic retinopathy marked by retinal edema, hemorrhage, exudates and capillary microaneurysms.
2) Preproliferative retinopathy- Shows preponderance of soft exudates or cotton wool spots heralding progressive retinal ischaemia.
3) Proliferative retinopathy- Retinal and vitreoretinal neovascularisation occurs, which predisposes to blinding complications like vitreous hemorrhage and tractional retinal detachment.

Diabetic retinopathy is a disorder of the retinal vasculature characterized by thickening of the endothelial basement membrane, loss of pericytes, micro aneurysm formation, capillary closure and neovascularisation. The clinical features of diabetic retinopathy-edema, exudates, hemorrhage and cotton wool spots are secondary to these retinovascular changes and are due to breakdown of the blood retinal barrier.

Primary Retinal Changes

1) *Basement membrane thickening*- Earliest change in the retinal vessels is thickening of the basement membrane of the capillary endothelium, which is evident by narrowing of the lumen of the vessel. Evidenced by increased PAS positive staining.

2) *Loss of pericytes*- is directly related to hyperglycemia. Normal retinal capillaries are composed of endothelial cells and pericytes in a ratio of 1:1. Endothelial cells have oval pale staining nuclei. Pericytes have round dark staining nuclei. Pericytes have contractile properties that regulate capillary caliber and the flow within the retinal microcirculation. They are found in capsules in the perivascular basement membrane. Pericytes are lost preferentially in the early stages of retinopathy thus permitting formation of hypercellular microaneurysms. Totally acellular areas of retinal capillary bed devoid of both endothelial cells and pericytes are also found. HPE of such areas reveals inner ischaemic retinal atrophy.

Retinal capillary pericyte loss results in retinal capillaries losing their ability to autoregulate, leading to changes in retinal blood flow. In addition,

pericytes have an inhibitory effect on vascular endothelial proliferation, which is mediated by transforming growth factor beta. Loss of this inhibitory effect may stimulate endothelial cell proliferation and neovascularisation.

3) Microaneurysms- Earliest recognizable clinical feature of diabetic retinopathy. Occur most abundantly in the posterior pole and surrounding acellular capillary beds. They are a potential site for plasma leakage, which can accumulate within the retina producing macular edema and lipid exudates. They consists of fusiform or saccular dilatations at the capillary level, 50 to 100 microns in size.

   HPE- Microaneurysms progress from a hypercellular endothelially lined state to an acellular hyalinized state. They occur due to weakening of the capillary wall secondary to focal pericytes loss. Walls of microaneurysm may break down producing intraretinal hemorrhages.

4) Acellular capillaries (capillary closure) - Trypsin digest preparations of retina in diabetes reveal that portion of the capillary bed consists of acellular basement membrane tubes which are due to obliteration of the lumen of the vessel by muller cell processes gaining access to the inside of these acellular tubes.

5) Intraretinal microvascular abnormalities (IRMA) - they are flat, intra-retinal vessels that extend from the terminal arterioles and venules towards zones of acellular capillaries. They may be single vessels or may be arranged in branching patterns or arcades. They have a markedly increased number of endothelial cells.

6) Neovascularisation- It is the defining characteristic of proliferative diabetic retinopathy and may develop intraretinally, preretinally or on the optic nerve head. The latter two are important clinically because these are the sites where vitreous traction on the neovascularisation may occur and subsequently result in vitreous hemorrhage and tractional retinal detachment.

Angiogenesis or formation of new vessels is stimulated by hypoxia due to closure of the retinal capillary bed and is mediated by various growth factors and inhibitors present in the retina and vitreous. Factors that play a role in neovascularisation in diabetes are-

1) Vascular endothelial growth factor (VEGF).
2) Basic fibroblast growth factor.
3) Acidic fibroblast growth factor.
4) Platelet derived growth factor.
5) Insulin like growth factor.
6) Endothelial stimulating angiogenic factor.
7) Transforming growth factor beta.
8) Various inhibitors of neovascularisation.

VEGF has been identified in ocular fluid from patients with active retinal and anterior segment neovascularisation. Neovascularisation originates from the venules and extends into the preretinal space. The new blood vessels become intimately associated with the collagen of the cortical vitreous.

The adherence of the adventitia of the new blood vessels to the vitreous collagen promotes preretinal and vitreous hemorrhage at the time of posterior vitreous detachment. The blood vessel growth is accompanied by the migration of other cellular elements into the vitreous cavity, many of which contains intracytoplasmic actin and is capable of cell-mediated contraction. Because the neovascular complex is tethered at one end by the retina and to the vitreous at the other, cell mediated contraction can result in tractional retinal detachments.

Secondary Retinal Changes

1) *Edema and exudates:* Retinal edema is due to leakage of plasma through microaneurysms and other vascular abnormalities. Water and electrolytes in the leakage are removed by RPE and retinal vascular endothelial transport mechanisms. But lipid remains in the retina in the form of "*hard exudates*". Microglial cells phagocytose lipid. Both edema and exudates are most abundant in the outer plexiform layer. Also present in inner nuclear and ganglion cell layer.
2) *Hemorrhages*: Hemorrhages in the nerve fibre layer of retina are *linear* because the extravascular blood becomes aligned parallel to the axons within this layer. Round *dot-and-blot* hemorrhages are seen in the nuclear and plexiform layer where they displace the neurons and glial cells and are limited at the periphery by undamaged neuronal and Muller cells. Clinically preretinal or vitreous hemorrhage is the most important type of hemorrhage. This hemorrhage may initially be limited to the preretinal space, however, it usually diffuses into the vitreous cavity. Blood within the vitreous cavity is broken down into hemoglobin globules and ghost cells. The ghost cells gain access to the anterior chamber and result in ghost cell glaucoma.
3) *Cotton wool spots:* They are microinfarctions of the nerve fibre layer. The ganglion cell layer and nerve fibre layers are thickened by a sharply circumscribed lesion and contain *cytoid bodies*, which are globular structure 10-20 microns in diameter. After resolution of the cotton wool spots, inner retinal ischemic atrophy may produce a local area of retinal thinning.
4) *Traction retinal detachment:* Persistent traction on the retina by a partially detached vitreous or cell mediated contraction from preretinal neovascular complexes can result in
   a) Schisis cavities within the retina.
   b) Avulsion of a retinal vessel.
   c) Detachment of the retina from the underlying RPE producing a tractional retinal detachment.

With prolonged detachment of the retina, the photoreceptor layers atrophy and the glial cells proliferate.

Histopathology *of diabetic retinopathy treatment following argon laser photocoagulation:*

There is variable destruction of the inner retina, destruction of the RPE and occlusion of choriocapillaris. These lesions heal by proliferation of the adjacent RPE to heal the defect and by glial scarring. Bruch's membrane is ruptured in few cases. Choroidal neovascularisation is a rare complication. Xenon photocoagulation results in full thickness retinal injury.

Surgical Treatment

Following vitrectomy to remove vitreous hemorrhage or to repair a traction detachment of the fovea, there is atrophy of the neovascular complexes on the optic nerve head and in the posterior retina. The peripheral vitreous is never completely removed. In some cases there is neovascularisation of the remaining anterior vitreous- anterior hyaloidal fibrovascular proliferation, which can extend across the posterior lens capsule and be the source of post vitrectomy hemorrhage and anterior tractional retinal detachment.

Changes In Iris In Diabetes Mellitus

There is non-progressive neovascularisation, which usually starts near the pupillary border and in the angle producing mild ectropion uvea. Neovascularisation may be more severe after vitrectomy resulting in peripheral anterior synechiae formation and subsequent development of neovascular glaucoma,

HPE- shows the presence of fine vessels on the anterior iris surface and ectropion uvea. With time the iris surface vessels may become covered with a basement membrane structure resembling Descemet's membrane. This membrane can extend across the trabecular meshwork creating a "pseudo angle". In chronic hyperglycemia, there is accumulation of glycogen in cystoid spaces within the iris pigment epithelium- *lacy vacuolization.*

Ciliary Body

There is thickening of basement membrane of the pigmented ciliary epithelium.

Choroid

Choroidal vascular changes in diabetes mellitus are-

a) Capillary dropout.
b) Beaded capillaries.
c) Arteriovenous anastomosis.
d) Neovascularisation.

Cornea

There is thickening of the corneal epithelial basement membrane, which can predispose to sheet like desquamation of corneal epithelium during vitreoretinal surgery. Patients are predisposed to recurrent corneal erosions and healing of epithelial defects is prolonged.

Risk of Infection

Mucormycosis can complicate diabetes mellitus. Mucor is normally a saprophytic fungus and can become pathogenic in poorly controlled diabetics who are acidotic. Infection begins in the paranasal sinuses and invades the orbital tissues secondarily. It can cause central retinal artery occlusion- unilateral or even bilateral.

Descriptive Terminology In Histopathology Reports

Acanthosis: Thickening of prickle cell layer of epidermis. It is a reactive phenomenon to underlying inflammatory, infectious or neoplastic processes.
Acantholysis: Intercellular spaces between squamous cells are widened due to intercellular and intracellular edema.
Atrophy: Decreased thickness of epidermis or dermis.
Atypia: Abnormal atypical appearance of the nuclei of individual cells in a disease process. Nuclear enlargement, hyperchromasia, irregularity of nuclear outline, prominent nucleoli, coarse granularity of intranuclear chromatin and enlarged nuclear to cytoplasmic ratio.
Dyskeratosis: Abnormal premature keratinisation of epithelial cells. Normal keratin is elaborated at the epidermal surface by mature squamous epithelial cells.
Dysplasia: Disordered arrangement of epithelium. Term is applied to a population of cells and should not be used in reference to a single cell. Dysplastic epithelium is characterized by a disturbance of the normal keratinocytic maturation sequence and is a common finding in actinic keratosis.

The individual cells within a dysplastic population is not necessarily atypical.
Hyperkeratosis: Increased thickening of the anucleate stratum corneum.
Hyperplasia: Increase in the number of cells in any given tissue.
Hypertrophy: Increase in cell size in any given tissue.
Papilloma: Any nipple shaped growth caused by an upward proliferation of subepidermal papillae e.g., seborrheic keratosis and verruca vulgaris. It shows fibrovascular core surrounded by acanthotic epithelium (Fig. 14.6).
Parakeratosis: Increased thickening of stratum corneum with incomplete keratinisation. It is a histologic evidence of rapid cell turnover. So epithelial cells retain their nuclei in contrast to hyperkeratosis. It is associated with underdevelopment or absence of the granular layer.

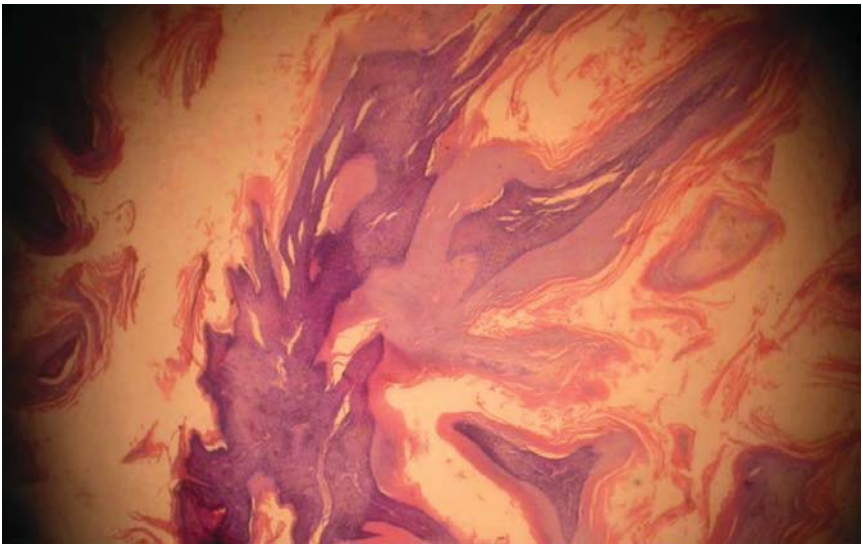


Fig. 14.6: Verruca vulgaris showing hyperkeratosis, papillomatosis and acanthosis with fibrovascular core

Pseudo epitheliomatous (carcinomatous hyperplasia): Irregular downward extension of acanthotic epidermis that mimics carcinoma.

BENIGN LESIONS OF EYELID

Benign Epidermal Lesions

True cysts—they have a circumscribed cavity lined by some type of epithelium and a cyst lumen that collects desquamated cells, cellular debris and cellular products (sebum, keratin, hair).

Epidermal inclusion cyst (Epidermoid cyst, Infundibular cyst)

It is the most common cystic eyelid lesion. Due to occlusion of infundibulum adjacent to the eyelash or accidental or surgical trauma, there is a sequestrum of epidermis trapped beneath the epidermal layer. Viable surface epithelium continues to shed keratin flakes and desquamated cells into the expanding unilocular cyst lumen. Cyst is lined by surface epithelium. The chronic mechanical effect of the cyst enlargement flattens the epithelial lining and promotes pseudocapsule formation. If the cyst ruptures, it usually incites a host foreign body giant cell inflammatory reaction.

Milia

They are small epidermal inclusion cysts less than 2 mm in diameter that arise at the base of the infundibulum of vellus hairs at the level of sebaceous duct. Histologically, similar to epidermal inclusion cysts.

Dermoid Cysts (Fig. 14.7)

It is due to trapping of surface ectoderm within the apposing sutures of the orbital bones. This heterotopic cyst is lined by epidermis and skin appendages. There is a cyst cavity filled with skin cells, keratin, sweat and sebaceous secretions and hairs. Ruptured cyst elicits an intense granulomatous foreign body tissue reaction.

Sudoriferous Cysts (Hidrocystoma)

They are benign cystic dilatations of sweat gland origin. They contain clear fluid and are transilluminant. With hemorrhage there is discoloration of the cyst. There are two types:

1) Apocrine hidrocystoma- It arises from sweat glands of Moll near the lid margin. It appears as single or multiple elevated translucent nodules. HPE shows a complex arborizing lumen lined by monolayer or bilayer of cuboidal epithelium, showing decapitation or apical snouting.
2) Eccrine hidrocystoma—it represents tissue retention cyst of sweat gland duct. It appears as solitary dilated cyst lumen with regular, smooth lining epithelium. Increased fluid pressure within may flatten the lining epithelium.

Squamous Papilloma (Skin Tag, Fibroepithelial Polyp)

Sessile or pedunculated proliferation of benign epidermis and is the most common benign eyelid lesion. It may be single or multiple and present on periorbital skin and lid margins. HPE- shows elongated tongues of vascularised loose connective tissue. They are covered by acanthotic squamous epithelium.

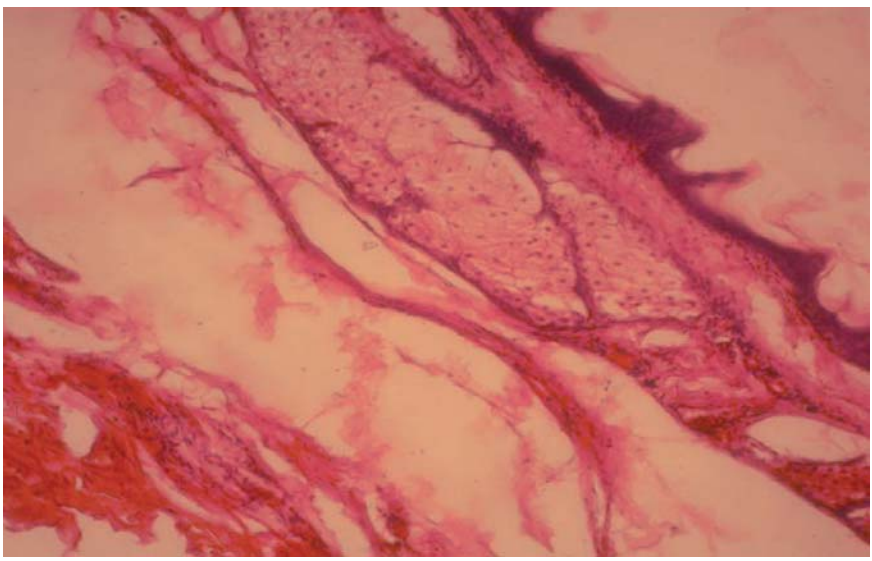


Fig. 14.7: Dermoid cyst: Cyst lined by epidermis and shows sebaceous lobules in the wall

Seborrheic Keratosis

It is seen in elderly patients as sharply demarcated superficial lesions that sit anterior to the plane of surrounding epidermis- like a button on the skin surface. It shows a greasy or scaly, "stuck on" appearance. It is often sessile and pigmented.

HPE shows upward papillomatous proliferation of basaloid cells resembling normal epidermal basal cells situated above the plane of surrounding skin. Under low power appears blue in color. Thick surface layer of keratin is present. Within the acanthotic epithelium circular spaces filled with keratin called "horn cysts" or pseudohorn cysts are found. They represent invagination of surface keratin - hyperkeratotic variant. In the adenoid variant, dermis shows interweaving bands of benign basal cells. Other histologic type is acanthotic type. Most tumors show combination of all three histologic types (Fig. 14.8).

Inverted Follicular Keratosis (basosquamous Cell Acanthoma)

It is considered to be an irritated form of seborrheic keratosis. It shows pseudocarcinomatous hyperplasia and has inverted cup shaped configuration and surface invaginations.

HPE shows marked hyperkeratosis. Proliferating basaloid cells are situated adjacent to concentric whorls of squamous epithelium (squamous eddies). Intercellular spaces between squamous cells are widened due to intracellular and intercellular edema, referred to as acantholysis.

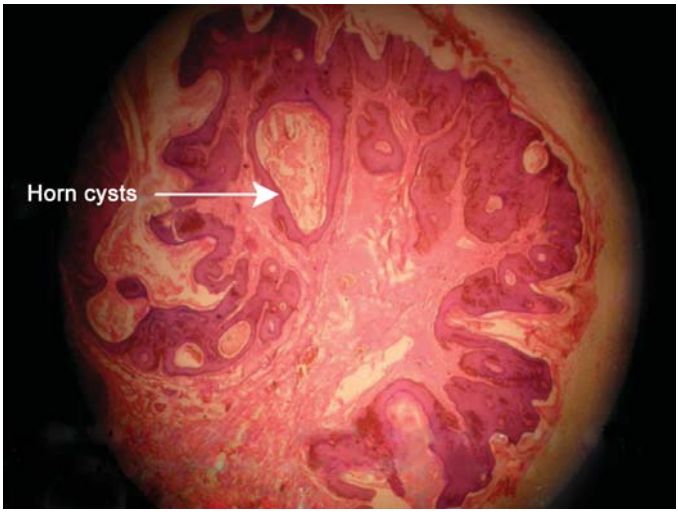


Fig. 14.8: Seborrheic keratosis: Shows squamous papilloma with hyperkeratosis, basilar acanthosis and intraepithelial horn cysts

Keratoacanthoma

It is a crater shaped squamous cell lesion and arises rapidly in elderly patients. It then undergoes spontaneous resolution. It was classified in the past as pseudocarcinomatous hyperplasia. It is now considered as 'deficient squamous cell carcinoma' that tends to involutes spontaneously. Rare cases invade deeply like squamous cell carcinoma. It is important to correlate the clinical history with tissue histopathology. It can arise along any hairy skin surface including periorbital skin. There is an increased incidence in AIDS patients.

Clinically a firm dome shaped nodule surrounds keratin filled excavation. There is rapid growth, which usually peaks by 8 weeks, then slowly involutes over a period of upto 12 months. Many patients will not tolerate the wait and justifiably seek its removal. It should be completely excised, because histopathologically it is similar to squamous cell carcinoma.

HPE shows central crater filled with densely packed keratin, enclosed by markedly acanthotic squamous epithelium, that typically extends like a lip or buttress over the side of the crater. The abnormal epidermal cells have pale, glassy cytoplasm. The presence of intraepidermal neutrophilic abscesses are helpful in distinguishing Keratoacanthoma from squamous cell carcinoma. Underlying dermis also shows intense band of inflammation. The base of a well-developed lesion appears regular and well demarcated. Margins are relatively smooth and pushing rather than infiltrative. Striking invasive acanthosis with abundant mitosis, focal atypia and dyskeratosis may predominate towards the periphery of the lesion. An incisional biopsy from this area will mislead to malignant interpretation.

A paradoxical finding for this benign lesion is perineural invasion. A helpful feature in diagnosis is that kratoacanthomas sit atop the undisturbed dermal sweat glands. An excision biopsy with adequate tumor free margins is ideal.

Pseudocarcinomatous Hyperplasia

It is a benign proliferation of epidermis stimulating an epithelial neoplasm. It is seen at the edge of burns or ulcers, near basal cell carcinoma, malignant melanoma and around chronic inflammation.

HPE- there is irregular invasion of the dermis by squamous cells. It may show mitotic figures. There is no dyskeratosis or atypia. Neutrophils are seen admixed with squamous cells.

Melanocytic Lesions of Eyelid

Junctional nevus Proliferation of benign nevus cells within the epidermis appearing as nests. They are darkly pigmented macules. Nevus cells are seen in the epidermal-dermal junction. Intradermal macrophages with ingested melanin seen (melanophages).

Compound nevus: It is lighter in colour and slightly elevated. Most congenital

nevi are compound nevi. Maturing nests of nevus cells descend into the dermis, so nevus cells appear within both the epidermis and the dermis. As the dermal migration continues, nevus cells lose their nesting tendency. Deep in the dermis, they appear as sheets of bland small blue cells resembling mature lymphocytes. Individual cells become compact with reduction of cytoplasm and melanin synthesis.

Intradermal nevus: It is pale tan in colour and more elevated. Nevus cells are totally confined to the dermis. A thin band of collagen is often seen between the intra dermal nevus and the uninvolved epidermis. Maturing nevus cells shrink, are less cohesive, exhaust their pigment and may resemble lymphocytes. Clinically it may present as a nodule or a sessile papule with hairs.

Blue nevus (Dermal melanocytoma): It is composed of slender, wavy, dendritic melanocytes. It rarely exceeds 1 cm in diameter. These cells produce abundant melanin pigment and extracellular pigment is ingested by dermal melanophages. Increased number of neighboring fibroblasts leads to focal dermal fibrosis.

Nevus of Ota (Oculodermal melanocytosis): It is a blue brown cutaneous discoloration seen along distribution of ophthalmic and maxillary division of trigeminal nerve, including eyelid skin, periorbital skin and ipsilateral conjunctiva and sclera, and is always unilateral. HPE shows increased number of melanocytes in the dermis.

Invasive malignant melanoma arising in eyelid skin is rare and is less than 1% of all primary eyelid malignancies.

Adnexal Lesions of Eyelid

Sebaceous adenoma

It is a well circumscribed benign lesion composed of multiple irregular glandular lobules with a biphasic cell population of mature sebaceous epithelium and lesser extent of basaloid appearing germinative cells (primitive adnexal epithelium) .

Sebaceous carcinoma (Meibomian gland carcinoma)

It can present as chalazion within the tarsal plate and is more common in women and Asians, so recurrent chalazia should be submitted for HPE. It rarely presents as chronic unilateral keratoconjunctivitis due to intraepithelial spread of tumor, which replaces conjunctival epithelium in a pagetoid fashion. 2/3 rd of cases arise in upper eyelid (because of greater mass of Meibomian gland tissue in upper eyelid). It is rare before 40 years. Incidence is same as squamous cell carcinoma of eyelid.

Gross: It is yellow in colour with nodular appearance resembling chalazion. It is well circumscribed, sometimes encapsulated or may be ulcerated or fungating infiltrative growth. It may completely replace the meibomian gland in the tarsus.

HPE-Tumor is seen as lobules and in sheets. Lobules resemble normal

sebaceous glands, in size and shape. There is no peripheral palisading. Nuclei of cells are larger, pleomorphic, hyperchromatic and more atypical. Mitotic figures are common and atypical. Cytoplasm of tumor cells has foamy vacuolated appearance. Large lobules show necrosis centrally- comedo carcinoma pattern. Tumor invades and replaces eyelid skin and conjunctival epithelium in pagetoid fashion (Fig. 14.9).

Spread is by direct extension to regional lymph nodes and to distant sites.

Benign Sweat Gland Tumors

Syringoma

It arises from eccrine sweat ducts and appears as multiple small soft papules involving lower eyelid. HPE- Dermis shows dilated sweat ducts, embedded in dense fibrous stroma. Ductal elements are round, oval or teardrop shaped and lined by attenuated bilayer of cuboidal epithelium. Lumen may contain amorphous material.

Pleomorphic Adenoma

It presents as a small firm, immobile dermal nodule and may also be seen in eyelid skin. HPE- Cords and nests of basaloid epithelium seen within a mucoid stroma. Tubular lumina are cystically dilated and surrounded by epithelial bilayer—inner cuboidal and outer-spindled myoepithelial layer. Myoepithelial layer produces stromal elements like cartilage, hyaline and dense fibrosis.

Other benign sweat gland tumors are—syringocystadenoma papilliferum, eccrine spiradenoma and clear cell hidradenoma.

Pilar Tumors

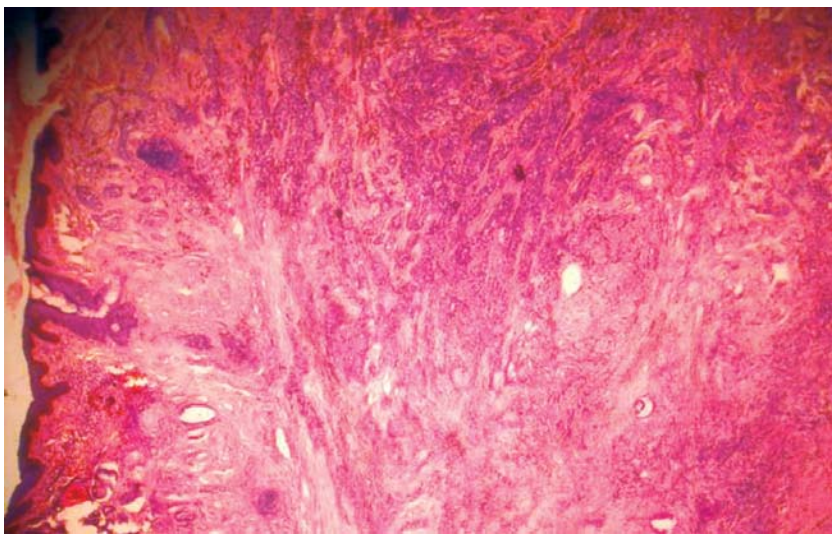


Fig. 14.9: Meibomian gland carcinoma—Lobules of malignant epithelial cells involving full thickness of eyelid.

They are tumors of hair forming elements.

Trichoepithelioma (Brooke's tumor) , trichilemmoma, trichofolliculoma, pilomatricoma (Benign calcifying epithelioma of Malherbe).

Fibrous tumors—fibrous histiocytoma, juvenile fibromatosis, angiofibroma.

Neural Tumors:

Neurofibroma

It may be isolated, diffuse or plexiform. Isolated neurofibroma is a haphazard accumulation of bland, elongated spindled cells with oval, wavy nuclei. Mast cells may be seen. Plexiform neurofibroma shows sharply demarcated nests of proliferating endodermal fibroblasts and Schwann cells in a myxoid background.

Vascular Tumors

Capillary hemangioma: It is seen in children as non-capsulated proliferation of capillaries and pericytes. Larger collecting vessels are also present (Fig. 14.10).

Cavernous hemangioma: It is seen in adults and composed of large dilated, endothelium lined vascular spaces filled with blood and separated by delicate septa that contain smooth muscle (Fig. 14.11).

Lymphangioma

It has endothelium lined vascular spaces without pericytes. Vascular endothelium is attenuated and incompletely lines the spaces. Significant stromal lymphoid aggregates or lymphoid follicles with germinal centers are seen. Blood or serous fluid may be seen within the irregular vascular spaces (Fig. 14.12).

Kaposi sarcoma in periocular region is indicative of full blown AIDS.

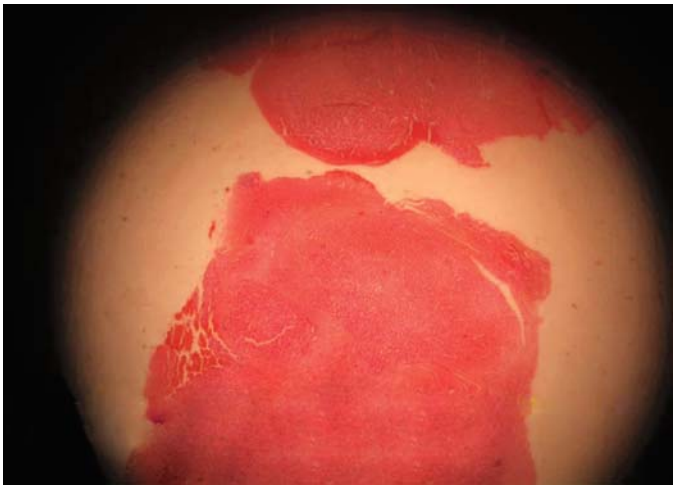


Fig. 14.10: Capillary hemangioma-non encapsulated proliferation of capillaries and larger collecting vessels

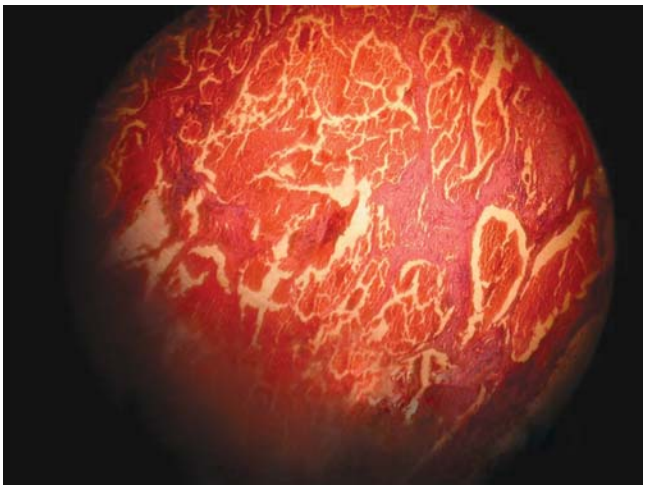


Fig. 14.11: Cavernous hemangioma—pseudocapsulated vascular lesion with large blood filled spaces separated by delicate fibrous septa

Fig. 14.12: Lymphangioma—lymphoid aggregates she with in fibrous sepfa that divide irregular thin walled endothelial lined fluid shones

Lymphoid Tumors

Benign reactive lymphoid hyperplasia shows polymorphous proliferation of mature lymphocytes, plasma cells and histiocytes.

Atypical lymphoid hyperplasia shows monotonous proliferation of lymphocytes with cytological irregularities.

Non-Hodgin's + lymphoma shows monotonous proliferation of neoplastic lymphocytes.

Multiple myeloma- In disseminated multiple myeloma eyelid and periorbital involvement is seen. Epidermis is spared and dermis shows sheets of atypical plasma cells with abundant Russell bodies.

Miscellaneous lesions- Metastatic cancer.

- Merkel cell tumor (Neuroendocrine carcinoma of skin) .

Pyogenic Granuloma (Fig. 14.13)

It shows exuberant proliferation of granulation tissue following accidental or surgical trauma. It is an exaggerated tissue healing response. It is a rapidly growing smooth, red purple lobulated mass. HPE- It is composed of radiating capillaries and fibroblasts with a mixed inflammatory infiltrate of lymphocytes and polymorphs.

Premalignant Condition of Eyelid

Actinic Keratosis - Senile keratosis

Actinic keratosis or solar keratosis is a premalignant squamous cell lesion. It develops on sun exposed skin of fair skinned middle-aged individuals.

Clinically it appears as scaly, keratotic, flat-topped or erythematous nodules. It sometimes shows a nodular, horny or warty configuration and measures only few mm.

HPE- epidermis is thickened and is replaced by atypical squamous cells. Parakeratosis is usually present and granular layer is absent. Irregular buds of atypical keratocytes extend into papillary dermis at the base of some lesions. Disease spares the opening of pilosebaceous units. Underlying dermis shows solar elastosis (elastotic degeneration similar to pterygium and pinguecula) and moderate lymphoplasmacytic infiltrates.

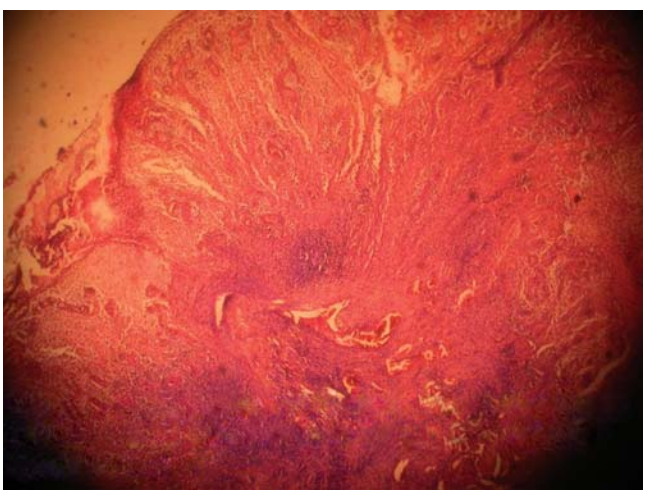


Fig. 14.13: Pyogenic granuloma—exuberant granulation tissue composed of fibroblasts, capillaries and a mixed inflammatory infiltrate

Patients often have other cutaneous premalignant and malignant lesions, including squamous cell, basal cell and adnexal carcinomas and malignant melanoma. If untreated 12 to 13% develop squamous cell carcinoma. Prognosis of squamous cell carcinoma arising in actinic keratosis is excellent because metastases very rarely occur (0.5%).

Important histologic feature in diagnosis is presence of actinic keratotic changes at the lateral epidermal margins of the invasive squamous cell carcinoma. Solar keratosis with squamous cell carcinoma is a separate non-aggressive entity distinct from squamous cell carcinoma arising de novo, which is capable of metastasis.

Histological types are:

- Hypertrophic
- Atrophic
- Bowenoid
- Solitary lichen planus like keratosis

Bowen's Disease of Eyelid

It is seen in fair skinned individuals at average age of 55 years. It is more common in men.

Clinically it appears as erythematous, pigmented, crusty, scaly, fissured keratotic plaques. Plaques are round, sharply demarcated, occasionally show heaped up margins. 2/3 rd of patients have single lesion and average size of lesion is 1.3 cm. 5% of cases show clinical and microscopic evidence of invasive carcinoma. 42% develop other cutaneous and mucocutaneous premalignant and malignant lesions—Actinic keratosis, basal cell carcinoma, squamous cell carcinoma and adenoid cystic (mucinous) carcinoma. 25% have primary internal or extra-cutaneous cancers. Early adequate excision of cutaneous lesions does not prevent subsequent development of systemic premalignant and malignant lesions. In order of frequency systemic lesions are respiratory system, gastrointestinal, genitourinary, reticuloendothelial, oral cavity, breast, endocrine, soft tissues and mucous membrane of conjunctiva and lip.

Lesions develop 6-7 years after Bowen's disease is noted or may co-exist at anytime. 75% of patients with Bowen's disease on follow up show evidence of primary systemic cancer. Strong evidence suggests inorganic arsenic (strong chemical carcinogen) as a cause of Bowen's disease. In addition, hereditary predisposition, exposure to petroleum by-products, trauma and cutaneous injury from ionizing radiation have been suggested.

HPE shows hyperkeratosis, plaque like acanthosis and hypogranulosis. Epidermis shows loss of normal polarity and architecture- atypical epithelial proliferation with hyper chromatic nuclei, multinucleated cells and vacuolated cells. Abnormal keratinocytic maturation is evidenced by abnormal mitotic figures and malignant dyskeratotic cells. The above changes occur at all levels

of the epidermis. It is confined by an intact dermo-epidermal basement membrane and the lesion represents intraepidermal squamous cell carcinoma (carcinoma in situ). The changes resemble that of actinic (solar) keratosis.

Difference- In Bowens serial sections shows involvement of the ducts of hair follicles and sebaceous glands. Lesions involve outer sheaths of hair follicles and eventually replace the sebaceous gland and cells. Sometimes, extensive skip areas of uninvolved epidermis are present. Presence of atypical vacuolated cells may be confused with sebaceous gland carcinoma.

Malignant Lesions of Epidermis of Eyelid

Squamous Cell Carcinoma

It is relatively rare compared to basal cell carcinoma. It affects elderly, fair skinned individuals and is common in lower lid margin. In upper eyelid and outer canthus, squamous cell carcinoma is more common than basal cell carcinoma. It may arise de novo or from intra epithelial carcinoma or actinic keratosis. It may also follow radiotherapy and in patients with xeroderma pigmentosum.

Clinically, It is an elevated, indurated plaque or nodule that tends to ulcerate and shows irregular borders. Well-differentiated tumors have grayish white granular appearance due to masses of keratin. Advanced cases metastasize to pre auricular and submandibular lymph nodes.

HPE—well differentiated tumors show polygonal cells with abundant eosinophilic cytoplasm. Dyskeratotic cells with formation of keratin pearls are seen. Nuclei are prominent, pleomorphic and hyperchromatic (Fig. 14.14). Spindle cell variant is rare. Cells are pleomorphic and hyperchromatic and spindle shaped, resembling fibroblasts. Cells extend into sub epithelial tissues from deeper layers of surface epithelium.

Basal Cell Carcinoma

It is the most common malignant tumor affecting periocular skin. It comprises 90% of all malignant eyelid tumors. It affects fair skinned adults and can occur in younger persons. It arises in sun-exposed skin and is thought to be caused by actinic damage. Basal cells normally contain 3 types of cells-

1) Basal cells.
2) Melanocyte cells- develop into nevi and malignant melanoma.
3) Primary epithelial germ cells—seen in the deepest layer of epidermis are derived from surface ectoderm and develop into basal cell carcinoma.

Basal cell carcinoma is 15-40 times more common than squamous cell carcinoma on eyelids. The site of occurrence in order of frequency is lower eyelid, inner canthus, upper eyelid and outer canthus.

Variants

1) Nodular type: It is a well circumscribed tumor with pseudo capsule and a firm elevated pearly white nodule with superficial telangiectatic vessels.

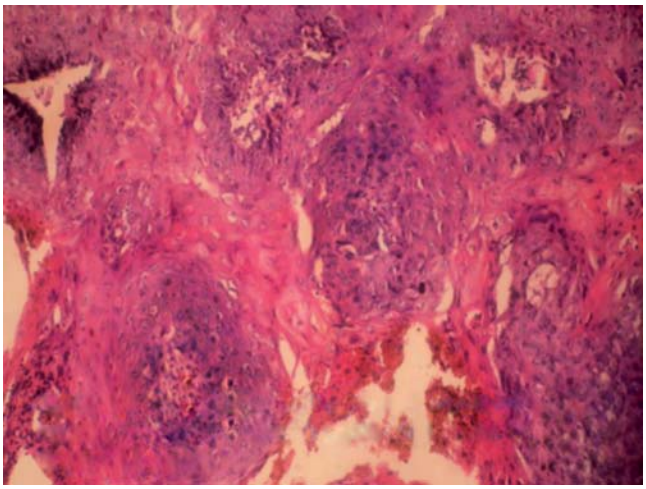


Fig. 14.14: Squamous cell carcinoma shows eosinophili nests of malignant squamous epithelial cells and foci of keratinization

HPE- however large the tumor, it does not extend deeply into the dermis or ulcerate the epithelium.

2) *Ulcerative type:* There is erosion of eyelids with absence of eyelashes. Advanced lesions have enlarging ulcers with prominent rolled out pearly white borders with distinct margins. HPE shows loss of epithelium centrally and invasion into dermis. *Nodulo ulcerative* type starts as nodular and as nodules increase in size central ulceration occurs.
3) *Sclerosing or morpheaform type:* It is a pale indurated plaque and resembles morphea (circumscribed form of scleroderma). It has indistinct margins and does not ulcerate till late. It shows widespread superficial multicentric involvement. HPE shows discrete islands of tumor cells encased in dense connective tissue network beneath an intact epithelium. Invasion into deeper structures of eyelid.
4) *Multicentric type:* It has diffuse irregular nodular surface with telangiectatic vessels. HPE shows diffuse multicentric involvement of epidermis extending into the superficial dermis. Subepidermal nests of tumor cells that are distinct from most of the tumor are also seen.

Histopathology of Basal Cell Carcinoma

Under light microscope it appears 'blue' and 'below' the surface. Neoplastic basaloid cells are arranged in large masses or fields, smaller nests or cords. Periphery of tumor lobules show characteristic peripheral palisading of nuclei (picket fence appearance). Empty spaces or clefts may be seen between tumor lobules and surrounding stroma due to shrinkage of mucin rich stroma during processing. Fibrous stroma separating tumor lobules are more fibrotic and denser than normal dermis- (desmoplasia) due to stimulation of fibrosis by tumor (Fig. 14.15).

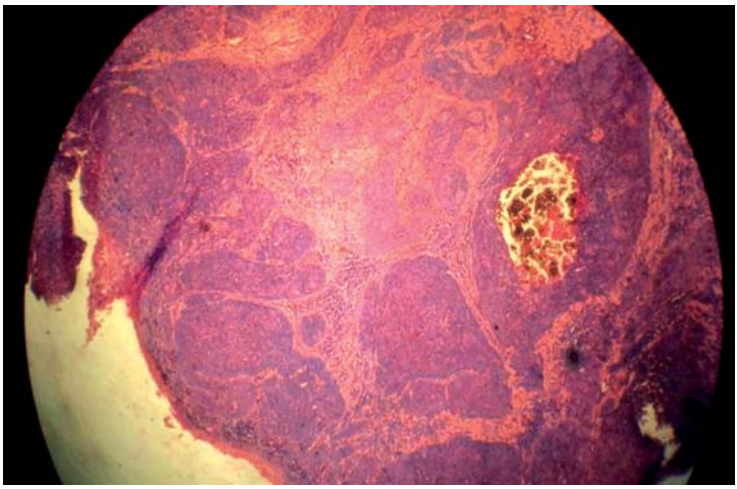


Fig. 14.15: Basal cell carcinoma. Shows nests, cords and lobules of neoplastic basaloid cells with peripheral palisading of nuclei

Histologic Variants

Tumor shows differentiation towards various epidermal appendages—

1) Adenoid cystic variant- mucin production is evident.
2) Keratotic basal cell carcinoma—keratinised horn cysts- pilar differentiation.
3) Sebaceous differentiation.
4) Pigmented basal cell carcinoma—cells contain melanin pigment.
5) Pseudo-cystic basal cell carcinoma- Necrobiosis in centre of a large mass of tumor cells.
6) Morpheaform basal cell carcinoma- Slender cords or tendrils of tumor cells embedded in densely fibrotic stroma.
7) Solid type.
8) Meta typical or basosquamous variant—more aggressive and shows infiltrative pattern.

Lesions in advanced cases shows extensive areas of ulceration called 'rodent ulcer'. It produces disfiguring facial destruction. Basal cell carcinoma rarely metastasizes. Metastatic tumors are of metatypical or basosquamous type. Deeply infiltrative, ulcerative lesions invade orbital bones and meninges causing secondary meningitis, which may be fatal.

In 0.7% of cases, multiple basal cell carcinomas occur on face and body of young patients. Nevoid basal cell carcinoma syndrome- basal cell nevus or Gorlin Goltz syndrome.

Other lesions in Gorlin Goltz syndrome-

Odontogenic keratocysts of jaw.

Bifid ribs.

Neurologic abnormalities.

Endocrine disorders.

Basal cell carcinoma in such patients may contain spicules of bone.

Skin Manifestations of Xeroderma Pigmentosum:

There are three stages-

1st stage - slight erythema with scaling and freckles.
2nd stage - mottled pigmentation with telangiectasia.
3rd stage - malignant neoplasms like squamous cell carcinoma, basal cell carcinoma, malignant melanoma and sarcoma develop.

HPE—first stage shows hyperkeratosis, thinning of malphigian layer and irregular hyperpigmentation throughout basal cell layer. Focal lymphocytic infiltration seen in upper dermis. In second stage, irregular patches of hyper pigmentation with acanthosis and atypical downward cellular proliferation are seen.

Malignant Melanoma of Eyelid

Constitutes 1% of malignant neoplasms of eyelid. Arises from epidermal melanocytes. There are four types-

1) Lentigo maligna (Hutchinson's melanotic freckle).
2) Superficial spreading melanoma.
3) Nodular melanoma.
4) Acral lentiginous melanoma.

Initial melanocytic proliferation is first horizontal (non invasive horizontal growth phase), followed by invasive vertical growth phase.

Clark's Prognostic Classification Based on Level of Invasion

Level 1 - tumor confined to epidermis with intact epithelial basement membrane.
Level 2 - tumor extends beyond basement membrane with early invasion of papillary dermis without abutting reticular dermis.
Level 3 - tumor fills entire papillary dermis and reaches interface between papillary and reticular dermis.
Level 4 - tumor penetrates reticular dermis.
Level 5 - tumor invades sub cutaneous tissues.

Malignant Melanoma of Uvea

It is the commonest primary intra ocular malignancy in adults. Men and women are equally affected and black persons are rarely affected. It is frequent in elderly and may arise from pre-existing nevi or de novo. The most common site is choroid and near the posterior pole.

Classification of Choroidal Melanoma: (Based on Largest Basal Diameter of Tumor)

1) Small—diameter is 10 mm or less and appears as focal discoid or oval area of choroidal thickening.
2) Medium—size is 11- 15 mm.
3) Large—more than 15 mm. Actively growing choroidal melanoma frequently rupture through Bruch's membrane into sub retinal space giving collar

button or mushroom configuration (diagnostic of uveal melanoma). Ruptured ends of Bruch's membrane exert a compressive effect on the waist of the tumor causing vascular congestion in the apex.

4) Diffuse flat choroidal malignant melanoma—occasionally malignant melanoma thickens the choroid without forming an elevated mass. It is less well differentiated, extends extraocularly invades optic nerve and has poorer prognosis.

Color and pigmentation

Commonly lesions are unpigmented or amelanotic. Most are light brown to grey and less commonly jet-black in color. Degree of pigmentation varies within different sites of same tumor and both heavily pigmented cells and relatively amelanotic cells may be seen.

HPE- two major types are seen-

1) Spindle cells are syncytium bipolar fusiform cells with long tapering processes and grow as parallel fascicles. They form a syncytium. The cytoplasmic margins of individual cells are indistinct. There are two sub-categories

   Spindle A and spindle B cells. Spindle A cells have bland, slender, cigar shaped nuclei with finely dispersed chromatin and indistinct nucleoli. Longitudinal fold in nuclear membrane is seen as a chromatin stripe or line in many cells. Spindle B cells are less differentiated than spindle A cells. Nuclei are plumper, oval and have distinct nucleolus and coarser chromatin pattern.

2) Epithelloid cells—they are the least differentiated of uveal melanoma cells. Tumors rich in epithelioid cells have poorer prognosis. Cells have abundant cytoplasm, are polyhedral with distinct cytoplasmic margin and poorly cohesive. Nuclei are larger, round or oval and vesicular. Typically, they have large reddish purple nucleoli. Nuclear chromatin is coarse and clumps along the inner side of nuclear membrane (peripheral margination of chromatin).

Variants of Epithelioid Cells

Small uniform epithelioid cells, highly anaplastic tumor giant cells and occasionally spindle shaped epitheloid cells can be seen.

Based on cell cytology, uveal melanomas are of 5 types—Callender classification—based on prognosis from best to worse-

1) Tumor composed entirely of nevus cells or benign spindle A cells- spindle cell nevus.
2) Spindle melanomas—composed of malignant spindle A cells, spindle B cells or mixture of spindle A and B cells.
3) Fascicular melanoma—predominantly spindle B cells arranged in the form of fascicles or interlacing bundles.

4) Melanoma of mixed cell type—mixture of spindle and epitheloid cells. Most large tumors treated by enucleation are mixed cell melanoma (Fig. 14.16).
5) Epithelioid melanoma—shows only epithelioid cells. It is rare and has the poorest prognosis.
6) Totally necrotic melanomas—behave like mixed cell type.

Prognosis depends on tumor size and cell type. Mortality is greater if tumor contains epithelioid cells. Large melanomas have poorer prognosis than small and medium size tumors. Small melanomas are likely to be spindle cell tumors with better prognosis. Vascular loops and network of loops indicate poorer prognosis.

Spread—it rarely invades optic nerve and extends out of the eye through emissarial canals of vessels and nerves in the sclera via lumina of vortex vein. Haematogenous spread to liver occurs.

Ciliary Body Melanoma

It is less common than choroidal tumors. Has more spherical shape. It is large when first detected as they are hidden behind iris.

Iris:

Most melanocytic lesions of iris are benign nevi or low-grade spindle cell tumors. Features suggesting that pigmented iris tumor is a melanoma are - large size, documented growth, increased IOP, hyphema and tumor vascularity.

Differential Diagnosis of Malignant Melanoma of Choroid-

1) Carcinoma of breast (adenocarcinoma).
2) Bronchogenic carcinoma (tumor shows squamous cell differentiation or oat cell carcinoma appearance).

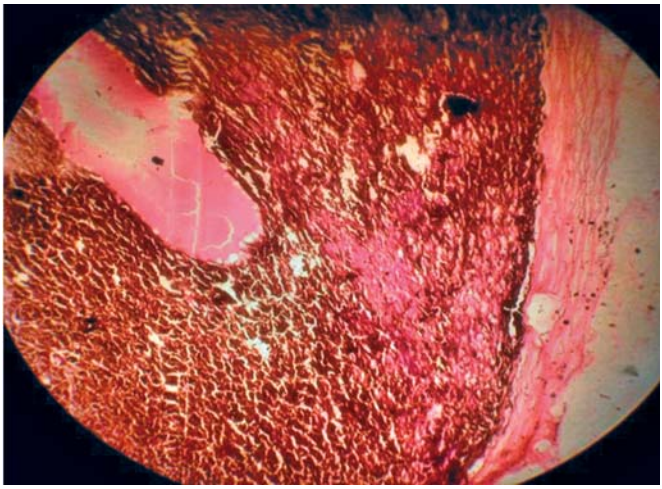


Fig. 14.16: Choroidal melanoma showing spindle and epithelioid melanoma cells

RETINOBLASTOMA

Histogenesis

It is derived from cells of sensory retina. Cellular differentiation is neural or mixed neuroglial. Suffix "blastoma" emphasizes the presence of primitive immature embryonic appearing blastemal cells that form the tumor. It is frequently bilateral. It often shows multicentric growth within the same eye. Multiple primary tumors in single eye differ from multiple deposits of tumor along the retinal surface because of seeding of the tumor.

Gross appearance is white, encephaloid or brain like. Lighter flecks of calcification may be seen—gritty to cut.

It may be

1) Exophytic.
2) Endophytic.
3) Mixed.
4) Diffuse infiltrating growth pattern.

Endophytic variety arises from inner layers of retina. Retina remains attached and seeding of vitreous and anterior chamber occurs. Differential diagnosis-resembles uveitis and ocular toxocariasis.

Exophytic variety causes outward growth of tumor toward subretinal space. Differential diagnosis—Coat's disease with exudative retinal detachment.

Most tumors have mixed endo-exophytic growth pattern and 1.4% of Retinoblastomas diffusely thickens the retina without forming distinct mass-diffusely infiltrating type seen in older children (6 years) .

HPE- Retinoblastoma arises from and destroys the retina. Under low power tumor appears blue, pink and purple. Blue areas represent viable parts of tumor and are composed of poorly differentiated neuroblastic cells with basophilic nuclei and scanty cytoplasm. Retinoblastoma cells are seen as sleeves or cuffs of viable cells measuring 90-100 microns in radius, generally around blood vessels. Retinoblastoma cells readily outgrow their blood supply and undergo necrosis.

Pink areas represent necrotic tumor cells which lose their basophilic nuclear DNA and appear pink or eosinophilic.

Purple areas are necrotic parts of tumor which show dystrophic calcification, and appear reddish purple on H & E, stained sections.

Many mitotic figures are seen and intensely basophilic DNA released from necrotic tumor cells may deposit in vessel walls, iris stroma, trabecular meshwork, walls of Schlemm's canal, lens capsule and retinal internal limiting membrane. Tumor cells collect on inner surface of Bruch's membrane forming focal detachment of RPE. Extensive seeding from involved vitreous and inner and outer surfaces of retina make it difficult to diagnose multifocal retinoblastoma (Fig. 14.17A and B).

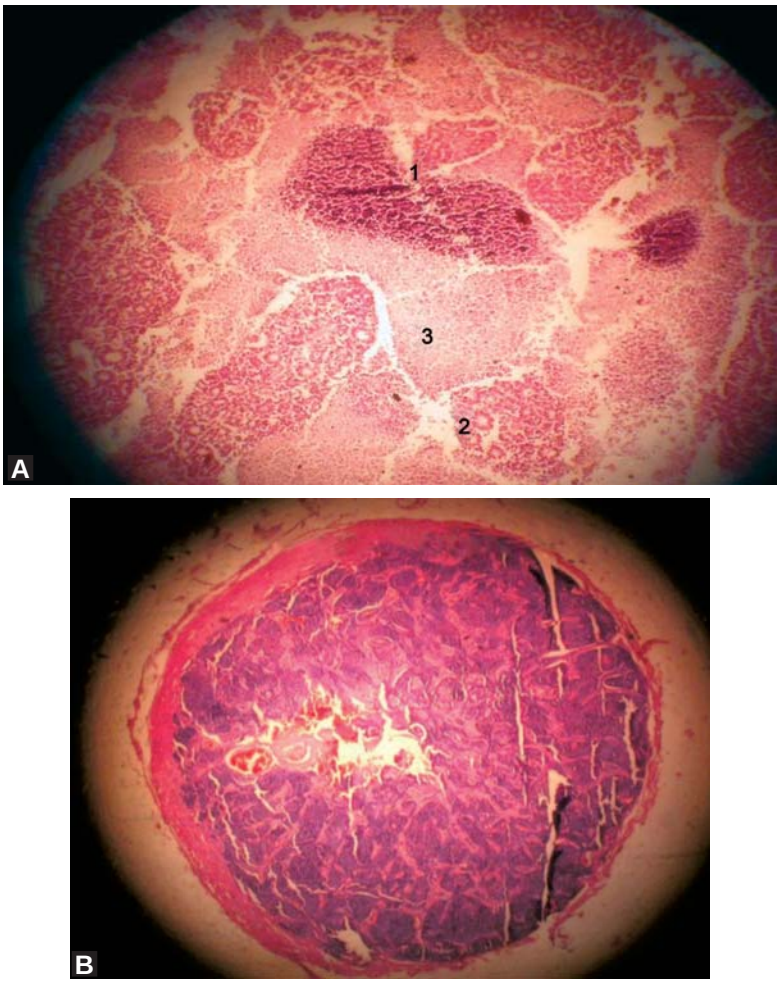


Fig. 14.17A and B: Retinoblastoma - showing 1. dystrophic calcification 2. flexner wintersteiner rosettes 3. necrotic tumor cells (A), Cross section of optic nerve showing extensive infiltration by retinoblastoma cells

Retinoblastoma shows varying degrees of differentiation-

1) Homer—Wright rosettes—indicates neuroblastic differentiation. There is no central lumen and constituent cells encompass central tangle of nerve filaments. They are non-specific and also seen in neuroblastoma and medulloblastoma.
2) Flexner Wintersteiner rosettes- are characteristic of retinoblastoma and represent early retinal differentiation. They have central lumen, which corresponds to subretinal space and are also seen in malignant medulloepithelioma (Diktyoma) and pineal tumors.
3) Fleurettes—represent photoreceptor differentiation and cells have prominent eosinophilic cellular processes. They may show small bouquet of tumor

cells aligned along a segment of neoplastic external limiting membrane. Tumors composed entirely of Fleurettes are called retinocytomas or retinomas and thought to be benign tumors. The cells are bland, have low nuclear/ cytoplasmic ratio, finely dispersed chromatin, fem mitosis and absent necrosis.

Calcification is seen in viable parts of the tumor and thought to be due to retinoblastoma that has undergone spontaneous regression. They have fish flesh appearance and contain abundant calcification likened to cottage cheese appearance. Annulus of RPE depigmentation typically surrounds the tumor.

Occasionally retinoblastoma undergoes spontaneous regression, resulting in degenerated phthisical eye with foci of calcified tumor cells in a matrix of glial scar tissue and extensive intraocular necrosis.

Optic nerve invasion is a characteristic feature of retinoblastoma and prognosis depends on depth of optic nerve invasion. Mortality rates are—

1) No optic nerve invasion- 8%.
2) Tumor invaded upto lamina cribrosa- 15%.
3) Tumor with retrolaminar invasion- 44%.
4) With extension beyond surgical margin- 64%.

So, a long segment of optic nerve should be removed with enucleated eye and sent for HPE.

Extraocular extension of tumor

1) Reach brain by infiltration of optic nerve, through orbital bones or foramina from areas of orbital involvement.
2) By CSF to subarachnoid space of brain and spinal cord.
3) Blood borne metastasis to lungs, bones and brain.
4) To pre-auricular and cervical lymph nodes if tumor reaches lymphatics in conjunctiva.

Metastasis occurs within 2 years of treatment. Histologic parameters of aggressive clinical behavior-

1) Lack of differentiation (absence of rosette formation).
2) Vitreous seeding.
3) Invasion into choroid.
4) Invasion into optic nerve.

Section 4

Pharmacology

15 Routes of Administration of Drugs for Ocular Conditions

There are various ways to administer drugs to the diseased site.

Eye Drops: In the eye, drugs can be administered easily as drops into the conjunctival sac. This is of great advantage as the systemic absorption is very minimal. But there are some problems like — very little of the drops applied i.e., about 20% is retained in the eye. If the drops cause stinging sensation, there will be watering and further dilution of the drug. As there is continuous secretion of tears, whatever drug is retained gets diluted further. Only 50% of the drug that was retained in the conjunctival sac remains after 4 minutes. This is the reason for applying antibiotic drops every five minutes initially to build up the concentration of antibiotics in the conjunctival sac. If more than one drop is applied it will be a waste, as the excess amount will overflow. Hence, if two drops have to be applied atleast five minutes interval must be given between the two.

Compression at the medial canthus also will prevent exit of tears through the naso lacrimal duct. This will also reduce absorption of the drugs through the nasal mucosa. Another important matter that has to be remembered is that the epithelium and the endothelium of the cornea are impermeable to the drugs as they have tight intercellular junctions. If there is any disruption of the epithelium drugs can penetrate the cornea in a better manner. Various methods are undertaken to get over this hurdle. Upto a limit the concentration of the solutions can be increased. Increasing the viscosity will increase the contact time and may help in penetration.

Complications of drops: Short contact time, inconsistent delivery of drug, frequent contamination and injury while applying the drops. Solutions are water-soluble and suspensions are for lipid soluble drugs. When suspensions are used they must be shaken well before use, which is not done properly. The suspended particles also may clog the dropper tip.

Lipid solubility: Both epithelium and endothelium are lipid barriers. The stroma allows water-soluble elements. Lipid solubility of the drugs is more important to make the drug get through the corneal epithelium. To achieve this, drugs are prepared in a slightly alkaline base. Addition of acetates and alcohol increases lipid solubility.

Surfactants: The preservatives that are added to the eye drops act on the epithelium of the cornea in the same way it affects the bacterial cell wall. These preservatives will increase the absorption of drugs. The preservatives inhibit growth of organisms or kill them e.g. quarternary ammonium compounds like benzalkonium to which EDTA can be added for enhancing the effect, mercurials (thiomersal) and alcohols (chlorbutanol) and esters of para hydroxy benzoic acids. But the preservatives are toxic to the epithelium and reduce the lipid part of tear film.

Addition of tear substitutes: If there is a stinging sensation after application of any drops the tearing will dilute the drops. Hence, tear substitutes are added to reduce irritation.

Change of base: Oil-based ointments contain petrolatum and mineral oil. These two substances are lipid solvents and help in better absorption of drugs. But the drugs must be lipid soluble to diffuse through the ointment and water-soluble to dissolve in the tears, e.g, Fluoromethalone, chloramphenicol and tetracycline.

Ointments should not be used when surgical wound opposition is not good, when the corneal ulcer is deep and when there is impending perforation.

Subconjunctival and subtenon's injections: These injections act as a depot and stay in the eye for a longer time. The drug also can enter the eye without encountering the conjunctival and corneal hurdles. Drugs, which are not lipid soluble, must be given subconjunctivally. But these injections are painful. It may perforate the eye causing retinal detachment and preretinal membrane formation.

Anterior subtenon's injections are similar to subconjunctival injections and have a greater danger of perforation. But in severe uveitis corticosteroids are, given in this site.

Posterior subtenon's injection is also given for delivering steroids. Here a canula is inserted under the conjunctiva and Tenon's anteriorly and then pushed backwards to deliver the drug in the posterior quadrant.

Intravitreal injections: Indications for intra vitreal injections are for endophthalmitis where the drug can be given directly into the site where it is needed. Triamcinolone is also given intra-vitreally for diabetic maculopathy. Only very low concentration of the drugs can be given in this method. Polymyxin B can be given in the anterior chamber but only 0.1mg can be given.

Retrobulbar injections are given to deliver anaesthetics, vasodilators, steroids and alcohol.

Peribulbar injections: Due to the danger of perforating the eyeball or the optic nerve during retro bulbar injection and also respiratory arrest due to the drug entering the subarachnoid space now peribulbar injections are given to deliver anaesthetics. Retrobulbar haemorrhage is also less with peribulbar injections. Here the drug seeps into the muscle cone instead of being directly delivered there.

Retrobulbar and peribulbar injections also cause ecchymosis, exposure keratitis, increased intra ocular tension, bradycardia, CRA, CRV occlusion, optic atrophy, decreased visual acuity, chemosis, pain shock and perforation.

Systemic drugs: Similar to blood brain barrier the retinal vessels which are nonfenestrated will not allow the drugs to enter the eyeball. Choroidal and ciliary body blood vessels are fenestrated but the pigmentary epithelium will prevent any drug from entering the eye. Lipid soluble drugs can overcome this barrier e.g., Chloramphenicol. If drugs are bound to plasma proteins e.g., Sulfonamides, they cannot overcome this barrier.

Intravenous injections: Continuous IV infusion can maintain high intra-ocular levels. Antibiotics like ampicillin, erythromycin, and chloramphenicol penetrate the eye and maintain high levels for four hours if given as one IV injection. Inflammation will help in better penetration. When drugs are given intravenously, high levels are found only in the conjunctiva and sclera. Smaller amounts are found in the iris and ciliary body. Some amount is seen in cornea, aqueous humor, choroid and retina. Very low levels are seen in the lens and vitreous.

Prodrugs: Are compounds that are inactive until enzymatically activated e.g., Dipivefrin is a prodrug for epinephrine. Prodrugs penetrate the eye better and after entering become the active compound. Because of better penetration the concentration needed is low. Consequently systemic side effects are also low.

Soft drugs: When inactive or nontoxic metabolite of a drug is instilled it becomes active at the site where it is needed. Then it becomes inactive.

Sustained release devices and gels: If more than required amount of drug is given to achieve higher concentration more side effects will be seen. To prevent this, drugs must be released at the required site at a steady level. Various methods are available to achieve this.

Ocuserts: This method is commonly used for antiglaucoma drugs like pilocarpine and timolol maleate. The ocusert is 13mm / 5.7mm in size. They are of two types. Pellets of hydroxy propyl methyl cellulose, which absorbs the tear fluid, and from the conjunctival sac and the capillaries. It then softens and dissolves slowly releasing the methylcellulose into the conjunctival sac. The second type contains the drug in between two membranes, which controls the release of the drug. There is a white ring around the device, which helps in handling the device. As there is a sustained release the patients are not dependent on others for instillation of drops.

When pilocarpine is given as an ocusert it releases 40 micro gram of the drug /hour which is equal in effect to 2% drops given q.i.d. So, only a small amount of the drug is used to get an equal and consistent effect. This results in lesser amount of induced miosis and accommodation, which is very disturbing to young patients.

The sustained release timolol preparation contains heteropolysaccharide that becomes a gel when it comes in contact with tear film and acts as a local reservoir.

Ocuserts can get displaced and lost. They also create blurring of vision after sometime.

Gancyclovir is an intravitreal sustained release drug. The gancyclovir sustained release intravitreal device (GIOD) has the drug in ethyl vinyl acetate disc coated with polyvinyl alcohol. This device is suspended from the sclera into the vitreous cavity. Polyvinyl alcohol allows only a minimal amount of drug to be released.

Gels contain carboxy methylcellulose sodium. Gels of lubricating drops contain agents, which have a high water binding capacity. Once instilled the gel becomes liquid. Blurring of vision is less with gels than in ointments.

Collagen shields: The scleral tissue of pigs or cattle is molded into shields, which look similar to contact lenses. Drugs can be incorporated into the collagen matrix or absorbed into the shield during rehydration of the shield. Drops can also be applied on the shield after application. The drugs will be retained for a long time in this method. Before application the shield must be soaked in some solution for 3 minutes. The duration of the presence of the shield in the eye depends on the type of manufacturing process. Usually they dissolve after 12, 24 or 72 hours. They create some irritation, which is a disadvantage.

They are used in bacterial ulcers, after surgery, trauma and erosions. It should not be used in bullous keratopathy and chemical injuries, as it will trap the polymorphs and metalloproteinases causing worsening of the condition.

Liposomes: These are synthetic microspheres in which drugs can be incorporated. A water-soluble drug can be incorporated inside with a single or multilayered lipid membrane envelope. A lipid soluble drug is incorporated into the liposomal wall of the sphere.

Drugs impregnated in filter paper strips are used in staining. The strips allow us to use minimum amount of drug in a sterile form.

16 Anti-inflammatory Agents

Ocular inflammation is controlled by drugs, which can be administered either locally as drops subconjunctival injections or as subtenons injections. It can be given orally and systemically also.

The types of drugs used are:

a. Steroids.
b. Non-steroidal anti-inflammatory drugs.
c. Antihistaminics.
d. Histamine release blockers.
e. Antifibrotics.
f. Immunosuppressive agents.

STEROIDS

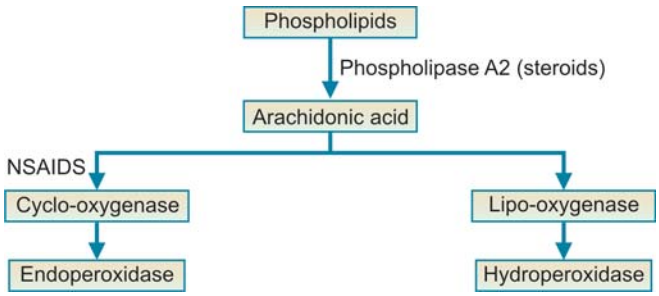
Steroids are the most widely used anti-inflammatory drugs. They are used to control allergic conjunctivitis, vernal conjunctivitis, episcleritis, scleritis, chemical injuries, keratitis, iritis, uveitis, retinal inflammations, optic neuritis, etc. Steroids can bring about rapid reduction in inflammation especially during acute attacks. But there are many side effects when they are used long term. Newer drugs are developed to bring about anti-inflammatory action with minimal side effects. The anti-inflammatory action is seen whatever may be the cause for it i.e., allergic, traumatic or infectious.

The steroids:

a. Inhibit migration of neutrophils into the diseased area.
b. Prevent adherence of neutrophils to the vascular endothelium.
c. Inhibit macrophages.
d. Interfere with lymphocytic activity.
e. Decrease the number of B and T lymphocytes.
f. Affect protein production in immunologically competent cells.
g. Decrease histamine release.
h. Inhibit phospholipase A2 thereby preventing biosynthesis of arachidonic acid and subsequent formation of prostacyclin, thromboxane A, prostaglandins, PGE2, PGF2 and leucotrienes.
i. Suppresses release of lytic enzymes from lysozomes.
j. Decrease capillary permeability and fibroblast formation.
k. Decrease collagen deposition.

The last two qualities will affect wound healing.

Steroids are available as drops, ointments, oral and intramuscular preparations. For systemic administration, preparations with minimal mineralocorticoid activity must be used. Methyl prednisolone, which does not have sodium-retaining activity, is preferred for systemic use. When prednisolone is given orally 20-40 mgs are given in the beginning, if there is severe inflammation 40-60 mgs are given for 2 days and the dose is increased if there is no response. Steroids must always be tapered off after clinical improvement unless very small dose has been given for a short period. Reduction is by 10mgs for larger doses or 5mgs for smaller doses at 3-4 days intervals. If there is recurrence of inflammation it should be increased again.



Side Effects

Long-term steroid use will give rise to osteoporosis, hypertension, peptic ulcer, and muscle weakness, adrenal insufficiency, cushing's syndrome, diabetes, increase of infection, delayed wound healing, mood changes and inhibition of growth in children. To reduce the side effects oral steroids can be given on alternate days as a single dose so that the body can recuperate during the interval. This can be done only with prednisolone, which has a short half-life. The single dose has to given at about 8.00am when the cortisol level of the body is also high. But this method will not be as effective as daily dose.

Because of the above side effects topical administration is preferred in anterior segment inflammations. Frequent application of drops and subconjunctival injections should be given for additional effect.

Different Types of Steroids in Use

In general, acetate and alcohol derivatives are more effective whether the epithelium is intact or not. Alcohol derivatives are in suspension form.

Prednisolone: Available as 0.125% and 1%. The acetate form is better attached to the receptor and hence has a better effect.

Dexamethasone: 0.1% solution, suspension or ointments which contain 0.5% of the drug. It has a long half-life and the drug is found in the anterior chamber even after 12 hours. The above two drugs are synthetic cortisol derivatives.

Fluoromethalone: 0.1% is an analog of progesterone. It does not elevate the intraocular tension as it is metabolized quickly in the anterior chamber. The acetate derivative is metabolized slightly slowly compared to alcohol and is more effective in treatment of conjunctival and scleral inflammation.

Medrysone is also a progesterone derivative. As it penetrates the cornea poorly it does not elevate the intraocular pressure. But because of this it is not suited for treating intra ocular inflammation.

Loteprednol etabonate is a soft drug, which becomes transiently active in the place where it is needed. It gets activated in the cornea, aqueous, iris and ciliary body. It is useful in treating giant papillary conjunctivitis, allergic conjunctivitis, and postoperative iridocyclitis.

Rimoxalone also is a derivative of progesterone and is available as 1 % suspension.

Side Effects in the Eye

This is usually due to long term use and complications like glaucoma are seen more in susceptible people. Posterior capsular cataract, increase in infection, secondary infection, defective wound healing, reduction in collagen formation which leads on to corneal and scleral thinning, uveitis and epithelial erosions are some of the other complications. The cause for glaucoma is not known but in these cases, endoreplication of DNA and aberrant polypeptide production are seen. The drug may directly affect the trabecular meshwork and alter outflow facility. Mydriasis and ptosis are sometimes seen perhaps due to the vehicles.

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

The anti-inflammatory effect of NSAIDS are lesser compared to steroids but the complications are also less. These drugs by blocking the cyclooxigenase pathway prevent formation of prostaglandins from arachidonic acid.

Examples:

Salicylates _____ aspirin, mefenamic acid, tolfenamic acid.

Indoles _____ indomethacin.

Phenyl alkanoic derivatives _____ diclophenac, flurbiprofen, ketoralac, piroxicam.

Pyrozolones _____ oxyphen butazone, phenyl butazone.

To understand the effect of NSAIDS some knowledge about the agents causing the inflammation is essential.

PGD2 is present in the mast cells. It increases conjunctival microvascular permeability. This causes erythema, chemosis and accumulation of eosinophils.

PGD2 is more potent than histamine.

PGE2 causes erythema and itching.

PGI2 causes itching.

PGF2 alpha increases uveo scleral outflow.

After any injury PGE2 and PGF2 alpha will be released.

It must be remembered that the cyclooxigenase has two types of action. Cox 1 in normal conditions and Cox 2 during inflammation. Both are inhibited by NSAIDS. Newer drugs inhibit only the Cox 2 action. These drugs have lesser gastro intestinal problems.

Protaglandin inhibitors are used to prevent intra operative miosis, post-operative inflammation, cystoid macular edema and allergic conjunctivitis. In diabetics, miosis is not prevented effectively by these drugs.

In allergic conjunctivitis, disruption of mast cells occur. This causes release of preformed mediators like histamine and eosinophil chemotactic factor, and initiation of prostaglandin synthesis. NSAIDS help in breaking this activity. Ketorolac is effective in reducing itching. It also reduces pain in conditions like corneal erosion, corneal ulcer and post-surgical conditions.

In uveitis and postoperative conditions the usage of steroids can be reduced if NSAIDS are added.

Side effects: Stinging sensation and delayed wound healing. Lysis of keratocytes is also noticed in some patients. Inhibition of prostaglandins can give rise to asthma and nasal polyp.

ANTIFIBROTIC AGENTS

Drugs like 5 flurouracil and mitomycin C are used to prevent excessive fibrosis after antiglaucoma surgeries.

5 flurouracil inhibits cellular proliferation, which occurs following inflammation and reduces the chances of failure of the bleb due to fibrosis.

Mitomycin C inhibits DNA synthesis and fibroblast proliferation. It is prepared from the fungus Streptomyces caespitosus. It is used after pterygium surgery as drops and during glaucoma surgery as a single application. But it can cause wound leakage, hypotony and scleral melting.

For pterygium, topical application of 0.02%—0.04% drops is used 4 times daily for one or two weeks. It can cause corneal edema, perforation, iritis and cataract formation.

IMMUNOSUPPRESSANTS

Human body requires minimum reaction to bacteria, virus etc., to protect the tissues. But if itself is recognized as an antigen; an autoimmune reaction results. This will destroy tissues. To prevent this immunosuppressants are needed.

Steroids and NSAIDS are milder forms of immuno suppressants. If these fail other substances which are more potent but have more side effects may have to be used. They control the immune reactions by:

a. Blocking lymphocyte proliferation—cytotoxic drugs.
b. Blocking synthesis of lymphokines-immunomodulators.
c. Reducing the inflammatory response.

IMMUNOSUPPRESSIVE (ANTI-NEOPLASTIC) AGENTS

These agents primarily developed for the treatment of neoplastic disease can also be employed in the treatment of autoimmune diseases. In ophthalmology be they have been primarily used for resistant uveitis cases and occasionally used in Grave's disease, pseudotumor of the orbit. Triethylenemelamine (TEM) has been used as adjuvant in the treatment of retinoblastoma and anti-neoplastic are used for ocular neoplastic disease.

They can be classified as

- Alkylating agents
- Antimetabolites
- Antibiotics
- Vinca alkaloids
- Radioactive isotopes

Basically these drugs act by blocking protein synthesis at different levels - precursor stages leading to RNA - DNA synthesis or subsequent activity of RNA and DNA. The commonly used drugs in ophthalmology are discussed below.

Fluorouracil(5- fluorouracil)

This fluorinated pyrimidine blocks the methylation reaction of deoxyuridylic acid to thymidylic acid by inhibiting thymidylate synthase resulting in interference in the synthesis of DNA and RNA. It has been used to prevent closure of conjunctival filtering blebs after glaucoma surgery, and for the prevention of massive preretinal proliferation and epiretinal membrane formation. Gastrointestinal and hematopoietic reactions are common. Subconjunctival injection of 3 mg are given after glaucoma surgery and at repeated intervals to prevent the closure of subconjunctival filtration blebs. For prevention of preretinal membranes, intravitreal dose of 1.25mg for several successive days have been tried.

Mitomycin C

Isolated from Streptococcus calspitosus, mitomycin reacts with DNA in ways similar to alkylating agents. It cross-links DNA and inhibits its synthesis. It is a highly effective antimitotic agent. The major systemic side effect is myelosuppression.

The ocular indications for mitomycin C are recurrent pterygium and glaucoma surgeries. Single application of mitomycin C, in a sponge, at the end of pterygium surgery and at the end of glaucoma surgery.

Potential complications of topical mitomycin C ocular therapy appear to be limited to instances of abuse and negligence, to instances in which drug dosage error occurred, and to instances in which the drug was used in patients with ocular surface disorders such as sicca syndrome and ocular rosacea.

Mitomycin C is antineoplastic antibiotic that is active against gram positive bacteria. It is very cytotoxic because it causes cross-linking of DNA. Mitomycin C also inhibits RNA and protein synthesis.

Topically applied mitomycin (1.0 mg/ml) caused conjunctival irritation, excessive lacrimation, and mild superficial punctate keratitis; these adverse effects were minimized with the lower (0.4 mg/ml) dosage; this lower dose was equally effective in preventing pterygium recurrence, and no systemic toxicity after topical administration was noted.

Mitomycin C in doses of 0.2 to 0.5 mg/ml has been used in glaucoma filtering surgery to inhibit fibroblast proliferation. Limbus based conjunctival flap is made and mitomycin C is applied in a sponge avoiding the edges of conjunctival flaps. O.2 mg is placed for 45 - 60 seconds and 0.4 mg is applied for around 30 seconds, and the area is completely irrigated with copious amount of normal saline. Adverse effects include sequelae of wound healing inhibition, such as bleb and wound leaks.

Bleb failure is heralded by a gradual but definite increase in the IOP, with flattening of the bleb occurring at any time during the postoperative period. When this occurs at an early stage, the inflammatory response with thickening and vascularity of the bleb eventually results in poor control of IOP. There are many ways that a bleb can fail; Tenon's cyst has been described as a stage in bleb failure. The initial stage of fibrovascular proliferative processes occurs early in the postoperative filtering state but can be altered by the use of mitomycin-C intraoperatively and 5-FU postoperatively. The possibility of sclerectomy obstruction must be evaluated any time when bleb failure is anticipated. Careful gonioscopy can reveal the presence of iris or a ciliary process prolapse into the sclerectomy, and laser or surgical removal is necessary. A clear membrane can occasionally be perforated by Nd.YAG laser.

Cytotoxic agents

Cyclophosphamide: Is an alkylating agent of nitrogen mustard type. By suppressing both T suppressor cells and T helper cells both humoral and cell mediated immune responses are reduced by this drug. It is useful in treating scleritis, cicatricial pemphigoid, Behcet's syndrome, Mooren's ulcer, rheumatoid arthritis, Wegener's granulomatosis.

Dose: 2 mg / kg / day, oral or intravenous.

Side effects: Nausea, vomiting, dizziness, liver toxicity, increased pigmentation and ulcers on the mucosal surfaces.

Chlorambucil: Suppresses T and B-lymphocytes and is used in Behcet's syndrome and sympathetic ophthalmia.

Dose: 0.1mg / kg /day.

Side effect: Bone marrow toxicity.

Methotrexate: Is a cytotoxic agent with antifolate activity. It affects synthesis of purines and thymidylate by inhibiting enzyme dihydro folate reductase.

This inhibits T and B cell proliferation. It may also reduce chemotaxis of polymorphs. This action is helpful in reducing inflammation in chronic uveitis and Mooren's ulcer where methotrexate is used for 6 weeks.

Side effects: Nausea, vomiting, liver damage and bone marrow depletion. In the eye it can cause irritation, photophobia, watering and exacerbation of seborrhoeic blepharitis.

Azathioprine: Is a purine analog, which is converted to mercaptopurine containing nucleotides. This affects synthesis of purines, which affects lymphocytic proliferation in the bone marrow. It is used along with steroids in uveitis, sympathetic ophthalmia and cicatricial pemphigoid.

Dose: 1-2mg /kg/ day oral.

Side effects: Nausea, vomiting, bone marrow depression, secondary infection, aloepecia and lymphoma.

IMMUNOMODULATORS

Cyclosporin A : Is a highly selective agent. As it does not interfere with DNA metabolism bone marrow depression does not occur. It inhibits cytokine production by helper T cells, interleukin-2, interleukin-4, interferon gamma and tumor necrosis factor.

Dose: Depends on the disease. 5mg / kg / day to 15 mg/ kg / day oral.

Side effects: Interstitial fibrosis, renal tubular atrophy, hypertension gingival hyperplasia, hyper trichosis nausea and vomiting.

It must be remembered that drugs like erythromycin, ketoconazole and amphotericin B will affect the metabolism of the drug and increase its toxicity. Phenytoin, phenobarbitol and rifampicin will accelerate the degradation of the drug.

Topically cyclosporin A is used as 10% ointment in chronic uveitis, Behcet's syndrome and sympathetic ophthalmia. 2% topical solution in olive oil is used in high-risk keratoplasties along with steroids. 0.5% drops is used in keratoplasty when steroids have to be stopped due to development of glaucoma and in cases like vernal conjunctivitis, rheumatoid arthritis with corneal complications and kerato conjunctivitis sicca.

Tacrolimus: (FK-506) Also selectively inhibits T cell activation. It is more potent than cyclosporine and used in uveitis.

Dose: 0.05-0.1mg / kg/ day.

Side effects: Less than cyclosporine. It is nephrotoxic and causes headache, tremor, insomnia, pain, hypertension, nausea and diarrhea.

Dapsone: Is similar to sulfonamides and acts by blocking synthesis of folates. The exact mechanism by which it reduces inflammation is not known. It probably stabilizes lysosomes, reduces cytotoxicity of polymorphs or prevents activation of complement pathway. It is used in cicatricial pemphigoid.

Dose: 25-30mg /day.

Side effects: Nausea, vomiting, anorexia, skin rashes, hepatitis, renal dysfunction and neuropathy.

17 Antiallergic Drugs

ANTIALLERGIC DRUGS

As the conjunctiva contains millions of mast cells allergic symptoms are very common in the eye. When an allergen binds with one of the innumerable receptors on the surface of the mast cell, it releases many mediators. They are:

a. Histamine.
b. Tryptase.
c. Prostaglandin D2.
d. Leukotriene C4.
e. Eosinophil chemotactic factor.
f. Platelet activating factor and others.

These substances cause the typical symptoms seen during an attack of allergy.

Antihistamines: Are very useful in allergic reactions, as histamine is a major cause for allergic reactions. It is released from basophils and mast cells. This causes Type 1 allergic reaction. It results in itching, watering of the eyes besides chemosis, papillary hypertrophy and congestion of the conjunctiva. High concentration of histamine is seen in lids, conjunctiva, episclera and limbus. During anaphylaxis histamine causes bronchospasm, angioneurotic edema and shock.

Heat and trauma also can release histamine from mast cells.

There are two types of histamine receptors.

a. H1 is found in bronchi, blood vessels and intestine.
b. H2 is found in eyes, heart and pulmonary blood vessels

Common antiallergic agents block H1 receptors e.g., Diphenhydramine, phenergan, chlorpheniramine and cetrizine. H2 receptors are blocked by cimetidine, ranitidine etc.

Antihistamines reversibly bind to the histamine receptors and prevent allergic reactions. H1 receptor blockers give relief from itching, watering and congestion of the mucosal linings of the eye, nose and bronchi. They cannot eliminate the conditions already present. Itching is relieved by the local anaesthetic effect.

Among oral antihistamines, the first generation antihistamines like chlorpheniramine, diphenhydramine and promethazine cause side effects like sedation and anticholinergic activity. The second-generation drugs are less

lipid soluble. Hence they cannot cross the blood brain barrier and do not cause sedation. These drugs inhibit histamine release and hence more effective. Terfenadine and astemizole cause ventricular arrhythmia. Some of these drugs like diphenhydramine and promethazine have anti emetic activity also. As these drugs are CNS depressants they should not be taken with alcohol and other depressants.

Side effects: Nausea, vomiting, anorexia, diarrhea or constipation, reduced secretion in the throat and dysuria. In the eye they will reduce tear secretion, cause allergic reaction, and reduce accommodation, cause mydriasis and precipitate angle closure attack.

Rarely, antihistamines cause convulsions. It may progress to coma, cardio-respiratory failure and death. Diazepam will control the convulsions.

TOPICAL PREPARATIONS

First Generation: Pheniramine maleate and antazoline phosphate are used along with naphazoline an adrenergic agonist.

Second Generation: Kitotifen, azelastine, olopatadine etc. Levocabastin is a synthetic H1 receptor antagonist used as 0.05% drops.

Side Effects: Stinging, burning, allergy and acute angle closure glaucoma. Mast cell stabilizers: These drugs prevent mast cell degranulation.

Examples, Cromolyn sodium prevents calcium influx into the mast cells and also may inhibit other leucocytes. It also prolongs tear break up time and reduces itching and hyperaemia. It will take 7 days for these effects to set in. Available as 2% and 4% drops.

It is used in allergic conjunctivitis like vernal catarrh, allergic kerato-conjunctivitis; giant papillary conjunctivitis i.e., in IgE mediated allergies.

Lodoxamide is more potent than cromolyn as they inhibit CD4 cells.

Available as 0.1% solution.

DECONGESTANTS

Drugs like phenylephrine, naphazoline, oxymetazoline and tetra hydrazine are used for decongestion of mucosa.

Phenylephrine is a synthetic sympathomimetic drug. When applied in low concentrations it will stimulate the alpha-receptors in the conjunctival vessels and constrict them. It can be combined with naphazoline for additive effect. If tetra hydralazine (0.05%) is also added to this the intra ocular pressure is also lowered to a small extent.

Dose: 0.12%

Side Effects: Rebound congestion, epitheliopathy and rarely mydriasis.

Besides decongestion, oxymetazoline will reduce itching, irritation, burning and watering. But it can also cause epithelial erosion of the cornea, pupillary dilatation and upper lid retraction. Systemically it will cause respiratory depression and bradycardia. Hence, must be used with caution in cardio vascular diseases, hyperthyroidism and diabetes.

18 Drugs Used for Dry Eyes and Corneal Edema

Conditions like dry eye, blepharitis and meibomianitis cause burning, irritation, watering and even corneal ulceration and scarring which is called ocular surface disorder.

Tear Substitutes: Ideal artificial tears should contain the following characteristics.

a. They must contain all the electrolytes in the tears.
b. Should be of the same pH as the tears.
c. Must not interfere with vision.
d. Should remain in the eye for a long time.
e. Should be chemically inert and non-toxic.
f. Should not disintegrate on sterilization.
g. Should not form precipitates.
h. Should not cause stinging and burning.

Artificial tears are water based and they need not cross the epithelium and enter the eye. To this base polymers are added to enhance viscosity, lubrication, retention time and stability of the tear film. But just because a solution is more viscous it does not mean that it will stay for a longer time in the eye. Natural tears have glycoproteins and other surfactant macromolecules, which reduces surface tension and other minerals, which must be replaced by the artificial tears atleast partly.

Commonly used polymers in the artificial tears

Methylcellulose (0.25% to 1%) and derivatives like hydroxy ethyl cellulose, hydroxy propyl cellulose (0.5%), hydroxy propyl methyl cellulose and carboxy methyl cellulose(1%) polyvinyl alcohol, polyvinyl pyrolidone or povidone.

These drugs increase the corneal surface wettability and reduce friction. The above substances are added to regular eye drops also to prolong contact time and to reduce irritation. It can also be used for gonioscopy to facilitate application of the device and to prevent damage to the epithelium.

Minerals like sodium chloride and potassium chloride are added to this to make it similar to tears. Preservatives like benzalkonium chloride, chlorbutanol, thiomerosol and EDTA are added. Nowadays preservatives are avoided as they affect the corneal epithelium.

Viscoelastics like sodium hyaluronate have a high molecular weight polysaccharide polymer. They are hydrophilic and increase tear film stability. They also lubricate and protect the epithelium of the cornea.

Dose: Hyaluronic acid -0.1%, chondroitin sulphate 1%.

Vinyl derivatives

Examples, Polyvinyl alcohol, polyvinyl pyrollidone, polyvinyl chloride. These are water-soluble polymers. When added to artificial tears they enhance the wetting property of the preparation due to their adsorptive effect. They act like mucin and increase the tear break-up time. They are useful for both aqueous and mucin deficiency. They are less viscous than methylcellulose.

Artificial tears must be either isotonic or hypotonic. Electrolytes like sodium chloride and potassium chloride are added to the artificial tears. Buffers like phosphates, phosphate acetate, phosphate citrate, bicarbonates, borate and sodium hydroxide are added to control the pH. In tears, besides bicarbonates phosphates, proteins and ammonia are present. The buffers are added to get a slightly alkaline pH, which makes the solution more soothing to the patient.

As preservatives are toxic to the epithelium and affect the lipid layer of the tear film thereby reducing the tear break up time, they must be avoided. Substitutes like sodium perborate produce low levels of hydrogen peroxide. After exposure to light it breaks down to water and oxygen. Sodium chlorate breaks down to sodium chloride and water on exposure to light.

Nutrients needed for the corneal epithelium like vitamin B12, Vit C, sodium lactate and citrate are supplied only by the tear fluids. Vitamin A is very essential for the integrity of the epithelium all over the body including the cornea and conjunctiva. It also increases the mucus production of goblet cells. Hence, topical tretinoin (all- trans retinoic acid) ointment 0.01% is tried, but its effect on dry eyes is not satisfactory. Besides, this Vit A solution can cause irritation and burning sensation.

Mucolytic agents: Improve the quality of the mucin produced by the goblet cells and also soften the mucus threads produced by dry eyes.

Examples, Acetyl cysteine, methyl cysteine and bromhexine. 10 or 20% acetyl cysteine solution is diluted to 2% and used. The solution has a bad smell.

Ocuserts are also available with tear substitutes. But these can be used only if some amount of tears secretion is available to dissolve the ocusert. In addition to ocuserts artificial tears also must be used but with lesser frequency.

Ointments: Create a lipid layer. Esters of fatty aids like petroleum will serve as the lipid layer of the tear film and lubricate the dry eye surface. But ointments blur vision and can affect healing of any epithelial wounds.

Tear stimulation: Many drugs are being tried to stimulate tear secretion by the lacrimal gland. This is done only when atleast part of the gland is functional and the ducts are patent.

Examples, Pilocarpine is parasympathomimetic. The muscarinic effect will stimulate secretions from glands. It has to be used orally.

Hormones: Both estrogens and androgens are needed for tear secretion. Local estrogens are being tried for dry eyes. Systemic androgens maintain the health of lacrimal glands and also protect them from immune reactions. It may also help in meibomian gland secretion.

3- isobutyl-1-methyl xanthine increases secretion from the accessory lacrimal glands by inhibiting cAMP dependent phosphodiesterase activity by preventing break down of cAMP.

Cyclosporine: Prevents autoimmune damage of lacrimal tissues. It reduces inflammation thereby increasing tear production. It is used as 0.05% or 0.1% ophthalmic preparations.

HYPEROSMOTIC AGENTS

The above drugs help in moistening the ocular surface. The hyperosmotic agents draw water from the cornea and reduce edema of the corneal epithelium and to some extent the stroma. Any hyperosmotic agent can reduce edema, but only sodium chloride and glycerin are clinically used.

Dose: 5% eye drops or 6% ointment of sodium chloride are available. Drops are applied 4 or 5 times during the day and ointment is applied at night. Stinging and burning sensation are common.

Glycerin is applied as 50 or 100% solution. Its effect is transient and is used for examining the angle of eyes with oedematous cornea. Topical anesthetic agent must be applied before instilling glycerin.

19

Antibiotics

ANTIBIOTICS

Bacteria are susceptible to antimicrobials. The antimicrobials act in various ways.

a. The bacteria have a cell wall. The drugs act on the cell wall either by destroying it or by affecting the synthesis of the cell wall. Without the protection of the cell wall the organism will die.
b. Some drugs act on the cell membrane.
c. The protein synthesis is affected by some drugs. They bind to the ribosomes in the bacterial cell and affect their replication.
d. Folic acid synthesis that is done by the bacterial cells will be altered by some drugs.
e. There are drugs, which can inhibit bacterial DNA gyrase, which in turn will affect the supercoiling of DNA during replication and transcription of DNA.

Drugs Affecting the Cell Wall

Examples, Penicillin, cephalosporins, bacitracin and vancomycin.

Penicillins: The nucleus of penicillins contains a thiozolidine ring and a β lactam ring connected to a side chain. Intact beta lactam is necessary for the drug to act against the cell wall synthesis. The side chains determine the antibacterial spectrum.

Penicillin G and penicillin V are effective against gram positive bacteria. G form is derived from the fungus penicillium notatum. Penicillin V is not destroyed by gastric acid and can be used orally. Some bacteria have started producing penicillinase or beta lactamase, which makes them resistant to penicillins. Organisms like pneumococci producing penicillin binding proteins will decrease affinity for penicillins.

Penicillin is not used locally as they produce allergic reaction.

To overcome the effect of penicillinase the structure of penicillin is altered. So, methicillin, cloxacillin, oxacillin and nafcillin are not affected by penicillinase. Wherever infections by staphylococci are seen cloxacillin can be used e.g., hordeolum internum, preseptal and orbital cellulitis.

Methycillin resistant organisms are resistant to cephalosporins, amino glycosides and erythrocin. In these cases vancomycin must be used.

Ampicillin and amoxacillin are modified penicillins and act against Hemophilus, morexella and other gram positive cocci, E coli, Klebsiella and Enterobacter. Addition of lactamase inhibitors like clavulanate and sulbactam will inactivate beta lactamase.

Penicillins like carbenicillin, piperacillin, ticarcillin, etc. act against Pseudomonas.

Side effects: Anaphylaxis, urticaria, angioneurotic edema, hemolytic anemia, nephritis, vasculitis, contact dermatitis and Steven Johnson's syndrome. Intravenous injections can cause phlebitis and thrombophlebitis. Nausea, vomiting and diarrhea also can occur.

Cephalosporins: Are similar to penicillin in structure, antibacterial activity and to some extent resistance patterns.

1st generation cephalosporins—Cefazolin and cephalexin act against gram positive and some of the gram-negative organisms. For corneal ulcer, cefazolin 50 mgs/ ml with garamycin, tobramycin or ciprofloxacin can be given as initial therapy locally.

2nd generation: Cefamandazole, cefaclor, cefproxil, cefoxitin and cefuroxime are more effective against gram negative enteric bacteria.

3rd generation: Ceftrioxone, cefixime, cefoperazone, ceftazidime, etc. are more active against gram negative organisms and less active against gram positive organisms compared to first and second generation penicillins. Ceftazidime is very active against Pseudomonas and can be given intra-vitreally.

Ceftrioxone when given IV or IM is active against N.gonorrhea, which is resistant to penicillin. It can be given for ophthalmia neonatorum. These drugs are more toxic to the kidneys.

Bacitracin: Also inhibits cell wall synthesis but the action is slightly different from the penicillins. It is active against gram positive cocci and actinomyces.

This drug is mainly used topically, as it is nephrotoxic. It is commonly available in combination with neomycin and polymyxin B so that the antibacterial spectrum can be widened, as the other two drugs are active against gram-negative organisms. It is used for blepharitis.

Vancomycin: Acts against Clostridium, C diphtheriae and Gonococcus besides gram-positive cocci. Topically it is used for blepharitis and corneal ulcers caused by resistant organisms. As the solution is acidic the pH must be altered when used topically. It is given intra vitreally for endophthalmitis.

It is highly toxic to kidneys and ear. It can permanently affect both organs.

DRUGS AFFECTING CYTOPLASMIC MEMBRANE

Polymyxin B and gramicidin are drugs that will interact with the cell membrane and affect the osmotic control of the cell.

Polymyxin B is effective against gram negative organisms and is used along with bacitracin. It is neurotoxic and nephrotoxic. Locally, it can cause allergic reactions and can cause necrosis if given subconjunctivally.

Gramicidin acts against gram-positive organisms. It is used along with polymyxin b and neomycin.

DRUGS AFFECTING PROTEIN SYNTHESIS

These drugs inhibit protein synthesis by binding to the bacterial ribosomes and more effective against gram-negative bacilli e.g, Aminoglycosides, tetracyclines, macrolides, chloramphenicol and clindamycin.

Aminoglycosides: E.g., streptomycin, neomycin, gentamycin, tobramycin and amikacin. These drugs are active against gram negative bacilli. Neomycin is active against gram positive organisms also but not against Pseudomonas aeroginosa.

These drugs are given either topically or parenterally. Penicillins will inactivate aminoglycosides if given together.

Resistance to the drug is caused by

a. Enzymatic inactivation of the drug. This is common.
b. Alteration of bacterial ribosomes.
c. Decreased antibiotic uptake by the organisms.

Neomycin: used along with bacitracin and polymyxin B. Contact dermatitis is common.

Gentamycin is active against gram negative bacilli and some gram-positive organisms. It is used as drops for conjunctivitis. Fortified preparation (14 mg/ml) is used for corneal ulcers. It is effective against Staphylococci and Hemophilus egyptius.

Tobramycin is similar to gentamycin and is effective against Pneumococci and pseudomonas, which are resistant to gentamycin.

Amikacin is more effective than even gentamycin and tobramycin as they are not affected by the enzymes that inactivate the aminoglycosides. It is used along with vancomycin as intra vitreal injection.

Side effects: All aminoglycosides are ototoxic and nephrotoxic. Systemic gentamycin can cause benign intra cranial tension. Locally it can cause chemosis, hyperaemia and corneal erosions. Intra-vitreal gentamycin can cause macular infarction even in low doses.

Tetracyclines: Are also produced by Streptomyces. Tetracyclines are active against a large range of organisms. Gram +ve, –ve, aerobic, anerobic bacteria, spirochetes, mycoplasma, rickettsia, chlamydia and some protozoa, brucella and leptospira are susceptible to tetracyclines.

Ointments are available to be used topically but they must be combined with systemic administration to be effective against most of the conditions for which they are given. Tetracyclines are prescribed for chlamydial infections, acne rosasea, meibomianitis and hordeola. When applied for blepharitis and

meibomitis it reduces free fatty acids in the oily secretion. Tetracyclines have anticollagenolytic activity when given systemically and are prescribed for chemical injuries to the cornea.

Side effects: Anaphylaxis and urticaria occur only rarely. Photosensitivity is more common. Anorexia, nausea and vomiting also can occur. It should not be given along with anti-acids, food or dairy products and iron containing products. Newer drugs like doxycycline are not affected by food. As they can affect the growth of teeth and bones they must be avoided during pregnancy and in children. Benign intracranial hypertension and blood dyscrasias are seen rarely.

Tetracyclines should not be used along with penicillins and anticoagulants.

Macrolides: Erythromycin, clarithromycin and azithromycin belong to this group. These drugs affect protein synthesis by binding to 50 S ribosomal subunit of bacteria.

These drugs are active against gram positive cocci and bacilli, Neisseria, Mycoplasma, Treponema, Rickettsia, Chlamydia and to some extent hemophilus influenza. Bacteria develop resistance by changing ribosomes. Erythromycin ointment is used for hordeola ophthalmia neonatorum (both for prophylaxis and treatment) and for chlamydial infections.

Clarithromycin is more effective against Chlamydia, Hemophilus, Mycobacterium chelonae and fortuitum keratitis.

Azithromycin: Taken, as once daily dose is effective against Hemophilus, Mycobacterium catarrhalis, Pneumococci, Staphylococci, Chlamydia and Gonococci.

Side effects: Nausea, vomiting, diarrhea, hepatotoxicity, ototoxicity and nephrotoxicity.

Chloramphenicol: Has high lipid solubility. Hence, it is used systemically in treatment of endophthalmitis. As it can cause aplastic anemia even when used topically it is rarely used now. This occurs only in susceptible people but it is irreversible.

It is active against gram positive and negative organisms, Rickettsia, Chlamydia, Spirochetes and Mycoplasma. It is not effective against Pseudomonas.

Clindamycin: Is active against gram positive and anaerobic gram negative bacteria. It is the drug of choice for gram negative and penicillin resistant anaerobic bacteria and ocular toxoplasmosis. It is active against encysted form of toxoplasma. The dose is 300 mg q.i.d. for 4 weeks combined with sulfa and steroids.

It is available as oral, IV and IM preparations.

Side effects: Pseudomembranous colitis, mild diarrhea, pruritis and urticaria.

DRUGS AFFECTING INTERMEDIARY MECHANISM

Examples, Sulfonamides, Pyrimethamine, Trimethoprim. These drugs are only bacteriostatic. They inhibit synthesis of folic acid by the bacteria, which in turn will affect synthesis of nucleic acid and protein. As human cells get folic acid from the diet these are not affected. This action will be inhibited by drugs containing PABA. The sulfonamides will not act in the presence of pus and necrotic tissue.

Sulfonamides: Are active against gram positive, gram negative organisms, actinomyces, chlamydia, plasmodia, pneumocystis and toxoplasma. But many organisms are resistant now.

Topically sulfonamides are applied as eye drops. In toxoplasmosis, sulphadiazine with pyrimethamine or sulfamethoxazole and trimethoprim combination is used.

Side effects: Anorexia, nausea, vomiting, diarrhoea, urticaria, rash and the dreaded Steven Johnson's syndrome. Blood dyscrasias like haemolytic anemia, aplastic anemia and agranulocytosis also are seen. Transient myopia can occur during treatment.

Locally it can cause irritation, burning and photosensitisation leading on to sunburn of lid margins and eye lid skin. As sulfonamides will increase the hypoglycaemic effect of tolbutamide and chlorpropamide, and anticoagulant effect of coumarin, it should be used with caution in, patients taking these drugs.

Pyrimethamine and trimethoprim: Act by inhibiting folic acid synthesis at a different level. Hence, they have an additive effect when given along with sulfa. They are used in the treatment of Pneumocystis carini infection in AIDS patients. A combination of trimethoprim and polymyxin B is used topically for Pneumococcal and H. influenza conjunctivitis.

Side effects: Depression of white blood cells, red blood cells and platelets causing megaloblastic anemia due to folic acid deficiency. To counteract this folic acid must be added during treatment with these drugs.

DRUGS ACTING ON BACTERIAL DNA SYNTHESIS

Examples, Fluroquinolones like ciprofloxacin, ofloxacin, levofloxacin, norfloxacin, sparfloxacin.

They prevent replication of the bacteria by inhibiting bacterial DNA gyrase. They have a broad spectrum of action against gram positive and negative organisms including pseudomonas and H. influenza. They are active to some extent on M. Chelonae and M. fortuitum. But many organisms have become resistant to these drugs.

Ciprofloxacin and ofloxacin are effective against gonococci. Cipro flox and Norflox are effective against E.coli, Salmonella, Shigella and Campylobacter.

When ofloxacin is given intra venously it can reach the vitreous. So it can be used for intraocular infection.

Side effects: Oral administration of fluroquinolones can cause convulsions. As it can affect the cartilages it should not be used in children below 18 years. But topical preparations can be used after 1 year of age.

Drugs active against Gram positive organisms:

Penicillin, ampicillin, amoxacillin, vancomycin.
Cephalexin, cephazolin.
Tetra cycline, doxycycline.
Azithromycin.
Cotrimoxazole.
Ciprofloxacin and norfloxacin.

Drugs active against Gram negative organisms:

Cefotoxime, ceftriaxone, ceftazidime.
Gentamycin, tobramycin, amikacin.
Doxycycline, cotrimoxazole.

Drug	*Topical dose*	*S/c*	*I/vit Mg/0.1ml*	*organism*
Penicillin G	1 lakh units/ml			gram +ve and -ve.
Cipro/oflo	0.3%			wide spectrum
Polymyxin B	50,000 units/ml			gram -ve
Genta/tobra	14mg/ml	20mg	0.1	gram -ve bacilli
Amikacin	2%	50mg	0.2-.4 In 0.08 ml	gram -ve bacilli
Chloramphenicol	0.5%	100mg		wide spectrum
Cephazolin	5%	100mg	2.25	gram +ve
Ceftriaxone	10%	100mg		gram -ve > +ve
Ampi/ amox	oral			gram +ve, --ve, Hemophilus, E.coli, klebsiella Enterobacter
Bacitracin				gram +ve cocci
Vancomycin	2-5%	25 mg	1 mg	gram +ve cocci Gonococcus
Erythromycin				gram +ve and -ve.

20 Antivirals

ANTIVIRALS

Viruses contain either RNA or DNA with a capsid a protein coat. They are intracellular which makes it difficult for the drugs to kill the organisms as that will affect the human cells also. The antivirals now available affect the viral nucleic acid synthesis thereby preventing their replication. The commercially available drugs are useful against Herpes group of viruses like Herpes zoster, Herpes simplex, Epstein Barr virus and Cytomegalovirus and the human immunodeficiency virus.

Drugs active against herpetic infections:

Pyrimidine analogs: Iodoxuridine, vidarabine and trifluridine.

Idoxuridine: Is a nucleoside analog of thymidine. It inhibits thymidine kinase, thymidilate kinase and DNA polymerase. It is incorporated into the viral DNA, which alters the character of the DNA. This in turn will prevent further replication of the viruses. Human epithelial cells also can take up iodoxuridine, which is the reason for its toxicity. It is effective only for epithelial involvement and not for uveitis.

Dose: 0.1% drops every hour and 0.5% ointment at night for 2 weeks by which time improvement will be noticed. Then the drug is tapered off.

Side effects are burning, diffuse punctate keratitis, subepithelial infiltrates, stromal ulceration and obliteration of the canaliculus. All these complications will mimic worsening of the disease inducing the treating surgeon to increase the dosage and prolong the treatment, which is detrimental to the corneal epithelium. This must be avoided.

Vidarabine: An adenosine arabinoside also inhibits viral DNA. As it is not soluble in water it is available only as an ointment. It is metabolized to arabinoside hypoxanthine, which is more soluble in water but less effective.

Dose: 3% ointment five times daily for 2 to 3 weeks. The side effects are similar to IDU.

Trifluridine: Triflurothymidine inhibits thymidylate synthetase and gets incorporated into viral DNA. This affects the transcription and translation of viral genome. It is more effective than IDU and can act against resistant strains. As it penetrates the epithelium it can be used in uveitis cases also.

Dose: 1% drops 9 times daily.

Side effects: Epithelial erosions, epithelial microcysts, edema of the stroma, contact dermatitis and punctal occlusion. Resistance is rare.

Purine Analogs

Acyclovir: Is a purine analog, which acts on HSV 1 and 2. It is less toxic to epithelium as the conversion of the drug to its active form is done by viral thymidine kinase only. Viral thymidine kinase induces phosphorylation of acyclovir, which makes it active. Resistant strains are already seen. Active compound inhibits DNA polymerase. It can penetrate the cornea and reach the aqueous.

Dose: 3% eye ointment 5 times daily. 400 mg tablets can be given 5 times daily for keratitis, endothelitis and uveitis. Systemic drugs are also used after keratoplasty to prevent recurrence of the disease in the graft. Oral acyclovir is given to children with chicken pox, and adults with herpes zoster infection. It shortens the duration of infection and reduces incidence of hyperesthesia and paresthesia. But it does not reduce neuralgia. The dose for adults with Herpes zoster ophthalmicus is 800 mg 5 times daily for 7-10 days. It must be started within the first 72 hours for good effect. This will reduce ocular complications like keratitis and uveitis.

Intravenous acyclovir is used in retinal necrosis, retinal arteritis and vitritis.

Side effects: Nausea, vomiting, diarrhoea, headache, skin rash, fatigue, sore throat, paresthesia and lymphadenopathy.

Valacyclovir: Is L-valyl ester of acyclovir, which is metabolized to acyclovir. It should be given three times daily.

Famcyclovir: Is converted to pencyclovir in the eye and it inhibits DNA polymerase. It is well absorbed when given orally.

Pencyclovir has to be applied every two hours for 4 days.

Gancyclovir: Is an analog of deoxyguanine, which inhibits DNA polymerase. It is very effective against CMV infections.

For CMV infections intravenous gancyclovir is given initially followed by oral maintenance therapy. It is also given intra-vitreally as once a week injection or as intra vitreal device which releases the drug slowly for about 6 months after which it has to be replaced.

Side effect: bone marrow depression.

Foscarnet: is a pyrophophate analog. It inhibits viral DNA polymerase and RNA polymerase. It can be given along with gancyclovir for CMV infections. When both drugs are given together the dose can be reduced. Foscarnet can be used along with zidovidine as it has anti HIV activity besides acting against Herpes zoster and simplex.

Side effects: Fever, nausea, vomiting and nephrotoxicity. It can affect the serum calcium, phosphorus and magnesium levels leading to seizures. Hence, the plasma levels of these minerals must be regularly checked. 5 - (2 bromo vinyl)-2 deoxyuridine BVDU is also phosphorylated by viral thymidine kinase into active form. It is more active against HSV 1.

Drugs like (S)-1-(3 hydroxy-2 phosphonyl methoxy propyl) cytosine (HPMPC, cidofovir is a DNA polymerase inhibitor and active against adenovirus and ribavirin and fluoro-deoxy-arabino furanosyl-isocytosine (FIAC) are being investigated for use against viral infections in human beings.

Dihydroxy propoxy methyl guanine (DHPG) is used for CMV retinitis and HSV keratitis.

Fomivirsen: Is an antisense oligonucleotide that blocks viral proteins necessary for replication. It can be given intravitreally for CMV retinitis along with foscarnet and gancyclovir. One injection is given on the first day followed by the second on the fifteenth day and then once every month.

Antiviral drugs for HIV infections

Zidovudine is a thymidine analog. It has to be phosphorylated by the cellular enzymes to active triphosphate form. This will inhibit reverse transcriptase and terminate viral DNA elongation. It is used in iridocyclitis due to HIV infection.

Side effects: Bone marrow depression, nausea, vomiting, hepatitis, malaise and myositis.

Didanosine, Zalcitabine, Stavudine and Lamivudine are nucleoside reverse transcriptase inhibitors. These drugs can cause pancreatitis and peripheral neuropathy.

Nevirapine and delaviridine are nonnucleoside inhibitors, which act by disrupting reverse transcriptase by binding to it.

Side effects: Nausea, vomiting, fever, headache and rashes.

Protease inhibitors: E.g. Saquinavir, Indinavir, Ritonavir, Relfinavir inhibit HIV specific protease. This will result in immature HIV particles, which are non-infectious.

Side effects: Nausea, vomiting, diarrhea, anemia, hepatitis, renal stones and renal failure.

Combination of reverse transcriptase inhibitors and protease inhibitors (HAART therapy) can reduce HIV levels in the blood.

INTERFERONS

Are substances produced by cells infected with virus to protect other cells. This can be used along with other drugs for better healing as it has an additive effect.

21 Antifungal Agents

ANTIFUNGAL AGENTS

There are three groups of antifungal drugs. They are polyenes (amphotericin, natamycin and nystatin), imidazoles (miconazole, ketaconazole, etc.) and flucytosine.

Polyenes act by binding to the sterols in the cell membrane of the fungi but not the human cells. This will damage the cell wall and cause leakage of the cellular contents and electrolyte imbalance. As cell wall is damaged other antifungal agents also enter the cell causing further damage. Thus, they will have an additive effect.

Amphotericin B binds to ergosterols of the fungal cell membrane. It is effective against candida, cryptococcus, aspergillus and to some extent against fusarium. It is an unstable compound and is affected by water and light. So, it can be stored only for a short time and that too only in a dark container away from light and heat. It is available as a powder, which should be dissolved in distilled water and diluted with glucose solution. Sodium chloride will cause precipitation.

0.1-0.2% i.e. 1 to 2 mg per ml is used as drops. The drug can bind with cholestrol in the human cell. This makes it very toxic. Drops will cause irritation, chemosis and corneal erosion. It is used systemically for involvement of other organs with fungal infections.

Natamycin: Is less toxic compared to amphotericin and more effective as it can penetrate intact epithelium to some extent. It is active against both candida and filamentous fungi. It is also more effective against fusarium compared to amphotericin.

It is available as 5% suspension to be applied every hour. White deposits form after a few applications, which causes irritation. But these deposits mixed with the mucus secretion form a depot, which acts for a longer time.

Nystatin is available for dermatologic candida infections. It can be used for candida infection of the cornea.

Imidazoles: Inhibit synthesis of ergosterol and are fungistatic.

Ketaconazole is water-soluble and is absorbed well when taken orally. It can be given topically as 1-2% drops. Ketaconazole tablet is mixed with artificial tears to get these drops.

Micanazole is also active against yeasts and some filamentous fungi. It is effective to a limited extent against fusarium and aspergillus. It can be used topically, sub conjunctivally and systemically.

Clotrimazole is active against Aspergillus and is given as 1-% suspension.

Flucytosine is a fluorinated pyrimidine. When absorbed by fungi it is converted to flurouracil which will prevent thymidine synthesis. It is effective against Candida, Cryptococcus, Aspergillus, Penicillium and Cladosporium. It is not effective against fusarium. It is better to use it along with amphotericin as the fungi develop resistance to the drug and is not useful when used alone.

Available as 1% solution (10 mg/ml). Orally 50-150 mg/kg/day is given in divided doses. It is toxic to bone marrow and liver.

Antiamebic Drugs

Diamidines like propamidine is used along with polymyxin B, neomycin and gramicidin. It has antifungal and antibiotic activity. Treatment should be continued for a long time. It should be applied every hour and tapered off to four times daily which should be continued for 1 year.

Polymeric biguanides: Polyhexamethylene biguanide (PHMB) is used to disinfect swimming pools. It is effective against both cysts and trophozoites when used as 0.02% solution. It should be combined with propamidine and neomycin for better effect.

Chlorhexidine solution also is effective against acanthamoeba when given as drops. 2.5 micrograms in one ml is used every hour.

22

Antihypertensives

ANTIHYPERTENSIVES

Hypertension (HT) is defined as the presence of a blood pressure elevation to a level that places patients at increased risk for target organ damage in several vascular beds including the retina, brain, heart, kidneys, and large arteries. Of all hypertensive patients 90 % have essential HT, the remaining have HT secondary to causes such as renal parenchymal disease, renovascular, pheochromocytoma and Cushing syndrome, etc.

Classification of B.P for adults

	Systolic (mm Hg)	***Diastolic (mmHg)***
Normal	<120	< 80
Prehypertension	120 - 139	80 - 89
Hypertension stage I	140 - 159	90- 99
Hypertension stage II	>160	>100

Measurement of BP should be done on multiple occasions. This or more abnormal readings are required to start treatment. Hypertension is diagnosed if patient's systolic BP is greater than 140 and diastolic BP is greater than 90 mm Hg. The main aim of treatment is to prevent long term target organ damage. Another entity called as malignant hypertension occurs when systolic BP exceeds 210 and diastolic BP exceeds 130 mm Hg, and presents as papilledema.

Nonpharmacological therapy should be practiced initially. It includes lifestyle modification including reduction in body weight, cessation of smoking and adequate mineral and vitamins.

Antihypertensive agents commonly used

I ***Diuretics:-***

Diuretics are effective by causing natriuresis and reduce intravascular volume. They produce vasodilatation by inhibiting sodium entry into smooth muscles. They are subclassified depending on the site of action.

a) Thiazides block Na^+ reabsorption in distal convoluted tubule by inhibiting Na/K^+ cotransporter. e.g. Hydrochlorthiazide and chlorthalidone.

b) Loop diuretics - block Na reabsorption in thick ascending loop of Henle. e.g., Frusemide, ethacrynic acid.

c) Potassium sparing agents–act competitively by inhibiting actions of aldosterone. E.g. spironolactone, triamterene.

Side effects of diuretics include weakness, cramps and impotence. They cause hypokalemia, hypomagnesemia, hyperlipidemia and some cause ototoxicity (loop diuretics).

II ***Sympatholytic agents:-***

a) β antagonist:

They act by competitive inhibition of catecholamines at beta receptors and thereby decrease heart rate and cardiac output. They also have CNS mediated antihypertensive effect.

They can be classified as cardioselective(affects ß1) and non-selective (β1and β2 blocker). Some agents have intrinsic sympathomimetic effects—cause less bradycardia.

1) Cardio selective - Atenolol, Betaxolol, Metoprolol.
2) Non-selective - Propranolol, Timolol.
3) ISA - Carteolol, Pinodolol.

Side effects include AV block, heart failure, Raynaud's and impotence. Those drugs which cross CNS cause insomnia and depression. They also increase triglyceride level.

b) Selective α antagonist:

They block post synaptic α receptors producing arterial and venous vasodilatation. e.g., Prazosin, terazosin.

They cause postural hypotension, dizziness, headache and drowsiness. They decrease triglyceride level.

c) Both α and β blockers:

They block the effects of catecholamines at beta receptors and alpha1 receptors. e.g., Labetolol, carvedilol.

Side effects include liver damage, postural hypotension and tremors.

d) Centrally acting:

They stimulate pre synaptic α_2 receptors in CNS and decrease the sympathetic tone and reduce vascular resistance. E.g. Clonidine, Methyl Dopa.

Side effects include bradycardia, dry mouth, postural hypotension, and galactorrhea. Acute withdrawal (rebound hypertension) may occur on abrupt cessation of drug.

III ***Calcium channel antagonist:-***

Calcium channel antagonists cause arteriolar vasodilatation by blockade of calcium channels in vascular smooth muscle. E.g. Verapaamil, Diltiazem. Nifedipine. Verapmil and Diltiazem should be used cautiously in cardiac conduction abnormalities and this can worsen cardiac failure.

Side effects include nausea, head ache, postural hypotension, constipation. Sublingual Nifedipine produces wide fluctuation and excessive reduction in BP and should be avoided in acute management of hypertension.

IV ***Inhibitors of Renin Angiotensin system:-***

1) ACE inhibitors: They block the production of angiotensin II by inhibiting ACE competitively leading to arterial and venous vasodilatation. This reduces aldosterone secretion producing natriuresis. E.g., Enalapril, Captopril.

   These drugs reduce rate of death, myocardial infarction and stroke. Side effects include dry cough, hypotension, taste disturbances, leucopenia and proteinuria. It may worsen renal function in patients with decreased renal perfusion. They cause hypercalcemia, and should be used with caution in patients on potassium sparing diuretics.

2) Angiotensin receptor blockers: They block vasoconstrictor effects of angiotensin II on smooth muscle and on glomerulus. This reduces peripheral resistance.

V ***Direct acting vasodilators:-***

They are used for refractory hypertension, hypertension in pregnancy. They produce direct arterial vasodilatation. E.g., Hydralazine, Minoxidil.

Minoxidil relaxes smooth muscle by stimulating ATP dependent K+ channel. They should be used with caution in ischemic heart disease because of reflex sympathetic hyperactivity.

Side effects include headache, nausea, tachycardia and postural hypotension. Hydralazine may include SLE like syndrome. Minoxidil may cause weight gain, hypertrichosis and pericardial effusion.

VI ***Parenteral antihypertensive agents:***

They are used for immediate reduction of BP in hypertensive emergencies. E.g., sodium nitroprusside, nitroglycerin, labetolol, esmolol, diazoxide, hydralazine.

Sodium nitroprusside is a direct acting arterial and venous dilator—drug of choice for most hypertensive emergencies. Duration of action is short and should be monitored to avoid hypotensive response. Side effects include paresthesia, tinnitus, delirium or seizures. It should be used with caution in hepatic dysfunction due to accumulation of cyanide.

Nitroglycerin is used where sodium nitroprusside is relatively contraindicated like, severe coronary insufficiency, hepatic or renal disease. It is used with caution in inferior myocardial infarction.

Labetolol is the drug of choice in hypertensive emergencies that occur during pregnancy. Patients should be treated in supine position since postural hypotension occurs.

Esmolol is a cardioselective beta antagonist useful in aortic dissection. It can be used along with sodium nitroprusside.

Hydralazaine is used in pregnancy related emergencies and has better safety.

ANTIHYPERTENSIVES

Drugs	*Initial dose*
I) Diuretics:	
Chlorthiazide - thiazide	500 mg qid
Chlorthalidone	25 mg qid
Frusemide	20 mg qid
Ethacrynic acid	50mg qid
Amiloride - potassium sparing diurectics	5 mg qid
Triamterene	50 mg qid
Spironolactone	50mg qid
Adrenergic antagonist	
a) beta blocker	
– Atenolol – selective	50mg qid
– Betoxolol – selective	10mg qid
– Betaprolol – selective	50mg qid
– Propranolol – non-selective	40mg qid
– Nadolol – non-selective	40mg qid
– timolol – non-selective	10mg bd
– Cartexolol – ISA	2.5 mg qid
– Pinodolol – ISA	5 mg qid
– labetolol – α and β receptors	100mg qid
– carvedilol – α and β receptors	6.25 mg qid
b) alpha antagonist	
Prazosin	1mg bd
Terazosin	1mg bd
Calcium antagonist	
Amolidipine	5mg qid
Diltiazem	30mg qid
Nicardipine	20mg qid
Nifedipine	10mg qid
Verapamil	80mg qid
ACE inhibitors	
Enalapril	5mg qid
Captopril	25mg qid
Lisinopril	10mg qid
Benazepril	10mg qid
Ramipril	2.5mg qid
Moexipril	7.5mg qid
Quinopril	10mg qid
Angiotensin II receptor blocker	
Losartan	25mg qid
Valsartan	80mg qid
Canderastan	8mg qid
Centrally acting adrenergic drugs	
Clonidine	0.1mg bd
Guanfacine	1mg qid
Methyl dopa	250mg bd
Direct acting	
Hydralazine	10mg qid
Minoxidil	5mg qid
Reserpine	0.5 mg

23 Parasympathomimetics, Mydriatics and Mydriolytics

PARASYMPATHOMIMETICS

Cholinergic agonist can be classified according to their mechanism of action as direct acting (activate cholinergic receptors at neuroeffector junctions—sphincter pupillae and ciliary body) and indirect acting (inhibiting cholinesterase inhibitors).

1. Direct acting — acetyl choline, methacholine, pilocarpine, carbachol.
2. Indirect acting
   i) *Reversible* — Neostigmine, physostigmine, edrophonium, demecarium.
   ii) *Irreversible* — Echothiophate.

Pilocarpine

It is a natural alkaloid plant Pilocarpus microphyllus. It is a direct acting cholinergic agonist with a dominant action at both peripheral and central muscarinic sites. The response of intraocular smooth muscle to pilocarpine is pupillary constriction, spasm of accommodation and decrease in intra ocular pressure.

Direct stimulation of longitudinal muscle of ciliary body which, in turn, causes the scleral spur to widen the intertrabecular spaces and increase the outflow thereby decreasing IOP. Pilocarpine appears to decrease IOP to the same degree (15% approximately) in both healthy and glaucomatous eyes (including the ocular hypertension patients). Darkly pigmented eyes demonstrate relative resistance to action of pilocarpine. It causes pupillary constriction and varying degree of accommodation. Long-term therapy alters the iris muscle activity and may cause permanent miosis resulting from loss of iris radial muscle tone and fibrosis of sphincter muscle.

Its onset of action is within 15 minutes and reaches maximum in 30 - 60 minutes.

Preparation: Topical 0.25% - 10% (Pilocarpine HCl, Pilocarpine nitrate). Applied 4 times a day. There is some additive action seen with PG analogues.

Ocusert: It is a type of sustained release system (membrane bound drug delivery system) which deliver at rates of 20 or 40 µg/ hr. Maximum effect is achieved in 2 hours after insertion of device and lasts for 7 days. Miosis with Ocusert is always less intense than solution. It provides less frequent, less intense, fewer fluctuations of vision. Disadvantage: difficulty with retention

and unnoticed loss of device from the eye, rupture of the membrane resulting in excessive delivery and high cost.

Gel form - 4% Gel.- ½ inch ribbon applied once daily at bed time.

In acute angle closure with high pressure, the ischemic sphincter is unresponsive to pilocarpine. Topical beta blocker, apraclonidine or systemic agents are indicated initially to bring the pressure below 50 mmHg before pilocarpine is administered. The reduction in vitreous volume by a systemic hyperosmotic agent prevents forward movement of the lens caused by pilocarpine. Pilocarpine is also useful during laser iridotomy to facilitate stretching of iris.

Side Effects

Ocular

i) Accommodative spasm 2-3 hours after instillation especially in young patients.
ii) Miosis.
iii) Hastens development of cataract.
iv) Follicular conjunctivitis.
v) Pupillary block and secondary angle closure (forward displacement of iris- lens diaphragm).
vi) Allergic blepharoconjunctivitis.
vii) Band keratopathy.
viii) Conjunctival injection.
ix) Lid myokymia.
x) Retinal detachment (anterior displacement of lens- iris diaphragm, leading to vitreoretinal traction or tractional tears with or without posterior vitreous detachment). Pre-existing retinal lesion/ break should be prophylactically treated before prolonged therapy.

Systemic

i) Headache.
ii) Browache.
iii) Marked salivation.
iv) Profuse perspiration.
v) Nausea / vomiting.
vi) Bronchospasm / Pulmonary / edema.
vii) Systemic hypotension / bradycardia
viii) Muscle weakness.
ix) Abdominal pain / Diarrhea.
x) Respiratory paralysis.

Contraindications

- Presence of cataract (especially nuclear sclerosis / posterior subcapsular cataract).
- Less than 40 years (accommodative spasm).

- Neovascular glaucoma/ Uveitic glaucoma (breakdown of blood aqueous barrier).
- H/O retinal detachment.
- Asthma.
- Acute angle closure —further shallow anterior chamber-promotes peripheral anterior synchiae and angle closure.

Carbachol

It is a direct acting cholinergic agonist (both muscarinic and nicotinic). It is completely resistant to hydrolysis by cholinesterase.

Clinical use

It is available as chloride salt for topical application (0.75%, 1.5%, 2.25%, 3.0 %), and requires less frequent application(once in every 8 hours). It is available for intracameral use as 0.01% sterile balanced salt solution in 1.5 ml glass disposable vial and 0.5 ml is used by gentle irrigation.

Side effects

Systemic toxicity is uncommon. Ocular toxicity — miosis, transient conjunctival and ciliary injection and ciliary spasm. Allergic reactions are rare.

Echothiophate

It belongs to organophosphorus group and acts by inhibiting the cholinesterase enzyme. Onset of action is within 10 - 30 minutes and accompanying decrease of IOP is maximal after 24 hours and lasts for days or weeks. Ocular hypotensive effect has been attributed to an increase in facility of aqueous outflow similar to that produced by pilocarpine and carbachol.

Clinical use

Available as iodide salt in 0.03, 0.06, 0.125,and 0.25 %. It has a very short shelf life - so prepared fresh and supplied as a powder to be reconstituted with diluent containing buffer and preservative. After reconstitution, refrigerated solution is useful upto 6 months (room temperature- 1 month).

Side effects

Ocular - cataract (characterized by anterior subcapsular opacities), greater risk of retinal detachment, reversible iris cyst.

Systemic - diarrhea, vomiting, respiratory paralysis, intestinal cramp, CNS symptoms, cardiac arrest, hypotension.

Nasolacrimal duct occlusion is mandatory after instillation in patients who are exposed to OPC fertilizers / insecticides to minimize side effects.
(OPC - Organo Phosphorous Compounds)

Contraindications

i) Phakic glaucoma.
ii) Patient with peripheral retinal disease.

iii) Discontinue drug several weeks before elective surgical procedures in which succinyl choline is used.
iv) Should be discontinued 4 - 6 weeks before intra ocular surgery to minimize inflammatory effects and to diminish conjunctival and episcleral bleeding.
v) Avoid in uveitic glaucoma because of vasodilatation / aggravation of uveitis.
vi) Patients exposed to carbamates or OPC should be warned of additive effects and preventive measures such as wearing respiratory masks and frequent washing and clothing should be recommended.

MYDRIATICS

Phenylephrine

It is a synthetic sympathomimetic amine which is structurally similar to adrenaline which acts primarily on alpha 1 receptors with no effect on beta receptors. After topical application it contracts the iris dilator muscle and smooth muscle of conjunctival arterioles resulting in pupillary dilatation and blanching of conjunctiva. It acts on Muller's muscle of upper lid resulting in widening of palpebral fissure; it also reduces IOP in normal eyes and in eyes with open angle glaucoma.

It is available as 2.5 % and 10 % phenylephrine hydrochloride solution which is available as clear and colourless solution which is subject to oxidation on exposure to air, light or heat. In order to prolong its half life, sodium bisulphate an antioxidant, is added to the vehicle.

Clinical uses

Phenylephrine 2.5 % / 10 % on instillation results in maximum dilatation in 45 - 60 minutes and recovery occurs in 6-7 hours. Its cycloplegic action is less than that of Tropicamide. Corneal penetration of phenylephrine is increased by topical anaesthetics and loss of corneal integrity. Phenylephrine and tropicamide are mixed together for routine pupillary dilatation. Only 2.5 % solution is used in infants and elderly.

Other than mydriasis phenylephrine has several other uses like

1. Breaking posterior synechiae.
2. Used along with echothiophate to prevent formation of miotic cyst.
3. Used in Ptosis due to Horner's syndrome.
4. It may improve vision in cataract by mydriasis.
5. Used as diagnostic test in Horner's syndrome. Here 1% phenylephrine dilates the pupil with post ganglionic sympathetic denervation whereas, it may cause minimal or no dilatation in normal eyes. In central / preganglionic lesion pupil does not respond as there is no denervation hypersensitivity.

Side Effects

Ocular

1. It causes transient pain, lacrimation and keratitis.
2. Allergic dermatoconjunctivitis occurs which causes scalded appearance around the eye.
3. Release of pigment granules from the eye occur which appears as aqueous floaters after instillation of 2.5 % or 10% solution. This disappears after 12 - 24 hours. Pigment granules are thought to be released from rupture of the pigmented epithelial cells of iris.
4. Rebound miosis and conjunctival congestion occurs in patients above 50 years.

Systemic

1. Systolic hypertension.
2. Occipital headache / subarachnoid hemorrhage.
3. Ventricular arrhythmias, tachycardia, reflex bradycardia
4. Blanching of skin.

Contraindications

1. It potentiates the effect of tricyclic antidepressants, MAO inhibitors.
2. In cardiac disease, aneurysms and idiopathic orthostatic hypotension.
3. Patients on reserpine, guanthedine, and methyl dopa show increase adverse effects.

EPINEPHRINE

It is used since 1920 to reduce IOP. It is a non-selective alpha and beta receptor agonist. Epinephrine and Dipivefrine have relatively low therapeutic index, high potential ocular and systemic side effects.

Ocular

It causes irritation, lacrimation, conjunctival hyperemia, and allergic blepharoconjunctivitis. Adrenochrome pigmentation of conjunctiva and staining of soft contact lens may occur. Causes pupillary dilatation and elevation of IOP and cystoid macular edema.

Systemic side effects

Severe headache, palpitation, tachycardia, premature ventricular contractions, HT crisis and anxiety.

MYDRIOLYTICS

Dapiprazole and thymoxamine are the mydriolytics clinically available. Dapiprazole is in clinical use to reverse diagnostic mydriasis.

Dapiprazole

Topical instillation produces miosis and reduces IOP. It blocks alpha receptors in the iris dilator muscle. It is available in the concentration of 0.12% to 1.5%. Miosis is concentration dependent and effect lasts for 6 hours. IOP can be reduced for upto 6 hours.

Clinical use

It is a safe miotic for reversing phenylephrine induced mydriaisis. 2 drops followed 5 minutes later by 2 drops causes nearly complete reversal of phenylephrine induced dilatation. Miosis begins 10 minutes after instillation and since it is due to alpha receptor blockage there is no shifting of iris-lens diaphragm and no subsequent shallowing of anterior chamber. As with Thymoxamine, iris color can affect the rate of pupillary constriction. It is slower with brown iris than green / blue iris.

It is also used for reversal of iatrogenically induced mydriasis produced by adrenergic agents or anticholinergic agents.

It is used as a weak miotic agent to reduce peripheral distortion after refractive surgery. Another theoretical use is in the treatment of pigment dispersion glaucoma, because alpha blocking effect causes miosis and iridoplegia. There is reduced shedding of pigment from the posterior iris causing less obstruction of aqueous outflow.

Drugs in the concentration of 0.25% - 0.5% are effective in angle closure glaucoma.

Side effects

1. It causes transient burning and conjunctival hyperaemia.
2. Corneal-edema, superficial punctate keratitis, chemosis, lid erythema, dry eye- these are due to effects of dilatation of conjunctival blood vessels.
3. Ptosis occurs due to alpha receptor blockade in Muller's muscle.

Contraindications

Acute anterior uveitis, hypersensitivity to any drug component.

24 Cycloplegics

CYCLOPLEGICS

Atropine

It is a naturally occurring alkaloid isolated from plant Belladonna (Atropha Belladonna). It is an non-selective muscarinic antagonist. The pH of atropine is 9.8, so at physiological pH it is primarily ionized and the ionized state makes corneal penetration difficult. It is the most potent mydriatic and cycloplegic, commercially available as a sulphate derivative as 1% ointment and solution. Maximum mydriasis is achieved after 30 - 40 minutes and the drug effect lasts for 7 - 10 days and paralysis of accommodation (cycloplegia) is maximum after 60 - 180 minutes. The effect lasts for 7 - 12 days. In heavily pigmented eyes, atropine exhibits a relatively slow onset and prolonged duration of cycloplegic effect.

Clinical use

1. Refraction - especially in young actively accommodating children with suspected latent hyperopia or accommodative esotropia.
2. Uveitis
   a) Relieves pain by relaxing ciliary muscle spasm.
   b) Dilates pupil - prevents posterior synchiae.
   c) Decreases the excessive permeability of inflammed vessels and thereby reduce cells and protein in the anterior chamber(flare).
3. Myopia - It has been suggested that topical ocular use of atropine may prevent or slow progression of myopia. By placing ciliary muscle at rest, accommodation is relaxed and the tension that produces elongation of eye may be reduced.
4. Amblyopia - as an alternative to direct occlusion in the treatment of amblyopia, referred to as 'penalization' for mild to moderate amblyopia.

Side effects

Ocular

- Direct irritation from drug preparation.
- Allergic contact dermatitis / allergic papillary conjunctivitis / allergic keratitis.
- Risk of angle closure glaucoma.

- Elevated IOP in open angle glaucoma patients (mechanism of pressure rise is not completely understood. Pressure elevation appears not to the degree of mydriasis attained but rather to a decrease in facility of aqueous outflow.
- Systemically administered atropine may also cause mydriasis and increase IOP in patients with open angle glaucoma.

Systemic side effects

- Dose dependent.
- Dry mouth, facial flushing, decreased sweating.
- CNS - convulsions, cognitive impairment, delirium.
- Patients with Down's syndrome have an cardio accelerated response to IV administration of atropine, mechanism of increased sensitivity to the vagolytic action of atropine is not clear.

Treatment of atropine overdose - prevent dehydration and hyperpyrexia. Physostigmine is given in severe toxicity.

Contraindications

- Hypersensitivity.
- Tendency towards IOP elevation.
- Angle closure / open angle glaucoma.
- Child with Down's syndrome- hyperactive pupillary response to topical atropine.

Can be given in breast feeding women, but caution is to be exercised.

Homatropine

It is approximately 1/10 as potent as atropine. It is partially synthetic and partially from Solanaecea plants and commercially available as 2% and 5 % hydrobromide salt. Mydriatic effect is reached after 40 - 60 minutes of application and the effect lasts for 1 - 3 days. Paralysis of accommodation is reached after 30 - 60 minutes of application and the effect lasts for 1- 6 days. The amount of cycloplegia produced by Homatropine is less than that produced by comparable doses of atropine and cyclopentolate. Duration of cycloplegia obtained with Homatropine is longer than cyclopentolate.

Hyosine/Scopolamine

It is a non-selective antagonist derived from Hyoscyances niger and scopolia carniolia plant. It is commercially available as 0. 25% hydrobromide salt. The effect of mydriasis is maximally reached after 20-30 minutes and lasts for 3-7 days, the effect of cycloplegia is reached maximum after 30- 60 minutes and last for 3- 7 days. There is higher incidence of idiosyncratic reactions to scopolamine than to other anti cholinergic agents, so it is not preferred as first choice. Use is reserved for patients who exhibit sensitivity to atropine.

Cyclopentolate

It is a stable water soluble ester. Its pH is 8.4, so primarily ionized at physiological pH. Commercially available as 0.5%, 1% and 2% solution. The effect of mydriasis and cycloplegia is maximum after 20 - 45 minutes, mydriasis and cycloplegic effect lasts for 1 day.

Uses

It is used for refraction especially in infants and young adults. Cycloplegia is superior to Homatropine (almost close to atropine), with faster onset and shorter duration. Unlike atropine onset of maximum cycloplegia generally approximates the onset of maximum mydriasis. Thus, when pupil is fully dilatated cycloplegia is adequate for refraction. However, the time cause of mydriasis and cycloplegia are not the same. Pupil dilatation typically lags behind loss of accommodation.

In uveitis (more frequent instillation is necessary).

Side effects

- Transient stinging is the most common side effect.
- Allergy (quite rare).
- Toxic keratitis - epithelial punctate keratitis with conjunctival hyperemia.
- Precipitates acute angle closure of glaucoma.

Systemic

- Cerebellar dysfunction.
- Visual/ tactile hallucinations, drowsiness, ataxia, disorientation, restlessness, incoherent speech, emotional disturbances.

Peripheral side effects of atropine not observed (like flushing, increase in temperature pulse rate and blood pressure).

Tropicamide

It is a synthetic derivative of tropic acid and non selective muscarinic antagonist (moderate selectivity for M4 receptors). It is commercially available as 0.5%,1% solution. Its pH is 5.37, hence only 2.3% is ionized. So unionized molecules readily penetrate the corneal epithelium with greater concentration of drug reaching the muscarinic receptor sites, so there is greater diffusibility, there is faster onset and shorter duration of action. The mydriatic action peaks after 20 - 35 minutes and cycloplegic action in 20 - 45 minutes. Both last for 6 hours. Pupil dilatation is less dependent on iris pigmentation (in DM patients - pupils are resistant to dilatation by anticholinergics. But show Supersensitivity to adrenergic agonist. So, cyclosyn to be used).

For premature infants 0.5% tropicamide and 2.5% Phenylephrine is also recommended.

Side effects

- Transient stinging effect.
- Increase in IOP in POAG patients due to decrease in aqueous outflow
- Angle closure glaucoma

Systemic reactions are rare - as decrease in affinity for systemic muscarinic receptors. Safest for systemic hypertension / angina and other cardiovascular patients and in neonates. Patients with hypersensitivity to belladonna exhibit cross sensitivity to tropicamide.

25 Local Anesthetics

LOCAL ANESTHETICS

Local anesthetics are drugs which cause reversible loss of sensory and motor impulses without loss of consciousness. Local anesthetics can be classified as ester linked and amide linked.

Amide linked agents produce more intense, long lasting anesthesia and rarely cause hypersensitivity reaction, whereas the ester linked LAs, have short duration and less intense anesthesia and is used on mucous membrane.

Ester linked LAs—Cocaine, Procaine, Tetracaine.

Amide linked LAs—Lidocaine, Bupivacaine, Ropivacaine.

Injectable anaesthesia also can be classified as:

1. Low potency - Procaine, Chlorprocaine.
2. Intermittent potency and short duration - Lidocaine.
3. High potency and long duration - Bupivaccine, Tetracaine, Ropivacaine.

In 1884, Carl Koller for the first time used Cocaine for topical anesthesia. It was used as 1% - 4 % topical solution. Cocaine is epitheliotoxic and used to remove corneal epithelium in severe epithelial edema. Because of its epithelial toxicity it was later discarded. Knapp used Cocaine as retrobulbar anesthesia for enucleation. Van Lint used LAs for orbicularis akinesia. Nowadays topical anesthesia with Proparacaine, Tetracaine are used for clear corneal phacoemulsification.

Mechanism of Action

LAs cause reversible nerve conduction block by decreasing the Na^+ uptake thereby decreasing the depolarization of nerves and slowing the conduction. Sensory and motor fibres are equally sensitive. Sensitivity is determined by diameter of nerve fibres, smaller fibres are more sensitive. Autonomic fibres are generally more susceptible than somatic fibres.

LAs are used either as topical, regional infiltration or specific nerve block in ophthalmology.

TOPICAL ANESTHESIA

DRUG	*Maximum dose (in mg%)*	*Onset of action (min)*	*Duration(min)*
Cocaine(1% - 4%)	20	1	20-30
Tetracaine (0.5%, 1%)	5	1	15
Proparacaine (0.5%)	10	1	15
Lidocaine (4 %)	3	1	15

Cocaine is a natural alkaloid from Erythroxylon coca. It is a good surface anesthetic, which is rapidly absorbed from mucous membranes. It is a protoplasmic poison and produces tissue necrosis. In the periphery it stimulates sympathomimetic effect. It causes constriction of conjunctival vessels, clouding and rarely sloughing of cornea due to drying and tissue toxicity.

Proparacaine is the first synthetic LA used. It has least bactericidal topical activity so it can be used as surface anaesthesia for diagnostic corneal scraping.

Tetracaine (Amethiocaine) — is more potent and more toxic due to slow metabolism. It is used both as surface and infiltrative anesthetic. Its absorption from mucous membrane is fast. Tetracaine and Proparacaine are the least toxic to corneal epithelium.

Benoxinate (0.4%) an ester anesthetic, is currently most frequently used as topical agent because of its high degree of safety.

Lidocaine is the most widely used LA. It is used both for surface and infiltrative anesthesia. It produces nerve block within 3 minutes. CNS side effects like drowsiness, mental clouding and tinnitus may occur. Overdose results in twitching, convulsions, arrhythmias and respiratory arrest.

Regional Infiltration

	Onset (min)	**Duration**	**Maximum dose (mg/Kg)**
Lignocaine (2%)	5	45 min	5
Lignocaine with adrenaline	5	1-2 hrs	7
Bupivacaine (0.25 - 0.75%)	7 - 10	2hrs	200

Bupivacaine is a long acting local anesthetic. It is used as infiltrative anesthetic which produces a delayed but prolonged effect. It is prone to cause tachycardia and cardiac depression. Ropivacaine, bupivacaine congener is less cardiotoxic and is equally long acting.

Adverse effects of topical ocular anaesthetics are:-

1. Epithelial toxicity—healing is delayed after an epithelial defect. Lignocaine does not affect healing.
2. Alteration of lacrimation and tear film stability.
3. Endothelial toxicity occurs when associated with penetrating trauma and Benzalkonium is used as a preservative.
4. Contact dermatitis can occur and is common with Proparacaine.
5. Surface keratopathy can occur.
6. Systemic effect may occur particularly with cocaine.

Adrenaline, a vasoconstrictor is used in concentration of 1: 100,000 to 1: 200,000. The advantage is

1. Prolongs the duration of LAs.
2. Reduces the systemic toxicity of LAs.

Disadvantages

1. It makes injection more painful.
2. May raise BP, cause arrhythmias in susceptible individuals.
3. Increases local tissue edema and delays wound healing by reducing the oxygen supply.

Hyaluronidase is an enzyme that hydrolyzes hyaluronic acid, an essential component of the connective tissue cement substance. As a result, tissue permeability is increased, and injected solutions and local accumulations of fluid spread more rapidly and are absorbed faster. It reduces the amount of anaesthetic used and enhances quick onset of action of drug. Preparations are as follows: lyophilized powder, 150NF(TR)units and 1500NF(TR)units per vial: solution for injection, 150USP units per ml.It is used in concentration of 7.5 to 10units/ml of anaesthetic.

OCULAR ANESTHESIA

Ocular anesthesia can be classified as those for:

1. Intraocular surgery.
2. Extraocular surgery.

Local anesthesia for Extraocular surgery (Fig. 15.1).

Lacrimal Sac

Regional anaesthesia for Dacrocystectomy and Dacrocystorhinostomy is produced by:

1. Anaesthesia of nasal mucosa by packing with ribbon gauze soaked in lignocaine 4 % plus adrenaline 1: 100,000. Injection of lignocaine 0,5 ml is done to raise nasal mucosa from bone.
2. Blocking the nasociliary nerve by injection at junction of roof and medial wall of orbit for 3 cm depth.
3. Another infiltration over anterior lacrimal crest towards the nasolacrimal duct.

Anaesthesia of Upper Lid

Supraorbital nerve is found by palpating superior orbital notch at junction of medial 1/3rd and lateral 2/3rd of superior orbital rim. Infiltration of 1-2 ml is done by pushing the needle backward and slightly downwards piercing the orbital fascia. It is redirected medially and laterally to involve supratrochlear and infratrochlear nerve. Injection of lacrimal nerve completes akinesia of upper lid.

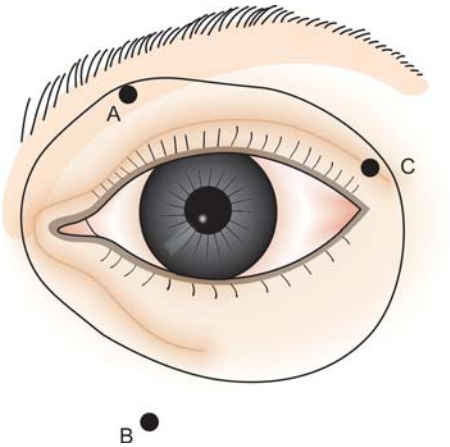


Fig. 15.1

A modified single injection by Hildreth and Silver is given by a 4 cm needle injected in midline of orbit following roof and about 0.5 ml is injected. This gives anaesthesia of upper lid except extreme nasal and temporal ends.

Infraorbital nerve block

The infraorbital foramen lies 4-5 mm below lower orbital margin in the same vertical plane as supraorbital notch. About 2 ml of anesthetic is injected at this point and directed laterally and posteriorly.

It can be also done by passing a needle along the junction of floor and lateral wall for 1.5-2.5 cm to reach inferior orbital fissure. Injection of 1-2 ml is made around infra orbital nerve as it enters the orbit. Anaesthesia for intraocular surgery involves akinesia of orbicularis oculi, extraocular muscles and sensory interruption of ciliary nerves.

Akinesia of orbicularis oculi

Orbicularis oculi is supplied by the facial nerve. Facial nerve passes from stylomastoid foramen into substance of parotid gland and divides into temporofacial and craniofacial branches. Temporofacial division passes in front of the neck of the mandible and divides into temporal and zygomatic branch and supplies the orbicularis oculi. Facial nerve and its temporofacial branch can be blocked in its course by

1. Nadbath - Ellis
2. O'Brein
3. Atkinson's
4. Van Lint.

Nadbath-Ellis involves injection at area of stylomastoid foramen where facial nerve emerges. 25- 26 G, 5/8th inch needle is used and 4 ml of anesthetic is injected perpendicularly in that area. Jugular foramen with vagus, glossopharygeal and spinal accessory nerve is located near stylomastoid foramen and accidental involvement may lead to dysphagia, dysphonia and respiratory arrest.

O'Brien's block: It is the commonly employed method by blocking the proximal trunk of facial nerve. The condyloid process of mandible is located by palpation just in front of the tragus. 27G, 1 inch needle is inserted upto the periosteum and 3-5 ml of anesthetic is injected. Injection may injure the temporomandibular joint, or external carotid artery which lies at a deeper level.
Atkinson's block: Temporofacial branch is blocked as it crosses the zygomatic arch. Injection is made with 23G, 1.5 inch needle from inferior edge of zygomatic bone and directed upwards across zygomatic arch towards the top of the ear. This avoids damage to cervicofacial branch.
Van Lint akinesia: Van Lint block is made by injecting vertically towards temporal fossa from 1cm lateral to lateral canthus. It is then injected subcutaneously along entire extent of both eyelids. Disadvantage is ballooning of lids which can be reduced by adding hyaluronidase which aids in rapid diffusion.
Akinesia of EOM and anesthesia of orbital contents:

RETROBULBAR (INTRACONAL) ANESTHESIA

Injection is made within the posterior muscle cone. It is a blind procedure in which dull tipped 1.25 inch 25-27 G needle is used.

The position of eye during the procedure is important. Patient is asked to look in primary gaze during injection at junction of lateral 1/3rd and medial 2/3rd of lower orbital margin. Surgeon should recognize when the needle has passed through orbital septum and then through intermuscular septum into muscle cone. 1.5 - 2 ml of anaesthetic mixture is injected which paralyses the nerves in the muscle cone and the ciliary ganglion. Trochlear nerve which is outside the muscle cone is spared and there is some amount of torsion seen. Depth of 31mm from orbital rim should not be exceeded to prevent injury to optic nerve.

Complications which occur during the procedure

1. Globe perforation.
2. Retrobulbar hemorrhage.
3. Optic nerve trauma.
4. CNS spread by piercing the meninges.
5. Retinal vascular occlusion.
6. Strabismus.
7. Ptosis.

Globe perforation occurs more commonly in myopic and deep set eyes. It results in marked pain, hypotony and poor red reflex. Retrobulbar hemorrhage may be either venous or arterial. Arterial hemorrhage causes proptosis, elevated IOP, massive ecchymosis of lids and conjunctiva, and inability to separate the lids. Increased IOP may affect the perfusion of optic nerve and may result in late optic atrophy. Venous hemorrhage spreads slowly. Surgery should be postponed.

Direct spread to CNS through subdural space may occur. This results in mental confusion, vomiting, dysphagia, dyspnea and respiratory depression. This can be prevented by maintaining primary gaze during injection and not penerating more than 31 mm.

Strabismus may occur after injection of anesthetic mixture into muscle. This mostly affects inferior rectus muscle due to progressive contracture of muscle.

PERIBULBAR (PERIOCULAR) ANESTHESIA

Peribulbar anesthesia has largely replaced retrobulbar anesthesia for intraocular surgery. Various methods are described by different texts.

It is given at the junction of lateral 1/3rd and medial 2/3rd of inferior orbital rim using ¾ th inch, 25 - 27 G needle in the peribulbar space. 1 ml of solution is injected in orbicularis muscle, the needle is pierced and 3 ml is injected at the equator and another 3 ml behind the equator. This creates mild proptosis and chemosis of conjunctiva which can be tackled by superpinke or Honan balloon.

Alternative method involves injecting, 5 ml of anaesthetic mixture at junction of lateral 1/3rd and medial 2/3rd of the lower orbital rim either transconjunctivally or transcutaneously into peribulbar space. Another 5 ml may be required as superior injection either temporally or nasally below the superior orbital rim.

Advantages

1. Separate facial nerve block is not required.
2. Less chance of retrobulbar hemorrhage, perforation of globe, optic nerve.

Disadvantages

1. Akinesia is slow.
2. Orbital pressure is more.
3. More amount of drug required.
4. Periorbital ecchymosis, conjunctival chemosis.

SUBTENON ANESTHESIA

The procedure is done with blunt cannula which is inserted into subtenon space in inferotemporal quadrant. The procedure can also be done with 27G needle. The degree of Akinesia is proportional to drug.

Disadvantage: conjunctival chemosis and haemorrhage (due to damage to vortex veins).

Advantage:

1. No rise of IOP.
2. Lack of elevation of blood pressure.

TOPICAL ANAESTHESIA

Topical anaesthesia is gaining popularity for clear corneal phacoemulsification. It requires a very cooperative patient. Midazolam is injected about ½ hour prior to surgery.

Topical anaesthetic tetracaine 0.5%, 0.75% bupivacaine or 4 % lignocaine can be used by instillation 5-10 minutes before surgery. A piece of cotton impregnated with mixture of lignocaine, bupivacaine and hyaluronidase is placed in the superior and inferior fornix. Topical anesthesia can be combined with intracameral lidocaine (1%).

Intracameral lidocaine provides uveal anesthesia for pressure changes which act at the root of the iris and ciliary body. It is used as 1% isotonic non preserved lidocaine 0.3 ml.

Advantage is, it is cost effective, there is immediate visual recovery and avoidance of complications of retrobulbar and peribulbar anesthesia.

Disadvantage is awake and talkative patient. No Akinesia of eye and if difficulties or problem occur the anesthesia may not be adequate.

Respective advantages of regional and general anesthesia

Regional anesthesia	*General anesthesia*
Simple technique	Complete control of patient
Less postoperative nausea and vomiting	No risk of retrobulbar hemorrhage
Faster recovery	No risk of globe perforation
Postoperative analgesia superior	No risk of myotoxicity
Blockade of oculocardiac reflex	Applicable to all ages
Absence of respiratory depression	Preferable for teaching of surgery
Full mental status retained	
No loss of 'control' for patient	
Potential for reduced stress	
No risk of toxic hepatitis	
Not contraindicated with low serum K+	
No risk of malignant hyperthermia	
Easily applicable in high altitude	

Contraindications to regional and general anesthesia (in decreasing order of significance)

Regional anesthesia	*General anesthesia*
Reversible medical condition, uncorrected	Reversible medical condition
Anesthetist inexperience	History of serious adverse effect from GA
True allergy to LA	History different airway
Emergency surgery (open eye wound)	Known problems or disease state
Prolonged surgery (more than 2 hours)	c. malignant hyperthermia
Children upto age of early teens	d. muscle disease: dystrophia myotonica, myasthenia gravis
Unsuitable psychological status	e. haemoglobinopathies
Mental retardation	f. chronic obstructive pulmonary disease caution with
Senile dementia	g. history of porphyria
Head movements or tremors	h. atypical pseudocholinesterase
Inability to lie flat	i. patients on MAO inhibitors
Intractable cough	j. interactions with regular medications
Communication barrier	k. patient on anticoagulants
a. language	
b. deafness	
moderate to severe arthritis	
neurological disease	
claustrophobia	
caution with patients on anticoagulants	

DOs and DON'Ts of ophthalmic regional anesthesia

DON'Ts	DOS
Use the 'up and in' globe position for inferotemporal intraconal blocking.	Use primary gaze position, or consider 'down and out' and 'down and in'.
Attempt without anatomical knowledge.	Use a precise technique based on sound anatomical knowledge.
Fail to check the axial length.	Measure axial length (thinner sclera with long ovoid myopic eyes).
Fail to check for globe anomalies of previous surgical intervention.	Take extra caution in patients known to have staphyloma, coloboma or scleral buckle.
Inject with other than tangential alignment	Align needles tangentially to the globe for all injections.
Use the superonasal quadrant	Use avascular injection sites.
Inject at the orbital apex using long needles	Inject no deeper than 31 mm from the inferior orbital rim.
Use dull coarse needles	Use fine sharp needles in combinations with good anatomical knowledge for better tactile discrimination, patient comfort and a lower incidence of tissue trauma and hemorrhage
Inject into extraocular muscles	Avoid extraocular muscles.
Use unnecessarily high concentrations of anesthetic agents.	Use a well - thought technique, volume and concentration of chosen anesthetic agent.

26 Fluorescein Sodium and Other Dyes

FLUORESCEIN

It is a yellow acid dye made of xanthene. It is generally available as sodium salt with molecular weight of 376 Kd. Straub in 1888 first clinically used fluorescein for detection of corneal ulcers and in 1910. Burk reported use of fluorescein to detect retinal disease.

Depending on the pH of the solution it exists in various ionic states.

pH less than 2- cationic form predominates with weak blue-green fluorescence.

pH 2 - 4 - cation dissociates to neutral molecules.

pH 7 - negative ions predominate with brilliant yellow- green fluorescence.

Factors Altering the Fluorescein

1. Concentration.
2. pH of the solution.
3. Presence of other substances.
4. Intensity and wavelength of absorbed light.

Clinical Use

It is available as topical drops, fluorescein impregnated filter paper strips which was developed by Kimura, and as injection form for IV use. Pseudomonas aeruginosa grows easily in presence of flourescein. Preservatives are used to prevent growth of microorganism in fluorescein solutions (like chlorbutanol, thiomersal).

Topical Preparations

2% freshly prepared fluorescein is commonly used. Uses are-

1. For assessment of ocular surface integrity. Because of its high degree of ionization at physiological pH, fluorescein neither penetrates intact corneal epithelium nor forms a firm bond with any vital tissue. Breakdown of epithelium allows stromal penetration. When observed with cobalt blue filter of slit lamp the epithelial defects appear outlined in vivid green fluorescence because the yellow orange dye diffuses freely through the intercellular spaces and accumulates in the defect.

Exact mechanism of fluorescence is not known. Havener suggests that a break in the epithelial barrier permits penetration of fluorescence into the Bowman's layer and stroma. Dye makes contact with alkaline interstitial fluid from aqueous and this causes change in colour of flourescein.

2. Siedel's test- a drop of fluorescein is placed on the superior bulbar conjunctiva in an attempt to detect a surgical wound leakage after cataract extraction. If leak is present a bright green rivulet of aqueous flows through the flourescein on the cornea. It is best seen in cobalt blue light or cobalt blue filter of slit lamp.
3. Contact lens fitting and management. It detects corneal epithelial damage. In soft contact lens usage if limbal epithelial hypertrophy occurs it leads to pooling of fluorescein around limbus.
4. Lacrimal system evaluation- it is used to evaluate the integrity of pre-corneal tear film and patency of lacrimal drainage system. Tear break up time is defined as the interval between the last complete blink and the development of first randomly distributed dry spot in the tear film. Tear break up time less than 10 seconds indicates unstable tear film. It is also used to evaluate epiphora. 2% solution is used for patency of lacrimal system.
5. It is used to record IOP by applanation tonometry. O.25% solution is used for this purpose.

Intravenous Fluorescein

Fluorescein is excreted by a wavelength of 465nm and emits a wavelength of 525 nm. In bloodstream it binds to albumin and redblood cells. 80% of Fluorescein in blood is bound to albumin and remaining is present in free form unbound to albumin which passes through blood ocular barriers when it is damaged. This property of Fluorescein is made to use of fluorescein, to outline the vasculature of retina and to delineate lesions within the layers.

Fluorescein is available as 5%,10% and 25% solution. Depending on the concentration, quantity needed differs, i.e. 10ml of 5%, 5ml of 10%, 3ml of 25%. 25% solution is used when media is more opaque. After injection through antecubital vein the dye reaches retinal circulation (central retinal artery) in 10 - 15 seconds. The passage of dye through the circulation is divided into various stages. There may be hyperfluorescence or hypofluorescence depending on the pathology.

Hyperfluorescence Occurs Due To

i) Window defect — due to RPE atrophy and resultant unmarking of choroidal fluorescence.
ii) Pooling of dye — in subretinal and sub RPE space.
iii) Leakage — abnormal vasculature.
iv) Staining — Drusen.

Hypofluorescence Occurs Due To

i) Blockage of fluorescence by retinal or choroidal lesion.
ii) Filling defects due to vascular occlusion.

Uses

1) Delineate vascular abnormalities of fundus.
2) In occlusive carotid disease by comparing the arm to retina circulation of both sides. A difference of more than 1 second is significant.
3) Iris angiography - for visualization of iris tissues and vessel infarcts.
4) Aqueous flow assessment.

VITREOUS FLUOROPHOTOMETRY

It is a non-invasive method of measuring fluorescein in vitreous. This is used to study the integrity of blood- retinal barrier.

Fluorescein can also be administered orally as powder form(1-2 gm) mixed in fruit juices. The dye appears in fundus in 15 minutes.

Side Effects

i) Nausea and vomiting are the most common and it depends on concentration of dye.
ii) Allergic reaction like urticaria, pruritis or pulmonary edema.
iii) Cardiovascular toxicity like hypotension and shock.
iv) Pain at site of injection and paresthesia of tongue and lips.
v) Temporary discolouration of skin and veins.
vi) Topical preparations cause transient urticaria of cornea or conjunctiva.

Since hypersensitivity may occur; emergency medication should be available. It may stain soft contact lens, therefore should be reinserted after 1-2 hours of fluorescein application.

FLUOREXON

Fluorexon is N, N - bis - aminomethyl fluorescein tetrasodium salt. It is pale, yellow brown in colour. It stains both epithelial defects and devitalized tissues. It is less absorbed by contact lens and is more useful in evaluating contact lens fit. It is also particularly useful in evaluating hybrid design contact lens.

Topical application may produce mild stinging sensation. It is not used with highly hydrated soft lens because of discoloration.

ROSE BENGAL

It is mostly used in diagnosis of ocular surface disease. It is a derivative of Fluorescein and stains only degenerated or dead cells and mucous strands. It is formulated as 1% solution, and available as sterile filter paper strips.

It is a photoreactive compound which reacts with light and oxygen to form singlet oxygen which causes damage to single stranded DNA and cell membranes. Therefore, it is not a vital dye.

Uses

i) Evaluation of corneal ulcer.
ii) Diagnosis of keratoconjunctivitis sicca. George et al developed a grading scale for quantifying the severity of dry eye.

Grade	*Description of severity*	*Punctate spots*
0	Absent	0
1	Minimal	3-10
2	Mild	11-30
3	Moderate	31-80
4	Severe	>80

Rose Bengal is used intravenously in animal studies, as it initiates photothrombosis. This is used in occluding the corneal new vessels.

Topical application causes stinging sensation which may be reduced by topical anaesthesia. The ability to stain is dose related, and it stains skin, and contact lenses. Intravenous administration may cause liver toxicity.

INDOCYANINE GREEN

ICG is a water soluble, tricarbocyanine dye with peak absorption in near infrared spectrum of 805nm and maximal emission at 835nm. On intravenous administration 98% of the dye is protein bound; it does not leak through choriocapillaris and gives outline of choroidal circulation.

Uses

1. Fluorescent dye for retinal and choroidal angiography. It is mostly used to identify ischemic, inflammatory and degenerative disorders. It is used most frequently to identify choroidal neovascularisation.

   It is administered by dissolving the dye in an aqueous solvent at a concentration of 12.5mg/ml for a total dose of 50 mg. It is administered via the antecubital vein at a rate of 1ml/sec. A serial of images are taken by infrared scanning technique.

   It is as safe as fluorescein. It may cause discoloration of skin, and mucous membrane. Since, it contains iodine it is not given to patients allergic to iodine.

   Other less commonly used dyes are methylene blue (5 %) and lissamine green(1%). Methylene blue stain devitalized cells, mucus and corneal nerves. It is used to stain lacrimal sac before DCR. Lissamine green is a vital stain which stains degenerated cells and mucus like Rose Bengal.

Botulinum Toxin

It is produced by bacteria clostridium botulinum. Botulinum A toxin serotype is used therapeutically. Botulinm selectively interferes with release of acetyl choline from nerve terminals. It causes dose dependent paralysis that lasts for 9 months.

Clinical uses

i) Correction of strabismus.
ii) Nystagmus
iii) Blepharospasm
iv) Hemifacial spasm
v) Lower lid entropion.

In strabismus it is most effective in small angle deviation, transient sixth nerve palsy, overcorrection and residual deviation after squint surgery.

Adverse effects are diplopia, ptosis, hemorrhage, pupillary dilatation, reduced accommodation, local swelling, ecchymosis, dry eye, ectropion and ectropion after treatment of facial spasm.

27 Irrigating Solutions

Irrigating solution refers to any aqueous solution that could be used to clean a tissue while maintaining its moisture. Both intraocular and extraocular solutions are available. Intraocular irrigating solution must supply nutrition to anterior segment particularly the sensitive corneal endothelium.

The irrigating solution should have desirable properties to prevent damage to ocular tissues. They should have appropriate osmolality, pH and required concentration of minerals, glucose. It also depends on the metabolic demands of the tissues it comes in contact with. Corneal epithelium obtains its nutrients from tear film and limbal vessels; therefore, extraocular solutions need not be exacting in nature. Corneal endothelium requires glucose for its nutrients and this is supplied by aqueous humor, therefore intraocular irrigating solutions should have glucose, calcium and magnesium which are required for cell adherens. Uvea, retina obtain their nutrition from ocular blood vessels and this is not dependent on irrigating solutions.

The irrigating solutions should have the required osmolality. All commercial ophthalmic solutions have about 300mosm. Hypertonic solution causes cell shrinkage due to water loss and a hypotonic solution causes edema and even destruction of cells.

Specific salts like calcium, magnesium should be added to maximize cell adhesions and cellular transport during surgery. An ideal pH of 7.4 is required. Preservatives are added for extraocular irrigating solutions for their antimicrobial effects.

Extraocular irrigating solutions which contains salt of sodium, potassium, magnesium, calcium with preservatives are used for

- First aid, in case of chemical injury.
- After tonometry for removal of fluorescein.
- After gonioscopy.
- After removal of foreign body.
- Diagnostic nasolacrimal duct irrigation.
- Washing out mucus, purulent discharge.

Intraocular irrigating solution like normal saline and Ringer lactate which were used earlier cause corneal edema after prolonged usage(more than 35 minutes).

These cause early breakdown of corneal endothelial cells because these solutions lack calcium and magnesium.

Balanced salt solution(BSS) is significantly less traumatic. It contains calcium and magnesium salts. It is useful for short term intraocular surgery as corneal edema occurs on prolonged use as it contains only salts and no glucose and bicarbonate ions.

BSS plus contains glucose and bicarbonate ions. It contains Glutathione as a reducing agent which prevents damage to endothelial cells by oxidants. BSS contains citrate acetate buffer. BSS plus should be prepared fresh every time. If surgery is done on compromised corneal endothelium or surgery exceeds longer than 60 minutes a complete irrigating solution is needed.

Glucose fortification of solution is needed to prevent cataract formation when vitrectomy is performed on diabetic individuals.

Components	*Ringer lactate*	*BSS*	*BSS plus*
pH	6.6	7.4	7.4
NaCl	102	109.5	122.2
KCl	4	10.1	5.1
CaCl	3	4.3	1.1
MgCl		1.5	1.00
Sodium phosphate			2.8
Sodium bicarbonate			25
Glucose			51
Glutathione			0.3
disulfate		28.6	
Sodium acetate		5.8	
Sodium citrate	28		
Sodium lactate			
Sodium gluconate			

28 Vitreous Substitutes

The success of surgery for retinal detachment is dependant on attaining closure of a retinal break, relieving traction on the retina by thorough dissection of epiretinal membranes or scleral buckling, or both, and minimizing the recurrence of traction. The use of intravitreally injected gaseous and liquid materials as adjuvant agents to vitreoretinal surgery plays a vital role in facilitating retinal reattachment. These materials are used as

i) Intraoperative instruments to reestablish intraocular volume.
ii) Assist in separating membranes adherent to the retina.
iii) Manipulate the retinal detachment.
iv) Mechanically flatten detached retina.
v) In the long, intravitreal gases and silicone oil maintain the neural retina in apposition to retinal pigment epithelium post-operatively.

Intra Operative Use of Vitreous Substitute Material

During surgery, substances are injected into the vitreous cavity to maintain intraocular volume and they are also used for their mechanical properties to function as 'soft instruments'. These liquids assist in separation of membranes or in hypokinetic manipulation of the retina and are used in membrane dissection, such as delamination and retinotomy. Finally at the end of the procedures, a material of high surface tension is injected to maintain closure of the retinal break until the maturation of chorioretinal adhesion is attained. A variety of gaseous and liquid substances are currently used intraoperatively.

Air and Other Gases

Air was the first gas to be employed in retinal surgery. Air can be used intraoperatively during buckling surgery to restore intraocular volume after drainage of subretinal fluid, to flatten 'fish mouth tears' that form radial folds, and to unroll the posterior flap of large retinal tear. The tamponade lasts only for 24- 48 hours.

Viscoelastic Fluids

The Viscoelastic fluids used in vitreoretinal surgery include sodium hyaluronate, chondroitin sulfate or hydroxypropyl methyl cellulose which are having molecular weights ranging from 30,000 to 4 million Daltons. At zero shear

rate (steady state) these materials exhibit high viscosity, whereas at high shear rates (shearing occurs when a fluid is made to flow) the polymers undergo temporary and reversible deformation (pseudoelastic behavior), with the result that the viscosity of material decreases. This behavior makes the material to be injected through the small gauge cannulas and yet it will regain its shape after injection.

Sodium hyaluronate is most favorable viscoelastic used in vitreoretinal surgery.

It can be used to

i) gently separate an embedded foreign body from the retinal surface or choroid.
ii) separation of epiretinal membranes found in proliferative diabetic retinopathy
iii) unfold the retina during repair of giant retinal tear and to manage hemorrhage

Low Viscosity Perflurocarbon Liquids

Perflurocarbon liquids have properties of high density, surface tension, low viscosity and are optically clear. They can be easily injected and aspirated through small - gauge microsurgical instruments. The specific gravity of perflurocarbon liquids is almost twice the density of water, hence the force exerted by perflurocarbon liquids against the retina is greater than of silicone oil and flurosilicone oil. The interfacial tension of perflurocarbon liquids with water is roughly equivalent to silicone oil, and the material tends to be cohesive, so that liquid remains in one large bubble. Perflurocarbon liquids have some deterrence to passage through a break and are virtually inert.

Eg. - perflurotributylamine

- perflurodecalin.
- Peflurophenanthrene.
- Perflurooethylcyclohexane.
- Perflurooctylbromide
- Perfluro - n- ocatane

Perfluro - n - ocatane is preferred because, it is highly purified, has relatively lower boiling point and higher vapor pressure than other perflurocarbon liquids.

Indications

- retinal detachment with severe proliferative vitreoretinopathy
- traumatic retinal detachment
- giant retinal tears
- management of sub retinal hemorrhage, vitreous hemorrhage, dislocated lens and intraocular lenses and retinal detachment secondary to macular hole.
- To locate the peripheral retinal breaks that were not seen preoperatively.
- Aid in the removal of foreign body

Silicone Liquid

Silicone liquid injection needs high pressure because of its high viscosity, bimanual manipulation is needed because of its lower density than that of water. It has been used during pars plana vitrectomy

- To reposition the retina
- Removal of the epiretinal membrane
- Unroll the flaps of retinal tears.

Fluorosilicone has higher specific gravity than silicone oil and has been used as an alternative intraoperative tool in the management of inferior retinal detachment.

Intraocular Gas

Intraocular gases are mainly employed for pneumatic retinopexy and are used in conjunction with scleral buckling and drainage of sub retinal fluid. In addition, for complicated forms of retinal detachments requiring vitrectomy, such as proliferative vitreoretinopathy or giant retinal tears, gases are used to provide internal tamponade post operatively. Two properties of a gas bubble are of particular importance in the repair of retinal detachment.

i) High surface tension between the gas bubble and the thin layer of aqueous covering the retina serves to tamponade a retinal break by blocking the flow of fluid from the vitreous cavity.

ii) Specific gravity of air or any gas is lower than that of water, the gas bubble exerts a buoyant force that pushes the neural retina against the pigment epithelium.

The gases commonly used for intraocular use are

- Sulfur hexafluoride (SF6)
- Perfluromethane (CF4)
- Perfluroethane (C2F6)
- Perfluropropane(C3F8)
- Octafluorocyclobutane(C4F8)
- Perfluoro - n- butane(C4F10)

Sulfur hexafluoride reaches its maximal expanded volume by 24 to 48 hours after injection. Perfluropropane reaches its maximal expanded volume by 72 to 96 hours after injection. These expansile substances are avoided in eyes with preexisting glaucoma or synechial angle closure. Intraocular gases are more useful in superior retinal breaks, in cases of superior and inferior retinal breaks intraoperative gases are mixed with silicone oil for better apposition.

Hydrophilic polymers(hydrogel) are used experimentally for apposition of retinal breaks. They can be used for superior and inferior retinal breaks. They are optically clear and special positioning of the patients are not required. Drawbacks of hydrogels are, they may react with ocular tissue and they are difficult to inject.

Complications

Silicone oil use can lead to cataract due to mechanical obstruction of diffusion of nutrients to the lens. Therefore the patient has to avoid prolonged supine position. It can lead to glaucoma ma in aphakic eyes by pupillary block and emulsification of silicone oil leads to trabecular meshwork obstruction. Pupillary block is avoided by doing inferior iridectomy. Silicone oil in the anterior chamber may cause decompensation of corneal endothelium.

Intraocular gases can lead to cataract, pupillary block glaucoma, and corneal decompensation. Excessive gas expansion may lead to central retinal artery occlusion. It can cause optical disturbances due to scattering of light.

29 Medical Management of Glaucoma

There is a tremendous change and introduction of newer drugs in the management of glaucoma in the recent past.

The treatment is aimed at lowering the IOP, which is the major factor in the disease process.

The reduction of pressure could be achieved either by reducing the aqueous production or by facilitating the aqueous outflow. The pressure is brought down to the required levelcalled as Target pressure at which there is no progression of the symptom complex.

The newer concept in management is Neuroprotection. These are various drugs, which are being tried to protect the nerve damage and are still in the experimental stage.

Gene therapy is also gaining popularity in the recent past.

CLASSIFICATION OF ANTI-GLAUCOMA DRUGS

Older Classification

Based on the site of action :
Cholinergic drugs.
Adrenergic drugs dopaminergic drugs.
Carbonic anhydrase inhibitors.
Hyperosmotic agents.
Neuro protective drugs.
Alternative group of drugs.

Newer Classification

Based on the Mode of action of the drugs:
A) Drugs increasing the aqueous outflow.
B) Drugs reducing the aqueous production.

OCULAR CHOLINERGICS

Ocular cholinergics have been the main stay in medical management of Glaucoma, both primary or secondary.

They can be classified as direct acting and indirect acting depending on the site of action.

DIRECT ACTING CHOLINERGICS

These are the group of Cholinergics, which directly stimulate the effector muscle (Sphincter Pupillae) by acting on the parasympathetic neuromuscular junction.

They are—

Pilocarpine.
Carbachol.

Among these Pilocarpine is the most commonly used directly acting anticholinergic drug and has been time tested.

INDIRECT ACTING ANTICHOLINERGIC DRUGS

These drugs are also known as anticholinesterases. Main action of these drugs is to prevent destruction of acetylcholine at neuromuscular junction by acetylcholine esterase, and thus making available more of neurotransmitter at neuromuscular junction and stimulating parasympathetic system.

These group of drugs can be divided into

- Reversible cholinesterase inhibitors.
- Irreversible cholinesterase inhibitors.

Reversible Cholinesterase Inhibitors

The Carbamate Inhibitors

i) Physiostigmine.
ii) Neostigmine.
iii) Pyridostigmine.
iv) Demecarium Bromide.

Irreversible Cholinesterase Inhibitors

i) Ecothiophate.
ii) Di Isopropyl Pyrophosphate (DPP)

PILOCARPINE

It is a directly acting anticholinesterase drug mainly acting on sphincter muscle directly.

Derived from the plant Pilocarpus microphyllus, dispensed in hydrochloride or nitrate salt. Pilocarpine stimulates only muscarinic receptors.

Mechanism of Action

It is a parasympathomimetic drug acting on muscarinic receptors causing

- Constriction of Pupillary sphincter.
- Contraction of ciliary muscle.

Due to constriction of ciliary sphincter, there is no crowding of peripheral iris in narrow angle patients, thus facilitating the outflow.

Due to contraction of ciliary muscle it produces a pull over the scleral spur, by contraction of longitudinal muscle, thus opening the trabecular meshwork beams and facilitating the outflow.

Uses

1) Primary open angle glaucoma.
2) Primary angle closure glaucoma.

Dosage and Concentration

Available in 1%, 2%, and 4% concentrations.

Dosage is 4 - 6 times per day

Pilocarpine is less effective in higher-pressure ranges, due to ischemia of sphincter pupillae. Therefore, always used with other pressure lowering agents if the IOP is in the higher range.

Various Forms of Pilocarpine Available

1) **Pilocarpine Solution Eye drops:** Concentrations available are 1%, 2%, and 4% dissolved in water-soluble base preservative.
2) **Ocusert: (Membrane Controlled Devices):** This is a controlled release of pilocarpine system with concentration of 20 and 40 micrograms of drug released per hour.

   Advantage is improved compliance due to decreased frequency of application.

   Applied once a week
3) **Pilocarpine Gel:** This is also a controlled release system of pilocarpine with ease of once daily once administration.
4) **Soluble Insert Devices:** Impregnated with pilocarpine provides IOP control for 24 hrs.

   It is inserted in the cul-de-sac. Another advantage is that it can be removed when desired effect is achieved.
5) **Soft Contact Lenses:** Soft contact lenses soaked in pilocarpine increase the corneal contact time. Therefore greater aqueous levels and longer IOP control are attained.
6) Subconjunctival injections of pilocarpine can be tried, but are not used commonly because of corneal edema.

Adverse Effects

1. Decreased vision in low illumination.
2. Induced myopia.
3. Headache, supraorbital pain, browache.
4. Conjunctival congestion.
5. Lens opacities.
6. Retinal detachment.
7. Iris cyst.
8. Increased inflammation due to break in blood retinal barrier. Hence, not used in inflammatory glaucomas.
9. Cicatrical pemphigoid—rare.

Systemic Side Effects

Nausea, vomiting, diarrhoea, diaphoresis, lacrimation, salivation, smooth muscle contraction, bronchospasm.

Indirect Parasympathomimetics

Physiostigmine, ecothiopate iodide, rarely used now-a-days.

ADRENERGIC STIMULATORS

Epinephrine, Dipivefrine, Apraclonidine, Brimonidine.

Epinephrine

This direct acting sympathomimetic stimulates both Alpha and Beta Adrenergic receptors.

Mechanism of action can be divided into three phases:

Phase -I Decrease in aqueous production, due to initial vasoconstrictive effect thus slowing the ultrafiltration procedure.

Phase - II Increase in aqueous outflow. This forms the major bulk, which is responsible for decrease in IOP.

Phase - III Late increase in aqueous outflow facility.

Administration: It is available in concentrations of 0.5%, 1%, and 2%. Administered twice daily.

Available with various bases like hydrochloride, borate and bitartarate.

Dipivefrine

This is a prodrug of epinephrine, which undergoes pharmacological conversion into epinephrine into the body when administered.

Mechanism of action is same as that of Epinephrine.

Drug Interaction

Has got additive effect when used along with miotics.

Has additive effect when used with or pretreatment with Beta-blockers. Betoxolol > Timolol.

Adverse Effects

Local

1) Itching and burning sensation.
2) Reactive hyperaemia. Less with Dipivefrine.
2) Adrenochrome deposits on the lower palpebral conjunctiva, In case of bullous keratopathy deposits are seen on the cornea leading to Black Cornea. Deposits may be either in Stag Horn shape or in a branching pattern.
3) Tearing.
4) Photophobia.
5) Punctal epidermalisation and occlusion.
6) Madarosis.

7) Mydriasis.
8) Epinephrine maculopathy.
9) Corneal epithelial damage.

Clinical Therapeutic Uses

Useful as second line of drugs in combination with Miotics or Carbonic anhydrase inhibitors in POAG.

Apraclonidine

Apraclonidine is α_2 Adrenergic agonist

Mechanism of action

Apraclonidine reduces the IOP by reducing the aqueous production.

Added advantage of Apraclonidine is that, it does not penetrate the blood brain barrier like its parent compound clonidine, which causes systemic hypotension.

Side Effects

Transient dry nose and dry mouth, follicular conjunctivitis, contact dermatitis. Eyelid retraction in rare cases.

Therapeutic Uses

Apraclonidine is mainly used in the treatment of transient rise of IOP. As in—

1. Post laser procedures of anterior segment, where there is a transient spike in the IOP, as in Post-Iridotomy, Post Laser iridoplasty, and Post Laser Yag capsulotomy etc.
2. It is also used in chronic maintenance treatment of open angle glaucoma. For transient rise of IOP, apraclonidine is given before and after the procedure.

For long term use Apraclonidine is used twice daily. Available in 1% and 0.5% concentrations.

Brimonidine (0.2%)

Brimonidine tartarate has selective Alpha 2 agonist mechanism of action lowering of the IOP is by

i) Decrease in aqueous production.
ii) Increase in uveoscleral outflow without affecting conventional outflow and episcleral venous pressure.

Recently it has been found that Brimonidine also increases the optic nerve blood flow thus assuming a role in neuroprotection.

Adverse Reaction: Conjunctival hyperemia and burning sensation are the major side effects of this drug, which may force the patient to discontinue the drug.

Uses: Used as second line drug in treatment of POAG.

ADRENERGIC INHIBITORS

Beta Adrenergic Blockers

Nonselective adrenergic inhibitors:

Timolol, Levobunolol, Carteolol, Metipranolol, Metaprolol, Pindolol, Nadolol, Befunolol, Penbutolol.

Selective beta adrenergic blockers:

Betoxolol is the only selective Beta adrenergic blocker in clinical use.

Timolol

It is a non-selective adrenergic beta 1 and beta 2 antagonist

Mechanism of Action

Timolol reduces the IOP by reduction in aqueous production with its direct action on the ciliary processes and ciliary perfusion thus affecting the ultrafiltration.

It is available in 0.25% and 0.5% concentrations.

Administered as twice daily dosage for effective and sustained lowering of IOP.

Efficacy

Short-term Escape: In the initial phase of treatment, patients respond very well with good control of IOP which continues for 3-4 weeks and then a plateau is reached. This short term escape from high IOP is due to initial increase in the receptors.

Long-term Drift: In some patients after long term therapy with timolol, the patient stops responding to the drug and IOP starts raising slowly. This phenomenon is explained by tachyphylaxis and down regulation of receptors.

This can be prevented by changing the drug for 60 days in a year and then restarting the drug. This is known as Timolol holiday.

Adverse Reactions

Ocular

Itching, burning sensation, superficial punctate keratitis, corneal anesthesia, reduced tear production; Timolol is usually well tolerated.

Systemic

Timolol being a nonselective β blocker, it is contraindicated in patients with

Cardiovascular system disorders - Bradycardia, ↑ heart block, heart failure, decreased nocturnal blood flow, decreased exercise tolerance.

Respiratory: - COPD, Induces bronchospasm

Diabetes Mellitus: - It masks hypoglycemia in Insulin dependent diabetes mellitus.

CNS - causes depression, anxiety, confusion, hallucinations, aggravation of myasthenia gravis, nausea, diarrhea, abdominal cramps.

Uses

It can be used in control of IOP in all forms of Glaucoma.

1) Primary open angle glaucoma.
2) Acute attack of angle closure glaucoma.
3) Postoperative -Glaucoma in Aphakia /Pseudophakia.
4) Initial management of pediatric glaucomas.
5) It is also useful in Ocular Hypertensives to prevent progression to frank glaucoma and prevent damage.

Levobunolol

It is an analog of propranolol. It is a non-selective β- blocker.

Advantage of Levobunolol over Timolol is its ease of administration as once daily doses, which effectively controls the IOP comparable to Timolol.

Adverse effects - similar to that of Timolol.

Clinical uses- similar to that of Timolol. In addition, it has shown effective control of IOP in postoperative spikes.

Carteolol - similar to other β - blockers.

Metipranolol- Similar to other β - Blockers, Granulomatous anterior uveitis is commonly encountered following use of this drug.

Betaxolol

It is a cardioselective Beta 1 antagonist but it has some action on beta 2 receptors which produces the lowering of IOP by decrease in aqueous production without affecting the outflow.

Therefore reduction in IOP is less compared to Timolol.

Available in: 0.25% and 0.5% concentration.

Indications:

Treatment of ocular hypertension.

Treatment of POAG.

Used in combination therapy along with other drugs.

Side Effects

Reduced corneal sensitivity, itching.

Systemic side effects are similar to Timolol except for respiratory side effects, which are minimal or nil.

Alpha Adrenergic Inhibitors

Thymoxamine
Dapiprazol
Bunazosin
Prazosin

Thymoxamine

It is an alpha antagonist, which competes with norepinephrine for alpha-receptors.

Mechanism of action: It produces miosis by inhibiting the dilator muscle without affecting the ciliary muscle.

Clinical Applications

1) Reversal of mydriasis in case of phenylephrine induced mydriasis.
2) Management of angle closure glaucoma- it causes miosis, inspite of ischemia.
3) Differentiating between angle closure glaucoma with open angle glaucoma with narrow angles (Thymoxamine breaks the angle closure attack, but it has no effect on the open angle glaucoma).
4) Management of Pigmentary glaucoma.
5) Management of Eyelid retraction.

CARBONIC ANHYDRASE INHIBITORS

Carbonic anhydrase inhibitors belong to sulphonamide group of drugs.

This group of drugs, reduces IOP by reducing the aqueous humor formation.

Mechanism of Action

Sodium movement into the posterior chamber is directly linked to bicarbonate synthesis, which in turn results in movement of isotonic fluid into posterior chamber, thus aqueous humor is formed by active secretion.

If bicarbonate synthesis is obstructed then there is reduced aqueous production. Carbonic anhydrase inhibitors contain free sulphonamide, which competes with the bicarbonate ion in binding to enzyme carbonic anhydrase. Thus it blocks the bicarbonate synthesis and aqueous humor formation.

$CO_2 + H_2O \rightarrow H_2CO_3 + H^+$

Various carbonic anhydrase inhibitors available are:

Acetazolamide.

Acetazolamide sustained release capsules.

Methazolamide - Metabolised by liver hence contraindicated in liver disease.

Ethoxazolamide.

Dichlorphenamide.

Dorzolamide.

Acetazolamide induces metabolic acidosis and reduces the IOP and also increases the visual function in glaucomatous eyes by increasing the blood flow to the optic nerve.

Hypotensive effect of CA inhibitors starts 2 hrs after administration and lasts for 6 hrs.

Dosage and Administration

Acetazolamide:

Dosage 250 mg every 6 hrs orally.

500mg sustained release tablets bid orally.

Dichlorphenamide

25 - 50 mg 1 - 3 times daily orally.

Methazolamide

50 - 100 mg 2-3 times daily orally.

Other uses of acetazolamide

1. Increases the absorption of subretinal fluid.
2. Increases the adhesion between RPE and neurosensory retina.
3. Used in macular oedema in patients with retinal pigment epithelial disease.

Clinical uses

1) Acute angle closure glaucoma.
2) Secondary glaucomas.

Routes of administration

Intravenous/oral/topical - Oral is the most common route used.

Side Effects

Ocular

Transient myopia, noted in few persons.

Systemic

Metabolic acidosis, hypokalemia, malaise, fatigue, weight loss, metallic taste, abdominal discomfort, nausea, taste alteration, epigastric burning, renal calculi, blood dyscrasias, thrombocytopenia, agranulocytosis, aplastic anemia, exfoliative dermatitis, hypersensitive nephropathy.

Contraindications

1) Adrenal Insufficiency.
2) Severe Liver or Renal impairment.
3) Hyperchloremic metabolic acidosis, renal calculi.
4) Sulphonamide hypersensitivity.

Topical CA Inhibitors

(a) Dorzolomide: 2%. It is a topical carbonic anhydrase inhibitor.
(b) Brinzolamide 1%.
(c) Sezolamide.
(d) Acetazolamide.

They are effective topical CA Inhibitors, administered two - three times daily dosage.

Mechanism of action is similar to other CA inhibitors. They cause reduction in IOP by reducing the aqueous production.

Maximum IOP reduction is within 2 hours after the instillation of drops.

Side effects

Topical CA inhibitors are well tolerated than systemic CA inhibitors. Frequent side effects noted are - Burning and stinging sensation in the eyes. Brinzolamide has got less ocular discomfort as compared to Dorzolamide.

Superficial punctate keratitis has been noted in some patients.

Patients allergic to sulphonamides may have anaphylaxis, erythema multiforme, Steven Johnsons syndrome, bone marrow depression, hemolytic anaemia, thrombocytopenic purpura, leucopenia etc.

HYPEROSMOTIC AGENTS

Systemic hyperosmotic agents are used for the immediate control of IOP.

Hyperosmotic agents available are:

1) Glycerine.
2) Isosorbide.
3) Mannitol.

Mechanism of Action

These agents increase the plasma osmolarity, thus causing the water from the eye mainly vitreous to be drawn into the systemic circulation, thereby reducing the intraocular volume and thus decreasing the IOP.

Indications

These agents are used as adjunctive therapy to lower the IOP rapidly :

i) Acute attack of glaucoma.
ii) Prior to ocular surgery.
iii) In Phacomorphic glaucoma.

Contraindications

- Anuria.
- Severe dehydration.
- Acute pulmonary edema.
- Cardiac failure or decompensation.

Glycerine

Glycerine is available as 50% and 75% lime flavoured oral solution.
Dosage - 1.0 to 1.5g /Kg body weight.
Has got high caloric value therefore should be avoided in patients with diabetes.

Isosorbid

Isosorbide is available as 45% mint flavoured solution
Dosage 1-2 g/Kg of body weight.

Mannitol

Available in 5 - 25 % solution.
Dosage 1-2 g / kg of body weight, given over a short period of time, i.e., 30 minutes.

If used preoperatively, it should be given 1 - 1½ hours prior to surgery to get maximum effect.

Disadvantages: - Mannitol solution when exposed to low temperature will crystalize.

Adverse Reactions

Systemic hypertension, nausea, ↑ vomiting, marked diuresis, confusion, congestive cardiac failure, ↑ acidosis, ↑ dry mouth, ↑pulmonary edema, hyperglycemia.

Contraindicated in oliguria and anuria.

Prostaglandin Analogues

The drugs that come under this group include:

1) Latanoprost - 0.005% (One drop once daily preferably in the evening).
2) Bimatoprost - 0.05% (One drop once daily preferably in the evening).
3) Unoprostone -0.12% (One drop twice daily).
4) Travoprost -- 0.03% (One drop once daily preferably in the evening).

Latanoprost

It is prostaglandin PGF2 alpha analogue.

Latanoprost is a prodrug which is absorbed through cornea, where it is hydrolysed into the active form.

It decreases the IOP by increase in Uveoscleral outflow.

Effect of prostaglandin Uveoscleral outflow is caused by two probable mechanisms:

i) Relaxation of ciliary muscle
ii) Remodeling of extracellular connective tissue matrix around the ciliary muscle cells.

Latanoprost also has neuroprotective action by interfering with cyclo-oxygenase and nitric oxide synthetase activity thereby decreasing apoptosis of retinal ganglion cells.

Dosage and Administration

0.005% (50 mg / ml) once daily administration, which increases the patient compliance drastically.

Adverse Effects

Conjunctival hyperemia, superficial punctate keratitis, increased pigmentation of iris (due to increase in number of melanin granules in the iris), increased growth of eyelashes, increased pigmentation of eye lashes, foreign body sensation and grittiness are the main side effects encountered.

Bimatoprost

It is a prostanoid derivative acting through independent receptors. It reduces the IOP by increasing the Uveoscleral outflow.

Ocular side effects are similar to that of Latanoprost. Conjunctival hyperaemia and foreign body sensation is less marked and drug is better tolerated.

It is also administered in once daily dosage.

Travoprost 0.03%

It is a prostaglandin analogue.

In the treatment of glaucoma one drug should never be substituted for another. If the response is inadequate, another should be added and the response to the addition of new drug noted.

Neuroprotective Agents

Now growing evidence points to the potential of neuroprotection of the optic nerve as these aim at vision saving by cell survival.

Retinal ganglion cell death is initiated due to various pathological events like ischemia, axonal injury and changes in the lamina cribrosa. This blocks the transport of neurotropins (growth factors from the brain to retinal ganglion cells). Blockage in these neurotropic factors initiates a cascade of events causing cell death.

Apoptosis

Apoptosis is defined as a programmed cell death, which is genetically determined.

It is a two hit mechanism in which first cells are triggered by the stimulus to activate the death gene. The second stimulus produces the chain of events leading to cell death. These triggered cells respond to noxious stimuli more easily than the non-triggered ones.

The aim of neuroprotective drugs is to increase the resistance of neurons to stresses that might trigger apoptosis and improving the cell survival signals.

Various neuroprotective agents are:

1. Alpha -2 agonist (Brimonidine).
2. Calcium channel blockers.
3. NMDA antagonists.
4. NMDA channel blockers.
5. Glutamate antagonists.
6. Nitric oxide inhibitors.
7. Neurotropic factors.
8. Free radical scavengers.

Alpha-2 agonists: Brimonidine

It binds to cell receptors and aids in neuroprotective functions; it preserves retinal ganglion cell survival and photoreceptor function. It is also known to increase the blood flow to the optic nerve.

Mechanism by which Brimonidine increases the cell survival is by up regulation of the expression of antiapoptotic gene bcl - 2 and bcl - x1, and has no effect on the expression of the apoptotic bax gene

It also upregulates the basic fibroblast growth factor (bfGF) which enhances the cell survival.

Calcium Channel Blockers

Calcium channel blockers such as diltiazem, nifedipine, nilvadipine and verapamil inhibit calcium influx in vascular smooth muscle decreasing the vascular tone and increasing the blood flow, these are particularly useful in patients with normotensive glaucoma.

NMDA Antagonists

It is directly acting non-IOP lowering neuroprotective agent.

N - Methyl D -Aspartate provides neuroprotection by blocking the glutamate, which drives cell death by facilitating calcium entry into the cell.

Glutamate is an important neurotransmitter in the retina. Under pathological conditions there is increase in the level of glutamate, which leads to cell death. Memantine—a non-competitive NMDA receptor antagonist derived from amantadine has got proven neuroprotective properties. Its non-competitive action blocks the toxic effects of glutamate without affecting the normal cell function.

Other drugs in research are—

Eliprodil: A non-competitive NMDA receptor antagonist.

Riluzole: Presynaptic Glutamate release inhibitor.

L - Deprenyl: Monoamine Oxidase inhibitor, which gives protection to retinal ganglion cells. It also inhibits apoptosis.

Nitrous oxide inhibitors, Antioxidants, Free Radical scavengers are general Neuroprotective agents which prevent secondary retinal cell and ganglion cell damage.

30 Viscoelastics

Viscoelastics refer to solutions that have dual properties—they act as viscous liquids as well as elastic solids or gels. In 1950's viscoelastic solutions were introduced as vitreous substitutes and in 1970's they were first used in anterior segment surgery. Balaz's introduced sodium hyaluronate as a replacement for aqueous and vitreous humor.

The ideal viscoelastic should be viscous enough to resist collapse of anterior chamber while at rest but liquid enough to be injected through small gauge cannula. The relevant characteristics of these agents are:

1. Viscoelasticity.
2. Viscosity.
3. Pseudoplasticity.
4. Surface tension.

Viscoelasticity is the ability of solution to return to its original shape after stress. Viscosity is resistance to flow determined by molecular weight. Pseudoplasticity is the ability to transform from a gel to liquid. Surface tension determines coatability, that is lower surface tension substances have better coatability.

The main functions of viscoelastics are:

1. Space maintenance.
2. Ease of injection and tissue manipulation.
3. Shock absorption.
4. Surface coating.

Space maintenance is dependent on solutions viscosity at rest. Higher molecular weight substances (HMW) have increased viscosity at rest and better space maintenance.

Tissue manipulation is dependent on pseudoplastic behaviour. HMW substances exhibit most pseudoplastic behaviour and better tissue manipulation.

Shock absorption is also dependent on molecular weight and pseudoplastic behaviour. Higher the elasticity, better the shock absorption.

Surface coating should prevent damage to endothelial cells during irrigation and other instrumentation. Surface coatability depends on surface tension. Lower surface tension has better coatability.

Depending on the above characteristics, viscoelastics can be classified as-

1. Cohesive.
2. Dispersive.

Cohesive viscoelastics adhere to each other are used for space maintenance, tissue manipulation, shock absorption and the easy to remove. They have high molecular weight. Dispersive viscoclastics have lower surface tension being less viscous are used for protecting corneal endothelium and coating instruments and intra-ocular lens.

Commonly used viscoelastics are:

1. Sodium hyaluronate.
2. Hydroxy propyl methyl cellulose.
3. Chondroitin sulphate.

Sodium hyaluronate is a biopolymer composed of two monosaccharide units found in aqueous and vitreous humor. It stimulates neutrophil function, cell proliferation, aggregation and migration facilitating wound healing. It is a mediator of ocular inflammation. It is available in higher and lower molecular weight concentrations. High molecular weight concentration has better space maintenance, and shock absorbing capabilities. Lower molecular weight substances are used in combination with other viscoelastics for their combined effect. It is produced by bacterial fermentation by genetic engineering.

Hydroxy propyl methyl cellulose 2% is a lower molecular weight substance derived from wood pulp. It is hydrophilic and can be irrigated from eye. It is viscoadherent with higher coatability and a dispersive viscoelastic.

Chondroitin sulphate is similar to hyaluronate and disappears from anterior chamber quicker than hyaluronate. It is derived from shark fin cartilage. It is mostly used in combination with hyaluronate (Sodium hyaluronate 4% and chondroitin sulphate 3%) so that, it has properties of both cohesive and dispersive agents.

Available viscoelastic agents are-

Hyaluronate 1.4%

Hyaluronate ↑ 4% and Chondroitin 3 %

HPMC ↑ 2 %.

The side effects are—

1. Increases IOP.
2. Occasionally increases intraocular inflammation leading to sterile endophthalmitis.

31 Diabetes Mellitus

It is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. It is classifed into two types—

1. Type I diabetes: Results from autoimmune mediated destruction of beta cells of pancreas. This is characterized by severe insulin deficiency which needs to be replaced.
2. Type II diabetes: This is more common (90%) and is due to obesity, insulin resistance and relative insulin deficiency. Insulin secretion is usually sufficient.

Gestational diabetes mellitus occur in 4% of pregnancies and resolves after delivery. Diagnosis of diabetes is made when—

1. Fasting blood glucose is > 126 mg/dl.
2. Random blood glucose >200 mg/dl.
3. Oral glucose tolerance test shows plasma glucose of 200 mg/dl or more after 2 hours with 75 gm glucose load.

The aims of treatment are—

1. Metabolic control.
2. Prevention of acute and long term complications.

Management of Diabetes Mellitus

1. Dietary modifications: It is more important in over weight persons. An intake of 10-20% of total calories should be proteins and less than 30% as fat.
2. Exercise: It improves insulin sensitivity, reduces fasting and post-prandial glucose.
3. Medications: It includes insulin and oral anti-hyperglycemic drugs.

Insulin Preparations

Type I diabetes requires life long insulin requirement. They differ by duration and peak activity.

1. Rapid acting insulin: These includes regular insulin, Lispro and Aspart insulin. They can be administered by intravenous, intramuscular and subcutaneous route.
2. Intermediate acting insulin: Isophane and lente insulin are released slowly from sub cutaneous site.

3. Long acting insulin: It provides a steady basal level of insulin and is absorbed slowly from injection site.

Insulin type	*Onset of action*	*Duration of activity*
Rapid acting		
Lispro, Aspart	20- 50 minutes	3- 5 hours.
Regular	1 hour	6- 8 hours.
Intermediate acting		
NPH	1- 2 hours	18- 24 hours.
Lente	1- 2 hours	18- 24 hours.
Long acting		
Ultra lente	4- 6 hours	24- 48 hours.
PZI	3- 8 hours	24- 48 hours.

Rapid acting insulin can be mixed with intermediate or long acting insulin. Premixed preparations are also available and this can be given subcutaneously in the abdominal wall thighs, arms. They are absorbed faster from abdomen due to the difference in blood supply.

Insulin dose is normally in the range of 0.5- 1units/kg/day. It is usually given as multiple daily insulin injections. 50% of insulin requirement is for basal insulin supply, which is by long or intermediate acting insulin and the remainder by rapidly acting insulin.

Insulin is administered before breakfast and before evening meal. 2/3 rd of daily dose is given in the morning.

Insulin secretagogues

1. Sulfonylureas: They lower blood glucose by augmenting insulin secretion. They should be taken 1/2- 1 hour before food. Dose should be started with lower concentration and gradually increased over several days. Hypoglycemia and weight gain are the common side effects.
2. Repaglinide: This stimulates insulin secretion and has a very short duration of action. They should be taken 1/2 hour before meals.
3. Nateglinide: It acts directly on pancreatic beta cells and stimulates insulin secretion. It should be taken 10 minutes before food. It controls postprandial hyperglycemia.

Sulfonylureas	*Dose*	*Duration*
First generation		
Tolbutamide	0.5-2 gm	12 hours.
Tolazamide	0.5-1 gm	12-24 hours.
Chlorpropamide	100- 500 mgm	36-72 hours.
Second generation		
Glipizide	5-40 mgm	12 hours.
Glimipiride	1-8 mgm	24 hours.
Rapid acting		
Nateglinide	180- 360 mgm	1- 2 hours.
Repaglinide	1- 16 mgm	1- 2 hours.

Biguanides

Metformin is currently in use. It inhibits glucose output by liver and stimulates glucose uptake by peripheral tissues. It is taken along with food. Lactic acidosis is the most serious side effect of the drug.

Metformin should be avoided in cardiogenic or septic shock. It is started with single dose of 500 mgm and is increased to 2000 mgm/ day.

Alpha Glucosidase Inhibitors

It acts by inhibiting carbohydrate digestion and decreases postprandial hyperglycemia. It is used along with other antihyperglycemic drugs.

Acarbose 75- 300 mgm/day.
Miglitol 75- 300 mgm.

Side effects are due to carbohydrate malabsorption like diarrhoea, abdominal cramps. Acarbose increases liver enzymes.

Thiazolidinediones

It increases insulin sensitivity in muscle, adipose tissues and liver.

Drugs	*Dose*	*Duration*
Rosiglitazone	2- 8 mgm/ day	12- 24 hours.
Pioglitazone	15- 45 mgm/day	24 hours.

Drug induced hepatoxicity can occur along with edema, and cytopenia from increased plasma volume. It may precipitate heart failure in patients who have cardio vascular diseases.

Rosiglitazone can be used alone or with other drugs. It can be taken with or without food. Pioglitazone can be used similar to Rosiglitazone. Both are started at a lower dose and gradually increased after several weeks. Regular monitoring of hepatic transaminases is done.

Section 5

Physiology

32 Physiology

ACCOMMODATION

Accommodation is a complex constellation of sensory neuromuscular, biophysical phenomenon, by which the overall refracting power of the eye changes rapidly to image objects at different viewing distances clearly on the retina. To accomplish this task the following strategies are required.

i) Changing the corneal curvature.
ii) Changing the distance between the cornea and retina.
iii) Placing another lens system between the cornea & the retina whose effective refracting power can be varied by changing its surface curvatures or its position within the globe.
iv) Changing the index of refraction of one or more components of the ocular media.
v) Having two or many separate optical pathways of different refractive power.

An interesting fact is that the raccoon can accommodate upto 20 diopters by moving the lens towards the cornea without changing the lens thickness.

In humans, accommodation is brought about by altering the form of the crystalline lens and this is accomplished by contraction of the ciliary muscle. The normal eye is so constructed that, when at rest, rays of light coming from infinity are focused on the retina. The refractive indices of the ocular media, the curvature of the refractive surfaces and the position of retina is such that the rays of light entering the eyeball parallel to the optical axis are focused on the sensitive outer layer of the retina i.e. the rods & cones. By definition an emmetropic eye is one in which the retina coincides with the posterior principle focus of the optical system when the eye is at rest. Rays of light coming from a distance of 6 meters or more are considered parallel and come, therefore, to a focus on the retina of the emmetropic eye.

The region through which an object may be moved in space without causing noticeable blurring of the image is called the depth of the field. This increases considerably as the pupillary aperture is narrowed. An emmetropic eye with a pupil of 2 mm has a depth of field from infinity of about 15 metres. But if the pupil becomes 4mm it is infinity to 30 meters.

The resolving power of the eye as an optical instrument is measured by the smallest visual angle at which two objects can be distinguished separately. This is called the minimum separable and is approximately 40 to 60 seconds of arc. In the emmetropic eye an object that subtends an angle of 60 seconds of arc has a retinal image of about 0.004 mm. An object may be seen even when it is out of focus as long as its diffusion image does not subtend an angle greater than the minimum separable (60 seconds of arc) i.e., as long as the image does not spread more than 0.004 mm. If the object is moved closer to the eye than the depth of focus permits, the blurred arc of diffusion falling on the percipient elements subtend an angle more than 60 seconds of arc. In order of obtain a clear focus under physiological conditions, these circumstances, either the dioptric power of the eye must increase or the eyeball must become large. It is obvious that the human eye cannot change its axial length under physiological condition; therefore, this change of focusing power must take place by changing the dioptric power of its refractory surface. Two objects cannot be in focus at the same time. Therefore, when the difference between the distances of each from the observer exceeds the depth of focus of the eye, the dioptric power of the eye must change.

The accommodative change whether it is one of relaxation or one of increased accommodation occurs with great precision and is generally completed in about half a second. The exact nature of the stimulus by means of which a blurred image on the retina gives rise to the accommodation reflex is not entirely known.

Anatomy of the Parts of the Eye Concerned with Accommodation

Accommodation is the result of a change in the form of the lens, brought about by the contraction of the ciliary muscles.

The Lens Capsule

It is a thin transparent membrane enclosing the lens and is composed of two layers. The outer layer is derived from the zonule and hence called the ‘ zonular lamella ’.

This becomes exfoliated in Glass Blower’s cataract and Glaucoma capsulare (Fig. 32.1).

The chief characteristic of the lens capsule is its elastic properties. The anterior capsule is much thicker than the posterior capsule and varies in thickness from the equator to the anterior pole (Fig. 32.2).

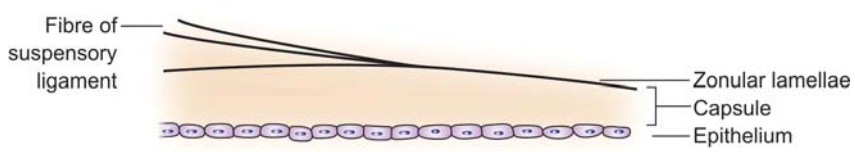


Fig. 32.1

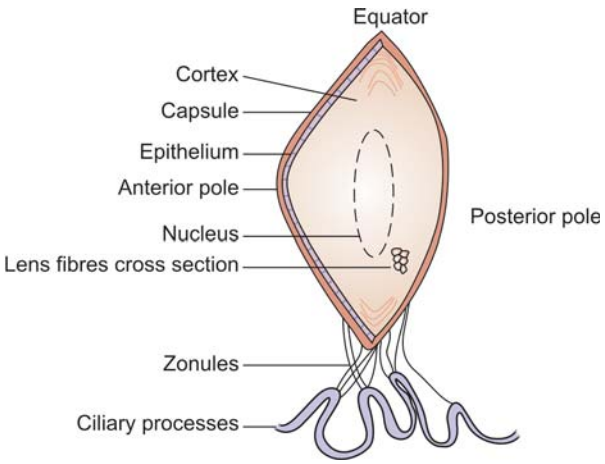


Fig. 32.2

Crystalline Lens and Zonular Ligaments

The Lens Substance

Anterior surface faces a layer of epithelial cells. There is no epithelium on the posterior surface. All the new fibres as they are formed arise at the equator and push the older fibres towards the centre as they form.

Growth

The lens continues to grow throughout life. There are two phases namely infancy and early childhood – rapid growth followed by a phase of slow, steady growth which continues throughout life. A loss of elasticity is associated with continued growth. Since the older fibres cannot be cast off as is usual with epithelial cells in other parts of the body, they are crowed together in the centre of the lens. Here they lose water and form a dense nucleus. This nucleus is of little physiologic or pathologic significance until the age of 30 years by which time it has become so large and dense that it interferes with the deformation of the lens during accommodation.

The growth of the lens with age is due to an increase in the thickness of the cortex.

Elasticity

The lens substance is not elastic. The posterior cortex has greater malleability than the anterior and the difference between the two becomes more marked with increase in age. The change from the soft cortex to the hard nucleus leads to an increase in refractive index of the lens. The more central fibres largely account for the refractive power of the lens.

Radii of Curvature

Anterior surface – 11 mm.
Posterior surface- 5.7 mm.

The Zonules

The lens is supported by the ciliary body. The zonules serve as the rope and appear to be made up of separate individual fibers when viewed at with slit lamp biomicroscope.

The Ciliary Muscle

Schlemm's has divided the ciliary muscle into three groups:

i) Meridional
ii) Radial
iii) Circular

Meridional Bundle

It is located on the scleral aspect of the muscle which is much thicker anteriorly than posteriorly.

Its origin is from the corneo-scleral junction just to the back of the Schlemm's canal. It inserts into the choriodal coat near the posterior pole.

Radial Portion

It is interposed with the connective tissue framework.

Circular Portion (Muller's Muscle)

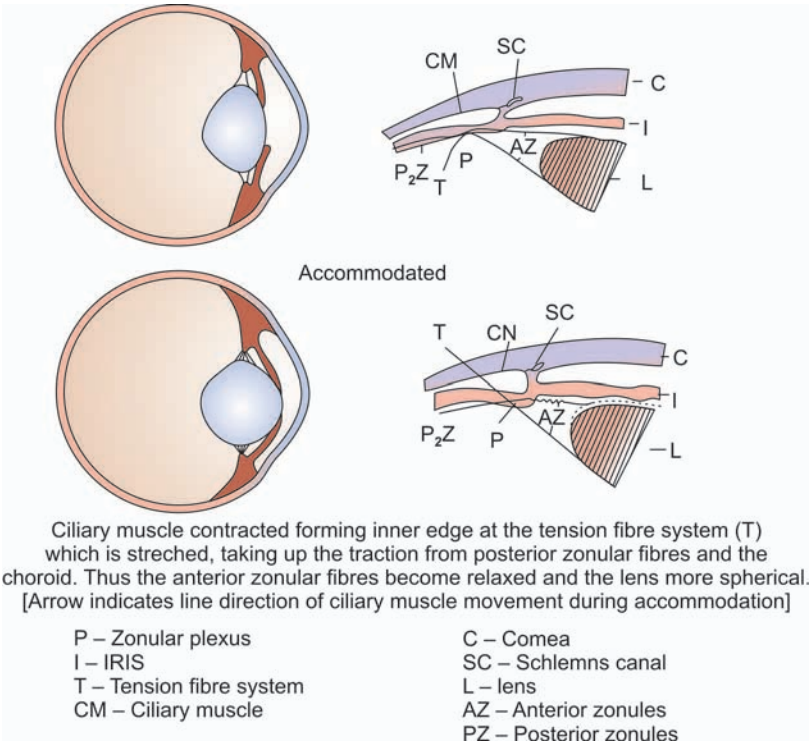
This forms a circular bundle at the inner anterior aspect of the ciliary body. It acts as a sphincter muscle and on contraction, narrows the ring formed by the ciliary processes. Always poorly developed in myopic eyes and well developed in hyperopic eyes which suggest that this bundle is important in accommodation.

Innervation of the Ciliary Muscle

i) IIIrd cranial nerve.
ii) Fibres from the sympathetic nervous system.

Changes in the Eye During Accommodation (Fig. 32.3)

i) Pupil contracts during accommodation and convergence. This is synkinesis and not a true reflex in that it does not depend upon either accommodation or convergence alone for its appearance.
ii) Anterior pole of the lens moves forwards carrying the iris with it. Hence, the anterior chamber becomes slightly shallow in the centre as the anterior pole of the lens approaches the back surface of the cornea. The posterior pole does not change it position.
iii) The anterior surface of the lens becomes more convex so that its radius becomes smaller. The posterior surface increases its curvature slightly
iv) Since the posterior pole remains fixed and the anterior pole moves forward, the thickness of the lens at the centre increases.
v) As the lens increases in axial thickness, it diminishes in diameter.
vi) Change occurs in the tension of the lens capsule. The anterior capsule becomes slack and separates from the posterior capsule.



Ciliary muscle contracted forming inner edge at the tension fibre system (T) which is streched, taking up the traction from posterior zonular fibres and the choroid. Thus the anterior zonular fibres become relaxed and the lens more spherical. [Arrow indicates line direction of ciliary muscle movement during accommodation]

P – Zonular plexus
I – IRIS
T – Tension fibre system
CM – Ciliary muscle
C – Comea
SC – Schlemns canal
L – lens
AZ – Anterior zonules
PZ – Posterior zonules

Fig. 32.3

vii) The lens is displaced in the direction of gravity during accommodation.
viii) Refractive power of the lens changes.

THEORIES OF MECHANISM OF ACCOMMODATION

Relaxation Theory of Helmholtz

Helmholtz considered that the lens was elastic and that in the normal state it was kept stretched and flattened by the tension of the suspensory ligament. In the act of accommodation, the contraction of the ciliary muscle lessened the circle formed by the ciliary processes and thus relaxed the suspensory ligament. The lens then assumed a spherical form. This is the 'Relaxation theory of Helmholtz'.

The relaxation theory assumes that when the eye is at rest or unaccommodated, the lens is compressed in its capsule by the zonules. In the compressed form, its surfaces are curved least and the dioptric power accordingly, is at minimum. The zonule is kept constantly stretched by its attachments to the ciliary body.

In the original theory of Helmholtz, it was supposed that the elasticity of the choroid sustained this. When the ciliary muscle contracts, two things happen. The choroid is pulled forwards relaxing the tension of the zonules and the ring

formed by the processes is narrowed by the sphincter or circular fibres of the ciliary muscle, thus making the zonules still more lax. When this occurs, the lens is freed of any compressory force and by virtue of elasticity of its capsule, it assumes the shape of a sphere. This increases the dioptric power of the eye.

It does not seem logical, however to expect the choroid to constitute a counter weight to such a continually acting force. The choroid being a richly vascular network is hardly a structure to be constantly stretched without showing pathological changes. To overcome this it was suggested that the traction of the zonules was borne chiefly by the radial and longitudinal portions of the ciliary muscles. According to Henderson, the ciliary muscle had functions mediated by different muscle bundles. The radial and longitudinal fibres maintain a constant postural activity which counterbalances the pull of the zonules, whereas sphincter or circular muscle overcomes the tension on the zonules and permits it to become slack. Thus according to him both parasympathetic and sympathetic innervations are concerned in accommodation. He considered that accommodation is accomplished by an inhibition of the postural activity of the longitudinal fibres of the ciliary muscles and by active contraction of the circular fibres. The former is due to 3rd nerve. He regarded the sympathetic as excitatory and 3rd nerve as inhibitory.

It has been shown already that during accommodation the lens is displaced in the direction of gravity and that both anterior and posterior capsules of the lens become slack, particularly the former. This is strong evidence in favour of the relaxation theory which demands that during accommodation both the surfaces of the lens become more convex.

One of the fundamental objections to the Helmholtz theory is that the choroid does not move forward when the longitudinal fibres of the ciliary muscle contract (Fig. 32.4).

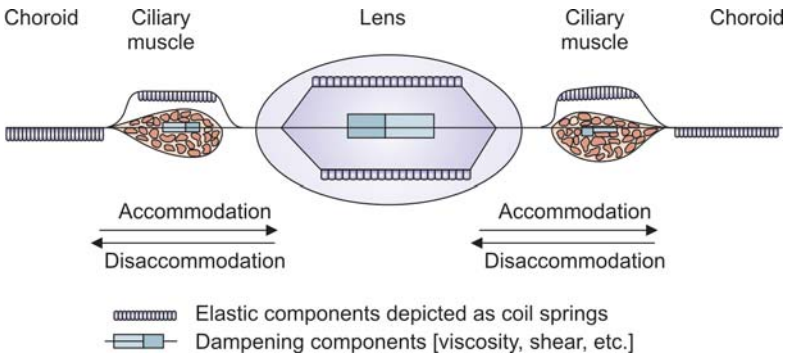


Fig. 32.4

The Tscherning Theory or Theory of Increase in Tension

Tscherning contended that in the act of accommodation the anterior surface of lens assumed, not a spherical but a hyperbolic form; and in order to account for the formation of the anterior lenticonus, he suggested that the contraction of the ciliary muscle tightened the suspensory ligament and compresses the lens against the vitreous, an action which caused its anterior surface to be bulged forwards. According to Fincham, the capsule of the lens particularly the anterior capsule does not have the same thickness throughout and he made use of this, to account for the conoidal during accommodation. If the lens capsule is elastic and exerts a compressory effect on the lens, the compression will be least where the capsule is at its thinnest. As a result, the lens bulges through. It is now generally accepted that the anterior surface of the lens becomes more convex as described by Tscherning and that during accommodation, it becomes conoidal in form.

When all the evidence for the two theories are viewed impartially, the weight of evidence is in favour of the relaxation theory. The general impression gained is that there is relaxation of the zonules during accommodation, with relaxation of the lens capsule to create greater curvature of the central surface of the lens. In addition these observations support the belief, during accommodation, the vitreous body does press on the periphery of the lens.

Aqueous Humor

The avascular structures of the anterior segment of the eye, the lens and the cornea depend upon a constant turnover of the surrounding aqueous to deliver nutrients and wash out metabolic waste products. So the important functions of the aqueous flow is to compensate the lens and the cornea for the lack of blood vessels and also to maintain the clarity of the optical media of the eye by constant production and drainage, maintaining intraocular pressure. In many respects, aqueous humor is comparable to cerebro spinal fluid. It fills the prelental space, (anterior, posterior) penetrates the gel-like vitreous humor and takes the place of the lymph that is absent within the eye as essential fluid. The combination of aqueous flow and the resistance of outflow routes ensure a relatively high pressure within the eye. The IOP is one of the highest tissue pressure in the body and may be a necessary requirement to maintain a stable form of the eye globe even when subjected to traction by the extraocular muscles.

Characteristics of Aqueous Humor

Physical Properties

Specific gravity – 1002 to 1012.
Refractive index- 1.3336 – 1.3370.
Surface tension – 72-73 dynes/cm at 18 °c

Viscosity

Normally with little protein, the viscosity is slightly greater than that of water and considerably less than that of blood (1.029 – 1.030).

Conductivity

Conductivity is greater than that of serum but lower than that of CSF due to the latter's greater concentration of Na^+ and Cl^- ions.

Osmotic Pressure

Aqueous is slightly hypertonic compared to plasma 3 – 5 mmols/li

Reaction

Slightly alkaline (pH 7.53).

Chemical Composition of Aqueous

Protein 0.02 gm%.
Urea 12 mg%.
Amino acids 9 mg%.
Creatinine 1.7 mg%.
Reducing substances 0.06%.
Organic acids – Ascorbic acid & lactic acid – higher than in plasma.

— Uric acid, pH, concentration of HCO3- lower than in plasma.

Hyaluronic acid present.
Inorganic acids- Na+ less than in plasma

— Cl- higher than in plasma
— Ca^{2+}, Mg^{2+}, K^+ lower than that in plasma.

Formation of Aqueous Humor

Anatomically the blood – aqueous barrier consists of

i) The anterior iris surface.
ii) The ciliary epithelium.
iii) The retina overlying the choriodal vessels anterior to the equator, where the retina is adequately nourished by choriodal circulation alone and diffusion through it into the vitreous presumably occur, and
iv) The capillary walls of the retinal vessels in the posterior half of the retina.

The main source of the intra ocular fluids is from the ciliary process (non–pigmented epithelium) . Histological findings point to the ciliary body as the main source. Recently the granules have been demonstrated in the ciliary epithelium whose structure is similar to those having known secretory activity.

The process involved in the formation of aqueous are complex and more than one mechanism must operate in order to account for the various discrepancies between the distribution ratio of the major constituents of plasma and aqueous.

Mode of entry of specific substances in the aqueous humor with regard to its composition must be influenced by the following processes:

i) Diffusion from the blood into the ciliary epithelium.
ii) Dialysis and ultra filtration.
iii) Active secretion by the ciliary epithelium into the posterior chamber (selective transport).
iv) A form of restricted leakage through spaces or pores present in the blood aqueous barrier.

Aqueous cannot be a simple plasma filtrate (diffusion) as it is slightly hypertonic to plasma.

Excess of ascorbate, chloride and sodium in the aqueous humor as compared with dialysate of plasma indicates secretory mechanism.

In examining the concentration ratios of these substances in the aqueous and plasma, the values are not compatible with the predicted values based on a distribution in accordance with GIBBS – equilibrium for a plasma dialysate.

Ultra Filtration: Also cannot explain this totally (dialysis in the presence of hydrostatic pressure), since the hydrostatic pressure differential between the aqueous and capillary bed in the ciliary processes is relatively negligible.

Pinocytosis: Movement of package substances by means of vesicle formation may be considered as a form of active transport.

Active Transport: Sodium and ascorbate are actively transported from blood to aqueous by the cells of ciliary epithelium, ultrafiltration playing a secondary role. Active transport of sodium is important because it may be considered as a primary process of aqueous secretion. Other ions follow passively the Na^+ ions and water is also moved from the plasma to aqueous as per osmotic considerations. The rate of Na^+ transport by ciliary body is sufficient to explain the rate at which water enters the eye.

Active transport is an energy requiring process. Various ATPases may play an important role in this. Na^+ activated ATPases has been found, intra cellularly in the cell membrane.

Oxidative phosphorylation is in some way connected with secretory process. Citric acid cycle may play a role in providing the energy (Fig. 32.5).

Membrane Permeability

Concentration of protein is determined by the sieve like properties of blood – aqueous barrier and by their relative molecular size.

On the other hand, penetration for lipid soluble substances is determined by their lipid solubility rather than molecular size.

Other factors affecting membrane permeability are a) Electrochemical potential on either side of the membrane b) Pore size.

Substances can move across the biologic membrane by means of simple or facilitated diffusion, filtration, active transport or a combination of all these.

In the eye, the biologic membrane forming blood – aqueous barrier in the posterior chamber is located at the site of ciliary epithelium. Capillary endothelium cannot be considered as a biologic membrane because it lacks active secretory process.

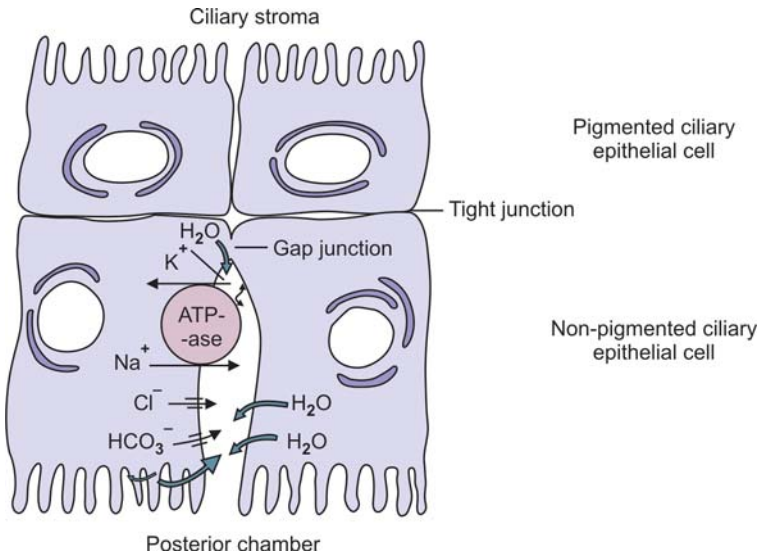


Fig. 32.5

Circulation of Aqueous

Metabolic interchanges between aqueous and various structures like iris, cornea and lens cause the movement of metabolites only and not circulation of aqueous. This is responsible for the regional differences in the aqueous of anterior chamber and posterior chamber. E.g., Phosphate is deficient compared to plasma because retina and lens use this for their metabolic activity. Glucose; 20% lower than that of plasma as it is continuously used by lens.

Bulk Flow

Fluid from the blood in capillaries of ciliary process passes into the capillary wall to the stroma of the process and from these into the ciliary epithelium ot the posterior chamber.

Posterior chamber-Pupil-Anterior chamber-Trabecular meshwork-Episcleral veins.

During this process from the entrance to exit, components are being continuously added and removed by border structures so that the fluid leaving the eye is chemically different from that entering the eye.

Thermal Circulation

A localized and relatively unimportant movement within the anterior chamber is caused by convention currents. Aqueous flows upwards in the region of iris and downwards in the region of cornea. It is a manifestation of difference in temperature between air cooled cornea and vascularised iris. It is purely a physical phenomenon and of little physiological importance. This circulation is responsible for the deposition of cellular elements and keratic precipitates on the posterior surface of the cornea.

Uveoscleral Outflow

Aqueous flow by means of extrascleral efferents through the episclera and to the superficial scleral plexuses has occasionally been noticed but without much significance.

Vascular arrangements in the wide capillaries of the ciliary body with their high pressure head and the connection of Schlemm's canal with venous exits for down the pressure gradient will determine the greatest inflow in the ciliary region and greatest outflow through the angle of anterior chamber while a constant incidental thermal circulation brings about a constant internal movement of aqueous humor.

Glass Rod Phenomenon

The normal intraocular pressure ranges upto 25 mm Hg (Schiotz) with a diurnal variation of 3 to 5 mm Hg. The pressure variations are dependant on pressure changes within the episcleral veins into which the Schlemm's canal drains; an effect that may be manifested in those eyes which have visible aqueous veins. It has been noted that these aqueous veins convey little or no aqueous during periods of the dry when intraocular pressure is rising but during the diurnal phases of decreasing intraocular pressure, they contain aqueous rather than blood; this suggests that the venous pressure enforce regulating effect on the outflow of aqueous if tributaries of aqueous vein are observed under the slit-lamp, one containing blood and the other aqueous. If the vein is occluded with a glass rod, the contents of the blood filled tributary will flow back into the aqueous filled tributary if this is under a lower pressure and vice – versa. (Glass Rod phenomenon) . This is a test of value in simple glaucoma.

Corneal Swelling and Transparency

Anatomic Basis

1. Absence of blood vessels.
2. Absence of pigments.
3. Regular arrangement of epithelial and endothelial cells.
4. The paucity of the cells in stroma.
5. Epithelial cells are not cornified.
6. Epithelial cells are covered with tears to form a regular refractive surface.
7. Epithelium, endothelium and Descemet's membrane are transparent because they have the same refractive index.
8. Regular arrangement of collagen fibrils in the stroma.

Physiologic and Metabolic Basis

About 75% of the cornea is composed of water. Transparency is extremely sensitive to the state of corneal turgescence. Even a small increase or loss in the water content of the cornea will result in some loss of transparency.

The morphological arrangement of the collagen fibrils within the stroma is also a basis of transparency.

Metabolic Basis

The swelling property of the stroma by imbibing water is due to

a) Lattice like and unwoven arrangement of collagen fibrils.
b) The mucopolysaccharides in the stroma.

Fluid can be imbibed from the tears fluid and aqueous humor. The metabolic activity of the epithelium and endothelium prevents the cornea from swelling by continuously extruding from it.

The energy derived from the corneal metabolism counteract the potential cornea by maintaining the deturgescing mechanism.

The intraocular pressure also contributes to the above mechanism by physically counteracting the swelling pressure. The intraocular pressure will counteract the inhibition of fluid from the tears but not from the aqueous, and this may in part account for the greater amount of swelling after damage to the endothelium than to the epithelium.

Influence of Epithelium and Endothelium

The epithelium and endothelium exert their major influence on corneal turgescence by an active rather than a passive process.

The epithelium is relatively impermeable to the passage of sodium ion, but the endothelium is 100 times permeable to sodium and other small ions.

So the sodium in the aqueous rapidly equilibrates with the tissue fluids in the cornea. Hence, a purely passive process like osmotic basis cannot maintain corneal deturgescence.

An active process driven by the metabolic energy present in the epithelium or endothelium is capable of removing the water that is entering the stroma from the fluids on the either side of the cornea.

Sodium and potassium activated ATPases present in the epithelium transports the sodium from the epithelium into the stroma.

A water pump present in the stroma removes the water and dissolved solute from stroma by a secretory process.

Pinocytosis: This may also play a role in the elimination of water from the cornea. Pinocytosis is defined as the movement of package substance by means of vesicle formation.

Membrane Permeability

This depends upon the properties within the membrane and on the characteristics of the penetrating substance.

The electrochemical potential on either side of the membrane determine the rate of diffusion of an ion.

The nature of the individual groups within a molecule, as well as its molecular weight is also important.

A molecule containing many polar groups will be water soluble and easily traverse the stroma.

A molecule containing many non polar groups will be lipid soluble and rapidly traverse the epithelial and endothelial cell membranes.

Color Vision

Colour is a sensory phenomenon (perceptual) not a physical attribute. Human awareness of colour arises out of subjective visual experiences in which given sensations are ascribed names. The perception of color varies complexly as a function of multiple parameters, including the spectral composition of light emanating from surrounding objects, the spectral composition of light adaptation in the subject just prior to viewing any given object.

Visible Spectrum

The index of refraction for an optical medium differs according to wavelength. The index of refraction for each wavelength must be individually specified by the symbol 'nm lambda'. This variable extent of refraction spreads a polychromatic white beam of light into its component wavelengths, a phenomenon referred to as spectral dispersion. The assay of individual wavelengths thus exposed is referred to as **Visible Spectrum**.

The sensation that these individual wavelengths evoke are called the spectral colours.

An object takes on a colour when it contains pigment capable of selectively reflecting or transmitting certain wavelengths of light within the visible spectrum (400-750 μm). Thus, a white object reflects most of the light rays incident upon it while a black object absorbs most of these rays. Blood is red because it contains pigments whose major absorption peaks are below 600 μm i.e., they absorb much of the violet, blue and green portions of the spectrum and transmit most of the light in the red end of the visible spectrum.

There are almost five times as many red or green cone receptors as blue due to which man sees poorly in blue light.

THEORIES OF COLOR VISION

Young Helmholtz Theory (Trichromatic)

In 1807, Thomas Young postulated the first colour vision theory known as the Trichromatic theory. He postulated that there are three sets of fibres in the retina sensitive to red, green and violet respectively. Helmholtz explained that different degree of excitation occurred in each of these sets when they were stimulated by light from various parts of the spectrum.

The peak of the violet wave is in the region of the spectrum which may be formed as blue. The term blue and violet are all rather used indiscriminately in explaining this theory.

Gradually this idea that there were three different sets of nerve fibres was changed to mean three different photoreceptors, and in general, these mean three different types of cones each with its own light sensitive material and

absorptive characteristics. The differences in these photoreceptors were later attributed to three different visual pigments. Thus, the theory which had original anatomic basis was changed into a chemical basis. This theory assumes the presence of three separate photoreceptors in the retina each transmitting a different type of cortical visual cells which receive these impulses, but these cells have never been demonstrated.

When these three photoreceptors are stimulated i.e. when all the photo pigments of cone absorb light energy, a sensation of white results. All other sensation of colour result from unequal stimulation of these photoreceptors. The sensation of black is probably due to an active process in the retina and not due to lack of any stimulation. It is not known whether there are any linkages between these various receptors or whether they send separate messages individually to the cortex, but it is probable that the fusion of color takes place in the cortex. The phenomenon by exposing each retina to a monochromatic frequency, for example yellow may be obtained by binocular fusion of red and green showing that it can be produced by cerebral fusion. This theory also explains the phenomenon of negative after images. If a retina exposed to green light is later is exposed to white light it has no longer the ability to respond to the green wavelength. The adjacent red and violet receptors which were not stimulated, respond to the red and violet part of the spectrum of white light.

The after image produced is a mixture of two color i.e. purple. Evidence suggests that the cone vision which is responsible to a great extent for color vision is more corticalised than the rod vision. It would appear that for the color vision, cone function is dependent on the integrity of the cortex and is not entirely a retinal function.

Hering's Theory of Color Vision

This theory assumes the presence of three photochemical receptors in the retina but they are of such a nature as to give rise to six different qualities of sensations. Visible light is supposed to breakdown a substance known as white black substance which sets up impulses in the optic nerve producing sensation of white light.

In the absence of light, this substance is synthesized into the original substance and the resynthesis produces sensation of blackness. There are two other substances red green and yellow blue, each of which produces sensation of one colour on breakdown of other on synthesis.

Photochemical substances	*Retinal process*	*Sensation*
White Black	Breakdown	White
	Resynthesis	Black
Red Green	Breakdown	Red
	Resynthesis	Green
Yellow Blue	Breakdown	Yellow
	Resynthesis	Blue

According to this theory, the complimentary colors are nearly antagonistic to each other and when they are exhibited simultaneously to the retina neutralize each other producing a sensation of white light. Furthermore, the theory does not recognize the doctrine of specific nerve energies since it says that the same fibre can signal two different sensations to the brain. Further, according to this theory; red and green when thrown on the retina simultaneously neutralize each other and produce the sensation of white. Indeed, they produce a sensation of yellow unless blue green and blue red are selected.

Ladd Franklin's Theory

This theory attempts a reconciliation between the above two theories and is not accepted.

Poly Chromatic Theory

Hart Ridge has postulated the presence of four secondary photoreceptors in addition to the three primary cones of the trichromatic hypothesis.

Enoch suggested a theory of wave guides whereby the cone, the diameter of which roughly corresponds to a wavelength of visible light transmits the light in the shape of patterns – "modes of transmission" depending on the wave length. If the pigments are arranged in the cones, each one will respond to a particular wave length. This is only theoretical speculation.

Four types of cones exist, three concerned with colour vision and one concerned with luminosity. The colour discrimination is based on different quality requirements of different cones. These things remain unestablished.

Binocular Vision

Binocular vision may be defined as the coordinate use of the two eyes which produce a single visual impression. (The vision is achieved by the coordinated use of both eyes so that the images which arise in each eye separately are appreciated as a single mental impression in the visual part of the cortex. There are many factors concerned with successful development and they are classified as sensory motor and central mechanisms).

Advantages of Binocular Vision

1. Optical defects present in one eye are made less obvious by the normal image of the other eye.
2. Uniocular visual field defect in one eye can be masked by the other. E.g. Blind spot, which cannot be detected in binocular visual field, since the object whose image falls on the blind area in one eye is perceived by the functioning retina in the other eye.
3. Binocular visual field is larger then either field alone.
4. Stereopsis and depth perception are made possible by binocular vision.

Requisites for Binocular Vision

1. There must be proper fixation with each eye by the coordinated extra-muscular movements which keep the object fixed by the corresponding retinal areas.
2. The visual fields of the two eyes must overlap to large extent.
3. Approximately similar images must be formed on the retina (size, shape, color intensity, etc.) When dissimilar objects are presented by a prism. retinal rivalry results in which one image followed by the other is presented to the consciousness.
4. Retina must posseses physiologically corresponding points which have common visual directions.
5. Semi decussation of fibres in the chiasma is a common attribute for binocular vision and stereopsis.
6. The eyes are coordinated by reflex activities at all times so that retinal receptors have a common visual direction receiving the same image (fusional movements).

Sensory Mechanisms

1. The factor of visual acuity value of the retinal receptors of each eye.
2. The factor of the normal correspondence of the retinal receptors of the two eyes.
3. The factor of semi – deccusations of the optic nerve fibres at the optic chiasma and the integrity of the apparent visual pathway.
4. The factor of the proprioceptive impulses of the extra ocular muscles.

The Visual Acuity Value of the Retinal Receptors of Each Eye

The development of binocular vision is dependent upon the presence of retinal receptors which have an adequate visual acuity value in each eye. The receptors of the fovea and Para fovea are represented only by cones which have a photopic type of perception (visual perception of form and colour) whereas the receptors of the rest of the retina comprises in addition to small number of cones. Large number of rods are concerned with scotopic vision (perception of light and movement). The development of binocular vision is dependent primarily on an adequate degree of central vision and this depends on a reasonable integrity of the foveal and macular elements and of the refracting media of the eye (cornea, aqueous, lens, vitreous) and also, a certain degree of comparability of the two eyes with regard to their refraction so that “size difference” of the two retinal images is not too great to prevent their fusion. An adequate degree of peripheral vision is also essential for the development of binocular vision.

The Factor of the Normal Correspondence of the Retinal Receptors of the Two Eyes

The significance of retinal correspondence is the fact that stimulation of corresponding retinal points results in the formation of a single mental impression. The retinal correspondence has a functional rather than anatomical basis. The two fovea may be regarded as corresponding retinal points and there are numerous other pairs of corresponding retinal points in the temporal retina of one eye and in the nasal retina of other eye which are more or less equidistant from their respective fovea.

Horopter

Binocular visual space

Binocular visual space can be divided into two regions

1. Horopter and Panum's fusional area
2. Region of physiological diplopia.

Horopter is defined as an imaginary surface passing through point of intersection of the two visual axes and is the sum of points in space, the images of which fall on corresponding retinal areas.

When the eyes are fixed on a point (fixation points remain stationary). Horopteric surface is defined by the intersections of the visual direction of the corresponding retinal points of the two eyes. It is not a plane, but is gently curved with concavity concave towards the object.

Panum's Area: An area just in front and behind the horopter is known as Panum's area. An object lying in this area is also capable of stimulating the corresponding retinal receptors in both the eyes and so will be seen singly.

Visual Direction

The line through the nodal point of the eye joining the object on which the eye is fixed and the fovea, is the visual axis. The mental projection on to the space along the visual axis is the principal visual direction.

In binocular vision the eyes are always more or less converged, and when the eyes converge so that the principal visual directions intersect at the object of regard.

(Cyclopean eye – Mental correlation of the two eyes into one is called as cyclopean eye)

Objects lying in areas other than Horopter and Panum's area are seen double. (Physiological diplopia) because it will stimulate the non corresponding retinal elements in both eyes.

If the object is in front of Panum's area the temporal retina is stimulated on both sides. Hence, the diplopia is heteronymous diplopia or crossed diplopia.

Similarly, if the object is lying farther from the fixation object in the horopter then it will also stimulate the non corresponding retinal elements in both eyes lying in the nasal side of fovea and projected to the left of fixation object in left

eye and to the right of fixation object in the right eye and hence produce homonymous or uncrossed diplopia.

The Factor of Semi Decussation of Optic Nerve Fibres at the Optic Chiasma

The development of binocular vision is dependent on a semi decussation of the afferent optic nerve fibres at chiasma since it enables the nerve fibres from corresponding retinal areas of the two eyes to become associated with one another ultimately in the visual area of the occipital cortex. Thus all retinal fibres from the temporal half of the fovea pass through the optic chiasma with out any decussation so that they enter ipisilateral optic tract, where as the retinal fibres from the nasal half of the retina with the nasal half of the fovea decussate in the chiasma and pass to the contralateral optic tract. It follows therefore the fibres from the corresponding retinal points (temporal retina of one eye and nasal retina of the other eye) travel in the same optic tract and terminate in the same lateral genicualte body and are relayed in the optic radiation to the striate area of the visual cortex in the occipital lobe.

The Factor of Proprioceptive Impulses from the Extraocular Muscles:

It is known that the extra ocular muscles provide the brain with sensory information of a proprioceptive nature and this is of some importance in the establishment of binocular vision.

Motor Mechanisms

The motor mechanism favouring binocular vision is concerned essentially with the maintenance of the two eyes in a correct positional relationship at rest and during movement. They may be considered in the following groups.

Anatomical Factors

Anatomical factors are concerned which determine the position of the eyes which are concerned with the structure of the bony orbit and their content with the structure of the eyes so that the eyes are able to be in the orbit in such ways that their visual axes are aligned correctly. There are several aspects of ocular development still to be completed after birth. They are 1) the retina and fovea are not fully developed at birth 2) the globe is only 73% of its adults size resulting in infantile hypermetropia. 3) the ciliary muscle is not fully developed until 3 years. 4) the medial recti are structurally more advanced muscle. By the age of 6 months structural development has progressed enough for the rudimentary formation of binocular vision to have been laid.

Physiological Factors

Physiological factors which determine the position of the eyes are of three types.

1. Postural reflexes which are independent of visual stimuli.
2. Fixation reflexes which are dependent of visual stimuli.

3. Kinetic reflexes which are concerned with accommodation & convergence relationship.

Postural reflexes: are concerned with the maintenance of the two eyes in their correct relative positions within the orbit so that the visual axes are aligned correctly to one another despite changes in movement of the head relative to body, or of the body relative to space in the absence of the intervention of any visual stimuli. In this way the effect is subjective orientation. They are part of a sub- cortical involuntary reflex mechanism and are entirely unconditioned. There are two groups of postural reflexes static, and stato-kinetic reflex.

Static reflexes: are initiated by changes in the position of head relative to body. They are controlled by part of the labyrinth and by proprioceptive impulses from neck muscles. E.g., Passive turning of head to right is followed by a conjugate movement of the eye to the left i.e. in direction opposite to the movement of the head (Doll's head phenomenon)

Stato kinetic reflexes: Are initiated by changes in position of the head relative to space. They are controlled by part of labyrinth (semicircular canal)

Fixation reflexes: Fixation reflexes are concerned with the maintenance of the two eyes in their correct position so that their visual axes are aligned correctly with one another despite changes in the movement of head relative to the body or of the body relative to space as a result of visual stimuli which reach the cortex by afferent visual pathway. They are part of cortical voluntary reflex mechanism.

a) Fixation reflex or orientation reflex and ability of each eye independently to fix a definite object.
b) Refixation Reflex: It is an elaboration of the fixation reflex and concerns the ability of the eye to retain fixation of a moving object or to change fixation from one object to another.
c) Conjugate fixation reflex: This is the application of the fixation to both eyes at the same time so that they retain fixation during the conduct of conjugate movements.
d) Disjunctive or vergence fixation reflex: This is the application of fixation reflex to both eyes at the same time so that they retain fixation during the conduct of a disjunctive movement.

Kinetic Reflexes

These are concerned with the maintenance of the two eyes in their correct relative position within the orbit as a result of a controlled accommodation – convergence relationship.

Central Mechanism

a) The factor fusion.
b) The factor of cortical motor control.

The factor of fusion: Even though an object is viewed separately by the two eyes, the visual cortex is able to perceive a single mental impression of the object and this is due to the cortical mechanism of fusion.

Cortical Motor Control

The centres in the frontal and occipital cortex and cranial nuclei concerned in the final efferent impulses to the extraocular muscles also play an important role in the maintenance of binocular vision.

Grades of Binocular Vision

a) Simultaneous perception.
b) Fusion.
c) Stereopsis.

THE PUPILLARY PATHWAY AND REACTION

The pupils are controlled by two muscles of ectodermal origin – the sphincter and dilator. The responses of these muscles to stimuli are very rapid, delicate and are easily observed. The constrictor centre possesses the 'tone' and is perpetually sending out impulses to the sphincter which keep the pupil slightly contracted.
Mydriasis – abnormal dilatation.
Miosis – abnormal contraction.

The Pupillary Pathways

The sphincter is supplied by cholinergic nerves of the parasympathetic system through 3rd cranial nerve. The fibres start in the Edinger- Westphal nucleus in the floor of aqueduct of Sylvius. This nucleus has connections with the dilator centre as well as with the frontal and occipital cortex. Then the fibres pass through 3rd nerve as far as the orbit. Here the fibres pass into branch supplying inferior oblique leaving it by the short root of ciliary ganglion. From this they pass through short ciliary nerves piercing the sclera around the optic nerve along the short ciliary arteries. They pass forwards in the choroid and ciliary body to the iris.

The dilator pupillae is supplied by the adrenergic fibres of the cervical sympathetic nerve. The fibres commence in the hypothalamus near the constrictor centre and also has got connections with cerebral cortex.

From the hypothalamic centre the fibres pass downwards through the medulla oblongata into the lateral columns of the cord by the ventral roots of the 1st dorsal and the last two cervical nerves, enter the rami communicantes and run to the 1st thoracic or stellate ganglion. From it to the cervical sympathetic by the anterior limb of the ansa of verusuens. Then in this nerve they pass upwards to the cervical ganglion. Then pass with the carotid plexus into the skull. They run over the anterior part of the semilunar ganglion and pass into the 1st ie ophthalmic division of the 5th nerve- nasociliary nerve –

long ciliary nerve (Thus, avoiding ciliary ganglion). Entering the eyeball by piercing sclera on either side of the optic nerve run along with the long ciliary arteries between the choroid and sclera, enter the ciliary body and thus, reach the iris.

Pupillary Reflexes

Reflexes that Constrict the Pupil

Light reflex: Whenever the intensity of illumination increases above a threshold value within a certain minimum period of time, the pupil constricts. The constriction of the pupil when light enters the eye is called the direct reflex. The constriction of opposite side pupil is called the consensual light reflex. This is an involuntary reflex, it has a threshold value in both time and intensity.

Direct Light Reflex

a) The chief factor that determines what effect a given change in illumination will have on the pupil is state of adaptation of the retina.
b) The second factor is the portion of the retina stimulated. Not all parts of the retina are of equal value in producing light reflex. The effectiveness of various portions of the retina in causing contraction of the pupil parallels the sensitivity to light. In the dark adapted eye the perimacular area is the most sensitive: in the light adapted, the fovea produces greatest pupillary response. This shows that the receptors for pupillo – motion are the same as or similar to light receptors.

The latent period of the light reflex is 0.2sec, with moderately strong stimulation. The response of the pupil depends upon the duration of the stimulus as well as on the intensity. Following maximum contraction the pupil dilates slightly. The contraction of the pupil to light may be attenuated by repeated stimuli, due to the adaptation of the retinal receptors. The rate of constriction shows considerable individual variation. In response to bright light, the average pupil reaches its maximum contraction in less than 5 seconds.

Consensual Light Reflex

The stimulation of one retina by light produces a contraction of the pupil in the opposite eye in all animals in which there is partial decussation of the optic nerve fibres in the chiasma. The latent period in mean is the same as that of direct reflex. If the light is thrown simultaneously into both eyes, the response is greater than that which results from stimulation of one eye, for the direct and consensual responses summate.

Afferent Pathway

The outer limbs of the rods and cones are the receptor cells and the fibres follow the course of visual fibres, decussate in the chiasma; travel in the optic tract near the lateral geniculate body where they leave the optic tract to enter the anterior colliculi and proceed to the pretectal region. Here the fibres synapse

with cells of the pretectal region whose axons connect the cells of the Edinger Westpal nucleus. The intercalated neurons from each side of the pretectum are distributed equally to the 3rd nerve nuclei of both sides. The intercalated fibres destined for the opposite 3rd nerve nucleus cross dorsal to the central aqueduct in the posterior commissure, then in company with axons arising on that side, arch ventrally to reach the 3rd nerve nucleus.

Efferent Pathway

The 3rd nerve nucleus consists of 3 parts:

1. Large celled nucleus typical somatic motor cells, subdivided into dorsal, ventral and scattered groups.
2. Peritia's nucleus, typical efferent cells lying between the right and left large celled masses.
3. Edinger Westpal nucleus cells of visceral motor type.

Accommodation–Convergence Reaction–Near Reflex

When a person is requested to direct his eyes to an object held close to the face, his pupils contract. It is not a true reflex but an associated movement or synkinesis. The three components, the sphincter pupillae, the ciliary muscle and the medial recti are dependent on supranuclear connections.

Trigeminal Reactions (Occulo Pupillary Reflex)

Constriction of the pupil is initiated in any way e.g., any marked irritation of the cornea or conjunctiva results in constriction of the pupil. Both the pupils may be constricted, but the pupil of the affected side is narrowed to a greater extent.

Reflexes that Dilate Pupil

1. **Withdrawal of Light:** The dilatation of the pupil in the dark is due to relaxation of the sphincter pupillae and constriction of dilator pupillae.
2. **Stimulation of Sensory Nerve:** causes dilatation of the pupil especially, if it is a painful stimulus due to simultaneous sympathetic activity and parasympathetic activity.
3. **Vestibular Stimulation:** Rotatory or caloric stimulation causes dilatation of the pupil during and for a short time after cessation of the stimulus, as some fibres of the sympathetic nerves connected with the iris muscle probably run through the middle ear.
4. **Psychic Stimulation:** Emotional states, such as fear and anxiety cause dilatation of the pupil caused by the stimulation of the sympathetic nerves and the inhibition of the Edinger Westpal nucleus, blood borne epinephrine may enhance the reaction.

Section 6

Microbiology

33

Microbiology

INTRODUCTION

Microbiology is the science of living organisms that are directly visible to the naked eye, but only under the microscope. Medical microbiology deals with the causative agents of infection, diseases, the ways in which they produce disease in humans body and essential information for diagnosis and treatment.

The credit for observation and description of bacteria goes to a Biologist Antony Von Leeuwenhook of Holland (1674). His microscopes consisted of a simple biconvex lens that magnified the object * 200.

Scientific development of microbiology was ushered by Louis Pasteur. He has done studies on fermentation, pasterurisation and vaccination. He was called as the “Father of Modern Microbiology”.

Joseph Lister introduced antiseptic technique in surgery (1867) for killing bacteria in wounds and in the air with carbolic acid. He is known as the “Father of Antiseptic Surgery”.

Robert Koch, a general practitioner perfected the bacteriological technique and introduced methods for isolation of pure strains of bacteria. The important discoveries of Robert Koch are Anthrax bacilli, Tubercle bacilli and Cholera vibrios. He is known as the "Father of Medical Microbiology”.

Other Scientists and their discoveries:-

1.	Malaria	-	Ronald Ross
2.	Antitoxin	-	Von Behring
3.	Theories of Immunology	-	Paul Ehrlich
4.	Anaphylaxis	-	Charles Ritchet
5.	Blood groups	-	Carl Landsteiner
6.	Sulphonamide	-	Gerhardt and Domagk
7.	Penicillin	-	Alexander Fleming
8.	DNA	-	Watson and Crick
9.	Australia antigen (HbsAg)	-	Blumberg

GENERAL MICROBIAL CHARACTERS OF BACTERIA

Morphology

Living material is organized in unit known as cell. Microorganisms are living forms of microscopical size and usually unicellular in structure. Originally microorganisms were classified under plant and animal kingdoms as they were found to contain combination of both animal and plant properties. This classification being unsatisfactory, a third kingdom " Protista" was proposed by Hachel in 1866.

Protista: They are mostly unicellular or coenocytic (many nuclei per cell) and each cell is capable of maintaining independent life. Protista is subdivided into 2 groups based on cellular organization and biochemical properties.

Classification of Protista:

A. Higher Protist (Eukaryocyte)
   1. Algae
   2. Protozoa
   3. Fungi

B. Lower Protist (Prokaryocyte)
   1. Bacteria

Structures	*Eukaryocyte*	*ProKaryocyte*
Nucleolus	Present	Absent
Nuclear Division	Mitosis	Binary fission
Chromosome	Many	One
Mitochondria	Present	Absent
Lysosome	Present	Absent
Muramic acid	Absent	Present
Sterols	Present	Absent

Bacteria are prokaryotic unicellular microorganisms which are divided by binary fission and do not possess chlorophyll and true branching except in the so called higher bacteria (Actinomycetes). Their size varies from 0.5 - 15 microns.

Microscopy

Optical or Light microscope: Bacteria can be studied by light microscope which generally uses 100* power objective (Oil immersion lens) along with 10* power ocular lens and magnifies the specimen 1000 times (Fig. 33.1).

Phase contrast microscope: Phase contrast microscope is used to study the internal structures of unstained living tissue, bacteria and protozoa.

Dark ground microscope (dark field illumination): DGI is used to visualise spirochetes. The bacteria looks luminous against a dark background.

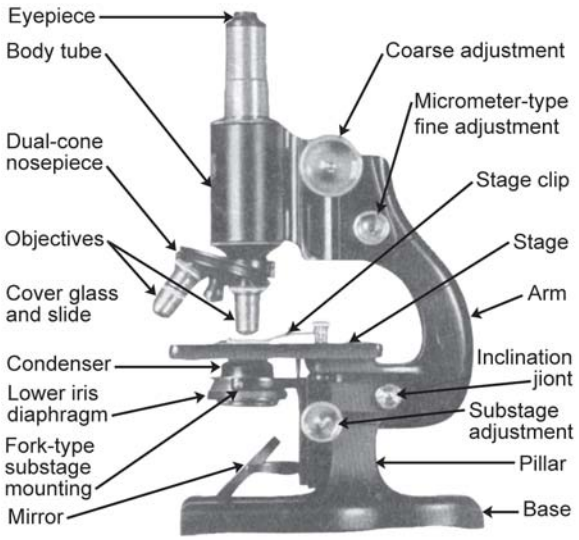


Fig. 33.1: The microscope

Fluorescent microscope: In this UV light is used. Bacteria stained with fluorescence dye (e.g. Auramine) becomes visible as bright objects against a dark background.

Immunofluorescence combines serology with fluorescence microscopy by using antibody labeled with fluorescent dyes (fluoroscein-isothiocyanate, Lissamine and Rhodamine) for detection of specific antigen and bacteria.

Electron microscope: A beam of electrons is focused by a circular electromagnet. When the electron beam passes through an object, the electrons get scattered producing an image in the built-in fluorescent viewing screen.

Adjustments to be made when viewing a stained smear (Gram's or Zeihl-Neelsen)

1. Oil immersion lens (100* objective) to be chosen.
2. Condenser should be raised fully.
3. Diaphragm should be completely opened.
4. Plane mirror should be used (if there is no self-illumination)
5. A drop of cedar wood oil should be placed on the smear.
6. 10* or 5* Eyepiece can be used for viewing.

Adjustments to be made when viewing a wet mount (saline, KOH or Lactophenol staining).

1. 10* (Low power) or 40 * (High power) objective to be chosen.
2. Condenser should be lowered.
3. Diaphragm should be partially opened.
4. Concave mirror should be used to focus the light.
5. The specimen either a drop of KOH or saline amount) should be covered by cover slip avoiding air-bubbles.
6. 10* or 5* Eyepieces can be used for viewing.

Gram's Stain Procedure

It is the most widely used stain in Bacteriology, The stain was originally devised by Christian Gram (1884).

Method

1. Heat fixed smear (from clinical material or culture) on a glass slide is flooded with a primary stain solution, e.g. Aniline dyes such as methyl violet, gention violet or crystal violet for one minute.
2. Pour the stain and add a solution of dilute Iodine (Gram's iodine) - Wait for a minute.
3. Wash with water.
4. Decolourise with a few drops of Acetone (10 seconds).
5. Wash with water
6. Counterstain with dilute carbol fuschin, safranin or neutral red for 30 seconds.

Gram positive bacteria will retain the primary stain and appear violet (Fig. 33.2).

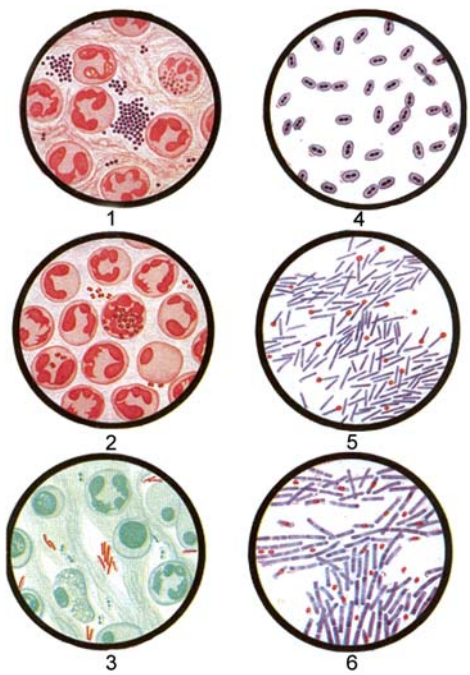


Fig. 33.2: Bacteria as seen with the oil-immersion lens

1. Staphylococcal in pus
2. Gonococci in pus
3. Tubercle bacilli in sputum
4. Pneumococci stained to show capsule
5. Cl. tetani stained to show spores
6. B. authrosis stained to show spores

Gram negative bacteria will take the counterstain and appear pink.

The exact mechanism is not understood. Cell wall permeability and pH of the protoplasm of the bacteria are believed to be responsible for these staining reactions.

Zeihl-Neelsen (Acid fast stain)

1. Heat fixed stain is stained by hot carbol fuschin (strong) for 10 minutes and washed in tap water.
2. The smear is decolourised by 20% sulphuric acid in 95% absolute alcohol for a minute
3. Then the smear is counterstained with 2% methylene blue or malachite green for 2 minutes and washed in water.

Mycobacterium will appear as pink colour whereas the background will appear as blue (Fig. 33.3).

Principle: Acid fastness has been attributed to the high content of lipids, fatty acids and higher alcohol and mycolic acid.

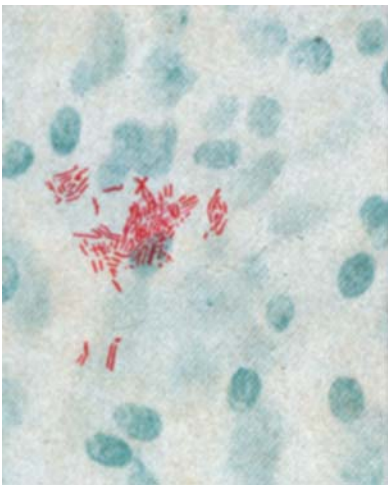


Fig. 33.3: Acid fast bacilli (pink against blue background (Zeihl-Neelsen)

Albert's Stain

Some bacteria, e.g. Corynebacterium diphtheriae possessing metachromatic granules stain better with Albert's stain.

Procedure

Heat fixed smear of throat/nasal swab is covered with Albert's stain I (a mixture of toludine blue and malachite green) and allowed to stand for 4-5 minutes. Pour off Albert's stain I and cover the smear with Gram's Iodine (Albert's II) for 1 minute.

Observation: The body of the bacilli will appear green and the metachromatic granules blue black.

The other stains which are less commonly used in microbiology are Giemsa, Giminez, Machivallo (for Chlamydiae), Silver Impregnation (Flagella), India ink (Capsule)and Leishman (peripheral blood) staining.

Classification of Bacteria: Bacteria can be classified into several varieties depending on their shape:

1. Cocci: e.g. Staphylococci and Streptococci
2. Bacilli: e.g. *Klebsiella, E. coli*
3. Vibrios: e.g. *Vibrio cholerae*
4. Spirochaetes: e.g. *Treponema pallidum, Leptospira*
5. *Actinomycetes*
6. *Mycoplasma*
7. Chlamydiae

Bacterial cell: The outer layer or cell envelope of a bacterial cell consists of two components. (a) a rigid cell wall proper and (b) underlying cytoplasmic membrane or plasma membrane. The cell wall encloses the protoplasm comprising of cytoplasm (Ribosomes, inclusion granules, mesosomes) and a single circular chromosome of DNA. Some bacteria may possess a capsule (Pneumococcus), flagella (organ of locomotion) and fimbriae (pili).

Structure of cell wall: The chemical structure of cell wall of gram-positive and gram-negative bacteria differ considerably. It is comparatively simpler in gram positive bacteria than that of gram-negative bacteria. The rigid part of the cell wall is a peptidoglycan and is the principal structural component of the cell wall. It is present in the cell wall of both gram-positive and gram-negative bacteria although it is abundant in gram-positive bacteria.

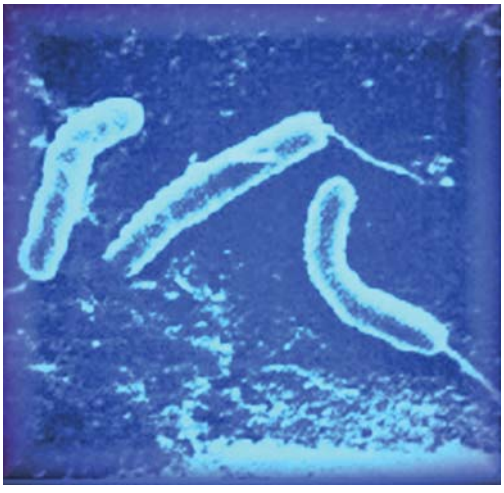


Fig. 33.4: Scanning electron microscopic picture of a bacilli with flagella

Peptidoglycan consists of alternating strands of N-acetyl muramic acid and N-acetyl glucosamine.

In addition to these structures gram-positive cell wall has some special components called teichoic and teichuronic acids.

Gram-negative cell wall is a complex structure and thinner than that of gram-positive cell. Outer membrane is made of phospholipids bilayer, proteins and lipopolysaccharides (LPS - Endotoxin).

Growth and Nutrition of Bacteria

The most important elements necessary for synthesis of bacterial structural components (Carbohydrate, lipid, protein, nucleic acid) are hydrogen, oxygen, carbon and nitrogen. In addition to these elements, phosphorus and sulphur are also required for bacterial growth.

Many bacteria produce toxins, enzymes and pigments. Toxins and enzymes play important role in pathogenicity. Toxins are of two types:

1. **Exotoxins:** Exotoxins are usually heat labile proteins secreted extracellularly by certain species of bacteria which diffuse into the surrounding medium. It is soluble in water and culture medium. Production of exotoxin is a physiological process and compatible with normal life of bacteria. It can be separated from culture by filteration. Exotoxins are potent in minute quantity. They are highly antigenic and can be converted into nontoxic toxoid by addition of formalin. Toxoids are nontoxic but antigenic, i.e. retain the ability to produce antibodies (antitoxins).
2. **Endotoxins:** Endotoxins are heat labile lipopolysaccharide-protein complexes which form structural components of the cell wall of gram-negative bacteria and liberated only on cell lysis or death of bacteria. Endotoxin is not separable from the elaborating microorganisms and their culture filtrate is nontoxic while the dead bacteria are equally toxic. Endotoxins are poor antigens and active only in relatively large doses.

STERILIZATION AND DISINFECTION

Sterilization is a process by means of which an article, surface or medium is made free from all living microorganisms including spores.

Disinfection is a process of destruction of vegetative forms of pathogenic organisms which are capable of producing infection.

There are different methods of sterilization:

1. Physical methods:

(a) Sun light (Natural source)

(b) Dry heat (Hot air-oven temp. 165 degree centigrade for one hour) - sterilizing metals, glass, cotton etc.

(c) Moist heat (Autoclave temp. 121 degree centigrade for 20 minutes) - sterilizing dressings, bin, culture medium etc.
(d) Filteration - HEPA filters in Laminar hood and seitz and membrane filters for sterilizing serum.
(e) UV radiation - in Laminar hoods and Bio-safety cabinets
(f) Ionising radiation - gamma radiation for disposable plastics

2. Gaseous methods

(a) Ethylene oxide - for disposable syringes and IV sets
(b) Formaldehyde - For sterilizing the operation theatres

3. Chemical methods

(a) Glutarldehyde - 2% (cidex) for endoscopes and cystoscopes

Following disinfectants are used widely:

1. Aldehyde:
   (a) Glutaraldehyde - for sterilizing Bronchoscopes
   (b) Formaldehyde - Wards, operation theatres and Instruments
2. Oxidising agents:
   (a) Hydrogen peroxide - 3-6% kills most of the organisms
3. Halogens: Bacteriocidal, used for water sterilization
   Sodium hypochlorite 5% can be used for
   Disinfecting HIV and Hepatitis virus
   (b) Iodine: such as povidone iodine for topical applications
4. Alcohols: Ethanol (Absolute alcohol) esp. 70 % and
   Iso-propyl alcohol are effective on skin.

The other disinfectants like phenol (cresol or Lysol), Chlorhexidine (savlon), Chloroxylenol (dettol) are also effective in killing the microorganisms.

Cultivation of Bacteria

Bacteria needs a culture medium for *in vitro* growth. There are plenty of culture medium available for cultivating the bacteria. The specimen to be cultivated is inoculated on a suitable medium and incubated at 37°C for 18- 24 hours.

The constituents of a basal culture media include water, electrolytes like sodium chloride, peptone (partially digested proteins from animal or plant source), Meat extract (lab lemco), yeast extract and agar.

Agar is derived from sea weed (algae). It has carbohydrates and fiber. It gives the solidifying effect to the solid media.

Simple or basal media: Basal media includes peptone water, Nutrient broth, Nutrient agar. Peptone water consists of 1% peptone and 0.5% sodium chloride and distilled water at a pH of 7.4 to 7.5

1. Nutrient broth is prepared from adding 1% meat extract to peptone water.
2. Nutrient agar is prepared by adding 2% agar to the nutrient broth.

Enriched medium: For the growth of certain fastidious bacteria, 5% sheep blood can be added to the Nutrient Agar (Blood agar). Other substances like cysteine, haemin can be added to chocolate agar (charred blood agar).
Enrichment medium: Certain liquid media which favours the growth of the particular bacteria and suppress the growth of commensals, e.g. Selenite F broth, tetrathionate broth for Isolation of Salmonellae.

Selective medium: Selective media are media that contain additives that enhance the growth of the desired organism by inhibiting other organism. DCA (Deoxy cholate citrate agar for Salmonella and Shigella spp.), LJ (Lowenstein Jensen) agar for Mycobacteria and TCBS (Thiosulphate, Citrate, Bile salt, Sucrose) for Vibrios.

Differential media: MacConkey agar for enterobactericeae. This contains lactose to differentiate lactose fermentors (*E. coli*) from non-lactose fermentors (proteus)

Transport medium: Used for transporting specimens, e.g. Stuart's medium for Gonococci and VR (Venkatraman Ramakrishnan) medium for Vibrios.

Identification of the bacteria after cultivation requires an array of biochemical tests to confirm.

The important and basic tests include catalase (Streptococcus are positive). Oxidase (Pseudomonas are positive), sugar fermentation reactions (e.g. Glucose, lactose etc.), Indole formation (*E. coli* is positive), Citrate utilization (Klebsiella is positive), coagulase (Staphylococcus is positive), Methyl red test (MR), Voges-Proskauer test (VP), urease (*Helicobacter pylori* + ve) and hydrogen sulphide production (Proteus).

SYSTEMATIC BACTERIOLOGY

Staphylococcus

Staphylococcus are gram-positive cocci that occur in grape like clusters. They are the commonest cause of localised suppurative lesion in man. Staphylococci were first observed in human pyogenic lesions by Von Reckling Lausen in 1871. Staphylococci are classified into 2 groups based on coagulase production. *Staph. aureus* which is coagulase positive, mannitol fermenting are pathogenic. They produce toxins. Most strain from golden yellow colonies.

Staph. epidermidis (*Staph. albus*) which is coagulase negative, mannitol non-fermenting are usually saprophytes. They do not produce toxins.Most strain form white colonies, though some may be pigment (*Staph. citreus*).

Morphology

They are spherical cocci approximately 1µ in diameter arranged in grape like cluster. Cluster formation is due to cells dividing sequentially in three perpendicular planes. They may also found singly or in pairs and in short

chain. They are nonmotile, non-sporing and non-capsulated. Sometimes capsules are demonstrated in young culture. They readily stain with aniline dyes and are uniformly gram-positive. The genus staphylococci has atleast 20 species.

Culture

They grow on nutrient agar incubated at 370°C for 24 hours. They form small white or golden brown colonies which are opaque. They can also be cultures on blood agar to demonstrate the type of hemorrhage. 5% Sheep blood is used to prepare blood agar plates. They grow in Maonkey's medium and produce small pink colonies due to fermentation of Lactose. Other media particular for Staphylococci are salt-milk agar (8-10 % of NaCl), Ludlam's medium.

Biochemistry

Staphylococci ferment a good number of sugar producing acid but no gas. They are catalase positive unlike Streptococci. They hydrolyse urea reduce nitrates to nitrites, liqueify gelatin and are MR and VP positive but indole negative. Phosphotase is produced.

Resistance

Staphylococci are more resistant of non-sporing bacteria. Dried on threads, they retain their viability for 3-6 months. They may withstand 600°C for 30 minutes. Some strains grown in the presence of 10-15% NaCl. Phenol and Mercury per chloride kills them. Staphylococci are uniformly resistant to lysozyme. They were originally sensitive to sulphonamides, penicillin and other antibiotics, but they develop drug resistive. So readily that most strain, especially from the hospital environment are now resistant to the commonly used antibiotic. Resistance may be due to Betalactamase production, e.g. penicillin. G, Ampicillin, Ticarcillin and similar drugs. Now many strains of *S. aureus* are even resistant to oxacillin and methicillin. They are coined as MRSA (Methicillin resistant Staphylococci aureus) and they are sensitive to vancomycin and teicoplanin.

Antigen Structure

Staphylococci contains protein, antigenic polysaccharides. Capsular antigen are found in mucoid strain. Peptidoglycan polysaccharide elicits the production of interleukin - 1 (endogenous progeny) and is responsible for the end toxic activity. Protein A is a cell wall component. It binds to the fact portion of Gig bound to protein A is free to combine with a specific antigen. This is called co-agglutination and is used in diagnostic lists.

Bacteriophage Typing

Staphylococci may be typed, based on their susceptibility to bacteriophage. The reference center for Staphylococcal phage typing in India is located in the Department of Microbiology, Maulana Azad Medical College, New Delhi. According to the information available, phage type 52/52A/80/81 is prevalent in most parts of India.

Toxic and Virulence Factors

Staph. aureus produce a number of exotoxins. The toxins are haemolysin, Leucocidin, Hyaluronidase fibrinolysin (streptokinase), nucleases, lipases. Pyogenic exotoxin is produced by *Staphylococcus* strain causing toxic shock syndrome.

Enterotoxin

This toxin is responsible for the manifestation of staphylococcal food poisoning, nausea, vomiting, diarrhea within six hours of consuming contaminated food.

Exfoliate Toxin

This epidermolytic toxin leads to a variety of exfoliate skin diseases including generalized exfoliation (Reiter's syndrome), toxic epidermal necrolysis, localized bullous impetigo and generalized scarlatini form eruption. This clinical picture is called Staphylococcal scalded skin syndrome.

Coagulase

Staph. aureus has the property of clotting human plasma. This is due to the enzyme coagulase, which along with an activator, the coagulase reacting factor (CRF) present in plasma, converts fibrinogen to fibrin. Coagulase promotes the virulence of the organism by inhibiting phagocytosis.

Pathogenicity

Staphylococci cause the majority of acute pyogenic lesions in man. Staphylococcal diseases may be classified as cutaneous and deep infections, acute toxaemia including food poisoning, exfoliative diseases and the toxic shock syndrome. Cutaneous lesions are furuncles, styes, boils, abscesses. carbuncles, impetigo and pemphigus neonatorum. Hospital-cross infections (Nosocominal) are common.

The commonest deep infection is acute osteomyelitis. In the respiratory track, it causes tonsillitis, pharyngitis, sinusitis and pneumonia. Hematogenous spread may lead to meningitis, endocarditis, renal abscesses and brain abscesses

Staphylococcal food poisoning results when food is contaminated with enterotoxins is consumed. The symptoms start within 6 hours.

Toxic shock syndrome is characterized by acute onset of high fever, hypotension, vomiting, diarrhea and scarlatiniform rash.

Epidemiology

Staphylococcal disease may follow endogenous or exogenous infection. The modes of transmission may be by contact, direct or through fomites, by dust or by air-borne droplets. They are the commonest cause of hospital acquired infections and postoperative wound infections, especially the MRSA strains.

Laboratory Diagnosis

The specimens to be collected are pus, sputum, faeces, blood, nasal swab (for carriers), CSF and urine (pylonephritis).

1. Direct microscopy. Gram stain. Group like clusters of Gram-positive cocci seen.
2. Culture of the organism can be done on blood agar plates, Nutrient agar and selective medium like salt-milk agar.The colonies on NA are small, opaque with golden brown color. On BA, beta-hemolysis seen. Smear from the culture are examined and subjected for coagulase test and other biochemical tests such as mannitol fermentation.

Treatment

As the drug resistance is so common in staphylococci, appropriate antibiotic should be chosen after antibiotic sensitivity tests. Penicillin is effective. If resistant , methicillin and cloxacillin are effective against pencillinase producing strain. For MRSA, Vancomycin and teicoplanin can be used.

Coagulase Negative Staphylococci

Staphylococcus epidermidis

These are coagulase negative staphylococci which are part of the normal flora. They may act as opportunistic pathogens causing septicemia, cystitis, UTI endocarditis and colonise the prosthetic valves of the heart.

Staphylococcus saprophyticus

This coagulase negative *Staphylococcus* has become clinically important as a common cause of acute urinary tract infection and is resistant to Novobiocin.

Micrococci

These are gram-positive cluster forming cocci which differ from staphylococci in fermenting sugars. They occur in tetrads or eight. They are saprophytes that may rarely cause opportunistic infection.

STREPTOCOCCI

Introduction

Streptococci are gram-positive cocci arranged in chains. They are important human pathogen causing pyogenic infection. They are also responsible for non-suppurative lesion like acute rheumatic fever and glomerulonephritis.

Classification

The Streptococci are classified based on their hemolytic properties.

α. Alpha-hemolytic streptococci produce a greenish discoloration with partial hemolysis around the colonies

β. Beta-hemolytic streptococci produce a sharply defined, clear, colorless zone of hemolysis.

γ. Gamma or non-hemolytic streptococci produce no change in the blood agar medium.

Most of the pathogenic streptococci (*Streptococcus pyogenes*) fall into the beta group and are called the hemolytic streptococci. The alpha streptococci are commensals of the throat (*Str. viridans*). The gamma streptococci include the fecal streptococci (*Str. faecalis* or enterococcus).

The hemolytic streptococci were classified by Lancefield (1933) serologically into groups based on the nature of a carbohydrate (c) antigen on the cell wall. These are known as Lancefield groups, 19 of which have been identified so far and named A-U (without I & J). The great majority of hemolytic streptococci that produce infections belong to group A (*Str. pyogenes*). These may be further divided into types based on the protein (M,T & R) antigen present on the cell surface (Griffith typing). About eighty types *Str. pyogenes* have been recognized so far.

Streptococci Pyogenes

They are spherical cocci having 0.5 - 1.0 μ in size. Gram positive and appear as chains.

Culture

It is an aerobe and facultative anaerobe, growing best at a temperature of 370°C (22–42°C). It is a fastidious organism which grows in culture medium containing blood or serum.

On blood agar, after incubation for 24 hours, the colonies are small (0.5 - 1.00 mm), circular, semi-transparent with an area of clear hemolysis around them. Growth and hemolysis are promoted by 10% CO_2. Strain with well marked capsules produce mucoid colonies. Biochemically they ferment most of the sugars produced acid but no gas. They are catalase negative and are not soluble in 10% bile, unlike Pneumococci.

Resistance

They are easily destroyed by heat. It is resistant to crystal violet. It is susceptible to penicillin and may antibiotics and unlike Staphylococci does not develop resistance to antibiotics.

Antigenic Structure

The cell wall is composed of an outer layer containing protein and lipoteichoic acid, a middle layer of group specific carbohydrate and an inner layer of peptidoglycan (mucoprotein) is responsible for cell wall rigidity. It has also some biological properties such as pyogenic and thrombolytic activity.

Toxins and Virulence Factors

Streptococcus pyogenes forms several exotoxins and enzymes which contribute to its virulence.

Hemolysins

Streptococci produce two hemolysis, Streptolysin 'O' and 'S' Streptolysin O is antigenic and antistreptolysin regularly appear in sera following Streptococcal infections. Estimation of ASO titer (Anti-streptolysin O) is a serological procedure of the retrospective diagnosis of infection with *Str. pyogenes* an ASO tube of >200 IU is significant.

Streptolysin S is responsible for the hemolysis seen around streptococcal colonies on the surface of blood agar plates. The other virulence factors enzyme include erythrogenic toxin, Streptokinase streptodornase, hyaluronidase and proteinase.

Pathogenicity

Respiratory Infections

The primary site of invasion of the human body by *Str. pyogenes* is the throat. Sore throat is the commonest of streptococcal infections. It may be localized to tonsils or pharynx.

Scarlet fever is a special variety of sore throat where infection is caused by the strain producing erythrogenic toxin.

Skin Infection

Str. pyogenes produce a variety of suppurative infection of the skin. For example, erysipelas and impetigo.

Non-suppurative Complication

Str. pyogenes infections lead to two important non-suppurative sequelae—Acute rheumatic fever and acute glomerulonephritis. The antigen of streptococci cross react with the glomerular antigen and heart tissue antigen.

Epidemiology

Streptococcal infection of the respiratory trait are more frequent in children 5-8 years of age. Crowding is an important factor in the transmission of infection.

Laboratory Diagnosis

The diagnosis is established by Gram stain of the pus, CSF or throat swab. Cultivation of the organism can be done on blood agar plates incubated at 37°C under 5-10% CO_2. The fluorescent antibody technique has been employed for the rapid identification of group A streptococci.

ASO titres are useful in the diagnosis of acute rheumatic fever and glomerulonephritis.

Prophylaxis

The indication for prophylaxis in streptococcal infection is only in the prevention of rheumatic fever. This is achieved by a long-term administration of penicillin in children who have developed early signs of rheumatic fever.

Treatment

All beta hemolytic group and streptococci are sensitive to penicillin G. in patients allergic to penicillin, erythromycin or Cephalexin may be used. Fluroquinolones such as ciprofloxacin, and ofloxacin are also effective.

The Viridians Group: (Str. viridians)

This group consists of Streptococci that produce alpha hemolysis on blood agar. They are the commensals of the mouth and throat. Generally they are non-pathogenic, but in persons with predisposing factors, such as valvular disease of the heart. They produce subacute bacterial endocarditis. Tooth extraction in such persons is dangerous and should be done under antibiotic— They are susceptible to penicillin.

Group G Streptococci (Enterococci)

Enterococci usually appears in pairs. They are predominantly present in intestine, genital tract and saliva.

They may be associated with urinary tract infection, subacute bacterial endocarditis, septicemia and peritonitis. They are sensitive to penicillin.

PNEUMOCOCCUS

Pneumococcus (Str. pneumoniae)

Pneumococci are gram-positive lanceolate diplococci which resemble the viridans streptococci. Pneumococci possesses a polysaccharide capsule which confers virulence to the organism. Pneumococci are normal inhabitants of the upper respiratory tract. They are the most prevalent simple bacterial agent in

pneumonia and in other media in children. They can also cause sinusitis, bronchitis, meningitis, and septicemia.

Morphology

Pneumococci are typically small (1μ) slightly elongated cocci presenting a flame shaped or lanceolate appearance. They are capsulated. They are strained by Gram stain and the capsule may be demonstrated by Indian ink staining (Fig. 33.5).

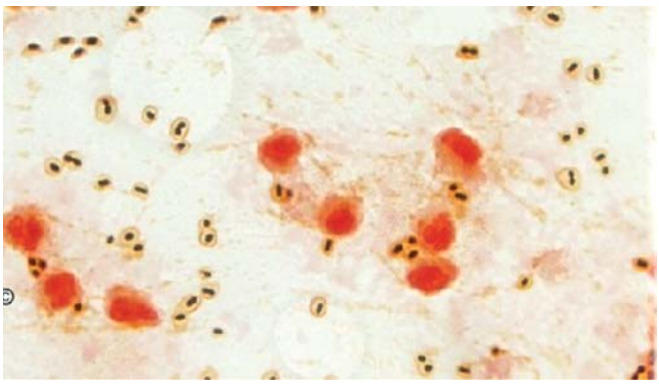


Fig. 33.5: Capsulated diplococci (Pneumococci) from sputum (Gram's stain)

Culture

Pneumococci have complete growth requirements and grow only in enriched media. Growth is improved by 5—10% CO_2.

On blood agar, after incubation for 18 hours, the colonies are small (0.5 - 1 mm) dome-shaped and glistening with an area of greenish discoloration (alpha hemolysis) around them.

Biochemically, the ferment several sugars forming acid only.

Pneumococci are bile soluble and they are catalase and oxidase negative.

Resistance

Pneumococci are delicate organisms that are readily destroyed by heat. They are sensitive to penicillin and other antibiotics. The sensitivity of Pneumococci to optochin useful in differentiating them from Streptococcus.

Antigenic Structure

The most important antigen of the pneumococci is the type specific capsular polysaccharide. Typing may be carried out by agglutination of the cocci with the type specific antigen.

Quellung Reaction

When a suspension of pneumococci is mixed on a slide with a drop of type specific antigen + methylene blue, the capsule become apparently swollen, sharply delineated and refractile.

CRP

An abnormal protein (beta globulins) that precipitate with the somatic 'C' antigen of Pneumococci and appear in the acute phase - of cases of Pneumococci and disappears during convalescence. It also occurs in some other pathological conditions. This is known as the 'C' Reactive Protein (CRP).

Diagnosis

1. Microscopy (Diplococci in Gram' stain)
2. Capsular stain (India ink stain)
3. Quellung reaction
4. Culture on blood agar (alpha hemolytic colonies)
5. Sensitivity to optochin antibiotic disc

Treatment: Pneumococci is sensitive to penicillin and most of the antibiotics.

Prophylaxis: The current polyvalent vaccine contains 23 different highly purified capsular polysaccharides extracted from pneumococci of the most prevalent types.

CORYNEBACTERIUM DIPHTHERIAE

Diphtheria is a localized infection of mucous membranes or skin caused by Corynebacterium diphtheriae. A characteristic pseudomembrane may be present at the site of infection. Some strains of *C. diphtheriae* produce diphtheria toxin, a protein that can cause myocarditis, polyneuritis, and other systemic toxic effects. Respiratory diphtheria is usually caused by toxinogenic *C. diphtheriae*, but cutaneous diphtheria is frequently caused by non-toxinogenic strains.

Morphology and Characters

C. diphtheriae is an aerobic, nonmotile, nonsporulating, irregularly staining, gram-positive rod. The bacteria are club-shaped and are often arranged in clusters (Chinese letters) or parallel arrays (palisades). *C. diphtheriae* forms gray to black colonies on selective media containing tellurite. The gravis, mitis, and intermedius biotypes are distinguished by colonial morphology and laboratory tests.

Toxoid

Treatment of diphtheria toxin with formaldehyde converts it to a non-toxic but immunogenic product (diphtheria toxoid). Immunization with toxoid elicits antibody (antitoxin) that neutralizes the toxin and prevents diphtheria.

Epidemiology

Humans are the principal reservoir for *C. diphtheriae*. Transmission occurs primarily by close personal contact. The risk is greater that *C. diphtheriae* will be transmitted to susceptible individuals from patients with diphtheria than from carriers. The incubation period for respiratory diphtheria is typically 2 to 5 days and rarely up to 8 days. Cutaneous diphtheria is usually a secondary infection whose signs develop an average of 7 days (range, 1 to >21 days) after the appearance of primary dermatologic lesions of other etiologies.

In temperate climates, diphtheria primarily involves the respiratory tract; occurs throughout the year, with a peak incidence in colder months. Treatment with antitoxin reduced the case-fatality rate to 5 to 10%.

In the tropics, cutaneous diphtheria is more common than respiratory diphtheria, occurs throughout the year, and often develops as a secondary infection complicating other dermatoses.

Pathology and Pathogenesis

C. diphtheriae infects mucous membranes, most commonly in the respiratory tract, and also invades open skin lesions resulting from insect bites or trauma. In infections caused by *C. diphtheriae*, initial edema and hyperemia are often followed by epithelial necrosis and acute inflammation. Coagulation of the dense fibrinopurulent exudate produces a pseudomembrane, and the inflammatory reaction accompanied by vascular congestion extends into the underlying tissues. The pseudomembrane contains large numbers of *C. diphtheriae* organisms, but the bacteria are rarely isolated from the blood or internal organs.

Diphtheria toxin acts both locally and systemically, and the lethal dose for humans is ~0.1 ug/kg. Toxin contributes locally to pseudomembrane formation; systemically, it can cause myocarditis, neuritis, and focal necrosis in various organs, including the kidneys, liver, and adrenal glands. Changes in the myocardium include cloudy swelling of muscle fibers and interstitial edema. These changes are followed within weeks by hyaline and granular degeneration (sometimes with fatty degeneration), progressing to myolysis and finally to the replacement of lost muscle by fibrosis. Thus, diphtheria can cause permanent cardiac damage. In diphtheritic polyneuritis, pathologic changes include patchy breakdown of myelin sheaths in peripheral and autonomic nerves, but recovery of nerve damage is the rule if the patient survives.

Diphtheria toxin is produced by *C. diphtheriae* as an extracellular polypeptide. Proteolytic cleavage forms nicked toxin consisting of fragments A and B. Fragment B binds to a plasma-membrane receptor (a precursor of a heparin-binding growth factor resembling epidermal growth factor), and the bound toxin is internalized by receptor-mediated endocytosis. Fragment A is translocated across the endosomal membrane and released into the cytoplasm, where it catalyzes the transfer of the adenosine diphosphate ribose moiety from nicotinamide adenine dinucleotide (NAD) to a modified histidine residue (diphthamide) on elongation factor 2 (EF-2), thereby inactivating EF-2 and inhibiting protein synthesis. One molecule of fragment A in the cytoplasm can kill a cell. Other metabolic alterations are secondary to inhibition of protein synthesis.

Clinical Manifestations

Patients with *C. diphtheriae* in the respiratory tract are classified as diphtheria cases if pseudomembranes are present and as diphtheria carriers if pseudomembranes are absent. The disease is graded as *tonsillar* if pseudomembranes are localized to the tonsils, as combined types or delayed diagnosis if more extensive pseudomembranes are present, and as severe if cervical adenopathy or cervical edema is also present. Onset is often gradual, but most patients seek medical care within a few days of becoming ill. Fever of 37.8° to 38.9°C (100° to 102°F), sore throat, and weakness are the most common symptoms, while dysphagia, headache, and change of voice occur in fewer than half of patients. Neck edema and difficulty breathing are noted in $\leq$10% of patients and are associated with an increased risk of death. Systemic manifestations are due primarily to toxic effects of diphtheria toxin. Patients without toxicity exhibit discomfort and malaise associated with local infection, whereas severely toxic patients may develop listlessness, pallor, and tachycardia that can progress rapidly to vascular collapse.

Primary infection in the respiratory tract is most often tonsillopharyngeal but may also be (in decreasing order) laryngeal, nasal, and tracheobronchial. Multiple sites are frequently involved, and secondary spread of pharyngeal infection upward to the nasal mucosa or downward to the larynx and tracheobronchial tree is much more common than primary infection at those sites. Systemic toxicity is usually most severe when extensive pseudomembrane extends from the tonsils and pharynx into contiguous regions. A small percentage of patients present with malignant or "bull-neck" diphtheria, with extensive pseudomembrane formation, foul breath, massive swelling of the tonsils and uvula, thick speech, cervical lymphadenopathy, striking edematous swelling of the submandibular region and anterior neck, and severe toxicity.

In tonsillopharyngeal diphtheria, isolated spots of gray or white exudate may appear first. These spots often extend and coalesce within a day to form a

confluent, sharply demarcated pseudomembrane that becomes progressively thicker, more tightly adherent to the underlying tissue, and darker gray in color. Unlike the exudate in streptococcal pharyngitis, the diphtheritic pseudomembrane often extends beyond the margin of the tonsils onto the tonsillar pillars, palate, or uvula. Dislodging the membrane is likely to cause bleeding. Laryngeal diphtheria often presents as hoarseness and cough. Demonstration of laryngeal pseudomembrane by laryngoscopy helps distinguish diphtheria from other infectious forms of laryngitis. Patients with nasal diphtheria may present with unilateral or bilateral serosanguineous nasal discharge associated with irritation of the nares or lip. Primary or secondary diphtheritic infection occasionally involves other mucous membranes, including the conjunctiva and the membranes of the genitourinary and gastrointestinal tracts.

Cutaneous diphtheria usually presents as an infection by *C. diphtheriae* of preexisting dermatoses involving the lower extremities, upper extremities, head, or trunk. The clinical features are similar to those of other secondary cutaneous bacterial infections. In the tropics, cutaneous diphtheria may present as a primary cutaneous lesion, typically with morphologically distinct "punched-out" ulcers that are covered by necrotic slough or membrane and have well-demarcated edges.

C. diphtheriae is an occasional cause of invasive infections, including endocarditis and septic arthritis. Risk factors for such infections include preexisting cardiac abnormalities, abuse of intravenous drugs, and alcoholic cirrhosis.

Complications

Obstruction of the respiratory tract can be caused by extensive pseudomembrane formation and swelling early in the disease or by sloughed pseudomembrane that becomes lodged in the airways later in the disease. The risk is greater when infection involves the larynx or the tracheobronchial tree and in children because of the small size of the airways. Myocarditis and polyneuritis are the most prominent toxic manifestations of diphtheria.

Bulbar dysfunction in diphtheritic neuritis typically develops during the first 2 weeks. Palatal and pharyngeal paralysis usually develops first. Swallowing is difficult, the voice is nasal, and ingested fluids may be regurgitated through the nose. Additional bulbar signs may develop over several weeks, with oculomotor and ciliary paralysis more common than facial or laryngeal paralysis. Peripheral polyneuritis typically begins from 1 to 3 months after the onset of diphtheria with proximal weakness of the extremities, which spreads distally. Paresthesia may occur, most often in a glove and stocking distribution. Polyneuritis usually resolves completely, with the time needed for improvement approximately equal to that elapsing from exposure to the development of symptoms.

Pneumonia occurs in more than one-half of fatal cases of diphtheria. Less common complications include renal failure, encephalitis, cerebral infarction, pulmonary embolism, and bacteremia or endocarditis due to invasive infection by *C. diphtheriae*. Serum sickness may result from antitoxin therapy.

Laboratory Diagnosis

1. Direct smear examination: Smears stained by Albert's stain show beaded slender rods in typical Chinese-letter pattern.
2. Culture: Swab is inoculated onto Loeffler's serum slope and Tellurite blood agar medium.
3. Animal inoculation in guinea pigs.
4. Elek's agar gel precipitation tests.

Treatment

The decision to administer diphtheria antitoxin must be based on the clinical diagnosis of diphtheria without definitive laboratory confirmation, since each day of delay in treatment is associated with increased mortality. Because diphtheria antitoxin is produced in horses, it is necessary to inquire about possible allergy to horse serum and to perform a conjunctival or intracutaneous test with diluted antitoxin for immediate hypersensitivity. Epinephrine must be available for immediate administration to patients with severe allergic reactions. Patients with immediate hypersensitivity should be desensitized before a full therapeutic dose of antitoxin is given. The dose of diphtheria antitoxin currently recommended by the Committee on Infectious Diseases of the American Academy of Pediatrics is based on the site of the primary infection and the duration and severity of disease: 20,000 to 40,000 units for disease that has been present for $\leq$ 48 h and involves the pharynx or larynx; 40,000 to 60,000 units for nasopharyngeal infections; and 80,000 to 100,000 units for disease that is extensive, has been present for $\geq$ 3 days, or is accompanied by diffuse swelling of the neck. Antitoxin is administered intravenously by infusion in saline over 60 min to neutralize unbound toxin rapidly. The ~10% risk of serum sickness is acceptable because of the established therapeutic value of antitoxin in decreasing mortality from respiratory diphtheria. The risk of systemic toxicity is lower in cutaneous diphtheria than in respiratory diphtheria and must be weighed against the potential adverse effects of antitoxin treatment; authorities are not unanimous in recommending antitoxin therapy for cutaneous diphtheria.

Antibiotics have little demonstrated effect on the healing of local infection in diphtheria patients treated with antitoxin. The primary goal of antibiotic therapy for patients or carriers is therefore to eradicate *C. diphtheriae* and prevent its transmission from the patient to susceptible contacts. Erythromycin, penicillin G, rifampin, or clindamycin is recommended by most authorities.

Prevention

Vaccines available in the United States for immunization against diphtheria include diphtheria and tetanus toxoids and pertussis vaccine adsorbed (DTP), diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed (DTaP), diphtheria and tetanus toxoids adsorbed (DT; for pediatric use).

OTHER CORYNEBACTERIA

Medically important coryneform bacteria (formerly called diphtheroids) include members of the normal flora that cause opportunistic infections, human pathogens of relatively low virulence, and animal pathogens that cause occasional zoonotic infections. Reported infections caused by coryneform bacteria have increased substantially in number over the past two decades. Isolates of *C. jeikeium* and *C. urealyticum* are often resistant to multiple antibiotics.

Laboratory Diagnosis

Because coryneform bacteria are potential pathogens, it is important not to dismiss them as constituents of the normal flora or as contaminants when they are found in clinical specimens. Laboratory differentiation of coryneform bacteria is important when they are isolated repeatedly, when they are recovered in pure culture or in large numbers, or when they form pigmented or hemolytic colonies.

The coryneform bacteria are a large, heterogeneous group of gram-positive, pleomorphic, irregularly staining bacilli or coccobacilli that superficially resemble *C. diphtheriae* and are difficult to identify and classify. The genus *Corynebacterium* is currently divided into three groups of species: the nonlipophilic, fermentative corynebacteria (including *C. diphtheriae*, *C. xerosis*, *C. striatum*, *C. minutissimum*, and others); the nonlipophilic, nonfermentative *corynebacteria* (including *C. pseudodiphtheriticum* and others); and the lipophilic corynebacteria (including *C. jeikeium*, *C. urealyticum*, and others). Coryneform bacteria also belong to many other genera (including Actinomyces, Arcanobacterium, and Rhodococcus).

Pathogenesis

C. jeikeium was recognized in 1976 as a cause of infections in immunocompromised hosts. This organism also causes infections in immunocompetent hosts, but severe infections continue to be most frequent in patients with hematologic malignancies and neutropenia. Skin colonization precedes clinical infection. Additional risk factors for nosocomial *C. jeikeium* sepsis include prolonged hospitalization.

C. urealyticum was identified as a significant cause of nosocomial urinary tract infections, including acute and chronic cystitis and pyelonephritis. The organism closely resembles *C. jeikeium* but differs from the latter by producing urease and failing to convert glucose to acidic metabolites. Hydrolysis of urea by urease causes alkalinization of the urine and formation of ammonium magnesium phosphate (struvite) stones.

A. haemolyticum causes pharyngitis and chronic skin ulcers; less frequently, it causes a variety of deep tissue infections, septicemia, and endocarditis.

C. minutissimum is frequently isolated from the lesions of erythrasma, a common superficial skin infection characterized by the presence in intertriginous areas of reddish-brown, scaly, pruritic, macular patches that exhibit coral-red fluorescence under a wood's light. The etiology of erythrasma appears to be polymicrobial; infection of the skin by *C. minutissimum* has been shown to follow the onset of maceration and scaling. Deep infections caused by *C. minutissimum* are rare and include abscesses, bacteremia, endocarditis, peritonitis, pyelonephritis, and infection of central venous catheters.

C. ulcerans infections in humans usually present as pharyngitis and can mimic respiratory diphtheria, whereas infections caused by *C. pseudotuberculosis* typically present as suppurative granulomatous lymphadenitis. Some strains of *C. ulcerans* and *C. pseudotuberculosis* produce diphtheria toxin.

C. pseudodiphtheriticum, a commensal of low virulence, is an uncommon cause of pneumonia in men with AIDS and of endocarditis, necrotizing tracheitis, tracheobronchitis, and urinary tract infection in patients without known immune deficiencies. Likewise, *C. xerosis and C. striatum* only occasionally cause human infections.

Diagnosis

1. Microscopy
2. Culture

Treatment

Most of the strains are susceptible to penicillin, azithromycin, quinolones and clindamycin.

GONOCOCCUS

Introduction

Gonorrhea is a sexually transmitted infection of epithelium and commonly manifests as cervicitis, urethritis, proctitis, and conjunctivitis. If untreated, infections at these sites can lead to local complications such as endometritis,

salpingitis, tuboovarian abscess, bartholinitis, peritonitis, and perihepatitis in the female; periurethritis and epididymitis in the male; and ophthalmia neonatorum in the newborn. Disseminated gonococcemia is an uncommon event whose manifestations include skin lesions, tenosynovitis, arthritis, and (in rare cases) endocarditis or meningitis.

Neisseria gonorrhea is a gram-negative, nonmotile, non-spore-forming organism that grows in pairs (diplococci). Each individual organism is shaped like a coffee bean, with adjacent concave sides seen on Gram's stain (Fig. 33.6). Gonococci, like all other Neisseria spp., are oxidase positive. They are distinguished from other Neisseria by their ability to grow on selective media and to utilize glucose but not maltose, sucrose, or lactose.

Modes of Transmission

Gonorrhea is transmitted from males to females more efficiently than in the opposite direction. The rate of transmission to a woman following a single unprotected sexual encounter with an infected man is on the order of 40 to 60%. Oropharyngeal gonorrhea occurs in ~20% of women who practice fellatio with infected partners. Transmission in either direction by cunnilingus is rare.

Clinical Manifestations

Gonococcal Infection in Males

Acute urethritis is the most common clinical manifestation of gonorrhea in males. The usual incubation period following exposure is 2 to 7 days, although the interval can be longer and some men remain asymptomatic. Urethral discharge and dysuria, usually without urinary frequency or urgency, are the

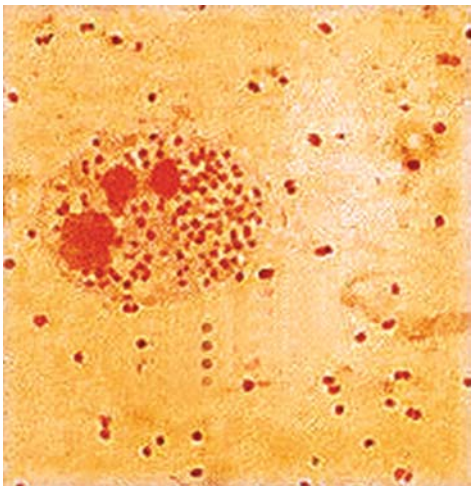


Fig. 33.6: Gram-negative diplococci (gonococci) in urethral discharge

major symptoms. The discharge initially is scant and mucoid but becomes profuse and purulent within a day or two. The clinical manifestations of gonococcal urethritis are usually more severe and overt than those of nongonococcal urethritis, including urethritis caused by *Chlamydia trachomatis;* however, exceptions are common, and it is often impossible to differentiate the causes of urethritis on clinical grounds alone. Most symptomatic males seek treatment and cease to be infectious. The remaining men, who are largely asymptomatic, accumulate in number over time and constitute about two-thirds of all infected men at any point in time. Together with men incubating the organism (who shed the organism but are asymptomatic), they serve as the source of spread of infection. Prior to the antibiotic era, symptoms of urethritis persisted for about 8 weeks. Epididymitis is now an uncommon complication, and gonococcal prostatitis occurs rarely, if at all. Other unusual local complications of gonococcal urethritis include edema of the penis due to dorsal lymphangitis or thrombophlebitis, submucous inflammatory "soft" infiltration of the urethral wall, periurethral abscess or fistulae, inflammation or abscess of Cowper's gland, and seminal vesiculitis. Balanitis may develop in uncircumcised men. After a decline in gonococcal infections among homosexual men early in the era of AIDS, a disturbing increase in gonorrhea was observed among young homosexual men in the 1990s, probably related to decreased condom use. The clinical features of anorectal and pharyngeal gonorrhea are discussed below.

Gonococcal Infections in Females

Women infected with *N. gonorrhoeae* usually develop symptoms. However, the women who either remain asymptomatic or have only minor symptoms may delay in seeking medical attention. Increased vaginal discharge and dysuria (often without urgency or frequency) are the most common symptoms. Although the incubation period of gonorrhea is less well defined in women than in men, symptoms usually develop within 10 days of infection and are more acute and intense than those of chlamydial cervicitis.

The physical examination may reveal a mucopurulent discharge (mucopus) issuing from the cervical os. The examiner may check for mucopurulent discharge by swabbing a sample of mucus from the endocervix and observing its color against the white background of the swab; yellow or green mucus suggests mucopus. However, only 35% of women with gonococcal cervicitis actually have a mucopurulent discharge defined by these criteria. Since Gram's stain is not sensitive for the diagnosis of gonorrhea in women, specimens should be submitted for culture or a nonculture assay (see below). Edematous and friable cervical ectopy as well as endocervical bleeding induced by gentle swabbing are more often seen in chlamydial infection.

Ocular Gonorrhea in Adults

Ocular gonorrhea in an adult usually results from autoinoculation from an infected genital site. As in genital infection, the manifestations range from severe to occasionally mild or asymptomatic disease. The variability in clinical manifestations may result from differences in the ability of the infecting strain to elicit an inflammatory response.

Infection may result in a markedly swollen eyelid, severe hyperemia and chemosis, and a profuse purulent discharge. The massively inflamed conjunctiva may be draped over the cornea and limbus. Lytic enzymes from the infiltrating PMNs occasionally cause corneal ulceration and rarely cause perforation.

Prompt recognition and treatment of this condition are of paramount importance. Gram's stain and culture of the purulent discharge establish the diagnosis. Genital cultures should also be performed.

Laboratory Diagnosis

A rapid diagnosis of gonococcal infection in men may be obtained by Gram's staining of urethral exudates. The detection of gram-negative intracellular diplococci (GNID) is usually highly specific and sensitive in diagnosing gonococcal urethritis in symptomatic males but is only ~50% sensitive in diagnosing gonococcal cervicitis. Samples should be collected with Dacron or rayon swabs. Part of the sample should be inoculated onto a plate of modified Thayer-Martin or other gonococcal selective medium for culture. It is important to process all samples immediately because gonococci do not tolerate drying. If plates cannot be incubated immediately, they can be held safely for several hours at room temperature in candle extinction jars prior to incubation. If processing is to occur within 6 h, transport of specimens may be facilitated by the use of nonnutritive swab transport systems such as Stuart or Amies medium. For longer holding periods (e.g. when specimens for culture are to be mailed), culture media with self-contained CO_2-generating systems (such as the JEMBEC or Gono-Pak systems) may be used. Specimens should also be obtained for the diagnosis of chlamydial infection.

Nucleic acid probe tests and PCR are now widely used for the direct detection of *N. gonorrhoeae* in urogenital specimens.

Treatment

Penicillin, Doxycycline, Ciprofloxacin are effective.

MOREXELLA

Moraxella Catarrhalis

The gram negative coccus now known as *Moraxella catarrhalis* has undergone three changes of name in as many decades. Originally called *Micrococcus*

catarrhalis, it was renamed *Neisseria catarrhalis* in the 1960s because of its morphologic similarity to *Neisseria* spp. Then, in 1970, it was elevated to the status of a distinct genus, Branhamella, on the basis of DNA homology. In 1979 this organism was placed into the genus Moraxella, of which Branhamella may be a subgenus. A component of the normal bacterial flora of the upper airways, *M. catarrhalis* has been increasingly recognized as a cause of otitis media, sinusitis, and bronchopulmonary infection.

BACTERIOLOGY

On Gram's staining, *M. catarrhalis* organisms appear as gram-negative cocci, sometimes occurring in pairs and retaining the side-by-side kidney-bean configuration of *Neisseria*. These cocci tend to retain crystal violet during the decolorizing step and may be confused with *Staphylococcus aureus*. *Moraxella* colonies grow well on blood or chocolate agar but may be overlooked because of their resemblance to Neisseria spp. (a major component of the normal pharyngeal flora). *Moraxella* is readily distinguishable from Neisseria spp. by biochemical tests.

OTITIS MEDIA AND SINUSITIS

M. catarrhalis has repeatedly been shown to be the third most common bacterial isolate from middle-ear fluid of children who have otitis media, being surpassed only by *Streptococcus pneumoniae* and nontypable *Haemophilus influenzae*. Recent studies have shown that this organism is also a prominent isolate from sinus cavities in acute and chronic sinusitis.

PNEUMONIA

M. catarrhalis causes acute exacerbations of chronic bronchitis (increased production and/or purulence of sputum), purulent tracheobronchitis (the latter also involving fever and leukocytosis), and pneumonia. The great majority of infected persons are >50 years old and have a long history of cigarette smoking and underlying chronic obstructive pulmonary disease (COPD).

Symptoms of M. catarrhalis infection have been regarded as modest in severity. Both cough and the amount and purulence of sputum are usually increased above baseline. Chills are reported in one-quarter of patients, pleuritic pain in one-third, and malaise in 40%. Most patients have peak temperatures of <38.3°C (<101°F), and peripheral white blood cell counts are <10,000/uL in nearly one-quarter of cases.

Diagnosis

Microscopic examination of a good sputum specimen following Gram's staining regularly reveals profuse organisms, and culture can be done.

Treatment

Treatment of *M. catarrhalis* infection with a penicillin/clavulanic acid combination seems highly appropriate. Cephalosporins, especially those of the second and third generations, are effective alternatives.

KLEBSIELLA

K. pneumoniae is the most important Klebsiella species medically, causing community-acquired, long-term-care, and hospital infections. *K. oxytoca* is primarily a pathogen in long-term-care and hospital settings. *K. rhinoscleromatis* and *K. ozaenae* are usually isolated from patients in tropical climates. Klebsiella species are broadly prevalent in the environment and colonize mucosal surfaces of mammals. In healthy humans, nurseries. The most common clinical syndromes are pneumonia, UTI, abdominal infection, surgical site infection, soft tissue infection, and subsequent bacteremia. *K. rhinoscleromatis* is the causative agent of rhinoscleroma, a slowly progressive (months to years) mucosal upper respiratory infection that causes necrosis and occasional obstruction of the nasal passages. *K. ozaenae* has been implicated as a cause of chronic atrophic rhinitis.

Diagnosis

Culture on McConkey agar reveals pink Lactose fermenting colonies which can be subjected for biochemical tests.

Treatment

Sensitive to Ampicillins, cephalosporins and quinolones.

PSEUDOMONAS AERUGINOSA

P. aeruginosa is a small, nonsporulating, aerobic gram-negative rod belonging to the family Pseudomonadaceae. It is motile by virtue of its single polar flagellum. More than half of all clinical isolates produce the blue-green pigment pyocyanin; this pigment is helpful in the identification of the organism and accounts for the species name aeruginosa, which refers to the distinctive color of copper oxide.

Most *P. aeruginosa* infections are acquired in the hospital, where intensive care units account for the highest rates of infection. According to the National Nosocomial Infections, the organism is transmitted to patients via the hands of hospital personnel or via fomites. While some infecting strains of *P. aeruginosa* appear to be endemic within the hospital, others are traced to a common source associated with a specific outbreak or epidemic. Epidemiologic investigation is facilitated by serotyping (immunotyping) of strains on the basis of differences

in lipopolysaccharide (LPS) structure and by the use of molecular techniques such as pulsed-field gel electrophoresis.

Pathogenesis

Infections caused by *P. aeruginosa* usually begin with bacterial attachment and superficial colonization of cutaneous or mucosal surfaces and progress to localized bacterial invasion and damage to underlying tissues. The infection may remain anatomically localized or may spread by direct extension to contiguous structures. This process may continue with bloodstream invasion, dissemination, the systemic inflammatory-response syndrome (SIRS), multiple-organ dysfunction, and ultimately death. Not only is local infection more likely to occur in immunocompromised hosts, such as those with profound neutropenia, but it is more likely to culminate in bloodstream invasion and dissemination. Endotoxin, which is a structural component of the bacterial outer membrane, is thought to play a pivotal role in the pathogenesis of the sepsis syndrome or SIRS.
P. aeruginosa produces a number of extracellular virulence factors.

Clinical Manifestations and Diagnosis

Respiratory tract infections: *Primary pneumonia*, or *non-bacteremic pneumonia*, results from aspiration of upper respiratory tract secretions; often develops in patients with chronic lung disease, congestive heart failure, or AIDS; and is most common in an intensive care setting in association with mechanical ventilator use. Fever, chills, severe dyspnea, cyanosis, productive cough, apprehension, confusion, and other signs of severe systemic toxicity characterize this acute, often life-threatening infection. Chest roentgenograms typically show bilateral bronchopneumonia with nodular infiltrates and small areas of radiolucency; pleural effusions are common; empyema is relatively uncommon; and lobar consolidation is occasionally seen. Cavitary lesions are particularly common in AIDS patients with *P. aeruginosa* pneumonia. Pathologic lesions include alveolar necrosis, focal hemorrhages, and microabscesses.

The clinical features of *P. aeruginosa* bacteremia are similar to those of other forms of bacteremia. Common primary sites of infection include the urinary tract, gastrointestinal tract, lungs, skin and soft tissues, and intravascular foci, including indwelling central venous catheters. Fever, tachypnea, tachycardia, and prostration are common. Disorientation, confusion, or obtundation may be evident. Hypotension can progress to refractory shock. Renal failure, adult respiratory distress syndrome, and disseminated intravascular coagulation occur as complications.

Endocarditis: *P. aeruginosa* infects native heart valves in injection drug users as well as prosthetic heart valves. The source of *P. aeruginosa* strains infecting drug users appears to be standing water contaminating drug paraphernalia. Foreign materials mixed with heroin may cause injury to valve leaflets or mural endocardium, with resulting fibrosis and an increased risk for valve infection. Exposure of the tricuspid valve to both trauma and bacteria apparently accounts for the high incidence of tricuspid involvement in association with injection drug use.

Ear infections: *P. aeruginosa* is often found in the external auditory canal, particularly under moist conditions and in the presence of inflammation or maceration (as in "swimmer's ear"). Moreover, this organism is the predominant pathogen associated with external otitis, a usually benign inflammatory process affecting the external auditory canal. The ear is painful or merely itchy, there is a purulent discharge, and pain is elicited by pulling on the pinna. The external canal appears edematous and is filled with detritus that often prevents visualization of the tympanic membrane.

P. aeruginosa occasionally penetrates the epithelium overlying the floor of the external auditory canal at the junction between bone and cartilage and invades underlying soft tissue. The ensuing invasive process, which involves soft tissue, cartilage, and cortical bone, is typically slow but destructive. Termed malignant external otitis, this condition occurs predominantly in elderly diabetic patients but is reported occasionally in infants with other underlying diseases and rarely in elderly nondiabetic patients.

Eye infections *P. aeruginosa* causes bacterial keratitis or corneal ulcer and endophthalmitis in the human eye. Keratitis due to *P. aeruginosa* may result from even minor corneal injury, which interrupts the integrity of the superficial epithelial surface and permits bacterial access to the underlying stroma. Corneal ulcer may complicate contact lens use, particularly when extended-wear soft contact lenses are involved. Contact lens solutions or the lenses themselves may be the source of the organism, which is probably inoculated into the eye at sites of minor lens-induced corneal damage. Patients who have sustained serious burns, have undergone ocular irradiation or tracheostomy, have been exposed to the intensive care environment, and/or are in a coma are also susceptible to *P. aeruginosa*-associated corneal ulcers. *P. aeruginosa* keratitis usually starts as a small central ulcer; spreads concentrically to involve a large portion of the cornea, sclera, and underlying stroma; and in some cases progresses to posterior corneal perforation.

The clinical manifestations of *P. aeruginosa* keratitis include a rapidly expanding, necrotic stromal infiltrate in the bed of an epithelial injury; surrounding epithelial edema; an anterior chamber reaction; and mucopurulent discharge adherent to the ulcer's surface. Corneal ulcer due to *P. aeruginosa* may advance rapidly to involve the entire cornea in $\leq$ 2 days or may evolve

subacutely over several days. Systemic symptoms are uncommon. Complications include corneal perforation, anterior chamber involvement, and endophthalmitis.

P. aeruginosa endophthalmitis is typically a rapidly progressive, sight-threatening condition that demands immediate therapeutic intervention. It may complicate penetrating injuries of the eye, intraocular surgery, hematogenous spread from other sites of *Pseudomonas* infection, or posterior perforation of corneal ulcers. Clinical manifestations may include eye pain, conjunctival hyperemia, chemosis, lid edema, decreased visual acuity, hypopyon, severe anterior uveitis, and signs of possible vitreous involvement. Panophthalmitis may result from this intraocular infection.

Urinary tract infections: *P. aeruginosa* is one of the most common causes of complicated and nosocomial infections of the urinary tract. These infections may result from urinary tract catheterization, instrumentation, surgery, or obstruction; they may arise from persistent foci (e.g. the prostate or stones) and may be chronic or recurrent. The urinary tract may be a target for bloodborne infection in patients with *P. aeruginosa* bacteremia but more often is the source of bacteremia. Chronic *P. aeruginosa* infections of the urinary tract are relatively common among patients with indwelling urinary catheters, altered urinary tract anatomy secondary to diversionary procedures, and paraplegia.

The clinical features of urinary tract infections due to *P. aeruginosa* are usually indistinguishable from those of other bacterial infections. However, *P. aeruginosa* infections exhibit a propensity for persistence, chronicity, resistance to antibiotic therapy, and recurrence. More unusual forms of urinary tract involvement peculiar to *P. aeruginosa* include (1) ulcerative lesions of the renal pelvis, ureters, and bladder that cause sloughing of vesical membranes in the urine; and (2) ecthyma-like lesions of the renal cortex that are seen in association with Pseudomonas sepsis.

Diagnosis

Culture on nutrient agar, blood agar and MacConkey agar. Pigmented colonies will be seen (Fig. 33.7).

Treatment

Aminoglycosides, Quinolones and Imepenem are effective. Usually 2 drug combination is effective.

BRUCELLA

Brucellosis is a zoonosis transmitted to humans from infected animals. Its clinical features are not disease specific. Brucellosis has many synonyms derived from the geographical regions in which the disease occurs

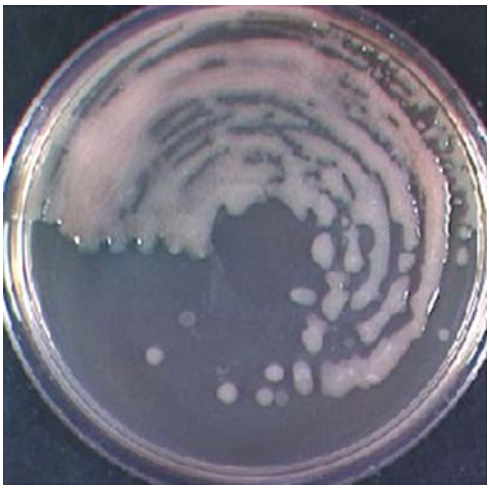


Fig. 33.7: Slimy pigmented colonies on nutrient agar which is oxidase positive

(e.g. Mediterranean fever, Malta fever, Gibraltar fever, Cyprus fever); from the remittent character of its fever (e.g. undulant fever); or from its resemblance to malaria and typhoid (e.g. typhomalarial fever, intermittent typhoid).

Etiology

Human brucellosis can be caused by any of four species: *Brucella melitensis* (the most common and most virulent cause of brucellosis worldwide) is acquired primarily from goats, sheep, and camels; *B. abortus* from cattle; *B. suis* from hogs; and B. canis from dogs. These small aerobic gram-negative bacilli are unencapsulated, nonmotile, non-spore-forming, facultative intracellular parasites that cause lifelong infection in animals. Brucellae are killed by boiling or pasteurization of milk and milk products. They survive for upto 8 weeks in unpasteurized, white, soft cheese made from goat's milk and are not killed by freezing. The organisms remain viable for upto 40 days in dried soil contaminated with infected-animal urine, stool, vaginal discharge, and products of conception and for longer periods in damp soil.

Pathogenesis

Serum opsonizes *Brucella* organisms for ingestion by polymorphonuclear leukocytes and activated macrophages. Brucellae resist intracellular phagocytic killing by mechanisms such as the suppression of the myeloperoxide-hydrogen peroxide-halide system and the production of superoxide dismutase. The pathogen-phagocyte interaction plays a key role in determining the severity and outcome of brucellosis. The organisms surviving within and escaping from the phagocytes multiply and reach the bloodstream via the lymphatics,

subsequently localizing in the liver, spleen, bones, kidneys, lymph nodes, heart valves, nervous system, and testes. In these organs, the bacteria are ingested by macrophages and survive by inhibition of phagosome-lysosome fusion. In infected tissues, inflammatory responses or noncaseating granulomas typically develop, and caseating granulomas and abscesses have been described.

Classification

Brucellosis is classified according to whether or not the disease is active (i.e. symptoms or progressive tissue damage and significantly raised Brucella agglutinin levels with or without positive cultures) and whether or not there is localized infection. The state of activity and the site of localization have a significant impact on recommended treatment. Classification of brucellosis as acute, subacute, serologic, bacteremic, or of mixed types serves no purpose in diagnosis and management.

Clinical Manifestations and Complications

Brucellosis is a systemic disease with protean manifestations. Its features may mimic those of other febrile illnesses. The incubation period lasts for about 1 to 3 weeks but may be as long as several months, depending on the virulence of the organisms, their route of entry, the infecting dose, and the host's preexisting health status. The onset of symptoms may be either abrupt (over 1 to 2 days) or gradual ($\geq$ 1 week). The most common symptoms are fever, chills, diaphoresis, headaches, myalgia, fatigue, anorexia, joint and low-back pain, weight loss, constipation, sore throat, and dry cough. Physical examination often reveals no abnormalities, and patients can look deceptively well. Some patients, in contrast, are acutely ill, with pallor, lymphadenopathy, hepatosplenomegaly, arthritis, spinal tenderness, epididymoorchitis, rash, meningitis, cardiac murmurs, or pneumonia. The fever of brucellosis has no distinctive pattern but may exhibit diurnal variation, with normal temperatures in the morning and high temperatures in the afternoon and evening.

Bones and joints: Although monoarticular septic arthritis occurs, 30 to 40% of patients have reactive asymmetric polyarthritis involving the knees, hips, shoulders, and sacroiliac and sternoclavicular joints. The total white cell count in synovial fluid ranges from 4000 to 40,000/mL, typically with about 60% polymorphonuclear leukocytes. The synovial fluid glucose concentration may be reduced and the protein concentration elevated; cultures of synovial fluid are positive in about 50% of cases.

Infection with Brucella organisms commonly causes osteomyelitis of the lumbar vertebrae, starting at the superior end plate (an area with a rich blood supply) and occasionally progressing to involve the entire vertebra, disk space, and adjacent vertebrae. Extraspinal Brucella osteomyelitis is rare. In Brucella

septic arthritis and osteomyelitis, the peripheral white cell count is typically normal, while the erythrocyte sedimentation rate may be either normal or elevated.

Heart: Cardiovascular complications of brucellosis include endocarditis, myocarditis, pericarditis, aortic root abscess, mycotic aneurysms, thrombophlebitis with pulmonary aneurysm, and pulmonary embolism. Brucella endocarditis may develop on valves previously damaged by rheumatic fever or congenital malformation but also occurs on previously normal valves.

Respiratory tract: Brucellae can produce respiratory symptoms. A flulike illness with sore throat, tonsillitis, and dry cough is common and usually mild. Hilar and paratracheal lymphadenopathy, pneumonia, solitary or multiple pulmonary nodules, lung abscess, and empyema have been reported.

Gastrointestinal tract and hepatobiliary system: Gastrointestinal manifestations of Brucella infection are generally mild and may include nausea, vomiting, constipation, acute abdominal pain, and/or diarrhea.

Genitourinary tract: The various genitourinary infections attributed to brucellae include unilateral or bilateral epididymoorchitis, which is rarely associated with testicular abscess. Prostatitis, seminal vesiculitis, dysmenorrhea, amenorrhea, tuboovarian abscess, salpingitis, cervicitis, acute pyelonephritis, glomerulonephritis, and massive proteinuria have also been documented. Brucella organisms have been cultured from the urine in upto 50% of cases of genitourinary tract infection.

Central nervous system: Neurobrucellosis is uncommon but serious and includes meningitis, meningoencephalitis, multiple cerebral or cerebellar abscesses, ruptured mycotic aneurysms, myelitis, Guillain-Barre syndrome, cranial nerve lesions, hemiplegia, sciatica, myositis, and rhabdomyolysis. Papillitis, papilledema, retrobulbar neuritis, optic atrophy, and ophthalmoplegia due to lesions in cranial nerves III, IV and VI may occur in Brucella meningoencephalitis.

Other manifestations: Conjunctival splashing with live attenuated *B. abortus* vaccine (S19) during animal vaccination may cause conjunctivitis, keratitis, and corneal ulcers, with progression to systemic disease in some cases. Uveitis, optic neuritis, retinopathy, retinal detachment, and endophthalmitis may result from hematogenous spread.

The bone marrow of *Brucella*-infected patients frequently contains non-caseating granulomas. Among the hematologic complications of brucellosis are anemia, leukopenia, and thrombocytopenia.

Endocrinologic findings reported in brucellosis include thyroiditis with abscess formation, adrenal insufficiency, and the syndrome of inappropriate secretion of antidiuretic hormone.

Diagnosis

The combination of potential exposure, consistent clinical features, and significantly raised levels of *Brucella* agglutinin (with or without positive cultures of blood, body fluid, or tissues) confirms the diagnosis of active brucellosis. The organism's identity is confirmed by phage typing, DNA characterization, or metabolic profiling. Use of a CO_2 detection system (such as BACTEC; Becton Dickinson, Sparks, MD) for blood culture provides a more sensitive and rapid culture result than standard methods, with positivity usually apparent after only 2 to 5 days of incubation. Serum antibodies to Brucella can be detected by several methods, including standard tube agglutinins (STA), the 2-mercaptoethanol agglutination test, Coomb' test, enzyme-linked immunosorbent assay, and polymerase chain reaction (PCR). *B. abortus antigens*, which are commonly used for serologic tests, cross-react with B. melitensis and *B. suis* but not with *B. canis*. The specific antigen required for assay of antibodies to *B. canis* is not commercially available.

A high titer of specific IgM suggests recent exposure, while a high titer of specific IgG suggests active disease. Lower titers of IgG may indicate past exposure or treated infection.

Cooperation and consultation with a clinical microbiology laboratory are important when brucellosis is suspected. It may be necessary to observe culture bottles for upto 6 weeks before organisms become detectable. Subcultures should be prepared on duplicate blood agar plates (with and without an atmosphere of 10% CO_2) and special media (such as a blood- or serum-enriched peptone-based medium) or with a rapid CO_2 detection system.

Treatment

Gentamicin, Netilmycin, Streptomycin are effective as well as Doxycycline.

MYCOBACTERIUM TUBERCULOSIS

Definition

Tuberculosis, one of the oldest diseases known to affect humans, is caused by bacteria belonging to the *Mycobacterium tuberculosis* complex (Fig. 33.8). The disease usually affects the lungs, although in upto one-third of cases other organs are involved. If properly treated, tuberculosis caused by drug-susceptible strains is curable in virtually all cases. If untreated, the disease may be fatal within 5 years in more than half of cases. Transmission usually takes place through the airborne spread of droplet nuclei produced by patients with infectious pulmonary tuberculosis.

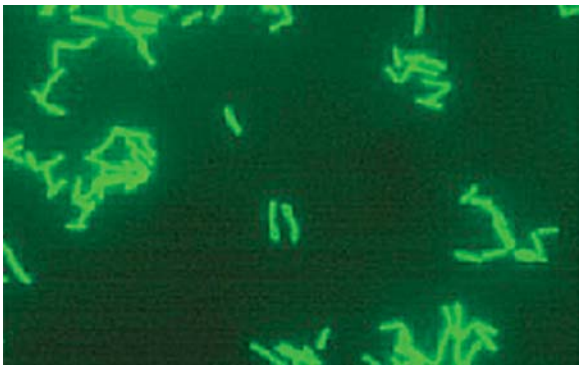


Fig. 33.8: *M. tuberculosis* seen under fluorescent microscope

Etiologic Agent

Mycobacteria belong to the family Mycobacteriaceae and the order Actinomycetales. Of the pathogenic species belonging to the *M. tuberculosis* complex, the most frequent and important agent of human disease is *M. tuberculosis* itself. The complex includes *M. bovis* (the bovine tubercle bacillus, once an important cause of tuberculosis transmitted by unpasteurized milk and currently the cause of a small percentage of cases in developing countries), *M. africanum* (isolated in a small proportion of cases in West and Central Africa), and *M. microti* (the "vole" bacillus, a closely related but rarely encountered organism).

M. tuberculosis is a rod-shaped, non-spore forming, thin aerobic bacterium measuring about 0.5 µm by 3 µm. Mycobacteria, including *M. tuberculosis*, do not stain readily and are often neutral on Gram's staining. However, once stained, the bacilli cannot be decolorized by acid alcohol, a characteristic justifying their classification as acid-fast bacilli (AFB). Acid fastness is due mainly to the organisms' high content of mycolic acids, long-chain cross-linked fatty acids, and other cell wall lipids.

Infection: M. tuberculosis is most commonly transmitted from a patient with infectious pulmonary tuberculosis to other persons by droplet nuclei, which are aerosolized by coughing, sneezing, or speaking. The tiny droplets dry rapidly; the smallest (<5 to 10 µm in diameter) may remain suspended in the air for several hours and may gain direct access to the terminal air passages when inhaled. There may be as many as 3000 infectious nuclei per cough. In the past, a frequent source of infection was raw milk containing *M. bovis* from tuberculous cows. Other routes of transmission of tubercle bacilli, such as through the skin or the placenta, are uncommon and of no epidemiologic significance.

Pathogenesis

The interaction of *M. tuberculosis* with the human host begins when droplet nuclei containing microorganisms from infectious patients are inhaled. While the majority of inhaled bacilli are trapped in the upper airways and expelled by ciliated mucosal cells, a fraction (usually fewer than 10%) reach the alveoli. There nonspecifically activated alveolar macrophages ingest the bacilli. Invasion of macrophages by mycobacteria may result in part from association of C2a with the bacterial cell wall followed by C3b opsonization of the bacteria and recognition by the macrophages. The balance between the bactericidal activity of the macrophage and the virulence of the bacillus (the latter being partially linked to the bacterium's lipid-rich cell wall and to its glycolipid capsule, both of which confer resistance to complement and free radicals of the phagocyte) determines the events following phagocytosis. The number of invading bacilli is also important.

In the initial stage of host-bacterium interaction, either the host's macrophages contain bacillary multiplication by producing proteolytic enzymes and cytokines or the bacilli begin to multiply. If the bacilli multiply, their growth quickly kills the macrophages, which lyse. Non-activated monocytes attracted from the bloodstream to the site by various chemotactic factors ingest the bacilli released from the lysed macrophages. These initial stages of infection are usually asymptomatic.

About 2 to 4 weeks after infection, two additional host responses to M. tuberculosis develop: a tissue-damaging response and a macrophage-activating response. The tissue-damaging response is the result of a delayed-type hypersensitivity (DTH) reaction to various bacillary antigens; it destroys nonactivated macrophages that contain multiplying bacilli. The macrophage-activating response is a cell-mediated phenomenon resulting in the activation of macrophages that are capable of killing and digesting tubercle bacilli. Although both of these responses can inhibit mycobacterial growth, it is the balance between the two that determines the form of tuberculosis that will develop subsequently.

Cell-mediated immunity is critical at this early stage. In the majority of infected individuals, local macrophages are activated when bacillary antigens processed by macrophages stimulate T lymphocytes to release a variety of lymphokines. These activated cells aggregate around the lesion's center and effectively neutralize tubercle bacilli without causing further tissue destruction. In the central part of the lesion, the necrotic material resembles soft cheese (caseous necrosis). Even when healing takes place, viable bacilli may remain dormant within macrophages or in the necrotic material for years or even throughout the patient's lifetime. These "healed" lesions in the lung parenchyma and hilar lymph nodes may later undergo calcification (*Ranke complex*).

In the early stages of infection, bacilli are usually transported by macrophages to regional lymph nodes, from which they disseminate widely to many organs and tissues. The resulting lesions may undergo the same evolution as those in the lungs, although most tend to heal. In young children with poor natural immunity, hematogenous dissemination may result in fatal miliary tuberculosis or tuberculous meningitis.

Cell-mediated immunity confers partial protection against *M. tuberculosis*, while humoral immunity has no defined role in protection. Two types of cells are essential: macrophages, which directly phagocytize tubercle bacilli, and T cells (mainly CD4+ lymphocytes), which induce protection through the production of lymphokines.

Clinical Manifestations

Tuberculosis is usually classified as pulmonary or extrapulmonary. Before the recognition of HIV infection, more than 80% of all cases of tuberculosis were limited to the lungs. However, up to two-thirds of HIV-infected patients with tuberculosis may have both pulmonary and extrapulmonary disease or extrapulmonary disease alone.

Pulmonary tuberculosis: Pulmonary tuberculosis can be categorized as primary or postprimary (secondary).

Primary disease: Primary pulmonary tuberculosis results from an initial infection with tubercle bacilli. In areas of high tuberculosis prevalence, this form of disease is often seen in children and is frequently localized to the middle and lower lung zones. The lesion forming after infection is usually peripheral and accompanied by hilar or paratracheal lymphadenopathy, which may not be detectable on chest radiography. In the majority of cases, the lesion heals spontaneously and may later be evident as a small calcified nodule (*Ghon lesion*).

Early in the course of disease, symptoms and signs are often nonspecific and insidious, consisting mainly of fever and night sweats, weight loss, anorexia, general malaise, and weakness. However, in the majority of cases, cough eventually develops—perhaps initially nonproductive and subsequently accompanied by the production of purulent sputum. Blood streaking of the sputum is frequently documented. Massive hemoptysis may ensue as a consequence of the erosion of a fully patent vessel located in the wall of a cavity.

Extrapulmonary tuberculosis: In order of frequency, the extrapulmonary sites most commonly involved in tuberculosis are the lymph nodes, pleura, genitourinary tract, bones and joints, meninges, and peritoneum. However, virtually all organ systems may be affected. As a result of hematogenous dissemination in HIV-infected individuals, extrapulmonary tuberculosis is seen more commonly today than in the past.

Genitourinary tuberculosis: Genitourinary tuberculosis accounts for about 15% of all extrapulmonary cases, may involve any portion of the genitourinary tract, and is usually due to hematogenous seeding following primary infection. Local symptoms predominate. Urinary frequency, dysuria, hematuria, and flank pain are common presentations.

Diagnosis

Microscopy: A presumptive diagnosis is commonly based on the finding of AFB on microscopic examination of a diagnostic specimen such as a smear of expectorated sputum or of tissue (for example, a lymph node biopsy). Most modern laboratories processing large numbers of diagnostic specimens use auramine-rhodamine staining and fluorescence microscopy. The more traditional method—light microscopy of specimens stained with Kinyoun or Zeihl-Neelsen basic fuchsin dyes—is satisfactory, although more time-consuming. For patients with suspected pulmonary tuberculosis, three sputum specimens, preferably collected early in the morning, should be submitted to the laboratory for AFB smear and mycobacteriology culture. If tissue is obtained, it is critical that the portion of the specimen intended for culture not be put in formaldehyde. The use of AFB microscopy on urine or gastric lavage fluid is limited by the presence of mycobacterial commensals, which can cause false-positive results.

Mycobacterial culture: Definitive diagnosis depends on the isolation and identification of *M. tuberculosis* from a diagnostic specimen—in most cases, a sputum specimen obtained from a patient with a productive cough. Specimens may be inoculated onto egg- or agar-based medium (e.g. Lowenstein-Jensen or Middlebrook 7H10) and incubated at 37°C under 5% CO_2.

Nucleic Acid Amplification, PCR are Latest diagnostic tools.

Skin testing: Skin testing with PPD is most widely used in screening for *M. tuberculosis* infection.

Treatment

Five major drugs are considered the first-line agents for the treatment of tuberculosis: isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin. Ofloxacin is the most widely used, but levofloxacin and sparfloxacin are the most active, although the latter drug is associated with high rates of photosensitization.

Prevention

BCG vaccination

IMMUNOLOGY

The term immunity is derived from Latin meaning free from burden. The burden is the disease caused by a variety of microorganisms (e.g. viruses, bacteria, fungi etc).

Immunity is defined as the state of resistance exhibited by the host to toxic molecules, microorganisms and foreign cells. Immunity may be natural (innate) or acquired (adapted).

Innate immunity is also known as native immunity. It is a resistance with which a person is born. This type of immunity is present for life.

Types of innate immunity

1. *Species immunity:* Animals of the same species exhibit uniform pattern of susceptibility to infections, e.g. Bacillus anthrax affects humans but not chickens. Birds are resistant to tetanus.
2. *Racial immunity:* Algerian race of sheep is immune to anthrax. Patients having glucose-6-phosphate dehydrogenase are less susceptible. Sickle cell patients are highly resistant to falciparum malaria.

Mechanism of innate immunity (natural resistance)

Natural resistance is by three factors namely physiochemical, humoral and cellular.

a. *Physiochemical barriers:* Skin not only provides a mechanical barrier to the invading microorganisms but also provides bacteriocidal secretions. The superficial microorganisms of the resident flora may be diminished by vigorous surgical scrubbing but the resident flora is rapidly replenished from sebaceous and sear glands.

   Nose, Nasopharynx , bronchioles of the respiratory tract act as mechanical barrier(cilia). Cough reflex plays an important defensive roll of respiratory tract. Mouth, stomach and intestinal tract (saliva, acidic Ph), conjunctivae (lysozyme) also play important roles in preventing the colonization of microorganisms.

b. *Tissue factors:* Cellular factors such as phagocytes accumulate at the site of infection along with outpouring of natural antibacterial substances. The cells of phagocytosis include macrophages(histiocytes, monocytes), Neutrophil polymorphonuclear leucocytes.

Phagocytic action can be divided into four stages:

1. *Chemotaxis:* Phagocytes reach the infection site.
2. *Attachment:* Adherence with the infecting agent
3. *Ingestion:* Cells engulf the foreign body into a vacuole (phagosome), the membrane of which fuses with a lysosome forming a phagolysosome. Lysosome contains hydrolytic enzymes and other bacteriocidal substances.
4. *Intracellular killing:* Done by hydrolytic enzymes.

Most bacteria are killed in the phagolysosome by the hydrolytic enzymes.

Acquired Immunity: Resistance acquired by an individual during life is called acquired or specific immunity. Acquired immunity is of two types: active and passive.

Active immunity: Active immunity is the resistance induced in an individual after effective contact with an antigen. It follows either natural infection or vaccination. Here, the immune system actively participates producing antibody and often cell mediated immunity also.

Mechanism of active immunity: The body's immune apparatus offers protection by sysnthesizing antibodies by B lymphocytes and plasma cells and/ or by T cells in response to an antigen. During the first encounter between the host and microbe, antibody forms only after a latent. This is called primary immune response.

When the individual encounters the same antigen subsequently, the immune response is more rapid and stronger. This is called secondary immune response.

Types of active immunity: Active immunity may be acquired either naturally or artificially.

Natural active immunity: It is acquired by natural infection or subclinical infections by microorganisms.

Artificial active immunity: It is the resistance produced by vaccination.

Passive immunity: The resistance induced in the recipient by transfer of preformed antibodies against an infective agent or toxin. There are two types of passive immunity:

a. Natural passive immunity: It is the resistance passively transferred from the mother to fetus
b. Artificial passive immunity: It is the resistance passively transferred to a recipient by the parentral administration of antibodies, e.g. Hepatitis B human immunoglobulin.

Local immunity: Natural infection or administration of live poliovaccine can initiate a local IgA response and this plays a major role in local(Gut) immunity.

Herd immunity: It refers to the collective resistance to the disease displayed by the community in its environmental setting. Epidemics can occur when the herd immunity in the community goes down.

Antigens and Antibodies

Antigen is a substance usually protein in nature and sometimes polysaccharide, which when introduced into a living body evokes specific immune response, either by producing specific antibody or specially sensitised T cells or both.

Antibodies are synthesized by host B lymphocytes and plasma cells when they come in contact with foreign antigen, e.g. Microbes. Antibodies are defined as serum proteins formed in response to an antigen and react specifically with that antigen or closely related to it. There are five classes of immunoglobulins IgG, IgM, IgA, IgD and IgE.

IgM is produced early in the disease and last for a few days when compared to IgG which lasts long. **IgM does not cross placenta.** IgE is the principal mediator of anaphylaxis. IgA is found in secretions and confers local immunity. IgD act as receptors for B cells.

Antigen antibody reactions: Important tests for antigen-antibody reactions include precipitation, agglutination, complement fixation, neutralization, immunofluorescence, enzyme immuno-assays and radioimmuno-assays.

Precipitation reactions: When a soluble antigen reacts with its specific antibody in presence of electrolyte (NaCl) at an optimal pH(7.4) and temperature (37°C), the antigen-antibody complex forms an insoluble precipitate. This reaction is called precipitation.

Techniques of precipitation: There are three principal types of precipitation technique

Simple precipitation tests:

1. Ring test: A ring is formed at the site of antigen-antibody reaction, e.g. Lancefield method of grouping beta-hemolytic streptococci
2. Flocculation test: VDRL test for syphilis. Here floccules are formed when the reactive serum is mixed with the antigen(cardiolipin).
3. Gel diffusion tests: Precipitation done in agar gel. When both antigen and antibody diffuses in the agar gel and a precipitate is formed. There are different kinds of gel diffusion techniques.
    a. Single diffusion
    b. Double diffusion
    c. Immunoelectrophoresis
    d. Counter immunoelectrophoresis

Agglutination is an antigen-antibody reaction in which an antibody combines with a particulate antigen in presence of electrolytes at optimal pH and temperature resulting in visible clumping of the particles. Particles like bacteria, erythrocytes, synthetic latex particles, etc. coated with antigen or antibody can be used to detect either soluble antibodies or the antigens, respectively. Agglutination reaction is better with IgM antibody than IgG antibody.

Different types of agglutination reactions:

1. Slide agglutination tests: Antigen (commercially available) is mixed with the patients serum suspected of having the disease. Immediate clumping occurs, e.g. Rapid Widal tests for Salmonella typhi.

2. `Tube agglutination tests: Agglutination occurs in fluid medium. Tube agglutination tests are more sensitive than the slide tests, e.g. Brucella agglutination test.

Complement Fixation Test

Principle: Antigen-antibody complex fixes the complement. The coupling of complement with Ag-Ab complex does not have any visible effect like agglutination/precipitation reactions. It is therefore used as an indicator system consisting of sheep red cells coated with anti-sheep red cell antibody (amboceptor). Complement lyses antibody coated red cells.

The use of CFT is considerably reduced with recent serological advances. However, CFT is still of value in virology in which CFT provides a retrospective diagnosis because complement fixing antibodies(IgG) appears in blood late (2 - 3 weeks after infection).

Neutralization tests: When an antitoxin combines with a toxin, the biological effects of the toxin are neutralized rendering it harmless to the body. Since toxin is an antigen in solution, it is also precipitated.

Radioimmunoassay (RIA)

Principle: RIA relies on the radioactivity of a specific isotope labeled antibody or antigen which is used to detect and quantify antigen or antibody in the test material. By this technique antigens upto picograms can be measured.

ELISA: Enzyme linked Immuno-sorbent Assay is now-a-days the widely used technique for diagnosis of most of the diseases (HIV, Hepatitis A,B and C), TORCH panel, Mycobacterium spp.etc.

Principle

1. The antigen is coated on to the surface of a microtiter plate (Polyvinyl or polystyrene) .
2. The patients serum (suspected to have antibodies) is added to it . Incubate for 30 minutes. Wash with distill water.
3. Then add an enzyme (horse radish peroxidase or alkaline phosphatase) which is labeled or linked to a specific antibody.
4. Finally add a substrate (p-nitrophenyl phosphate). The enzyme catalyse the substrate to give a color end point (yellow color). The intensity of the color gives an indication of the amount of bound antibody.

Immunofluorescence

Principle: Fluorescence is the property of certain dyes which absorb light in the ultraviolet region (200 - 400 nm). The popularly used fluorescin dyes such as isothiocyanate and Rhodamine can be conjugated to antibodies which serve

to locate and identify antigens in tissues. Fluorescin isothiocyanate exhibits blue green and rhodamine orange red fluorescence when viewed under fluorescent microscope, e.g. Mycobacterium tuberculosis in sputum.

HYPERSENSITIVITY REACTIONS

Hypersensitivity reactions are of two main types—immediate and delayed—based on time required by sensitized host to respond to the shocking dose of the antigen. Immediate form is mediated by humoral antibody and manifests in a few minutes to few hours while delayed form appears more slowly, usually after 24 hours which reaches a peak after 48-72 hours and is mediated by sensitized CD4 T cells.

Hypersensitivity reactions are of four types:

Type I (Immediate) Hypersensitive Reaction

Anaphylaxis is an immediate type I reaction and life threatening when not intervened properly. It is IgE mediated which develops quickly after introduction of a large shocking dose of antigen following one or more small sensitizing doses.

Mechanism of anaphylaxis: IgE is the major antibody responsible for anaphylaxis. IgE antibody usually binds to the mast cells and release the mediators of anaphylaxis namely, histamine, prostaglandin D_2, platelet activating factor, serotonin and SRS-A.

Which can produce vasodilation, bronchospasm, laryngeal edema, respiratory distress, shock and death.

Atopy is also a type I reaction that occurs spontaneously in response to substances encountered in the environment in everyday life. Atopy is typified by hay fever and asthma. About 10% of the population are prone to develop sensitization to various environmental antigens such as pollens, ragweed, grasses, foods or animal dander.

Type II (Cytotoxic) Reaction

It is a cytotoxic reaction mediated by antibodies directed towards antigens present on the surface of cell or other tissue components. The antibody binds to cell surface molecules and the subsequent activation of cytotoxic/cytolytic responses is brought about by the classic complement cascade or by cellular mechanisms.

Examples are:

a. ABO transfusion reactions
b. Auto immune hemolytic anemia
c. Thrombocytopenia
d. Drug reactions such as quinidine

Type III (Immune Complex) Reactions

It is a type of humoral antibody mediated hypersensitivity reaction characterized by deposition of Ag-Ab complexes in tissues (particularly on vascular endothelial surfaces), activation of complement and massive infiltration by polymorphonuclear leukocytes leading to tissue damage.

There are two types of type III hypersensitivity responses

a. Arthus reaction: When repeated subcutaneous injections of antigen (horse serum) into rabbits, a high level of precipitating antibody appears in blood. When the same antigen is injected subsequently in the same animal intense local edema and hemorrhage develop which reaches a peak in 3–6 hours. This type of reaction is called Arthus reaction.

Some important immune complex diseases include glomerulonephritis, rheumatic fever and rheumatoid arthritis.

Type IV Delayed Reaction (Cell Mediated Immunity)

Type IV hypersensitivity reaction or delayed hypersensitivity is a specially provoked, slowly evolving (24–72 hours), mixed cellular reaction involving lymphocytes and macrophages, where tissue damage events is mediated by CD4 T lymphocytes and not by antibody.

Examples of delayed hypersensitivity:

1. Tuberculin test (Mantoux test)
2. Lepromin test
3. Contact dermatitis.

MYCOLOGY

Introduction

Mycology is a part of the microbiological mainstream because of serious public health hazard created by them. The organisms in the kingdom fungi are

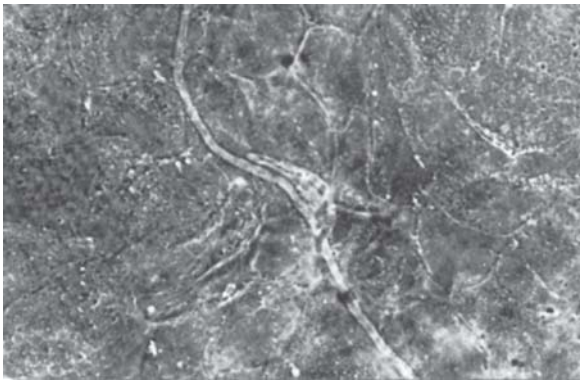


Fig. 33.9: 10 % KOH preparation shows fungal filaments from a corneal scrapping

eukaryotic cells and do not possess chlorophyll. The pathogenic fungi grow on dead and decaying organic matter and are true saprophytes, but under favorable conditions they can become pathogenic. Fungi are now emerging as important nosocomial pathogens in immune compromised patients including AIDS. Infections due to fungi are classified into (1) Superficial mycoses that are limited to the outermost layers of the skin and hair, (2) cutaneous mycoses are diseases that extend deeper into the epidermis, hair and nail, and (3) subcutaneous mycoses involve the deeper tissues include dermis, subcutaneous tissues, muscle and fascia.

Opportunistic Mycoses

Aspergillosis

Aspergillus fumigatus is the most common cause of aspergillosis, but A. flavus, A. niger, and several other species can also cause disease. Aspergillus is a mold with septate hyphae about 2 to 4 um in diameter. The fungus is identified by its gross and microscopic appearance in culture and Lactophenol cotton blue staining (Fig. 33.10).

Pathogenesis

All the common species of Aspergillus that cause disease in humans are ubiquitous in the environment, growing on dead leaves, stored grain, compost piles, hay, and other decaying vegetation. Inhalation of Aspergillus spores must be extremely common, but disease is rare.

Aspergillus can colonize the damaged bronchial tree, pulmonary cysts, or cavities of patients with underlying lung disease. Balls of hyphae within cysts or cavities (aspergillomas), usually in the upper lobe, may reach several

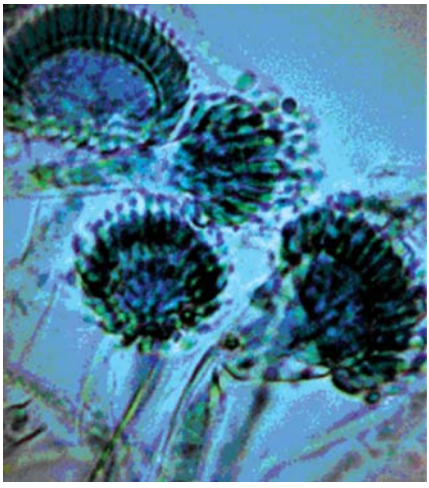


Fig. 33.10: Aspergillus spp. in lactophenol cotton blue stain

centimeters in diameter and may be visible on chest X-ray. Tissue invasion does not occur. The term allergic bronchopulmonary aspergillosis denotes the condition of patients with preexisting asthma who have eosinophilia, IgE antibody to Aspergillus, and fleeting pulmonary infiltrates from bronchial plugging.

Clinical Manifestations

Endobronchial saprophytic pulmonary aspergillosis presents as chronic productive cough, often with hemoptysis, in a patient with prior chronic lung disease, such as tuberculosis, sarcoidosis, bronchiectasis, or histoplasmosis. Aspergillus may be spread from its endocavitary or endobronchial site to the pleura during the course of bacterial lung abscess or surgery.

Aspergillus sinusitis in immunocompetent patients may take three forms. A ball of hyphae may form in a chronically obstructed paranasal sinus, without tissue invasion. Much less commonly, a chronic, fibrosing granulomatous inflammation associated with Aspergillus hyphae within tissue may begin in the sinus and spread slowly to the orbit and the brain.

Aspergillosis in HIV-infected patients most commonly involves the lung, presenting as fever, cough, and dyspnea. Typically, the CD4 cell count is below 50/uL. Roughly half of these patients have neutropenia or have recently been treated with glucocorticoids.

The growth of Aspergillus on cerumen and detritus within the external auditory canal is termed otomycosis. **Trauma to the cornea may cause Aspergillus keratitis. Endophthalmitis follows the introduction of Aspergillus into the globe by trauma or surgery**. Aspergillus may infect intracardiac or intravascular prostheses.

Diagnosis

Culture can be done on Saborauds Dextrose agar with a pH of 5.2, however, Aspergillus spp. can contaminate any basal medium.

Direct Koh examination of the specimen for fungal hyphae.

Lactophenol cotton blue stain to see the morphology.

Treatment

Treatment with intravenous Amphotericin B (1.0 to 1.5 mg/kg daily). Itraconazole (200 mg twice daily).

CANDIDIASIS

Candida albicans is the most common cause of mucosal candidiasis and is responsible for about half of all cases of candidemia in hospitalized patients. A small proportion of *C. albicans* isolates have been transferred to a new

species, *C. dubliniensis. C. tropicalis, C. parapsilosis, C. guilliermondii, C. glabrata (formerly Torulopsis glabrata), C. krusei*, and a few other Candida species also cause potentially fatal bloodstream infection. Many of these non-albicans species can enter the bloodstream through an intravascular catheter. Candida species, taken together, are the fifth most common cause of nosocomial bloodstream infections in the United States.

All Candida species pathogenic for humans are also encountered as commensals of humans, particularly in the mouth, stool, and vagina. These species grow rapidly at 25° to 37°C on simple media as oval, budding cells. In special culture media and in tissue, hyphae or elongated branching structures called pseudohyphae are formed. *C. glabrata* differs from other members of the genus in that it forms no true hyphae or pseudohyphae *in vitro* or in infected tissue. *C. albicans* and *C. dubliniensis* can be identified presumptively by their ability to form germ tubes in serum or by the formation of thick-walled large spores called chlamydospores (at 25 degree centigrade on corn meal agar). Final identification of all Candida species requires biochemical tests.

Pathogenesis

Candidiasis is often preceded by increased colonization of the mouth, vagina, and stool with Candida due to broad-spectrum antibiotic therapy. Additional local and systemic factors favor infection. Oropharyngeal thrush is particularly likely to occur in neonates and in patients with diabetes mellitus, HIV infection, or dentures. Vulvovaginal candidiasis is especially common in the third trimester of pregnancy. Candida from the perineum can enter the urinary tract via an indwelling bladder catheter. Cutaneous candidiasis most often involves macerated skin, such as that in the diapered area of infants, under pendulous breasts, or on hands constantly in water or covered by occlusive gloves. Candida can pass from the colonized surface into deep tissue when the integrity of the mucosa or skin is violated, as, for example, by perforation of the gastrointestinal tract through trauma, surgery, or peptic ulceration or by mucosal damage due to cytotoxic agents used for cancer chemotherapy. Although Candida is not normally a resident of the skin, secretions from the mouth, rectum, or vagina as well as drainage from surgical wounds or tracheostomy sites can contaminate the hub or skin site of a catheter in an umbilical or central vein. Intravenous drug abuse or third-degree burns can also provide a skin portal for Candida that can lead to deep candidiasis. Once Candida has passed the integumentary barrier, very low birth weight (in neonates) and neutropenia or glucocorticoid therapy (in any patient) markedly compromise host defense. Hematogenous seeding is particularly evident in the retina, kidney, spleen, and liver.

Clinical Manifestations

Oral thrush presents as discrete and confluent adherent white plaques on the oral and pharyngeal mucosa, particularly in the mouth and on the tongue. These

lesions are usually painless, but fissuring at the corners of the mouth can be painful. Unexplained oropharyngeal thrush raises the possibility of HIV infection. Oral thrush is common in acute HIV infection and becomes increasingly common as the CD4+ cell count falls. At CD4+ counts <50/uL, esophageal thrush also becomes common. HIV infection appears not to be an independent risk factor for vulvovaginal thrush.

Cutaneous candidiasis presents as red macerated intertriginous areas, paronychia, balanitis, or pruritus ani. Candidiasis of the perineal and scrotal skin may be accompanied by discrete pustular lesions on the inner aspects of the thighs. Chronic mucocutaneous candidiasis or candidal granuloma typically presents as circumscribed hyperkeratotic skin lesions, crumbling dystrophic nails, partial alopecia in areas of scalp lesions, and both oral and vaginal thrush.

Esophageal candidiasis is often asymptomatic but can cause substernal pain or a sense of obstruction on swallowing. Most lesions are in the distal third of the esophagus and appear on endoscopy as areas of redness and edema, focal white patches, or ulcers. Biopsy or brushing is required for diagnosis and for detection of concomitant infections, particularly herpes simplex in patients with hematologic malignancies and cytomegalovirus infection in AIDS patients. Esophagography (barium swallow) is diagnostically insensitive but may reveal spasm or mucosal irregularities. Candida esophagitis can cause bleeding and impaired alimentation. Hematogenous dissemination from the esophagus probably occurs in some neutropenic patients but is rarely reported in HIV-infected patients. Candida can cause cystitis and pyelitis.

Hematogenous dissemination can lead to brain abscess or chronic meningitis. Diagnosis of infections of ventriculoperitoneal shunts is difficult because symptoms are indolent and cultures of lumbar fluid are usually sterile.

Diagnosis

Demonstration of pseudohyphae on wet smear with confirmation by culture is the procedure of choice for diagnosing superficial candidiasis. Scrapings for the smear may be obtained from skin, nails, and oral and vaginal mucosa. Culture can be done on Saborauds Dextrose agar. White creamy colonies are formed at 37°C. When the colonies are stained by Gram'

Deeper lesions due to Candida may be diagnosed by histologic section of biopsy specimens or by culture of cerebrospinal fluid, blood, joint fluid, or surgical specimens. Blood cultures are useful in the diagnosis of Candida endocarditis and intravenous catheter-induced sepsis but are positive less often in other forms of disseminated disease. Serologic tests for antibody or antigen are not useful.

Serology is not useful in confirming the infection.

Treatment

Clotrimazole, miconazole, econazole, ketoconazole, sulconazole, and oxiconazole are available as creams or lotions. Candida vulvovaginitis responds better to an azole than to nystatin suppositories. There is little difference in efficacy among miconazole.

ZYGOMYCOSIS

Zygomycosis (mucormycosis): The clinical entry Zygomycosis, also called mucormycosis is an opportunistic infection often seen in patients with uncontrolled diabetes mellitus.

Morphology: The fungi grow rapidly on all laboratory media not containing cycloheximide. They produce coenocytic hyphae and reproduce sexually by producing sporangia. The sporangiospores develop within sporangia.

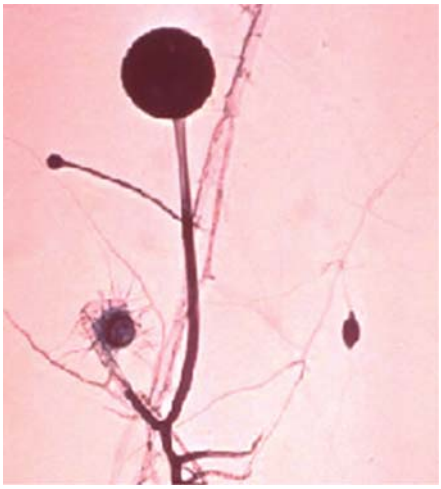


Fig. 33.11: Rhizopus species

Etiology: The etiologic agents of most cases of zygomycosis fall into three genera of fungi: Mucor, Rhizopus, Absidia.

Pathogenesis: The infection usually originates in paranasal sinus and involve the ocular orbit and palate extending into the brain. This infection usually occurs as a terminal event in diabetic acidosis and death follows in a few days.

Other clinical forms of zygomycosis are seen in immunocompromized patients, in malnutrition and diarrheal diseases involving lungs, gastrointestinal tract and subcutaneous tissues.

Clinical disease: The lesion present as cotton-like growths on the roof of the mouth or nares in diabetic patients.

Diagnosis

Diagnosis is usually made clinically and confirmation is made by culture (SDA) and microscopy by KOH examination (non-septate, ribbon-like hyphae)
Treatment: Amphotericin B

FUSARIUM (FIG. 33.12)

Several saprophytic fungi such as Fusarium, Aspergillus and Penicillium are occasionally encountered in nail infection usually as secondary invaders following trauma. Fusarium species also produce Keratitis, mycetoma, otomycosis and osteomyelitis following trauma.

Fig. 33.12: Fusarium

Diagnosis

Microscopical examination: The mycelium is septate, conidiospores are single or branching, occasionally producing whorls. The microphialoconidia are single celled and occur in balls. Culture on SDA shows woolly colonies which will turn into orange or violet color later.

PENICILLIUM

Penicillium sometimes produce Keratitis, penicillosis, otomycosis and rarely deep infections.

Morphology: The saprophytic fungi contains septate mycelium and bears flask shaped phial ides, which in turn supports chains of round phial conidia (Fig. 33.13).

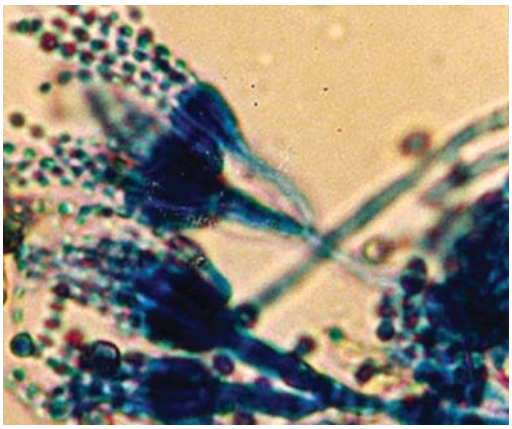


Fig. 33.13: Penicillium in lactophenol stain.
Culture on SDA shows velvety blue green colonies

RHINOSPORIDIOSIS

Rhinosporidiosis is a chronic granulomatous disease caused by agent Rhinisporidium seeberi.

Clinical Features

Rhinosporidiosis is characterized by formation of friable pedunculated or sessile polyp or wart-like lesions in the nasal or nasopharyngeal mucosa, and less often on the conjunctiva of the eye. Rarely other mucosal sites may be affected.

The mode of infection and transmission are not known but most infections occur in males with frequent contact with stagnant water or aquatic life.

Diagnosis

The fungus has not been cultivated. Laboratory diagnosis is made by demonstration of sporangia in tissues (HPE)

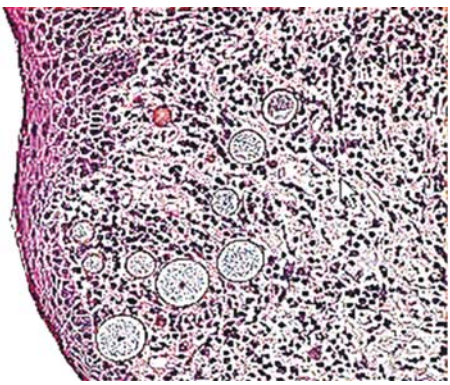


Fig. 33.14: Histopathology showing sporangiospores

DIMORPHIC FUNGI

Dimorphic fungi are fungi that grow as filamentous mould at 25 degree centigrade and transform to unicellular yeast in tissue and culture at 37 degree centigrade.

There are five types of dimorphic fungi:

1. Histoplasma capsulatum
2. Blastomyces dermatitidis
3. Coccidioides immitis
4. Paracoccidioides braziliensis
5. Cryptococcus neoformans

HISTOPLASMA CAPSULATUM

Etiologic Agent

Histoplasma capsulatum is a dimorphic fungus that grows as a mold in nature or on Sabouraud's agar at room temperature (Fig. 33.15). Hyphae bear both large and small spores, which are used for identification. Nucleic acid hybridization can also be used to identify the organism in culture. *H. capsulatum* grows as a small budding yeast in host tissue and on enriched agar, such as blood cysteine glucose, at 37°C. Despite its name, the fungus is unencapsulated. Coculture of isolates with opposite mating types can produce different sporulating structures in which genetic recombination occurs. When these structures, referred to as a *teleomorph* or the *perfect state*, are seen in culture, the name *Ajellomyces capsulatus* is used.

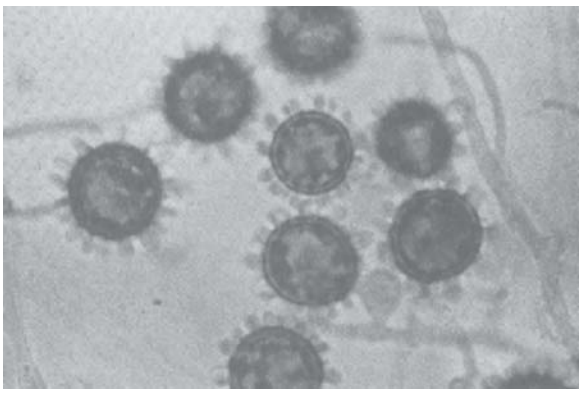


Fig. 33.15: Histoplasma capsulatum

Infection with *H. capsulatum* has been encountered in many areas of the world but is much more frequent in certain areas. Within the United States, infection is most common in the southeastern, mid-Atlantic, and central states.

Pathogenesis

Microconidia, or small spores, of *H. capsulatum* are small enough to reach the alveoli on inhalation and are transformed there to budding forms. With time, an intense granulomatous reaction occurs. Caseation necrosis or calcification may mimic tuberculosis. In children, the primary infection usually heals completely but may leave spotty calcification in the hilar nodes or lung. Transient dissemination may leave calcified granulomas in the spleen. In adults, a rounded mass of scar tissue, with or without central calcification, may remain in the lung. This mass has been called a histoplasmoma. Previous exposure is thought to confer some protection against reinfection, but infection in persons with prior positive skin tests clearly has occurred.

Clinical Manifestations

The vast majority of infections are either asymptomatic or mild, and the diagnosis is elusive. Cough, fever, malaise, and chest X-ray findings of hilar adenopathy with or without one or more areas of pneumonitis are typical features. Erythema nodosum and erythema multiforme have been reported in a few outbreaks.

Chronic pulmonary histoplasmosis is characterized by a gradual onset (over weeks or months) of increasing productive cough, weight loss, and sometimes night sweats. Chest X-ray reveals uni- or bilateral fibronodular apical infiltrates.

Diagnosis

Culture of the etiologic organism is the preferred method for diagnosis of histoplasmosis but is often difficult. Blood cultures are best done by the lysis-centrifugation technique, with plates held at 30°C for at least 2 weeks. Approximately 15 mL of blood should be cultured. Diagnosis based on Giemsa-stained smears of blood or bronchoalveolar lavage fluid or on methenamine silver staining of infected tissue. Neither skin testing nor serology has been predictive of histoplasmosis in patients infected with HIV.

Treatment

Itraconazole (200 mg/d), Fluconazole, Ketoconazole (400 to 800 mg once daily) and amphotericin B (0.6 mg/kg daily).

BLASTOMYCOSIS

Blastomyces dermatitidis is a dimorphic fungus that grows at room temperature as a white or tan mold but grows within the host or at 37°C as budding, round yeastlike cells. The fungus can be identified on the basis of its appearance, its dimorphism, the small spores borne on hyphae of the mold form, or the results

of nucleic acid hybridization. When isolates of the two opposite mating types are grown close together on special culture medium, such as yeast extract or soil extract agar, sporulating structures that characterize the perfect state (teleomorph), called *Ajellomyces dermatitidis*, appear.

Pathogenesis

Infection with B. *dermatitidis* appears to be acquired by inhalation of the fungus from soil, decomposed vegetation, or rotting wood. Several case clusters have resulted from participation in recreational activities in wooded areas along waterways. Infection is not transmissible from person to person. The initial pulmonary infection may either heal spontaneously or become chronic. Spread to other portions of the lung, cavitation, or endobronchial lesions may be found in patients with chronic disease.

Clinical Manifestations

A few patients have acute, self-limited pneumonia. Fever, productive cough, myalgia, and malaise usually resolve within a month. Pulmonary infiltrates clear slowly as *B. dermatitidis* disappears from the sputum.

In the vast majority of patients, blastomycosis has an indolent onset and a chronically progressive course. Fever, cough, weight loss, lassitude, skin lesions, and chest ache are common. Skin lesions favor exposed areas and enlarge over many weeks from pimples to well-circumscribed, verrucous, crusted, or ulcerated lesions. Pain and regional lymphadenopathy are minimal. Large chronic lesions may undergo central healing with scarring and contracture. Mucous membrane lesions resemble squamous cell carcinoma. Chest X-ray findings are abnormal in two-thirds of patients, with one or more pneumonic or nodular infiltrates. Calcification, hilar adenopathy, and large pleural effusions are rare. Osteolytic lesions may be found in nearly any bone and present as a cold abscess or a draining sinus. Extension to a contiguous joint may cause indolent swelling, pain, and restricted motion. Prostatic and epididymal lesions clinically resemble those of tuberculosis.

Diagnosis

The diagnosis of blastomycosis is made by demonstration of the fungus in a culture of sputum, pus, or urine. An expert can diagnose blastomycosis on the basis of the appearance of the organism in wet smear or histopathologic section. The fungus may be visible in a sputum cytology smear but is easily overlooked.

Treatment

Intravenous amphotericin B, itraconazole (200 mg twice daily with food).

COCCIDIOIDOMYCOSIS

Coccidioides immitis has two forms, growing as a white fluffy mold on most culture media but as a non-budding spherical form (a spherule) in host tissue or under special condition. The organism reproduces in host tissue by forming small endospores within mature spherules. After rupture of the spherule, the released endospores enlarge, become spherules, and repeat the cycle. The fungus is identified by its appearance and by the formation of thick-walled, barrel-shaped spores, called *arthrospores*, in the hyphae of the mold form (Fig. 33.16).

Pathogenesis

C. immitis is a soil saprophyte found in certain arid regions of the United States, Mexico, Central America, and South America. Within the United States, most cases of infection with *C. immitis* are acquired in California, Arizona, and western Texas. A few cases are acquired by exposure to fomites from endemic areas (e.g. in cotton bales).

Infection in humans and animals results from inhalation of wind-borne arthrospores from soil sites. This primary pulmonary infection is symptomatic in only 40% of cases, with symptoms ranging from a mild influenza-like illness to severe pneumonia. Mild self-limited infections may come to medical attention because of case clusters or hypersensitivity reactions: erythema nodosum, erythema multiforme, toxic erythema, arthralgia, arthritis, conjunctivitis, or episcleritis.

Pleural effusion may be the only manifestation of primary infection. Spontaneous healing of this form is common.

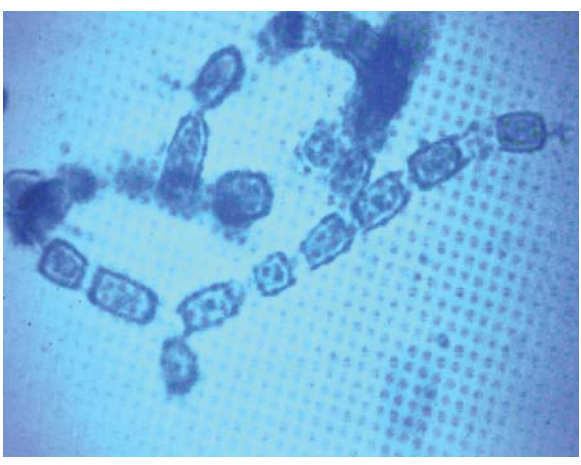


Fig. 33.16: Arthospores of *Coccidioides immitis*

Clinical Manifestations

Symptomatic primary pulmonary infection is manifested by fever, cough, chest pain, malaise, and sometimes the hypersensitivity reactions listed above. Chest radiographs may show an infiltrate, hilar adenopathy, or pleural effusion. Mild peripheral-blood eosinophilia may be found. Spontaneous improvement begins after several days to 2 weeks of illness and usually culminates in complete recovery.

The symptoms of a chronic thin-walled cavity include cough or hemoptysis in half of cases; the other half are asymptomatic. Chronic progressive pulmonary coccidioidomycosis causes cough, sputum production, variable degrees of fever, and weight loss.

Diagnosis

When coccidioidomycosis is suspected, sputum, urine, and pus should be examined for *C. immitis* by wet smear and culture. The laboratory request should indicate clearly that coccidioidomycosis is suspected, because the mold form must be handled with extreme care to prevent infection of laboratory personnel. On biopsy, smaller spherules must be distinguished from nonbudding forms of *Blastomyces* and *Cryptococcus*, but the appearance of the mature spherule is diagnostic.

Serologic tests are very helpful in the diagnosis of coccidioidomycosis. Latex agglutination and agar gel diffusion tests are useful in screening sera for antibody to Coccidioides. The complement fixation test is used for CSF determinations and for the confirmation and quantitation of serum antibody detected by screening tests.

Treatment

Amphotericin B or itraconazole can be used to treat the infection.

CRYPTOCOCCOSIS

Etiologic Agent

Cryptococcosis is an infection caused by the yeastlike fungus *Cryptococcus neoformans*. This fungus reproduces by budding and forms round, yeastlike cells. Within the host and on certain culture media, a large polysaccharide capsule surrounds each yeast cell. The fungus grows well in smooth, creamy-white colonies on Sabouraud's or other simple media at 20 to 37°C. Identification of the organism is based on gross and microscopic appearance, biochemical test results, and growth at 37°C. The results of nucleic acid hybridization or the formation of brown pigment on Niger seed agar can also be used for identification.

The fungus has four capsular serotypes, designated A, B, C, and D. There are also two mating types. Coculture of opposite mating types creates a transient diploid state called Filobasidiella neoformans var. neoformans for serotypes A and D and F. neoformans var. bacillispora for serotypes B and C. Organisms not cultured under mating conditions are designated C. neoformans var. neoformans for serotypes A and D and C. neoformans var. gattii for serotypes B and C; a simple color medium distinguishes the two varieties.

Pathogenesis and Pathology

Infection is thought to be acquired by inhalation of fungus into the lungs. Pulmonary infection has a tendency toward spontaneous resolution and is frequently asymptomatic. Silent hematogenous spread to the brain leads to clusters of cryptococci in the perivascular areas of cortical gray matter, in the basal ganglia, and, to a lesser extent, in other areas of the central nervous system. The inflammatory response around these foci is usually scant. In the more chronic cases, a dense basilar arachnoiditis is typical. Lung lesions are characterized by intense granulomatous inflammation. Cryptococci are best seen in tissue by staining with methenamine silver or periodic acid-Schiff. Although a strongly positive result on mucicarmine staining of tissue is diagnostic, staining varies from intense to absent.

Clinical Manifestations

Most patients have meningoencephalitis at the time of diagnosis. This form of the infection is invariably fatal without appropriate therapy; death occurs any time from 2 weeks to several years after the onset of symptoms. Early manifestations include headache, nausea, staggering gait, dementia, irritability, confusion, and blurred vision. Both fever and nuchal rigidity are often mild or lacking. Papilledema is evident in one-third of cases at the time of diagnosis. Cranial nerve palsies, typically asymmetric, occur in about one-fourth of cases.

Pulmonary cryptococcosis causes chest pain in about 40% of patients and cough in 20%. The chest X-ray shows one or more dense infiltrates, which are often well circumscribed. Cavitation, pleural effusions, and hilar adenopathy are infrequent. Calcification is not evident, and fibrotic stranding is rarely noticeable.

Diagnosis

An india ink smear of centrifuged cerebrospinal fluid (CSF) sediment reveals encapsulated yeast in more than half of cases, although artifacts can cause confusion.

Culture done on Saborauds dextrose medium.

Treatment

Ampotericin B, Fluconazole are effective.

PARASITOLOGY

The major groups of parasites infecting human beings are Protozoa and Helminths. A relationship between two species; where one species (parasite) derives food and shelter from another species (Host).

Hosts and parasites share a dynamic relationship. Generally, parasites cannot exist independently. They generally inflict injury, affecting the wellbeing of the host.

Intestinal Parasites

Most helminths and protozoa exit the body in the faecal strain. The patient or the patient's attendant should be instructed to collect faeces in a clean cardboard container (now disposable plastic containers are available) and to record the time of collection on the container.

Intestinal Nematodes

Nematodes belong to the Phylum Nematoda. They are non-separated, cylindrical worm that taper both the ends. They possess a shiny, tough, acellular, hyaline cuticle (skin) which may be smooth, spiral or ridged. Digestive system is complete. It consists of mouth, esophagus, intestine and anus. Sexes are separate (diecious). Male is smaller than female and its posterior end is curved ventrally. Both possess reproductive system. Females are either vivi-parous (produce larvae), oviparous (lay eggs) or ovo- viviparous (lay eggs which hatch immediately).

Ascaris Lumbricoides

It is the commonest intestinal nematode of human. It exists in 2 forms—Adult and larvae. Adult round worms are the largest intestinal nematodes. Freshly expelled worms are pink and cylindrical. They may measure upto 40 cm in length and have tapering ends. The anterior end when examined with a hand lens, shows a mouth surrounded by three finely toothed lips. The adult worm lives for 1 to 2 years (Fig. 33.18).

Adult male measures 15-30 cm in length and 2-4 mm in diameter.The posterior end is curved and has 2 copulatory spicules.

Adult female measures 20-40 cm in length and have a diameter of 3-6.

Their posterior end is straight and conical. The vulva lies at the junction of anterior and middle thirds of the body. This part of the worm is narrow and called vulvar waist. A lumbricoides females are oviparous. The mature female produce upto 2 lakh eggs a day which passes with the faeces. The size of the egg is 60 mm × 40 mm. They may be fertilized or unfertilized.

They are bile stained. The ovum is at the center of the fertilized egg having 2 clear spaces both sides. The egg shell is curved with albuminous coat called cortications. Eggs are resistant to drying but can be killed by sunlight.

Fertilized eggs develop[p in soil over 30 - 40 days and infect the human. Unfertilised eggs measure about 90 µm × 45 µm and are bile stained having an atrophic ovum.

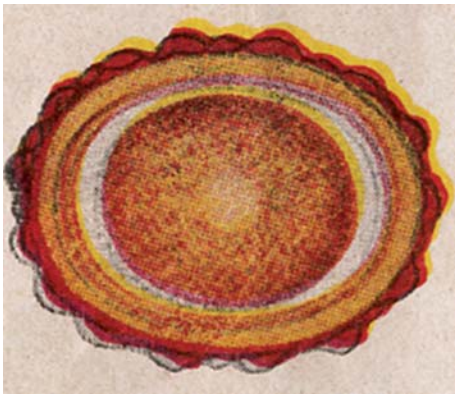


Fig. 33.17: Egg of *A. lumbricoides*

Life Cycle

Humans are the only host to complete its life cycle. The infection form is the fully developed egg containing a larva (Embryonated eggs). Eggs hatch in small intestine to release larvae. These mobile larvae penetrate mucosa to inter-portal circulation and reach liver. After 3-4 days these larvae enter systemic circulation to reach the lungs. After 10-14 days of development in lungs, these larvae penetrate through the capillaries and enter alveoli. From here the larvae ascend the bronchial tree, trachea, larynx and pharynx. Larvae then crawl over epiglottis are swallowed to the GI system . They finally reach small intestine and grow into adult worm in 6-10 weeks. After mating, the female lays fertilized eggs and thus the cycle is repeated.

Fig. 33.18: Adult worm of *A. lumbricoides*

Pathogenicity and Clinical Features

The disease is called ascariasis. The clinical signs and symptoms are both due to the adult worm and the migratory larvae. Due to migration of larvae to the lungs, patients develop cough (non-productive) and dyspnea. There may be hemoptysis. Fever, Abdominal pain and urticaria develop in most of the patients. The larvae induce an eosinophilia response which is referred as Loeffler's pneumonia.

The adult worm may be asymptomatic when present in large numbers especially in children. A lumbricoides interferes with absorption of food. This may contribute to protein energy malnutrition and vitamin A deficiency. Sometimes they even produce life-threatening emergencies causing industrial obstruction. They may even produce appendicitis, cholecystitis, pancreatic and liver absesses. They can cause peritonitis, if they penetrate a typhoid ulcer. Adult worm may reach the esophogus and larynx and can cause asphyxia. Rarely, hypersensitivity to the antigen may occur. Fever, urticaria, angio-neurotic edema, wheezing, and conjunctivitis can be seen in such patients.

Lab Diagnosis

Demonstration of Eggs.
Adult worm.
Peripheral smear for Eosinophilia.
X- ray, Barium study and USG.

Treatment

Mebendazole and Albendazole are effective. Pyrantel Palmoate, Piperazine Citrate are also effective.

Prevention

Proper sanitation, Washing of hands before eating food, Avoiding consumption of uncooked vegetables and fruits grown in soil.

ENTEROBIOUS VERMICULARIS

Enterobious vermicularis:- (Other names: Threadworm, Pinworm)
E. vermicularis is distributed worldwide. It is more prevalent in temperate climate.

Characteristic Features

E.vermicularis exists in two forms—adult and larvae. Adults habitate in Appendix, Caecum. The male worm measures 2 to 5 mm × 0.1 to 0.2 mm. It has a coiled tail with a single spicule. The female worm measures

8 × 13 mm × 0.3 to 0.5 mm. The posterior thread of its body is pointed like a pin. The gravid worm has egg filled uteri occupying the entire body. The female E. Vermicularis worm in oviparous. Eggs are colorless (not bile stained). They are Plano-convex and measure about 55 µm × 30 µm. Eggs contain a larva.

Life Cycle

It has a simple life cycle. Humans are the only host. The infective form of E.Vermicularis is the embryonated egg. Transmission is faeco- oral. Eggs hatch in the intestine to release the larvae. The larvae develops into mature forms. The male fertilized female and dies. The female worm migrate around the anal region and lays upto 10,000 eggs. The larvae present in the eggs develop fully in 6 hours. Such mature eggs are infertile. E. vermicularis eggs can reinfect the same host (autoinfection) or infect a different host (Fig. 33.19). They migrate through the anus upto caecum to develop them.

The eggs on the perianal region contaminate the under garments and the bedding. These contaminated clothes can transmit the infection. The life cycle of *E. Vermicularis* completes in one month.

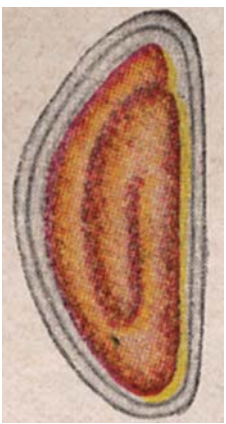


Fig. 33.19: Egg of *E. vermicularis*

Clinical Features

Perianal pruritis is the characteristic feature of Enterobiosis. Itching is worst at night. Excoriation and secondary bacterial infection may occur. In females they cause vulvovaginitis, salphingitis and pelvic or peritoneal granuloma. Another surgical complication of pin worm is appendicitis (acute or chronic).

Laboratory Diagnosis

1. Demonstration of eggs or adult worms in faeces.
2. Perianal swab can be used for demonstration of eggs.

3. Eggs can otherwise be demonstrated by applying transparent cellophane tape in the perianal region which is subjected for microscopy.
4. NIH (National Institute of Health) swab can be used for screening the eggs.

Treatment

Mebendazole, Pyrantal palmoate.
Household contacts should be screened and treated as well.

ANCYLOSTOMA DUODENALE

Ancylostoma Duodenale

Characteristic features: A. duodenale exist in 2 forms. Adult and Larval forms. Adults live in the small intestine (jejunum). They are reddish brown in color. A.duodenale is curved with concavity on the dorsal aspect. The anterior end is bent dorsally, hence the name Hookworm. The mouth of A.duodenale has 4 pointed teeth (2 on the dorsal side and 2 on the ventral side. Life span of an adult worm is about 6-8 years. The adult male measures 8 × 0.4 mm. Its posterior end has a conspicuous umbrella like copulatory bursa having 2 copulatory spicules. The bursa has 3 membranous lobes. These lobes supported by fleshy layers. The adult female measures 10 × 0.6 mm. The vulva opens ventrally at the junction of the middle and posterior thirds of the body. The female worm is oviparous and lays 10,000 eggs to 20,000 eggs per day that pass along the faeces. Eggs are oval and measure 60 × 40 Micro meters. They are colorless (not bile-stained) with a thin transparent hyaline shell membrane (Fig. 33.20). The ovum present inside is usually segmented with 4 blatomers. Freshly passed eggs are not infectious. Further development of eggs occurs in soil. These eggs hatch to produce rhabditiform larvae. The rhabditiform larvae develop into filariform larvae which is the infective form.

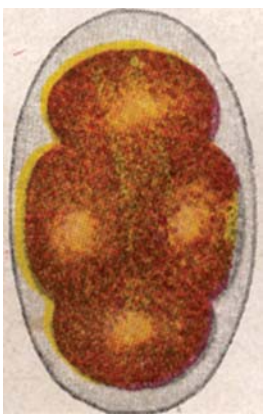


Fig. 33.20: Egg of *A. duodenale*

Life Cycle

Human is the only host. The infective filariform larvae enters human by penetrating the skin especially in bear footed walkers.They invade the blood stream to reach lungs. In lungs they penetrate through the capillaries to reach alveoli. From here the larvae ascend the bronchial tree, trachea, larynx and pharynx. Then they crawl over the epiglottis and are swallowed with saliva. On reaching jejunum, they attach to the mucosa and grow into adults in 3-4 weeks. The eggs are passed in the feaces after 3-4 weeks and develop in the soil. Rhabditiform larvae hatch out from the eggs in about 48 hours. They moult twice to develop into the infection filariform larvae to complete the cycle.

Clinical Features

A duodenale cause ancyclostomiasis or hookworm diseases. Most hookworm infections are asymptomatic. Symptomatic infections are either due to larvae or adults. Larvae produce maculo-papular dermatitis (ground itch) at the site of the skin penetration. Larvae migrating through the lungs can sometime produce pneumonitis. This pulmonary disease (loeffler pneumonia) is rarely seen in hookworm disease. Adult worm may produce epigastric pain, diarrhoea and vomiting.

The most important and serious manifestation of hookworm infestation is microcytic hyphochromic anaemia (Iron deficiency anaemia). The adult worm sucks blood at the site of attachment in the intestine. A single worm sucks about 0.2 ml blood per day. Development of virus deficiency anaemia is related to the chronicity of the infections. Malnourished individuals also have hypoproteinemia.

Laboratory Diagnosis

1. Finding the eggs in the faeces.
2. Stool concentration methods like Format Ether and Zinc Floatation techniques can be used.
3. Peripheral smear and hemoglobin assay.

Treatment

Mebandazole, Pyrantel palmoate or albendazole can be used.

Supplemental Iron to correct anemia.

Prevention and Control

Prevention of soil pollution with human faces. Adequate foot wear.

TRICHIURIS TRICHIURA (WHIPWORM)

This worm is moist and warm climates.

Characteristic Features

It exists in 2 forms: adult and larvae. The adults live in caecum and appendix of human like E. vermicularis.

They are pinkish white and have a whip like shape. Hence called as whip worm. The anterior three - fifths is thin, elongated and coiled. The posterior two - fifths is thick and fleshly resembling the handle of a whip. The adult worm lives for several years. The male is 30-40mm long. Its posterior end is coiled ventrally and has a simple sheathed spicule. Adult female is 40- 50 mm long. The posterior end is blunt and round. The female worm is oviparous. They lay about 5000 eggs per day. The eggs are oval shaped and measure 50 mm × 25 mm in size (Fig. 33.21). Mucous plugs project from both the poles. The eggs are bile stained. When passed in faeces the eggs contain unsegmented ovum. At this stage they are not infertile to human. Development of eggs occur in soil. The infertile larvae develops within the egg in 3 - 4 weeks.

Fig. 33.21: Egg of *Trichiuris trichiura*

Life Cycle

Man is the only host. The infective form is the egg containing the larvae. Man gets infection by ingesting the embryonated eggs along with food and water. The eggs hatch in small intestine and grow into adult worm. They migrated to caecum and appendix. The fertilized female lays eggs that pass along with the faeces. The entire process of acquiring infection to passage of eggs takes 3 months.

Clinical Features

T. trichiura causes Trichiuriasis. Infections may result in abdominal pain, bloody diarrhea. T.trichiura can cause anemia, malnutrition and growth retardation. Appendicitis and rectal prolapse may occur rarely.

Laboratory Diagnosis

Demonstration of characteristic Eggs with mucous plugs on both poles
Demonstration of adult worm in faeces.

Treatment

Mebandazole or Albendazole.

STRONGYLOIDS STERCORALIS

S stercoralis infections seen world wide.

Characteristic Features

Strongyloids stercoralis exists in adult and larval form. Adult males are not demonstrated in the humans because they are eliminated from bowel by the time females live in the mucosa of the small intestine. They measure 2.5 mm × 0.04 to 0.05 mm. The female S. stercoralis worm is ovo- viviparous. The eggs have a thin shell and measure about 55µm × 30µm. The eggs are laid in the tissues. A rhabditiform larvae hatches out immediately and move to the lumen of the intestine and passed with the faeces. They measure about 200 × 300 µm × 15µm. They are actively motile.

Life Cycle

Humans acquire Strongyloids following contact with faecally contaminated soil or by autoinfection. The infective form are the filariform larvae that penetrate intact skin or mucous membranes. After penetration, the larvae travel through the blood stream to the lungs, where they break into alveoli, ascend the bronchial tree, crawl over the epiglottis and are swallowed to reach the small intestine. Female lays eggs and the eggs hatch within the intestinal mucosa to release rhabdtiform larvae either passed with the faeces to the soil or develop into filariform larvae lead to auto infection by penetrating the mucosa or the perianal skin.

Rhabditiform larvae that have reached the soil can develop into 2 ways. They can mature into infective filariform larvae or develop into free living adult males and females.

The male fertilizes the female and eggs are laid in the soil. Rhabditiform larvae develop into filariform larvae and thus the cycle is repeated.

Clinical Features

Most cases of Strongyloidiasis are asymptomatic. Recurrent utricaria is the most common cutaneous manifestation. Migrating larvae (larva currens) produces a pruritic, raised, erythematous lessin that advances along the course of migration. Migrating larvae also produce hemorrhagic lesions in the lungs.

Adult parasites produce epigastric pain, nausea, diarrhea, gastrointestinal bleeding, colitis and Eosinophilia. In Immuno suppressed patients, pregnant women and malnourished children.

There will be hyperinfections with S. Stercoralis. The larvae may invade CNS, peritoneum, liver and kidney.

Septicemia, pneumonia or manifestation may result from secondary bacterial infections.

Strongyloids Fulebornis

It causes swollen belly syndrome in children.

Laboratory Diagnosis

1. Demonstration of rhabditiform larvae in faeces is diagnostic.
2. Analysing the duodeno-jejunal contents broncho-alveolar aspirates.
3. Biopsy, ELISA to detect antibodies.

Treatment

Thiabendazole

Prevention

Wearing foot wear.

CESTODES (TAPEWORMS)

Cestodes, or tapeworms, are segmented worms (Fig. 33.22). The adults reside in the gastrointestinal tract, but the larvae can be found in almost any organ. Human tapeworm infections can be divided into two major clinical groups. In

Fig. 33.22: Tapeworm (Tinea)

one group, humans are the definitive hosts, and the adult tapeworms live in the gastrointestinal tract (Taenia saginata, Diphyllobothrium, Hymenolepis, and Dipylidium caninum). In the other, humans are intermediate hosts, and larval-stage parasites are present in the tissues. Diseases in this category include echinococcosis, sparganosis, and coenurosis. For T. solium, the human may be either the definitive or the intermediate host.

The ribbon-shaped tapeworm attaches to the intestinal mucosa by means of sucking cups or grooves located on the head (scolex). Behind the scolex is a short, narrow neck from which proglottids (segments) form. As each proglottid matures, it is displaced further back from the neck by the formation of new, less mature segments. The progressively elongating chain of attached proglottids, called the strobila, constitutes the bulk of the tapeworm. The length varies among species. In some, the tapeworm may consist of more than 1000 proglottids and may be several meters long. As each proglottid becomes gravid, eggs are released. Since eggs of the different Taenia species are morphologically identical, differences in the morphology of the scolex or proglottids provide the basis for diagnostic identification to the species level. Most human tapeworms require at least one intermediate host for complete larval development. After ingestion by an intermediate host, an egg releases the larval oncosphere, which penetrates the intestinal mucosa. The oncosphere migrates to tissues and develops into an encysted form known as a cysticercus (single scolex), a coenurus (multiple scolices), or a hydatid (cyst with daughter cysts, each containing several protoscolices). Ingestion by the definitive host of tissues containing a cyst enables a scolex to develop into a tapeworm.

TAENIASIS SAGINATA

The beef tapeworm T. saginata occurs in all countries where raw or undercooked beef is eaten. It is most prevalent in sub-Saharan African and Middle Eastern countries.

Pathogenesis

Humans are the only definitive host for the adult stage of T. saginata. This tapeworm, which can reach 8 m in length, inhabits the upper jejunum and has a scolex with four prominent suckers and 1000 to 2000 proglottids. Each gravid segment has 15 to 30 uterine branches (in contrast to 8 to 12 for T. solium). The eggs are indistinguishable from those of T. solium; each measures 30 to 40 um and has a thick brown striated shell containing the embryo. Eggs deposited on vegetation can live for months to years until they are ingested by cattle or other herbivores. The embryo released after ingestion invades the intestinal wall and is carried to striated muscle, where it transforms into a cysticercus. When ingested in raw or undercooked beef, this form can infect humans. After the cysticercus is ingested, it takes about 2 months for an adult worm to develop.

Clinical Manifestations

Patients become aware of the infection most commonly by noting passage of proglottids in their feces. The proglottids are often motile, and patients may experience perianal discomfort when proglottids are discharged. Mild abdominal pain or discomfort, nausea, change in appetite, weakness, and weight loss can occur with T. saginata infection.

Diagnosis

The diagnosis is made by the detection of eggs or proglottids in the stool. Eggs may also be present in the perianal area; thus, if proglottids or eggs are not found in the stool, the perianal region should be examined with use of a cellophane-tape swab (as in pinworm infection). Distinguishing T. saginata from T. solium requires examination of mature proglottids or the scolex. Serologic tests are not helpful diagnostically. Eosinophilia and elevated levels of serum IgE may be detected.

Treatment

A single dose of praziquantel (5 to 10 mg/kg) is highly effective.

Prevention

The major method of preventing infection is the adequate cooking of beef; exposure to temperatures as low as 56°C for 5 min will destroy cysticerci. Refrigeration or salting for long periods or freezing at -10°C for 9 days also kills cysticerci in beef. General preventive measures include inspection of beef and proper disposal of human feces.

TAENIASIS SOLIUM AND CYSTICERCOSIS

The pork tapeworm *T solium* can cause two distinct forms of infection. The form that develops depends on whether humans are infected with adult tapeworms in the intestine or with larval forms in the tissues (cysticercosis). Humans are the only definitive hosts for *T solium*; pigs are the usual intermediate hosts, although dogs, cats, and sheep may harbor the larval forms. *T solium* exists worldwide but is most prevalent in Latin America, Africa, South and Southeast Asia, and eastern Europe. Cysticercosis occurs in industrialized nations largely as a result of the immigration of infected persons from endemic areas.

Pathogenesis

The adult tapeworm generally resides in the upper jejunum. Its globular scolex attaches by both sucking disks and two rows of hooklets. Often only one adult

worm is present, but that worm may live for years. The tapeworm, usually about 3 m in length, may have as many as 1000 proglottids, each of which produces up to 50,000 eggs. Groups of three to five proglottids are generally released and excreted into the feces, and the eggs in these proglottids are infective for both humans and animals. The eggs may survive in the environment for several months. After ingestion by the intermediate host, eggs embryonate, penetrate the intestinal wall, and are carried to many tissues, with a predilection for striated muscle of the neck, tongue, and trunk. Within 60 to 90 days, the encysted larval stage develops. These cysticerci can survive for long periods. Humans acquire infections that lead to intestinal tapeworms by ingesting undercooked pork containing cysticerci. Infections that cause human cysticercosis follow the ingestion of *T. solium* eggs, usually from fecally contaminated food. Autoinfection may occur if an individual with an egg-producing tapeworm ingests eggs derived from his or her own feces.

Clinical Manifestations

Intestinal infections with *T. solium* may be asymptomatic. Epigastric discomfort, nausea, a sensation of hunger, weight loss, and diarrhea are infrequent. Fecal passage of proglottids may be noted by patients.

In cysticercosis, the clinical manifestations are entirely different. Cysticerci can be found anywhere in the body, most commonly in the brain and the skeletal muscle. The clinical presentation of cysticercosis depends on the number and location of cysticerci as well as the extent of associated inflammatory responses or scarring. Neurologic manifestations are the most common. When inflammation surrounds cysticerci in the brain parenchyma, seizures are frequent. These seizures may be generalized, focal, or Jacksonian. Hydrocephalus results from obstruction of cerebrospinal fluid (CSF) flow by cysticerci and accompanying inflammation or by CSF outflow obstruction from arachnoiditis. Signs of increased intracranial pressure, including headache, nausea, vomiting, changes in vision, dizziness, ataxia, or confusion, are often evident. Patients with hydrocephalus may develop papilledema or display altered mental status. When cysticerci develop at the base of the brain or in the subarachnoid space, they cause chronic meningitis or arachnoiditis, communicating hydrocephalus, or strokes.

Diagnosis

The diagnosis of intestinal *T. solium* infection is made by the detection of eggs or proglottids, as described for *T. saginata*. In cysticercosis, diagnosis can be difficult. Diagnosis is made on the basis of a combination of clinical presentation, radiographic studies, serologic tests, and exposure history.

Treatment

Praziquantel (5 to 10 mg/kg).

ECHINOCOCCOSIS

Echinococcosis is an infection of humans caused by the larval stage of *Echinococcus granulosus (Fig. 33.23)*, *E. multilocularis*, or *E. vogeli*. Like other cestodes, echinococcal species have both intermediate and definitive hosts. The definitive hosts are dogs that pass eggs in their feces. Cysts develop in the intermediate hosts—sheep, cattle, humans, goats, camels, and horses for E. granulosus and mice and other rodents for *E. multilocularis*—after the ingestion of eggs. When a dog ingests beef or lamb containing cysts, the life cycle is completed.

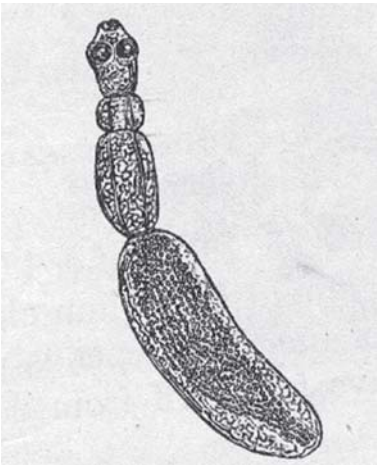


Fig. 33.23: *Echinococcus granulosus*

The small (5 mm long) adult *E. granulosus* worm, which lives for 5 to 20 months in the jejunum of dogs, has only three proglottids—one immature, one mature, and one gravid. The gravid segment splits to release eggs that are morphologically similar to Taenia eggs and are extremely hardy. After humans ingest the eggs, embryos escape from the eggs, penetrate the intestinal mucosa, enter the portal circulation, and are carried to various organs, most commonly the liver and lungs. Larvae develop into fluid-filled unilocular hydatid cysts that consist of an external membrane and an inner germinal layer. Daughter cysts develop from the inner aspect of the germinal layer, as do germinating cystic structures called brood capsules. New larvae, called protoscolices, develop in large numbers within the brood capsule. The cysts expand slowly over a period of years.

The life cycle of *E. multilocularis* is similar except that small rodents serve as the intermediate hosts. The cyst of *E. multilocularis*, however, is quite different in that the larval form remains in the proliferative phase, the hydatid cyst is always multilocular, and vesicles progressively invade the host tissue by peripheral extension of processes from the germinal layer (Fig. 33.24).

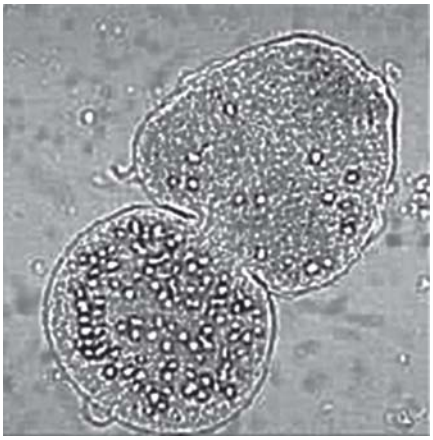


Fig. 33.24: Hydatid cyst

Clinical Manifestations

Slowly enlarging echinococcal cysts generally remain asymptomatic until their expanding size or their space-occupying effect in an involved organ elicits symptoms. The liver and the lungs are the most common sites of these cysts. Since a period of years elapses before cysts enlarge sufficiently to cause symptoms, they may be discovered incidentally on a routine X-ray or ultrasound study.

Patients with hepatic echinococcosis who are symptomatic most often present with abdominal pain or a palpable mass in the right upper quadrant. Compression of a bile duct or leakage of cyst fluid into the biliary tree may mimic recurrent cholelithiasis, and biliary obstruction can result in jaundice. Rupture of or episodic leakage from a hydatid cyst may produce fever, pruritus, urticaria, eosinophilia, or anaphylaxis. Pulmonary hydatid cysts may rupture into the bronchial tree or peritoneal cavity and produce cough, chest pain, or hemoptysis. Rupture of hydatid cysts may lead to multifocal dissemination of protoscolices, which can form additional cysts. Rupture can occur spontaneously or at surgery. Other presentations are due to the involvement of bone (invasion of the medullary cavity with slow bone erosion producing pathologic fractures), the central nervous system (space-occupying lesions), and the heart (conduction defects, pericarditis).

Diagnosis

Radiographic and related imaging studies are important in detecting and evaluating echinococcal cysts.

A specific diagnosis can be made by the examination of aspirated fluids for scoliceal hooklets, but diagnostic aspiration is not usually recommended because of the risk of fluid leakage resulting in either dissemination of infection or anaphylactic reactions. Serodiagnostic assays can be useful, although a negative test does not exclude the diagnosis of echinococcosis. Cysts in the liver elicit positive antibody responses in ~90% of cases, whereas up to 50% of individuals with cysts in the lungs are seronegative. Detection of antibody to specific echinococcal antigens by immunoblotting has the highest degree of specificity.

Casonis skin test

Treatment

Praziquantal

FILARIASIS

Filarial worms are nematodes that dwell in the subcutaneous tissues and the lymphatics. Eight filarial species infect humans; of these, four—*Wuchereria bancrofti*, *Brugia malayi*, *Onchocerca volvulus*, and *Loa loa*—are responsible for most serious filarial infections.

Usually, infection is established only with repeated and prolonged exposures to infective larvae. Since the clinical manifestations of filarial diseases develop relatively slowly, these infections should be considered chronic diseases with possible long-term debilitating effects. In terms of the nature, severity, and timing of clinical manifestations, patients with filariasis who are native to endemic areas and undergo lifelong exposure may differ significantly from those who are travelers or who have recently moved to these areas. Characteristically, the disease is more acute and intense in newly exposed individuals than in natives of endemic areas.

LYMPHATIC FILARIASIS

Lymphatic filariasis is caused by *W. bancrofti*, *B. malayi*, or *B. timori*. The threadlike adult parasites reside in lymphatic channels or lymph nodes, where they may remain viable for more than two decades.

Clinical Features

The most common presentations of the lymphatic filariases are asymptomatic (or subclinical) microfilaremia, hydrocele, acute adenolymphangitis (ADL),

and chronic lymphatic disease. In areas where *W. bancrofti* or *B. malayi* is endemic, the overwhelming majority of infected individuals have few overt clinical manifestations of filarial infection despite large numbers of circulating microfilariae in the peripheral blood. Although they may be clinically asymptomatic, virtually all persons with *W. bancrofti* or *B. malayi* microfilaremia have some degree of subclinical disease that includes microscopic hematuria and/or proteinuria, dilated (and tortuous) lymphatics (visualized by imaging), and—in men—scrotal lymphangiectasia (detectable by ultrasound). Despite these findings, the majority of individuals appear to remain clinically asymptomatic for years; relatively few progress to the acute and chronic stages of infection.

ADL is characterized by high fever, lymphatic inflammation (lymphangitis and lymphadenitis), and transient local edema. The lymphangitis is retrograde, extending peripherally from the lymph node draining the area where the adult parasites reside. Regional lymph nodes are often enlarged, and the entire lymphatic channel can become indurated and inflamed. Concomitant local thrombophlebitis can occur as well in the interdigital area, are common. DLA is often diagnosed as cellulitis.

Diagnosis

A definitive diagnosis can be made only by detection of the parasites and hence can be difficult. Adult worms localized in lymphatic vessels or nodes are largely inaccessible. Microfilariae can be found in blood, in hydrocele fluid, or (occasionally) in other body. Two tests are commercially available: one is an enzyme-linked immunosorbent assay (ELISA) and the other a rapid-format immunochromatographic card test. Both assays have sensitivities that range from 96 to 100% and specificities that approach 100%. There are currently no tests for circulating antigens in brugian filariasis.

Polymerase chain reaction (PCR)-based assays for DNA of *W. bancrofti* and *B. malayi* in blood have been developed. A number of studies indicate that this diagnostic method is of equivalent or greater sensitivity compared with parasitologic methods, detecting patent infection in almost all infected subjects.

Eosinophilia and elevated serum concentrations of IgE and antifilarial antibody support the diagnosis of lymphatic filariasis.

Treatment

Diethyl carbamazine is used to treat filariasis. Albendazole is found to be effective against microfilaria.

ONCHOCERCIASIS

Onchocerciasis ("river blindness") is caused by the filarial nematode O. volvulus, which infects an estimated 13 million individuals. The majority of individuals infected with O. volvulus live in the equatorial region of Africa extending from the Atlantic coast to the Red Sea. About 70,000 persons are infected in Guatemala and Mexico, with smaller foci in Venezuela, Colombia, Brazil, Ecuador, Yemen, and Saudi Arabia. Onchocerciasis is the second leading cause of infectious blindness worldwide.

Etiology

Infection in humans begins with the deposition of infective larvae on the skin by the bite of an infected blackfly. The larvae develop into adults, which are typically found in subcutaneous nodules. About 7 months to 3 years after infection, the gravid female releases microfilariae that migrate out of the nodule and throughout the tissues, concentrating in the dermis. Infection is transmitted to other persons when a female fly ingests microfilariae from the Host's skin and these microfilariae then develop into infective larvae. Adult *O. volvulus* females and males are about 40 to 60 cm and 3 to 6 cm in length, respectively. The life span of adults can be as long as 18 years, with an average of ~9 years. Because the blackfly vector breeds along free-flowing rivers and streams (particularly in rapids) and generally restricts its flight to an area within several kilometers of these breeding sites, both biting and disease transmission are most intense in these locations.

Pathology

Onchocerciasis affects primarily the skin, eyes, and lymph nodes. In contrast to that in lymphatic filariasis, the damage in onchocerciasis is elicited by microfilariae and not by adults. In the skin, there are mild but chronic inflammatory changes that can result in loss of elastic fibers, atrophy, and fibrosis. The subcutaneous nodules, or onchocercomata, consist primarily of fibrous tissues surrounding the adult worm, often with a peripheral ring of inflammatory cells. In the eye, neovascularization and corneal scarring lead to corneal opacities and blindness. Inflammation in the anterior and posterior chambers frequently results in anterior uveitis, chorioretinitis, and optic atrophy. Although punctate opacities are due to an inflammatory reaction surrounding dead or dying microfilariae, the pathogenesis of most manifestations of onchocerciasis is still unclear.

Clinical Features

Skin: Pruritus and rash are the most frequent manifestations of onchocerciasis. The pruritus can be incapacitating; the rash is typically a papular eruption that is generalized rather than localized to a particular region of the body.

Onchocercomata: These subcutaneous nodules, which can be palpable and/or visible, contain the adult worm. In African patients, they are common over the coccyx and sacrum, the trochanter of the femur, the lateral anterior crest, and other bony prominences; in Latin American patients, they tend to develop preferentially in the upper part of the body, particularly on the head, neck, and shoulders. Nodules vary in size and characteristically are firm and not tender. It has been estimated that, for every palpable nodule, there are four deeper nonpalpable ones.

Ocular tissue: Visual impairment is the most serious complication of onchocerciasis and usually affects only those persons with moderate or heavy infections. Lesions may develop in all parts of the eye. The most common early finding is conjunctivitis with photophobia. In the cornea, punctate keratitis—consisting of acute inflammatory reactions surrounding dying microfilariae manifested as "snowflake" opacities—is frequent in younger patients and resolves without apparent complications. Sclerosing keratitis occurs in 1 to 5% of infected persons and is the leading cause of onchocercal blindness in Africa. Anterior uveitis and iridocyclitis develop in ~5% of infected persons in Africa. In Latin America, complications of the anterior uveal tract (pupillary deformity) may cause secondary glaucoma. Characteristic chorioretinal lesions develop as a result of atrophy and hyperpigmentation of the retinal pigment epithelium. Constriction of the visual field and frank optic atrophy may occur.

Lymph nodes: Mild to moderate lymphadenopathy is frequent, particularly in the inguinal and femoral areas, where the enlarged nodes may hang down in response to gravity ("hanging groin"), sometimes predisposing to inguinal and femoral hernias.

Systemic manifestations: Some heavily infected individuals develop cachexia with loss of adipose tissue and muscle mass. Among adults who become blind, there is a three- to fourfold increase in the mortality rate.

Diagnosis

Definitive diagnosis depends on the detection of an adult worm in an excised nodule or, more commonly, of microfilariae in a skin snip. Skin snips are obtained with a corneal-scleral punch, which collects a blood-free skin biopsy sample extending to just below the epidermis, or by lifting of the skin with the tip of a needle and excision of a small (1- to 3-mm) piece with a sterile scalpel blade. The biopsy tissue is incubated in tissue culture medium or in saline on a glass slide or flat-bottomed microtiter plate. After incubation for 2 to 4 hours (or occasionally overnight in light infections), microfilariae emergent from the skin can be visualized by low-power microscopy.

Eosinophilia and elevated serum IgE levels are common but, because they occur in many parasitic infections, are not diagnostic in themselves. Assays to detect specific antibodies to Onchocerca and PCR to detect onchocercal DNA in skin snips are now in use in specialized laboratories and are highly sensitive and specific.

The *Mazzotti* test is a provocative technique that can be used in cases where the diagnosis of onchocerciasis is still in doubt (i.e., when skin snips and ocular examination reveal no microfilariae). A small dose of DEC (0.5 to 1.0 mg/kg) is given orally; the development or exacerbation of pruritus or rash within hours is highly suggestive of onchocerciasis.

Treatment

Ivermectin

LOIASIS

Etiology

Loiasis is caused by L. loa (the African eye worm), which is present in the rain forests of West and Central Africa. Adult parasites (females, 50 to 70 mm long and 0.5 mm wide; males, 25 to 35 mm long and 0.25 mm wide) live in subcutaneous tissues; microfilariae circulate in the blood with a diurnal periodicity that peaks between 12:00 noon and 2:00 P.M.

Clinical Features

Manifestations of loiasis in natives of endemic areas may differ from those in temporary residents or visitors. Among the indigenous population, loiasis is often an asymptomatic infection with microfilaremia. Infection may be recognized only after subconjunctival migration of an adult worm or may be manifested by episodic. Calabar swellings, evanescent localized areas of angioedema and erythema developing on the extremities and less frequently at other sites. Nephropathy, encephalopathy, and cardiomyopathy are rare. In patients who are not residents of endemic areas, allergic symptoms predominate, episodes of Calabar swelling tend to be more frequent and debilitating, microfilaremia is rare, and eosinophilia and increased levels of antifilarial antibodies are characteristic.

Pathology

The pathogenesis of the manifestations of loiasis is poorly understood. Calabar swellings are thought to result from a hypersensitivity reaction to the adult worm.

Diagnosis

Definitive diagnosis of loiasis requires the detection of microfilariae in the peripheral blood or the isolation of the adult worm from the eye or from a subcutaneous biopsy specimen from a site of swelling developing after treatment (Fig. 33.25). PCR-based assays for the detection of L. loa DNA in blood are now available in specialized laboratories and are highly sensitive and specific. In practice, the diagnosis must often be based on a characteristic history and clinical presentation, blood eosinophilia, and elevated levels of antifilarial antibodies, particularly in travelers to an endemic region, who are usually amicrofilaremic. Other clinical findings in the latter individuals include hypergammaglobulinemia, elevated levels of serum IgE, and elevated leukocyte and eosinophil counts.

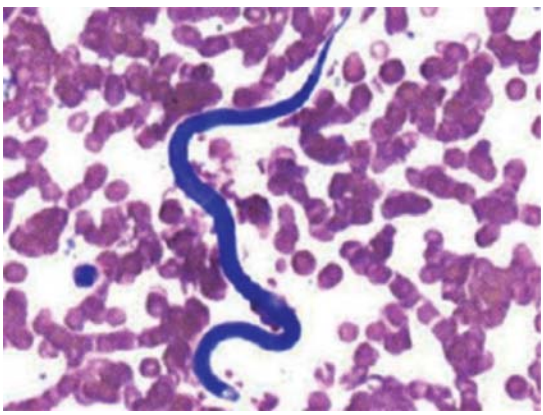


Fig. 33.25: Peripheral blood smear showing *L. loa*

Treatment

Diethyl Carbamazine, Albendazole, Ivermectin.

ACANTHAMOEBA

There are seven species of Acanthamoeba known to infect humans. Acanthamoeba culbertsoni is common.

Characteristic Features

Acanthamoeba spp. exists in two forms, trophozoite and cyst. Both the forms occur in infected tissue. Trophozoites are 15 microns to 45 microns in size. The nuclear characters are similar to Naegleria fowleri. They produce fine, tapering, hyaline pseudopodia called acanthopodia. Motility is sluggish. Aflagellate stage is absent in Acathamoeba spp. as against Naegleria fowleri. The cysts have double wall. The inner wall is smooth and the outer wall is wrinkled and ragged. They are round and measure 8-25 microns. The cyst survives in dust for many years.

Pathogenesis

Acathamoeba occur in soil and water. Trophozoites as well as cysts are infective to human beings (Fig. 33.26). The infection is through the contamination of traumatized skin or eyes and by inhalation. In primary infections the skin and lungs are involved.

Acanthamoeba can produce keratitis. Contact lens users are at an increased risk. Infection can occur in swimmers. Deeper corneal invasion with perforation and loss of vision may follow Acanthamoeba keratitis. Most of the non-ocular infections are due to and corneal lesions are usually due to A. polyphagia.

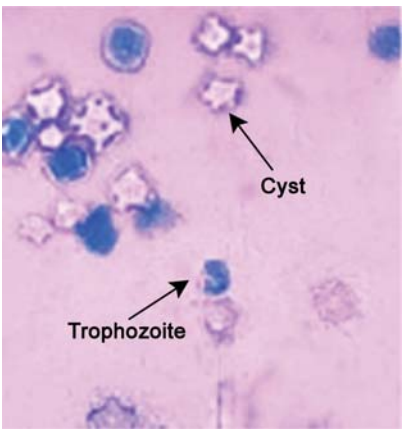


Fig. 33.26: Acanthamoeba

Lab Diagnosis

The cysts of Acanthamoeba are present in corneal scrapping

Treatment: Acanthamoeba keratitis respond to a combination of neomycin drops, dibromopropamid ointment and propamide isethionate.

VIROLOGY

Viruses consist of a nucleic acid surrounded by one or more proteins. Some viruses also have an outer-membrane envelope. Viruses differ from other replicating organisms in that they do not have ribosomes or enzymes for high-energy phosphate generation or for protein, carbohydrate, or lipid metabolism. Viruses are obligate intracellular parasites—that is, they require cells in order to replicate. Typically, viral nucleic acids encode proteins necessary for replicating and packaging the nucleic acids into new viral particles.

Viral Structure

Viruses have from a few to 200 genes. These genes may be embodied in a single-strand or double-strand DNA genome or in a single-strand sense, a single-

strand or segmented antisense, or a double-strand segmented RNA genome. Sense-strand RNA genomes can be translated directly into protein. Sense and antisense genomes are also referred to as positive-strand and negative-strand genomes, respectively. The viral nucleic acid is usually associated with one or more virus-encoded nucleoproteins in the core of the viral particle. The viral nucleic acid is almost always enclosed in a protein shell called a capsid. Because of the limited genetic complexity of viruses, their capsids are usually composed of multimers of identical capsomers. Capsomers are in turn composed of one or a few proteins. Capsids have icosahedral or helical symmetry. Icosahedral structures approximate spheres but have two-, three-, and fivefold axes of symmetry, while helical structures have only a twofold axis of symmetry. The entire structural unit of nucleic acid, nucleoprotein(s), and capsid is called a nucleocapsid. Many human viruses have a simple nucleocapsid structure. For these viruses, the outer surface of the capsid mediates contact with uninfected cells. Other viruses are more complex and have an outer envelope that is derived from membranes of the infected cell.

Stages of Infection

At the cellular level, viral infection proceeds in stages.

Viral Interactions at the Cell Surface First, virus adsorbs to a receptor on the cell surface. Adsorption is the consequence of a molecular interaction of a viral surface protein with a molecule on the cell's plasma membrane.

After adsorption, viruses penetrate through or fuse with the cell membrane, lose their sensitivity to neutralizing antibody, and become uncoated as they enter the cytoplasm. For all viruses, penetration and uncoating result in viral nucleocapsid or nucleoprotein entry into the cytoplasm. Penetration and uncoating as well as subsequent steps in viral replication depend on the cell's energy metabolism and on biochemical changes in the cell's plasma membrane and cytoskeleton.

Viral Gene Expression and Replication After uncoating and release of viral nucleoprotein into the cytoplasm, the viral genome is transported to a site for expression and replication. In order to produce infectious progeny, viruses must (1) replicate their nucleic acid, (2) produce structural proteins, and (3) assemble the nucleic acid and proteins into progeny virions.

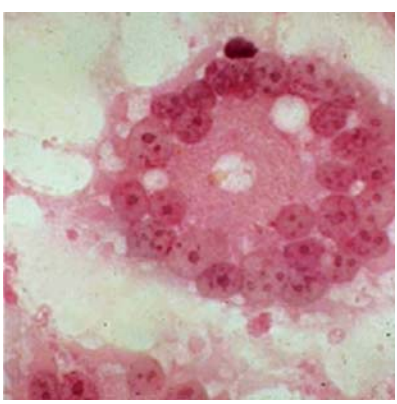


Fig. 33.27: Cytopathic effect in cell culture showing giant cell formation

HERPES VIRUSES

Definition

Herpes simplex viruses (HSV-1, HSV-2; Herpesvirus hominis) produce a variety of infections involving mucocutaneous surfaces, the central nervous system (CNS), and—on occasion—visceral organs. The advent of effective chemotherapy for HSV infections has made prompt recognition of these syndromes even more clinically important than in the past.

Etiologic Agent

The genome of HSV is a linear, double-stranded DNA molecule (molecular weight, ~100×10^6) that encodes more than 75 gene products. The genomic structures of the two HSV subtypes are similar.

Pathogenesis

Exposure to HSV at mucosal surfaces or abraded skin sites permits entry of the virus and initiation of its replication in cells of the epidermis and dermis. Investigators have identified several cell receptors that are ligands for HSV attachment proteins. Studies to define how these receptors influence viral replication and pathogenesis are under way. Initial HSV infection is often subclinical—i.e., without clinically apparent lesions. Both clinical acquisition and subclinical acquisition are associated with sufficient viral replication to permit infection of either sensory or autonomic nerve endings.

Clinical Presentation

HSV has been isolated from nearly all visceral or mucocutaneous sites. The clinical manifestations and course of HSV infection depend on the anatomic site involved, the age and immune status of the host, and the antigenic type of the virus. Primary HSV infections (i.e., first infections with either HSV-1 or HSV-2 in which the host lacks HSV antibodies in acute-phase serum) are frequently accompanied by systemic signs and symptoms, involve both mucosal and extramucosal sites, and have a longer duration of symptoms, a longer duration of virus isolation from lesions, and a higher rate of complications than recurrent episodes of disease. Both viral subtypes can cause genital and oral-facial infections, and the infections caused by the two subtypes are clinically indistinguishable. However, the frequency of reactivation of infection is influenced by anatomic site and virus type. Genital HSV-2 infection is twice as likely to reactivate and recurs 8 to 10 times more frequently than genital HSV-1 infection. Conversely, oral-labial HSV-1 infection recurs more frequently than oral-labial HSV-2 infection. Asymptomatic shedding rates follow the same pattern.

Oral-Facial Infections Gingivostomatitis and pharyngitis are the most frequent clinical manifestations of first-episode HSV-1 infection, while recurrent herpes labialis is the most frequent clinical manifestation of reactivation HSV infection. HSV pharyngitis and gingivostomatitis usually result from primary infection and are most commonly seen in children and young adults. Clinical symptoms and signs, which include fever, malaise, myalgias, inability to eat, irritability, and cervical adenopathy, may last from 3 to 14 days. Lesions may involve the hard and soft palate, gingiva, tongue, lip, and facial area. HSV-1 or HSV-2 infection of the pharynx usually results in exudative or ulcerative lesions of the posterior pharynx and/or tonsillar pillars. Lesions of the tongue, buccal mucosa, or gingiva may occur later in the course in one-third of cases. Fever lasting from 2 to 7 days and cervical adenopathy are common. It can be difficult to differentiate HSV pharyngitis clinically from bacterial pharyngitis, Mycoplasma pneumoniae infections, and pharyngeal ulcerations of noninfectious etiologies (e.g., Stevens-Johnson syndrome). No substantial evidence suggests that reactivation oral-labial HSV infection is associated with symptomatic recurrent pharyngitis.

Eye Infections HSV infection of the eye is the most frequent cause of corneal blindness in the United States. HSV keratitis presents with an acute onset of pain, blurring of vision, chemosis, conjunctivitis, and characteristic dendritic lesions of the cornea. Use of topical glucocorticoids may exacerbate symptoms and lead to involvement of deep structures of the eye. Debridement, topical antiviral treatment, and/or interferon therapy hastens healing. However, recurrences are common, and the deeper structures of the eye may sustain immunopathologic injury. Stromal keratitis due to HSV appears to be related to T cell-dependent destruction of deep corneal tissue. An HSV-1 epitope that is autoreactive with T cell-targeting corneal antigens has been postulated to be a factor in this infection. Chorioretinitis, usually a manifestation of disseminated HSV infection, may occur in neonates or in patients with HIV infection. HSV and varicella-zoster virus can cause acute necrotizing retinitis as an uncommon but severe manifestation.

Diagnosis

Staining of scrapings from the base of the lesions with Wright's, Giemsa's (Tzanck preparation, or Papanicolaou's stain demonstrates characteristic giant cells or intranuclear inclusions of herpesvirus infection. These cytologic techniques are often useful as quick office procedures to confirm the diagnosis. Limitations of the cytologic method are that it does not differentiate between HSV and varicella-zoster virus infections, that it is relatively insensitive, and that the correct identification of giant cells requires experience.

HSV infection is best confirmed in the laboratory by isolation of virus in tissue culture or by demonstration of HSV antigens or DNA in scrapings from

lesions. HSV causes a discernible cytopathic effect in a variety of cell culture systems

PCR is found to be more sensitive.

Treatment

Acylovir, Famciclovir are effective

VARICELLA ZOSTER

Varicella-zoster virus (VZV) causes two distinct clinical entities: varicella (chickenpox) and herpes zoster (shingles). Chickenpox, a ubiquitous and extremely contagious infection, is usually a benign illness of childhood characterized by an exanthematous vesicular rash. With reactivation of latent VZV (which is most common after the sixth decade of life), herpes zoster presents as a dermatomal vesicular rash, usually associated with severe pain.

VZV is a member of the family Herpesviridae, sharing with other members such structural characteristics as a lipid envelope surrounding a nucleocapsid with icosahedral symmetry, a total diameter of approximately 180 to 200 nm, and centrally located double-stranded DNA that is about 125,000 bp in length.

Pathogenesis

Primary Infection: Transmission is most likely to take place by the respiratory route; the subsequent localized replication of the virus at an undefined site (presumably the nasopharynx) leads to seeding of the reticuloendothelial system and ultimately to the development of viremia. Viremia in patients with chickenpox is reflected in the diffuse and scattered nature of the skin lesions and can be verified in selected cases by the recovery of VZV from the blood. Vesicles involve the corium and dermis, with degenerative changes characterized by ballooning, the presence of multinucleated giant cells, and eosinophilic intranuclear inclusions. Infection may involve localized blood vessels of the skin, resulting in necrosis and epidermal hemorrhage. With the evolution of disease, the vesicular fluid becomes cloudy because of the recruitment of polymorphonuclear leukocytes and the presence of degenerated cells and fibrin. Ultimately, the vesicles either rupture and release their fluid (which includes infectious virus) or are gradually reabsorbed.

Recurrent Infection: The mechanism of reactivation of VZV that results in herpes zoster is unknown. Presumably, the virus infects the dorsal root ganglia during chickenpox, where it remains latent until reactivated. Histopathologic examination of representative dorsal root ganglia during active herpes zoster demonstrates hemorrhage, edema, and lymphocytic infiltration.

Clinical Manifestations

Chickenpox: Humans are the only known reservoir for VZV. Chickenpox is highly contagious, with an attack rate of at least 90% among susceptible (seronegative) individuals. Persons of both sexes and all races are infected equally often. The virus is endemic in the population at large; however, it becomes epidemic among susceptible individuals during seasonal peaks—namely, late winter and early spring in the temperate zone. Children between the ages of 5 and 9 are most commonly affected.

The incubation period of chickenpox ranges between 10 and 21 days but is usually between 14 and 17 days. Secondary attack rates in susceptible siblings within a household are between 70 and 90%. Patients are infectious approximately 48 h prior to the onset of the vesicular rash, during the period of vesicle formation (which generally lasts 4 to 5 days), and until all vesicles are crusted.

Clinically, chickenpox presents as a rash, low-grade fever, and malaise, although a few patients develop a prodrome 1 to 2 days before onset of the exanthem.

The most common infectious complication of varicella is secondary bacterial superinfection of the skin, which is usually caused by *Streptococcus pyogenes* or *Staphylococcus aureus*. This complication may result from excoriation of skin lesions after scratching. Gram's staining of skin lesions should help clarify the etiology of unusually erythematous and pustulated lesions.

The most common extracutaneous site of involvement in children is the CNS.

Other complications of chickenpox include myocarditis, corneal lesions, nephritis, arthritis, bleeding diatheses, acute glomerulonephritis, and hepatitis. Hepatic involvement, distinct from Reye's syndrome and usually asymptomatic, is common in chickenpox and is usually characterized by elevated levels of liver enzymes, particularly aspartate and alanine aminotransferases.

Herpes Zoster: Herpes zoster, a sporadic disease, is the consequence of reactivation of latent VZV from the dorsal root ganglia. Most patients have no history of recent exposure to other individuals with VZV infection. Herpes zoster occurs at all ages, but its incidence is highest (5 to 10 cases per 1000 persons) among individuals in the sixth through the eighth decades of life. Recurrent herpes zoster is exceedingly rare except in immunocompromised hosts, especially those with AIDS.

Herpes zoster, also called shingles, is characterized by a unilateral vesicular eruption within a dermatome, often associated with severe pain. The dermatomes from T3 to L3 are most frequently involved. If the ophthalmic branch of the trigeminal nerve is involved, zoster ophthalmicus results.

CNS involvement may follow localized herpes zoster.

Laboratory Findings

1. Isolation virus in the tissue culture
2. PCR
3. ELISA for antibodies

Prophylaxis

A live attenuated varicella vaccine has been licensed and is recommended for administration to all immunocompetent children and adults at risk of infection.

Treatment

Acyclovir and Famciclovir is effective.

ADENOVIRUS

Adenoviruses are complex DNA viruses that measure 70 to 80 nm in diameter. Human adenoviruses belong to the genus Mastadenovirus, which includes at least 47 serotypes. Adenoviruses have a characteristic morphology consisting of an icosahedral shell composed of 20 equilateral triangular faces and 12 vertices. The protein coat (capsid) consists of hexon subunits with group-specific and type-specific antigenic determinants and penton subunits at each vertex primarily containing group-specific antigens.

Clinical Manifestations

In children, adenoviruses cause a variety of clinical syndromes. The most common is an acute upper respiratory tract infection, with prominent rhinitis. On occasion, lower respiratory tract disease, including bronchiolitis and pneumonia, also develops. Adenoviruses, particularly types 3 and 7, cause pharyngoconjunctival fever, a characteristic acute febrile illness of children that occurs in outbreaks, most often in summer camps. The syndrome is marked by bilateral conjunctivitis in which the bulbar and palpebral conjunctivae have a granular appearance. Low-grade fever is frequently present for the first 3 to 5 days, and rhinitis, sore throat, and cervical adenopathy develop. The illness generally lasts for 1 to 2 weeks and resolves spontaneously. Febrile pharyngitis without conjunctivitis also has been associated with adenovirus infection. Adenoviruses have been isolated from cases of whooping cough with or without Bordetella pertussis; the significance of adenovirus in that disease is unknown.

In adults, the most frequently reported illness has been acute respiratory disease caused by adenovirus types 4 and 7 in military recruits. This illness is marked by a prominent sore throat and the gradual onset of fever, which often reaches 39°C (102.2°F) on the second or third day of illness. Cough is almost always present, and coryza and regional lymphadenopathy are frequently seen.

Physical examination may show pharyngeal edema, injection, and tonsillar enlargement with little or no exudate. If pneumonia has developed, auscultation and X-ray of the chest may indicate areas of patchy infiltration.

Adenoviruses have been associated with a number of non-respiratory tract diseases, including acute diarrheal illness caused by types 40 and 41 in young children and hemorrhagic cystitis caused by types 11 and 21. Epidemic keratoconjunctivitis, caused most frequently by types 8, 19, and 37, has been associated with contaminated common sources such as ophthalmic solutions and roller towels. Adenoviruses also have been implicated in disseminated disease and pneumonia in immunosuppressed patients, including recipients of solid-organ or bone-marrow transplants and patients with AIDS. In the latter group, high-numbered and intermediate serotypes have been isolated, usually in the setting of low CD4+ counts, but their isolation frequently has not been clearly linked to disease manifestations. Adenovirus nucleic acids have been detected in myocardial cells from patients with "idiopathic" myocardiopathies, and adenoviruses have been suggested as causative agents in some cases.

Laboratory Diagnosis

1. Isolation of virus in tissue culture
2. Detection of antibodies by ELISA
3. PCR

Treatment

Only symptom-based treatment and supportive therapy are available for adenovirus infections, and no clinically useful antiviral compounds have been identified. Live vaccines have been developed against adenovirus types 4 and 7 and have been used to control illness in military recruits. These vaccines consist of live, unattenuated virus administered in enteric-coated capsules. Infection of the gastrointestinal tract with types 4 and 7 does not cause disease but stimulates local and systemic antibodies that are protective against subsequent acute respiratory disease due to those serotypes. Vaccines prepared from purified subunits of adenovirus are being investigated. Adenoviruses are also being studied as live-virus vectors for the delivery of vaccine antigens and for genes.

AIDS AND HIV

AIDS was first recognized in the United States in the summer of 1981, when the U.S. Centers for Disease Control and Prevention (CDC) reported the unexplained occurrence of *Pneumocystis carinii* pneumonia in five previously healthy homosexual men in Los Angeles and of Kaposi's sarcoma (KS) in 26 previously healthy homosexual men in New York and Los Angeles. Within months, the disease became recognized in male and female injection drug

users (IDUs) and soon thereafter in recipients of blood transfusions and in hemophiliacs. As the epidemiologic pattern of the disease unfolded, it became clear that a microbe transmissible by sexual (homosexual and heterosexual) contact and blood or blood products was the most likely etiologic agent of the epidemic.

Etiologic Agent

The etiologic agent of AIDS is HIV, which belongs to the family of human retroviruses (Retroviridae) . Nononcogenic lentiviruses cause disease in other animal species, including sheep, horses, goats, cattle, cats, and monkeys. The four recognized human retroviruses belong to two distinct groups: the human T lymphotropic viruses (HTLV) I and HTLV-II, which are transforming retroviruses; and the human immunodeficiency viruses, HIV-1 and HIV-2, which are cytopathic viruses. The most common cause of HIV disease throughout the world, and certainly in the United States, is HIV-1. HIV-1 comprises several subtypes with different geographic distributions (see below). HIV-2 was first identified in 1986 in West African patients and was originally confined to West Africa. However, a number of cases that can be traced to West Africa or to sexual contacts with West Africans have been identified throughout the world. HIV-2 is more closely related phylogenetically to the simian immunodeficiency virus (SIV) found in sooty mangabeys than it is to HIV-1.

Morphology of HIV

Electron microscopy shows that the HIV virion is an icosahedral structure containing numerous external spikes formed by the two major envelope proteins, the external gp120 and the transmembrane gp41 (Fig. 33.28). The virion buds from the surface of the infected cell and incorporates a variety of host proteins, including major histocompatibility complex (MHC) class I and II antigens into its lipid bilayer.

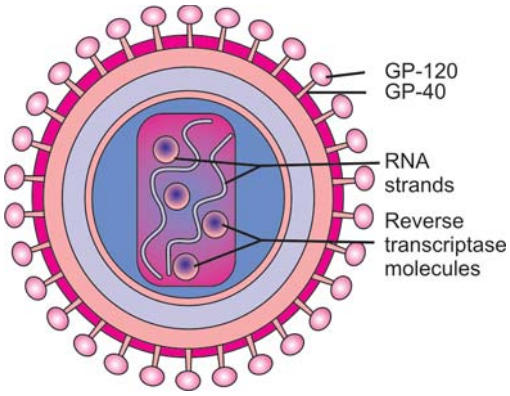


Fig. 33.28: Human immunodeficiency virus

HIV is an RNA virus whose hallmark is the reverse transcription of its genomic RNA to DNA by the enzyme reverse transcriptase. The replication cycle of HIV begins with the high-affinity binding of the gp120 protein via a portion of its V1 region near the N terminus to its receptor on the host cell surface, the CD4 molecule . The CD4 molecule is a 55-kDa protein found predominantly on a subset of T lymphocytes that are responsible for helper or inducer function in the immune system . It is also expressed on the surface of monocytes/macrophages and dendritic/Langerhans cells.

Cellular activation plays an important role in the life cycle of HIV and is critical to the pathogenesis of HIV disease (see below). Following initial binding and internalization of virions into the target cell, incompletely reverse-transcribed DNA intermediates are labile in quiescent cells and will not integrate efficiently into the host cell genome unless cellular activation occurs shortly after infection. Furthermore, some degree of activation of the host cell is required for the initiation of transcription of the integrated proviral DNA into either genomic RNA or mRNA. In this regard, activation of HIV expression from the latent state depends on the interaction of a number of cellular and viral factors. Following transcription, HIV mRNA is translated into proteins that undergo modification through glycosylation, myristylation, phosphorylation, and cleavage. The viral particle is formed by the assembly of HIV proteins, enzymes, and genomic RNA at the plasma membrane of the cells. Budding of the progeny virion occurs through the host cell membrane, where the core acquires its external envelope . The virally encoded protease then catalyzes the cleavage of the gag-pol precursor to yield the mature virion. Each point in the life cycle of HIV is a real or potential target for therapeutic intervention . Thus far, the reverse transcriptase and protease enzymes have proven to be susceptible to pharmacologic disruption.

Transmission

HIV is transmitted by both homosexual and heterosexual contact; by blood and blood products; and by infected mothers to infants either intrapartum, perinatally, or via breast milk. After approximately 20 years of scrutiny, there is no evidence that HIV is transmitted by casual contact or that the virus can be spread by insects, such as by a mosquito bite.

Sexual Transmission

HIV infection is predominantly a sexually transmitted disease (STD) worldwide. Although approximately 42% of new HIV infections in the United States are among men who have sex with men, heterosexual transmission is clearly the most common mode of infection worldwide, particularly in developing countries. Furthermore, the yearly incidence of new cases of AIDS attributed to heterosexual transmission of HIV is steadily increasing in the United States, mainly among minorities, particularly women in minority groups .

Transmission by Blood and Blood Products

HIV can be transmitted to individuals who receive HIV-tainted blood transfusions, blood products, or transplanted tissue.

Maternal-fetal/Infant Transmission

HIV infection can be transmitted from an infected mother to her fetus during pregnancy or to her infant during delivery. This is an extremely important form of transmission of HIV infection in developing countries.

Transmission by other Body Fluids

There is no convincing evidence that saliva can transmit HIV infection, either through kissing or through other exposures, such as occupationally to health care workers. HIV can be isolated from saliva of only a small proportion of infected individuals.

Pathogenesis

The hallmark of HIV disease is a profound immunodeficiency resulting primarily from a progressive quantitative and qualitative deficiency of the subset of T lymphocytes referred to as helper T cells, or inducer T cells. This subset of T cells is defined phenotypically by the presence on its surface of the CD4 molecule, ced HIV infection and cardiomyopathy.

Opportunistic Infections

1. Fungal infections like Candida, Cryptococcus, Histoplasma
2. Protozoal infections like Giardia, Cryptosporidia, Isospora
3. Bacterial infections like Shigella, Salmonella
4. Viral infections like CMV, Herpes etc.
5. Toxoplasma and Pneumocystis carinii is also common.

Treatment

The reverse transcriptase inhibitors include the *nucleoside analogues* zidovudine, didanosine, zalcitabine, stavudine, lamivudine, and abacavir and the *nonnucleoside* reverse *transcriptase inhibitors* nevirapine, delavirdine, and efavirenz . These were the first class of drugs that were licensed for the treatment of HIV infection.

CHLAMYDIAL INFECTIONS

Trachoma is a chronic conjunctivitis associated with infection by *C. trachomatis* serovar A, B, Ba, or C. It has been responsible for an estimated 20 million cases of blindness throughout the world and remains an important cause of

preventable blindness. Inclusion conjunctivitis is an acute ocular infection caused by sexually transmitted *C. trachomatis* strains (usually serovars D through K) in adults exposed to infected genital secretions and in their newborn offspring.

Epidemiology

Epidemiologically, two types of eye disease are caused by *C. trachomatis.* In trachoma-endemic areas where the classic eye disease is seen, transmission is from eye to eye via hands, flies, towels, and other fomites and usually involves serovar A, B, Ba, or C. In non-endemic areas, organisms of serovars D through K can be transmitted from the genital tract to the eye, usually causing only the inclusion conjunctivitis syndrome, occasionally with keratitis. Rarely, the eye disease acquired in this way progresses, with the development of pannus and scars similar to those seen in endemic trachoma. These cases may be referred to as paratrachoma to differentiate them epidemiologically from eye-to-eye-transmitted endemic trachoma.

Clinical Manifestations

Both endemic trachoma and adult inclusion conjunctivitis present initially as a conjunctivitis characterized by small lymphoid follicles in the conjunctiva. In regions with hyperendemic classic blinding trachoma, the disease usually starts insidiously before the age of 2 years. Reinfection is common and probably contributes to the pathogenesis of trachoma. Studies using PCR techniques indicate that chlamydial DNA is often present in the ocular secretions of patients with trachoma, even in the absence of positive cultures. Thus, persistent infection may be more common than was previously thought.

The cornea becomes involved, with inflammatory leukocytic infiltrations and superficial vascularization (pannus formation). As the inflammation continues, conjunctival scarring eventually distorts the eyelids, causing them to turn inward so that the inturned lashes constantly abrade the eyeball (trichiasis and entropion); eventually the corneal epithelium is abraded and may ulcerate, with subsequent corneal scarring and blindness. Destruction of the conjunctival goblet cells, lacrimal ducts, and lacrimal gland may produce a "dry-eye" syndrome, with resultant corneal opacity due to drying (xerosis) or secondary bacterial corneal ulcers.

Communities with blinding trachoma often experience seasonal epidemics of conjunctivitis due to H. influenzae that contribute to the intensity of the inflammatory process. In such areas the active infectious process usually resolves spontaneously in affected persons between 10 and 15 years of age, but the conjunctival scars continue to shrink, producing trichiasis and entropion and subsequent corneal scarring in adults. In areas with milder and less prevalent disease, the process may be much slower, with active disease continuing into adulthood; blindness is rare in these cases.

Eye infection with genital *C. trachomatis* strains in sexually active young adults presents as the acute onset of unilateral follicular conjunctivitis and preauricular lymphadenopathy similar to that seen in acute adenovirus or herpesvirus conjunctivitis. If untreated, the disease may persist for 6 weeks to 2 years. It is frequently associated with corneal inflammation in the form of discrete opacities ("infiltrates"), punctate epithelial erosions, and minor degrees of superficial corneal vascularization. Very rarely, conjunctival scarring and eyelid distortion occur, particularly in patients treated for many months with topical glucocorticoids. Recurrent eye infections develop most often in patients whose sexual consorts are not treated with antimicrobials.

Diagnosis

The clinical diagnosis of classic trachoma can be made if two of the following signs are present:

1. Lymphoid follicles on the upper tarsal conjunctiva
2. Typical conjunctival scarring
3. Vascular pannus
4. Limbal follicles or their sequelae, Herbert's pits

The clinical diagnosis of endemic trachoma should be confirmed by laboratory tests in children with more marked degrees of inflammation. Intracytoplasmic chlamydial inclusions are found in 10 to 60% of Giemsa-stained conjunctival smears in such populations, but isolation in cell cultures, newer antigen detection testing, or chlamydial PCR is more sensitive. Follicular conjunctivitis in adult Europeans or Americans living in trachomatous regions is rarely due to trachoma.

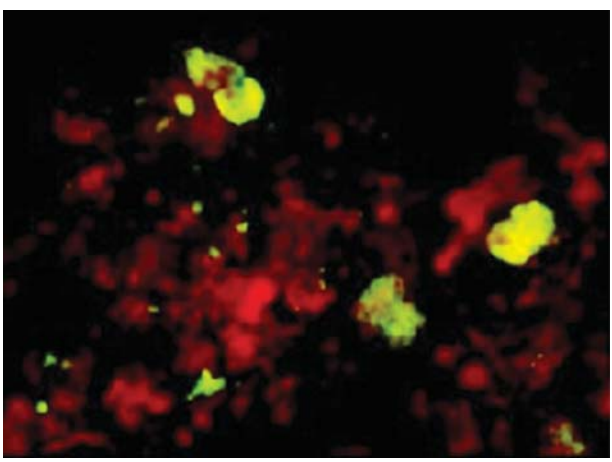


Fig. 33.29: Immunofluorescence staining of chlamydiae

Treatment

Erythromycin and Tetracylines are effective.

INDEX

A

B

C

D

E

F

G

H

I

K

L

M

N

O

P

R

S

T

U

V