3RD 4TH AND 6TH CRANIAL NERVES

CRANIAL NERVES

- There are 12 pairs of cranial nerves out of which the first two pairs from the forebrain and the next 10 pairs arise from the brainstem
- They are numbered from 1 to 12 in the craniocaudal sequence of their attachment on the brain
- The cranial nerves are generally designated by Roman numerals

i. OLFACTORY
ii. OPTIC
iii. OCCULOMOTOR
iv. TROCHLEAR
v. TRIGEMINAL
vi. ABDUCENT
vii. FACIAL
viii. VESTIBULO COCHLEAR/AUDITORY
ix. GLOSSOPHARYNGEAL
x. VAGUS
xi. ACESSORY
xii. HYPOGLOSSAL

OOOTTAFAGVAH

Functional components

- A cranial nerve consists of motor fibres(motor nerve) or sensory fibres (mixed nerve) or both the motor and sensory fibres

Motor fibres

1. General somatic efferent fibres(GSE)
   Which supply striated muscle

2. Special visceral efferent fibres(SVE)

   which supply the muscles which develop from the mesoderm of pharyngeal arches

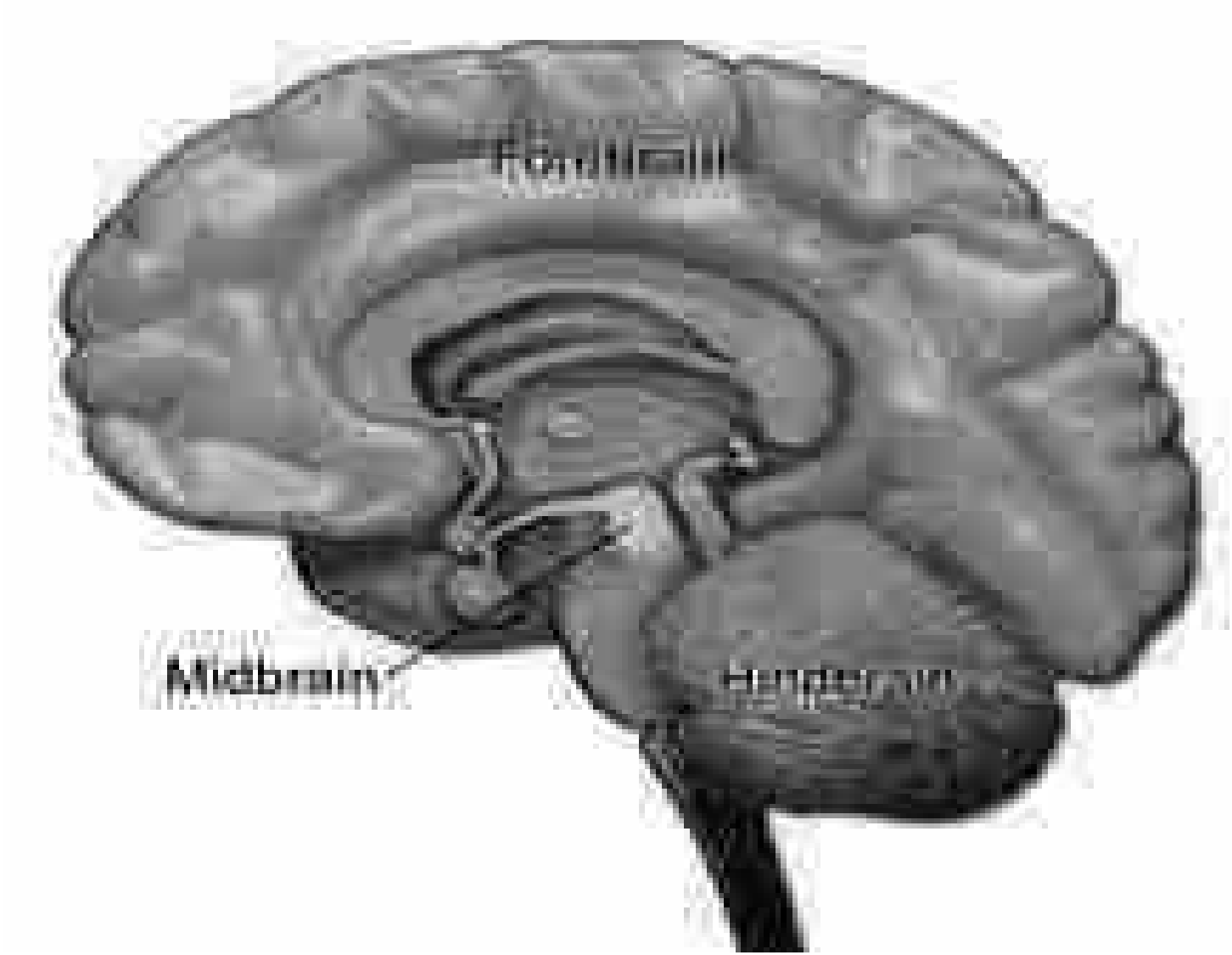
3. General visceral efferent fibres(GVE)

   which supply the glands , smooth muscle of viscera and vessels they are the preganglionic parasympathetic fibres

Sensory fibres

1. General somatic afferent fibres(GSA) – They carry general sensations of pain , touch , and temperature from skin and proprioceptive sensation of vibration and muscle and joint sense
2. General visceral afferent (GVA) fibres – They carry general sensation of distension and ischemic pain from viscera
3. Special visceral afferent fibre (SVA) carry special sensation of taste from tongue
4. SSA carry special sensation of smell hearing and balance

i. OLFACTORY - SOME
ii. OPTIC- SAYS
iii. OCCULOMOTOR – MONEY
iv. TROCHLEAR-MATTERS
v. TRIGEMINAL-BUT
vi. ABDUCENT-MY
vii. FACIAL-BROTHER
viii. VESTIBULO COCHLEAR/AUDITORY-SAYS
ix. GLOSSOPHARYNGEAL- BIG
x. VAGUS-BRAIN
xi. ACESSORY-MATTERS
xii. HYPOGLOSSAL- MORE


Midbrain

OCCULOMOTOR NERVE

INTRODUCTION

- It is the third cranial nerve
- It is purely motor
- As the name implies it moves the eye
- It supplies all the muscle of eye and plays a principle role in accomodation of the eye

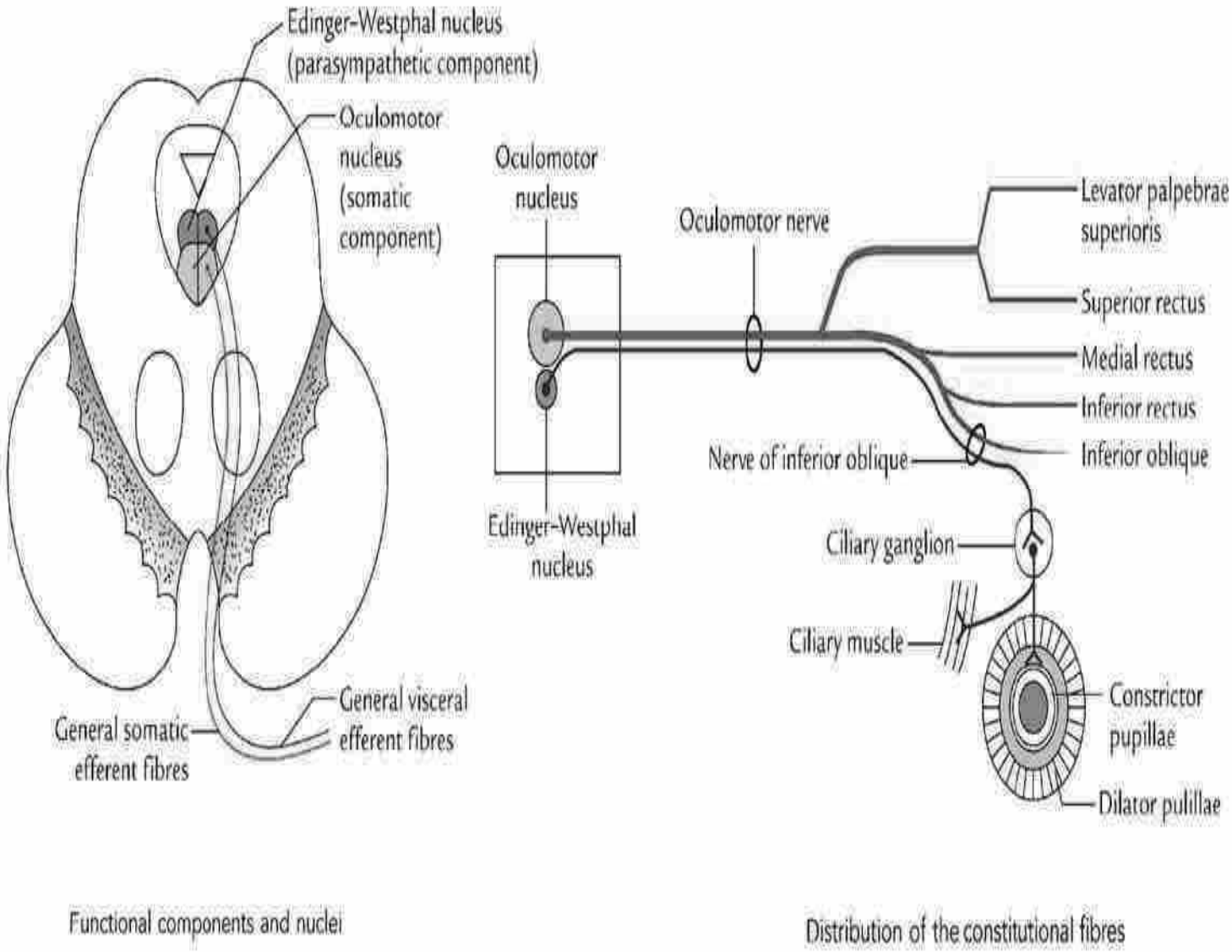
Functional component and nuclei

General somatic efferent fibres ;

- They supply all the extraocular muscles except lateral rectus (supplied by 6th cranial nerve) and superior oblique muscle (by 4th cranial nerve)
- The GSE fibres arising from the somatic component of oculomotor nucleus also called somatic motor nucleus

General visceral efferent fibres;

- They supply the sphincter pupillae muscle and ciliaris muscles(intraocular)
- They arise from the parasympathetic component of oculomotor nucleus also called Edinger – Westphal nucleus
- These are preganglionic parasympathetic fibres and relay in the ciliary ganglion
- The postganglionic parasympathetic fibres from the ganglion and supply the sphincter pupillae and ciliaris muscle



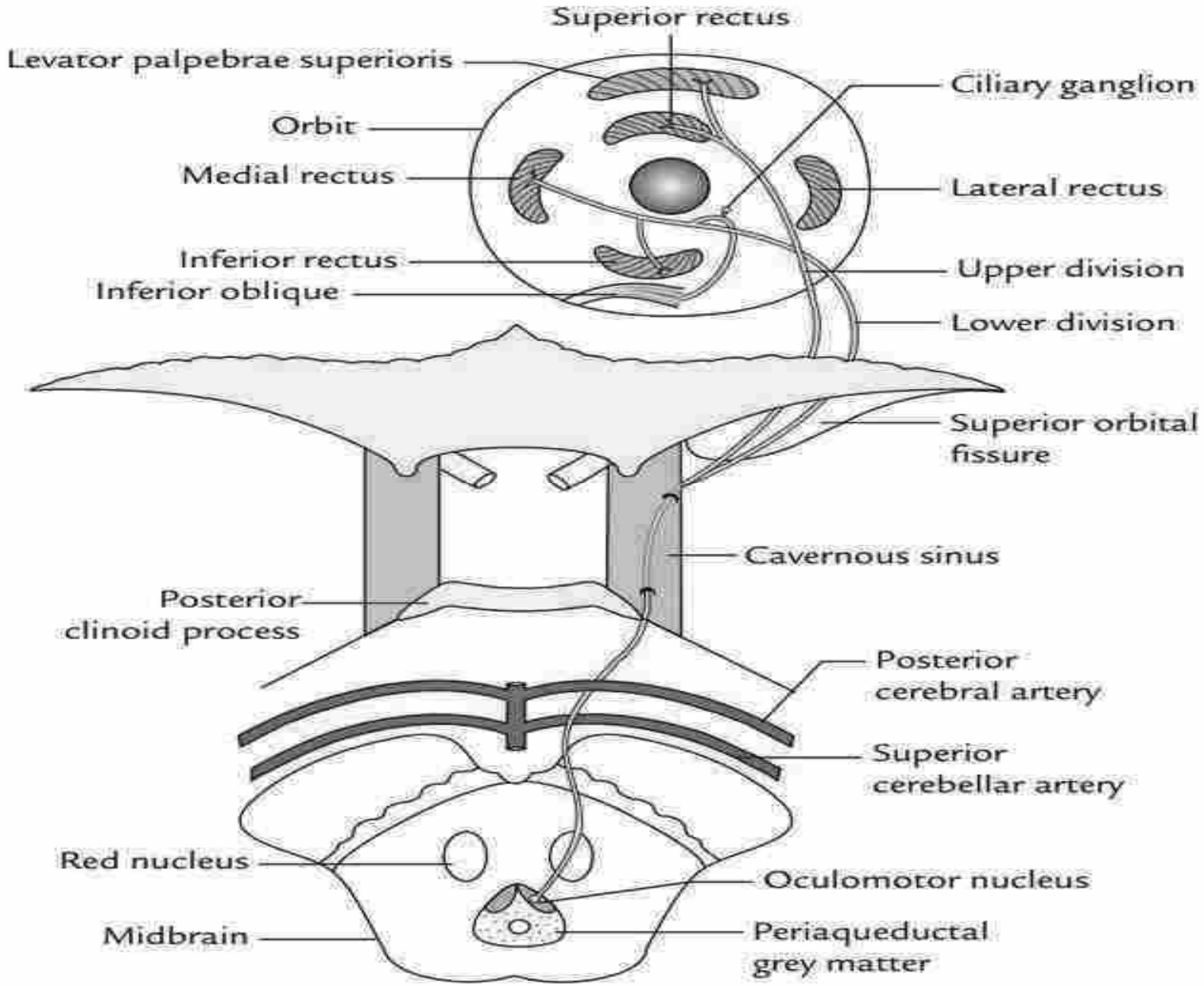
Functional components and nuclei

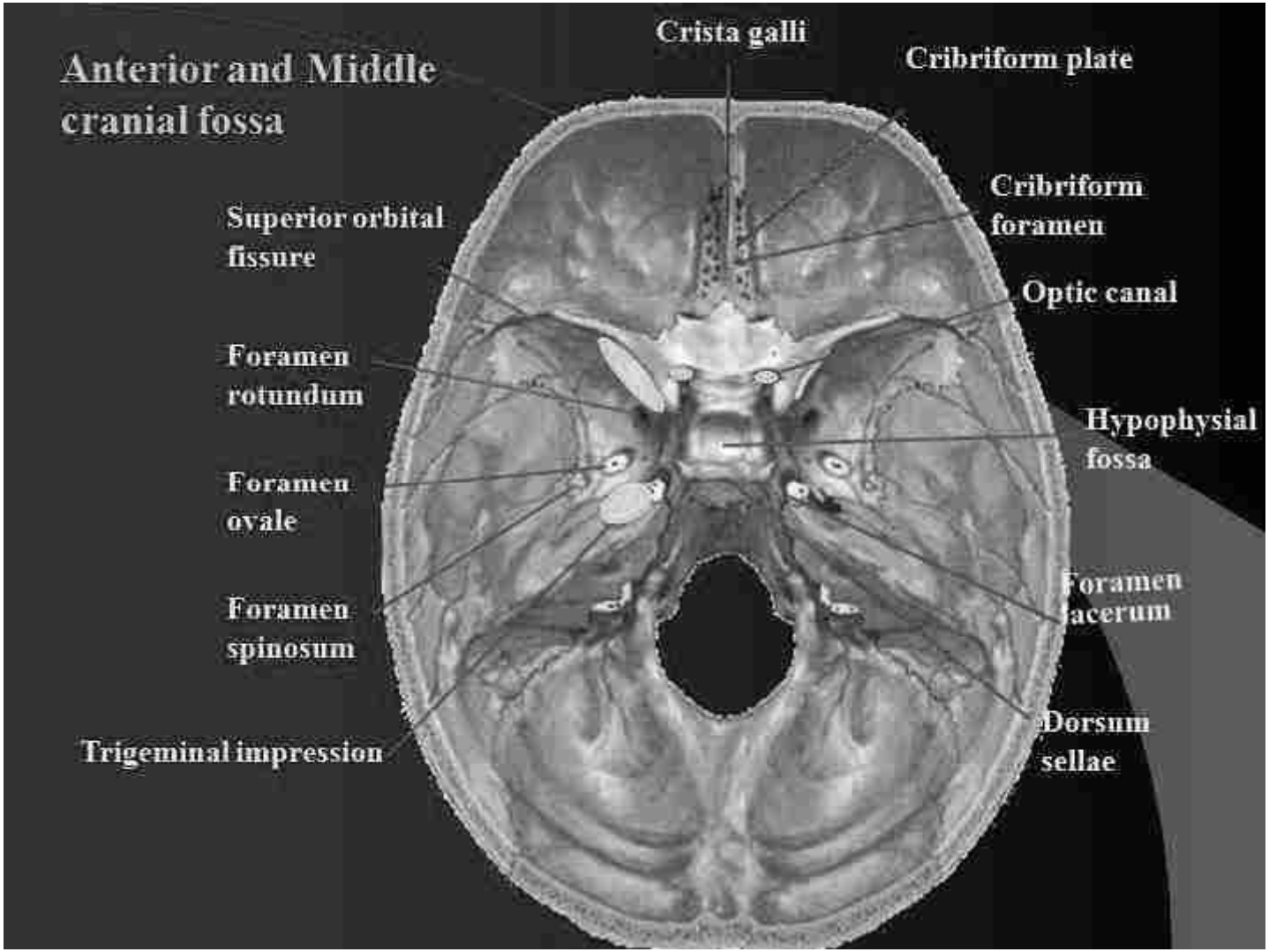
Distribution of the constitutional fibres

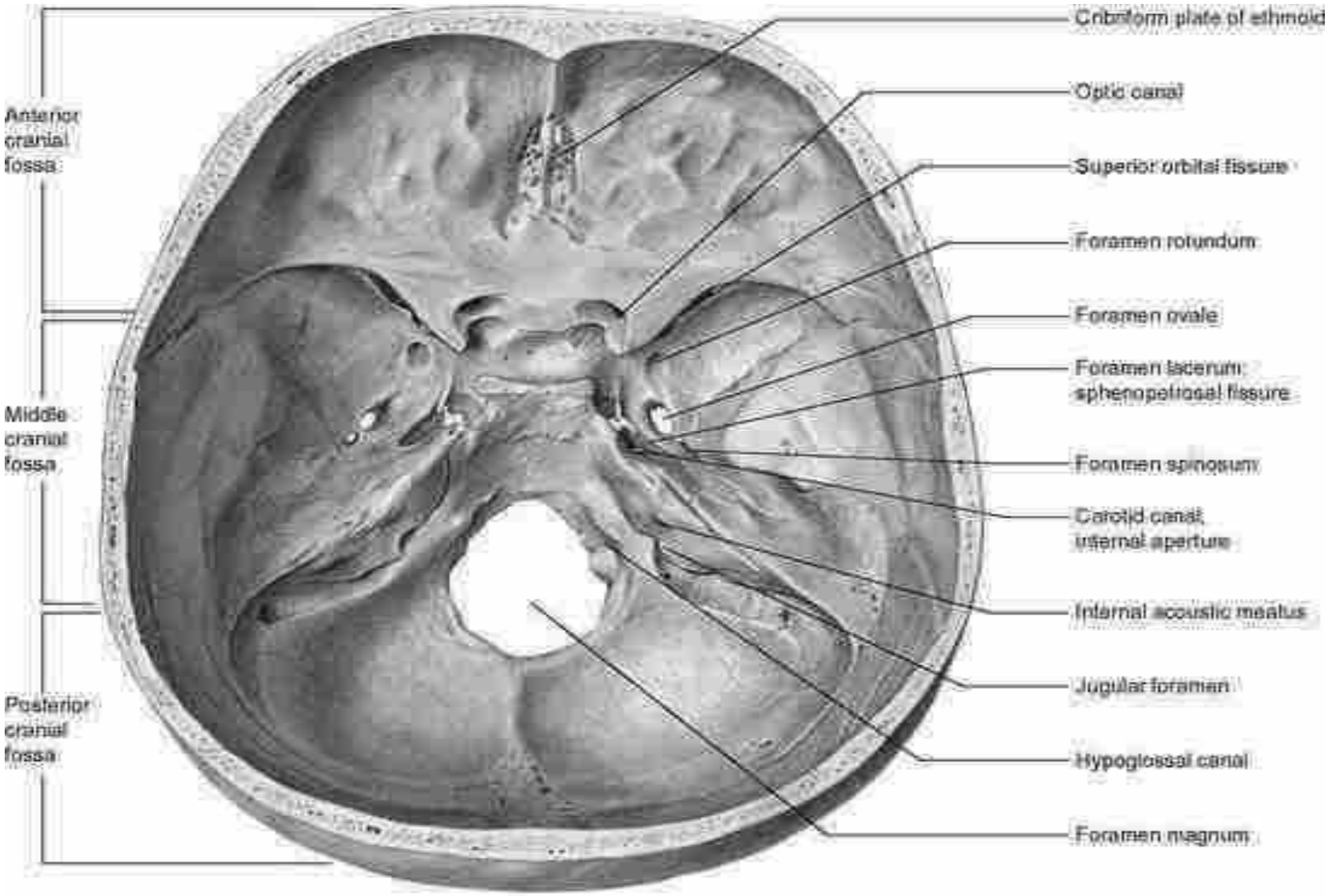
Course and Distribution

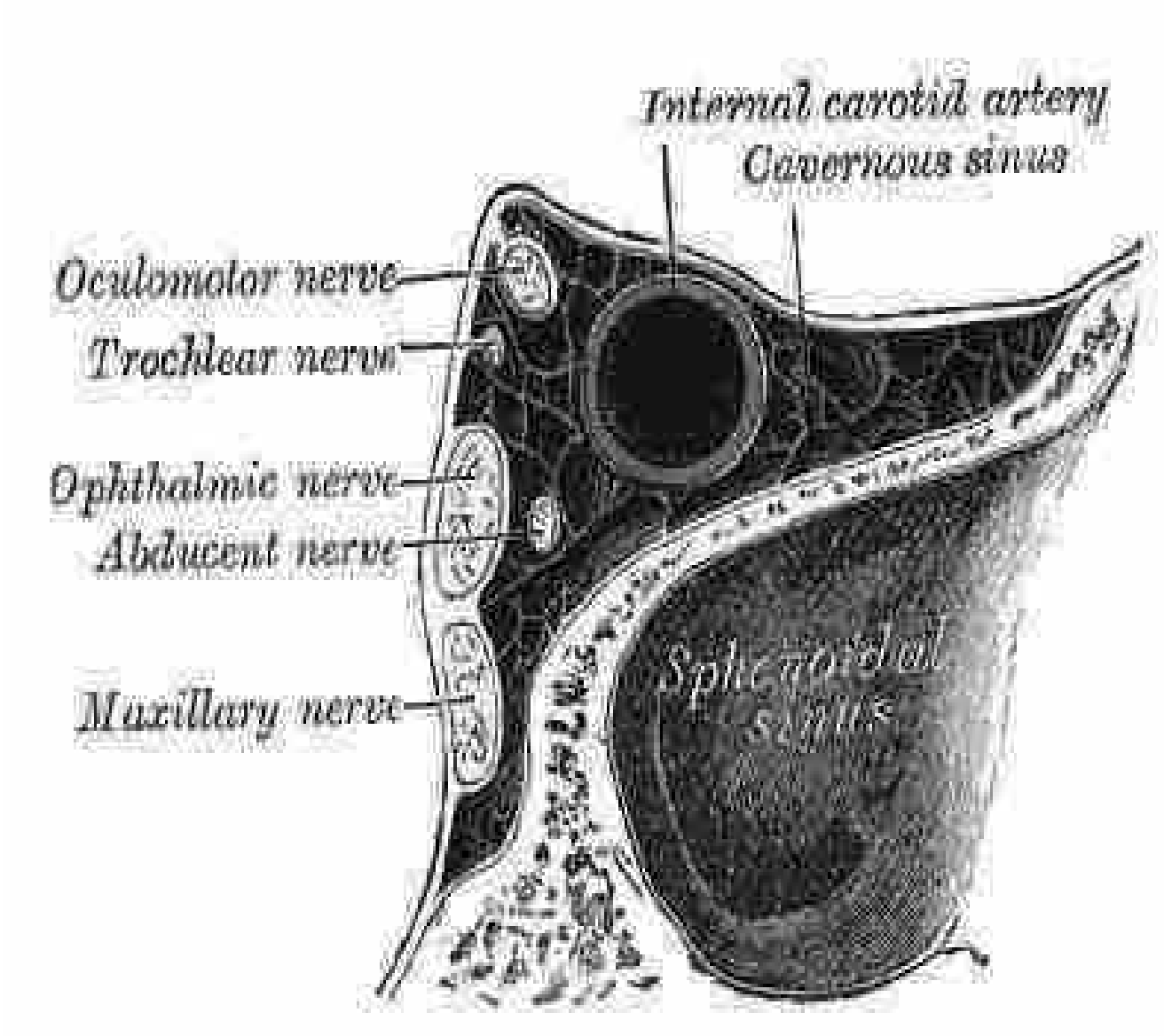
- The oculomotor nerve arises from the oculomotor sulcus on the medial aspect of the cerebral peduncle of the midbrain and appears in the interpeduncular fossa
- Runs forwards and laterally between the posterior cerebral and superior cerebellar arteries and lateral to the posterior communicating artery , passes through the tentorial notch of tentorium cerebelli to reach the middle cranial fossa

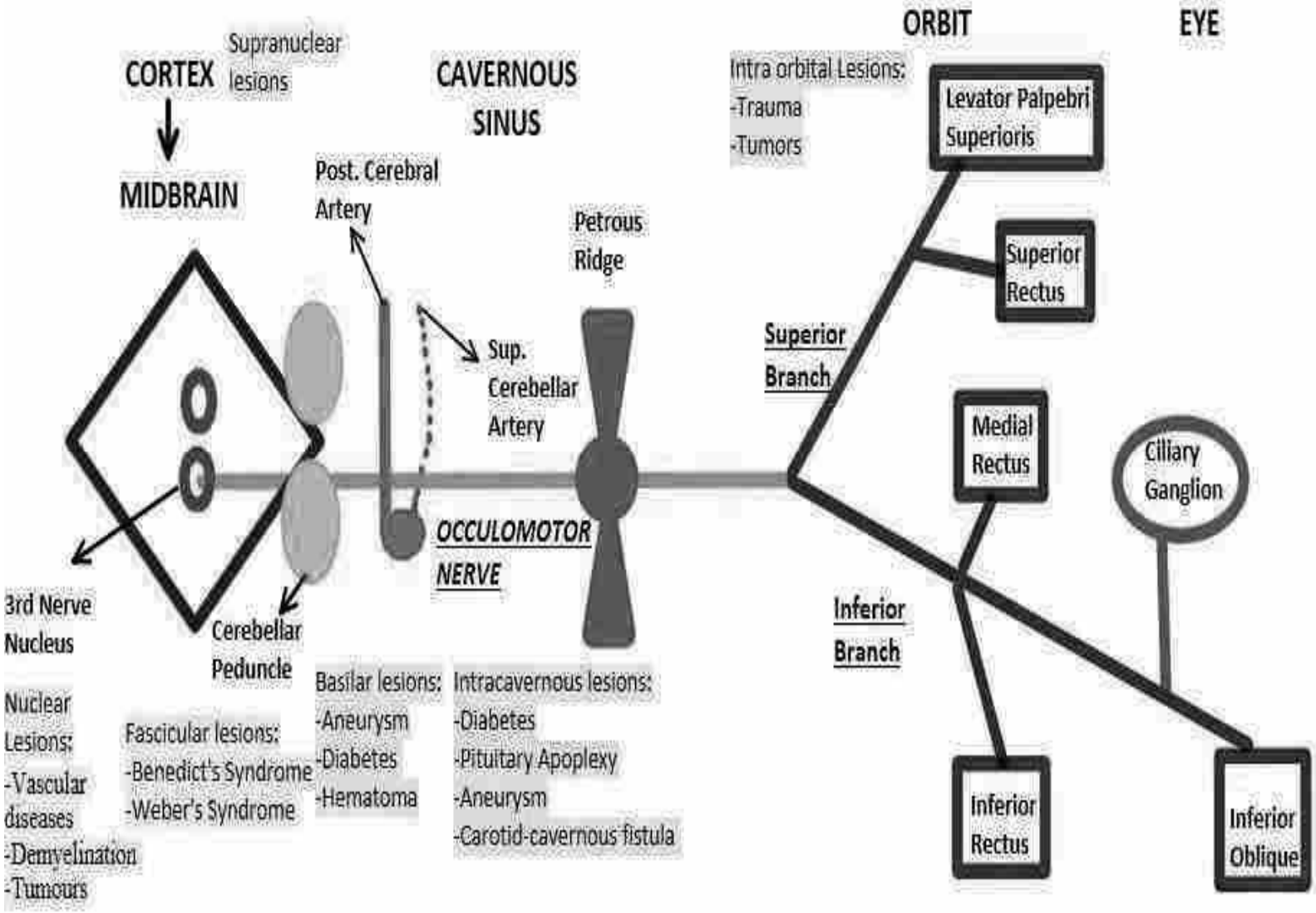
- In the middle cranial fossa it pierces the dura matter in the oculomotor triangle lying between the free and attached margin of tentorium cerebelli in the roof of cavernous sinus and enters the lateral wall of cavernous sinus
- In the lateral wall it lies superior to the trochlear opthalmic and maxillary nerves and lateral to the internal carotid artery
- In the anterior part of cavernous sinus it divides into upper and lower divisions


Superior rectus
Levator palpebrae superioris
Ciliary ganglion
Orbit
Medial rectus
Lateral rectus
Inferior rectus
Upper division
Inferior oblique
Lower division
Superior orbital fissure
Cavernous sinus
Posterior clinoid process
Posterior cerebral artery
Superior cerebellar artery
Red nucleus
Oculomotor nucleus
Midbrain
Periaqueductal grey matter

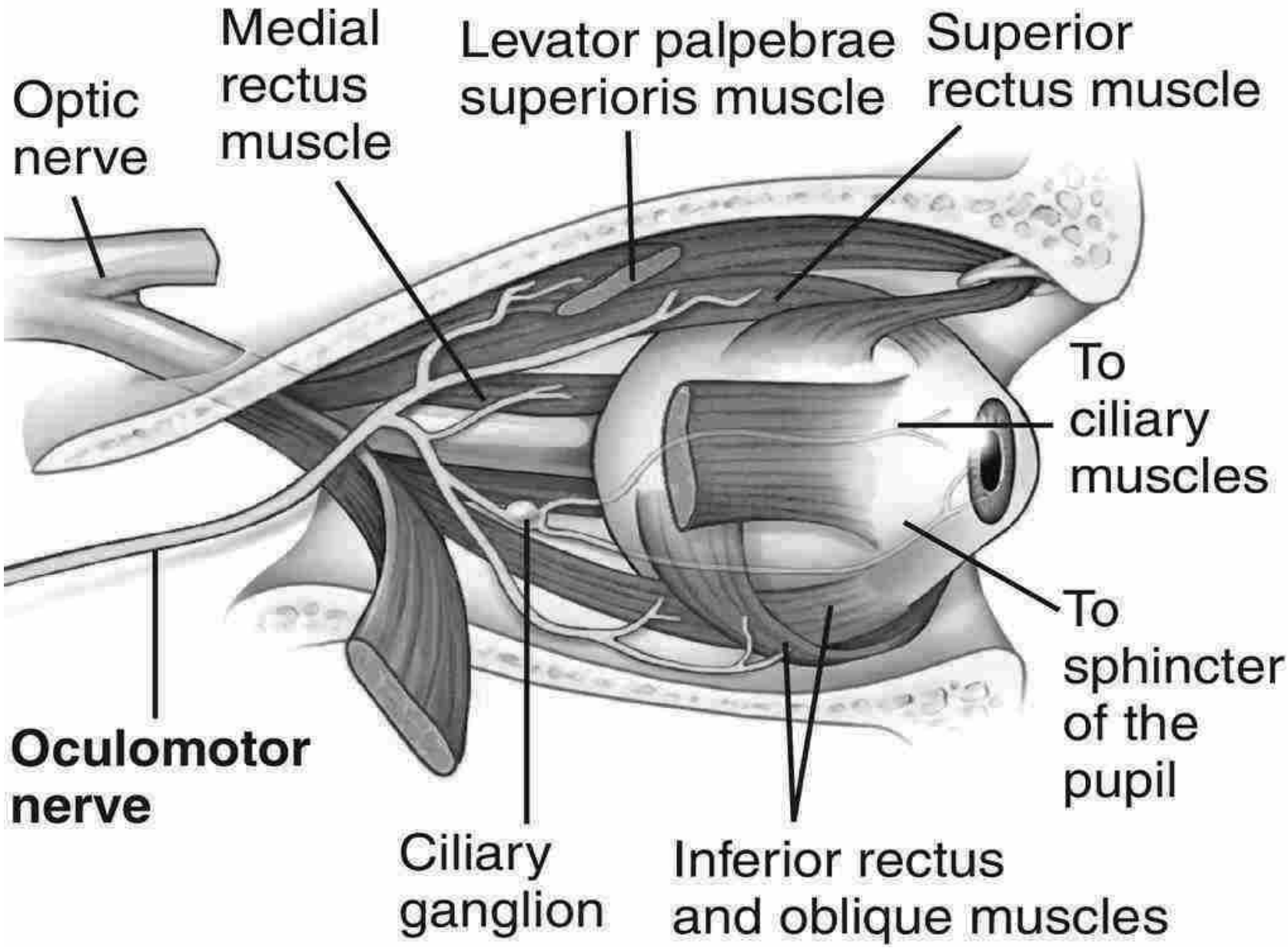

Anterior and Middle cranial fossa
Crista galli
Cribriform plate
Cribriform foramen
Optic canal
Hypophysial fossa
Foramen lacerum
Dorsum sellae
Superior orbital fissure
Foramen rotundum
Foramen ovale
Foramen spinosum
Trigeminal impression


Anterior cranial fossa
Middle cranial fossa
Posterior cranial fossa
Cribriform plate of ethmoid
Optic canal
Superior orbital fissure
Foramen rotundum
Foramen ovale
Foramen lacerum; sphenopetrosal fissure
Foramen spinosum
Carotid canal, internal aperture
Internal acoustic meatus
Jugular foramen
Hypoglossal canal
Foramen magnum

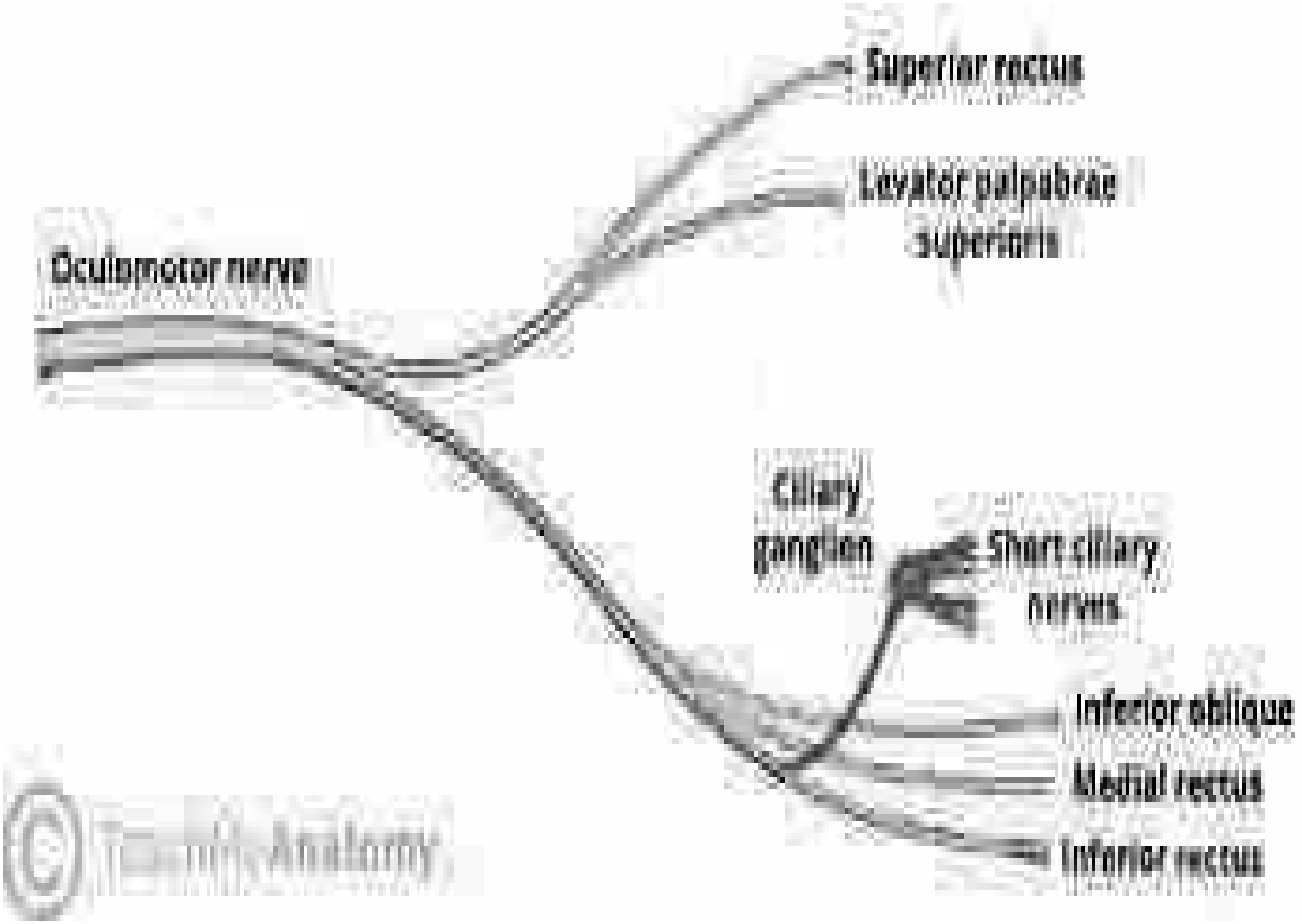

Internal carotid artery
Cavernous sinus
Oculomotor nerve
Trochlear nerve
Ophthalmic nerve
Abducent nerve
Maxillary nerve
Sphenoidal sinus


ORBIT
EYE
CORTEX
Supranuclear lesions
MIDBRAIN
CAVERNOUS SINUS
Intra orbital Lesions:
-Trauma
-Tumors
Levator Palpebri Superioris
Superior Rectus
Post. Cerebral Artery
Petrous Ridge
Sup. Cerebellar Artery
Superior Branch
Medial Rectus
Ciliary Ganglion
OCCULOMOTOR NERVE
3rd Nerve Nucleus
Cerebellar Peduncle
Inferior Branch
Nuclear Lesions:
-Vascular diseases
-Demyelination
-Tumours
Fascicular lesions:
-Benedict's Syndrome
-Weber's Syndrome
Basilar lesions:
-Aneurysm
-Diabetes
-Hematoma
Intracavernous lesions:
-Diabetes
-Pituitary Apoplexy
-Aneurysm
-Carotid-cavernous fistula
Inferior Rectus
Inferior Oblique

- The two divisions of nerve enters the orbit through superior orbital fissure within the common tendinous ring
- The nasociliary nerve intervenes between the two divisions
- The smaller upper division passes above the optic nerve on the inferior surface of the superior rectus and then passes through the superior rectus to supply the levator palpebrae superioris


Optic nerve
Medial rectus muscle
Levator palpebrae superioris muscle
Superior rectus muscle
To ciliary muscles
To sphincter of the pupil
Oculomotor nerve
Ciliary ganglion
Inferior rectus and oblique muscles

- The larger lower division of the oculomotor nerve passes below the optic nerve and immediately gives three branches which supply the medial rectus, inferior rectus and inferior oblique muscles
- The nerve to inferior oblique gives motor root to the ciliary ganglion located in the posterior part of orbit
- The postganglionic fibres from this runs through short ciliary nerve and supply the sphincter pupillae and ciliaris muscle


Superior rectus
Levator palpebrae superioris
Oculomotor nerve
ciliary ganglion
Short ciliary nerves
Inferior oblique
Medial rectus
Inferior rectus

Clinical anatomy

The lesion of oculomotor nerve

Causes;

- **Compression by aneurysm of the posterior communicating artery while it passes between posterior cerebral and superior cerebellar arteries**
- **Compression by aneurysm of internal carotis artery as it passes through the lateral wall of cavernous sinus**

- Compression by transtentorial uncal herniation as it passes through the tentorial notch

Damage of oculomotor clinically presents as

- Ptosis; Drooping of upper eyelid due to the paralysis of the levator palpebrae superioris
- Lateral strabismus;(Lateral squint) due to the paralysis of medial rectus and consequent unopposed action of the lateral rectus muscle

- Dilated and fixed pupil; Due to the paralysis of the sphincter pupillae and consequent unopposed action of dilator pupillae
- Loss of accomodation; Due to the paralysis of the medial rectus , sphincter pupillae and ciliaris muscle
- Double vision or dipolopia occurs on lookin medially inferiorly and supeirorly due to the paralysis of the medial rectus inferior rectus and inferior oblique muscles
- Proptosis; prominence of eyeball due to

TROCHLEAR NERVE

Introduction

- Trochlear nerve is the 4th cranial nerve
- It is purely motor and supply only one muscle – the superior oblique muscle of the eyeball

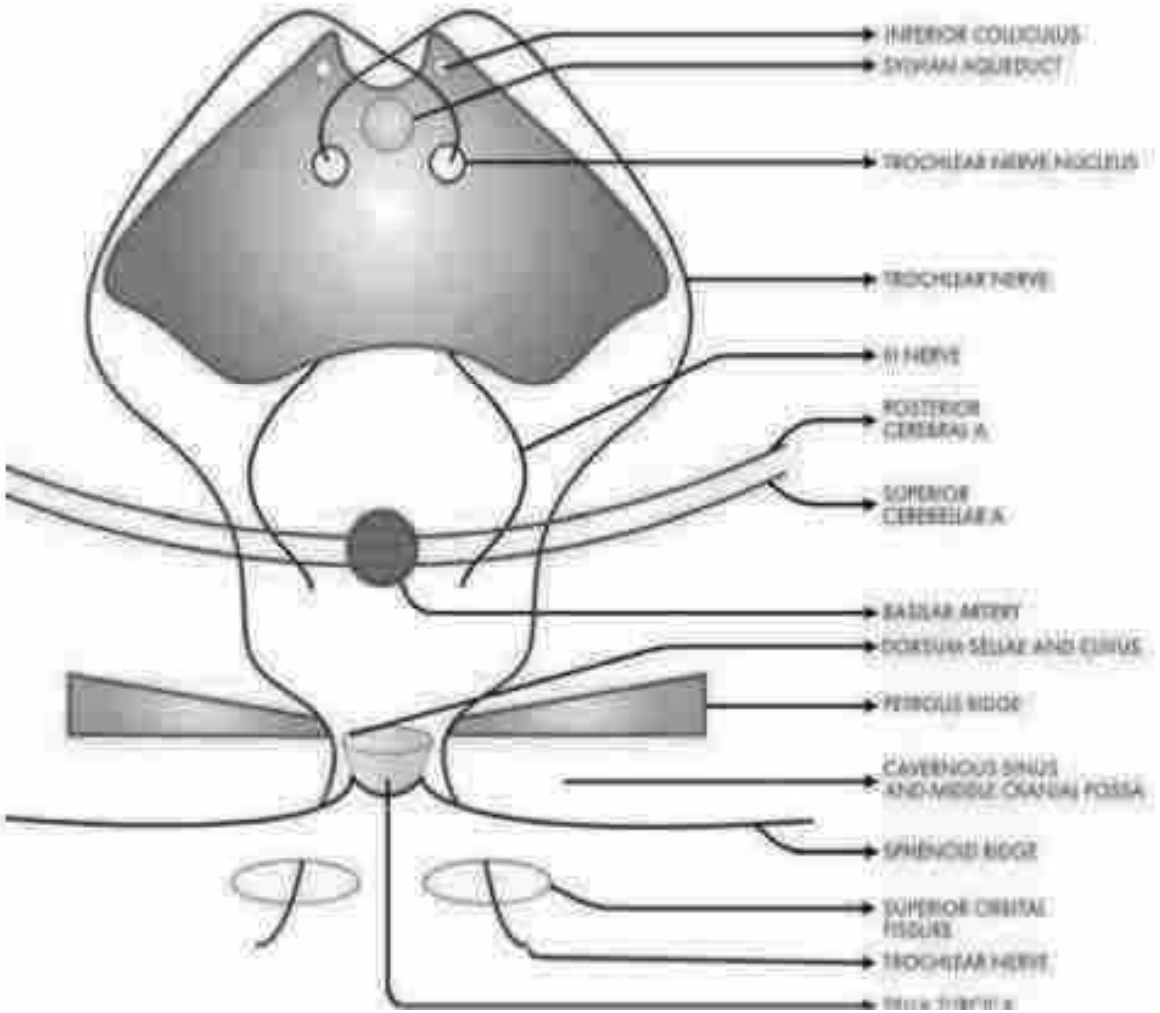
Unique features

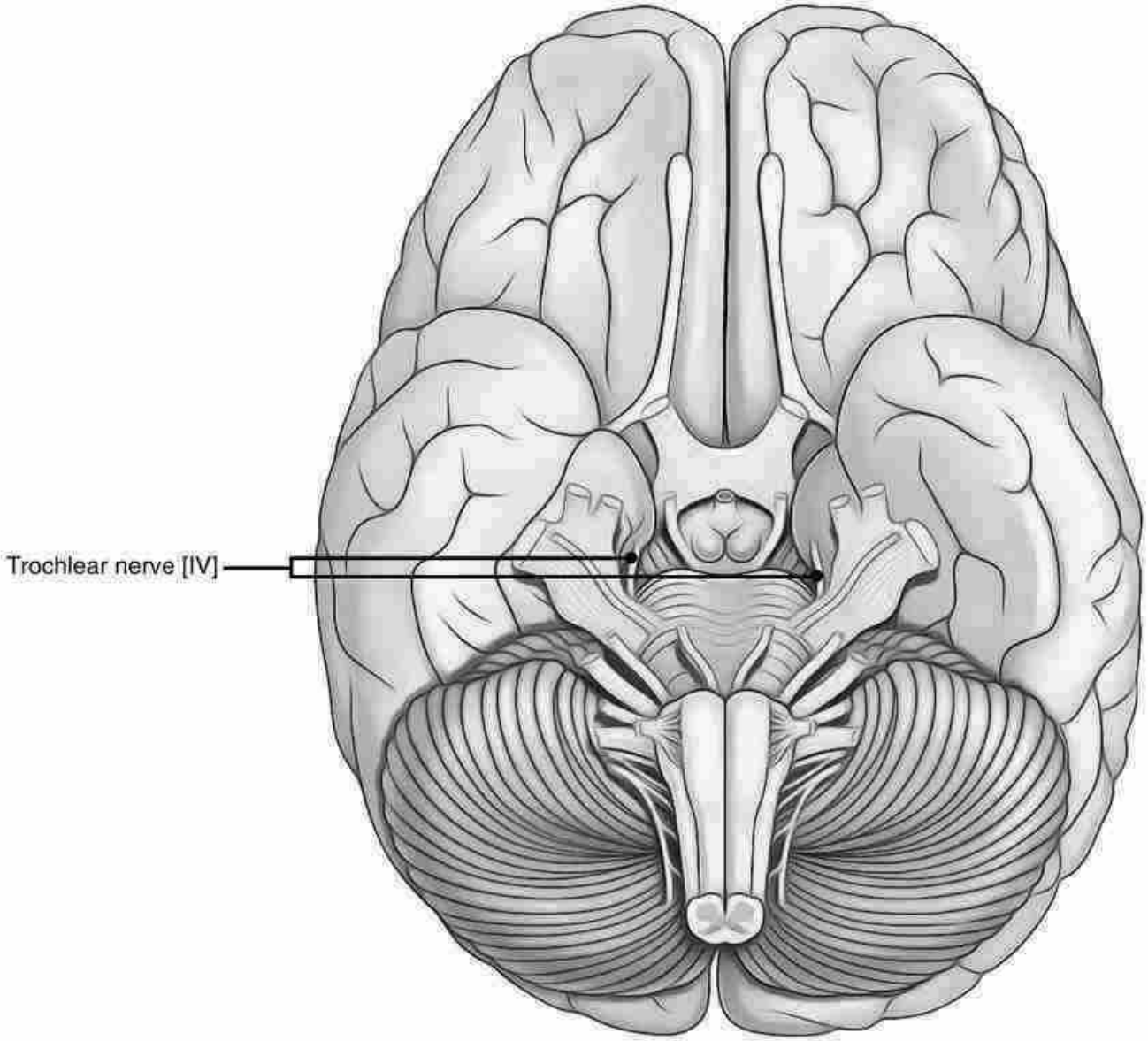
- It is the only cranial nerve which emerges on the dorsal aspect of the brain
- It is the most slender of all the cranial nerves
- It is the smallest cranial nerve
- It is the only cranial nerve whose nuclear fibres decussate before emerging on the surface of the brain
- Its nucleus receives only ipsilateral corticonuclear fibres

Functional component and nuclei

- General somatic efferent fibres; They arise from the trochlear nucleus in the midbrain and supply the superior oblique muscle of the eyeball

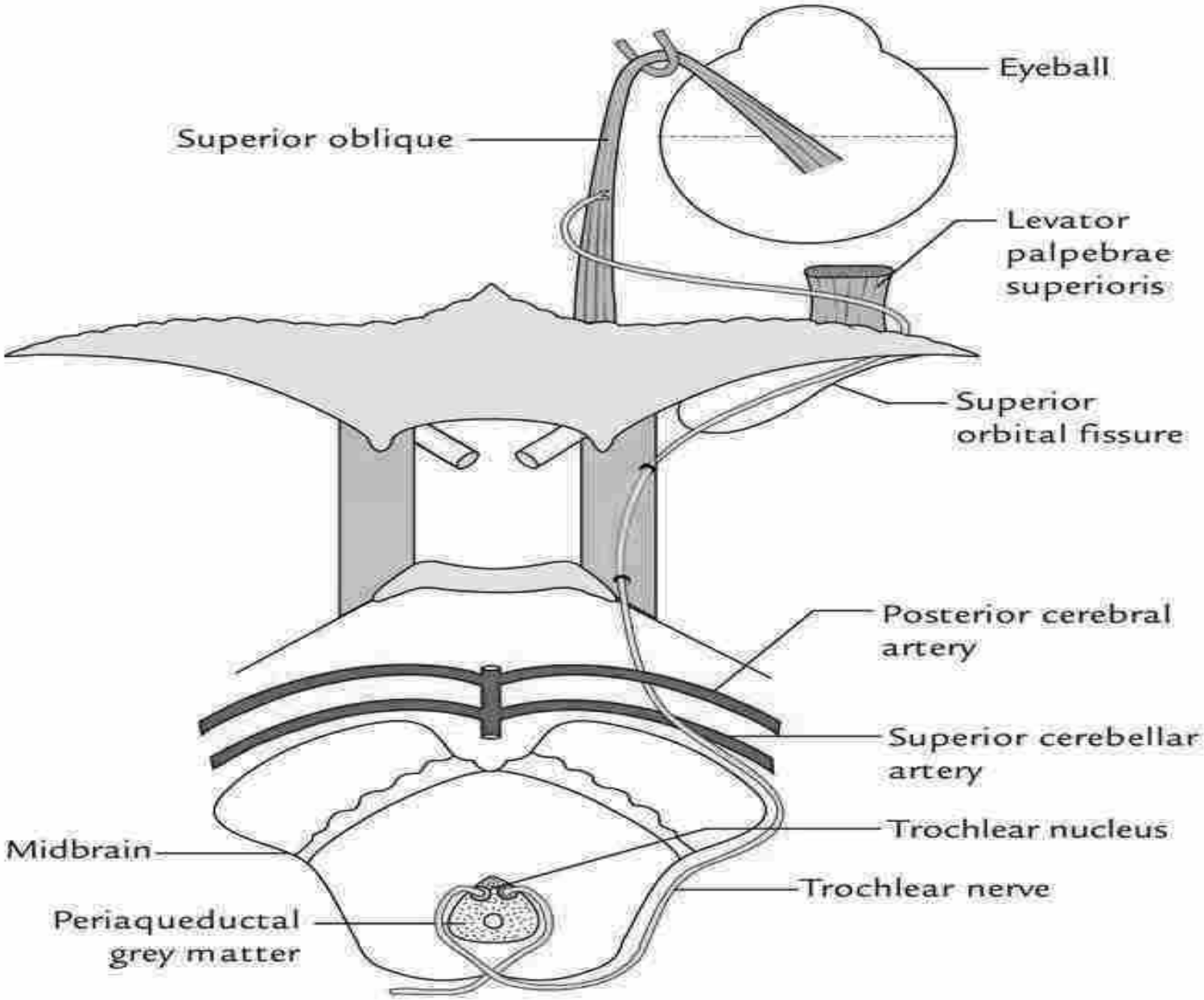
Course of Trochlear Nerve



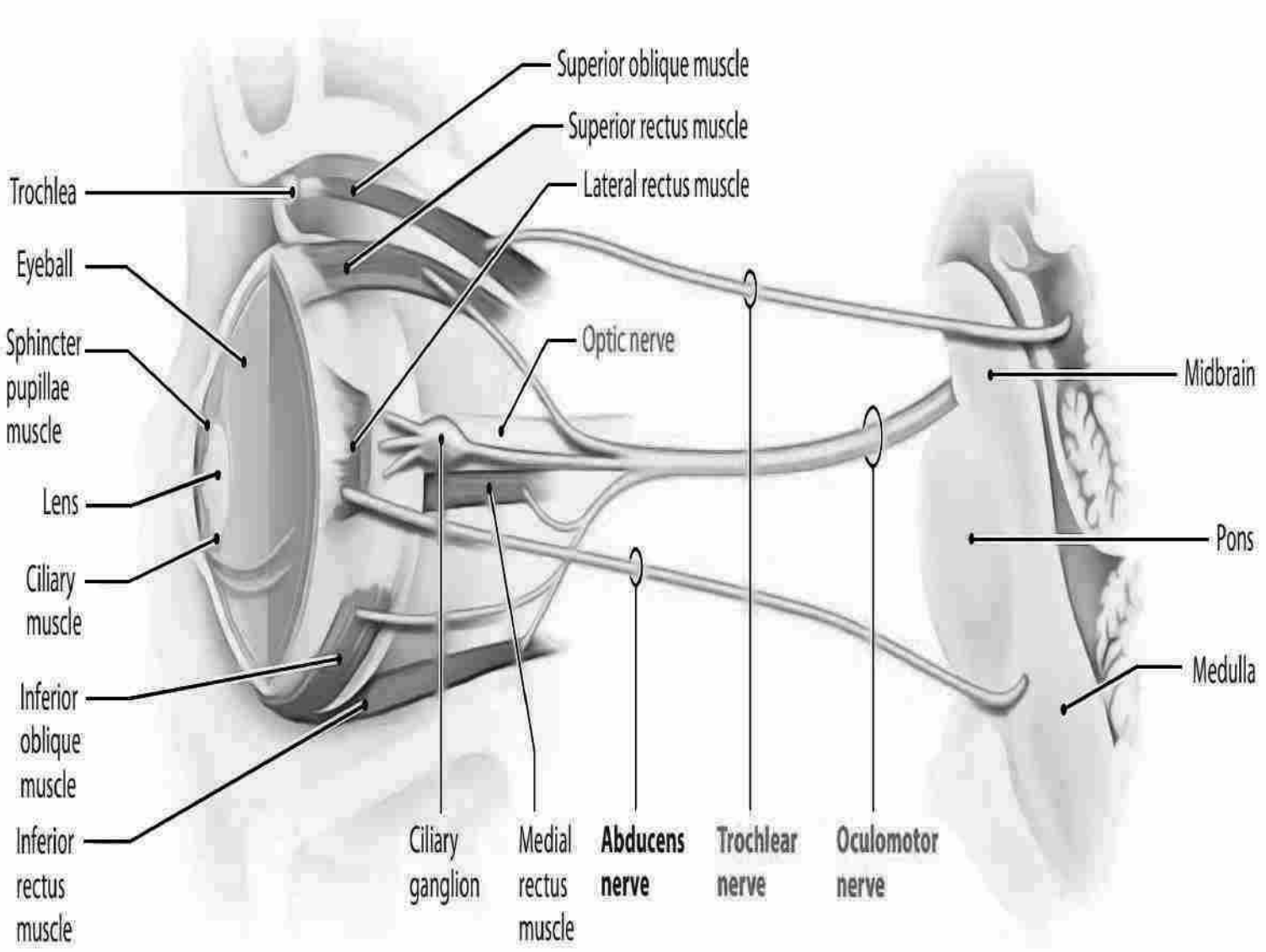

Trochlear nerve [IV]

Course and distribution

- The trochlear nerve arises from the dorsal aspect of the midbrain one on either side of frenulum veli
- After emerging from the brain the nerve winds around the superior cerebellar peduncle and cerebral peduncle just above the pons
- It then passes between the posterior cerebral and superior cerebellar arteries to appear ventrally


Eyeball
Superior oblique
Levator palpebrae superioris
Superior orbital fissure
Posterior cerebral artery
Superior cerebellar artery
Trochlear nucleus
Midbrain
Trochlear nerve
Periaqueductal grey matter

- It lies medial to and below the free margin of tentorium cerebelli
- The nerve enter the cavernous sinus by piercing the posterior corner of its roof
- In the cavernous sinus it runs forward in its lateral wall between the oculomotor and ophthalmic nerves
- In the anterior part of sinus it crosses over the oculomotor nerve and becomes lateral to it


Superior oblique muscle
Superior rectus muscle
Lateral rectus muscle
Trochlea
Eyeball
Sphincter pupillae muscle
Optic nerve
Midbrain
Lens
Pons
Ciliary muscle
Medulla
Inferior oblique muscle
Inferior rectus muscle
Ciliary ganglion
Medial rectus muscle
Abducens nerve
Trochlear nerve
Oculomotor nerve

- The nerve enters the orbit through the superior orbital fissure superolateral to the tendinous ring
- It runs medially above the levator palpebrae superioris to enter the orbital surface of the superior oblique which it supplies

Clinical anatomy

Lesions of trochlear nerve

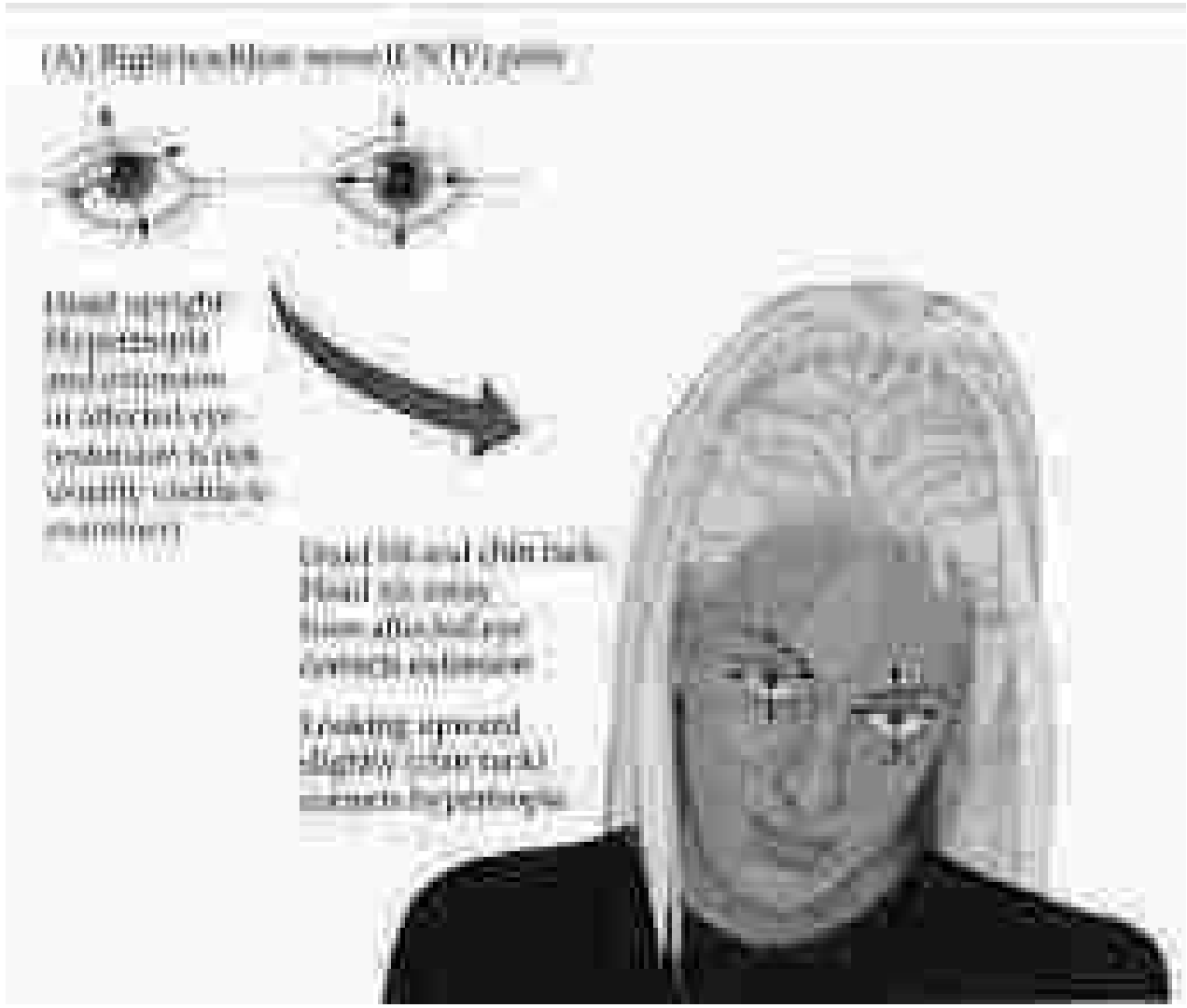
Causes;

- **The injury to trochlear nerve will cause paralysis of the superior oblique muscle of the eye ball**

clinically present as ;

- **Extorsion of eyeball and weakness of downward gaze . As a result the patient faces difficulty while going downstairs or reading newspaper**

- **Diplopia (double vision) which occurs when the patient looks laterally and in glances on looking downward . There is compensatory head tilting to the opposite side**



Lesions & Test of CN IV

- Lesion
- **the eye cannot look down as far as it should when turned in.**
- **The head is tilted towards the opposite shoulder to compensate for extorsion.**
- Test
- AS THE OCULOMOTOR NERVE !!

?

ABDUCENT NERVE

Introduction

- Abducent nerve is the 6th cranial nerve
- It is purely motor and supplies only one muscle – the lateral rectus of the eye ball
- It is so named because it abducts the eye
- It also called lovers eye because in ancient times the boy used to call the girls from a gathering by sending signal through the action of this muscle

Unique feature

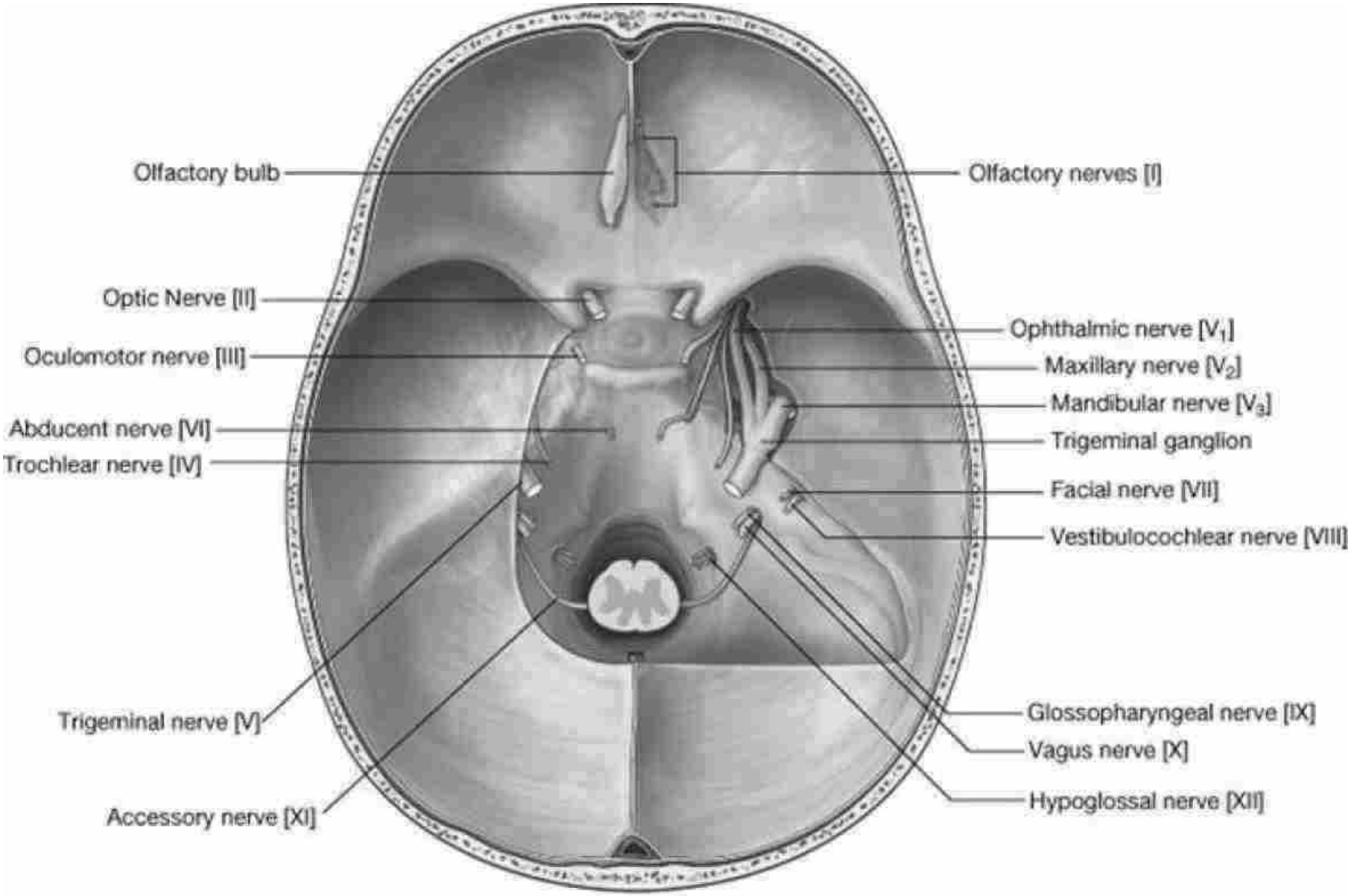
- **It is the most susceptible to damage of all the cranial nerve during increased intracranial pressure**

Functional component and nuclei

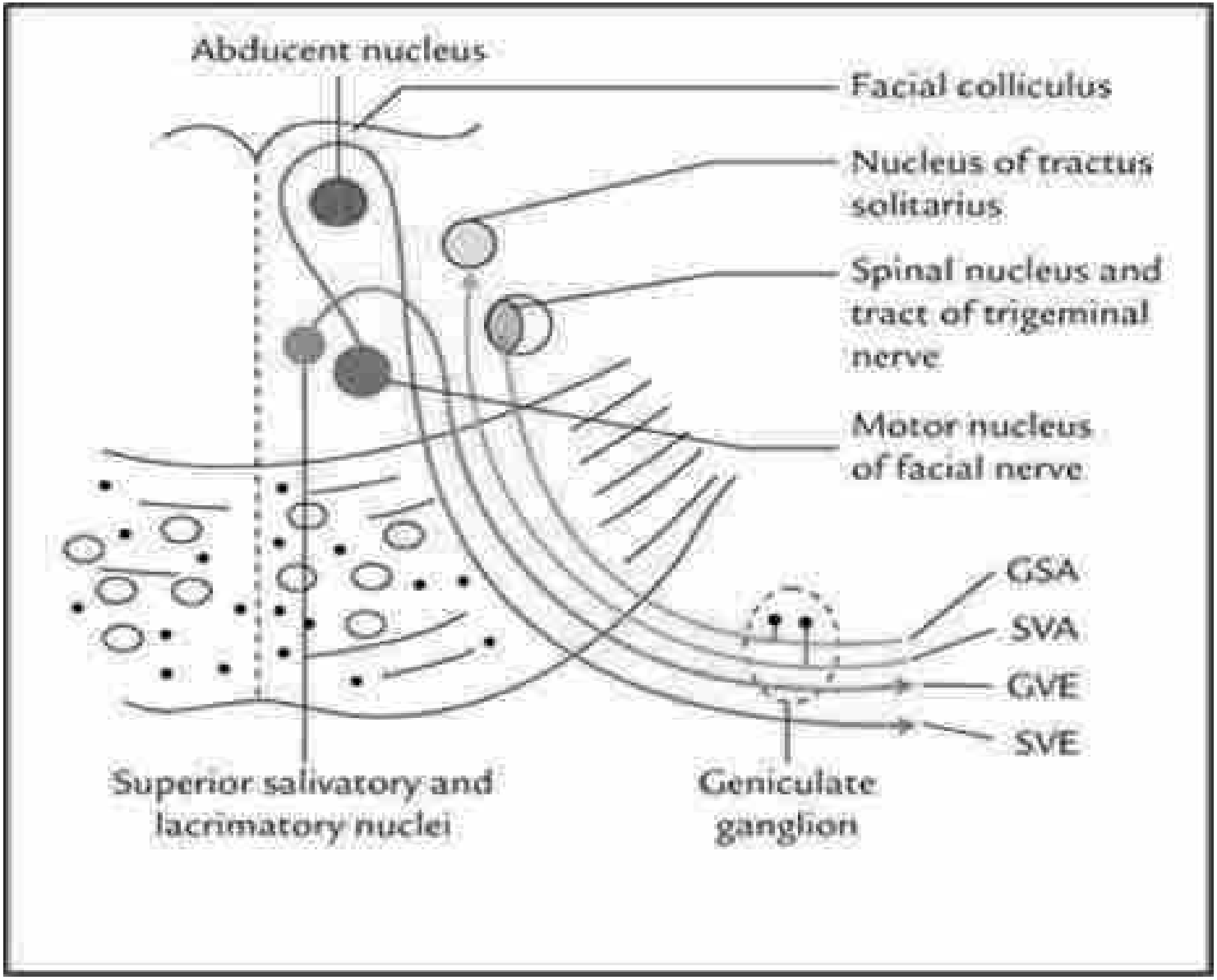
- General somatic efferent fibres; They arise from the abducent nucleus in the pons and supply the lateral rectus muscle of the eye ball
- General somatic afferent fibres; They carry proprioceptive sensations from the lateral rectus and terminate in the mesencephalic nucleus of the trigeminal nerve

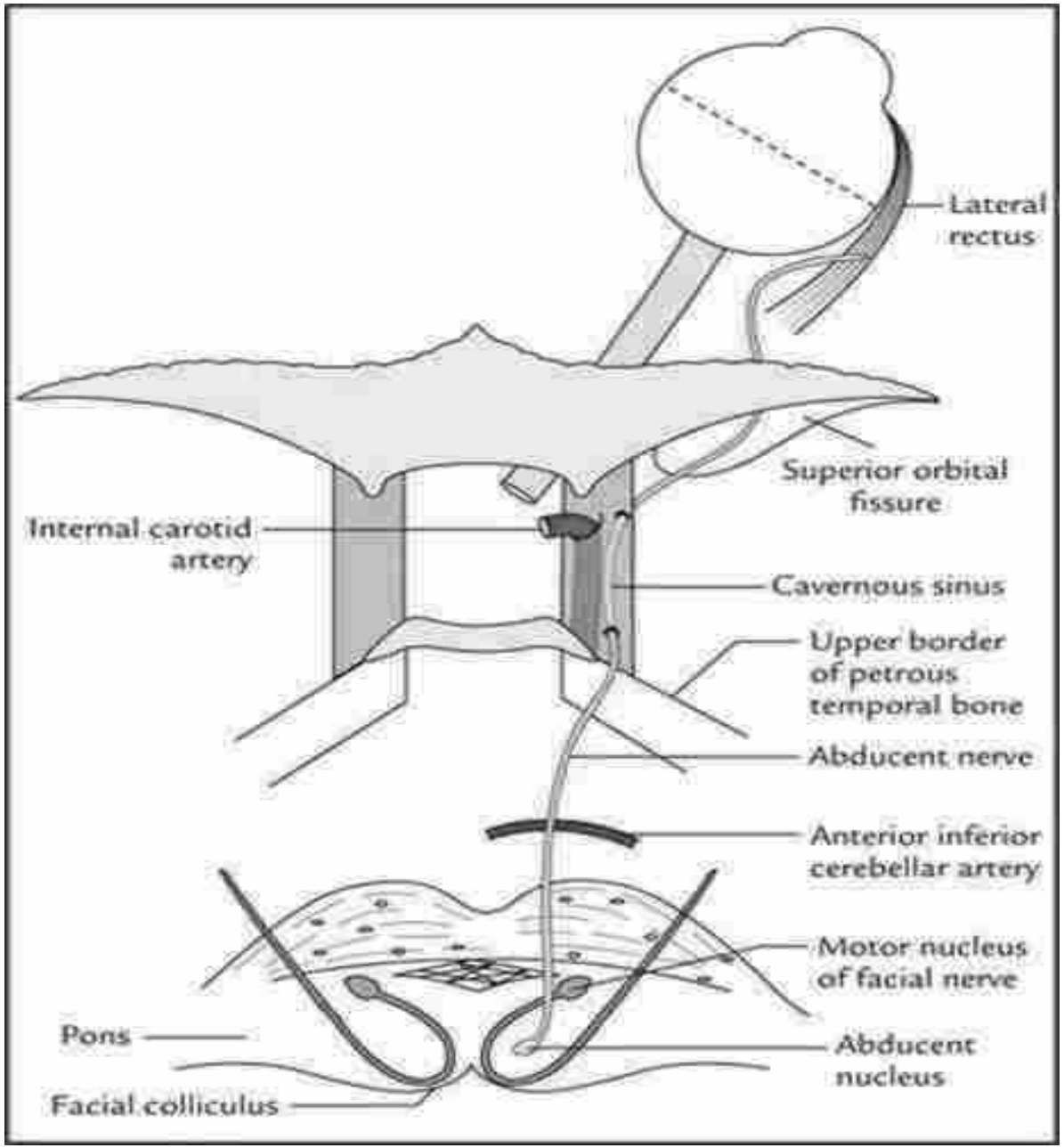
Course Relations and Distribution

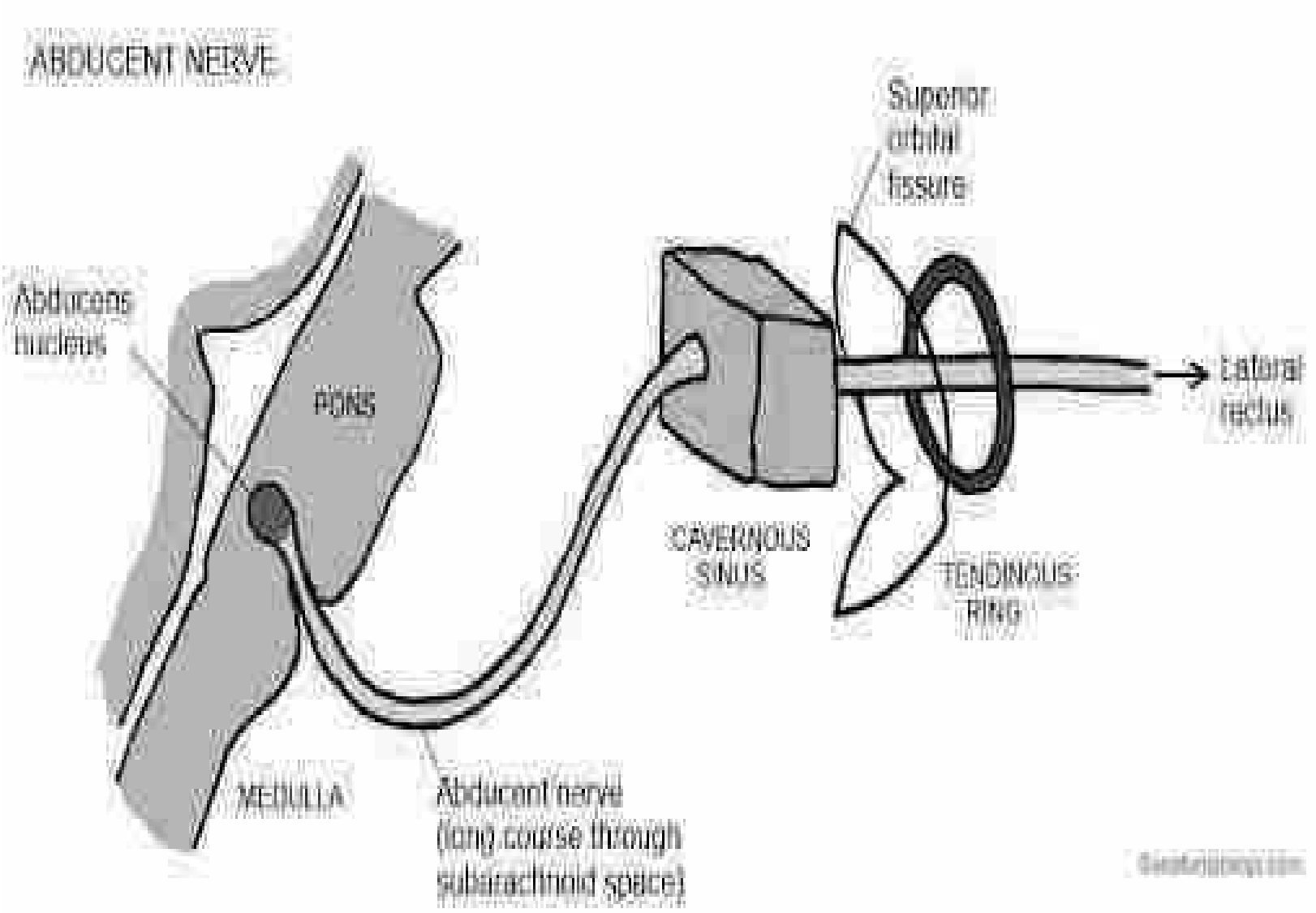
- The abducent nerve arises at the lower border of the pons opposite the pyramid of medulla
- The nerve runs upward , forward , and laterally dorsal to the anterior cerebellar artery and pierces the dura matter over the clivus inferolateral to the dorsum sellae



© Elsevier Ltd. Drake et al: Gray's Anatomy for Students www.studentconsult.com


Abducent nucleus
Facial colliculus
Nucleus of tractus solitarius
Spinal nucleus and tract of trigeminal nerve
Motor nucleus of facial nerve
GSA
SVA
GVE
SVE
Superior salivatory and lacrimatory nuclei
Geniculate ganglion


Lateral rectus
Superior orbital fissure
Internal carotid artery
Cavernous sinus
Upper border of petrous temporal bone
Abducent nerve
Anterior inferior cerebellar artery
Motor nucleus of facial nerve
Pons
Abducent nucleus
Facial colliculus


ABDUCENT NERVE
Superior orbital fissure
Abducens nucleus
PONS
Lateral rectus
CAVERNOUS SINUS
TENDINOUS RING
MEDULLA
Abducent nerve (long course through subarachnoid space)

- It then passes through the medial wall of the inferior petrosal sinus and arches forwards directly over the sharp ridge of the petrous temporal bone under the petro clinoid ligament and enters the fibro – osseous canal (Dorello's canal) formed by the apex of the petrous temporal bone and petroclinoid ligament (Gruber's ligament)
- The nerve than enters the cavernous sinus by piercing the posterior wall close to the floor of the sinus

- In the cavernous sinus it runs forward inferolateral to the internal carotid artery
- The nerve enters the orbit through the superior orbital fissure within the tendinous ring lateral to two divisions of oculomotor and nasociliary nerves
- In the orbit it runs forwards toward the lateral side to enter the side orbital surface of the lateral rectus muscle which it supplies

Clinical anatomy

Lesions of abducent nerve

- **The abducent nerve is generally damaged during increased intracranial pressure**
- **During increased intracranial pressure , the nerve is stretched due to descent of brainstem**
- **Consequently the nerve is cut by the sharp bony edge of the petrous temporal bone**

The paralysis of lateral rectus muscle following the injury to the abducent nerve leads to

- Convergent squint due to the unopposed action of medial rectus
- Inability to abduct the eye
- Diplopia (double vision) with maximum separation of two images while looking toward the paralyzed side

BRONCHOPULMONARY SEGMENTS

Definition

- Well defined anatomical, physiological and surgical sections of lungs are called as bronchopulmonary segments
- Both right and left lung consists of 10 bronchopulmonary segments

Broncopulmanory Segements

- **Right lung**

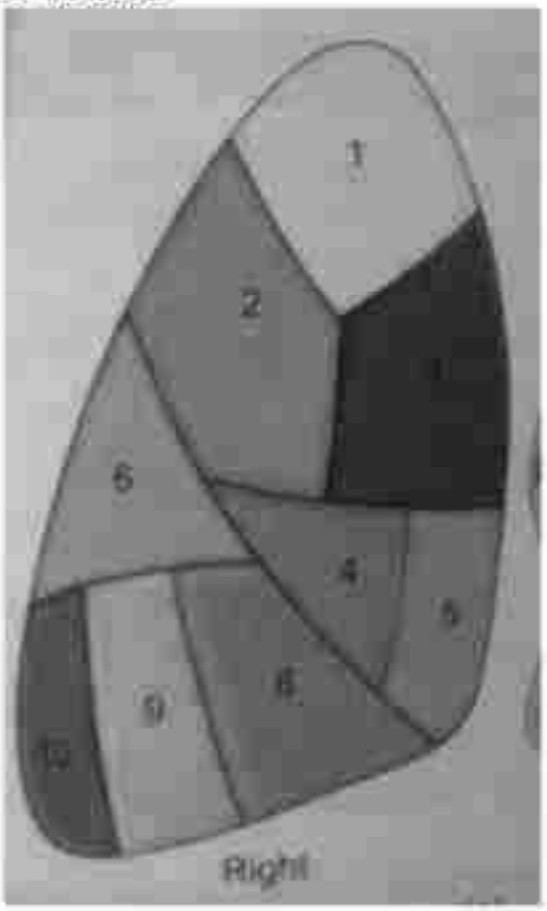
- Subdivided into **three** lobes with **ten segments:**

Right upper lobe
1. Apical
2. Posterior
3. Anterior

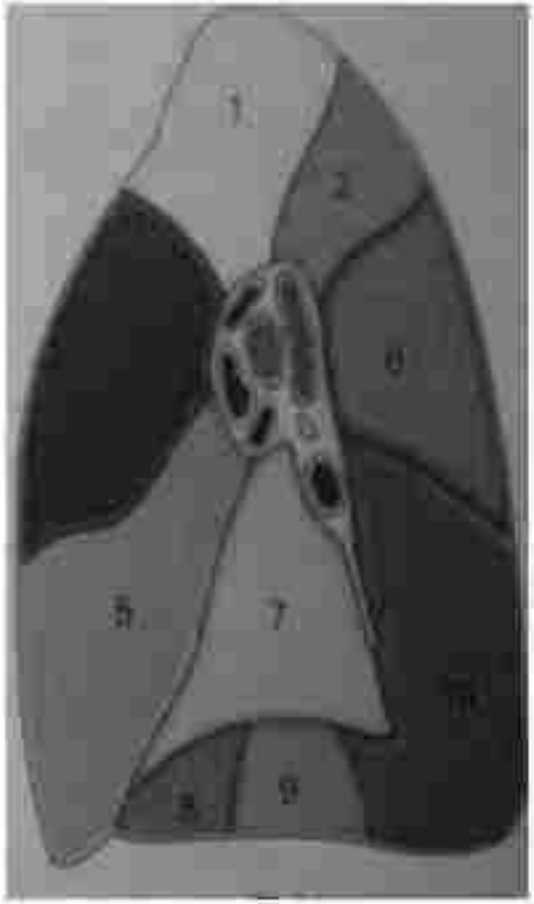
Right middle lobe
4. Lateral
5. Medial

Right lower lobe
6. Superior
7. Medial Basal
8. Anterior Basal
10 Posterior Basal
9 Lateral Basal

Costal aspect



Medial Surface



Broncopulmanory Segements

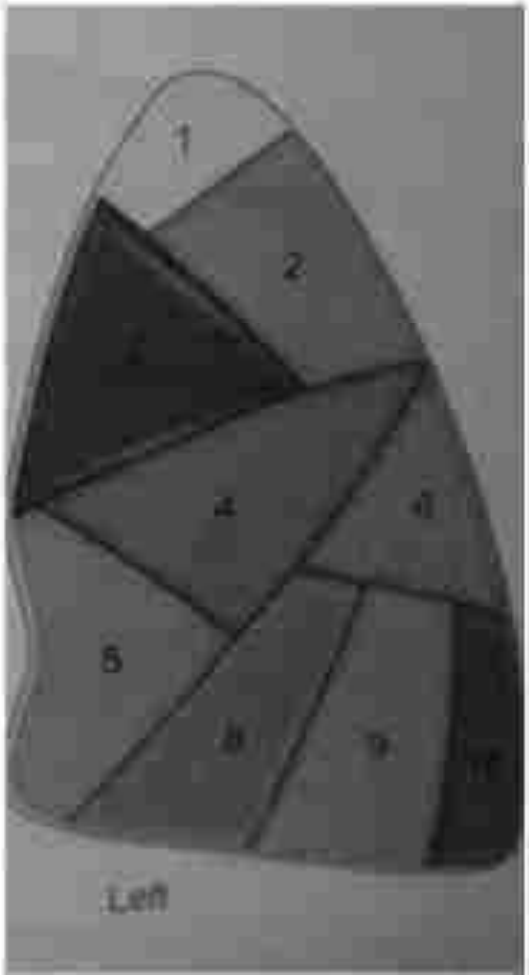
- **Left Lung**

- Subdivided Into Two Lobes With **Ten Segments** :

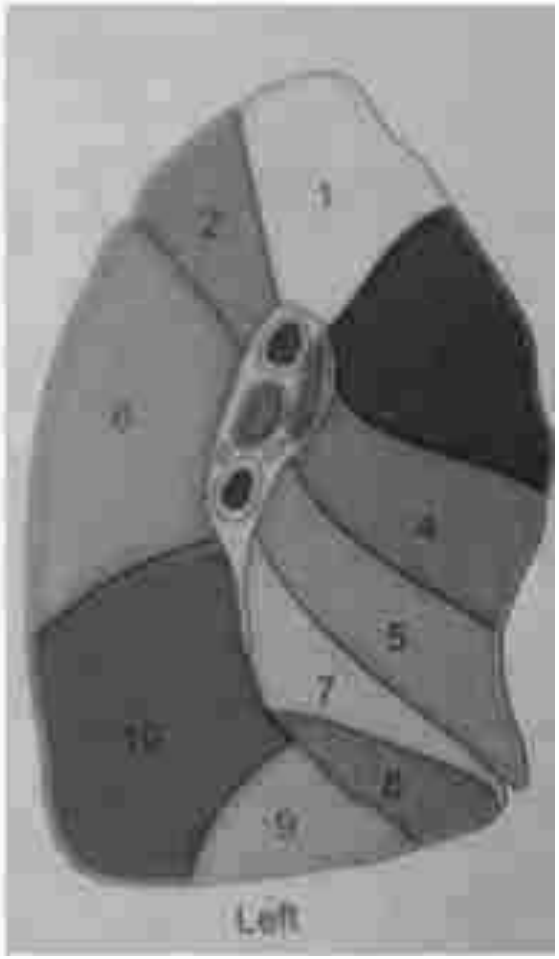
- **Left Upper Lobe**
  - 1. Apical
  - 2. Posterior
  - 3. Anterior
  - **4. Superior Lingular**
  - **5. Inferior Lingular**

- **Left Lower Lobe**
  - 6. Superior
  - 7. Medial Basal
  - 8. Anterior Basal
  - 10. Posterior Basal
  - 9. Lateral Basal

Costal aspect



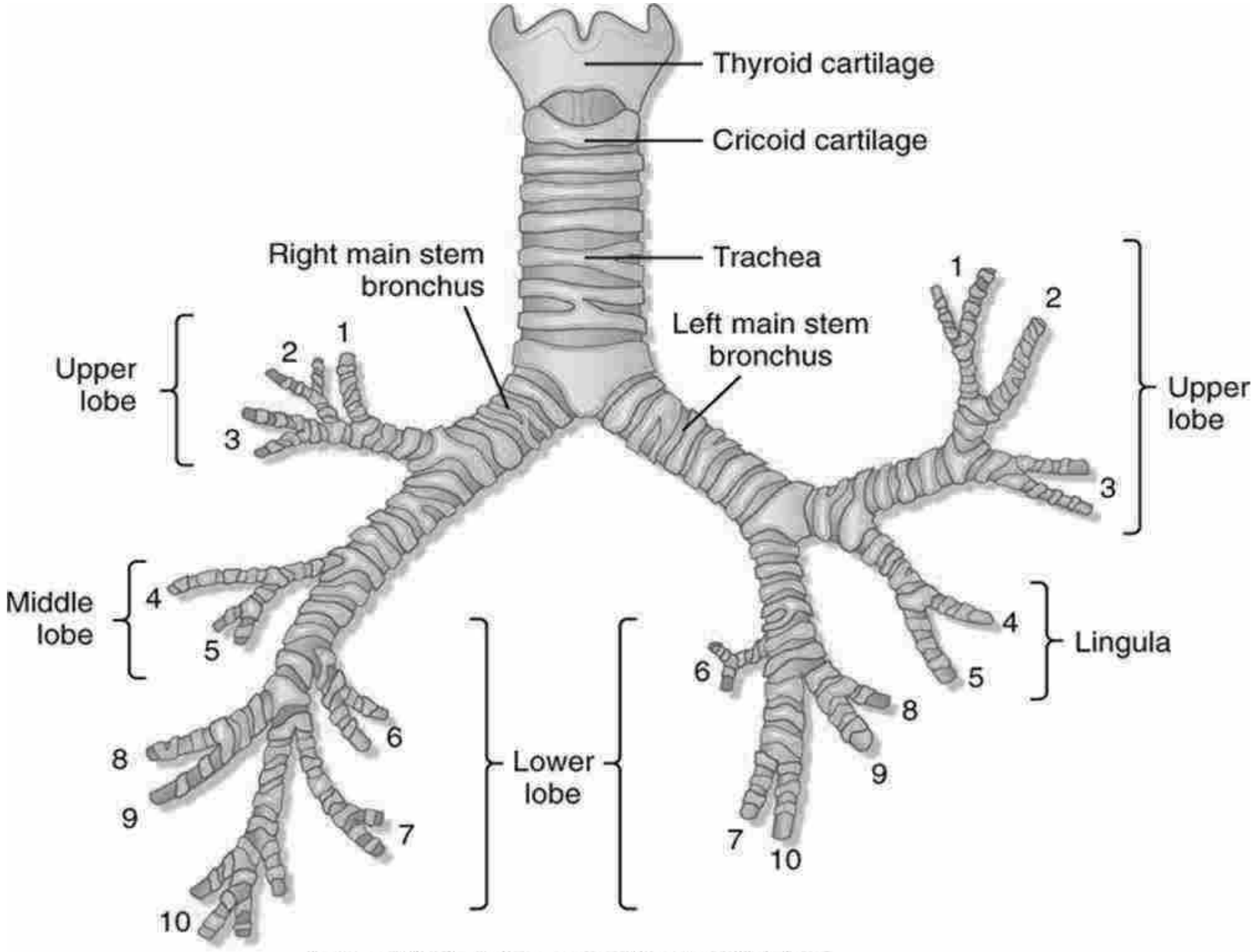
Medial Surface



Features

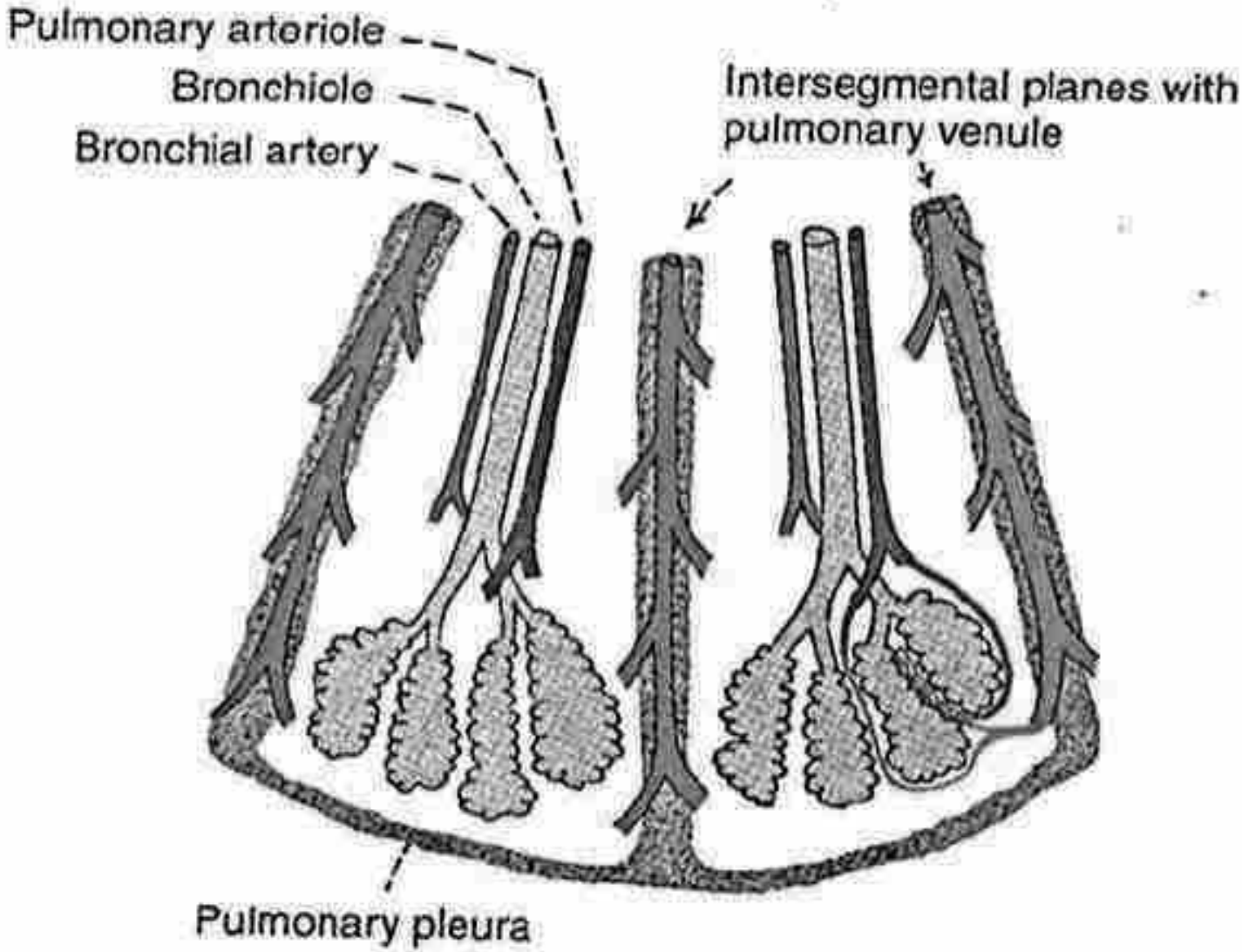
- Each one is aerated by segmental bronchus or tertiary bronchii
- Each segment is pyramidal in shape with its apex directed towards root of the lung

- Each segment has segmental bronchus segmental artery , autonomic nerves and lymph vessels
- The segment venules lies in the connective tissue between the adjacent pulmonary units of bronchopulmonary segment



Koeppen & Stanton: Berne and Levy Physiology, 6th Edition.
Copyright © 2008 by Mosby, an imprint of Elsevier, Inc. All rights reserved

Intersegmental course of pulmonary vein



Relations

To pulmonary artery

- The branches of pulmonary artery accompany the bronchi
- The artery lies dorsolateral to the bronchus, thus each segment has its own separate artery

To pulmonary vein

- The pulmonary vein accompany the bronchii or pulmonary arteries
- They run in intersegmental planes
- Thus each segment has more than one vein and each vein drains more than one segment

- Near the hilum it lies ventromedial to bronchus
- It is considered as bronchopulmonary segments not bronchovascular segment since it contain its own segmental bronchus not its own segmental veins

Significance

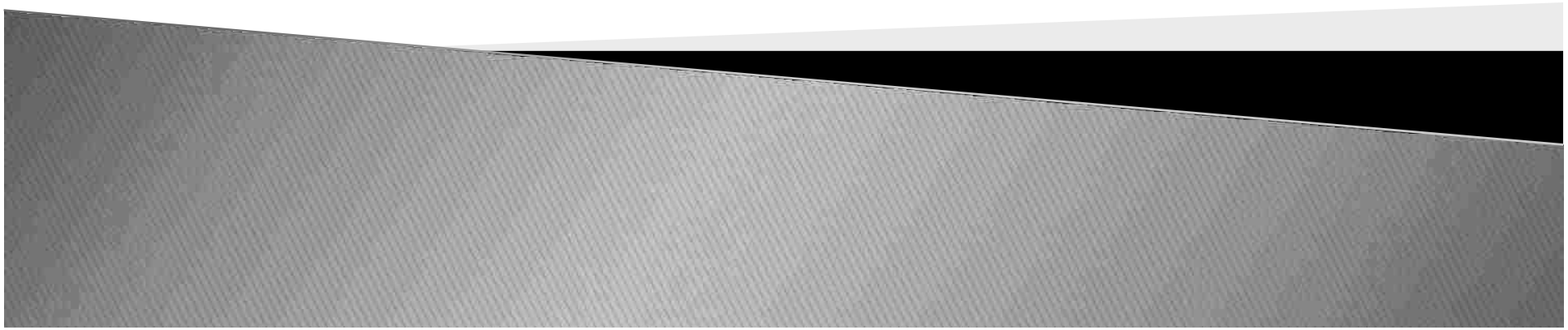
- Surgical/segmental resection; If any part of the lung is infected the surgeons removed only the particular segment of the affected area without affecting the other parts

Clinical anatomy

- **Infection**; Infection to any bronchopulmonary segment is restricted to that segment only not spread to other segments exceptions are **Tuberculosis** and **Bronchogenic carcinoma**
- **Bronchoscopy; To** visualise the interior of bronchi

THANKYOU

CONNECTIVE TISSUE



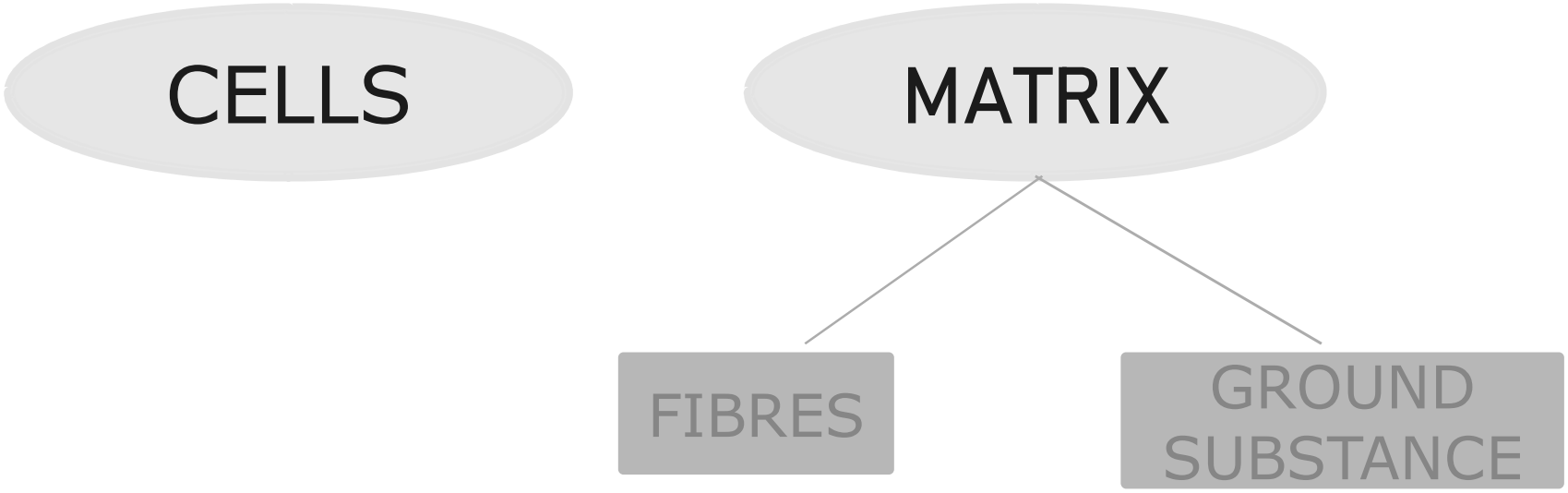
Definition

Connective tissue are a group of tissues which connects or binds other tissues in our body

FUNCTIONS

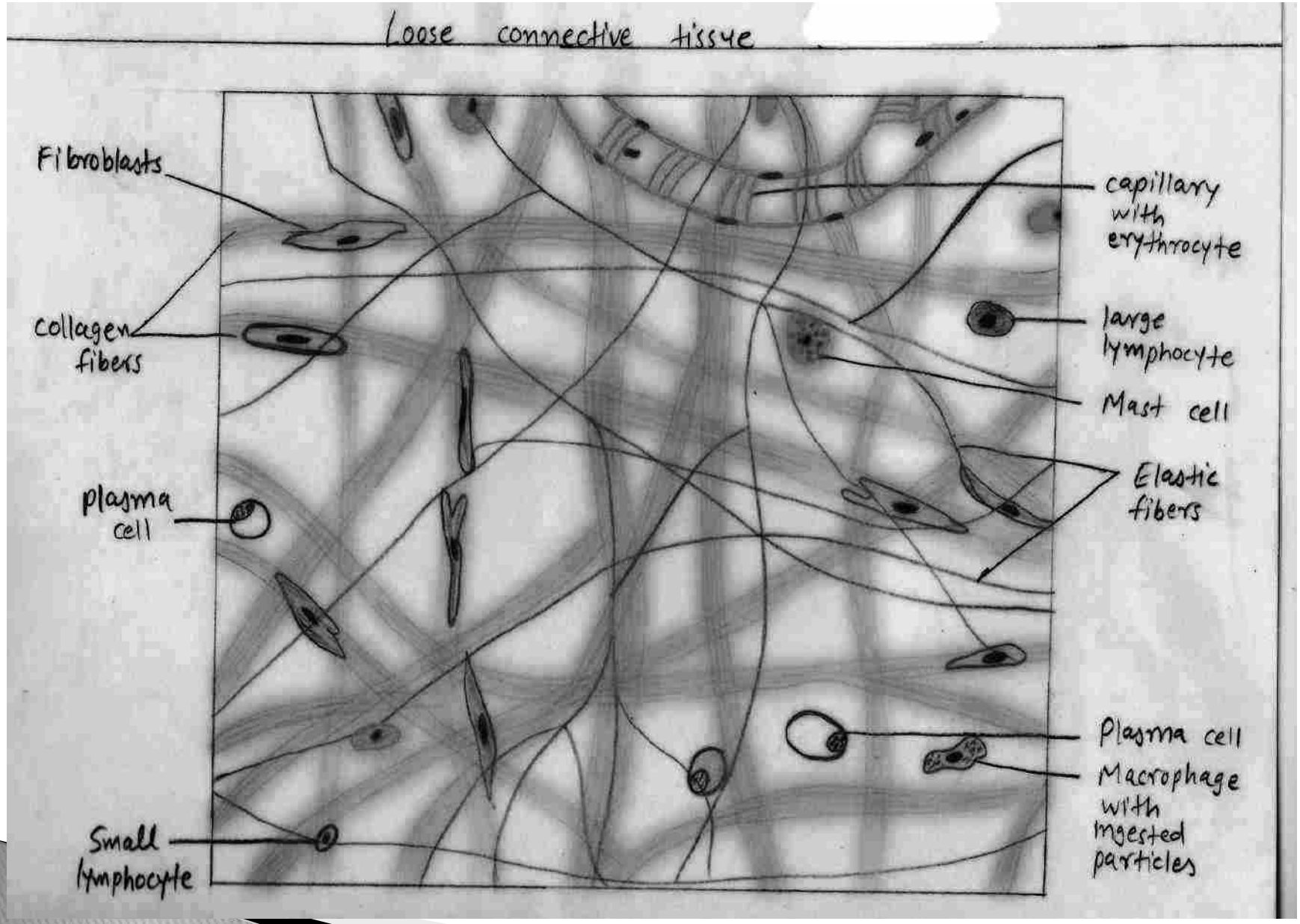
- Mechanical support
- Medium for exchange of nutrients and waste products
- Energy source and thermal insulations
- Defensive function: *Barriers, Engulf bacteria, Antibodies*

COMPONENTS



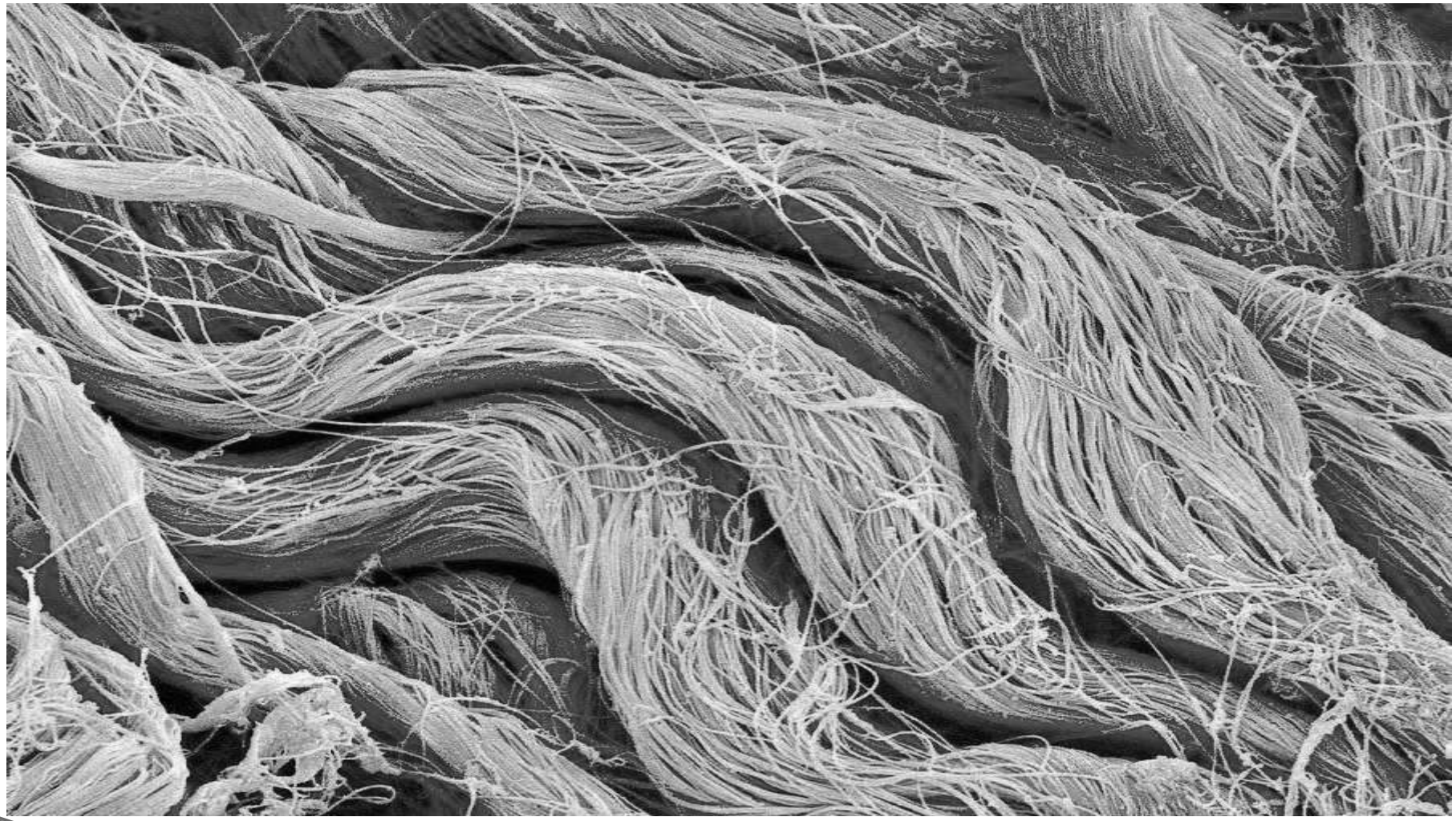
Fibres

- Collagen fibres
- Reticular fibres
- Elastic fibres


Loose connective tissue
Fibroblasts
collagen fibers
plasma cell
Small lymphocyte
capillary with erythrocyte
large lymphocyte
Mast cell
Elastic fibers
Plasma cell
Macrophage with ingested particles

COLLAGEN FIBRES

- Most abundant type of connective tissue fibres
- Within the light microscope these are seen in bundles
- Bundles may be wavy or straight depending how much they are stretched
- The bundles are made up of collection of individual fibres which which are 1-2 micro meter in diameter

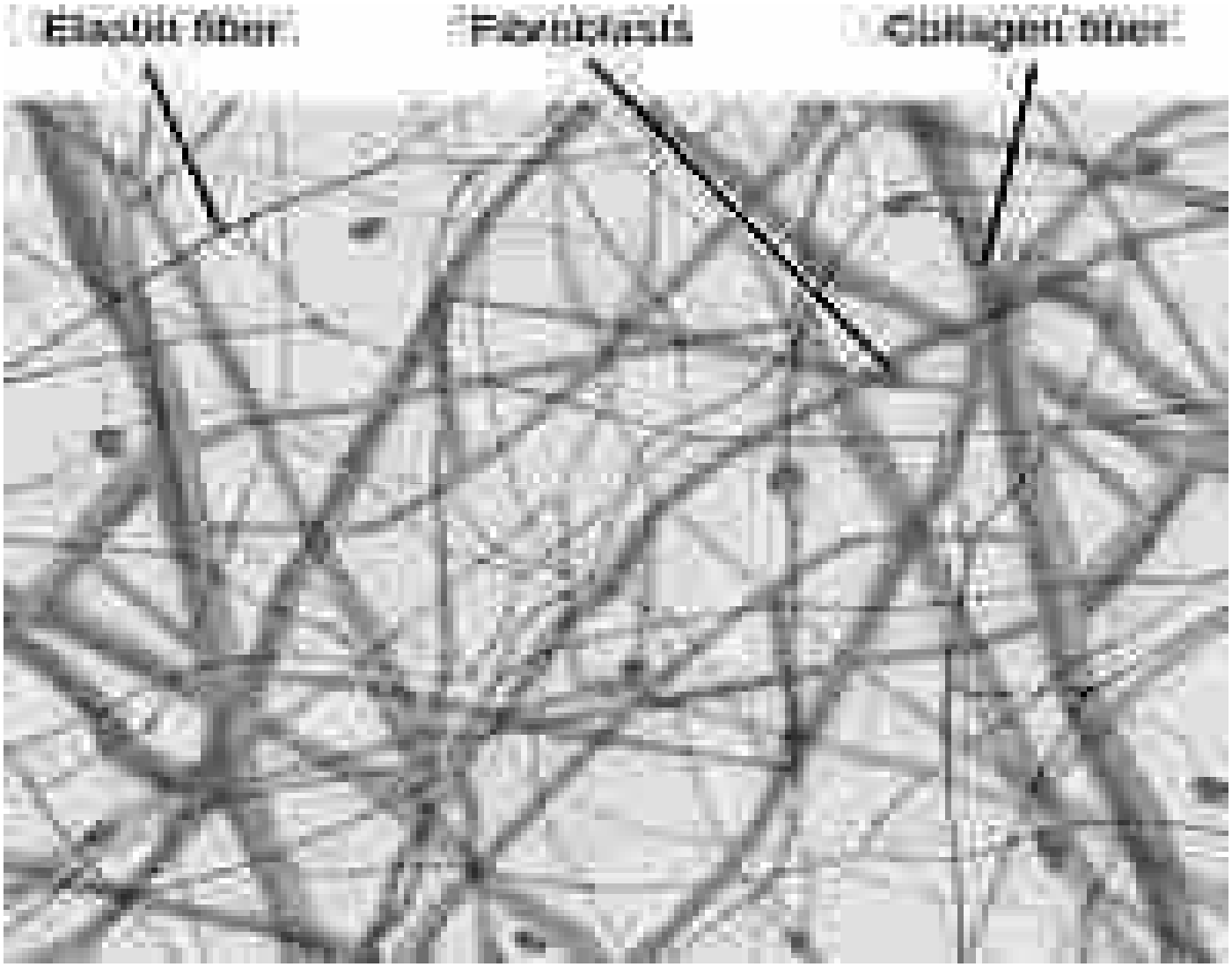


- Bundles of fibres white with unaided eye.

- Collagen fibres mainly made of a protein called collagen, which is made up of molecules called tropocollagen.
- It can resist considerable tensile forces without significant increase in their length.
- The are pliable and can bend easily
- It becomes swell and soft when treat with weak acids , destroyed by strong acids and converted into gelatin on boiling

- Tropocollagen is synthesised by *fibroblast* cells and released into extracellular space

  where they polymerised into collagen fibres.
- It is also synthesised by *chondroblasts* in cartilage,*osteoblast* in bone and smooth muscle in blood vessel


Elastin fiber
Fibroblasts
Collagen fiber

Types and distribution;

Type1;

- Found in connective tissue , tendon, ligaments, fasciae, aponeuroses etc..
- Also found in dermis of the skin and meninges
- They are of large diameter and have prominent cross striations
- These forms the fibrous basis of bones and fibrocartilage

Type2;

- These are of two subtypes. The larger of these are about 100nm in diameter while the narrower fibres are about 20nm
- Striation are less prominent than type1
- These forms the fibrous basis of hyaline cartilage
- Seen in hyaline cartilage

Type3;

- These forms the reticular fibres

Type4;

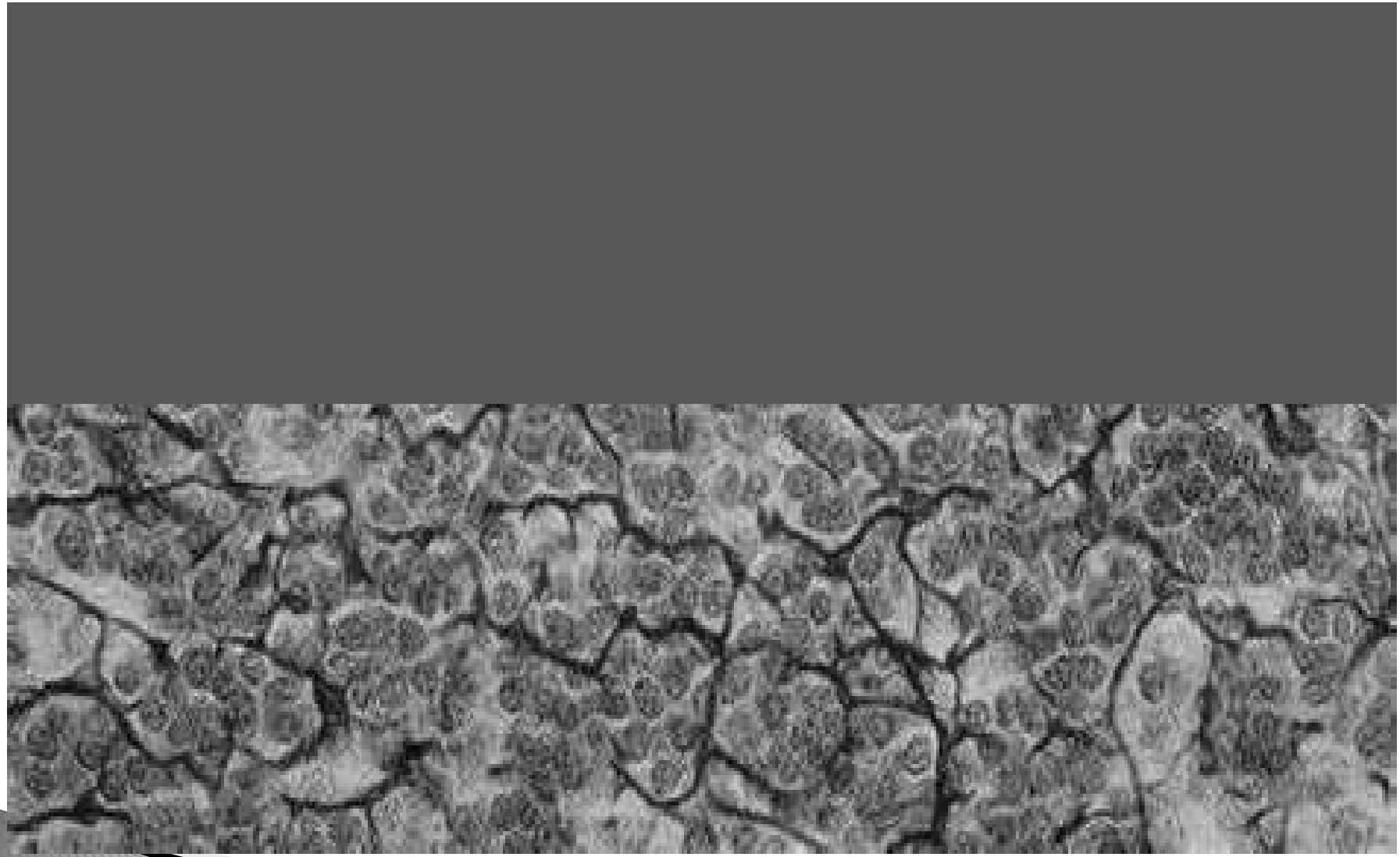
- Consists of short filaments that forms sheets
- It is present in the basement membrane , also seen in the lens capsule

Type5;

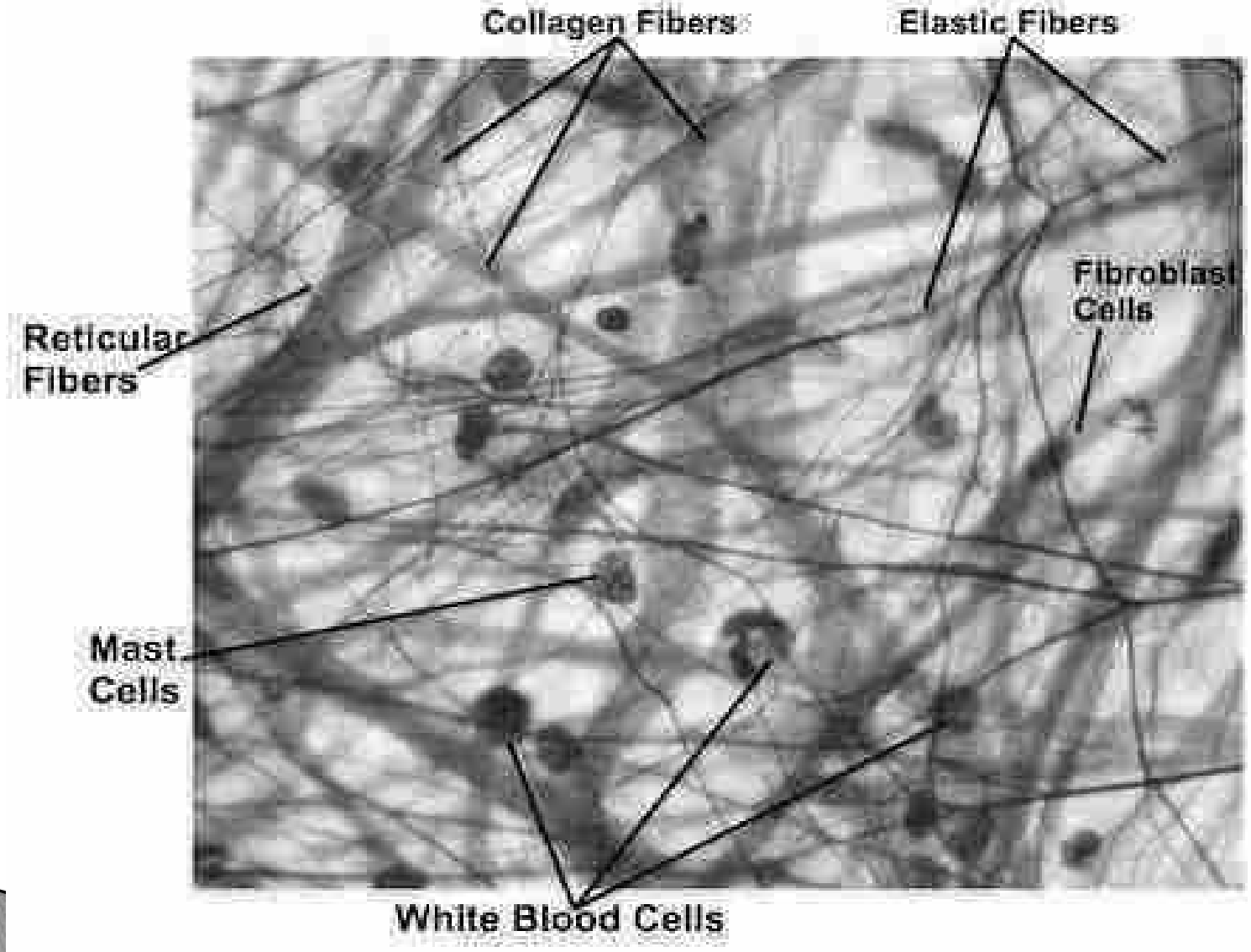
- These type of fibre are seen in blood vessels and foetal membranes

RETICULAR FIBRES

- These are a variety of collagen fibres and produced by type3
- They show striations of 67nm
- They are differ from typical collagen fibres as follows;
  - They are much finer and have uneven thickness
  - They form a network or reticulum by branching or anastomosing with each other



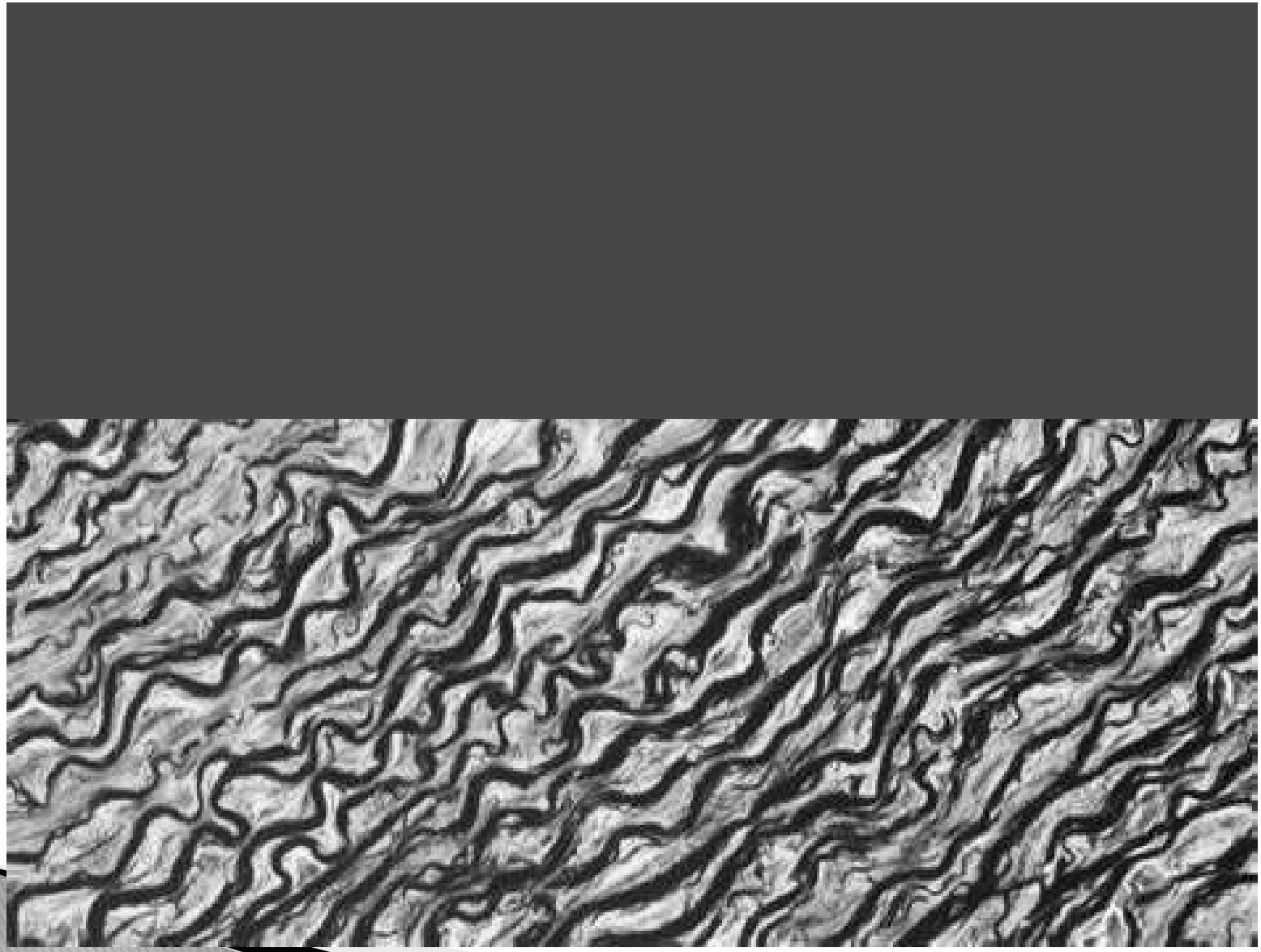
- They do not run in bundles
- They are stained by **silver impregnation** which render them black
- Reticular fibres contain more carbohydrates than type1 fibres
- They provide a supporting frame work in lymphoid organs like spleen , lymph nodes and bone marrow
- Also most glands include liver and the kidneys


Collagen Fibers
Elastic Fibers
Fibroblast Cells
Reticular Fibers
Mast Cells
White Blood Cells

- Reticular fibres forms the essential component of the all basement membrane
- The are also found in relation to the smooth muscle and nerve fibres
- They are synthesised by fibroblalst and reticular cells

ELASTIC FIBRES

- ✓ Elastic fibres are much fewer than collagen
- ✓ They run singly , branch or anastomosis with other fibres.
- ✓ Thinner than those of collagen fibres
- ✓ In some situations it is thick eg.*Ligamentum flava*
- ✓ In some situations they form fenestrated membranes eg. Walls of large arteries



- With the EM each elastic fibre is seen to have a central amorphous core and outer layer of fibrils
- The outer fibrils are made up of glycoprotein called *fibrillin*
- Periodic striations are not present in elastic fibres
- They are not stain with usual stains of collagen
- Stained with orcein andwith aldehyde fuschin

- Elastic fibre mainly consists of a protein called *elastin* that forms the central amorphous core
- Elastin is made up of smaller units called *tropoelastin*
- Elastic fibres are mainly produced by fibroblasts.
- In some situation it is also formed by smooth muscles
- As the name implies it can be stretched and return to original length when tension released

Cells

Cells that are intrinsic component

- Fibroblast cell
- Undifferentiated mesenchymal cells
- Pigment cells
- Fat cells

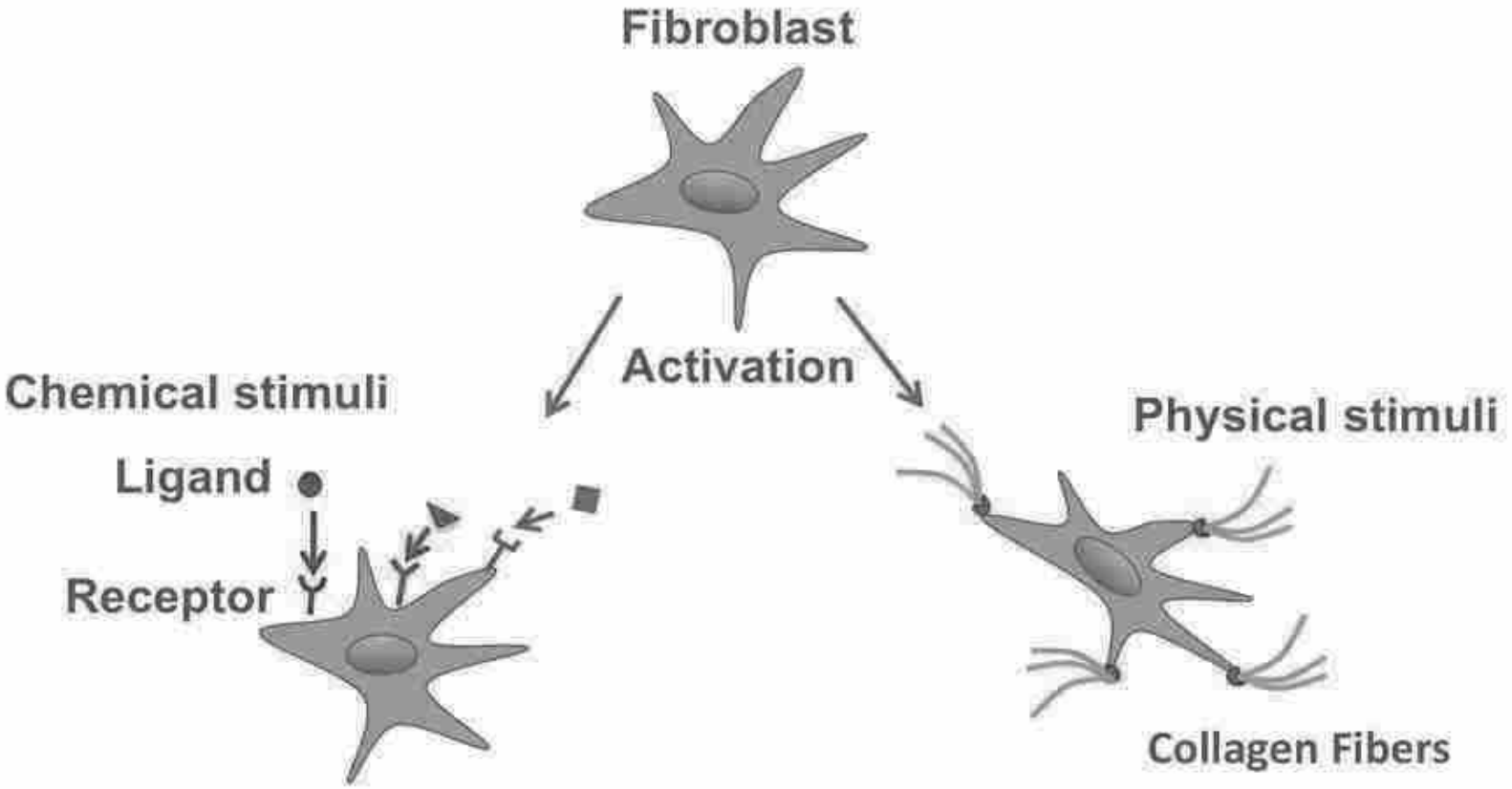
Cells belong to immune system

- Macrophage cells
- Mast cells
- Lymphocytes
- Plasma cells
- Monocytes
- Eosinophil

FIBROBLASTS

- Most numerous cells of connective tissue
- They are so called because they are concerned with the production of collagen fibre
- They are also produce reticular and elastic fibres
- This cells which produce reticular fibres are called as reticular cells
- They are present so close to the collagen fibres

FIBROBLAST ACTIVATION



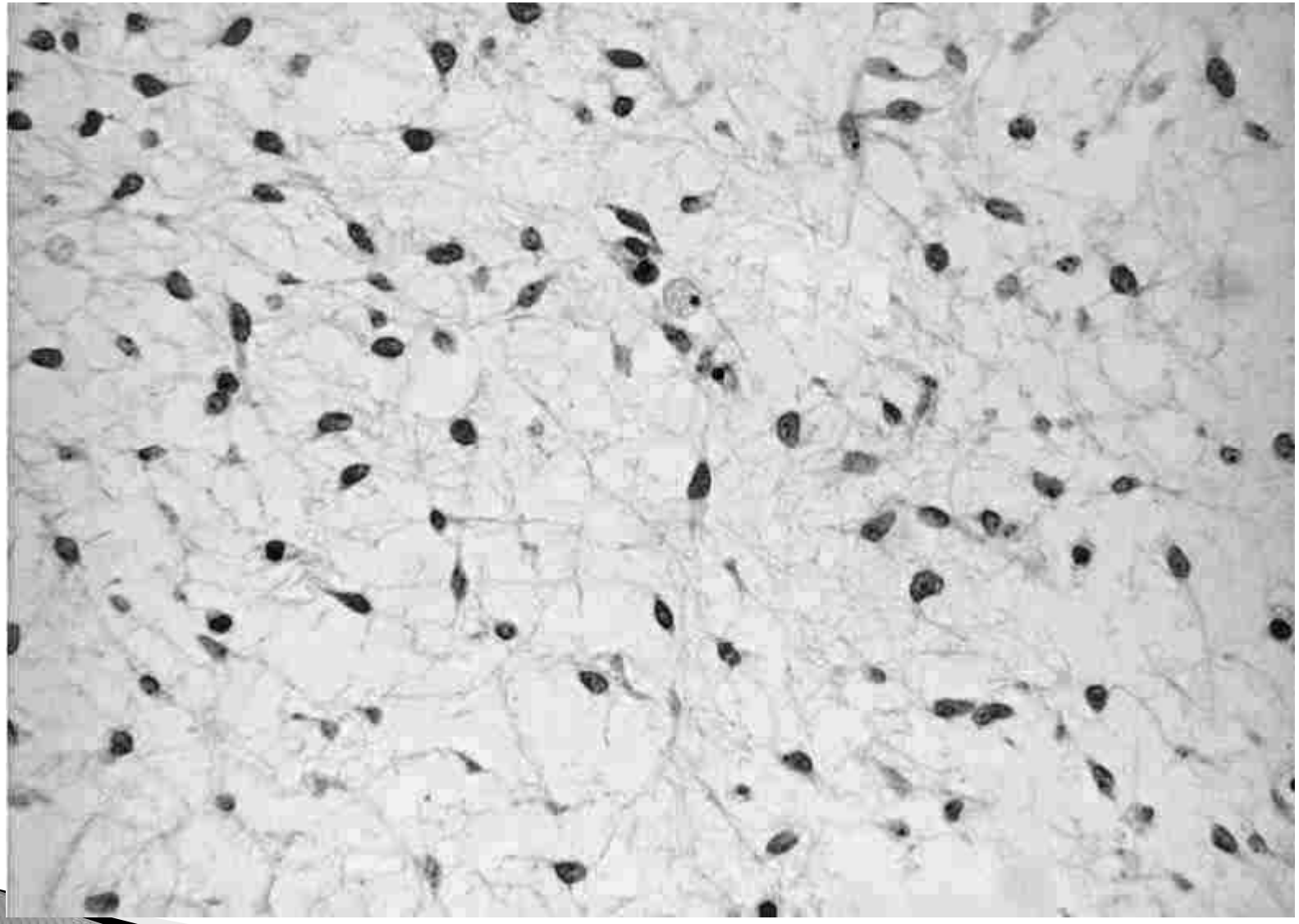
All rights reserved. © 2011 Copyright MINERVA Research Labs Ltd

- They are fixed cells
- IN tissue sections these appears to be spindle shaped and the nucleus appears to be flattened
- When seen from the surface cells show branching process
- Nucleus is large , euchromatic and has prominent nucleoli
- In inactive fibroblast cytoplasm is scanty cell oraganelles are few and nucleus become heterochromatic

- Inactive fibroblasts is often called as *fibrocytes*
- In active fibroblast, abundant cytoplasm and also more conspicuous cell oraganelles are also seen
- Fibroblast become active when there is need to lay down collagen fibre eg.wound repair

UNDIFFERENTIATED MESENCHYMAL CELLS

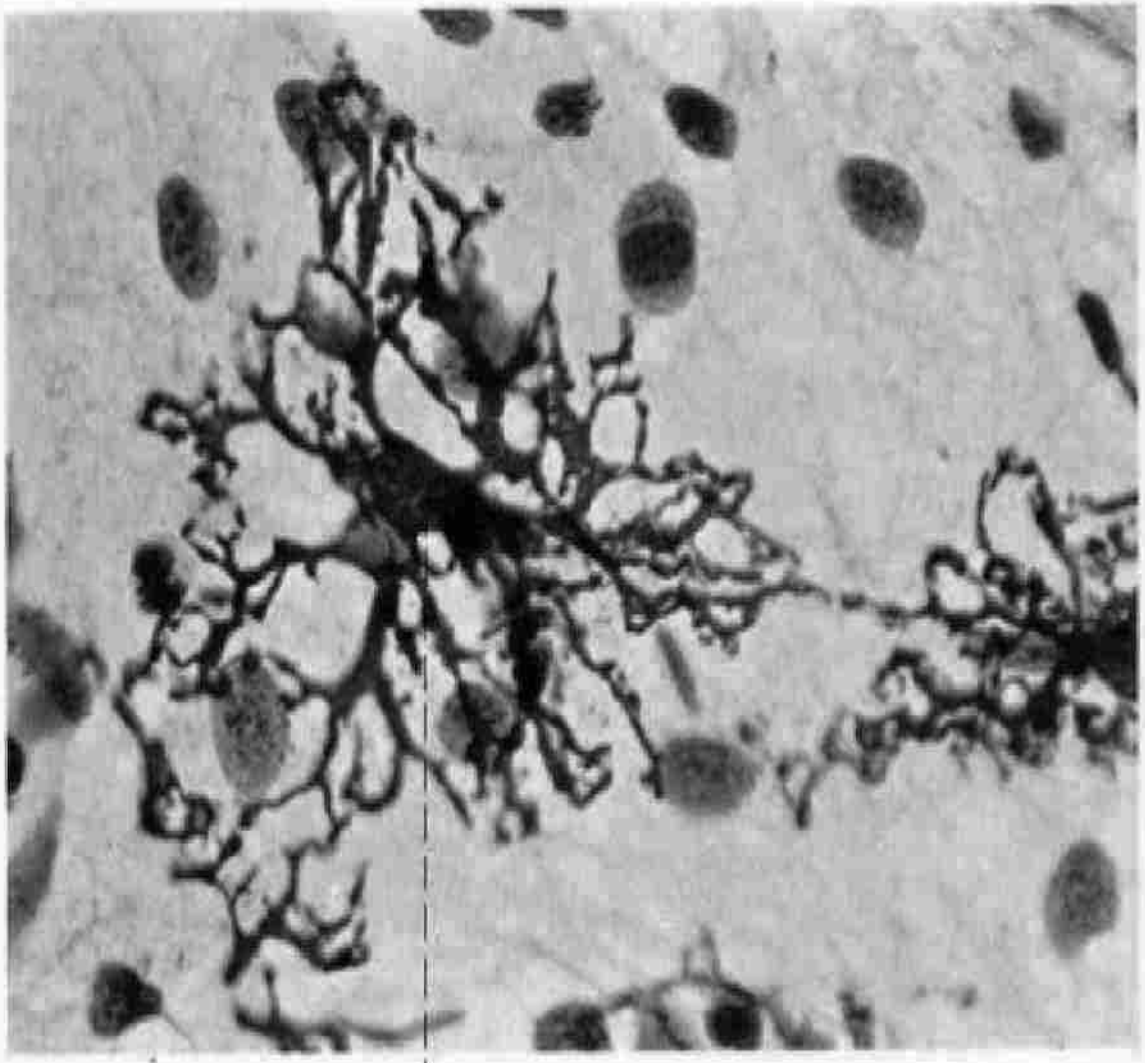
- Embryonic connective tissue is called mesenchyme
- It is made up of stellate small cells with slender branching process that join to form a fine network called as *undifferentiated mesenchymal cells*



PIGMENT CELLS

- They are easily distinguished as they contain brown pigment (melanin)
- They are most abundant in connective tissues of the skin and in the choroid and iris of the eyeball
- They give dark colour to skin , iris and choroid along with the pigment containing epithelial cells
- Variations in the number of pigment cells and the amount of pigment present differnces will be there in different races and in different individual

F. 121



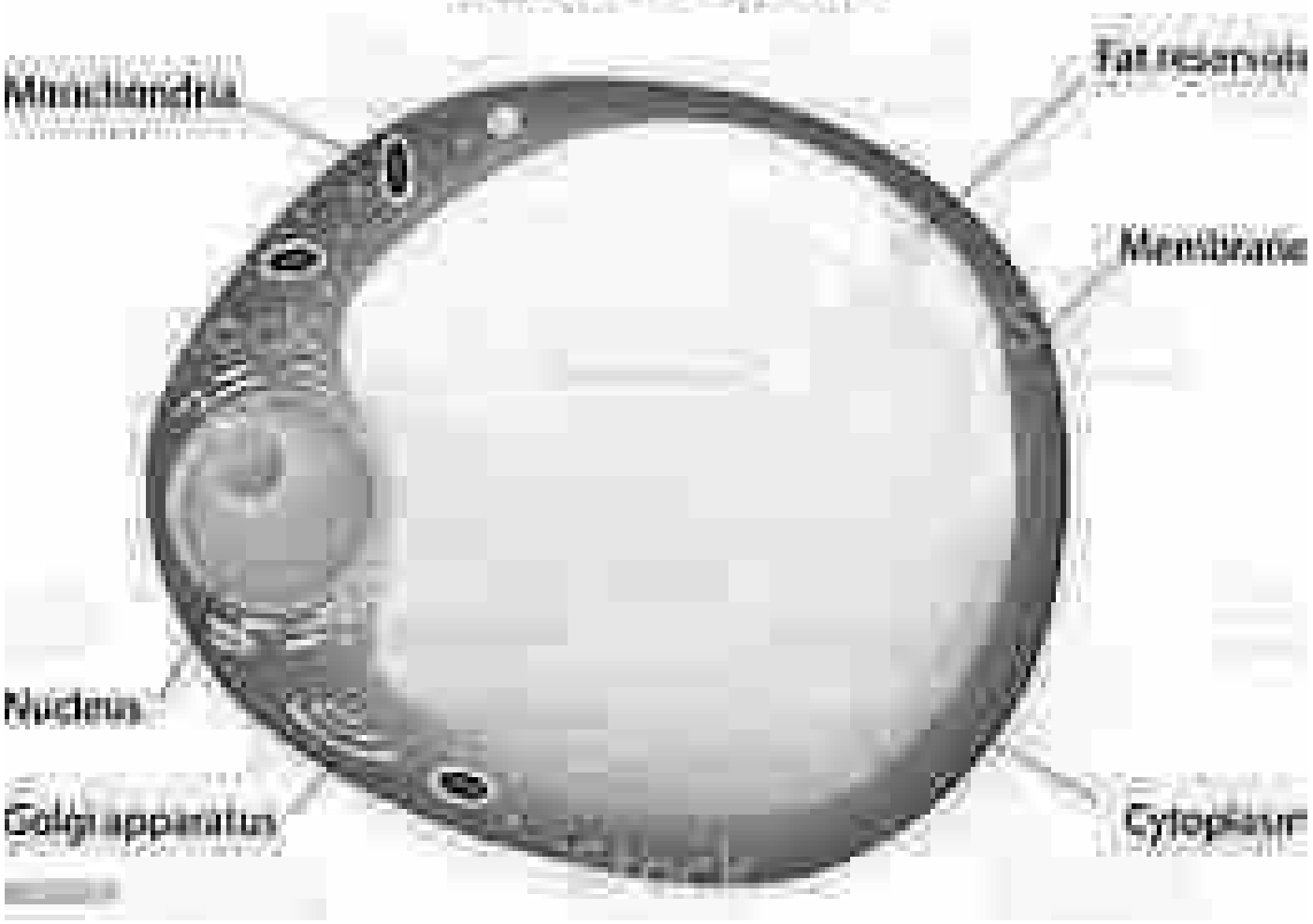
121 *Nucleus of pigment cell*

- Of the many cells that contain pigment in their cytoplasm only a few are actually capable of synthesising melanin such cells are called *melanocytes*
- Remaining cells engulfed the pigment that released by the other cells , such cells are called as *chromatophores* or *melanophores*

FAT CELLS

- Some cells store fat in large amount and become distended with it. These are called *fat cells or adipocytes*
- Each fat cell contain large droplet of fat that almost fills it
- As a result the cell becomes rounded
- The cytoplasm of the cell forms a thin layer just deep to the plasma membrane
- The nucleus is pushed against the plasma membrane and is flattened resembling a signet ring

ADIPOCYTE


Mitochondria
Fat reservoir
Membrane
Nucleus
Golgi apparatus
Cytoplasm

MACROPHAGE CELLS

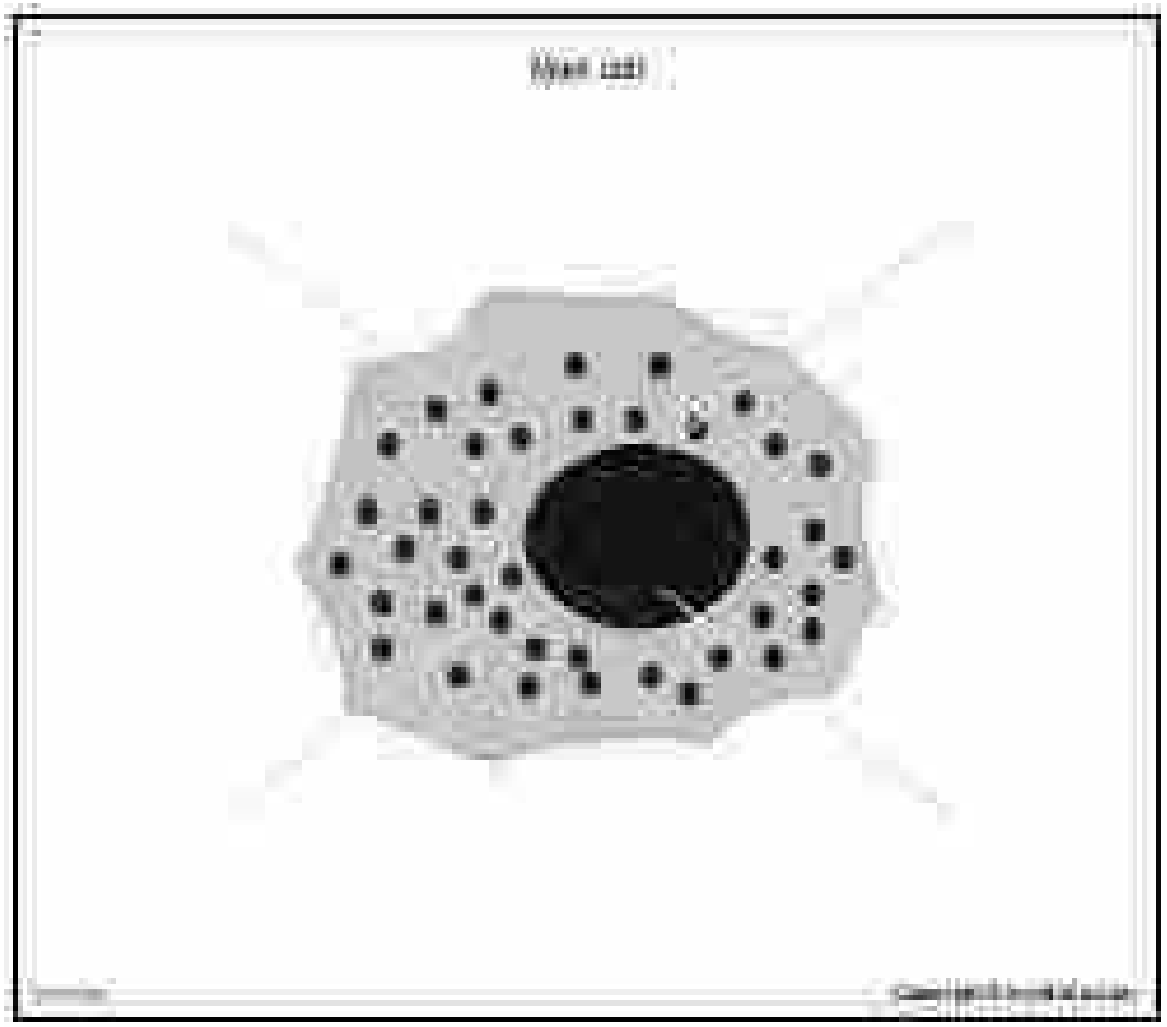
- These are a large series of cells present in the body that have similar functions. These collectively called as *mononuclear phagocyte system*
- Macrophage cells of connective tissue is also called *histiocytes* or *clasmatocytes*
- They have the ability to phagocytose the unwanted material such as bacteria which invades tissues and damaged tissues
- It also phagocytose the the inorganic particles injected into the body

Macrophage
© Can Stock Photo

- Macrophages are usually fixed but when they are attached to fibres they become free or motile
- Fixed macrophages resemble fibroblasts in appearance , but free macrophages are rounded
- The nuclei of macrophages are smaller and are of kidney shaped.
- Cytoplasm contains numerous lysosomes which help in digesting the phagocytosed material
- Sometimes macrophages may fuse together to form multinucleated giant cells

MAST CELLS

- These are small rounded or oval cells called *mastocytes* or *histaminocytes*
- Nucleus is small and centrally placed
- Irregular microvilli are present on the sell surface
- The distinguishing feature of these cells is the presence of numerous granules in the cytoplasm
- Granules stain with PAS STAIN

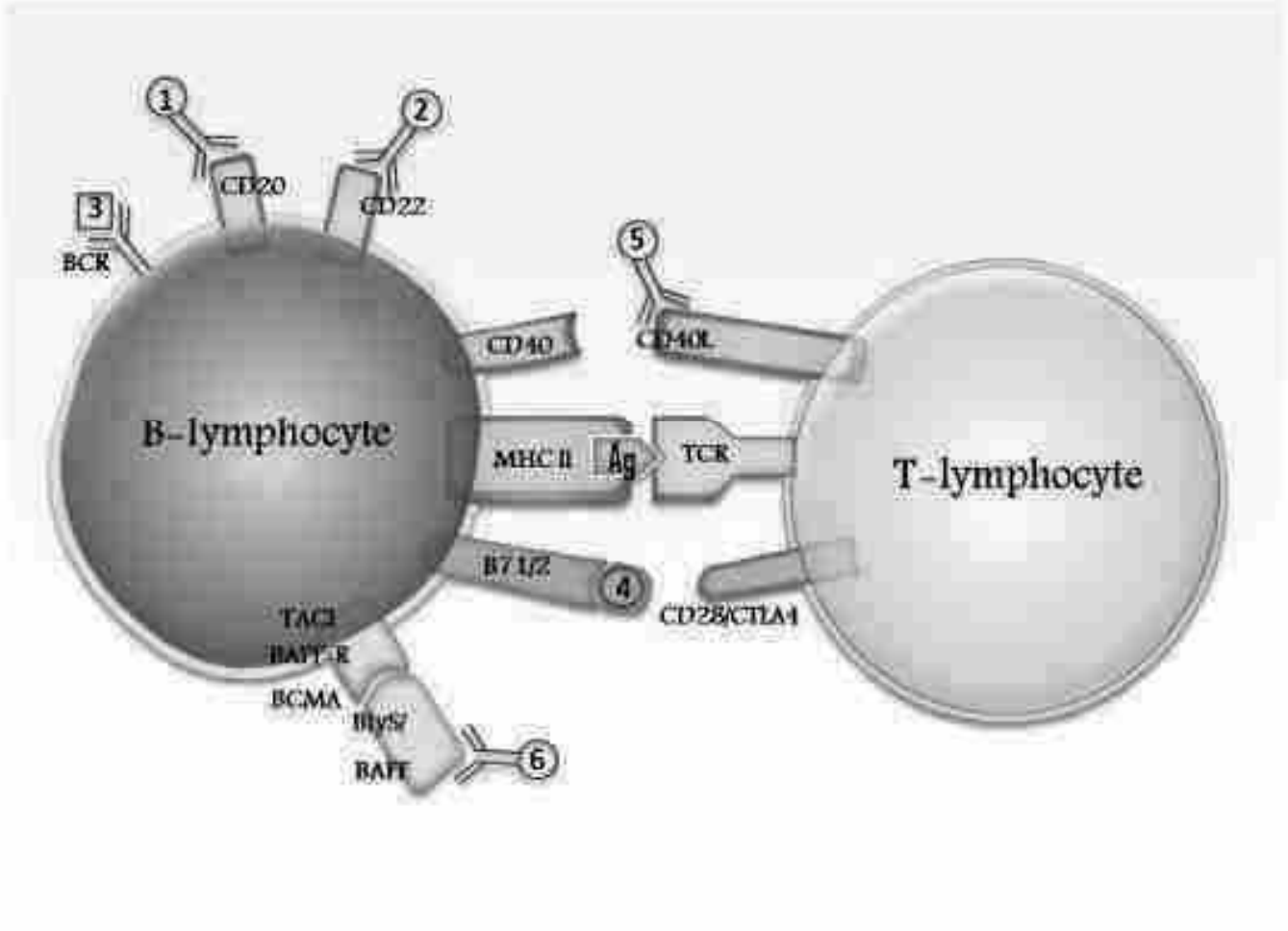


- With EM the granules are seen as vesicles which is surround by a membrane
- The vesicles contains various substance most important among them is *histamine*
- Histamine is associated with the production of allergic reactions when a tissue is exposed to an antigen which is sensitive
- They are most frequently seen around blood vessels and nerves

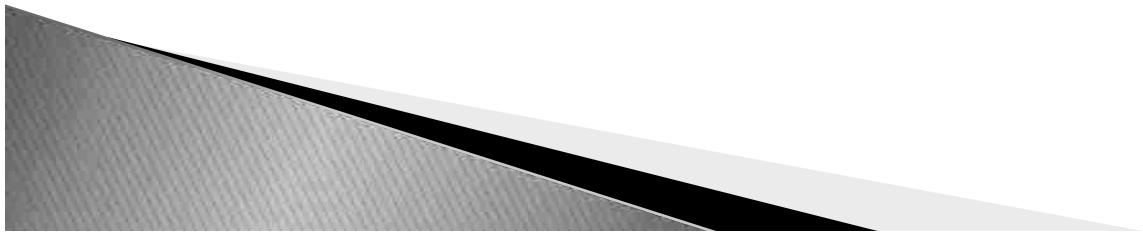
LYMPHOCYTES

- Represent one variety of leukocytes present in the blood
- Large aggregations of lymphocytes are present in lymphoid tissue and from here it reaches the connective tissue and are specially numerous when the tissue undergoes inflammation
- It plays an important role in defence of the the body against invasion of bacteria

T Lymphocyte B Lymphocyte



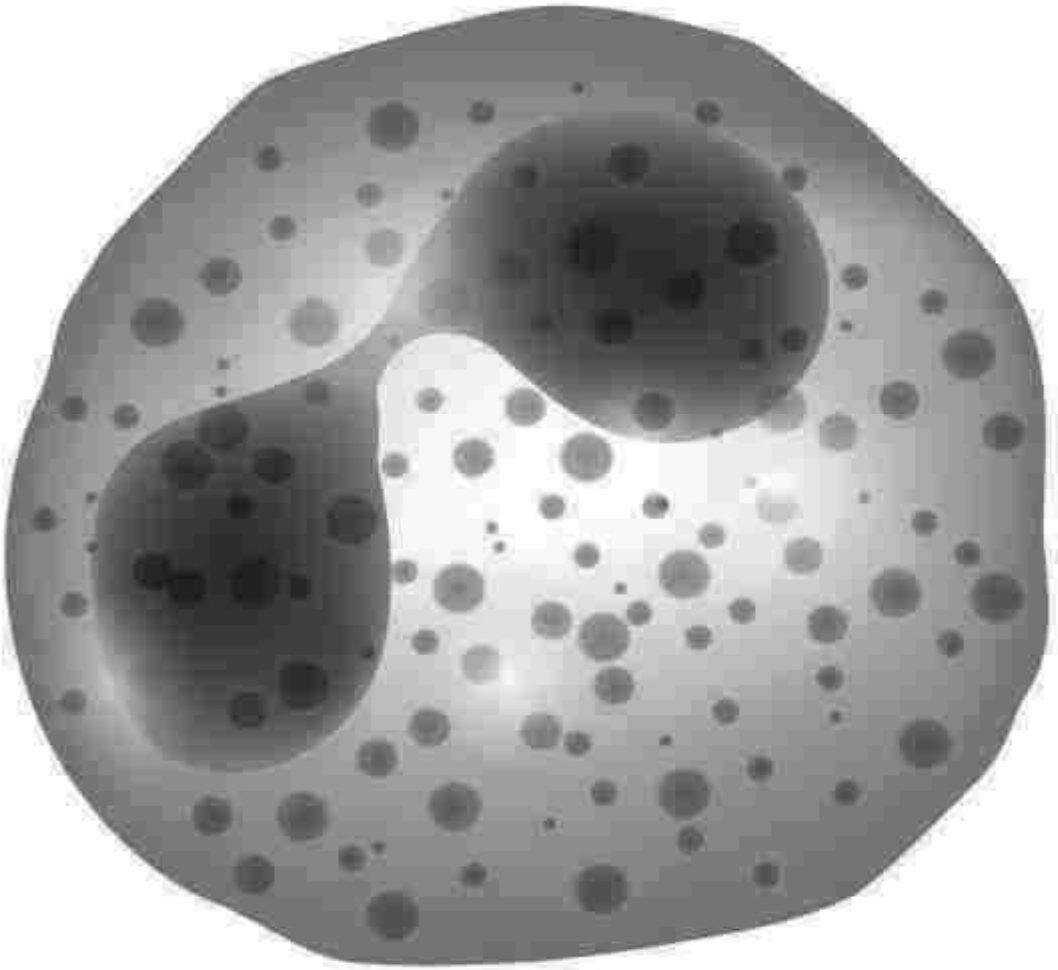
- They have the ability to identify the substances that are new to the host body and to destroy these by produce antibodies against them
- Two types; *B lymphocytes and*
  T lymphocytes



OTHER LEUKOCYTES

1. Monocytes ; closely related to macrophages
2. Eosinophil ; Eosinophilic granules in the cytoplasm and increase in their number in allergic disorders

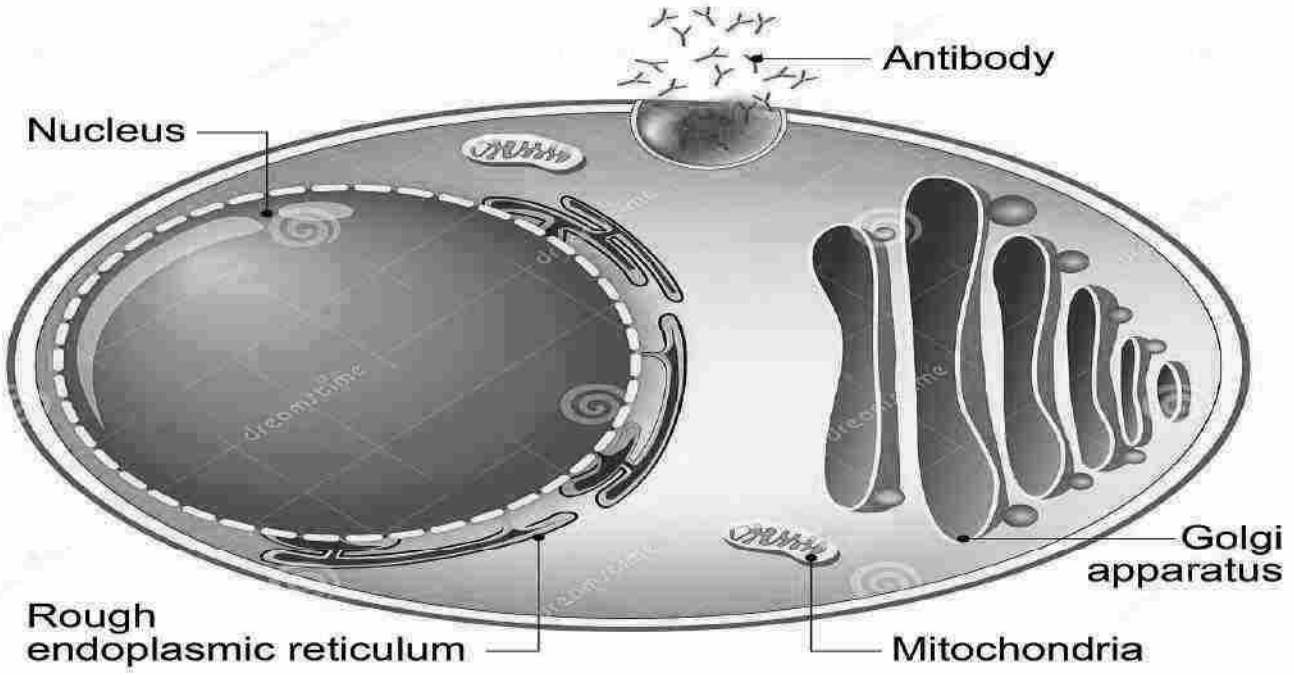
EOSINOPHIL



PLASMA CELLS

- A plasma cell is seen to be small and basophilic cytoplasm
- The chromatin in its nucleus forms four or five clumps near the periphery thus giving the nucleus a *cartwheel* appearance
- They produce antibodies that may be discharged locally may enter into the blood circulation or may be stored within the cell itself in the form of inclusion called *Russels bodies*

PLASMA CELL



dreamstime.com
ID 131694509 © Designua

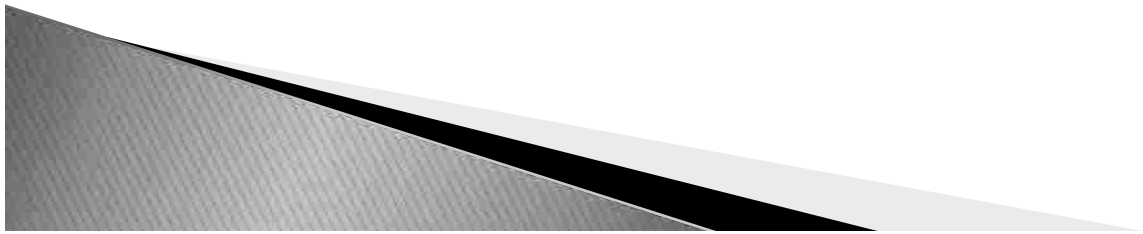
- Very few plasma cells seen in connective tissue but their number increases in the presence of certain inflammation
- It is believed that plasma cells represent B lymphocytes that have matured and have lost their power of further division

INTERCELLULAR SUBSTANCE

- It fills the space between cells and fibres
- A complex mixture of ***glycoproteins*** and ***proteoglycans***
- Participate in binding cells to the fibres of connective tissue
- Colourless and transparent
- Act as both a lubricant and a barrier to the penetration of foreign particles

Glycoproteins

- Fibronectin
- Laminin
- Enactin



Proteoglycans

- Hyaluronic acid/ GAG
- Chondroitin sulfate
- Dermatin sulfate
- Keratin sulfate
- Heparin sulfate

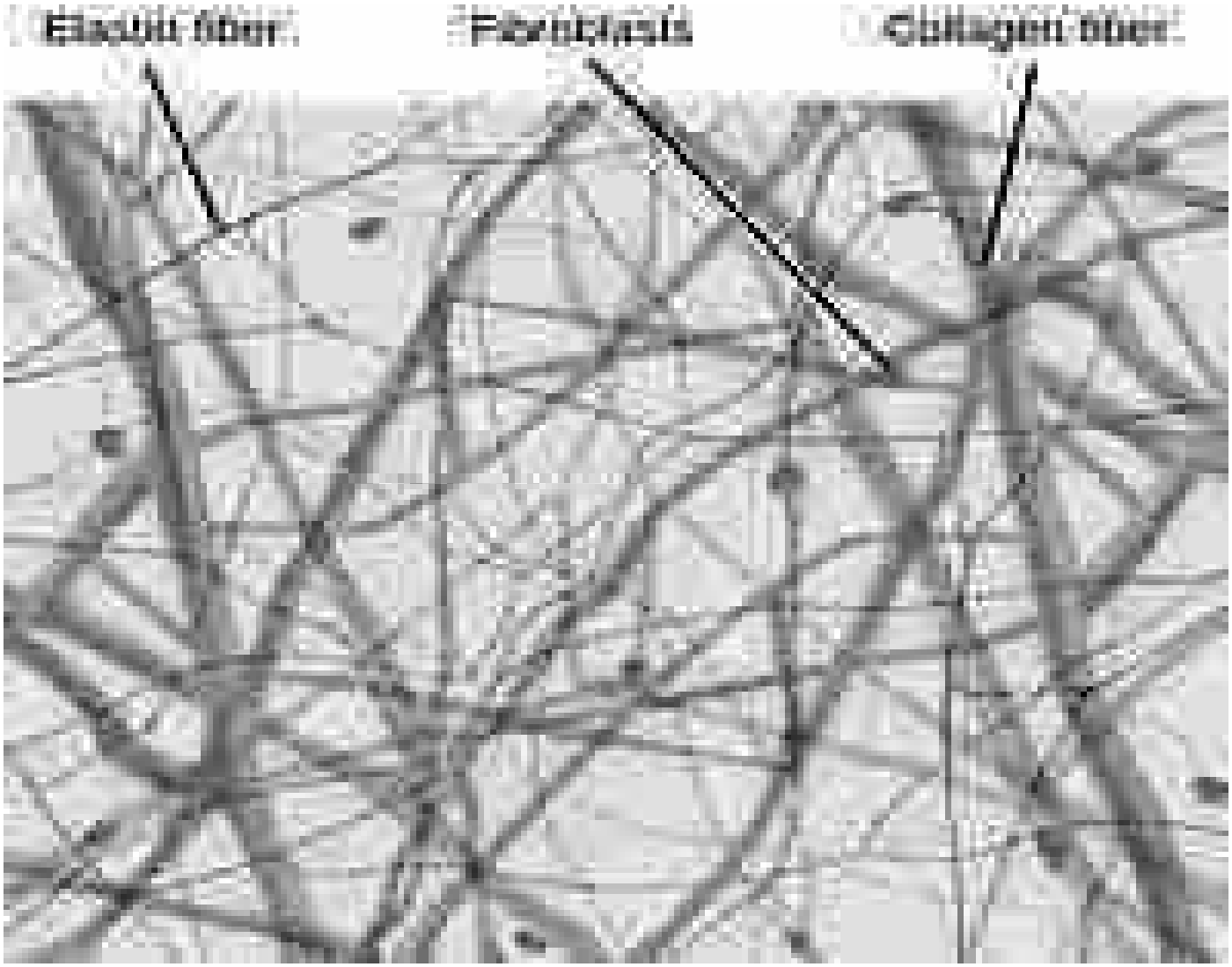
Different forms of C.T

- Loose connective tissue
- Dense connective tissue

Loose connective tissue

AREOLAR

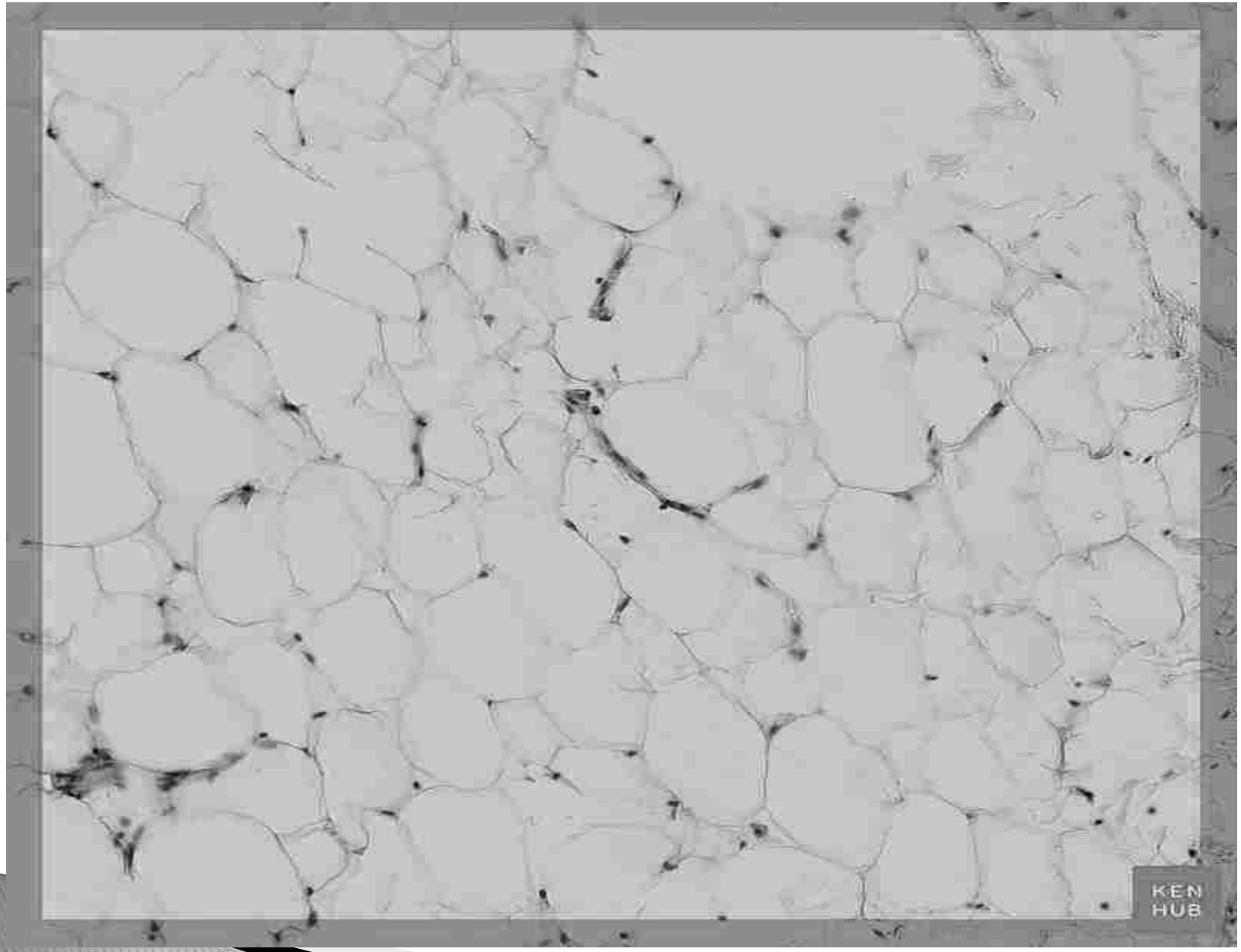
- They form a loose network
- Consists of collagen fibre, elastic fibre, reticular fibres and several kind of cells
- Location; Below the skin, fill space between muscles
- Functions; Gives strength elasticity and support to tissues


Elastin fiber
Fibroblasts
Collagen fiber

ADIPOSE CONNECTIVE TISSUE

- It consists of adipocytes which store fat
- Location; it present in subcutaneous layer deep in the skin around the heart and kidneys
- Functions; Prevent heat lose from the body

Act as a reservoir of energy, give shape to the limbs of body , protects underlying organs from injury


KEN
HUB

RETICULAR CONNECTIVE TISSUE

- Contains reticular fibres and cells
- Location; Present in the supporting framework of liver, spleen,lymphnodes, and bone marrow
- Functions; It binds together smooth muscle tissue cells filters and remove microbes in lymph node

Dense Connectivetissue

- In this fibres are closely or densely packed
- The fibre content is higher
- Cell content is lower

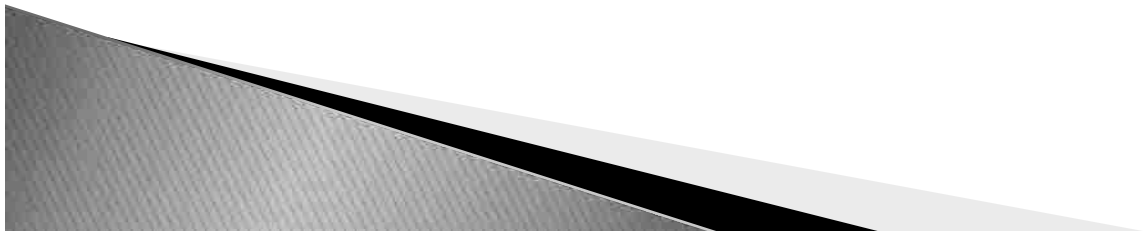
DENSE REGULAR CONNECTIVE TISSUE

- Bundles of collagen fibre are arranged in parallel patterns to provide strength to tissue
- Fibroblast appears in rows between the fibres
- Rough in nature
- Location; Tendons, and ligaments
- Provide strong attachment to structures

DENSE IRREGULAR CONNECTIVE TISSUE

- It contain collagen fibres which are arranged irregularly and few fibroblasts are appear in between rows
- Location; Dermis layer of skin, membrane capsule around kidneys
- It provide strength to different organs

THANKYOU



GENERAL ANATOMY AND HISTOLOGY OF CARDIOVASCULAR SYSTEM

GENERAL ANATOMY

INTRODUCTION

- The cardiovascular system is transport system of the body
- It comprises blood ,heart and blood vessels
- The system supplies nutrients to and remove waste products from various tissue of the body

FUNCTIONS

- Transport nutrients,hormones
- Remove waste products
- Gaseous exchange
- Immunity
- Heart pumps blood through blood vessels

HEART

- Heart is a four chambered hollow muscular organ approximately the size of your fist
- Each half of the heart receiving blood is called atrium and pumping blood is called ventricle

BLOOD VESSELS

- A close network of tubes
- These includes; Arteries, Capillaries,Veins

ARTERIES

- Blood vessels that carry blood away from the heart are called arteries
- They are the thickest blood vessels and they carry blood high in oxygen known as oxygenated blood except pulmonary artery

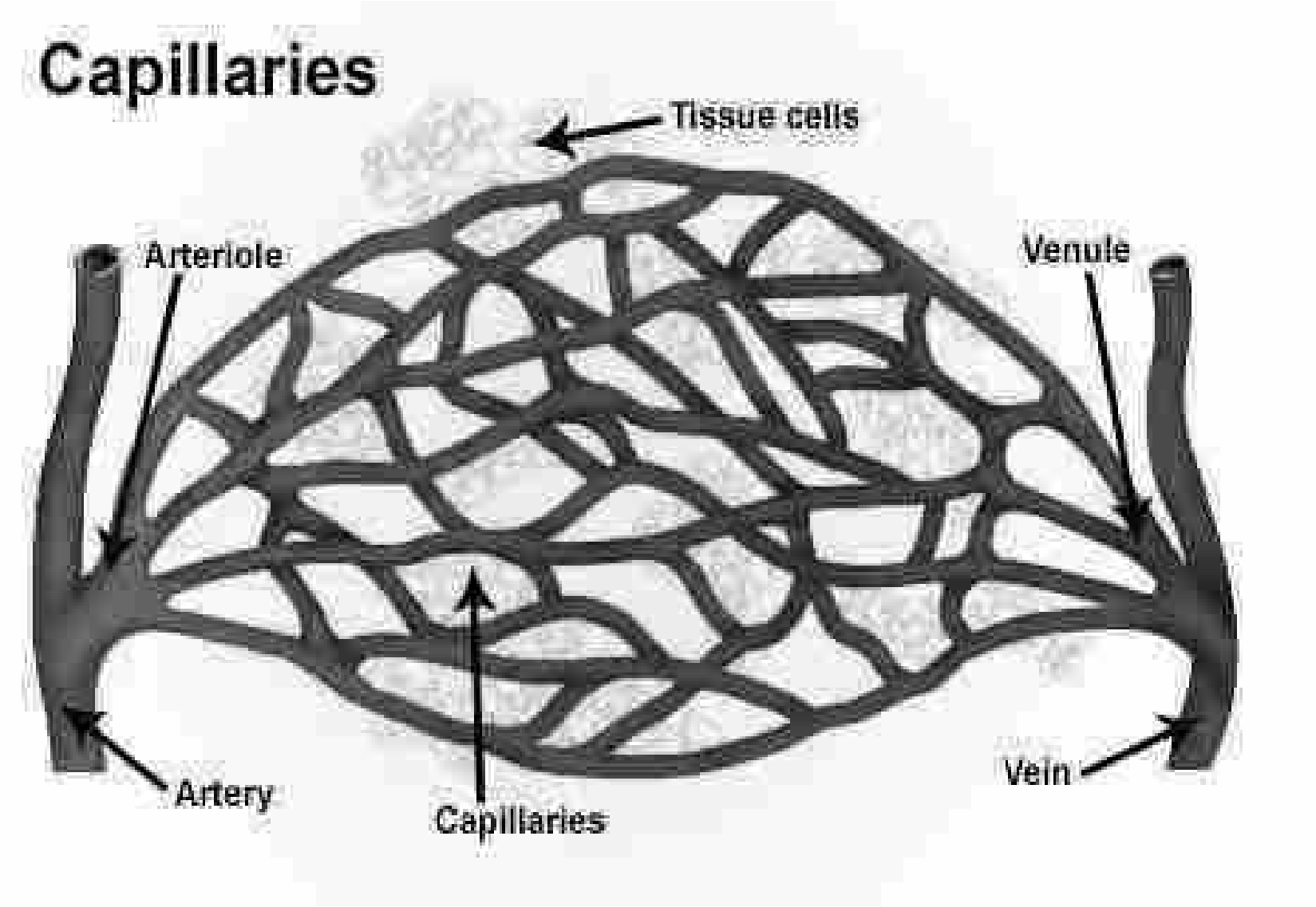
- Accompany by veins and nerves
- Lumen is small and thick walled
- No valves
- Repeated branching

Classification

- Elastic artery e.g. Aorta and its major branches
- Muscular artery e.g. Renal artery, Testicular, Radial and Tibial
- Arterioles; Terminal arterioles, Meta arterioles

CAPILLARIES

- The smallest blood vessels which connect the arteries and veins
- This is where the exchange of nutrients and gases occurs


Capillaries
Tissue cells
Arteriole
Venule
Artery
Capillaries
Vein

SINUSOIDS

- These are large irregular vascular space
- E.g. Liver, spleen, Bone marrow, suprarenal
- Their lumen is wider and thinner walls

VEINS

- Blood vessels that carry blood back to the heart are called veins
- They have valves which help the unidirectional flow of blood and not the backward flow
- They carry CO_2 rich blood called deoxygenated blood except pulmonary V.

- Thin walled
- Large irregular lumen
- Have valves
- Types; Large, Small
- Veins without valves are venae cavae, hepatic,renal,pulmonary,facial portal vein and all the veins which has less than 2mm in diameter

ANASTOMOSIS

Communication between vessels

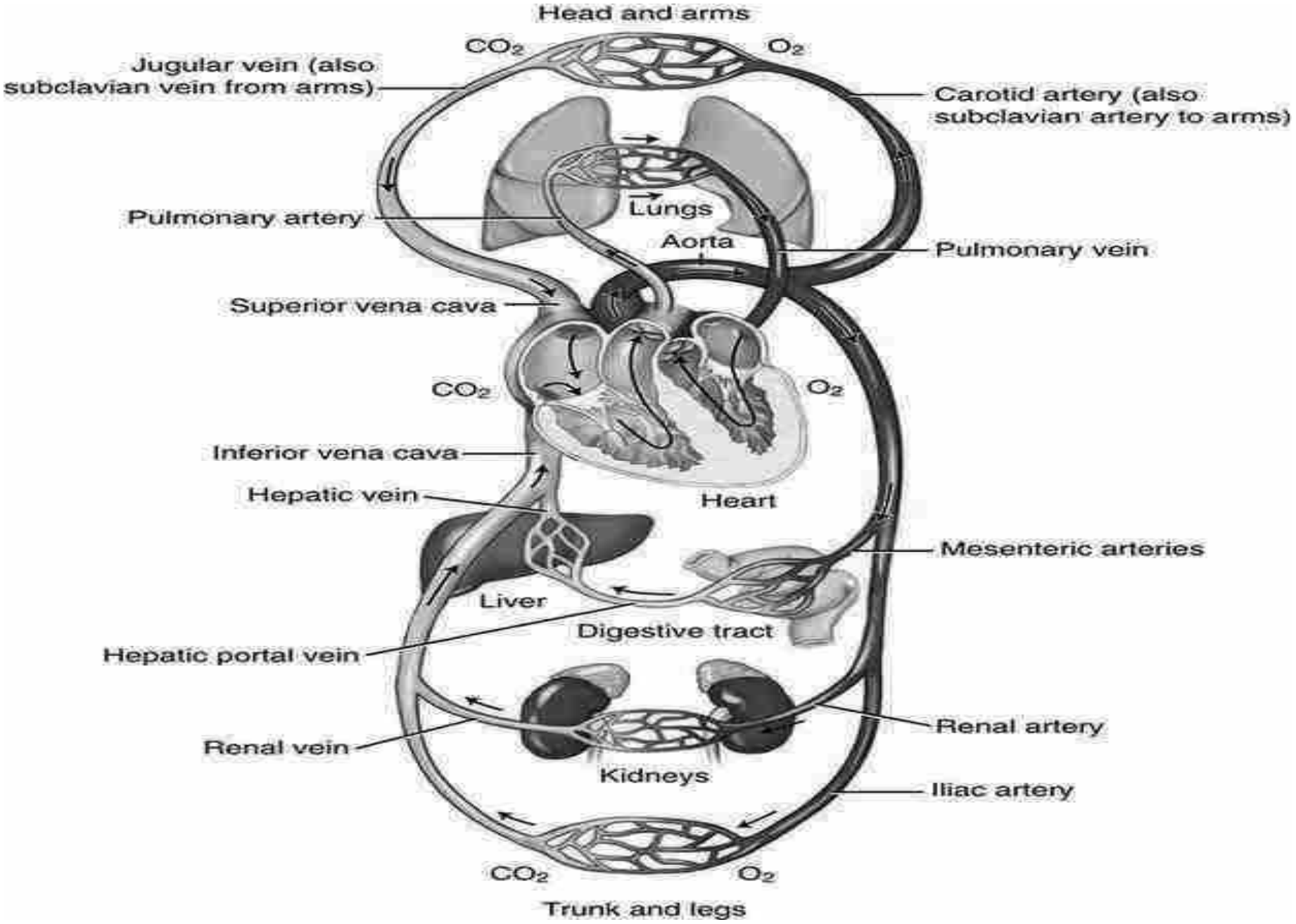
- Arterial anastomosis; communication between arteries

1. Actual- Arteries meet end to end
2. Potential- Communication between terminal arterioles

- Venous anastomosis; Communication between veins
- Arteriovenous anastomosis; Communication between an artery and a vein

CIRCULATION

- Pulmonary circulation
- Systemic circulation
- Portal circulation


Head and arms
CO_2
O_2
Jugular vein (also subclavian vein from arms)
Carotid artery (also subclavian artery to arms)
Pulmonary artery
Lungs
Aorta
Pulmonary vein
Superior vena cava
CO_2
O_2
Inferior vena cava
Hepatic vein
Heart
Mesenteric arteries
Liver
Digestive tract
Hepatic portal vein
Renal artery
Renal vein
Kidneys
Iliac artery
CO_2
O_2
Trunk and legs

- Aorta
- Muscular artery
- Arterioles
- Muscular arteriole
- Terminal arteriole
- Capillaries
- Small veins
- Large veins
- Venae cavae

HISTOLOGY

ENDOTHELIUM

- The inner surface of the heart and of all blood vessels are lined by flattened cells called endothelial cells or endotheliocytes
- Cytoplasm is sparse and contain cell organelles

BASIC STRUCTURE OF ARTERY

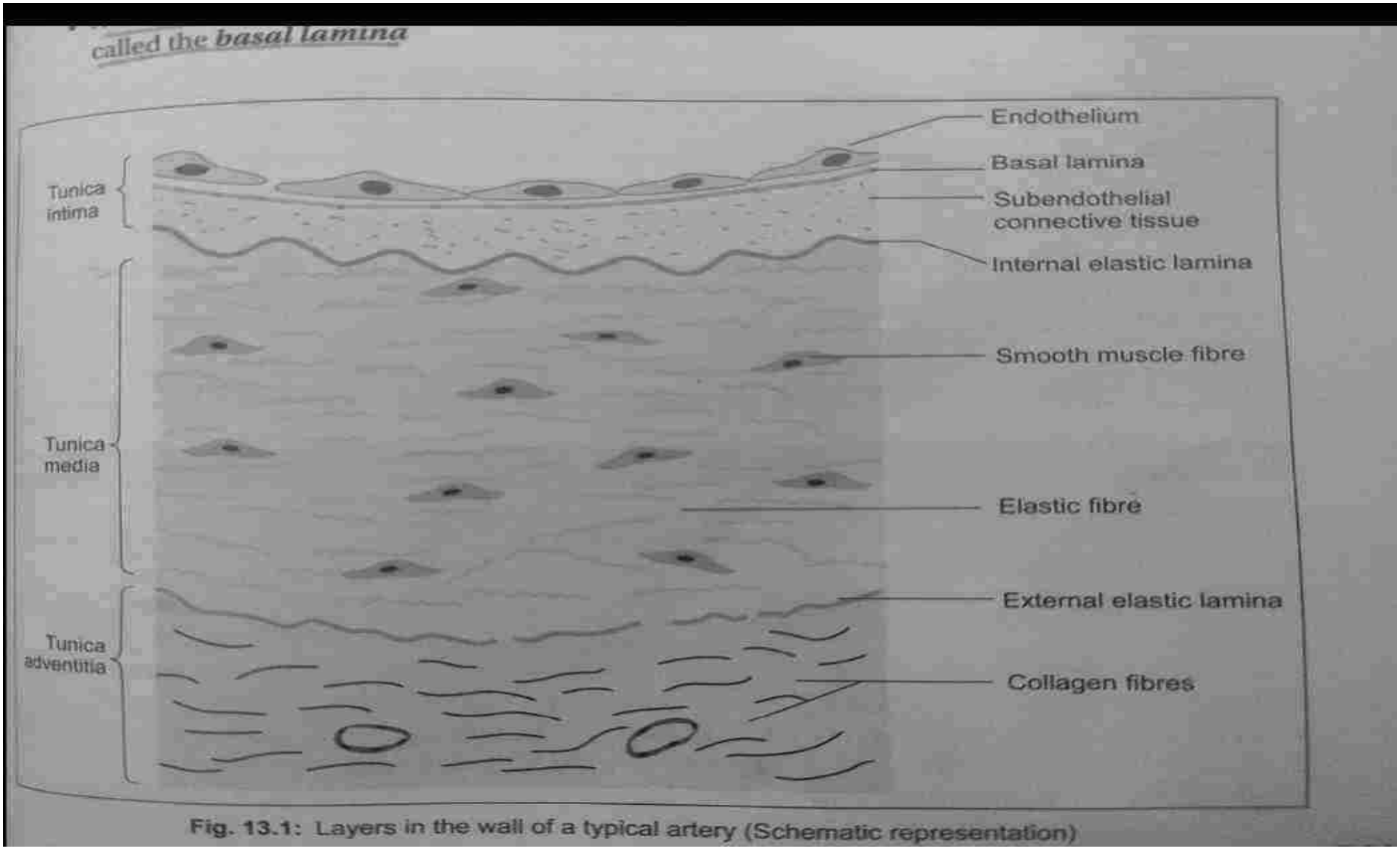


Fig. 13.1: Layers in the wall of a typical artery (Schematic representation)

Three layers;

1. **Tunica intima** (innermost layer) It consists of

- ❖ An endothelial lining
- ❖ A thin layer of glycoprotein- Basal lamina
- ❖ A delicate layer of sub endothelial connective tissue
- ❖ A membrane formed by elastic fibres called internal elastic lamina

2.Tunica media

- Elastic fibres or smooth muscle limited outside by external elastic membrane

3. Tunica adventitia

- Consists of connective tissue in which collagen fibres are prominent

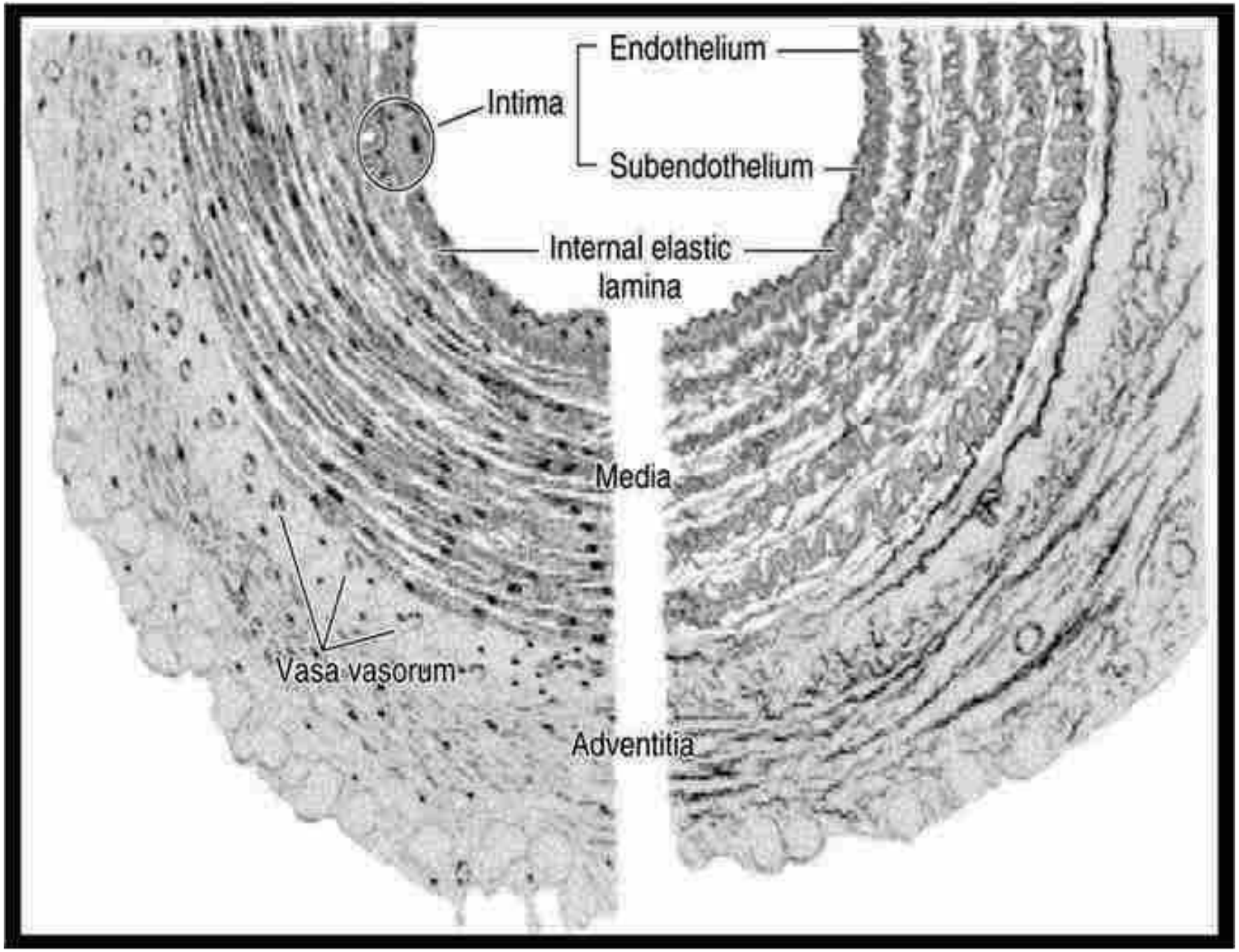
TYPES OF ARTERIES

On the basis of kind of tissue present in tunica media there are two arteries

- **Large or elastic artery**
- **Medium sized or muscular artery**

Elastic artery

- The largest artery in the body e.g. Aorta and its branches
- Tunica intima; Same to that of basic structure but internal elastic lamina is not distinct from the media as it has the same structure of the elastic membranes of the media

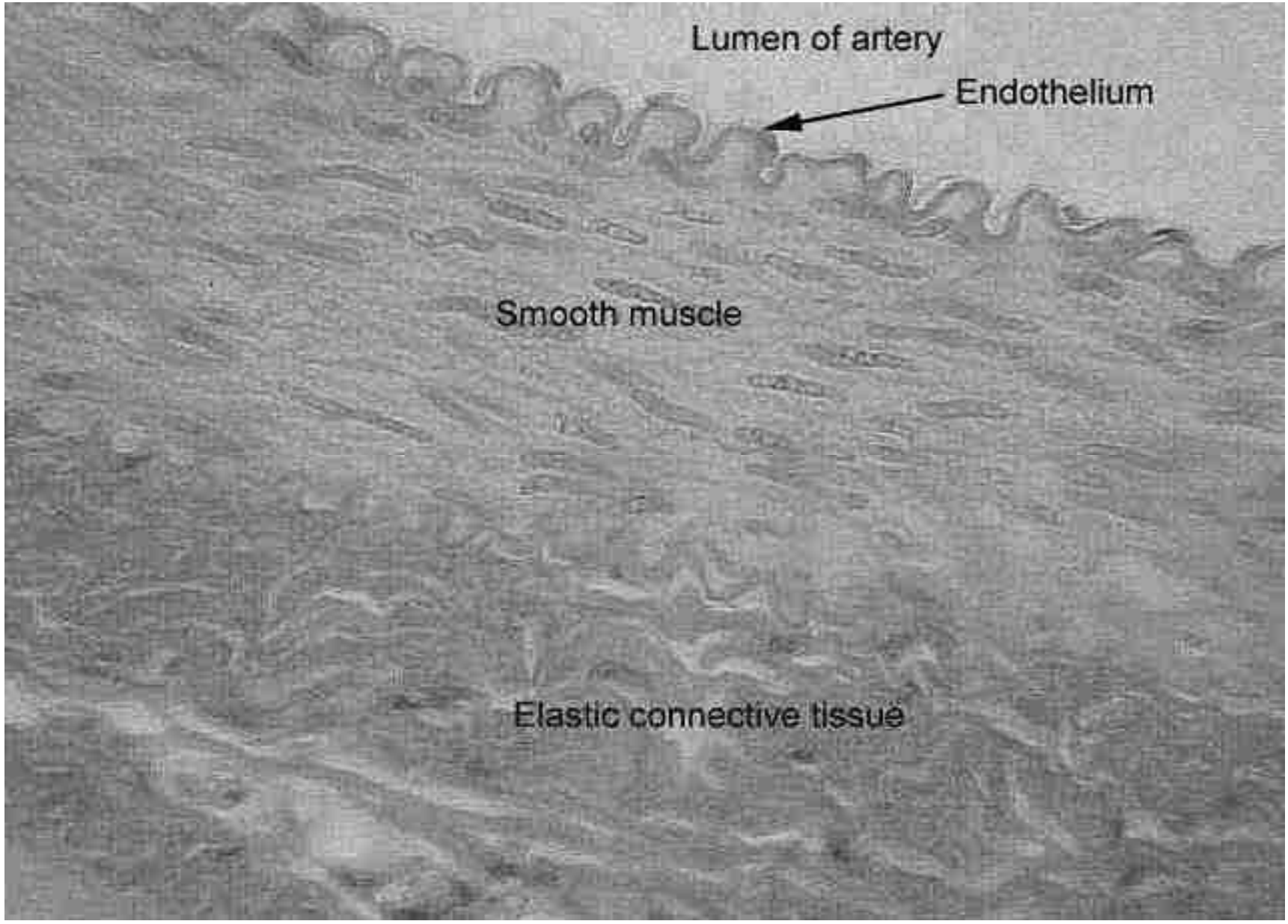

Endothelium
Intima
Subendothelium
Internal elastic lamina
Media
Vasa vasorum
Adventitia

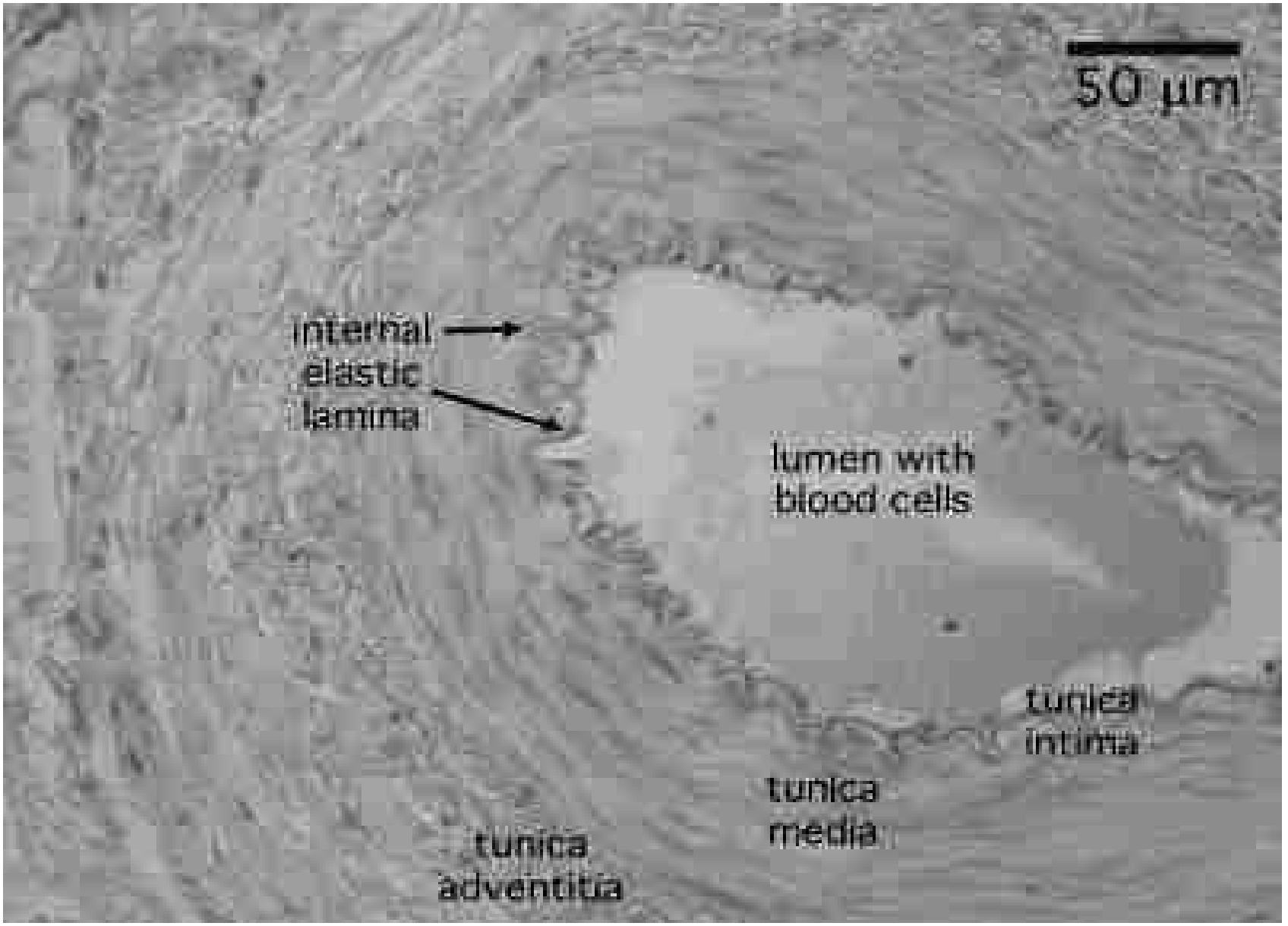
- Tunica media; Made up of elastic tissue. The elastic tissue arrange in the form of series of concentric membrane. Some smooth muscle may be present
- Tunica adventitia; Thin in large arteries in which great proportion of elastic fibres are present. The fibres merges with external elastic membrane

Muscular artery

- They are the continuation of elastic artery
- Internal elastic lamina seen distinctly from the media
- Predominent smooth muscles in the tunica media
- Muscle fibres are arranged circularly

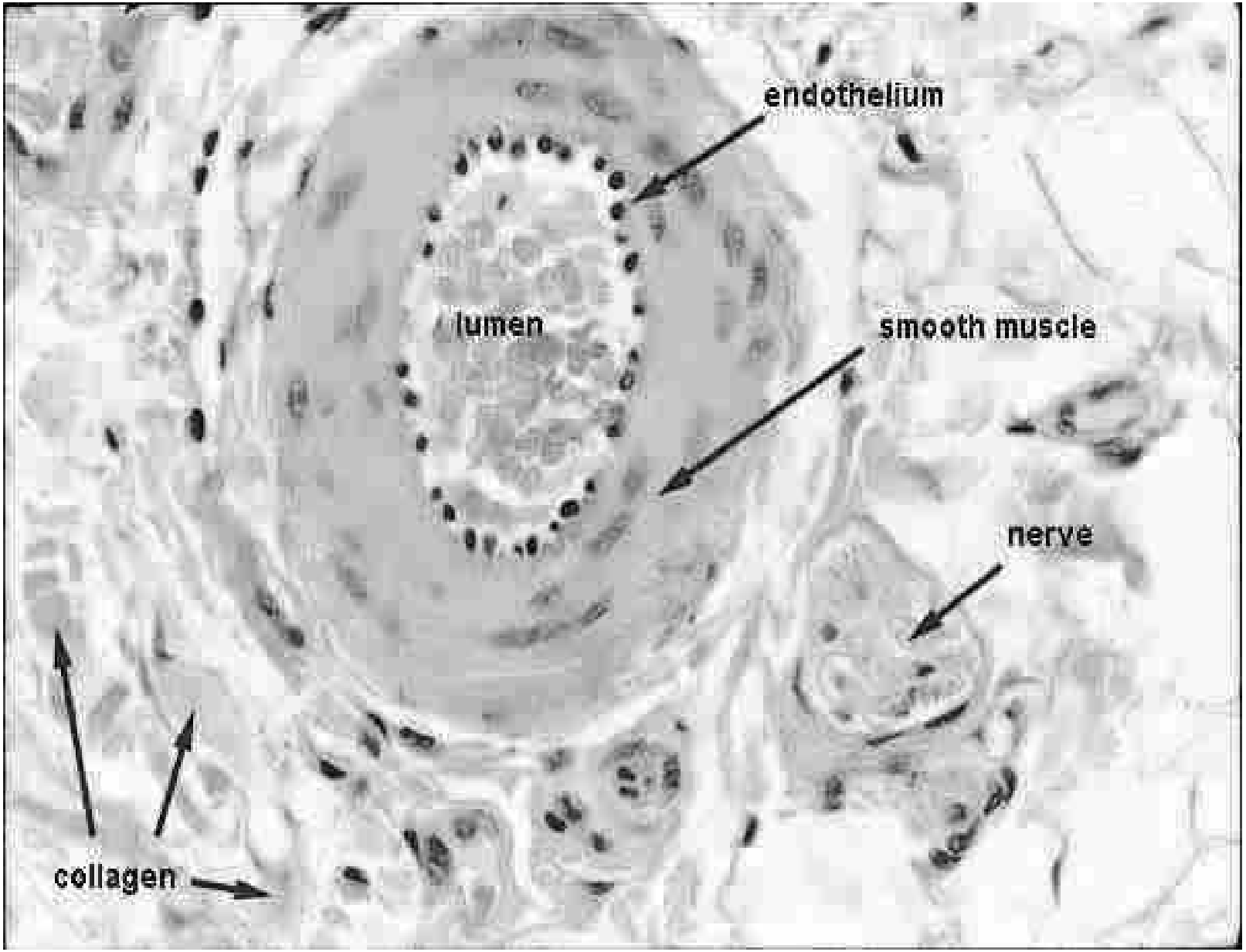
- Connective tissue are also present between the muscle fibres
- Tunica adventitia contain connective tissue and few elastic fibres


Lumen of artery
Endothelium
Smooth muscle
Elastic connective tissue


50 µm
internal
elastic
lamina
lumen with
blood cells
tunica
intima
tunica
media
tunica
adventitia

Arterioles

- The muscular arteries having diameter less than 100 micro meter is called arterioles
- Two types; Muscular arteriole (100-50) and terminal arteriole (less than 50 micrometer)
- Thin walled compared to arteries


endothelium
lumen
smooth muscle
nerve
collagen

Capillaries

- Terminal arteriole are continued into capillary plexus.
- In the wall of capillaries it is lined by endothelium
- Endothelial cells are lined by basal lamina
- Overlying basal lamina there may be perivascular cells called pericyte

- Continues capillary ; The edges of adjoining endothelial cells fuse e.g in muscle, skin , lung and brain
- Fenestrated capillaries; Aperture are present in endothelial cells e.g. Small intestine,endocrine glands and kidney

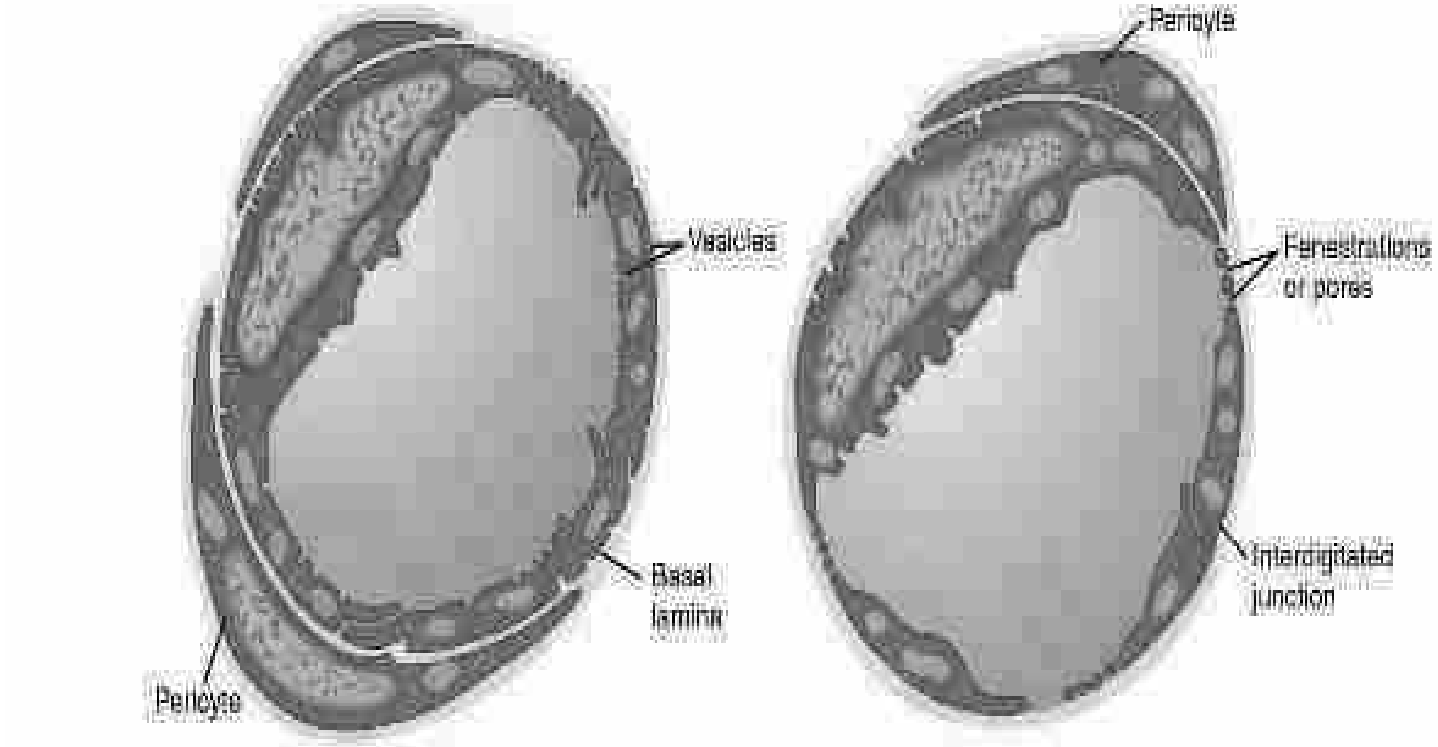
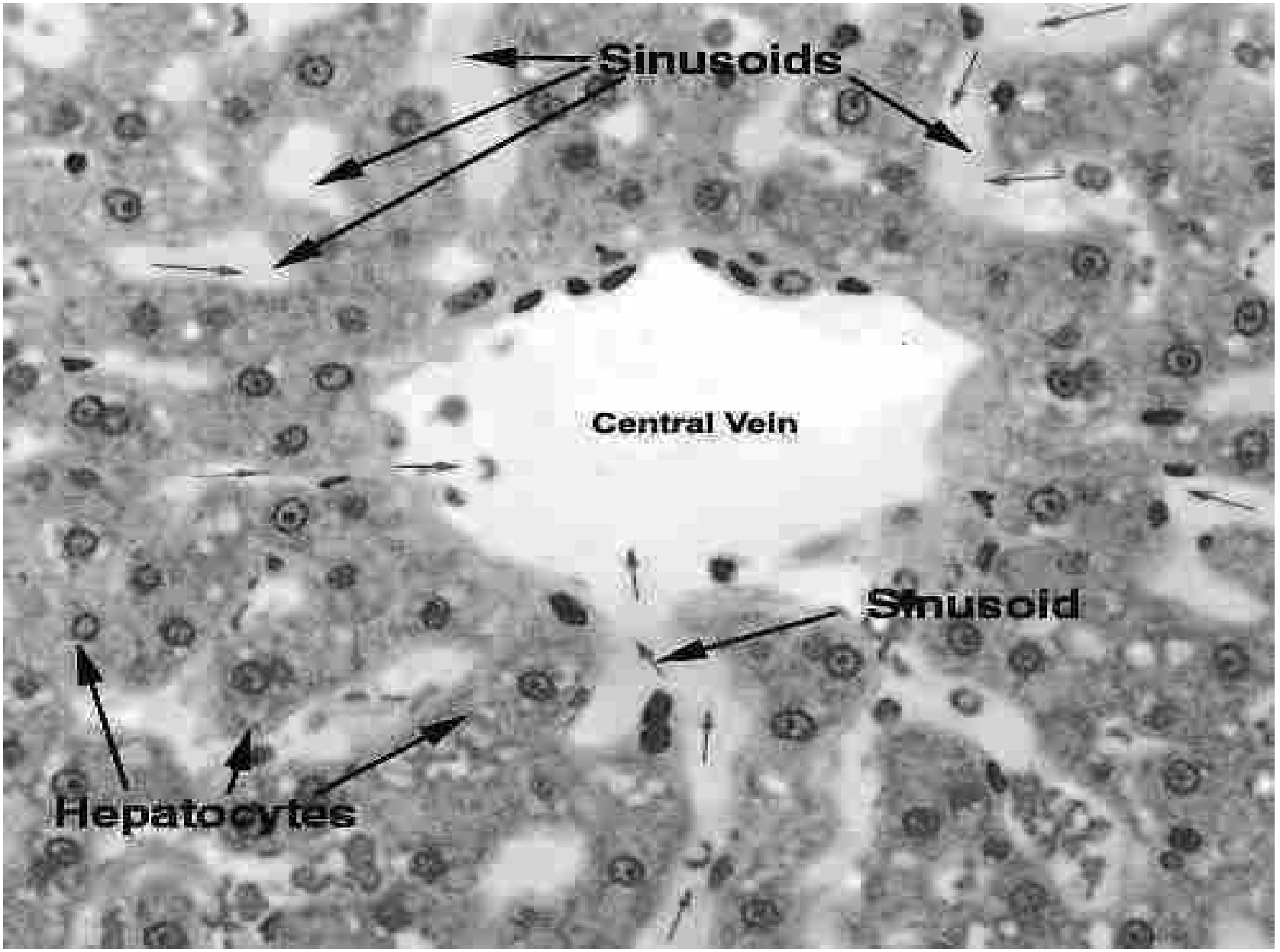


FIGURE 31–17 **Cross-sections of capillaries. Left:** Type of capillary found in muscle. **Right:** Fenestrated type of capillary. (Reproduced with permission from Ohhashi T and O Sieminski, editors: *Peripheral Blood Vessels*. Baltimore: Williams & Wilkins, 1997.)

Sinusoids

These are also network different from the capillaries

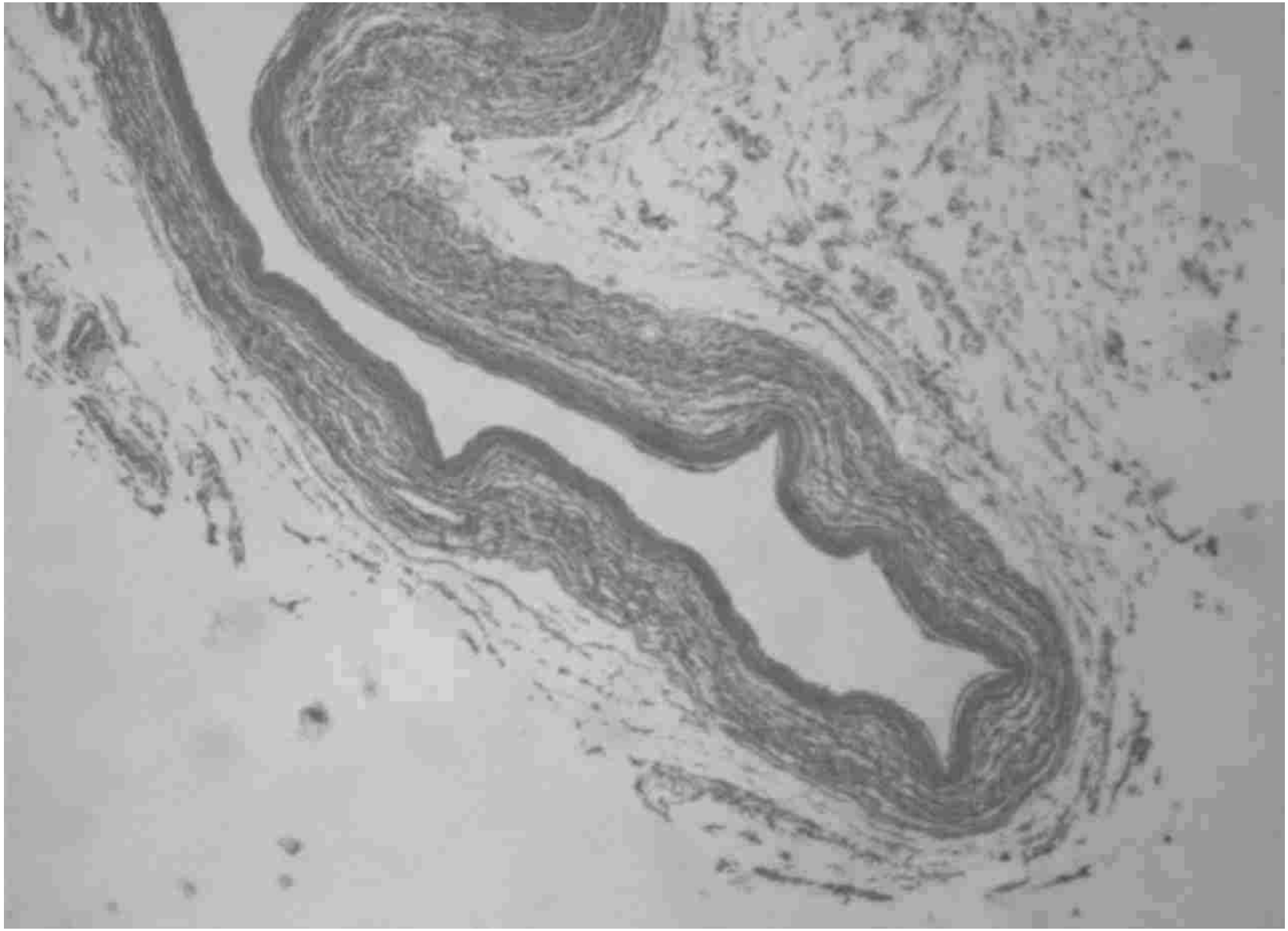
- The wall of sinusoids contains only the endothelial cells covered by a layer of connective tissue
- In some places the wall is incomplete
- The lumen is broader but irregular than the capillaries

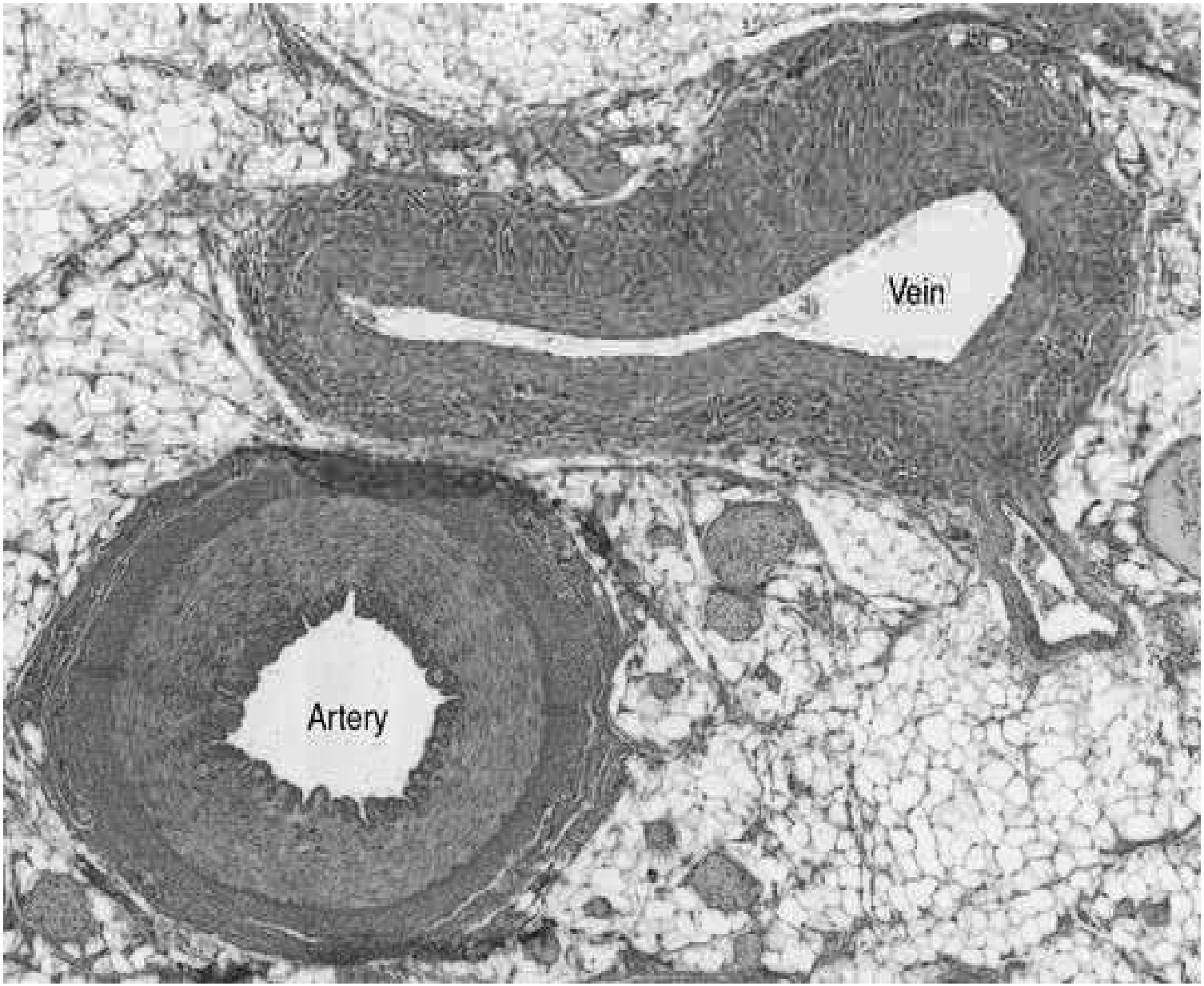

Sinusoids
Central Vein
Sinusoid
Hepatocytes

- The sinusoids are found in the organ in which made up of the cord cells e.g. spleen, liver, suprarenal gland and pituitary gland

VEINS

- The basic structure is same as artery
- The wall of vein is thinner
- Presence of abundant collagen fibres in media layer
- The smooth muscle fibres or elastic fibres reduced




Vein
Artery

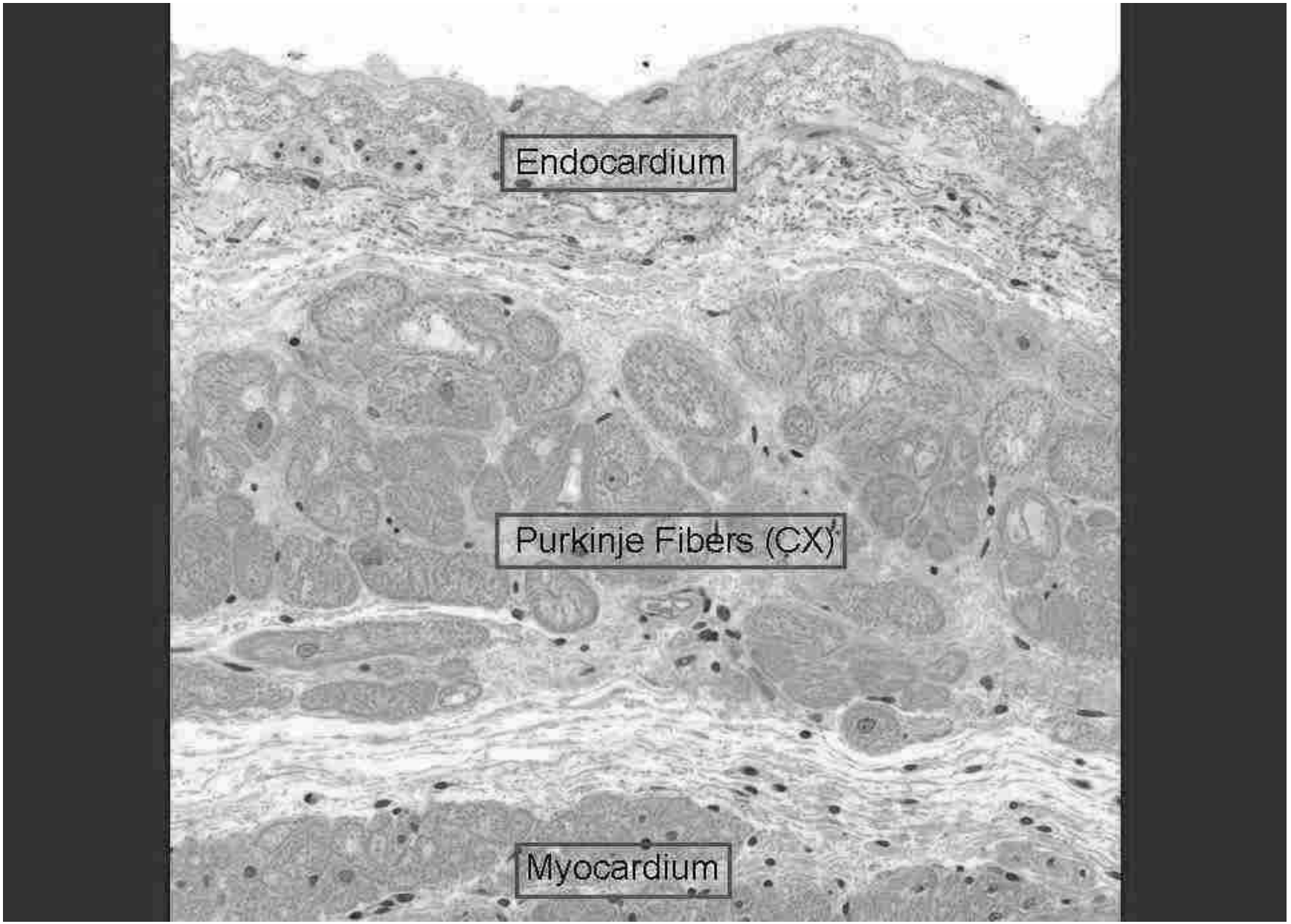
- The adventitia layer is thicker
- Significant amount of elastic fibres and also the muscle fibres in adventitia
- There is no distinction of 3 layers especially in small vein

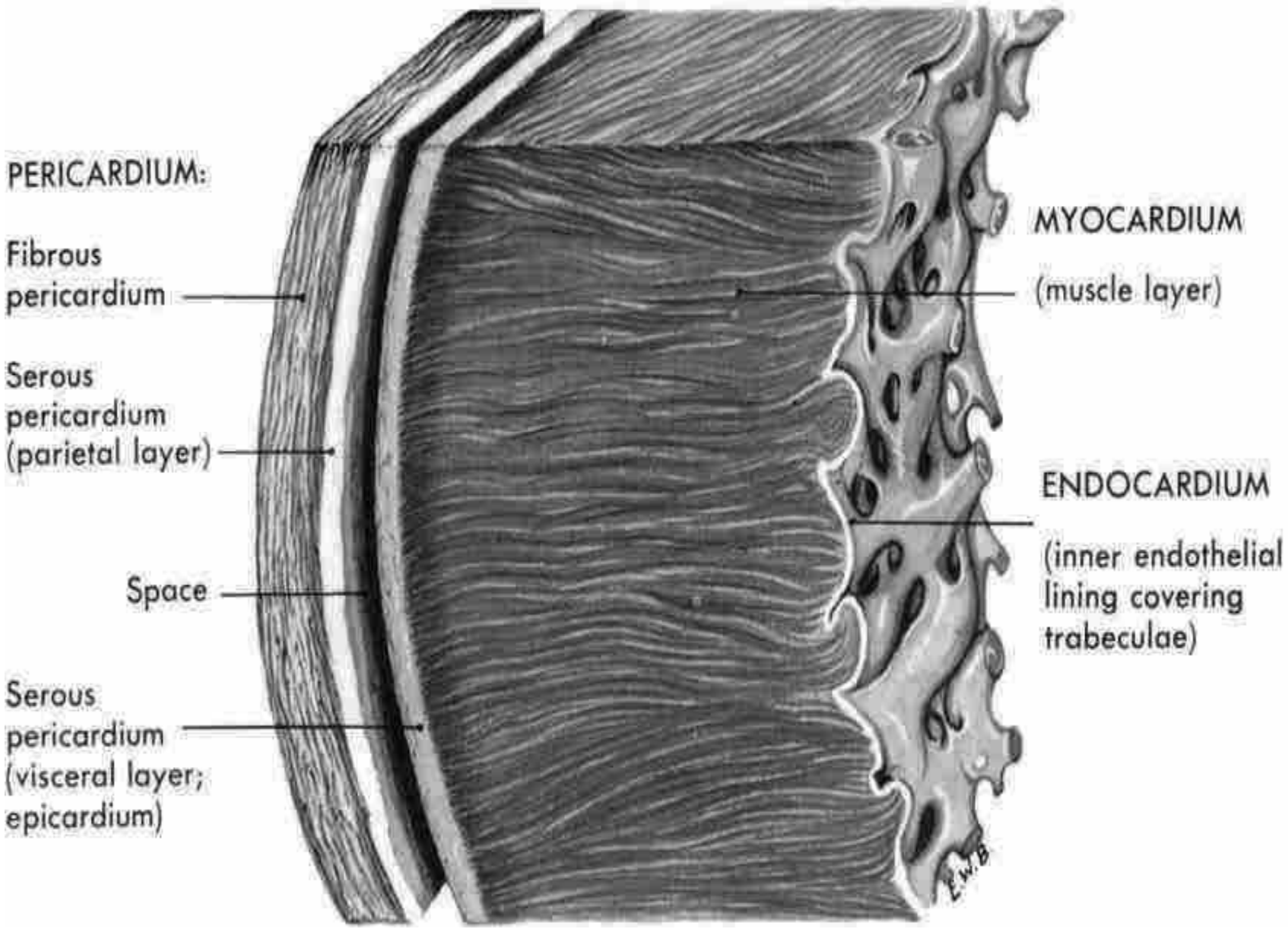
Venules

- Smallest vein which drain from capillaries are called the venule
- Diameter 20-30 micrometer
- The wall consists of endothelial lining, basal lamina, and thin adventitia
- Wall is very much permeable especially lymphocytes

HEART

- Made up of 3 layers
- Inner endocardium
- A middle myocardium
- An outer epicardium
- The endocardium; Simple squamous epithelium and a thin sub endothelial connective tissue


Endocardium
Purkinje Fibers (CX)
Myocardium


PERICARDIUM:
Fibrous pericardium
Serous pericardium (parietal layer)
Space
Serous pericardium (visceral layer; epicardium)
MYOCARDIUM
(muscle layer)
ENDOCARDIUM
(inner endothelial lining covering trabeculae)

- The myocardium; Thickest layer and it consists of cardiac muscle fibres
- The epicardium consists of squamous mesothelium and an underlying subepicardial layer of connective tissue
- The subepicardial layer contains coronary blood vessels nerves and adipose tissue

CARTILAGE

Definition

- Cartilage is a specialised type of connective tissue designed to give support bear weight and withstand tension , torsion and bending
- Firm ground substance

General features

- Support regions of the body that require flexibility
- Not supplied by blood vessels
- Non nervous structure
- Poor regeneration capacity
- Consists of all three components of connective tissue
- Usually surrounded by perichondrium

Components

- Cells – chondroblasts
- Fibres – collagen and elastic
- Ground substance – Mucopolysachrides (chondroitin sulphate , keratin sulphate and hyaluronic acid)

Perichondrium

- Cartilage is covered externally covered by a dense connective tissue sheath known as perichondrium except articular cartilage and fibro cartilage
- Has two layers outer fibrous layer and inner chondrogenic layer
- Has cells which can regrow cartilage to some extent if the cartilage is damaged

Cells

- They are derived from undifferentiated mesenchymal cells
- Young cells are small with branched cytoplasmic processes known as chondroblasts they multiply to chondrocytes
- Older and mature cells are known as chondrocytes

- Chondrocytes are bigger in size and are found in spaces called as lacunae
- They are found either in groups of 2-4 cells together known as cell nest or individual cells
- They are responsible for production of fibres and ground substance of the cartilage
- Old mature cells are incapable of mutipliation

Types

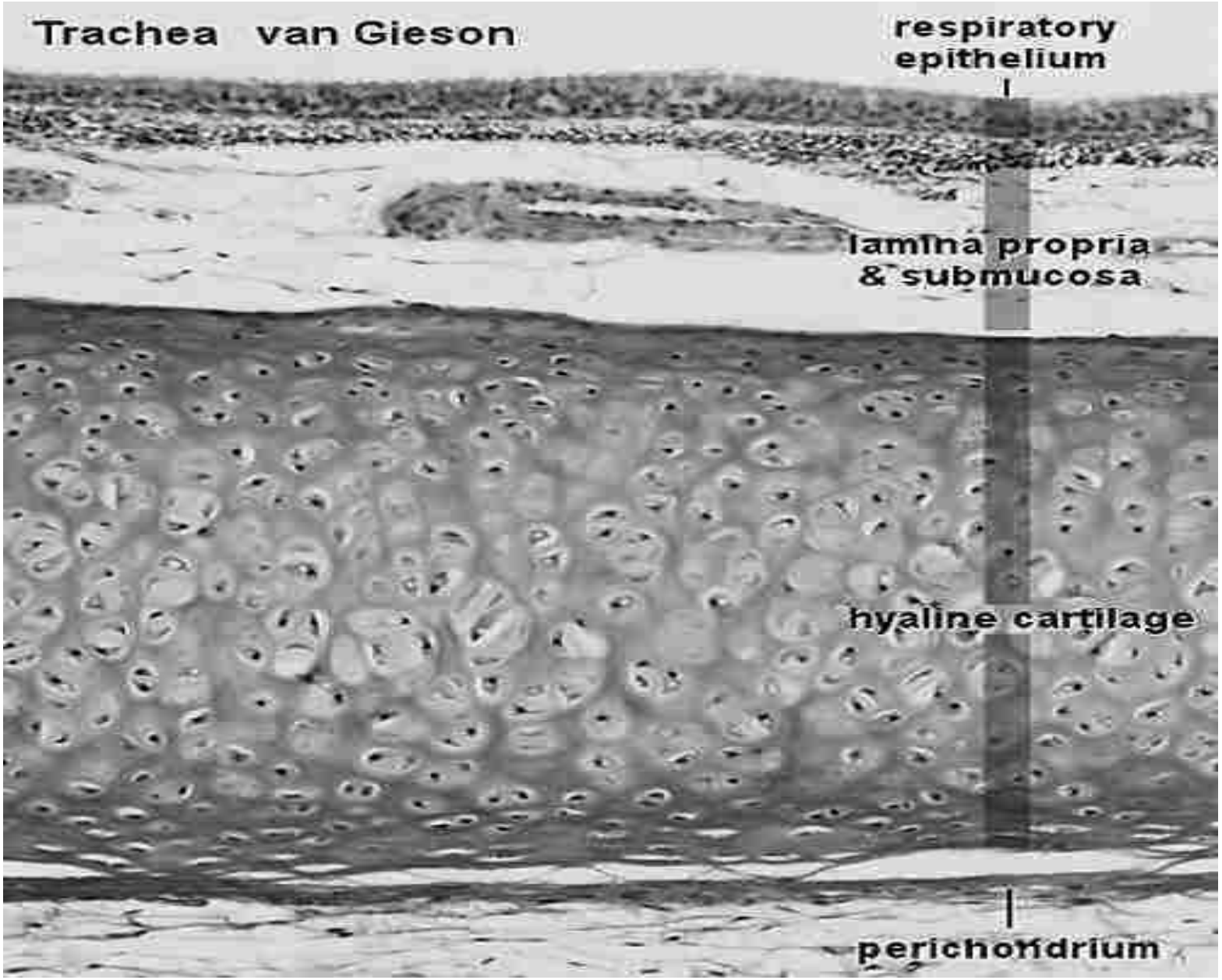
- HYALINE CARTILAGE
- ELASTIC CARTILAGE
- FIBRO CARTILAGE

Hyaline cartilage

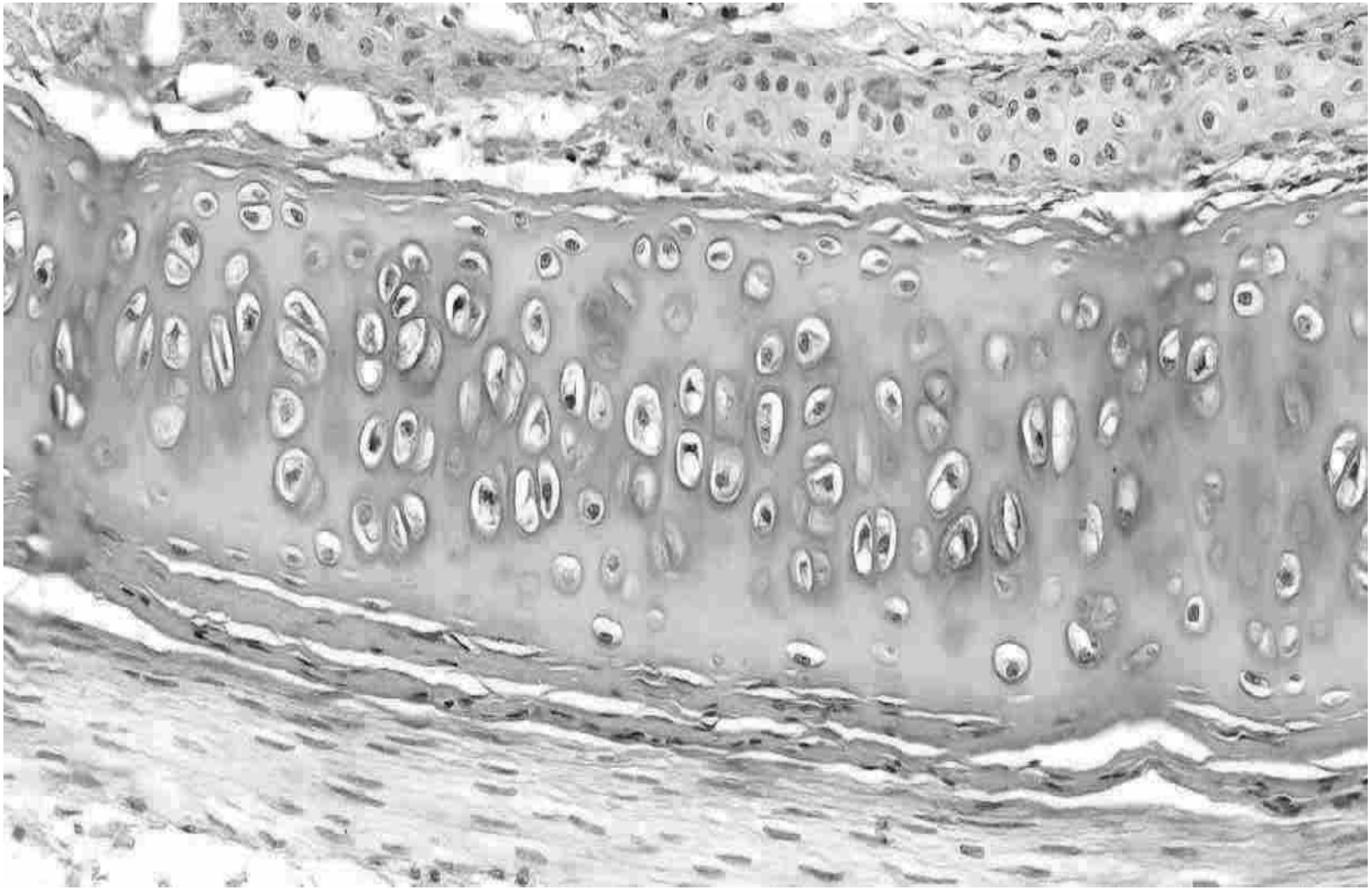
- Hyalos = glass
- Most abundant
- Perichondrium present
- Characterised by the presence of highly basophilic homogeneous transparent amorphous intercellular matrix
- Appear homogeneous as the collagen fibres present in them have the same refractive index to that of ground substance

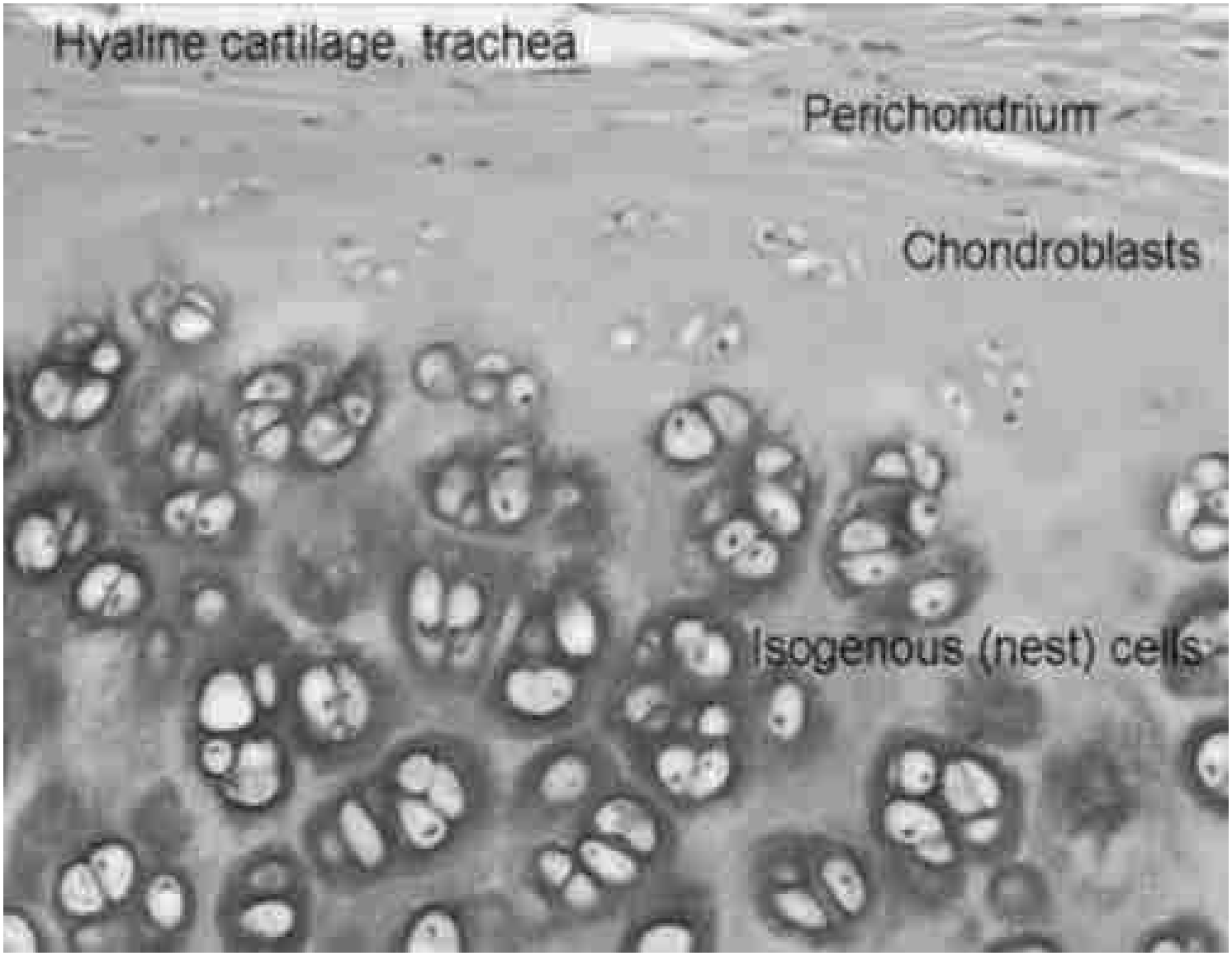
CELLS

- Chondrocyte
- Occupy in lacunae
- Cells are encapsulated in groups of 2 – 4 cells called cell nests
- Matrix around the cells is brighter and deep in colour than other areas this matrix is known as territorial matrix
- Two groups of cells are separated by a lightly coloured matrix known as interstitial matrix


Trachea van Gieson
respiratory epithelium
lamina propria & submucosa
hyaline cartilage
perichondrium

gettyimages
xia yuan
116891218D




Hyaline cartilage, trachea
Perichondrium
Chondroblasts
Isogenous (nest) cells

- Fibres present here is mainly type2 collagen fibres
- Collagen provides stability and strength
- Perichondrium present which as outer fibrous and inner cellular layer

EXAMPLES

- Foetal skeleton
- Nose
- Costal cartilage
- Trachea, bronchi , larynx

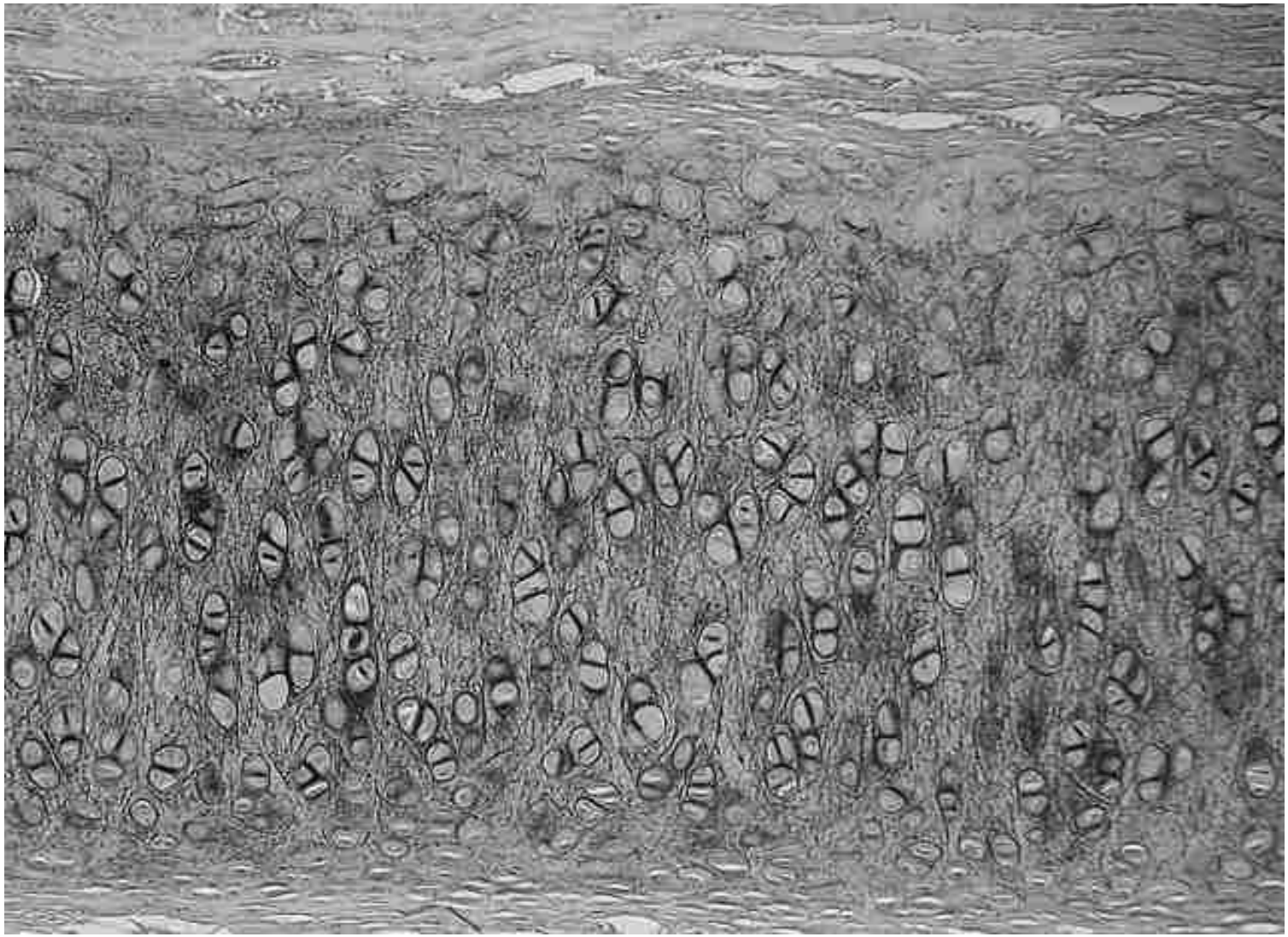
Elastic cartilage

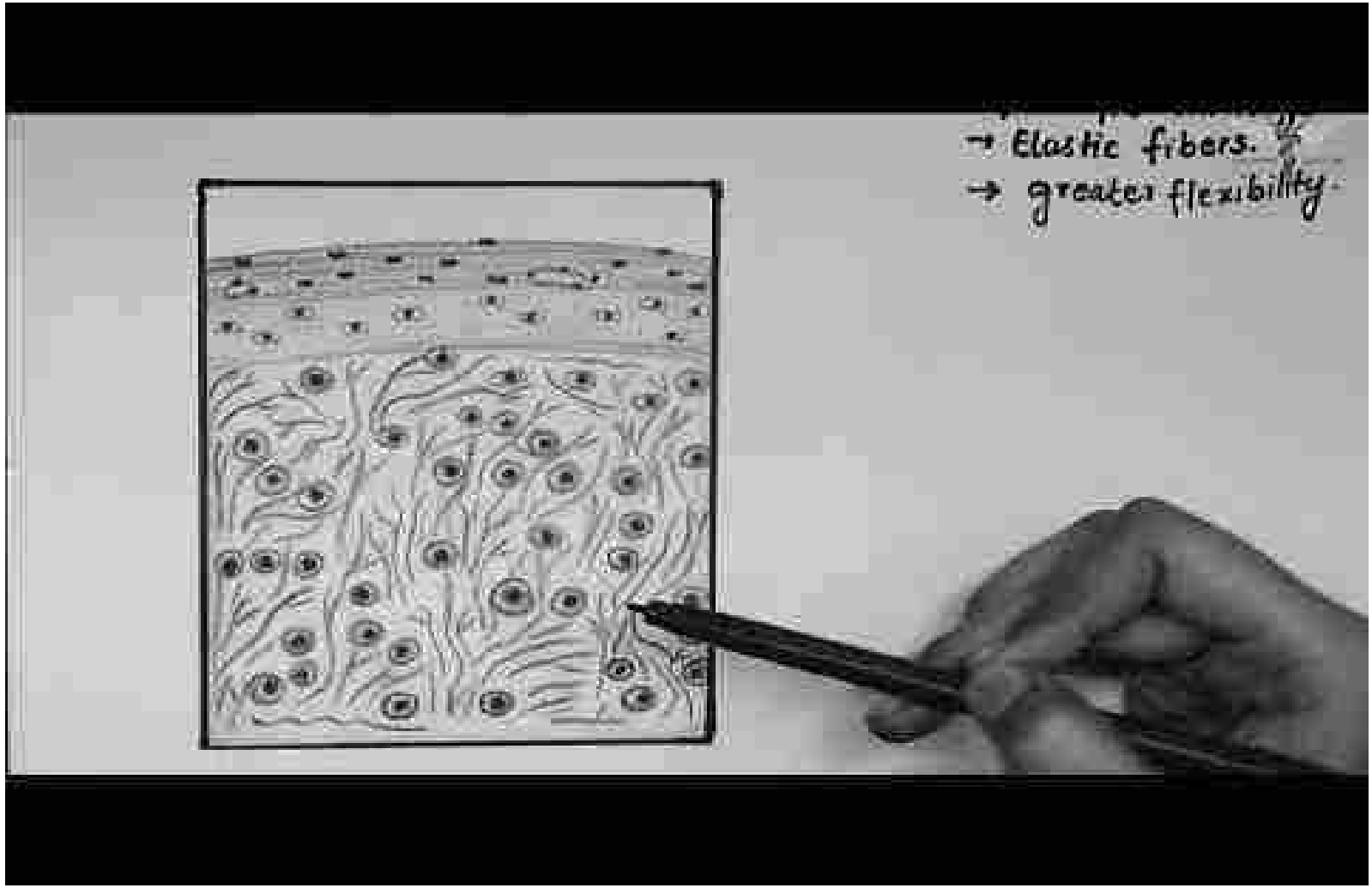
- Yellow fibrocartilage
- Perichondrium present
- Characterised by the presence of elastic fibres in abundance which ranch and anastomose
- Chondrocytes are larger than those of hyaline cartilage
- Chondrocytes are found singly or in group two in lacuna

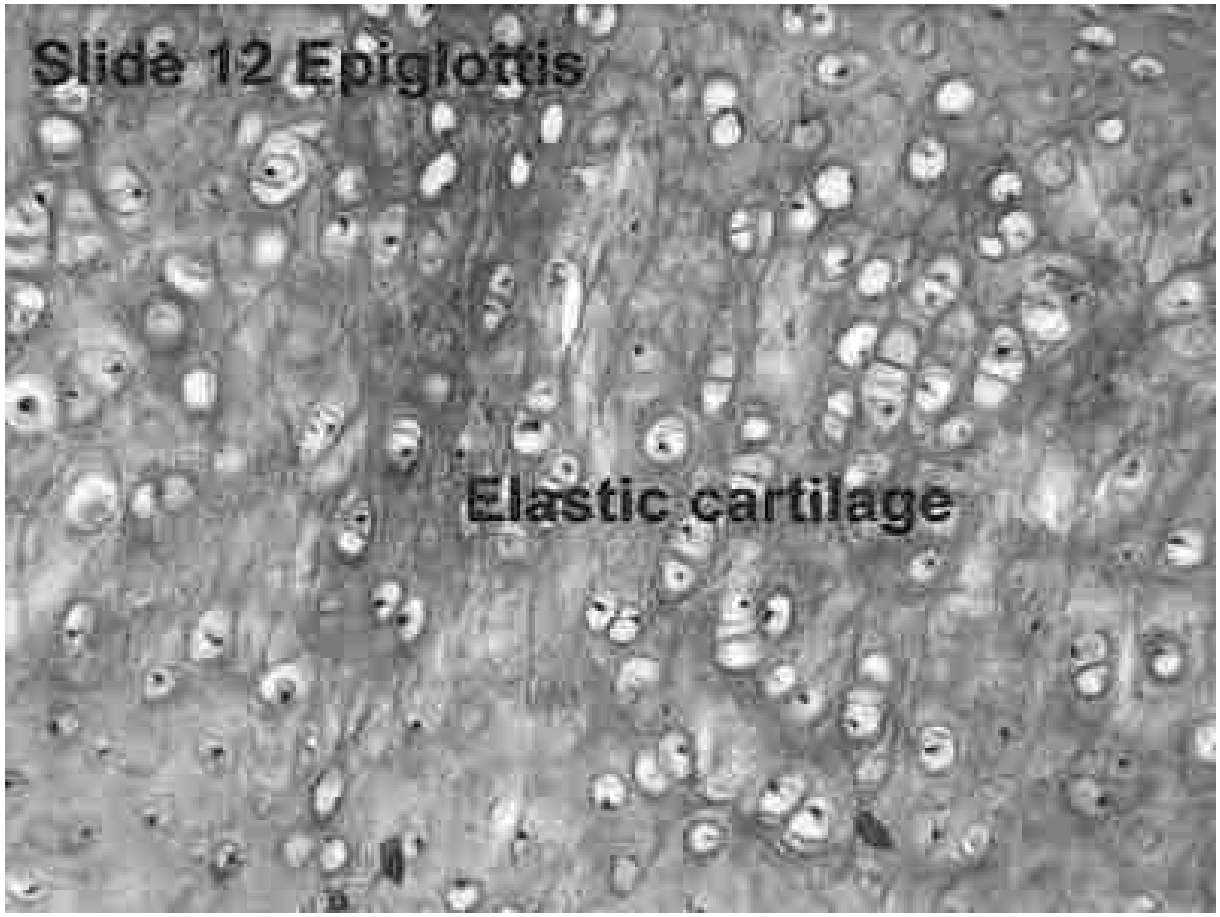
- Though the matrix of hyaline cartilage calcifies in old age the matrix of elastic cartilage doesn't calcify with age
- It provide shape and support to the organ with elasticity

EXAMPLES

- Auricle of pinna
- Epiglottis
- Corniculate and cuniform cartilage of larynx
- External auditory meatus




→ Elastic fibers.
→ greater flexibility.

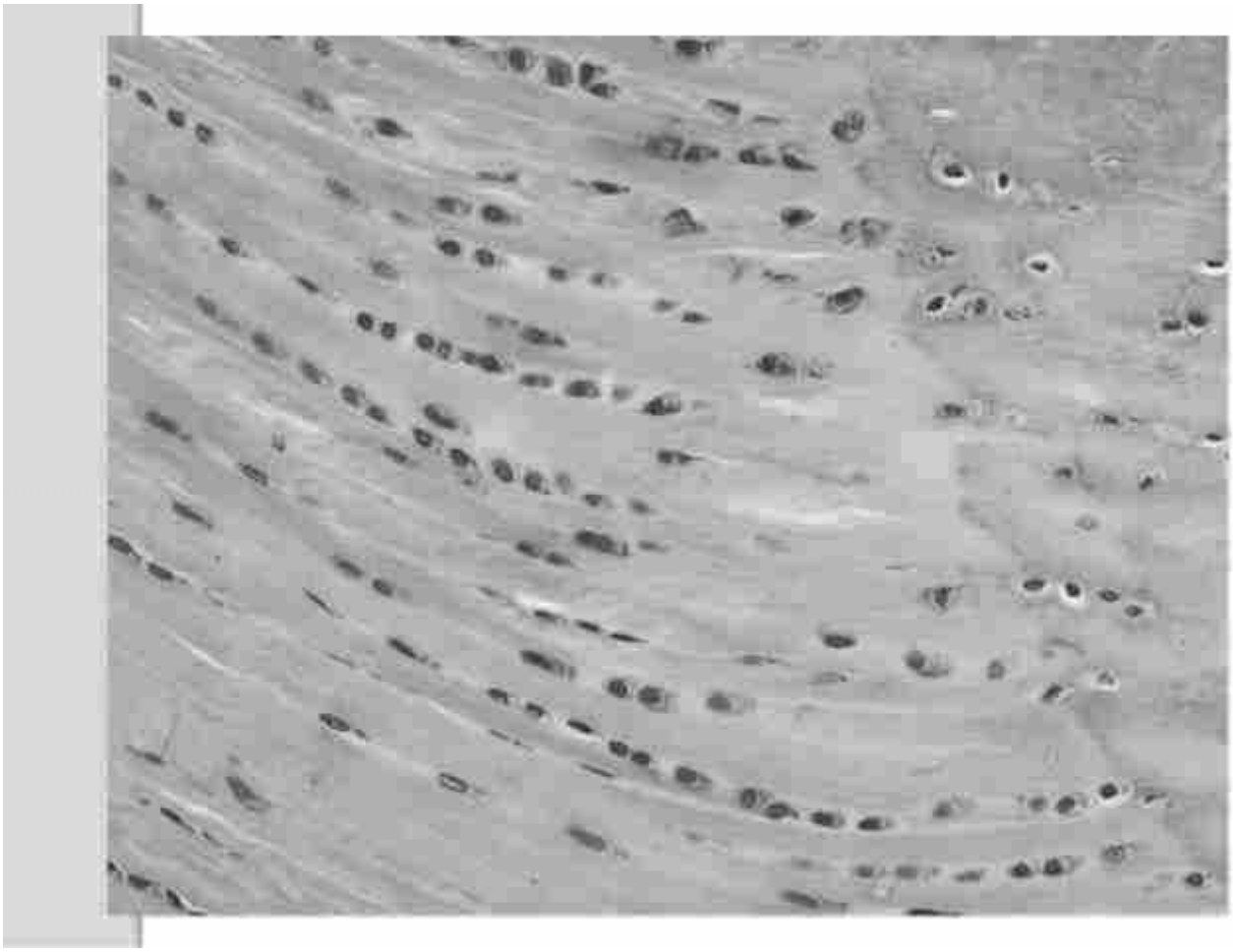

Slide 12 Epiglottis
Elastic cartilage

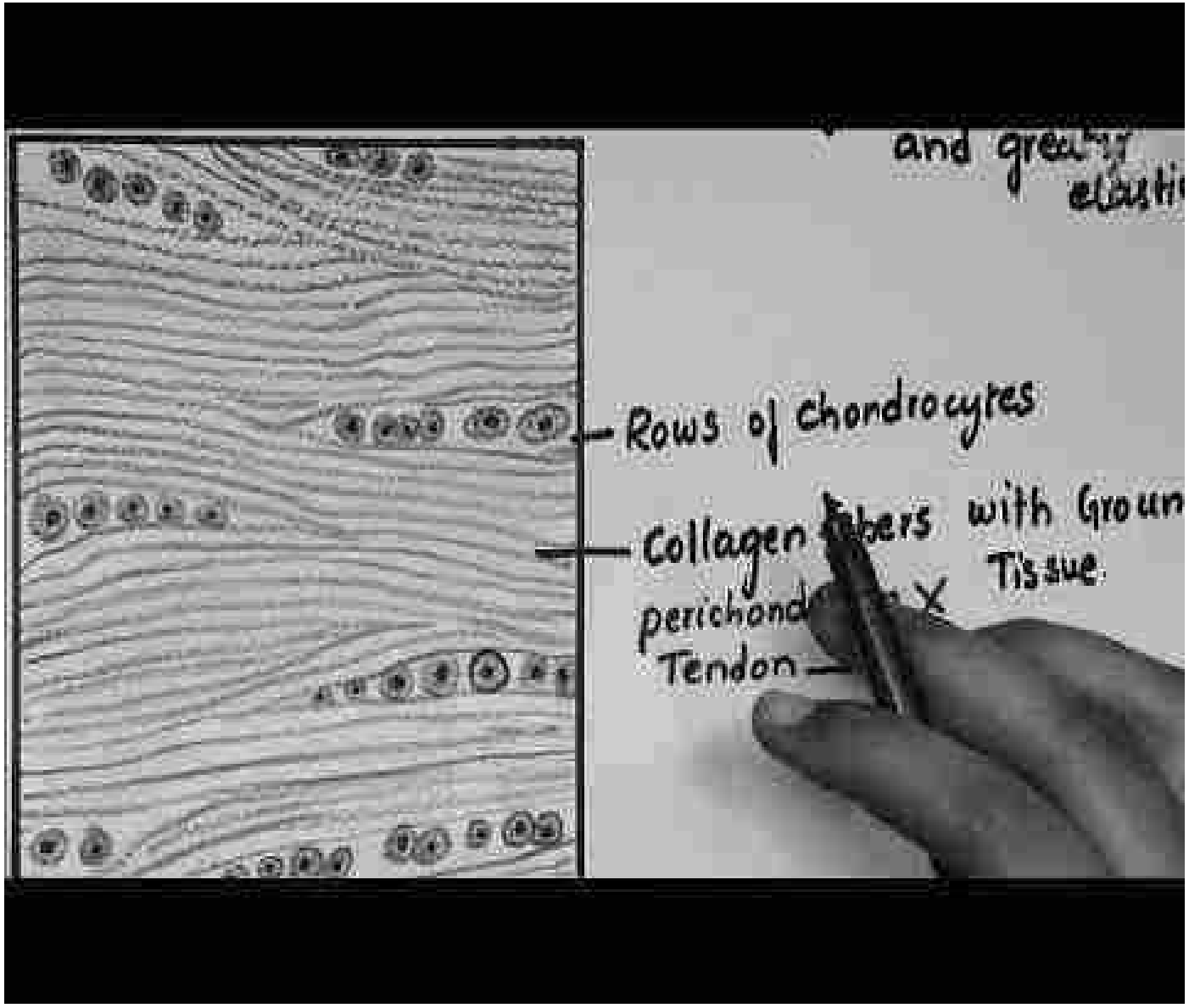
White fibrocartilage

- Perichondrium is absent
- Has thick bundles of colllagen fibres (type2)
- Minimal amount of ground substance , few cells
- Chondrocytes are seen between fibres in single or narrow rows

EXAMPLES

- **Pubic symphysis**
- **Manubriosternal joint**
- **Intervertebral discs**
- **Glenoidal labrum of shoulder joint**
- **Acetabular labrum of hip joint**




and great
elasti
Rows of chondrocytes
Collagen fibers with Groun
Tissue
perichond
Tendon

THANKYOU

INTERCOSTAL SPACE AND NERVES

Topics

- INTRODUCTION
- BOUNDARIES
- CONTENTS
- APPLIED

INTRODUCTION

- *Intercostal space* is the space or gap between two adjacent ribs
- There are 11 intercoastal space present on either side
- Two parts are there *intercartilaginous* part; space between two adjacent coastal cartilage and *interosseous* part; space between two adjacent ribs

BOUNDARIES

- *Superiorly* bounded by the lower border of upper rib
- *Inferiorly* by the upper border of the lower rib
- *Anteriorly* by the lateral border of coastal cartilage
- *Posteriorly* by the body of thoracic vertebra

Intercostal space at the posterior end or vertebral end is *narrow* and at the costochondral junction it is *widest*

CONTENTS

- Intercostal muscles
- Intercostal vessels- artery and vein
- Intercostal nerves

Intercostal muscles

i. External intercostal
ii. Internal intercostal
iii. Transverse thoracis;
    1. *Subcostalis*
    2. *Intercostalis intimi*
    3. *Sternocostalis*

EXTERNAL INTERCOSTAL MUSCLE

- Origin; Lower border of the rib above the space
- Insertion; Outer lip of the upper border of the rib below
- Action ;It elevates the rib during inspiration

INTERNAL INTERCOSTAL MUSCLE

- Origin; Floor of the coastal groove of the rib above
- Insertion; Inner lip of the upper border of the rib below
- Action ;It depress the rib during expiration

TRANSVERSE THORACIS

SUBCOSTALIS

- Origin; Inner surface of the rib near the angle
- Insertion; Inner surface of the two or three ribs below
- Action; Depress the rib
- It present in upper intercostal space and occupies the posterior 1/4th of the space

INTERCOASTALIS INTIMI

- Origin ; Middle two fourth of the ridge above the coastal groove
- Insertion; Inner lip of the upper border of the rib below
- Action; Depress the rib
- It occupies in all the intercostal space except 1st and occupies the middle 2/4th of the space

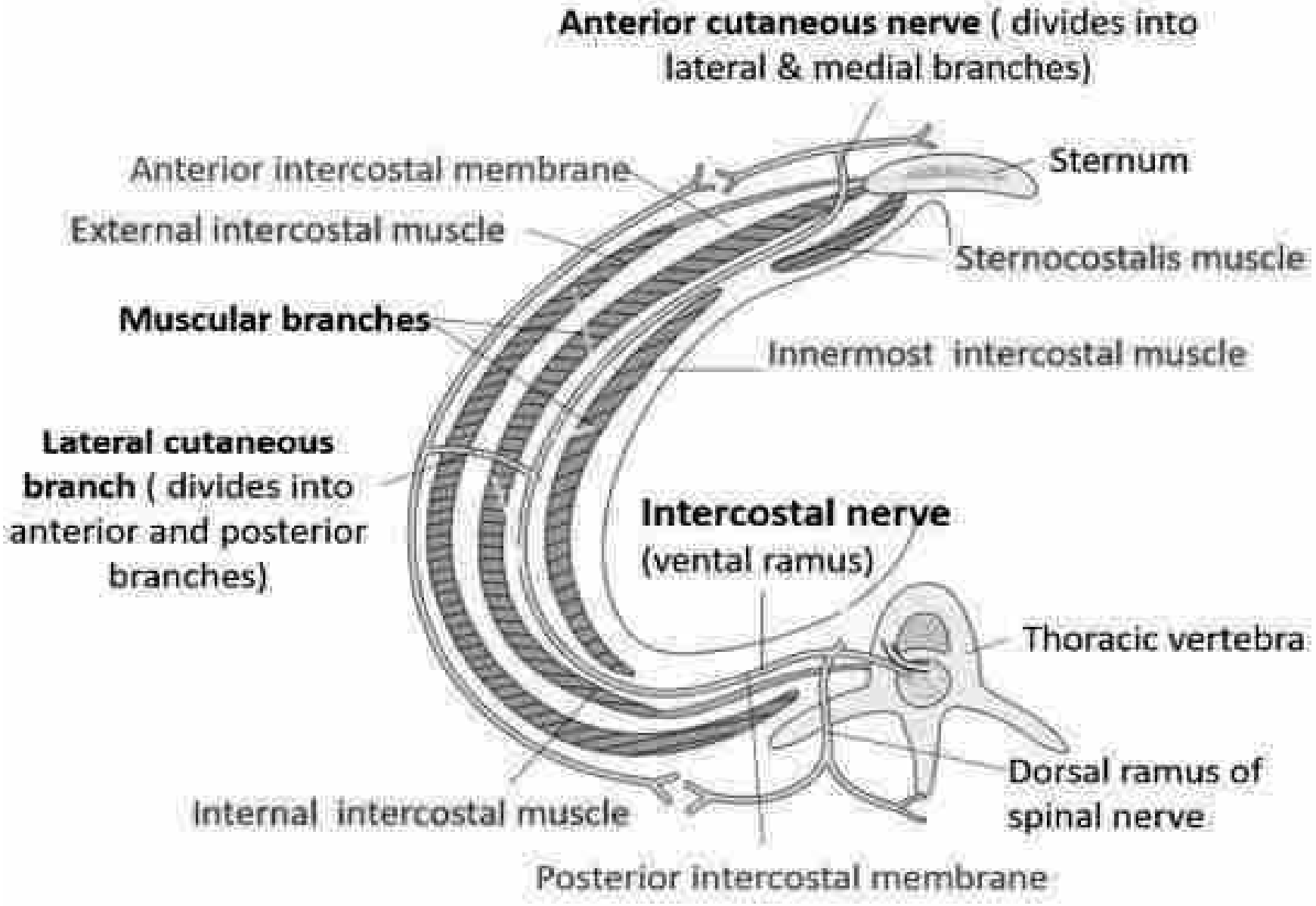
STERNOCOSTALIS

- Origin; Lower one third of the posterior surface of the body of sternum , posterior surface of the xiphoid process, posterior surface of the coastal cartilage of lower 3 or 4 true rib near the sternum
- Insertion; Coastal cartilage of the 2^{nd} to 6^{th} rib
- Action; Depress the rib
- It present in lower intercostal space and occupies the anterior $1/4^{th}$ of the space

EXTENT OF MUSCLES

- External intercostal muscle;Extend from the tubercle of rib to costochondral junction,from the costochondral junction it is extend to sternum as *external intercostal membrane or anterior intercostal membrane*
- Internal intercostal muscle; Extend from lateral border of sternum to the angle of rib , beyond the angle it is continous as *internal intercostal membrane*

- Subcostalis is confined to the posterior part of the lower intercostal space only
- Intercostalis intimi confined to the middle two fourh of all spaces
- The sternocostalis is present in the anterior part of upper intercostal space


Anterior cutaneous nerve (divides into lateral & medial branches)
Anterior intercostal membrane
Sternum
External intercostal muscle
Sternocostalis muscle
Muscular branches
Innermost intercostal muscle
Lateral cutaneous branch (divides into anterior and posterior branches)
Intercostal nerve (vental ramus)
Thoracic vertebra
Dorsal ramus of spinal nerve
Internal intercostal muscle
Posterior intercostal membrane

Direction of muscle fibres

- The fibres of external oblique run downwards, forwards and medially
- The fibres of internal oblique run downwards forwards and laterally
- The fibres of Transverse thoracis runs in the same direction as that of internal intercostal

INTERCOSTAL NERVES

INTRODUCTION

COURSE

BRANCHES

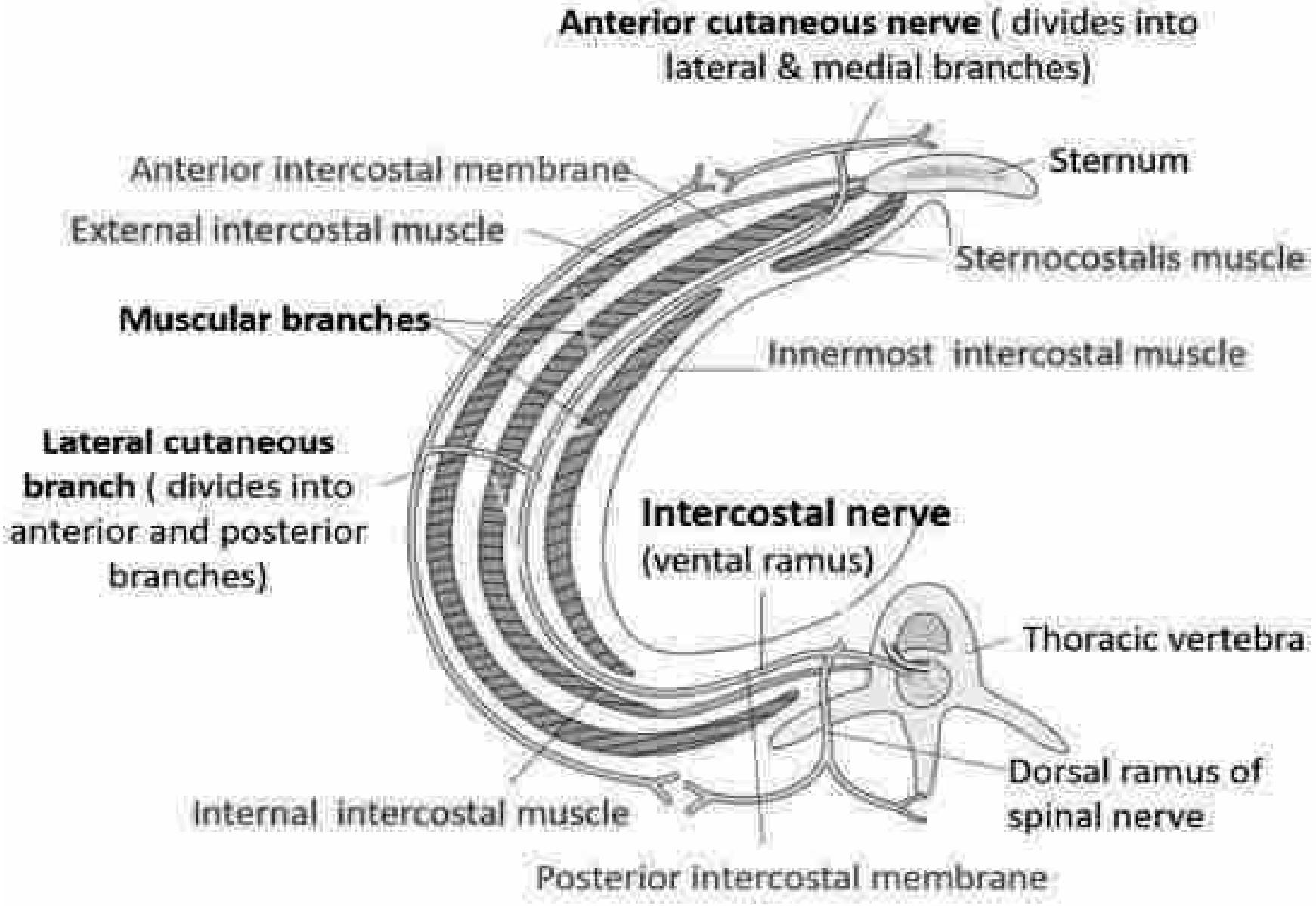
APPLIED

INTRODUCTION

- They are the anterior or ventral rami of *T1* – *T11* spinal nerves
- *T12* is not intercostal nerve since it passes below the 12^{th} rib so it is called as subcostal nerve

Types

- Typical intercostal nerve; The nerve only supplies the thoracic wall. From T3- T6
- Atypical intercostal nerve ; The nerve which supply the upper limb(T1,T2) and abdominal wall(T7-T12) along with thoracic wall


Anterior cutaneous nerve (divides into lateral & medial branches)
Anterior intercostal membrane
Sternum
External intercostal muscle
Sternocostalis muscle
Muscular branches
Innermost intercostal muscle
Lateral cutaneous branch (divides into anterior and posterior branches)
Intercostal nerve (vental ramus)
Thoracic vertebra
Dorsal ramus of spinal nerve
Internal intercostal muscle
Posterior intercostal membrane

COURSE AND RELATION

- In the upper part; It passes below the neck of rib between parietal pleura , endothoracic fascia and internal intercostal membrane and enters the costal groove
- In the costal groove it lies in between intercostalis intimi and internal intercostal muscle.It is related to the intercostal vessels here.It order from above downwards is *VAN*

- Near the sternum nerve crosses the vessels and sternocostalis muscle.It then pierces the internal intercostal muscle and external intercostal membrane and continous *anterior cutaneous nerve of thorax*

Branches

MUSCULAR BRANCH

1. Arising from the main trunk and supply muscles of the same space
2. *Collateral branch* arising from the angle of rib and runs along the lower part of the space along with neurovascular plane and at the costochondral junction it may communicate with the main trunk nor continous as the anterior cutaneus nerve of thorax and supplies muscles of space

CUTANEOUS BRANCHES

1. Anterior cutaneous branch; Termination of intercoastal nerve
2. Lateral cutaneous branch; It arises from the angle of rib and runs along the main trunk and at the mid axillary line it pierces the internal intercostal and external intercostal muscle and becomes and enterior and posterior branches

COMMUNICATING BRANCH

1. Each nerve communicate with the sympathetic ganglion
2. Each nerve communicates with adjacent intercostal nerve in the posterior part of intercostal space

APPLIED

- The irritation of intercostal nerve is referred in the front of the chest or abdomen.This is known as *root pain or girdle pain*
- Herpes virus may cause infection of intercostal nerve
- Pus formed in the vertebral column passes and reaches the thorax through the neurovascular line

INTERCOSTAL ARTERIES

Anterior intercostal artery

- 9 pairs
- Two in each space
- Origin; 1-6 from *Internal thoracic artery*,7-9 from *musculo phrenic artery*

Posterior intercostal ar.

- 11 pairs
- One in each space
- Origin;1,2 from *superior intercostal artery* branch of subclvian artery, 3-11 from *descending aorta*

COURSE

Anterior intercostal artery

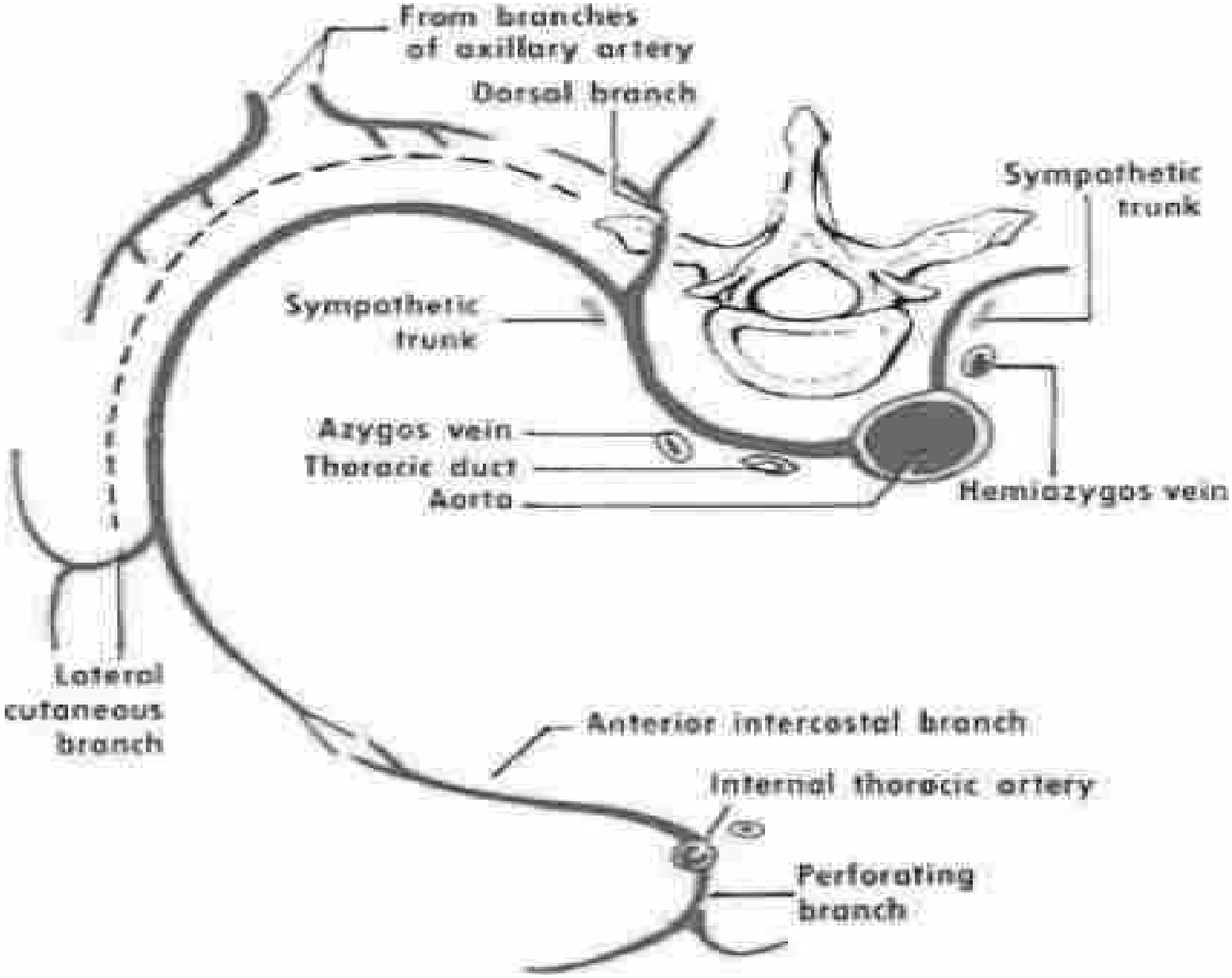
- The two anterior intercostal arteries reaches the costochondral junction and anastomosis with the collateral branch of posterior intercostal artery

Posterior intercostal artery

COURSE

- Right posterior intercostal arteries are longer than left
- Pass behind the oesophagus,thoracic duct, and azygos vein and sympathetic chain
- Left posterior intercostal arteries pass behind the azygos vein and sympathetic chain

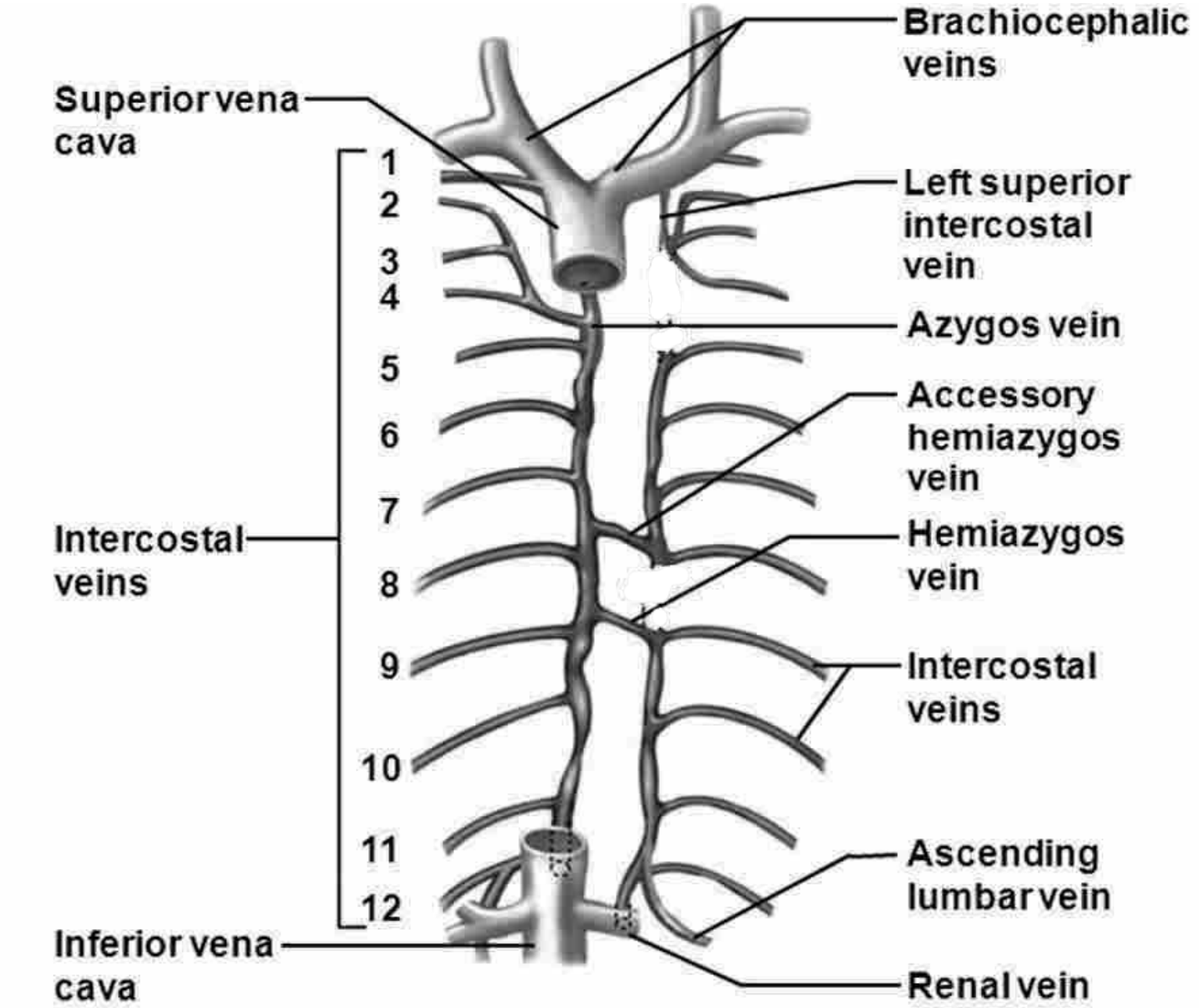
- In the intercostal groove it lies along with the intercostal nerve and vein(VAN) and in the groove upper part it lies between parietal pleura and internal intercostal membrane and middle part between intercostalis intimi and internal intercostal muscle
- When it reaches the costochondral junction it anastomosis with the upper anterior intercostal artery of the space


From branches
of axillary artery
Dorsal branch
Sympathetic
trunk
Sympathetic
trunk
Azygos vein
Thoracic duct
Aorta
Hemiazygos vein
Lateral
cutaneous
branch
Anterior intercostal branch
Internal thoracic artery
Perforating
branch

INTERCOSTAL VEIN

- There are 2 anterior intercostal vein and in each space
- Nine pairs of anyterior intercostal vein are there
- In 1-3 spaces vein drains into internal thoracic vein
- In 4-6 space it drains into venae comitantes accompanying internal thoracic artery
- Rest of the space it drians into venae comitantes accompanying musculophrenic arery

- There is one posterior and one collateral intercostal vein in each space accompanies the corresponding artery and lies most superior to the artery and nerve(VAN)


Brachiocephalic veins
Superior vena cava
1
2
3
4
5
6
7
8
9
10
11
12
Left superior intercostal vein
Azygos vein
Accessory hemiazygos vein
Intercostal veins
Hemiazygos vein
Intercostal veins
Ascending lumbar vein
Inferior vena cava
Renal vein

LYPHATIC DRAINAGE

- Lymphatic from anterior part of the space reaches the internal mammary node lies along the internal thoracic artery
- Lymphatics from the posterior part of the space reaches the posterior intercostal node which lies on the head and neck of the rib

HMP shunt Pathway

- Other names:

  Phosphogluconate Pathway

  Hexose Monophosphate Shunt

  - **Glucose is shunted through this pathway instead of glycolysis.**
  - **Intermediates are monophosphates only .**
  - **Reaction involves intermediate formation of pentose phosphates.**

Definition:

It is an alternative pathway for oxidation of glucose to provide **NADPH** and **ribose 5-phosphate.**

Site

Operates in Cytoplasm & Active in : liver, adipose tissue, adrenal cortex, thyroid, erythrocytes, testis and lactating mammary gland

Its activity is low in muscle

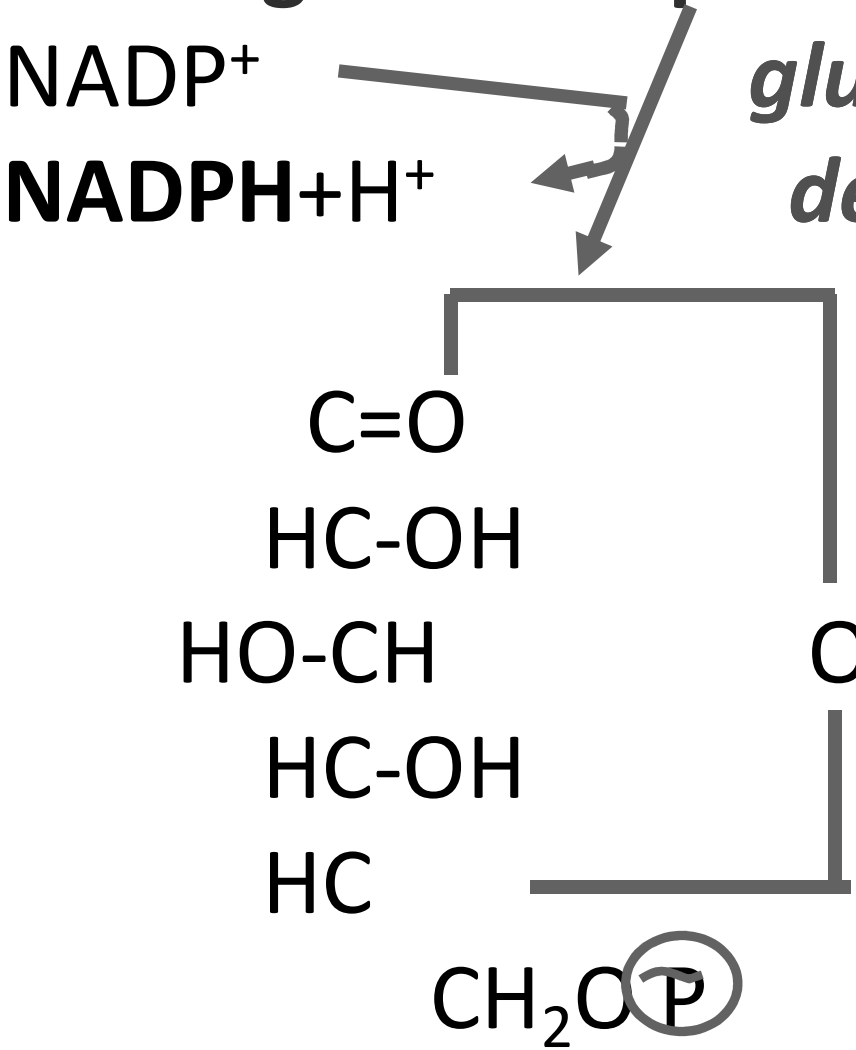
Reactions of the pentose phosphate pathway occur in the cytosol in two phases

- **Oxidative non-reversible phase**
- **Non-oxidative reversible phase**
- **$NADP^+$, not NAD^+, is used as hydrogen acceptor**

Oxidative Phase-

generates reducing power as NADPH

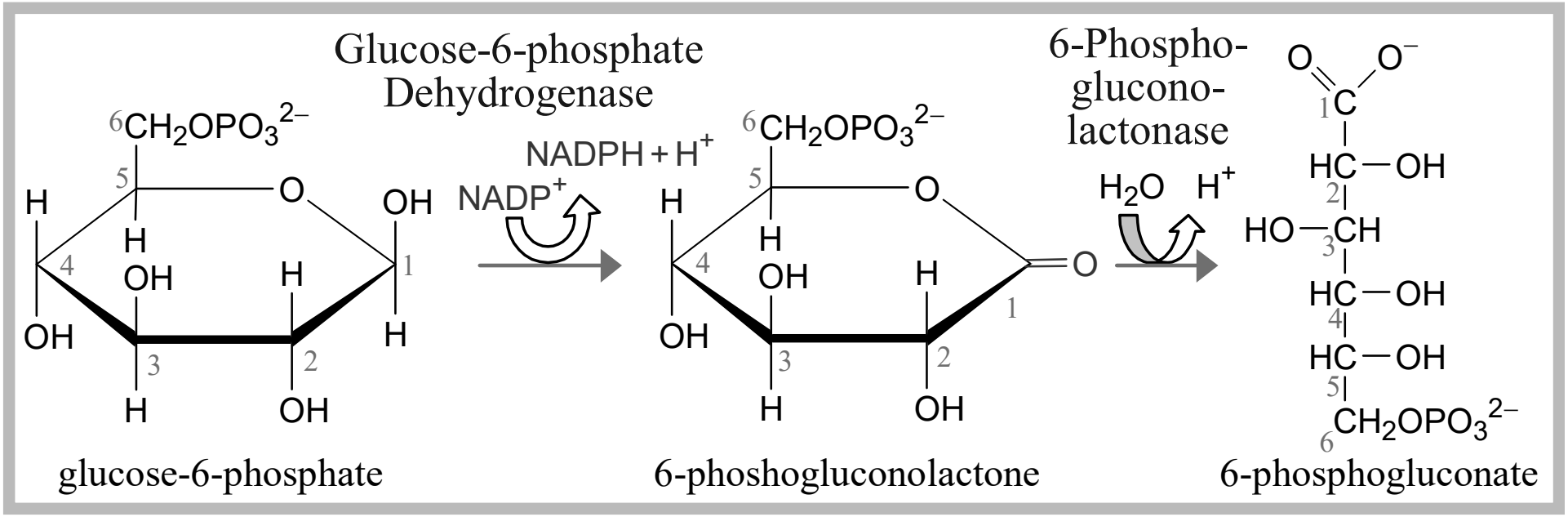
glucose-6-phosphate



glucose-6-phosphate dehydrogenase

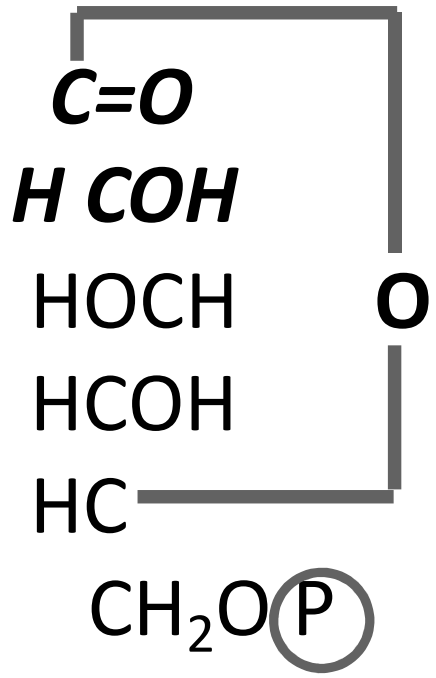
6-phosphoglucono -δ-lactone

unstable with a large release of energy upon hydrolysis



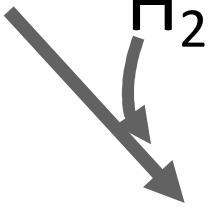
Glucose-6-phosphate Dehydrogenase catalyzes **oxidation** of the aldehyde (hemiacetal), at **C1** of glucose-6-phosphate, to a **carboxylic acid**, in ester linkage (lactone).

$NADP^+$ serves as electron acceptor.



6-phosphoglucono - δ-lactone

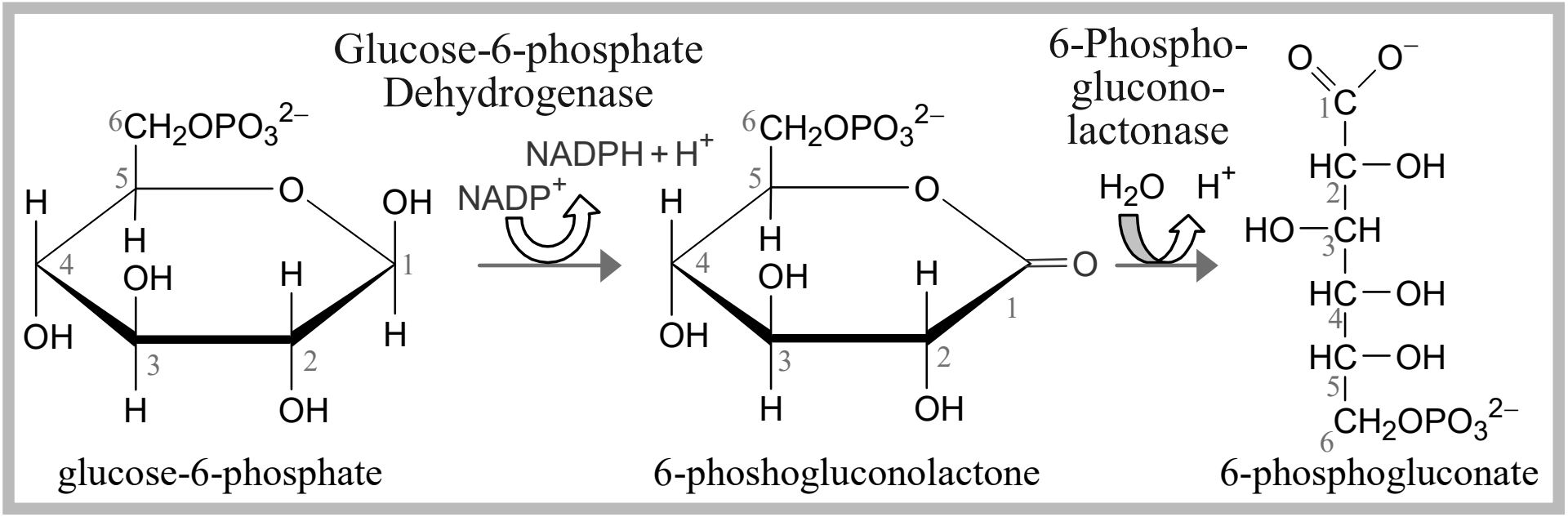
H_2O ***lactonase***



COO^-
CHOH
HOCH
CHOH
CHOH
CH_2O P

-pulls initial dehydrogenation

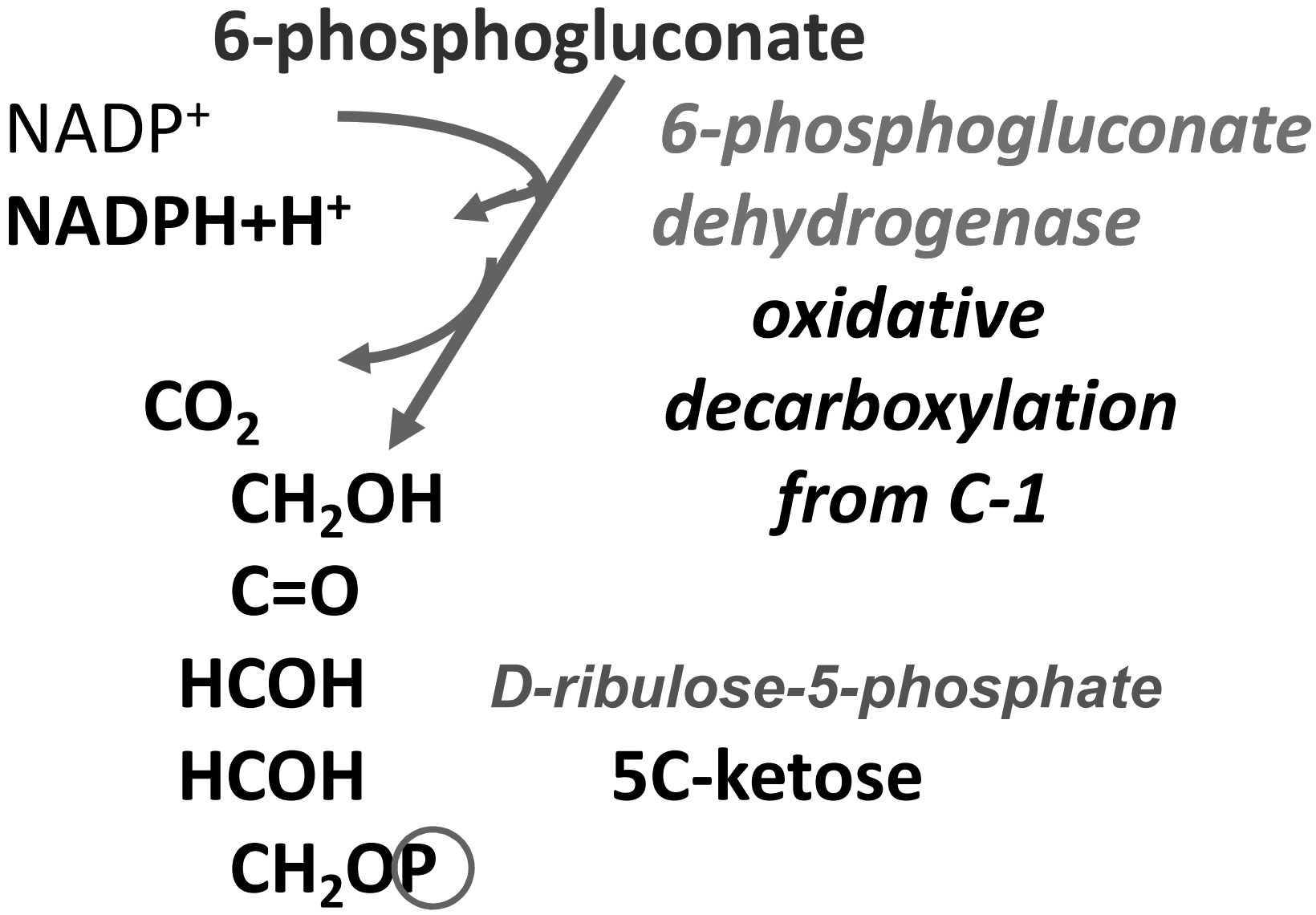
sugar acid-
6-phosphogluconate

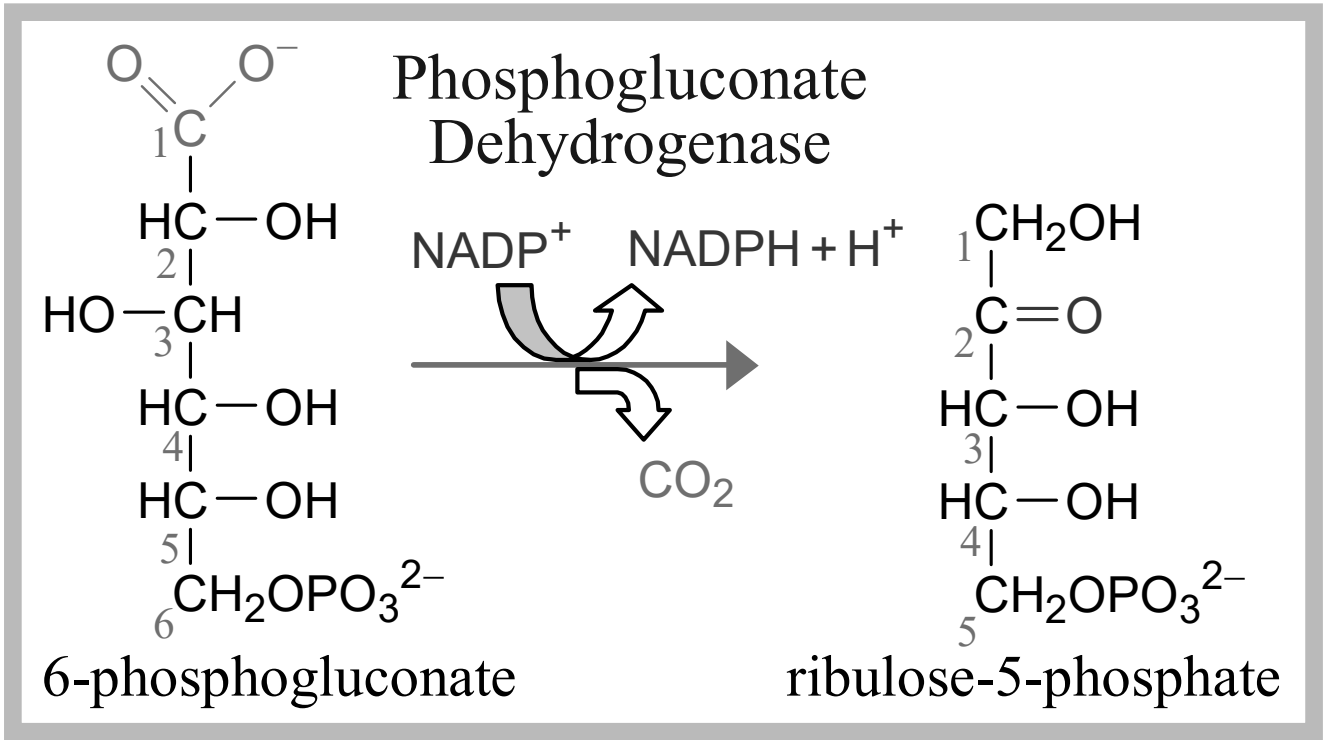


6-Phosphogluconolactonase catalyzes **hydrolysis** of the ester linkage, resulting in **ring opening**.

The product is **6-phosphogluconate**.

Although ring opening occurs in the absence of a catalyst, 6-Phosphogluconolactonase speeds up the reaction, decreasing the lifetime of the highly reactive, and thus potentially toxic, 6-phosphogluconolactone.


6-phosphogluconate
NADP+
NADPH+H+
6-phosphogluconate
dehydrogenase
oxidative
decarboxylation
from C-1
CO2
CH2OH
C=O
HCOH
HCOH
CH2OP
D-ribulose-5-phosphate
5C-ketose

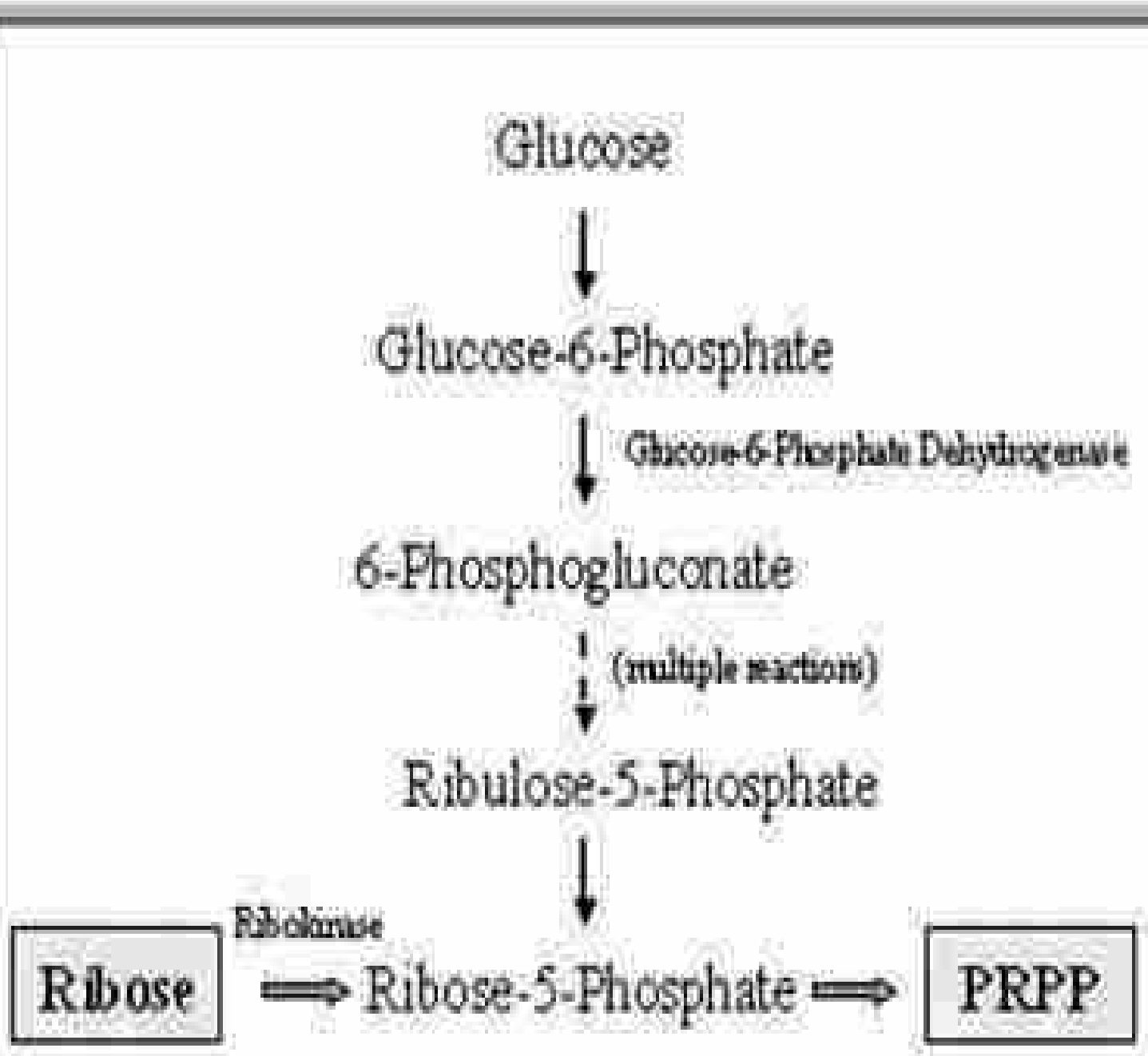


Phosphogluconate Dehydrogenase catalyzes **oxidative decarboxylation** of 6-phosphogluconate, to yield the **5-C** ketose **ribulose-5-phosphate**.

The **OH** at C**3** (C**2** of product) is oxidized to a **ketone**.

This promotes loss of the carboxyl at C**1** as **CO_2**.

$NADP^+$ serves as oxidant.


Glucose
Glucose-6-Phosphate
Glucose-6-Phosphate Dehydrogenase
6-Phosphogluconate
(multiple reactions)
Ribulose-5-Phosphate
Ribokinase
Ribose
Ribose-5-Phosphate
PRPP

Summary of First Phase

Overall reaction:

glucose-6-Ⓟ + 2 $NADP^+$ + H_2O → ribose-5-Ⓟ + CO_2 + 2 NADPH + $2H^+$

-- this reductive portion of the shunt has generated

- **reducing power** (NADPH) and
- **5-carbon sugars**

-- Most cells need much more NADPH than pentose phosphates

Nonoxidative Phase

-- in cells that need much more NADPH, three pentose phosphate molecules are transformed into two hexose phosphates and a triose phosphate.

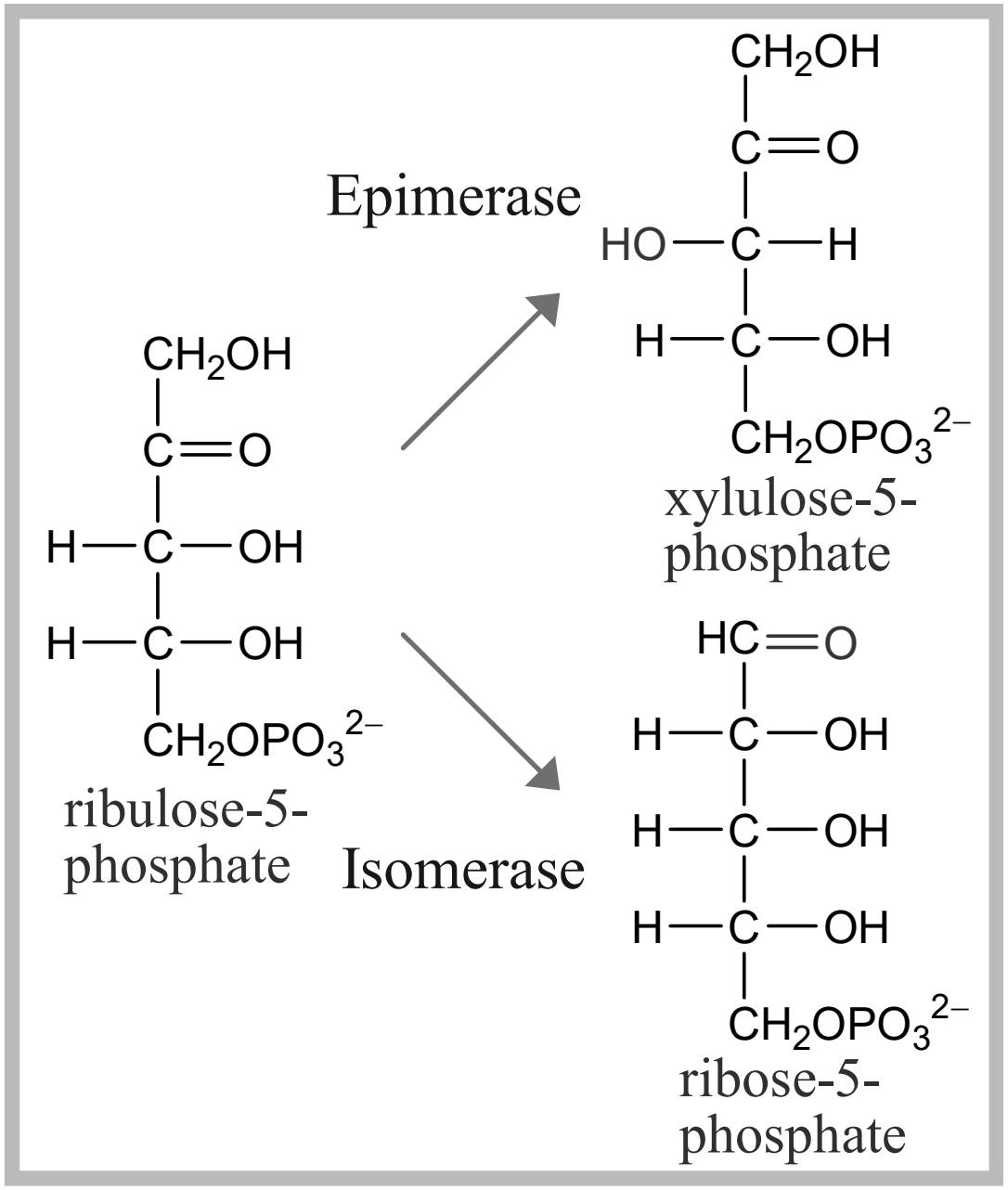
-- ribulose 5-phosphate→→
glyceraldehyde 3-phosphate +
2 fructose 6-phosphate

Epimerase inter-converts stereoisomers ribulose-5-P and xylulose-5-P.

Isomerase converts the ketose ribulose-5-P to the aldose ribose-5-P.

Both reactions involve deprotonation to an **endiolate** intermediate followed by specific reprotonation to yield the product.

Both reactions are reversible.



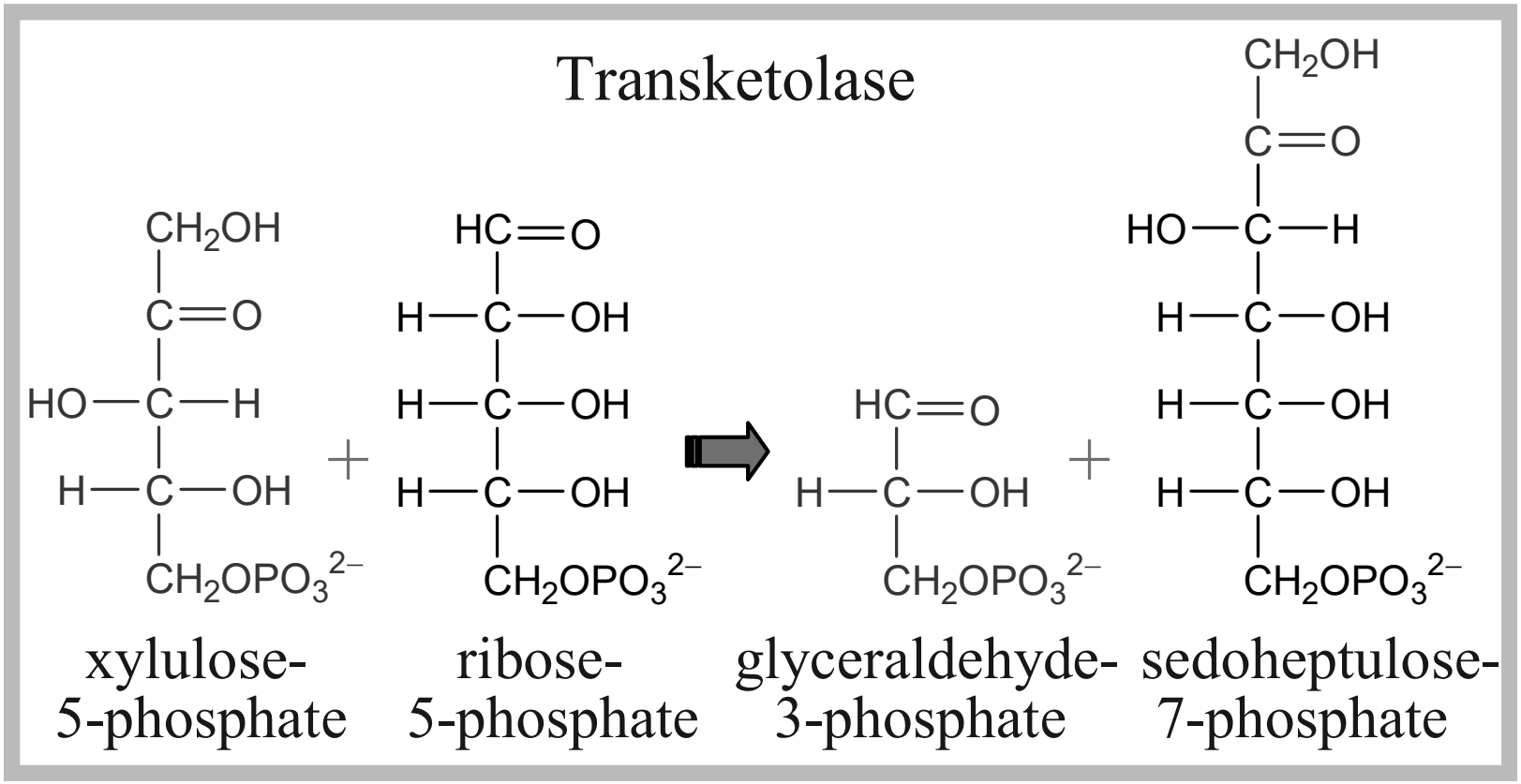
Transketolase & **Transaldolase** catalyze transfer of 2-C or 3-C molecular fragments respectively, in each case from a ketose donor to an aldose acceptor.

- Transketolase actually transfers an aldol moiety (glycoaldehyde), and
- Transaldolase actually transfers a ketol moiety (dihydroxyacetone).

1) During the remaining reactions, *transketolase* and *transaldolase* catalyze the interconversions of trioses, pentoses, and hexoses.

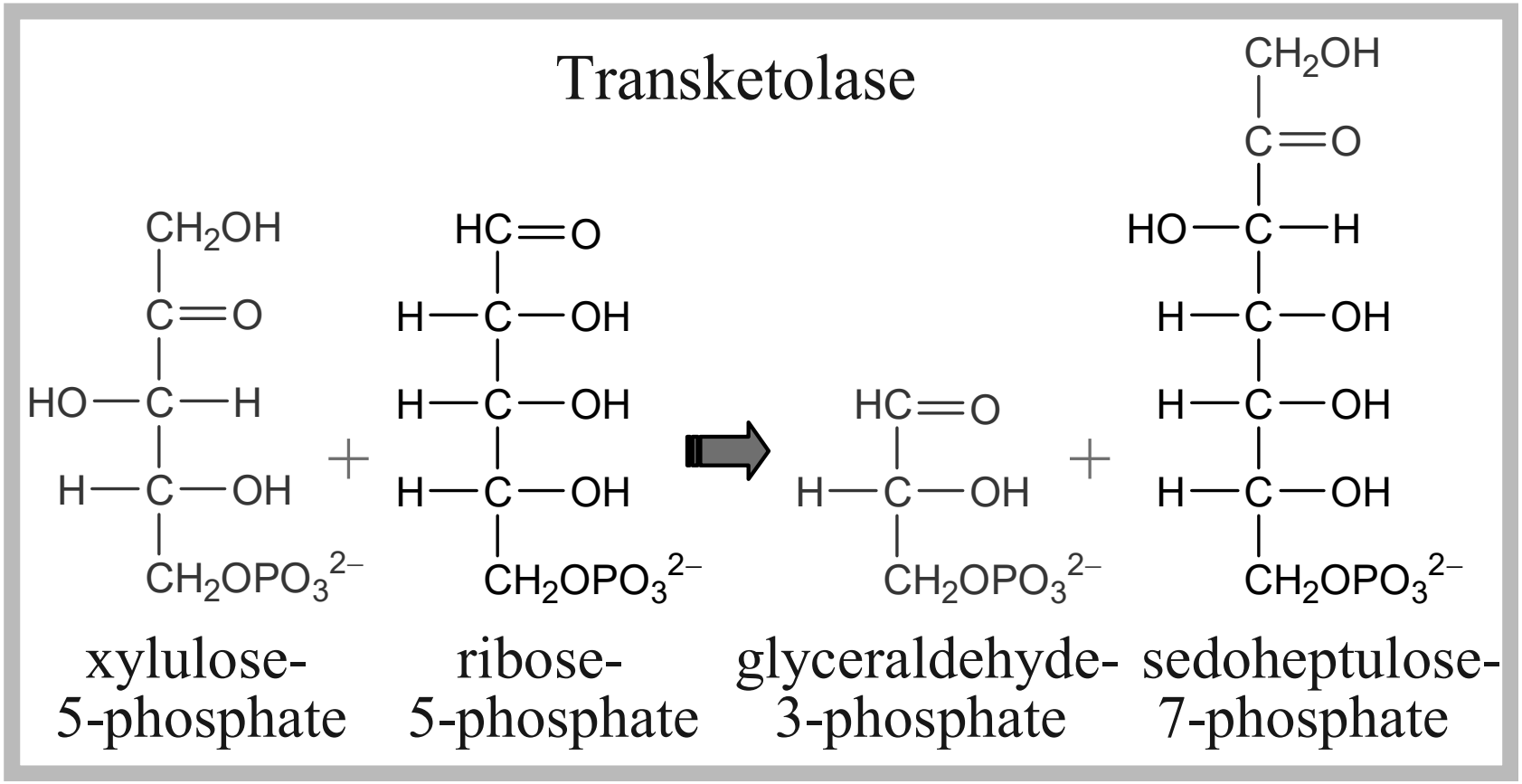
2) Transketolase is a thiamine pyrophosphate (TPP)-requiring enzyme that transfers 2-carbon units from a ketose to an aldose.

3) Transaldolase transfers 3-carbon units from a ketose to an aldose.

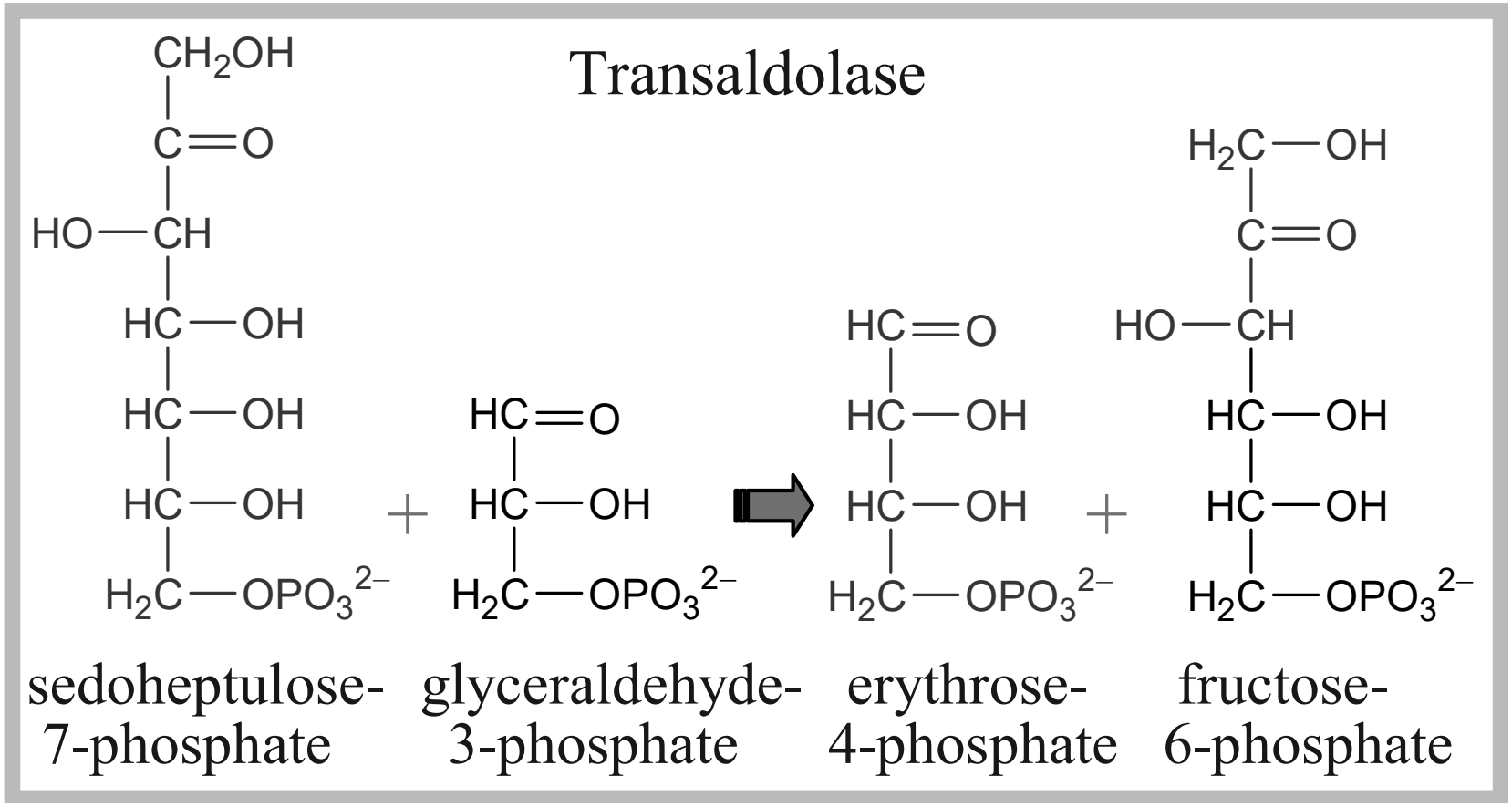


- **Transketolase** transfers a **2-C fragment** from xylulose-5-P to either ribose-5-P or erythrose-4-P.
- Transketolase utilizes as prosthetic group **thiamine pyrophosphate (TPP)**, a derivative of **vitamin B_1**.

  Pyruvate Dehydrogenase of Krebs Cycle also utilizes TPP as prosthetic group.

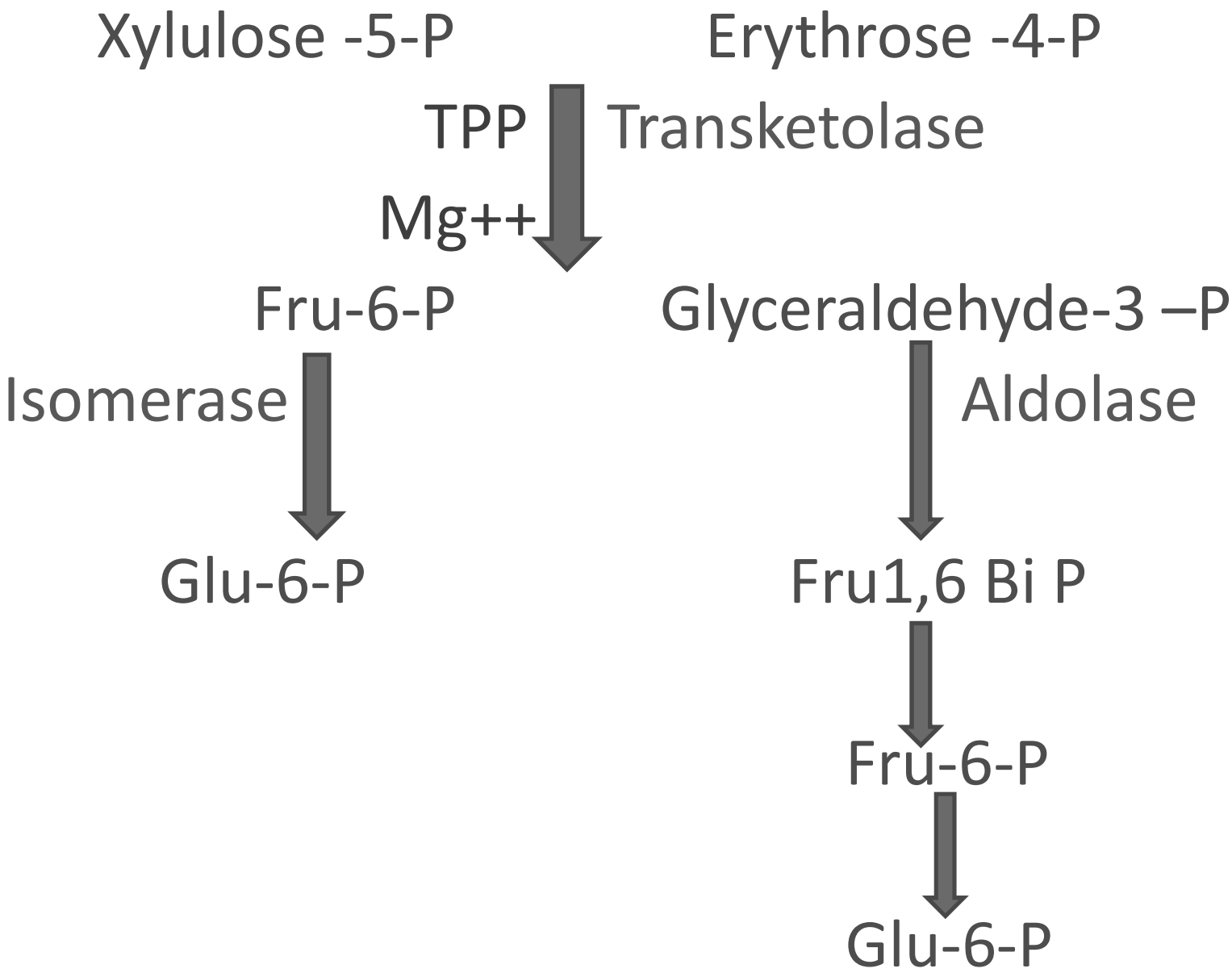


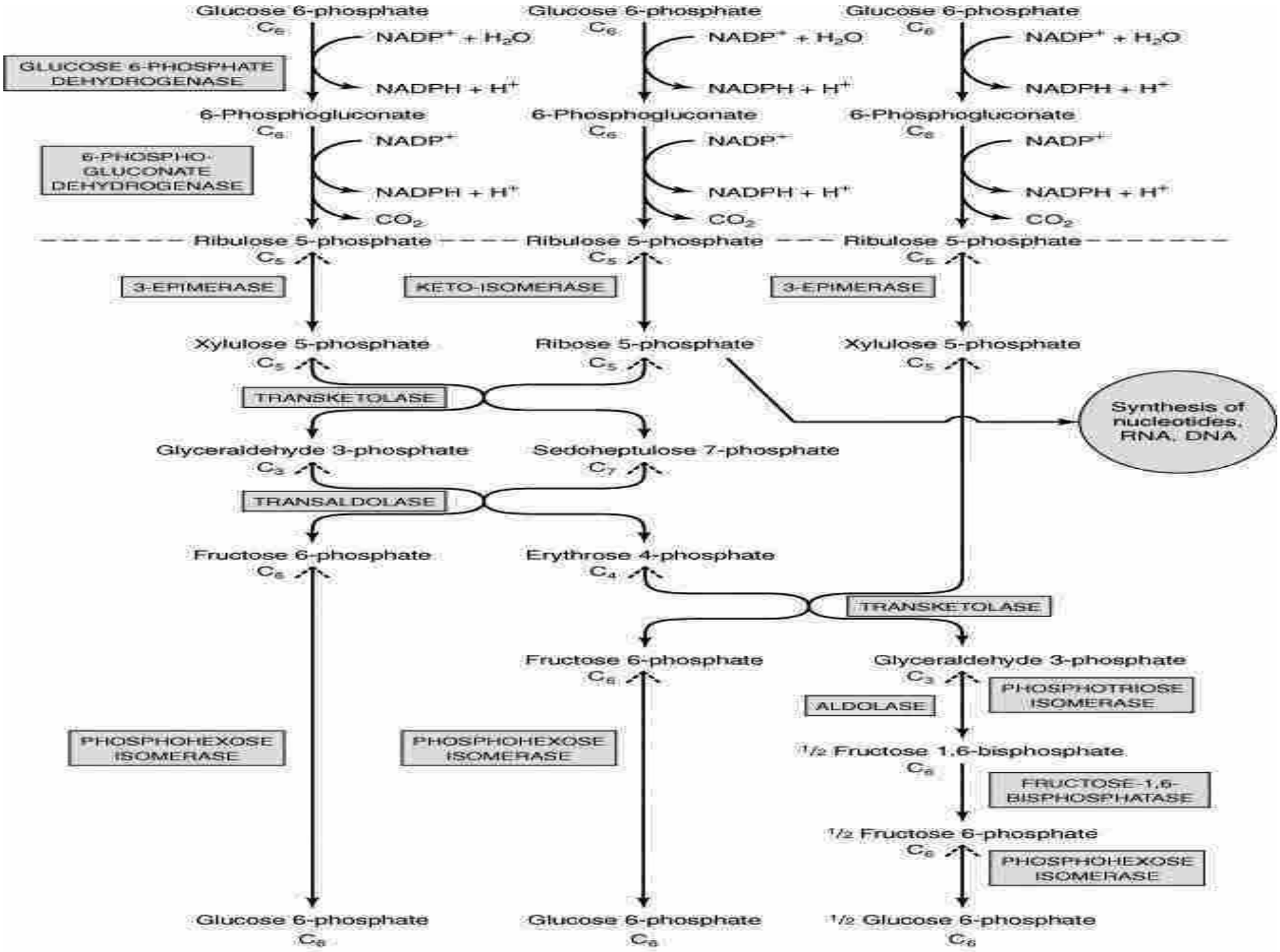
- Transfer of the 2-C fragment to the 5-C aldose ribose-5-phosphate yields sedoheptulose-7-phosphate.
- Transfer of the 2-C fragment instead to the 4-C aldose erythrose-4-phosphate yields fructose-6-phosphate.



Transaldolase catalyzes transfer of a **3-C** dihydroxyacetone moiety, from sedoheptulose-7-phosphate to glyceraldehyde-3-phosphate.

Transaldolase has an α,β **barrel** structure.


Xylulose -5-P
Erythrose -4-P
TPP
Transketolase
Mg++
Fru-6-P
Glyceraldehyde-3 –P
Isomerase
Aldolase
Glu-6-P
Fru1,6 Bi P
Fru-6-P
Glu-6-P


Glucose 6-phosphate
C6
NADP+ + H2O
NADPH + H+
GLUCOSE 6-PHOSPHATE DEHYDROGENASE
6-Phosphogluconate
C6
NADP+
NADPH + H+
CO2
6-PHOSPHO-GLUCONATE DEHYDROGENASE
Ribulose 5-phosphate
C5
3-EPIMERASE
KETO-ISOMERASE
Xylulose 5-phosphate
Ribose 5-phosphate
TRANSKETOLASE
Synthesis of nucleotides, RNA, DNA
Glyceraldehyde 3-phosphate
C3
Sedoheptulose 7-phosphate
C7
TRANSALDOLASE
Fructose 6-phosphate
Erythrose 4-phosphate
C4
PHOSPHOHEXOSE ISOMERASE
ALDOLASE
PHOSPHOTRIOSE ISOMERASE
1/2 Fructose 1,6-bisphosphate
FRUCTOSE-1,6-BISPHOSPHATASE
1/2 Fructose 6-phosphate
1/2 Glucose 6-phosphate

It is a multicyclic process in which 3 molecules of glucose -6-P gives rise to :

- 3 molecules of CO_2
- 3 molecules of 5 – carbon sugars.
- Latter rearranged to generate 2 molecules of glu-6-P and 1 molecule of glyceraldehyde-3-P.
- It does not generate ATP

Regulation

Glucose-6-phosphate Dehydrogenase:

- Glucose-6-phosphate Dehydrogenase is the **committed step** of the Pentose Phosphate Pathway. This enzyme is regulated by availability of the substrate **$NADP^+$**.
- As NADPH is utilized in reductive synthetic pathways, the increasing concentration of $NADP^+$ stimulates the Pentose Phosphate Pathway, to replenish NADPH.
- Insulin induce G-6-PD .
- Increases in high carbohydrate diet & reduce in DM & Starvation.

Significance of Pentose Phosphate Shunt

1) Generation of NADPH for reductive processes such as fatty acid , steroid biosynthesis ,Synthesis of sphingolipids & Neurotransmitter and antioxidant mechanisms.

2) Provision of ribose for nucleotide & nucleic acid biosynthesis .

3) In RBCs provides NADPH for reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH).

- HMP shunt in RBCs provides NADPH for reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH). Reactions as follows:

Glutathione reductase (FAD)

- $GSSG + NADPH + H^+ \longrightarrow 2GSH + NADP$

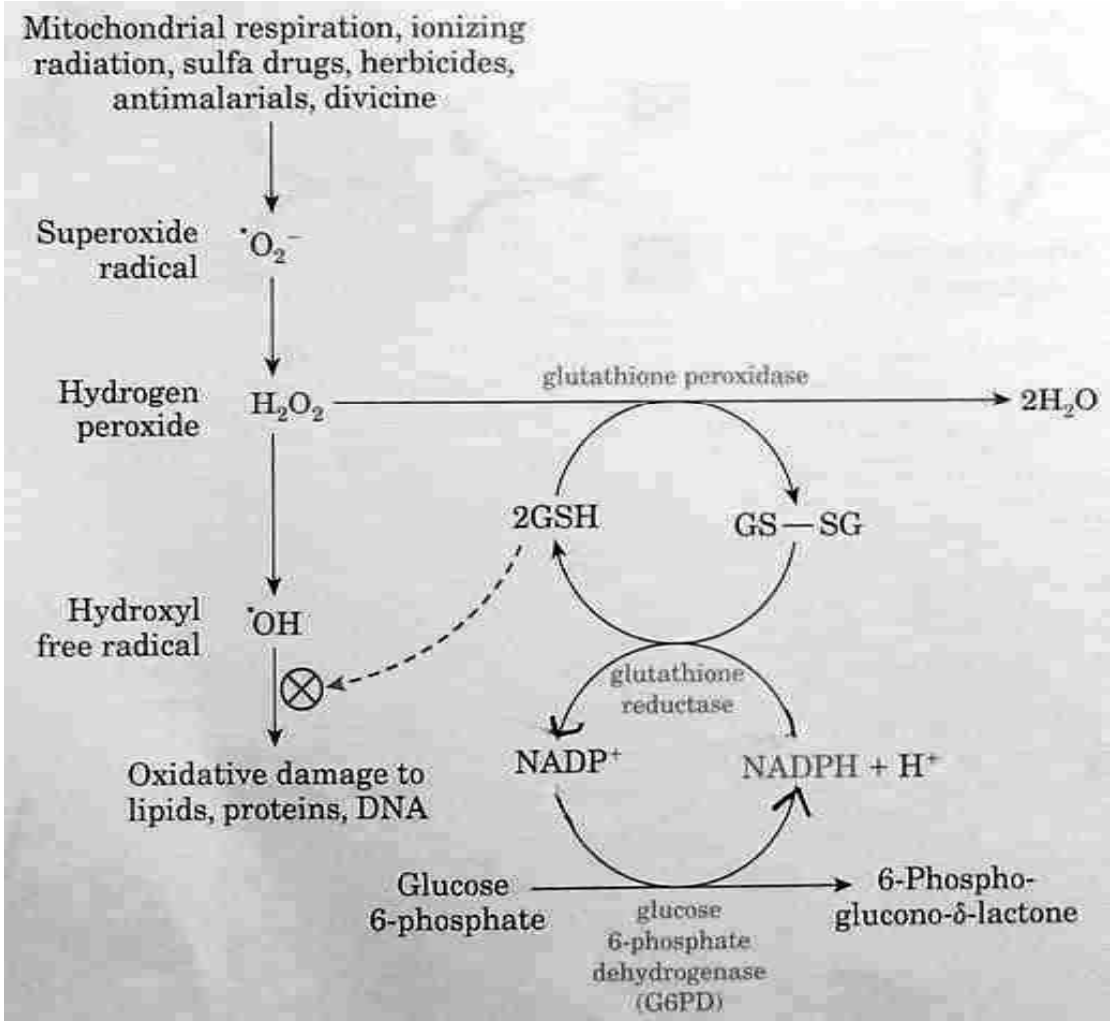
(oxidized glutathione) (reduced glutathione)

Glutathione peroxidase (Se)

- $2GSH + H_2O_2 \longrightarrow GSSG + 2H_2O$

Role of NADPH and glutathione in protecting cells against ROS

Role of NADPH and glutathione in protecting cells against highly reactive oxygen derivatives. Reduced glutathione (GSH) protects the cell by destroying hydrogen peroxide and hydroxyl free radicals. Regeneration of GSH from it oxidized form (GS-SG) requires the NADPH produced in the glucose 6-phosphate dehydrogenase reaction.



- This reaction is important in RBCs to prevent accumulation of H_2O_2 that may decrease the life span of RBCs by oxidizing Hb to MetHb.
- It also keep iron of Hb in reduced ferrous state & prevents the formation of MetHb

- Role in lens of eye – Maximum concentration of NADPH is in lens of eye. For preserving transparency of lens, NADPH is required.
- Role in Phagocytosis – NADPH is utilized by NADHP oxidase in producing superoxide aninos (O_2-) for destroying phagocytozed material.

THE PENTOSE PHOSPHATE SHUNT

NADPH and **ribose 5-phosphate**, both required for biosynthesis.

Note: ①Electron carrier **NAD^+/NADH** used primarily to *oxidize* substrates.

②Electron carrier **$NADP^+$/NADPH** used to *reduce* substrates & functions synthetically, e.g., steroids, cholesterol and fatty acid synthesis (anabolic). Also destroys toxic oxidants.

Glucose-6-phosphate dehydrogenase deficiency causes hemolytic anemia

- It is most common enzyme deficiency.
- X-linked condition.
- Mutations present in some populations causes a deficiency in glucose 6-phosphate dehydrogenase, with consequent impairment of NADPH production.
- Detoxification of H_2O_2 is inhibited, and cellular damage results - lipid peroxidation leads to erythrocyte membrane breakdown and hemolytic anemia.

- Reduced form of glutathione serves as sulfhydryl buffer that maintains cysteine residues of Hb& other red blood cell proteins in reduced state.
- In absence of enzyme , hemoglobin no longer maintained in reduced form.
- Hemoglobin molecules cross link with one another to form aggregates of Heinz bodies on cell membrane that leads to RBC lysis.

- Most G6PD-deficient individuals are asymptomatic - only in combination with certain environmental factors (sulfa antibiotics, herbicides, antimalarials, *divicine) do clinical manifestations occur.

  *toxic ingredient of fava beans

- Malarial parasite plasmodium is especially sensitive to oxidizing conditions, so any circumstances that lowers cellular antioxidant capacity inhibits the infection. Sickle-cell trait also confers resistance to malaria.

favism

- The haemolytic effect of fava beans ,the disease is called *favism in G-6-PD deficiency.*
- *Fava beans contain purine glycoside, vicine & isovarmil.These reacts with glutathione leading to reduced cellular level of GSH.*
- The Mediterranean variant of this genetic disease is particularly susceptible to Favism

Uronic Acid Pathway (Glucuronic acid cycle)

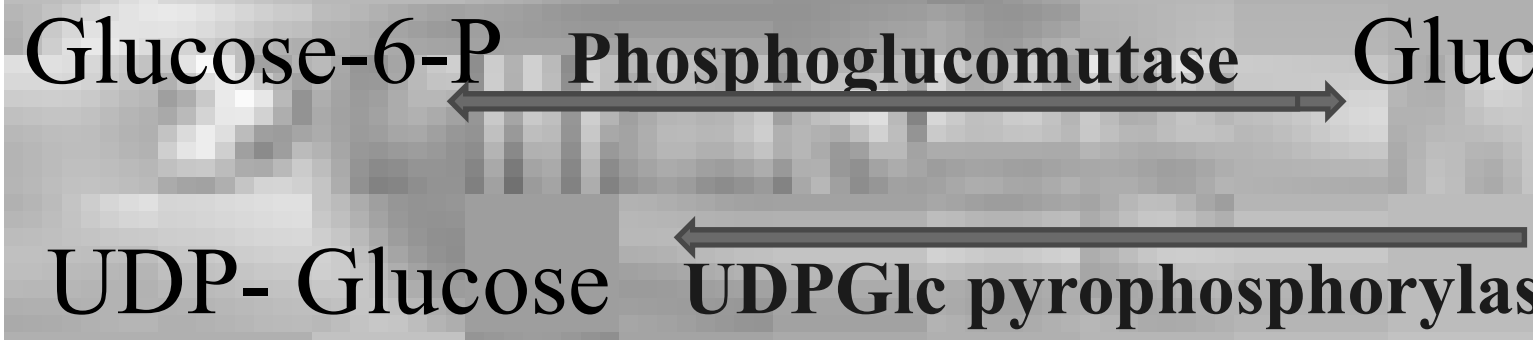
- It is an alternative oxidative pathway for glucose that, like the pentose phosphate pathway, does not lead to generation of ATP
- It includes oxidation of glucose to

1. **Glucuronic acid:** which is used in detoxication and enters in the formation of mucopolysaccharide.
2. **Ascorbic acid** (except in humans) &
3. **Pentoses**

- It occurs in liver cytoplasm, mainly

Formation of UDP-glucuronic acid

Glucose 6-phosphate is isomerized to glucose 1-phosphate, which then reacts
with uridine triphosphate (UTP) to form uridine diphosphate glucose (UDPGlc)
in a reaction catalyzed by UDPGlc pyrophosphorylase



UDPGlc is oxidized at carbon 6 by NAD-dependent UDPGlc dehydrogenase in a two-step reaction to yield UDP-glucuronate

UDP Glucose

2 NAD

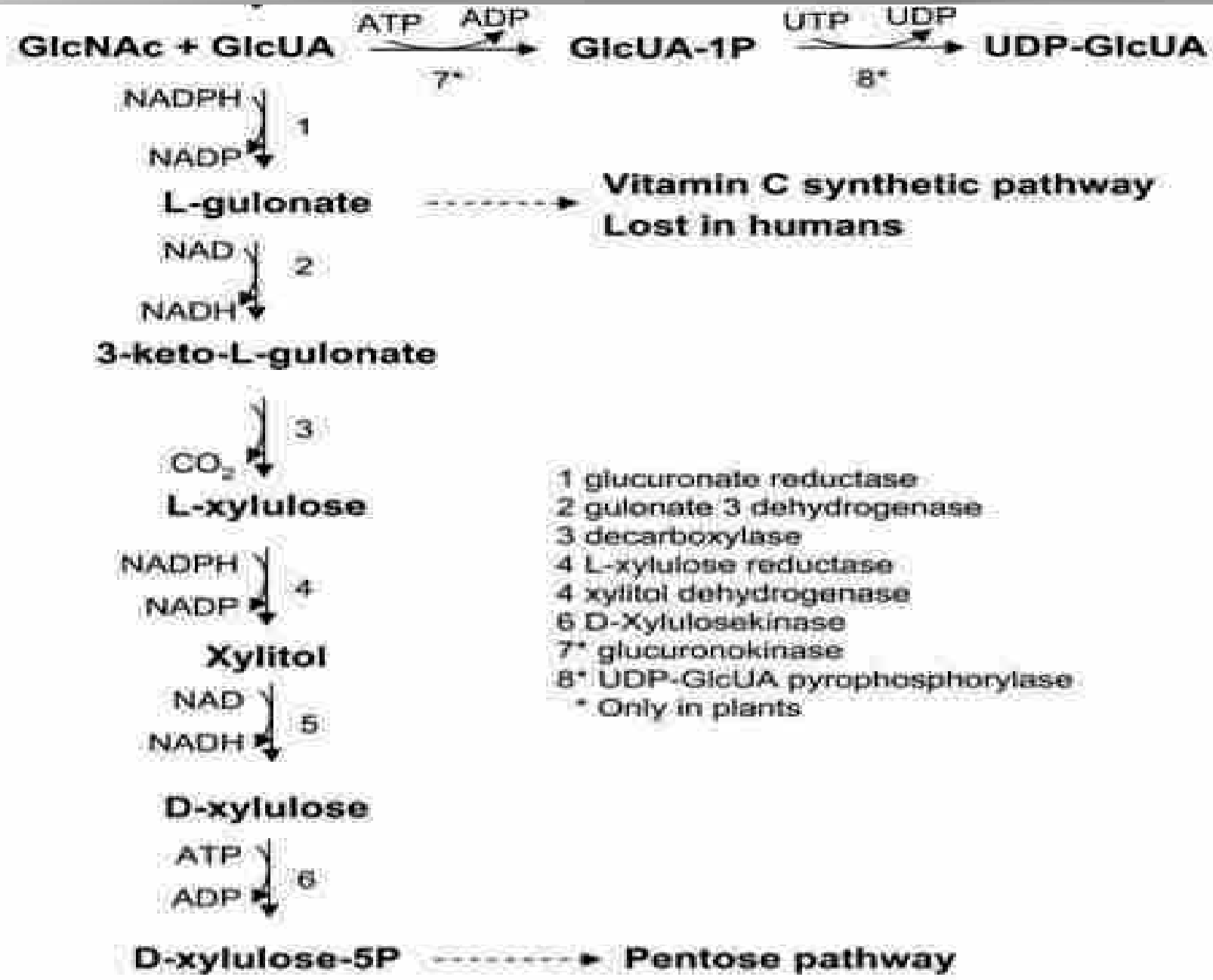
2 NADH + $2H^+$ **UDPG dehydrogenase**

UDP Glucuronic acid

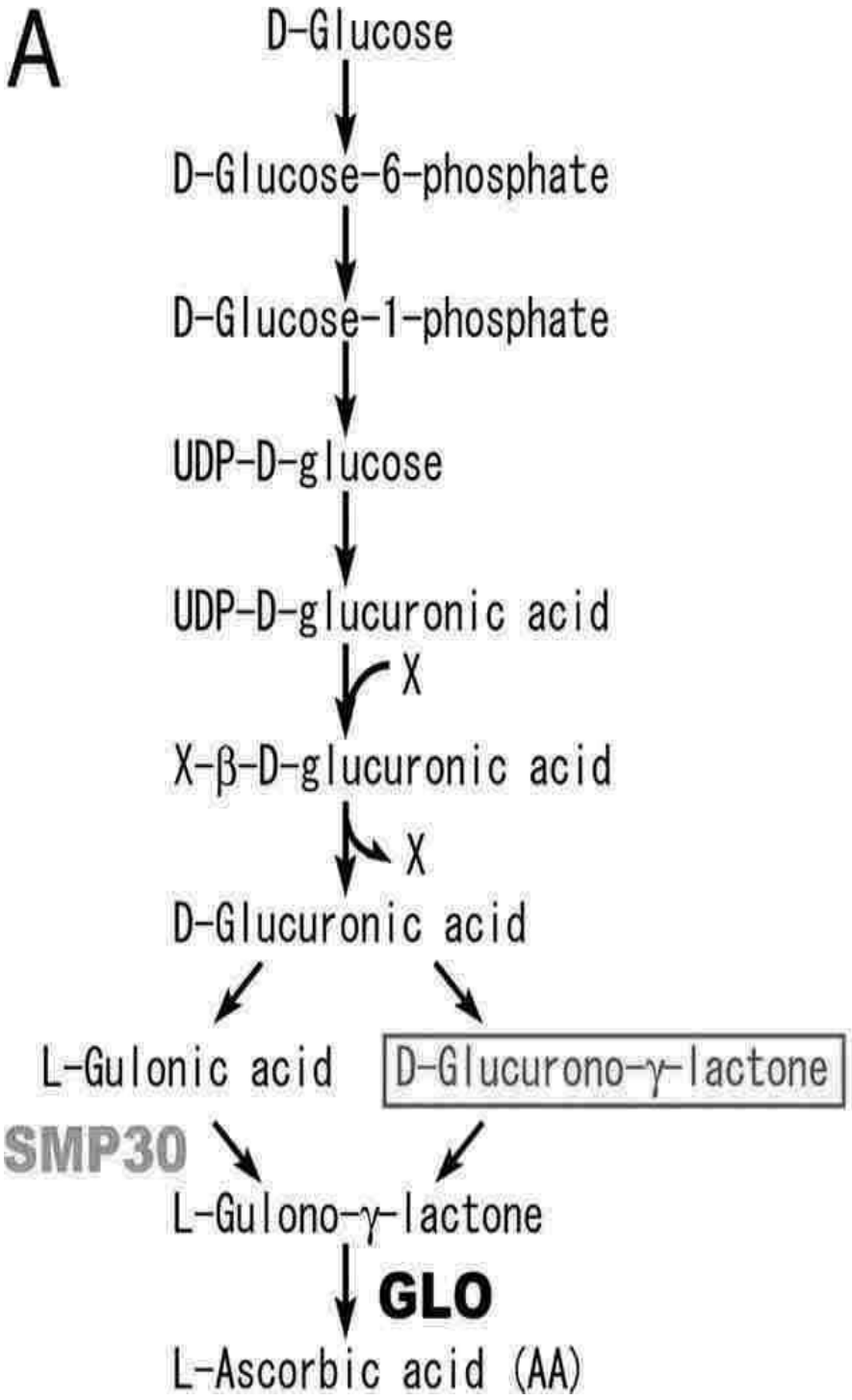
H_2O

UDP **Glucuronidase**

D- Glucuronic acid


GlcNAc + GlcUA
ATP
ADP
7*
GlcUA-1P
UTP
UDP
8*
UDP-GlcUA
NADPH
NADP
1
L-gulonate
Vitamin C synthetic pathway
Lost in humans
NAD
NADH
2
3-keto-L-gulonate
3
CO2
L-xylulose
NADPH
NADP
4
Xylitol
NAD
NADH
5
D-xylulose
ATP
ADP
6
D-xylulose-5P
Pentose pathway
1 glucuronate reductase
2 gulonate 3 dehydrogenase
3 decarboxylase
4 L-xylulose reductase
4 xylitol dehydrogenase
6 D-Xylulosekinase
7* glucuronokinase
8* UDP-GlcUA pyrophosphorylase
* Only in plants

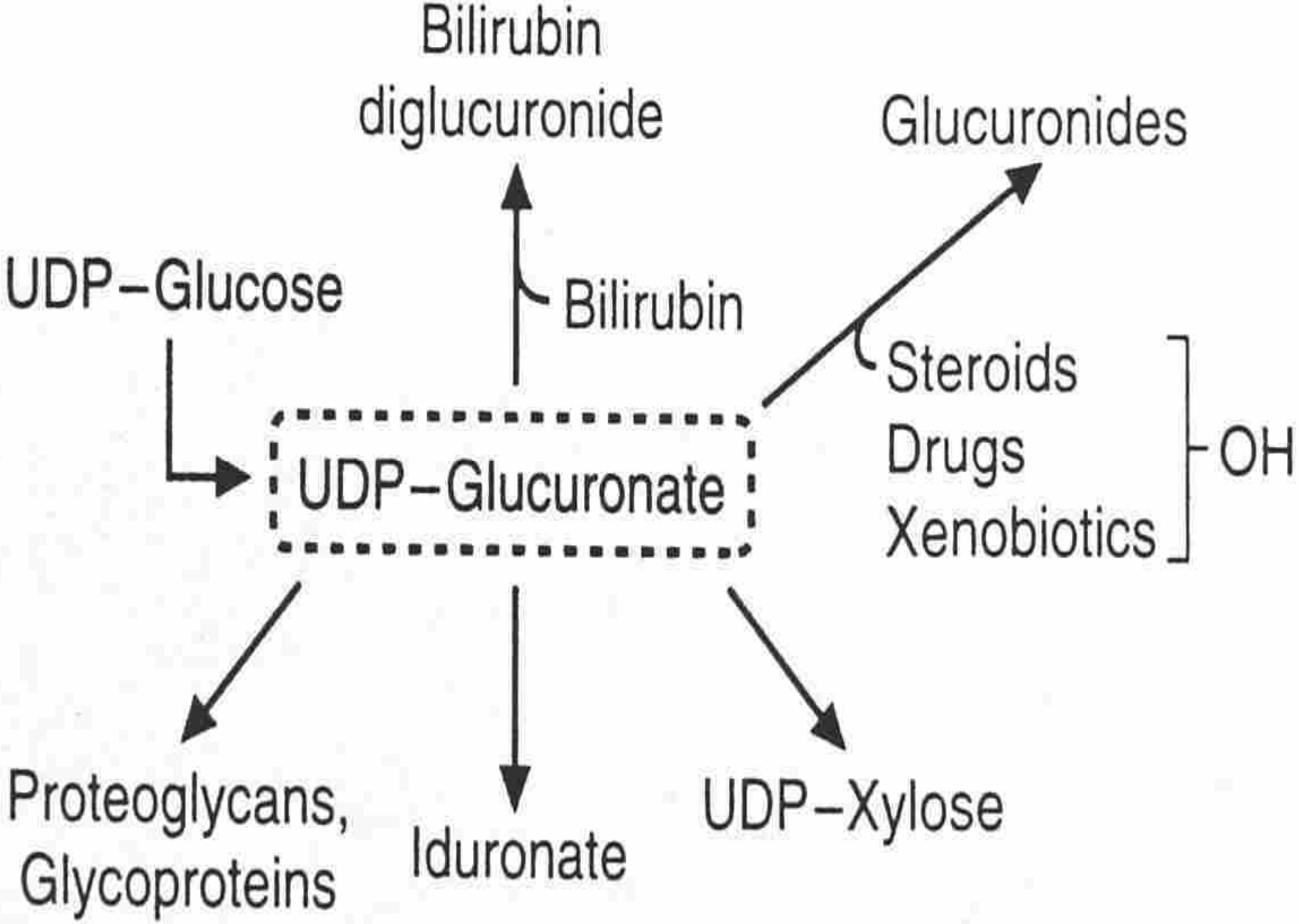
- L- Gulonate is precursor of ascorbate in animal synthesizing this vitamin.
- In humans, absence of enzyme L- gulonolactone oxidase .



Significance

1. **Source of UDP Glucuronic acid (active form)**
2. **Synthesis of glycosaminoglycans needed in:**(mucopolysaccharide) as heparin, hyaluronic acid & Glycoproteins.
3. Also precursor of UDP – xylulose & iduronate another sugar precursor of GAGs

4. **Conjugation to less polar compounds as bilirubin,** steroids and some drugs making them more water soluble .
5. Uronic acid pathway is source of UDP glucose , which is used for glycogen metabolism.
6. Necessary for formation of pentoses eg. D- xylulose.
7. The uronic acid pathway also provides a mechanism by which dietary **D-xylulose enter the central pathway.**


Bilirubin
diglucuronide
Glucuronides
UDP–Glucose
Bilirubin
Steroids
Drugs
Xenobiotics
OH
UDP–Glucuronate
Proteoglycans,
Glycoproteins
Iduronate
UDP–Xylose

Essential Pentosuria

- **ESSENTIAL pentosuria is a rare, recessive genetic metabolic disorder characterized by the excretion of gram quantities of L-xylulose.**
- It is associated with a deficiency of L-xylulose reductase.
- Enzyme reduces L- xylulose to xylitol.
- Drugs like aminopyrine, antipyrine, barbiturates increases L- xylulose excretion

Heteropolysaccharides

Definition

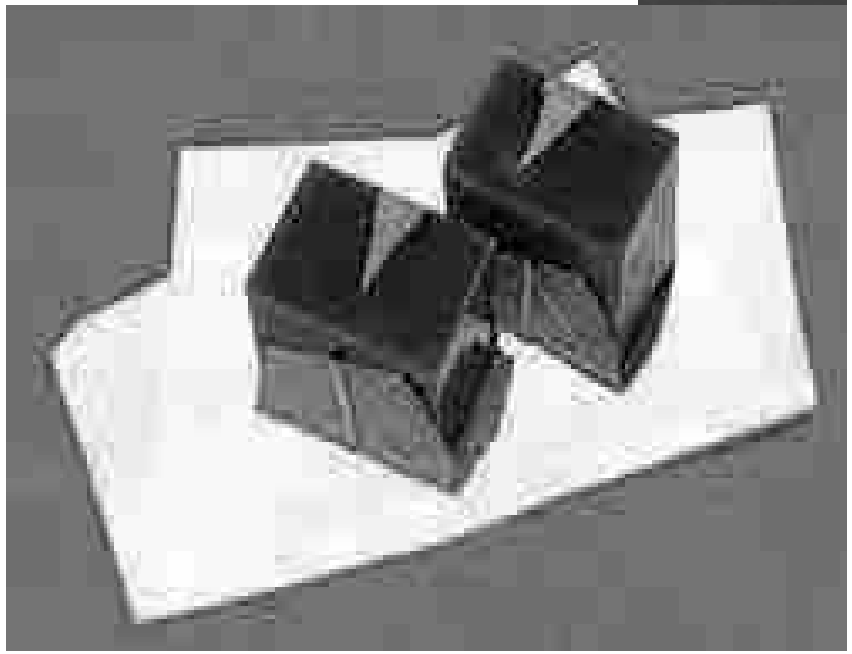
- These are polysaccharides containing more than one type of sugar residues.

Agar

- It is prepared from seaweeds or it accumulates in the cell walls of agarophyte red algae.
- contains galactose , glucose & other sugars.
- Agar consists of a mixture of agarose and agaropectin.
- The predominant component agarose is a linear polymer, made up of the repeating monomeric unit of agarobiose.

- Agar is used throughout the world to provide a solid surface containing medium for the growth of bacteria and fungi.
- Microbial growth does not destroy the gel structure because most microorganisms are unable to digest agar.
- At 100^0C it dissolves , which upon cooling sets into gel.

- Widely used as supporting agent to culture bacterial colonies.
- Agar (agar-agar) can be used as a laxative, a vegetarian gelatin substitute, a thickener for soups, in jellies, ice cream, and other desserts, as a clarifying agent in brewing, and for sizing paper and fabrics.



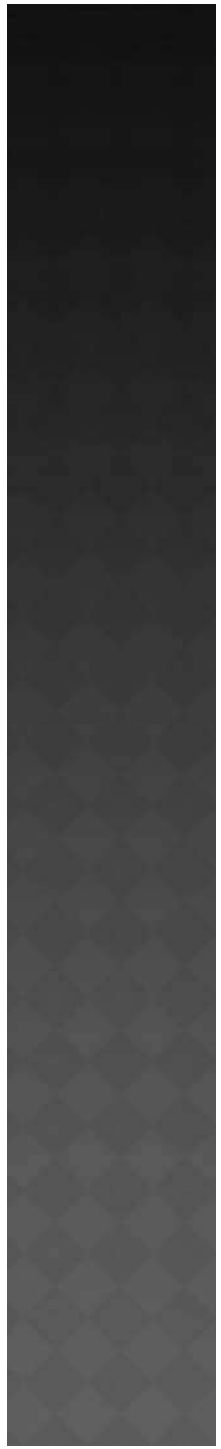
Gum & pectins

- Gum used as an adhesive, contains galactose, arabinose , glucuronic acid.
- Pectin in acid solutions used to prepare jellies in food industries.
- Occurs in citrus fruits, apple, carrots.

Glycosaminoglycan (GAGs) or Mucopolysaccharides

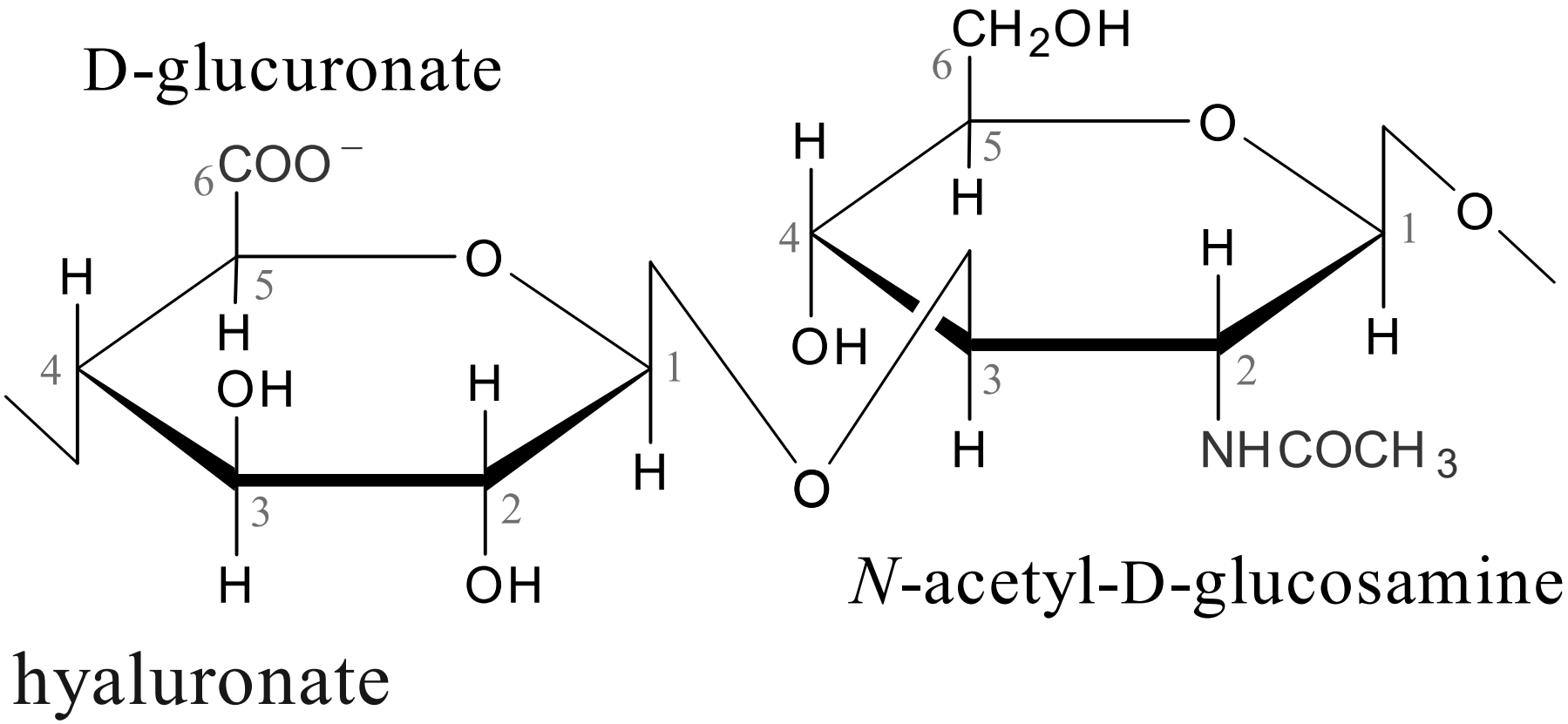
- They are long unbranched polysaccharides consisting of a repeating sugar unit.
- They contains uronic acid and amino sugars.
- Acetylated amino groups , sulphates and carboxyl groups also present.
- Because of charged groups, they attract water molecules and so they produce viscous solutions.

- 1.Hyaluronic acid
- 2.Chondroitin sulphateA
- 3.Keratan sulphate
- 4. Dermatan sulphate(chondroitin sulphate B)
- 5. Chondroitin sulphate C
- 6. Heparin



Hyaluronic acid

- It is an anionic, nonsulfated glycosaminoglycan.
- Present in connective tissues, tendons, synovial fluid , Vitreous humor & umbilical cord.
- Composed of repeating units of D-glucuronic acid and D-N-acetylglucosamine.



- linked via alternating β-1,4 and β-1,3 glycosidic bonds.

Functions

- Acts as lubricant in joints.
- Acts as cementing substance between tissues & capillary walls.
- Resists the penetration of bacteria
- Forms protective gel coat around ovum.

Functions

- It facilitates fertilization.
- Hyaluronidase enzyme in semen acts on hyaluronic acid around ovum & helps penetration of sperm in ovum.

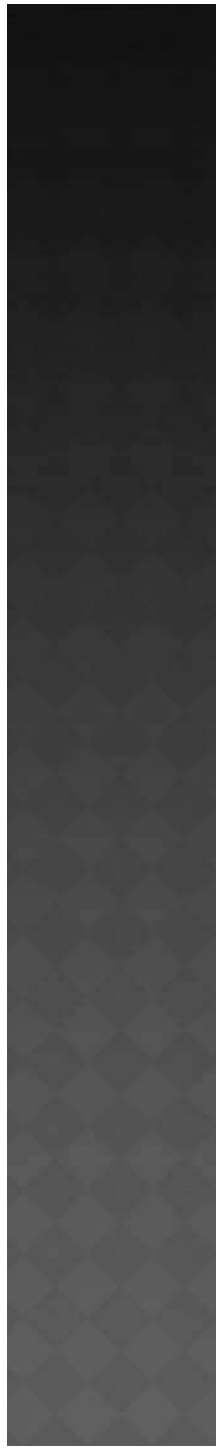
Chondroitin sulfate

- Unbranched polysaccharides composed of repeating units of D-glucuronic acid (GlcA) and N-acetyl-D-galactosamine (GalNAc).

- linked via alternating β-1,3 and β- 1,4 glycosidic bonds.

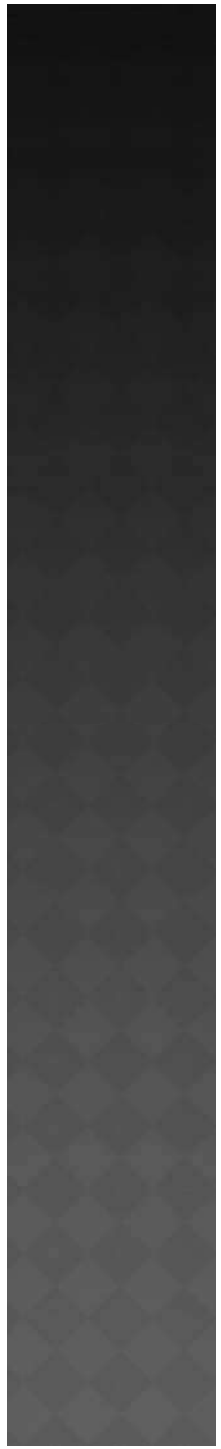
- **Chondroitin Sulphate A:** It is sulphated mucopolysaccharides,
- Distribution-Occurs in cartilage, cornea and adult bones
- Composition- repeating units of N-acetyl galactosamine 4 sulphate+ glucuronic acid , joined with β1-4 and β1-3 glycosidic linkage
- Functions- It holds water, helps in transperancy of cornea and responsible for shape of an eye.
- A major component of extracellular matrix, and is important in maintaining the structural integrity of the tissue

- **Chondroitin sulphate B (Dermatan sulphate)**
- Distribution-It occurs in skin, and heart valves,tendons,lungs
- Composition-repeating units of N-acetyl galactosamine4 sulphate and iduronic acid , joined with β1-4 and β1-3 glycosidic linkage
- Function- It's a structural component Dermatan sulfate may have roles in coagulation, cardiovascular disease, carcinogenesis, infection, wound repair, and fibrosis



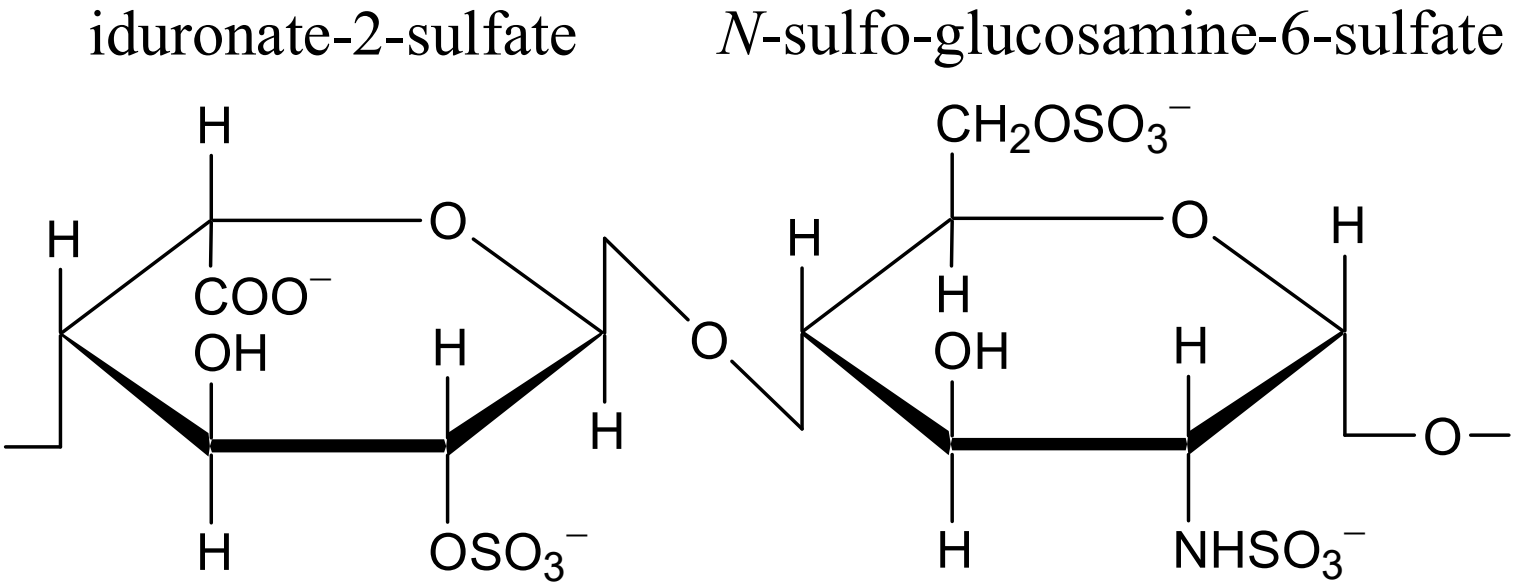
- **Chondroitin sulphate C:**
- Distribution-It occurs in cartilage and cornea
- Composition-consists of repeating units of N-acetyl galactosamine6 sulphate and glucuronic acid , joined with β1-4 and β1-3 glycosidic linkage
- Function- structural component.

- Keratan Sulphate
- Found especially in the cornea, cartilage, and bone.
- It is also synthesized in the central nervous system where it participates both in development·
- keratan sulfate is a linear polymer that consists of a repeating sugar unit The repeating units are galactose & N-acetyl glucosamine in β1-4 & β1-3 linkage.
- Keratan sulfates are large, highly hydrated molecules which in joints can act as a cushion to absorb mechanical shock.
- Functions in transparancy of cornea



Heparin

- Present in liver, lungs, spleen & monocytes.
- It contains repeating units of sulphated glucosamine & L- iduronic acid in α1-4 linkage & so on.



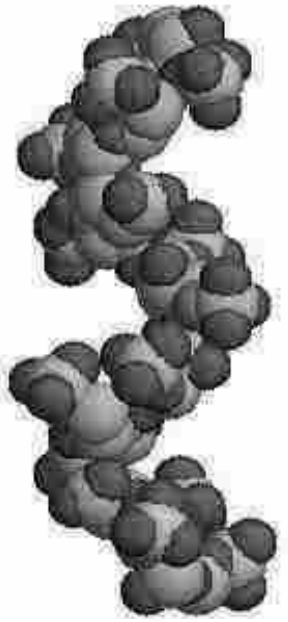
heparin or heparan sulfate - examples of residues

Heparin, a soluble glycosaminoglycan found in granules of mast cells, has a structure similar to that of heparan sulfates, but is more **highly sulfated**.

When released into the blood, it inhibits clot formation by interacting with the protein antithrombin.

Heparin has an **extended helical conformation**.

PDB 1RID



heparin: $(IDS\text{-}SGN)_5$

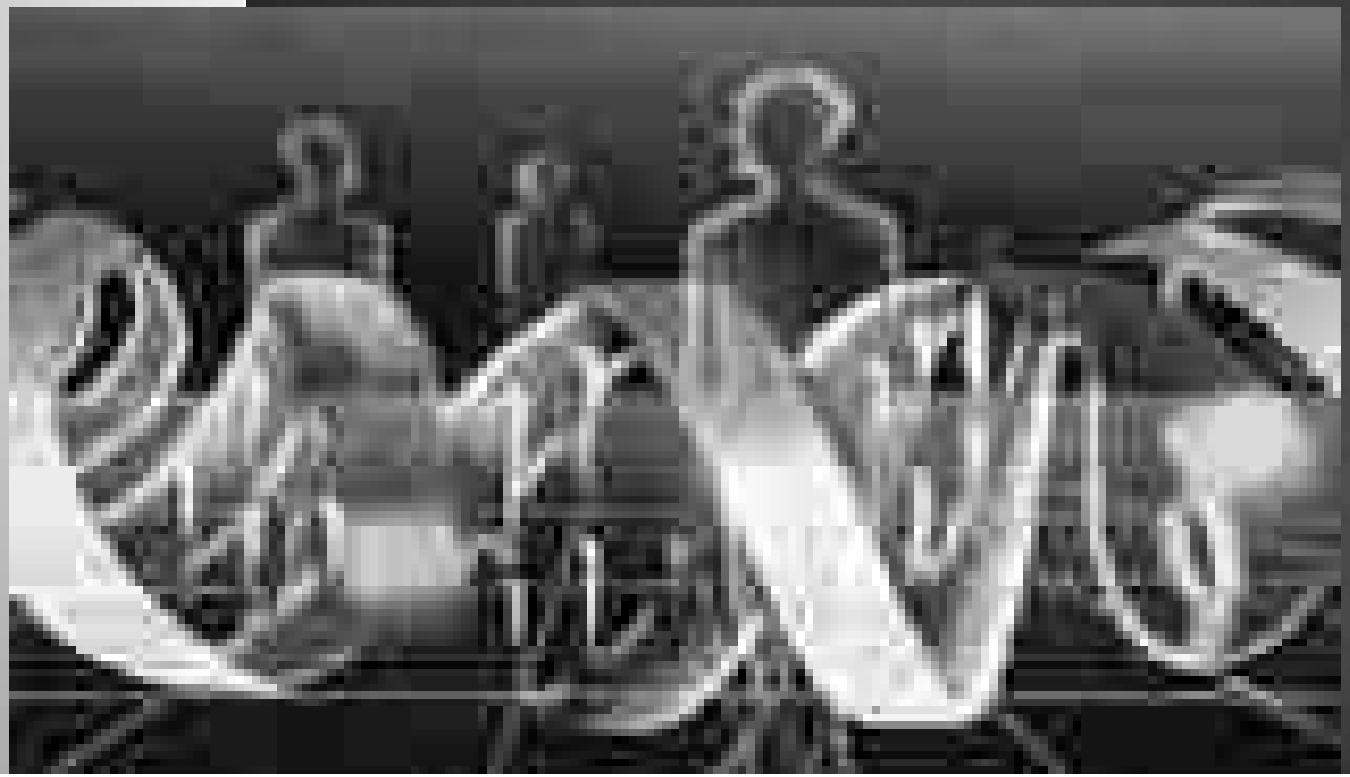
CONS

Medical use

- Heparin is a naturally-occurring anticoagulant produced by basophils and mast cells.
- Used therapeautically in conditions to prevent intravascular coagulation.
- The biologic effect of hepain is to bind and activate antithrombin III , which in turn inactivates thrombin, factor X and factor IX.

- Commercial preparation of heparin is from animal lung tissues.
- Heparin sulphate is present in skin, fibroblast.
- They are component of plasma membrane where they acts as receptor.

MUCOPOLYSACCHARIDOSIS



- GAGs are degraded in lysosomes.
- Lysosomes contains hydrolytic enzymes that are required for complete degradation of GAGs.
- These are hereditary disorders that are characteriszed by accumulation of GAGs in various tissues.
- Causing skeletal & extracellular matrix deformities.

- **Mucopolysaccharidoses** are a group of metabolic disorders caused by the absence or malfunctioning of lysosomal enzymes needed to break down molecules called glycosaminoglycans.

EXAMPLES

- Hunter syndrome
- Hurler syndrome
- Scheie syndrome
- Sanfilippo syndrome
- Morquio disease
- Maroteaux-Lamy syndrome

HURLER SYNDROME

TYPE I

(ALPHA-L-IDURONATE DEFICIENCY)

CAUSES OF THE HURLER SYNDROME

- Inherited as an autosomal recessive trait
- Metabolic defect: inability
  - The body's to make an enzyme:
    - lysosomal alpha-L-iduronase
    - Storage of abnormal quantities of this material (mucopolysaccharide) in different body tissues is responsible for the symptoms and appearance of the disease

SYMPTOMS

- Short stature
- Severe mental retardation
- Thick, coarse facial features with low nasal bridge
- Full lips with a thick, large tongue
- Increased body hair (hirsutism)
- Progressive corneal clouding, coronary disease
- Hearing loss

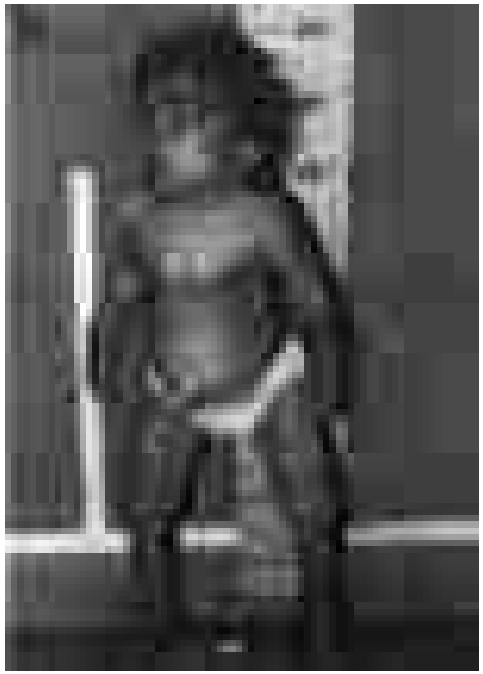
- Urinary excretion of Dermatan sulphate & heparan sulphate.

Prevention

- **Genetic counseling: important for parents with a family history of Hurler syndrome**
- **Prenatal diagnosis:**
- **An amniocentesis in the amniotic fluid are then cultured and the a-L-iduronidase activity in the cells is determined.**

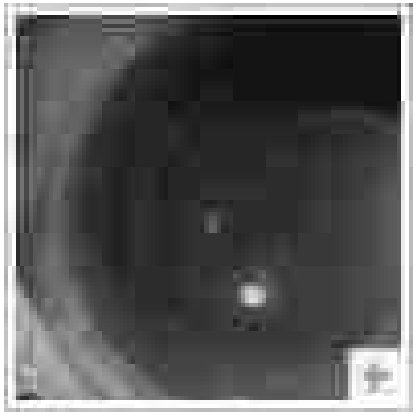
Hurler syndrome (type I)

Hurler syndrome type I



Mucopolysaccharidosis I (MPS I) Disease (Hurler, Hurler-Scheie, Scheie Syndromes)

Hernia



Corneal clouding

Coarse facial features

Claw hand

HUNTER SYNDROME TYPE II

(*SULPHO-IDORONIDE SULPHATASE DEFICIENCY*)

HUNTER SYNDROME TYPE II

(*SULPHO-IDORONIDE SULPHATASE DEFICIENCY*)

- X-linked
- Coarse, thick, facial features
- Progressive stiffness
- decreased mental development
- Hepatomegaly (liver enlargement)
- Splenomegaly (spleen enlargement)
- Abnormal bone x-rays

- Urinary excretion of Dermatan sulphate & heparan sulphate.

Symptoms

- Severe mental retardation
- Pulmonary hypertension
- Vulvular disease

HUNTER SYNDROME TYPE II

SANFILIPPO SYNDROME TYPE III

- Sanfilippo syndrome is one of the hereditary mucopolysaccharide storage diseases
- it is characterized by the absence of heparan sulphatase or N- acetyl glucosaminidase.
- This substance is called heparan sulfate, and in Sanfilippo syndrome, large amounts of it are excreted in the urine

CAUSES

- Coarse, thick, facial features
- Prominent dark eyebrows
- Progressive stiffness
- gait disturbances
- speech disturbances
- decreased mental development that progresses to severe mental retardation

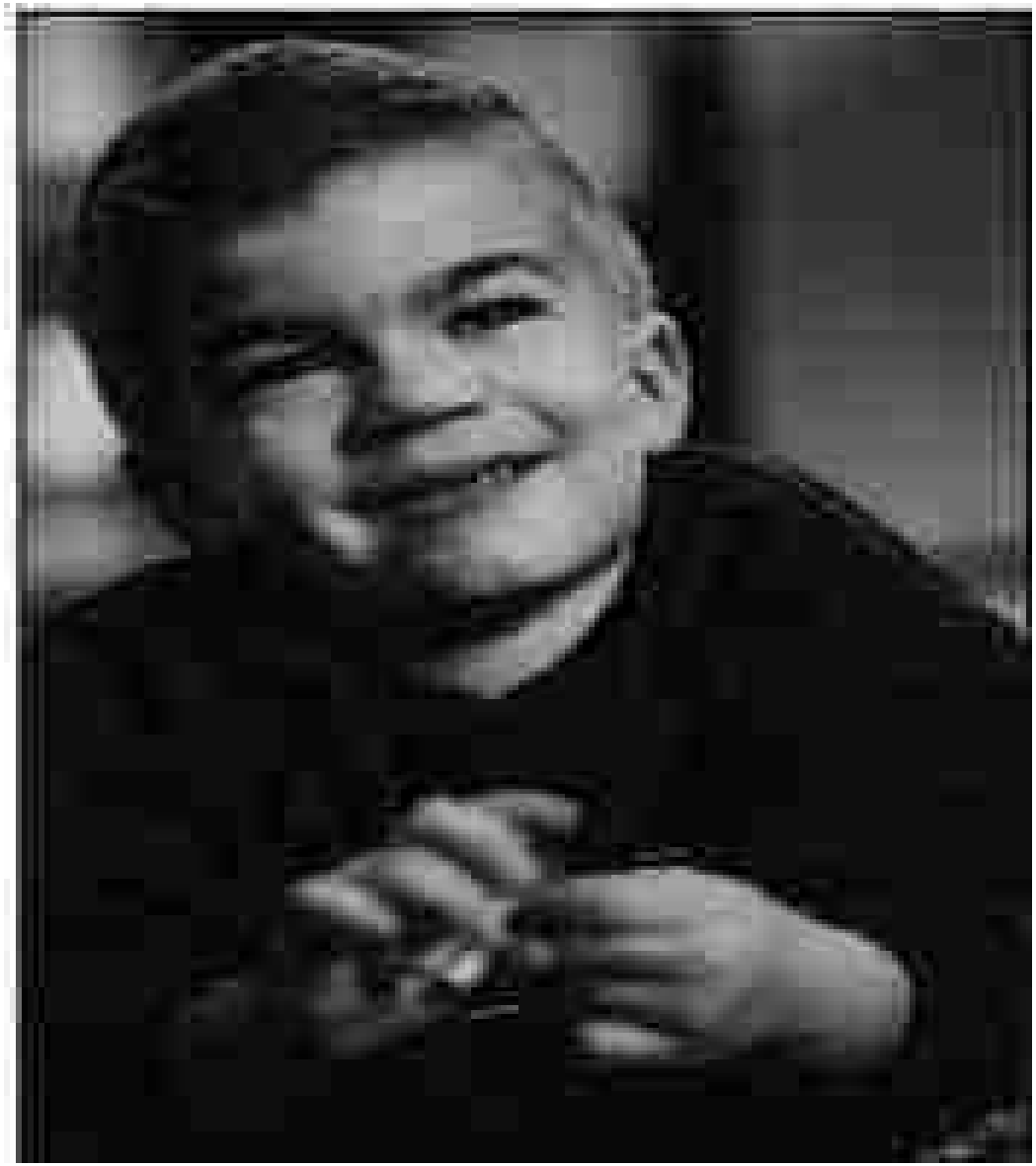
SANFILIPPO SYNDROME CAUSES

- an autosomal recessive trait
- It is possibly the most common of the mucopolysaccharide storage diseases
- It has a relatively late onset rather than during the first year of life

FEATURES AND CHARACTERISTICS CHILDREN WITH SANFILIPPO SYNDROME

- Occasional enlarged head
- Coarse facial features
- Coarse hair
- Excessive hair growth
- Joint stiffness

SANFILIPPO SYNDROME TYPE III



Sanfilippo syndrome type III

MORQUIO SYNDROME TYPE IV

subtypes A & B

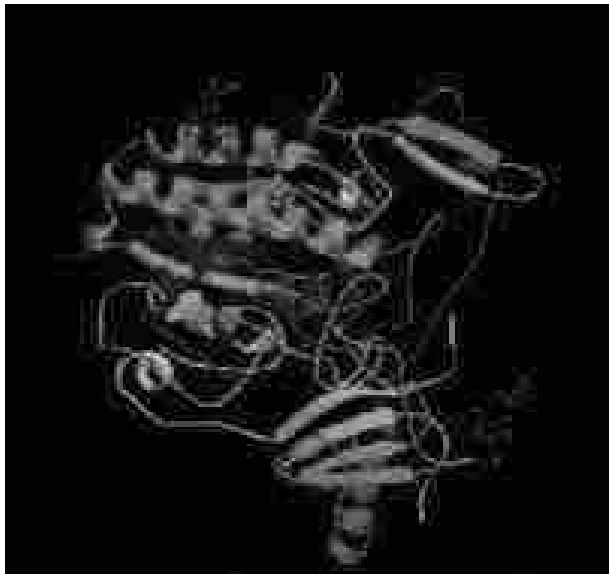
- **Type IVA: Galactose-6- sulfatase deficiency**
- **Type IVB: β–Galactosidase deficiency**

MORQUIO SYNDROME TYPE IV

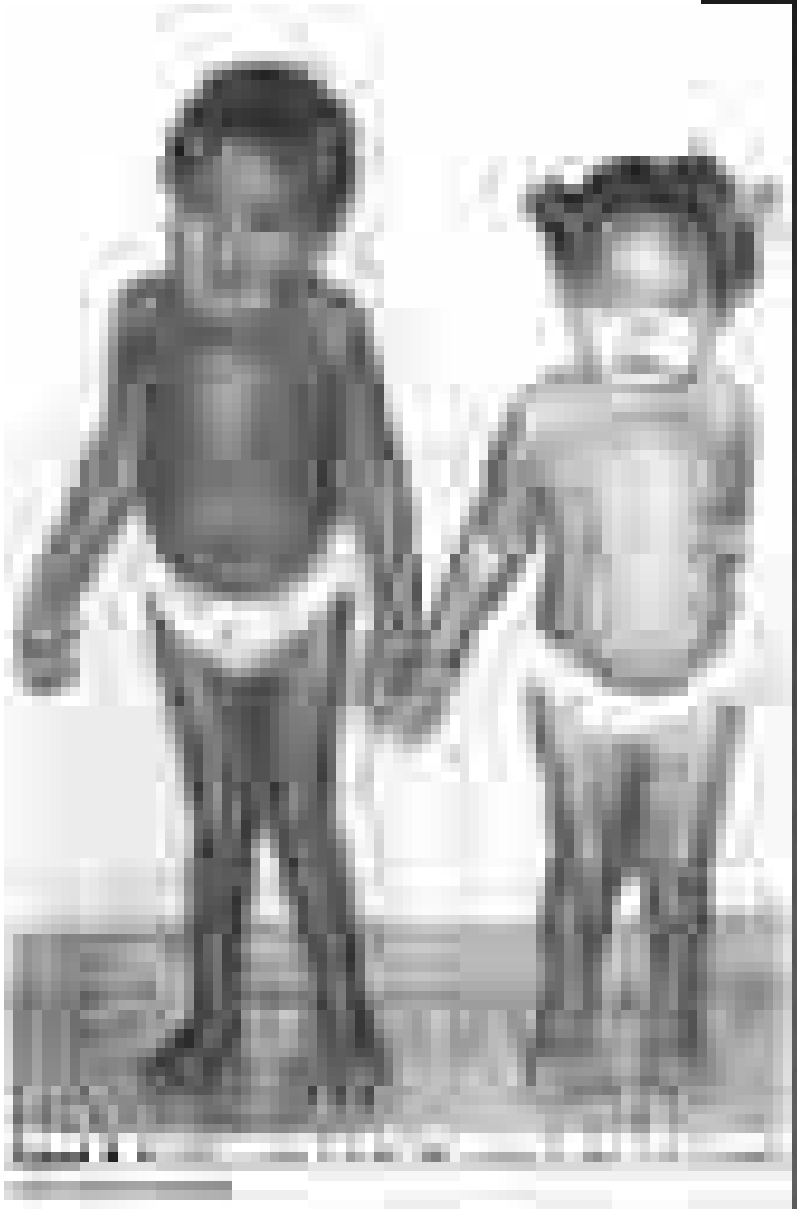
Skeletal abnormality - hand

MAROTEAUX-LAMY SYNDROME TYPE V

(N-ACETYL-GALACTOSE-AMIN-4-SULFATASE (ARYLSULFATASE B)



MAROTEAUX-LAMY SYNDROME TYPE V



FEATURES AND CHARACTERISTICS

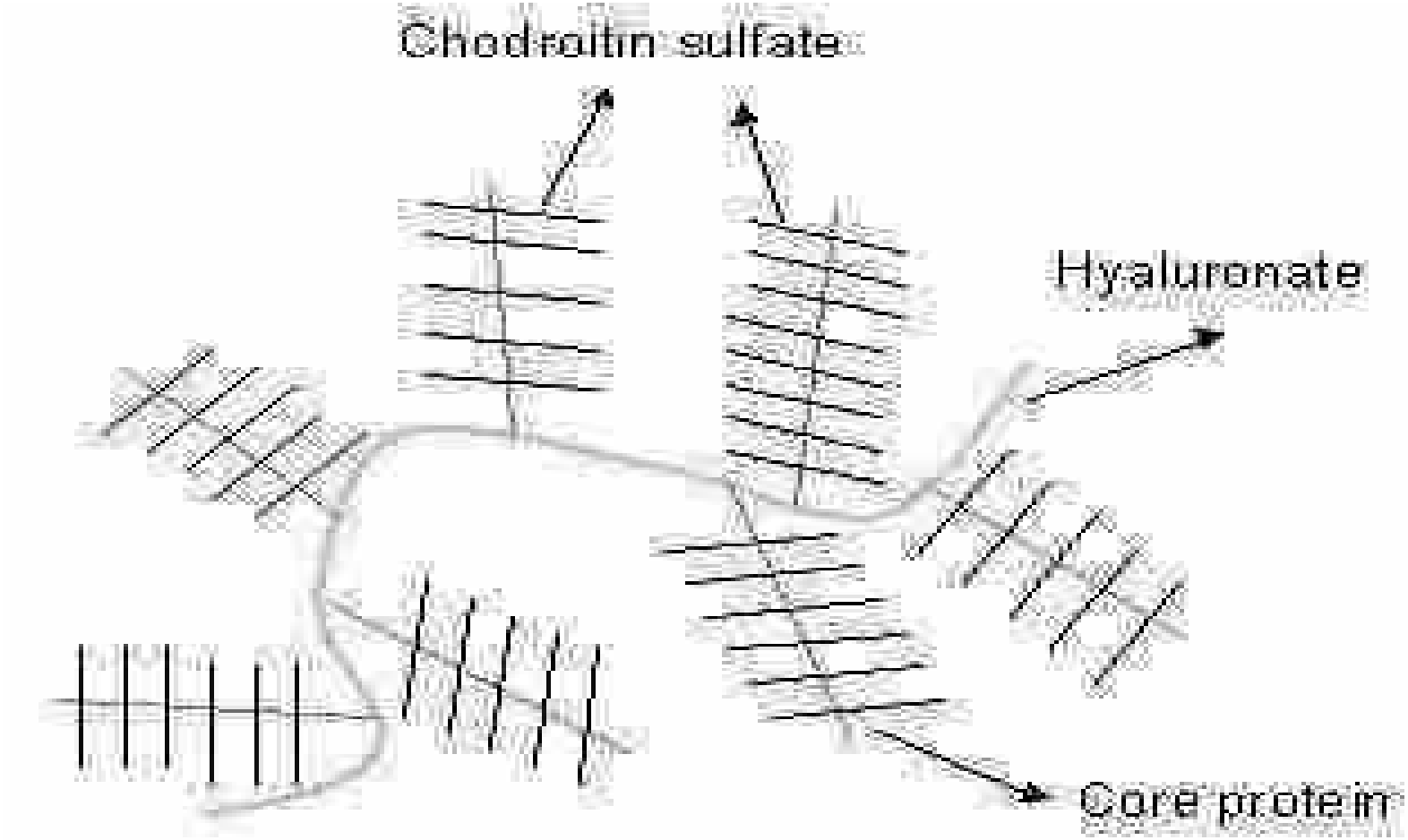
MAROTEAUX-LAMY SYNDROME

- Coarse facial features

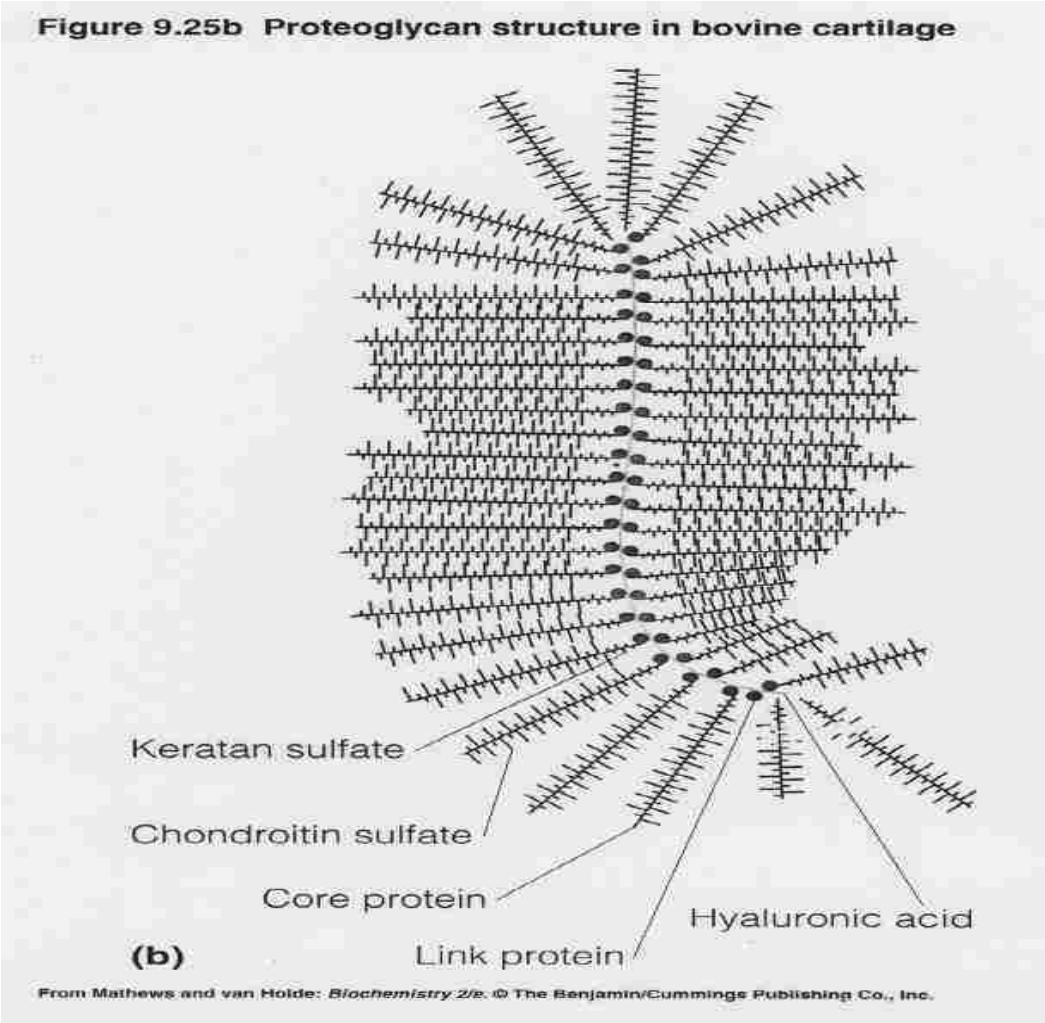
TREATMENT

- **At the present time, there is no cure for MPS disorders.**
- **Enzyme replacement therapy and gene therapy are the two treatments that researchers have been focusing on to eventually cure MPS diseases.**

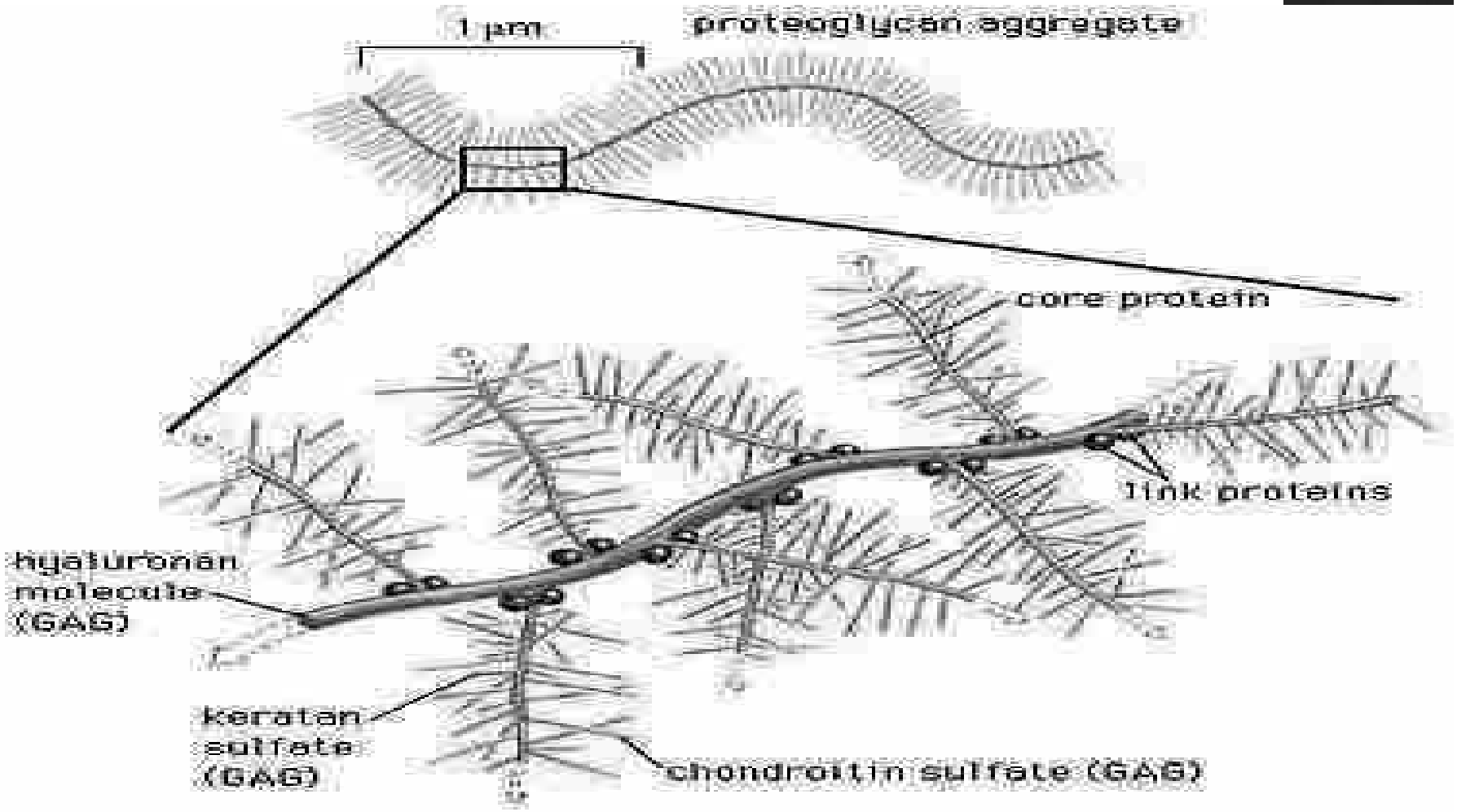
Proteoglycans



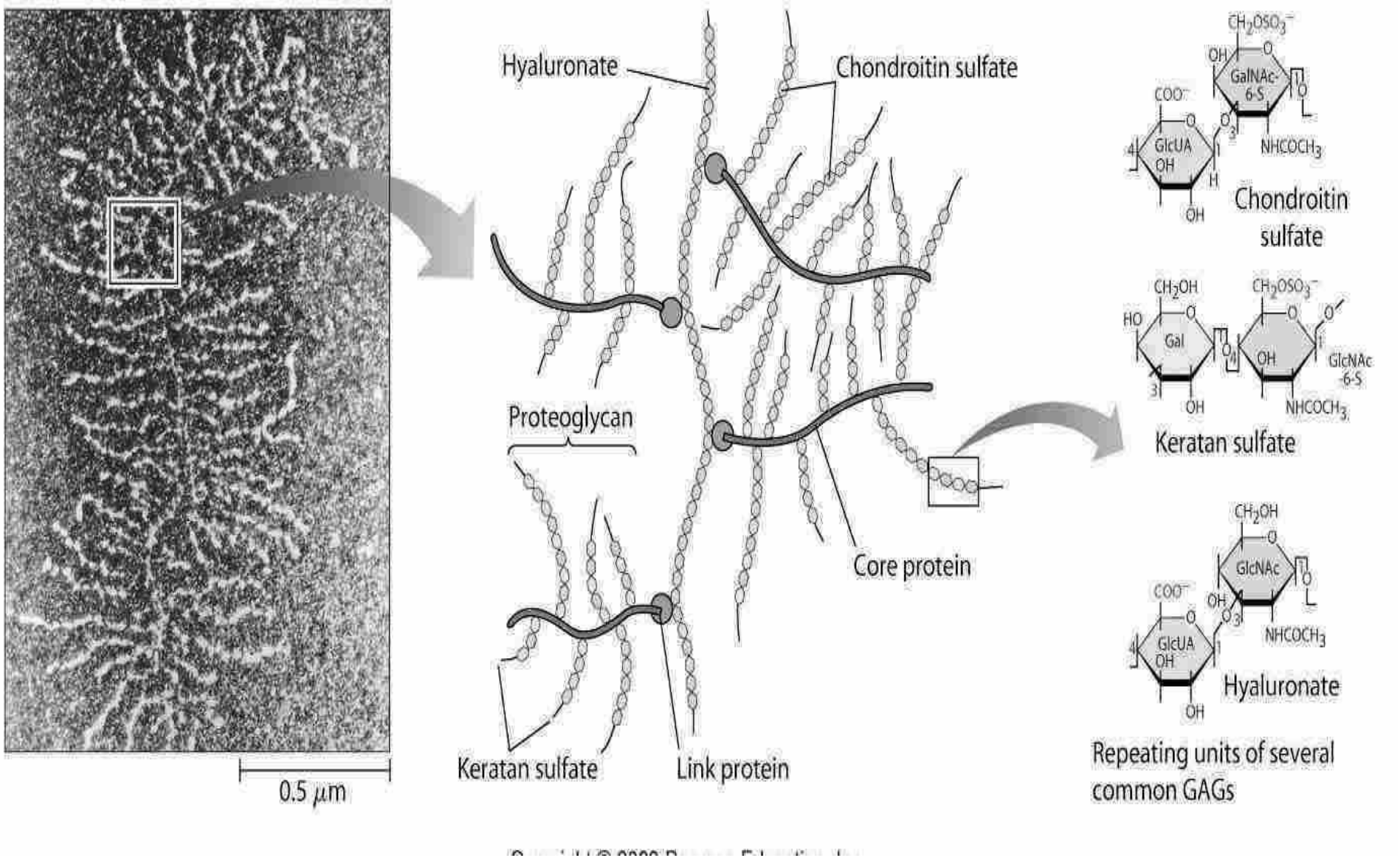
- GAG is a polymer of uronic acid – amino sugar.
- This polymer attached covalently to extracellular proteins called core protein to form proteoglycans.
- A resulting structure resembles " bottle brush"

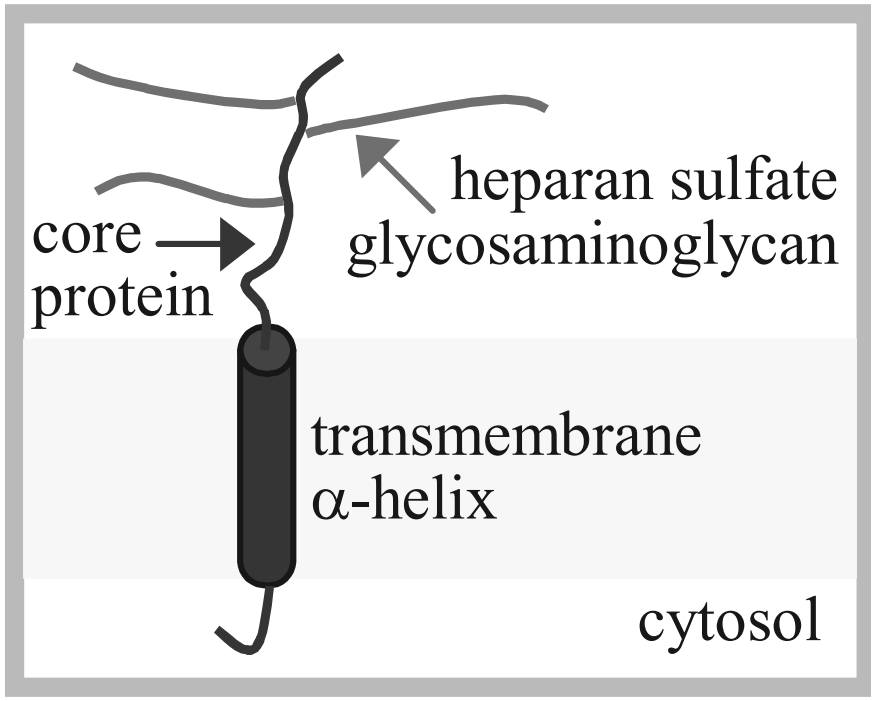


- Proteoglycan monomer associate with a molecule of hyluronic acid to form proteoglycan aggregates.
- This association through ionic interaction between core protein & hyaluronic acid.



- The association is stabilized by additional small protein called link proteins.





Proteoglycans are **glycosaminoglycans** that are covalently linked to serine residues of specific **core proteins**.

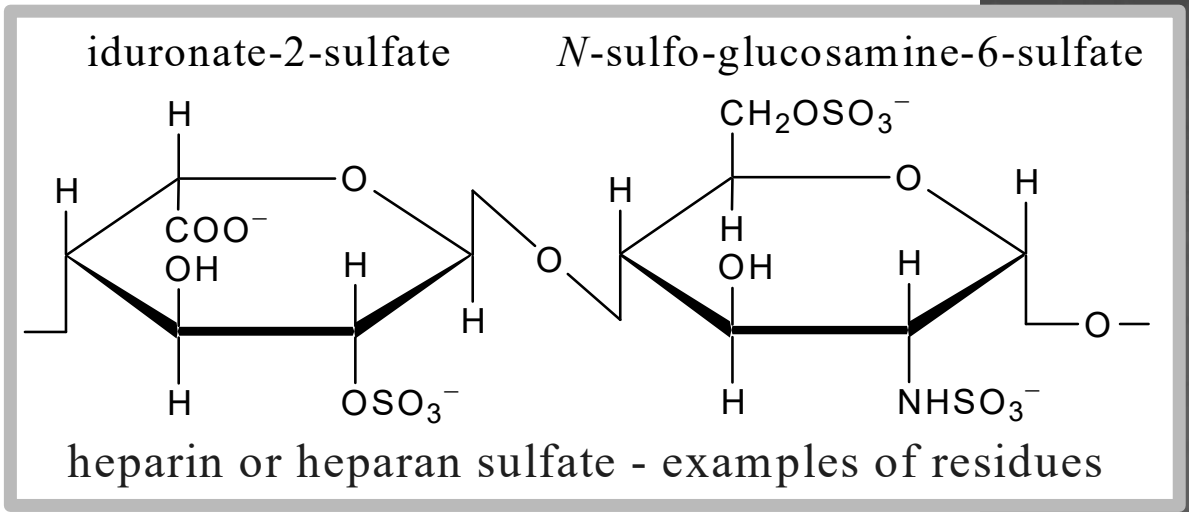
The glycosaminoglycan chain is synthesized by sequential addition of sugar residues to the core protein.

Proteins involved in **signaling** & **adhesion** at the cell surface recognize & bind heparan sulfate chains.

E.g., binding of some **growth factors** (small proteins) to cell surface receptors is enhanced by their binding also to heparan sulfates.

Regulated cell surface **Sulf** enzymes may **remove sulfate** groups at particular locations on heparan sulfate chains to **alter affinity** for signal proteins, e.g., growth factors.

Diagram by Kirkpatrick & Selleck.

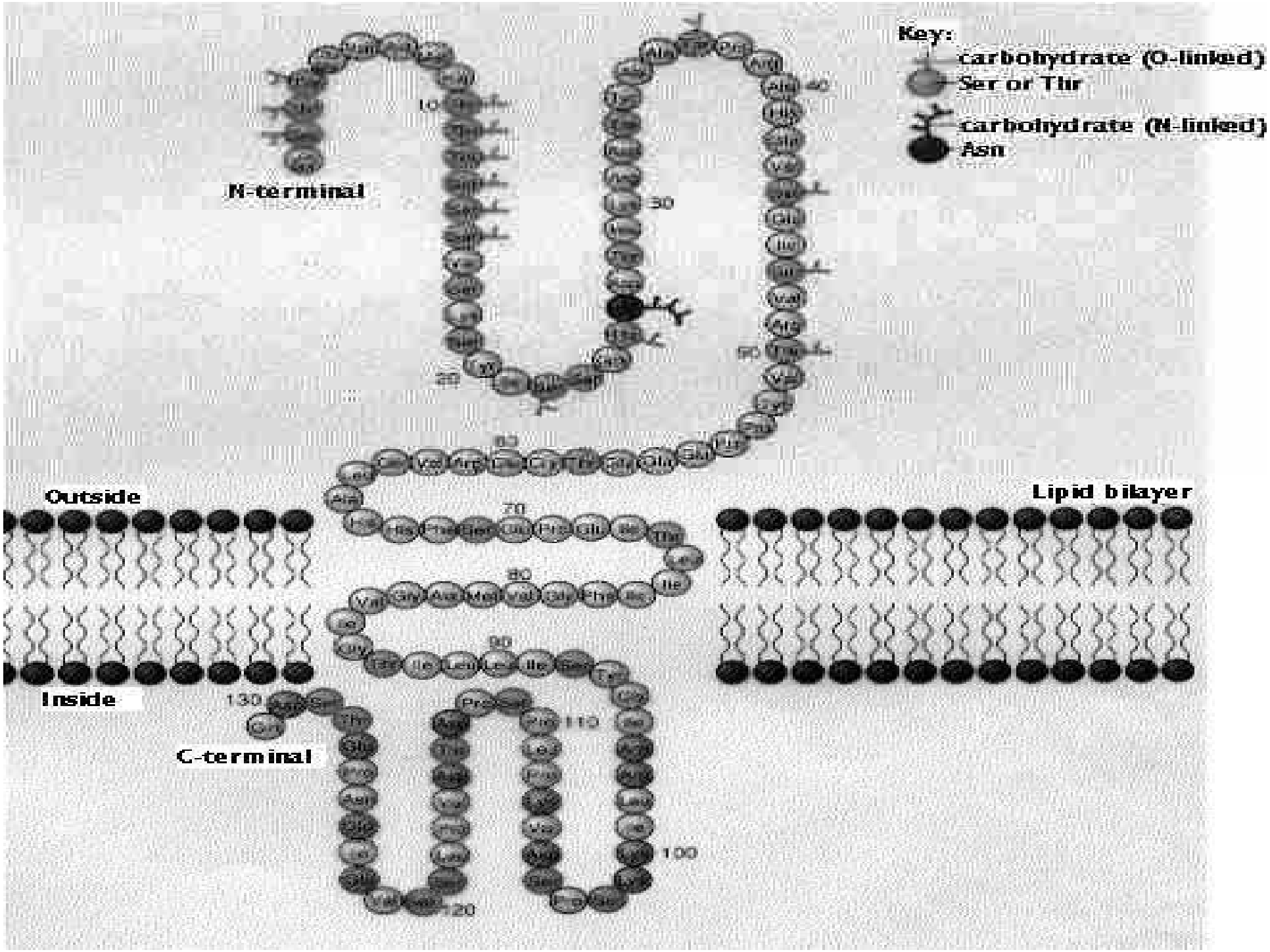


heparin or heparan sulfate - examples of residues

Glycoproteins

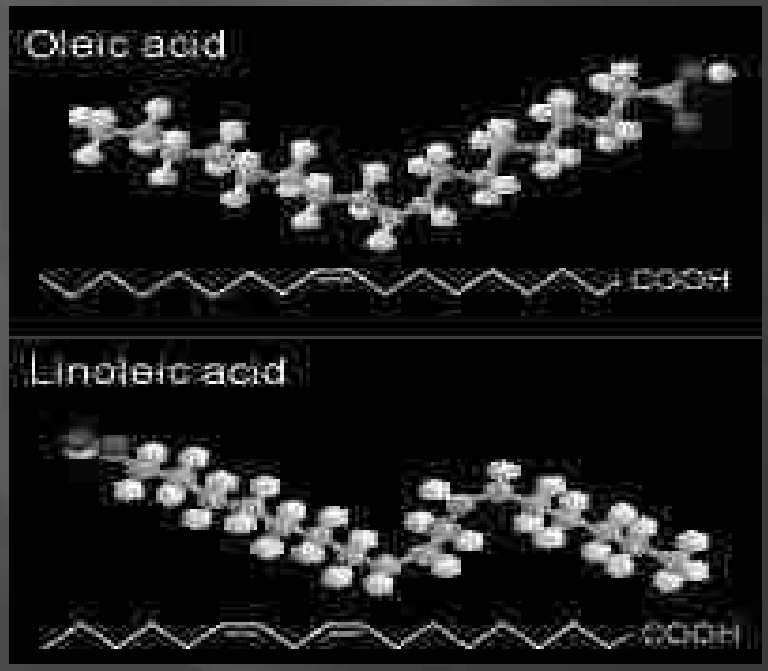
- Glycoproteins are proteins to which oligosaccharides are covalently attached to their peptide chain.
- Glycoproteins contains much shorter carbohydrate chain than proteoglycans.
- These oligosaccharide chains are often branched instead of linear & do not contain repeating disaccharides.
- The carbohydrate is attached to the protein in a cotranslational or posttranslational modification. This process is known as glycosylation.
- Glycoproteins are often important integral membrane proteins, where they play a role in cell–cell interactions. Glycoproteins also occur in the cytosol

- There are two types of glycoproteins:
- In N-glycosylation , the addition of sugar chains can happen at the amide nitrogen on the side-chain of the asparagine.
- In O-glycosylation, the addition of sugar chains can happen on the hydroxyl oxygen on the side-chain of hydroxylysine, hydroxyproline, serine, or threonine.
- glycoproteins found in the body is mucins, which are secreted in the mucus of the respiratory and digestive tracts.
- The sugars attached to mucins give them considerable water-holding capacity and also make them resistant to proteolysis by digestive enzymes.


Key:
carbohydrate (O-linked)
Ser or Thr
carbohydrate (N-linked)
Asn
N-terminal
C-terminal
Outside
Inside
Lipid bilayer
10
20
30
40
50
60
70
80
90
100
110
120
130

Function	Glycoproteins
Structural molecule	Collagens
Lubricant and protective agent	Mucins
Transport molecule	Transferrin, ceruloplasmin
Immunologic molecule	Immunoglobins, histocompatibility antigens
Hormone	Human chorionic gonadotropin (HCG), thyroid-stimulating hormone (TSH)
Enzyme	Various, e.g., alkaline phosphatase
Cell attachment-recognition site	Various proteins involved in cell–cell (e.g., sperm–oocyte), virus–cell, bacterium–cell, and hormone–cell interactions
Hemostasis (and thrombosis)	Specific glycoproteins on the surface membranes of platelets
Receptor	Various proteins involved in hormone and drug action

Derived Lipids



DERIVED LIPIDS

Obtained by hydrolysis of simple & compound lipids.

(A) Fatty acids

(B) Monoacyl glycerol and Diacyl glycerol

(C) Alcohols

(D) Fatty Aldehydes

(E) Ketone bodies

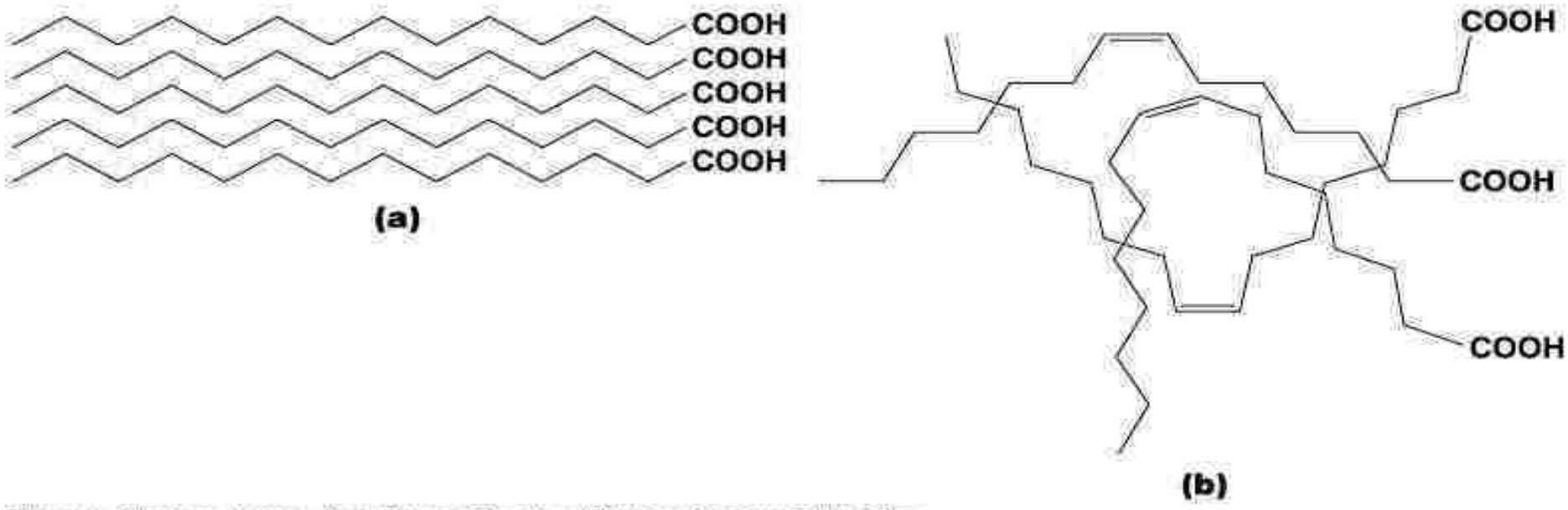
Fatty acids

- Most common component of lipids in the body.
- Free fatty acids are formed only during metabolism
- They are carboxylic acids with hydrocarbon chains (- CH_2 – CH_2- CH_2 -)
- **Have the general formula R-$(CH_2)_n$-COOH and mostly have straight chain**

- Defination

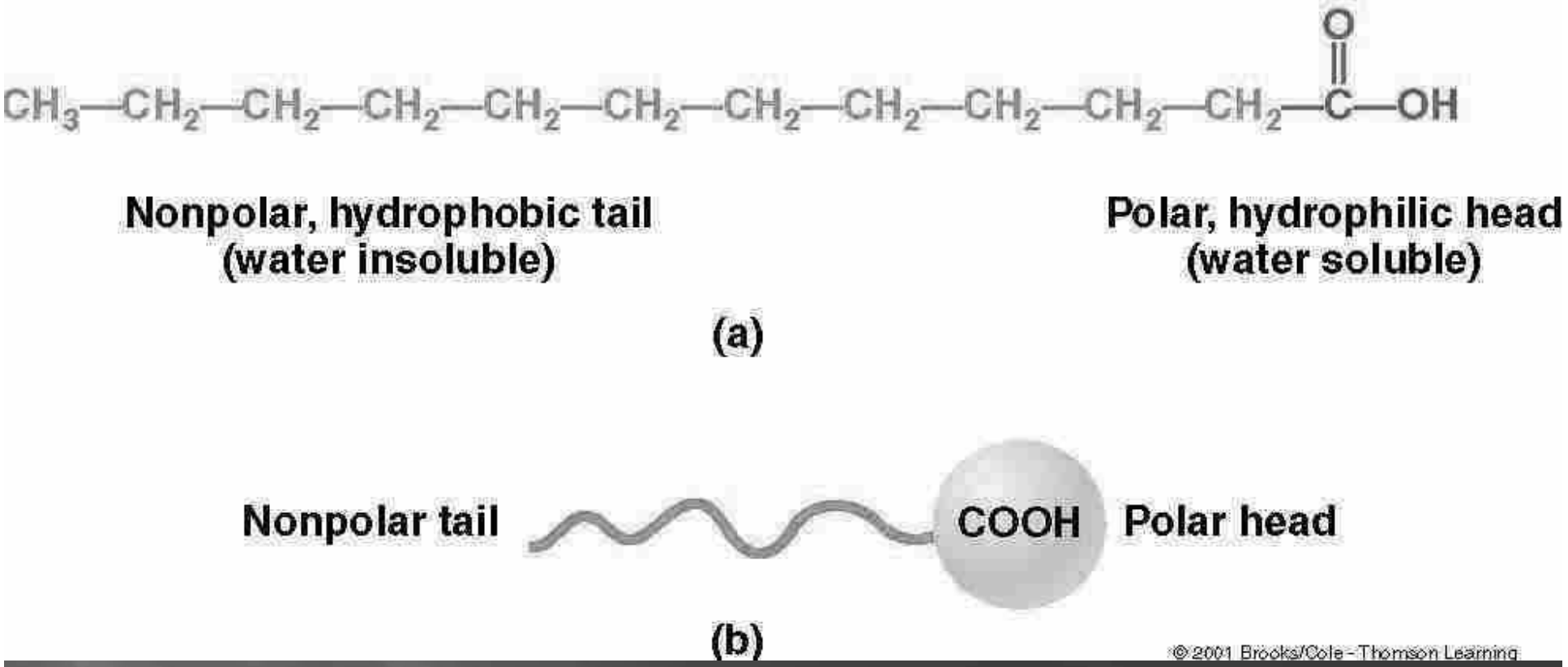
- Fatty acids are organic acids that occurs in a natural triglycerides & is a mono carboxylic acid.
- Ranging in chain length from C_4 to about C_{24} carbon atoms.

- R- stands for hydrocarbon chain.



Timberlake, Chemistry: An Introduction to General, Organic, and Biological Chemistry, Eighth Edition.
Copyright © 2003 Pearson Education, Inc., publishing as Benjamin Cummings.

- Amphipathic in nature
- Each has hydrophilic (COOH) and hydrophobic (hydrocarbon Chain) groups in the structure.



© 2001 Brooks/Cole - Thomson Learning

- Fatty acids occurs mainly as esters of glycerol in neutral fats and oils
- Serve as major fuel for most cells.
- They are precursors of all other classes of lipids
- They are in a low oxidation state (Carbon almost completely reduced as CH_2), and thus conversion to carbon dioxide and water is highly exergonic (lots of energy in fats!)
- Chains may be fully saturated, or have some unsaturated double bonds
- Chains are usually unbranched

Classification of fatty acids

(A) Straight chain

(B) Cyclic

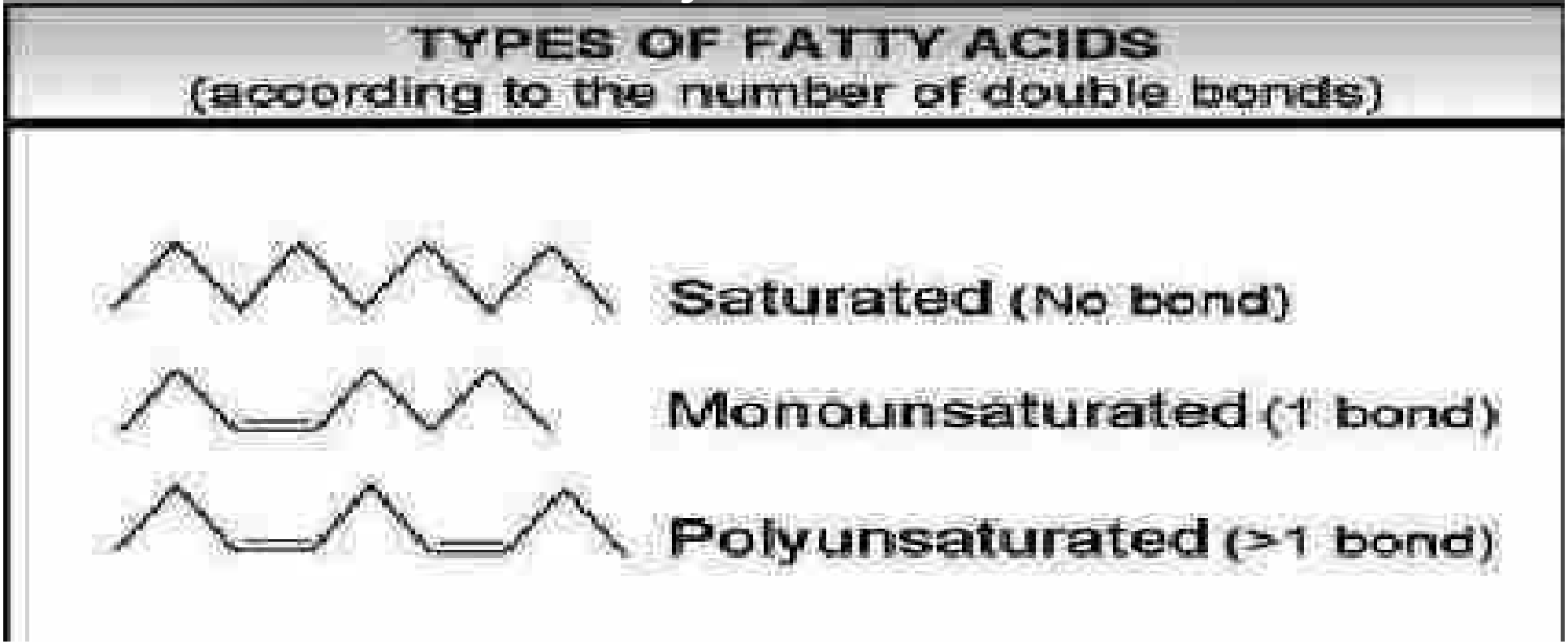
(C) Branched

(D) Substituted

Straight chain fatty acids

Carbons are arranges linearly, are subclassified into

- Saturated fatty acids
- Unsaturated fatty acids



Systematic Nomenclature

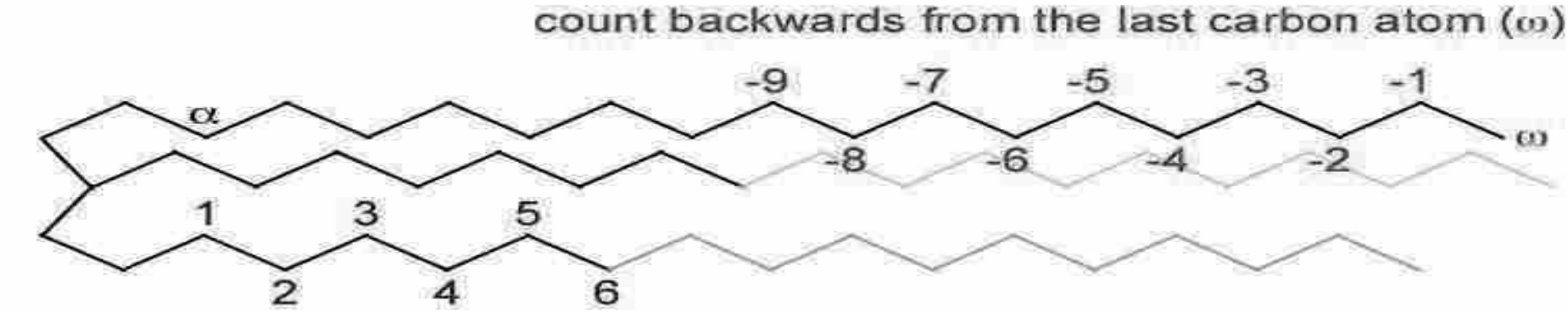
- Saturated fatty acids end with suffix anoic
- Eg C_{18} saturated fatty acid is octadecanoic acid because parent hydrocarbon is octadecane.
- Unsaturated fatty acids ends with suffix enoic
- Eg. C_{18} with one double bond called octadecenoic acid
- With two double bond octadecadienoic acid.

- FA Nomenclature
- C atoms are numbered from the CARBOXYL atom (C 1)
- Adjacent to C1 is **C2 = α**

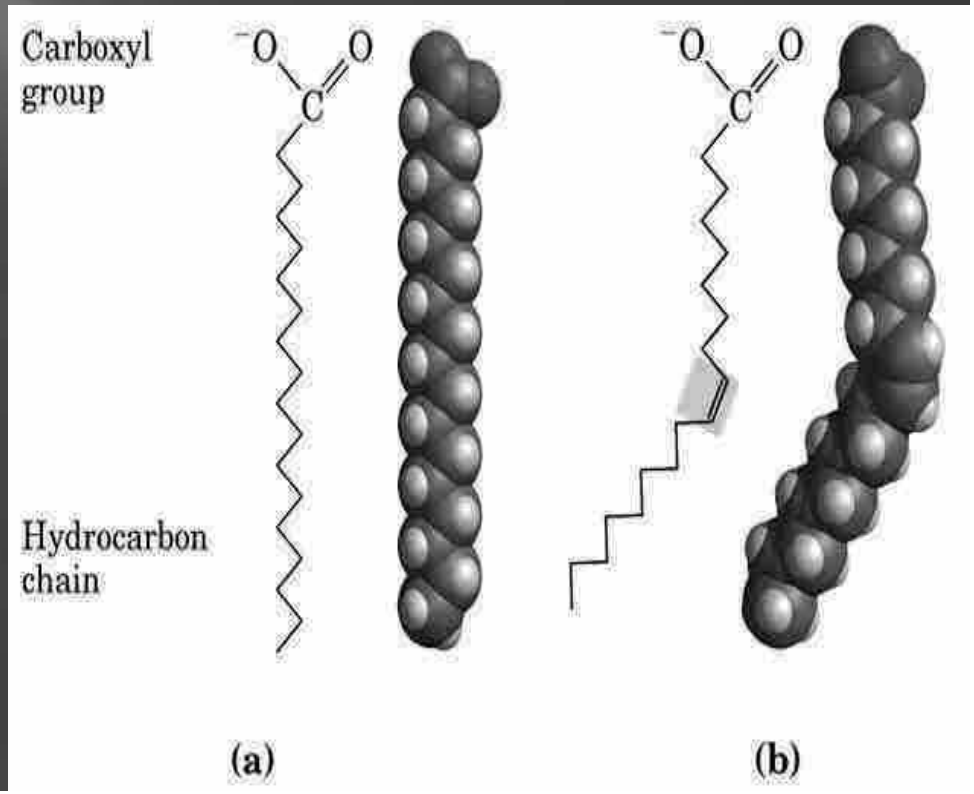
  C3 = β

  C4 = γ
- Methyl end: CH3 is known as **ω**

The numbering system:

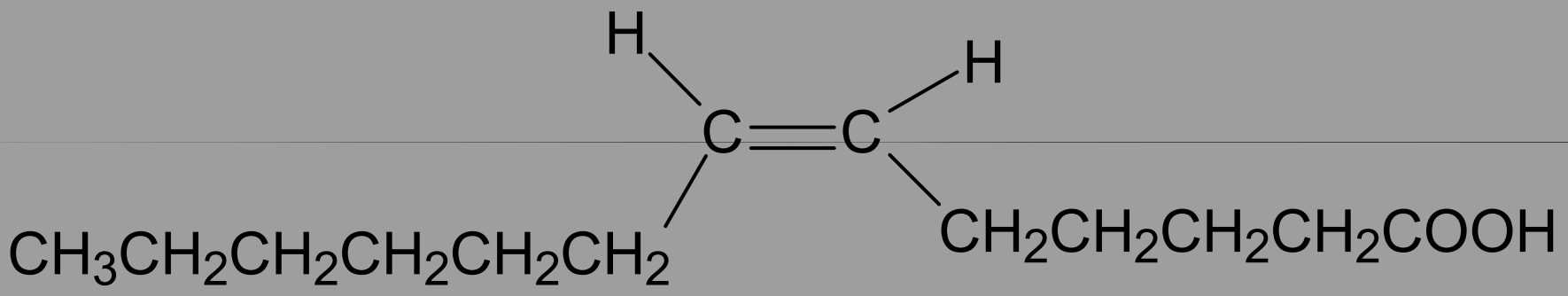


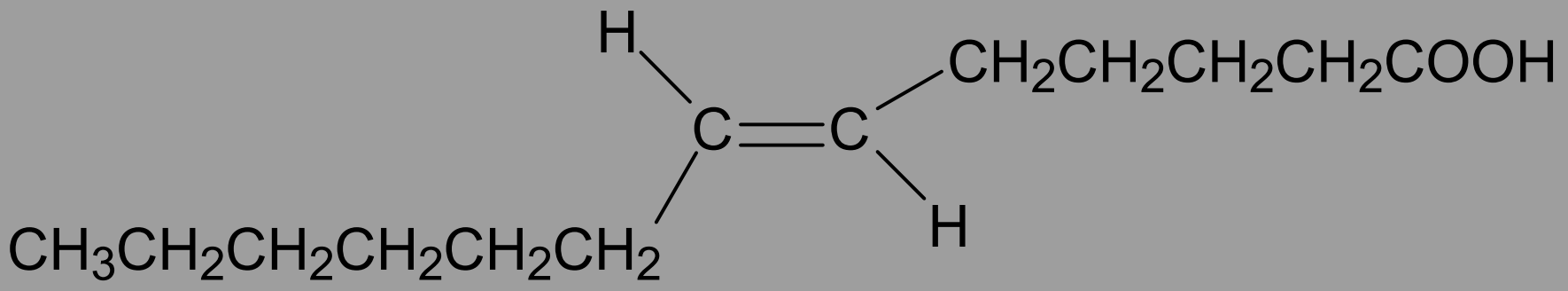
- FA Nomenclature
- Δ→ indicates the number and position of the double bond :ex Δ9 indicates a double bond between 9-10
- ω 9 → indicates a double bond on the ninth C based on the ω terminal
- 20:2($\Delta^{9,12}$) =20C: 2 double bonds, between C9-10, C12-13



Cis vs Trans Fatty Acids

- Cis fatty acids occur naturally
- Trans fatty acids occur during hydrogenation reactions and in animal fats.



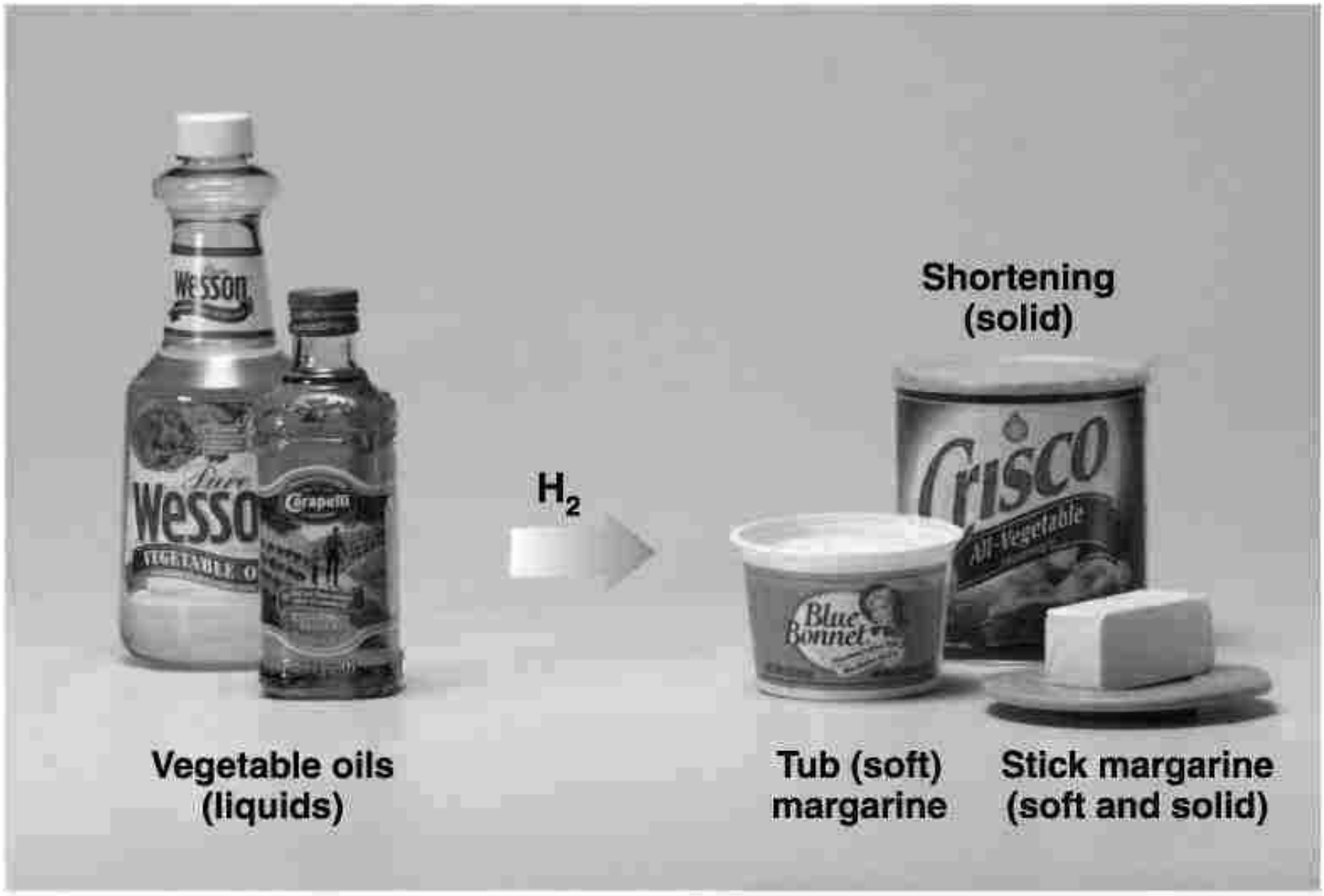


- Trans fatty acids
- Hydrogenation – is to change physical properties of oils into solid products , for easy manipulation.
- They are injurious to health
- Used in food industry as they increase shelf life of fried food.
- Fast foods preparations have high TFA content.
- Risk factor – system inflammation , endothelial dysfunction, insulin resistance , diabetes, adiposity.

Properties of fatty acids

- **Hydrogenation or hardening of oils**:
- **It is a type of addition reactions accepting hydrogen at the double bonds of unsaturated fatty acids.**
- **The hydrogenation is done under high pressure of hydrogen and is catalyzed by finely divided nickel or copper and heat.**
- **It is the base of hardening of oils (margarine manufacturing)**
- Linolenic $\xrightarrow{+2H}$ Linoleic $\xrightarrow{+2H}$ Oleic $\xrightarrow{+2H}$ Stearic
- **Excessive hydrogenation- very hard, of very low biological value and difficult to digest.**

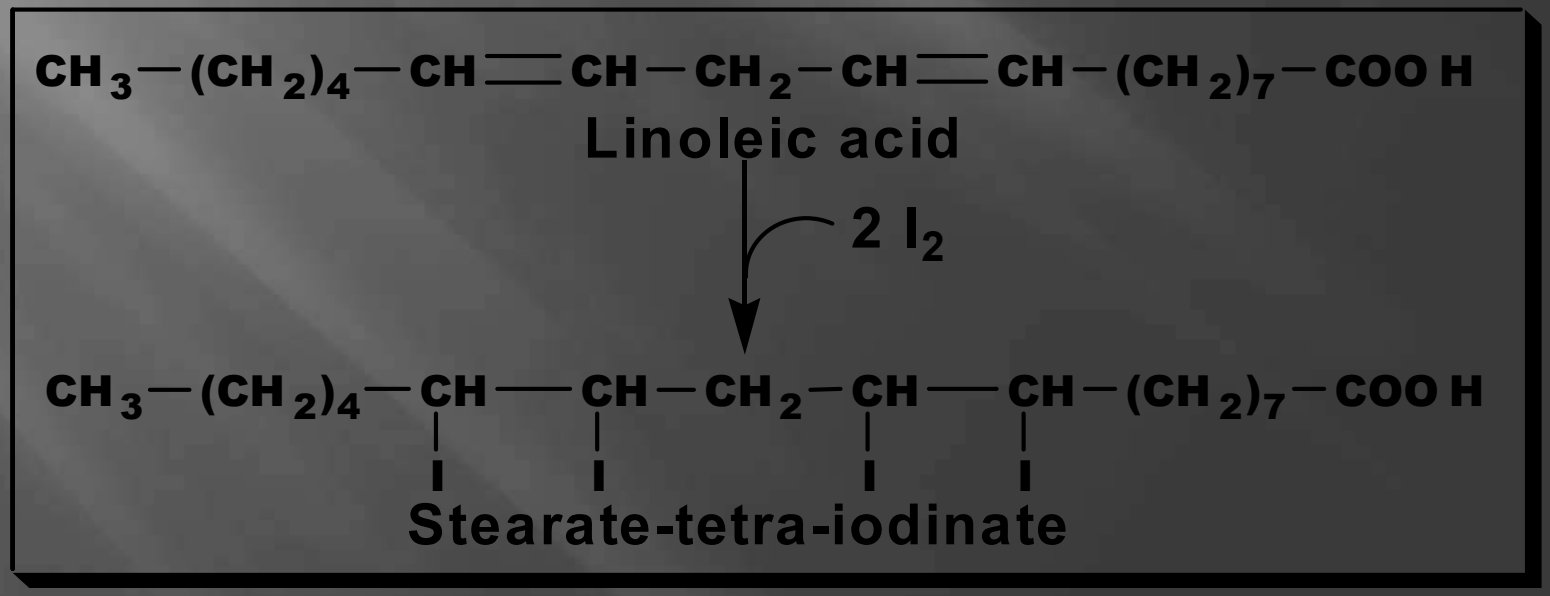
Hydrogenation of Oils



Timberlake, Chemistry: An Introduction to General, Organic, and Biological Chemistry, Eighth Edition.
Copyright © 2003 Pearson Education, Inc., publishing as Benjamin Cummings.

3-Halogenation

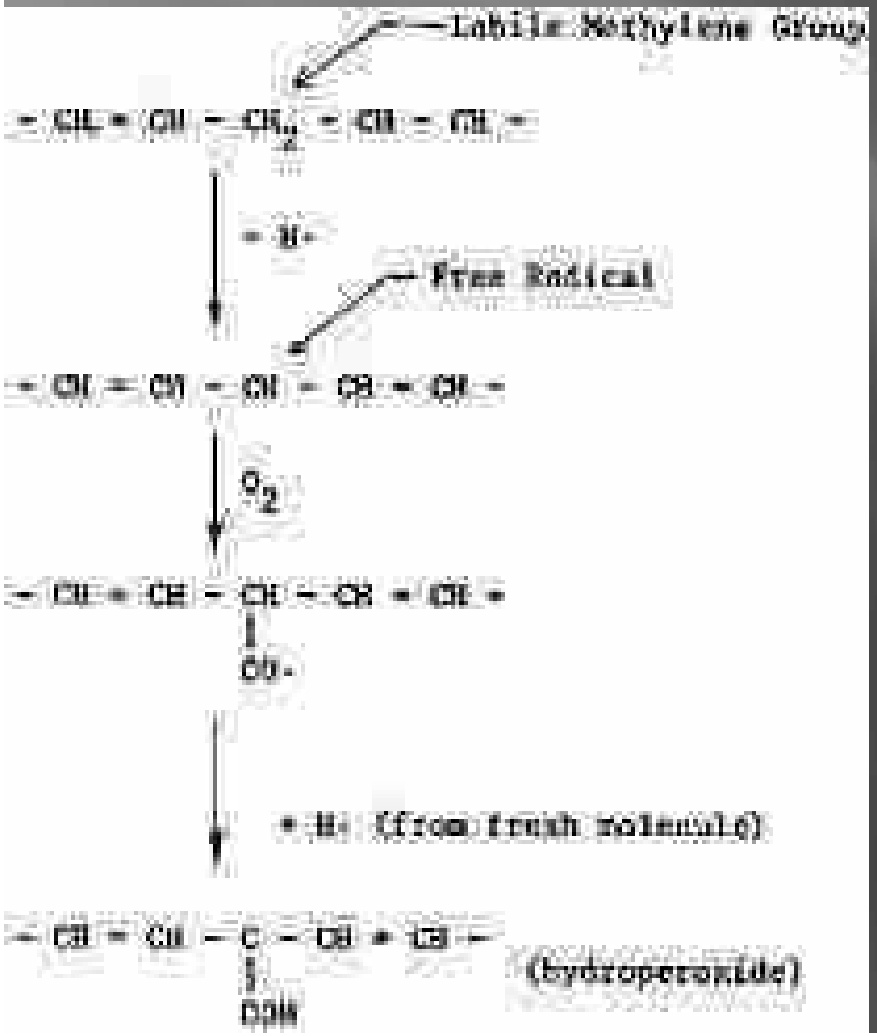
- Neutral fats containing unsaturated fatty acids have the ability of adding halogens (e.g., hydrogen or hydrogenation and iodine or iodination) at the double bonds.
- - It is a very important property to determine the degree of unsaturation of the fat or oil that determines its biological value

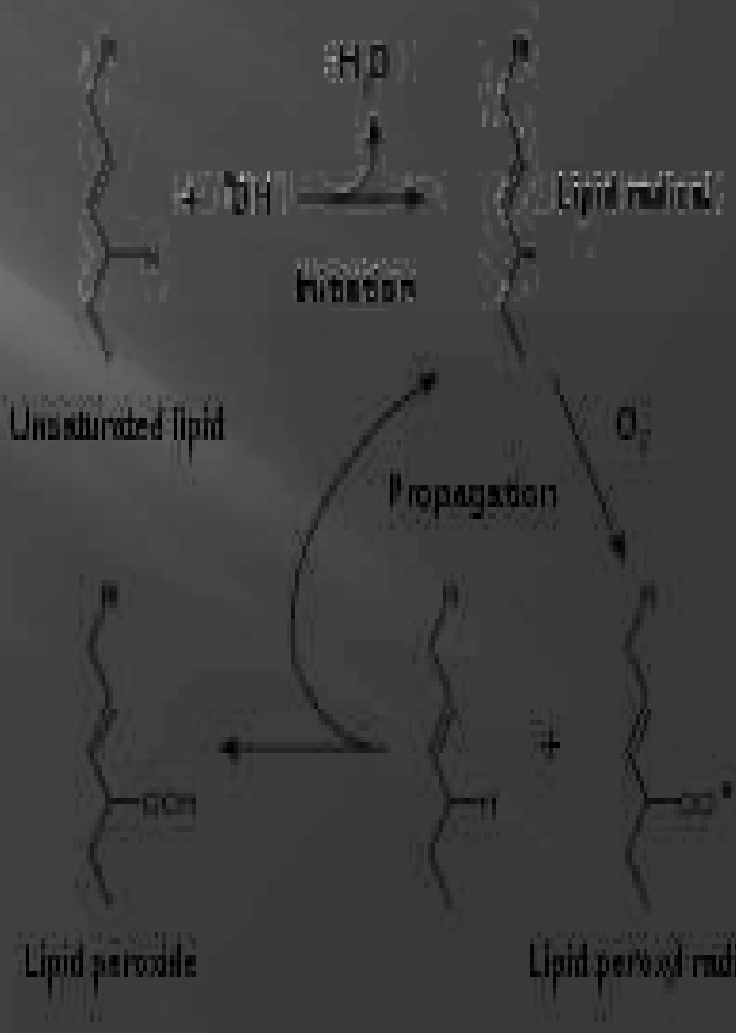


LIPID PEROXIDATION

- Auto- oxidation of lipids exposed to oxygen
- Deterioration of foods but also for damage to tissues in vivo.
- May be cause of cancer
- It is a chain reaction providing a continuous supply of free radicals that initiate further per oxidation.

LIPID PEROXIDATION





LIPID PEROXIDATION

- Control & reduce peroxidation by antioxidants.
- antioxidants prevents rancidity
- Natural antioxidants are – vit E (tocopherol) & β – carotene (provit.A), Vit.C
- Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BTH) are antioxidants used as food additives.
- In vivo – by haem compounds & by lipoxygenase found in platelets & leucocytes.

Saturated fatty acids

- No double bond in the hydrocarbon chain
- Are in extended conformation and can be packed together into a compact structure.
- Subclassified into
- Even carbon acids – even carbons

  eg palmitic acid & stearic acid
- Odd carbon chain – odd carbons eg propionic acid

- **They have the following molecular formula, $C_nH_{2n+1}COOH$**

A-Short chain Saturated F.A. (2-10 carbon).

a-Short chain Saturated volatile F.A.(2-6 carbon).

b- Short chain Saturated non volatile F.A.(7-10 carbon).

B-Long chain Saturated F.A.(more the10 carbon)

a-Volatile short-chain fatty acids:

- They are liquid in nature and contain (1-6) carbon atoms.
- water-soluble and volatile at room temperature, e.g., acetic, butyric, and caproic acids.
- Acetic F.A. (2C) CH_3-COOH.
- Butyric F.A. (4C) CH_3-$(CH_2)_2$-COOH.
- Caproic F.A. (6C) CH_3-$(CH_2)_4$-COOH

b-Non-volatile short-chain fatty acids:

- They are solids at room temperature and contain 7-10 carbon atoms.
- They are water-soluble and non-volatile at room temperature include caprylic and capric F.A.

- Caprylic (8 C) $CH_3-(CH_2)_6-COOH$.
- Capric (10 C) $CH_3-(CH_2)_8-COOH$

B-Long-chain fatty acids:

- They contain more than 10 carbon atoms.
- They occur in hydrogenated oils, animal fats, butter and coconut and palm oils.
- They are non-volatile and water-insoluble
- Include palmitic, stearic, and lignoceric F.A.

- Palmitic(16C) CH_3-$(CH_2)_{14}$-COOH
- Stearic (18 C) CH_3-$(CH_2)_{16}$-COOH
- Lignoceric (24C) CH_3-$(CH_2)_{22}$-COOH

Unsaturated Fatty Acids

They contain double bond

- monounsaturated

  they contain one double bonds .

  ($C_nH_{2n-1}COOH$)

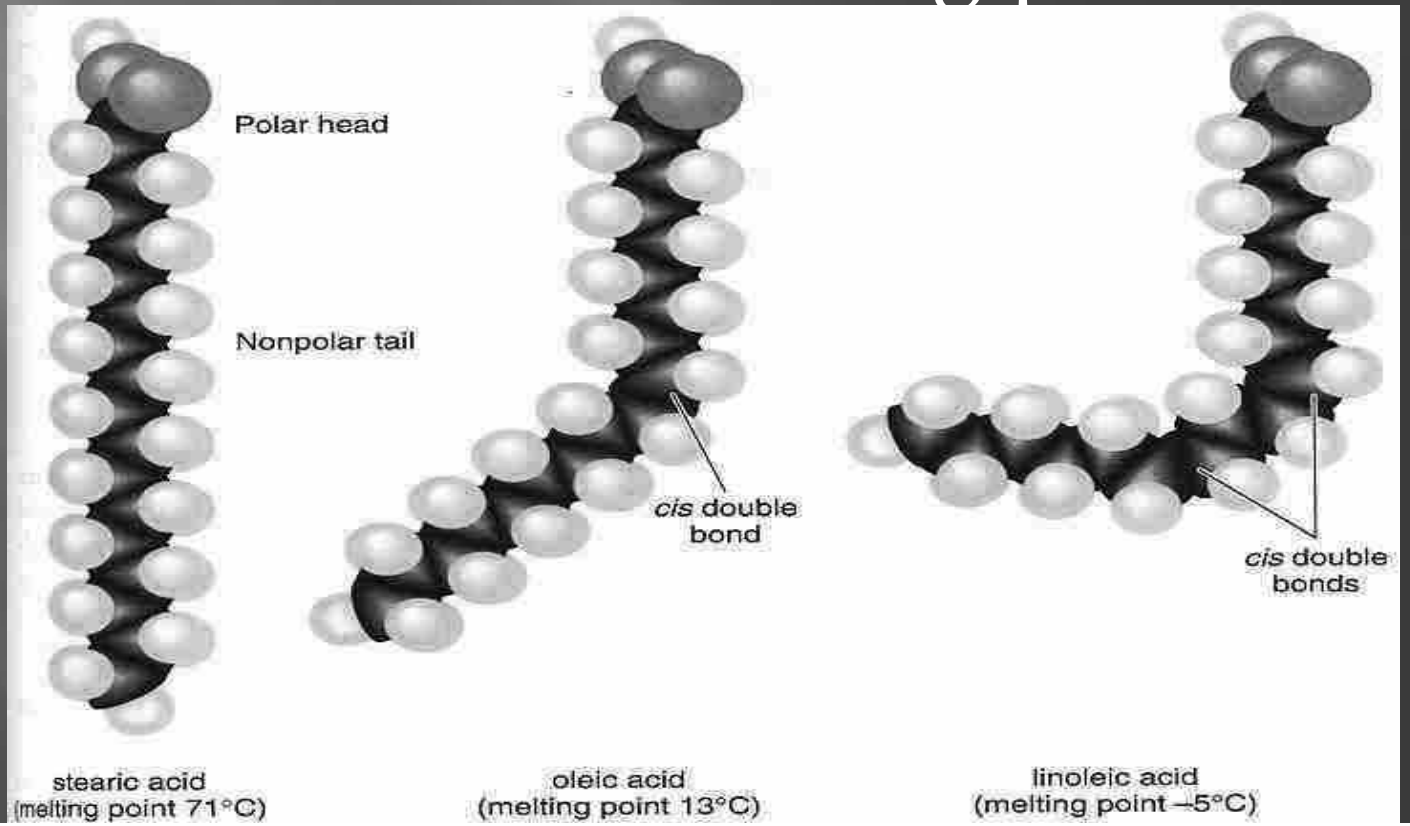
- polyunsaturated

  they contain more the one double bond

  ($C_nH_{2n-\text{more than }1}COOH$)

  Most double bonds are *cis*- bonds that cause a bend in the chain

- Prevents compact packing
- Unsaturated fatty acids are loosely packed , so easily disturbed by heat.
- This accounts low melting point



- Monoenoic acids (one double bond):
  - 16:1, 9 ω7: palmitoleic acid (***cis***-9-hexadecenoic acid)
  - 18:1, 9 ω9: oleic acid (***cis***-9-octadecenoic acid)
  - 18:1, 9 ω9: elaidic acid (***trans***-9-octadecenoic acid)
  - 22:1, 13 ω9: erucic acid (***cis***-13-docosenoic acid)
  - 24:1, 15 ω9: nervonic acid (***cis***-15-tetracosenoic acid)

Polyunsaturated fatty acid

- Dienoic acid:
  - 18:2; 9,12. ω6: Linoleic acid
- Trienoic acids (3 double bonds)
  - 18:3;6,9,12 ω6 : γ-linolenic acid (all ***cis***-6,9,12-octadecatrienoic acid)
  - 18:3; 9,12,15 ω3 : α-linolenic acid (all-***cis***-9,12,15-octadecatrienoic acid)
- Tetraenoic acids (4 double bonds)
  - 20:4; 5,8,11,14 ω6: arachidonic acid (all-***cis***-5,8,11,14-eicosatetraenoic acid)

ESSENTIAL FATTY ACIDS

- Fatty acids that cannot be synthesized in the body ; hence have to be supplied in diet called EFAs.
- Required for optimal health
- Human can biosynthesize many saturated & monounsaturated varieties.

EFA Cannot be synthesized because we lack the enzyme to insert double bonds beyond Δ^9. (ER)

- ω3 & ω6 series cannot be synthesized.
- Desaturase enzymes present are : Δ^4, Δ^5, Δ^6, Δ^9

  Δ^{12} & Δ^{15} Desaturase are present in plants.

EFAs are : 1. Linoleic Acid
2. Linolenic Acid
3. Arachidonic Acid

EFA

FUNCTIONS

- Synthesis of longer ω3 & ω6 series PUFAs.
- Synthesis of DHA Docosahexaenoic Acid – (22 : 6 ω3) present in high concentration is Brain, retina, tests.
- Synthesis of prostaglandins and leukotrienes from Arachidonic Acid.
- Arachidonic Acid is present is membranes (15%) and phospholipids.

DEFICIENCY DISORDERS

1. Phraenoderma : “Toad skin”.
   Dry, scaly skin.
2. Retinitis Pigmentosa : ↓ levels of DHA.
3. Retinopathy of prematurity :
   ↓ Δ^4 Desaturase → ↓ DHA from ω3 precursors.

Chemistry of Lipids

Dr.aarti

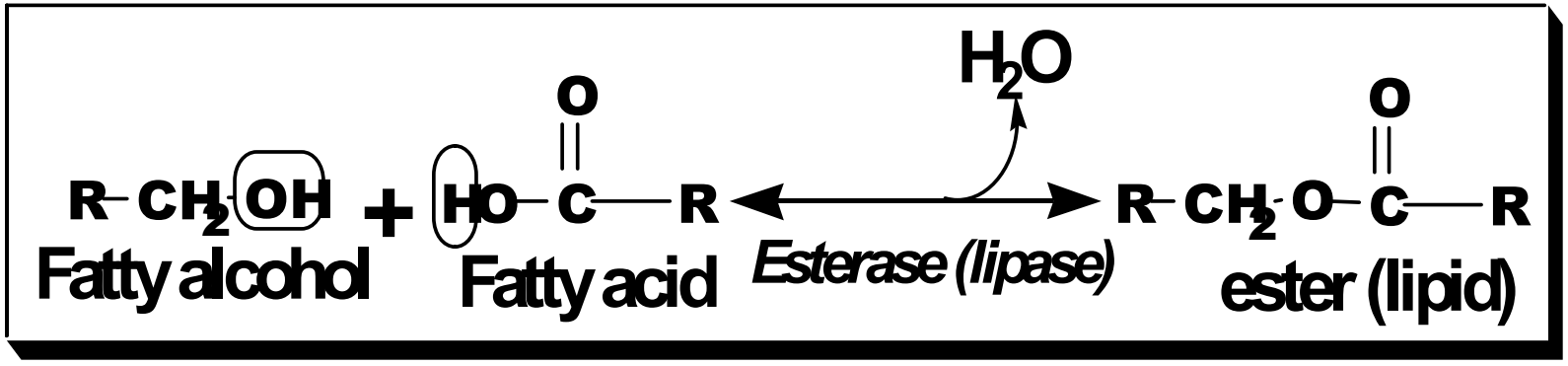
- - Lipids are insoluble in water, but soluble in fat or organic solvents (ether,chloroform, benzene, acetone).

- - Lipids include fats, oils, waxes and related compounds.

- They are widely distributed in nature both in plants and in animals.

Definition:

- **- Lipids are organic compounds formed mainly from alcohol and fatty acids combined together by ester linkage.**



Biological Importance of Lipids:

- Storage form of energy

- **They are more palatable and storable to unlimited amount compared to carbohydrates.**
- **They have a high-energy value (25% of body needs) and they provide more energy per gram than carbohydrates and proteins but carbohydrates are the preferable source of energy.**

- Supply the essential fatty acids that cannot be synthesized by the body.
- Supply the body with fat-soluble vitamins (A, D, E and K)

- Stored lipids "depot fat" is stored in all human cells acts as:

- A store of energy.
- A pad for the internal organs to protect them from outside shocks.
- A subcutaneous thermal insulator against loss of body heat

- Non polar lipids acts as electrical insulators, allowing rapid propagation of depolarization waves along myelinated nerves.
- Provide shape and contour to the body
- Act as metabolic regulators (steroid hormones)
- Tissue fat is an essential constituent of cell membrane and nervous system. It is mainly phospholipids in nature that are not affected by starvation

- **Lipoproteins - Combination of lipid and protein are important cellular constituents, occurring both in the cell membrane and in the mitochondria.**
- **Serving also as the means of transporting lipids in the blood.**

- **Cholesterol enters in membrane structure and is used for synthesis of adrenal cortical hormones, vitamin D3 and bile acids.**

LIPIDS

Simple Lipids

- Neutral Fats
  - -Fats
  - -Oils
- Waxes

Compound lipid

- -phospholipids
- -Glycolipids
- -Lipoproteins
- -Sulpholipids

Derived Lipids

- -Steroids
- -cholesterol
- --Bileacids
- -Vit D
- -sex hormones
- -steroid hormones

Classification of Lipids

1. **Simple lipids (Fats & Waxes)**
2. **Compound or conjugated lipids**
3. **Derived Lipids**
4. **Lipid-associating substances**

Simple Lipids:

- Esters of fatty acid with various alcohols
  - Fats: Esters of fatty acid with glycerol
  - Waxes: esters of FA; higher molecular wt monohydric alcohol

- **Complex Lipids:**
- **Esters of fatty acids containing groups in addition to alcohol and a fatty acid**.
  - Phospholipids:
  - phosphoric acid and a residue
  - They frequently have nitrogen containing bases and other substituents, eg in glycerophospholipid the alcohol is glycerol and in sphingophospholipids the alcohol is spingosine
  - Glycolipids :
  - Lipids containing a fatty acid, spingosine and carbohydrates
  - Other complex lipids: Sulpholipids & Aminolipids, Lipoproteins

Derived lipids:

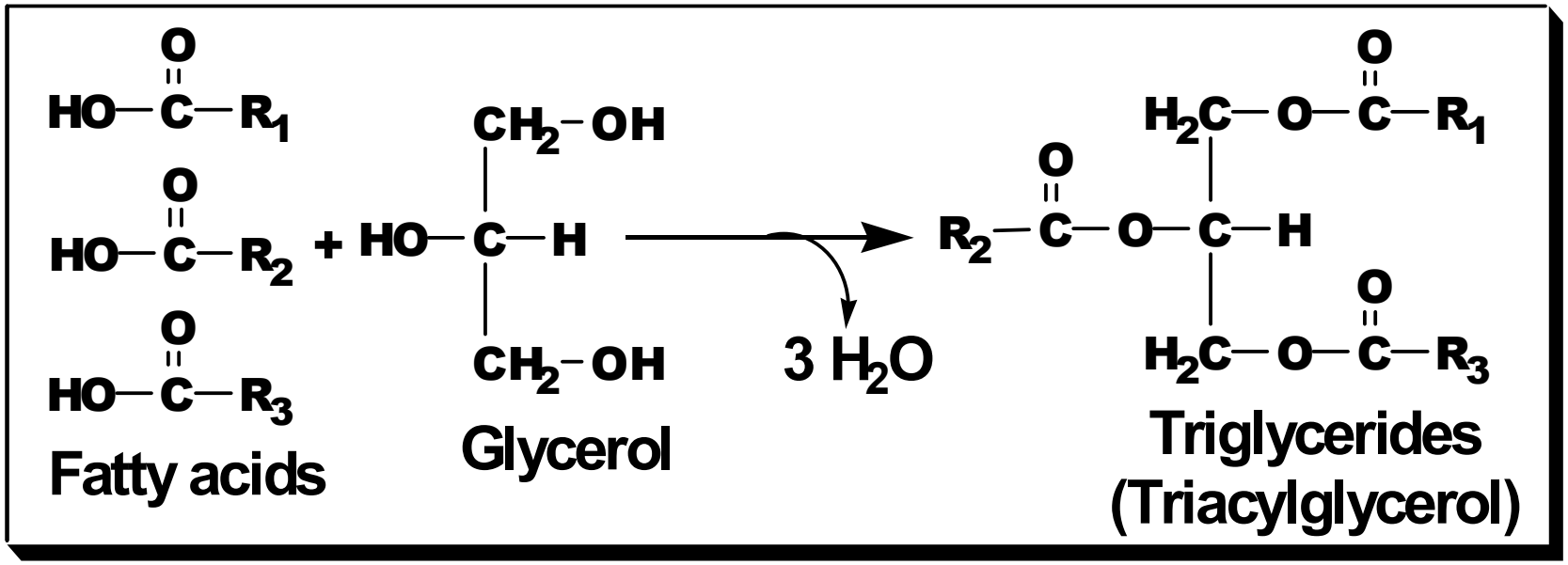
- Products obtained after the hydrolysis of simple & compound lipids Fatty acids.
- Glycerol
- Steroids
- Other alcohols
- Fatty aldehydes
- Ketone bodies
- Hydrocarbons, lipid- soluble vitamins and hormones

Neutral fats or triacylglycerol or triglycerides

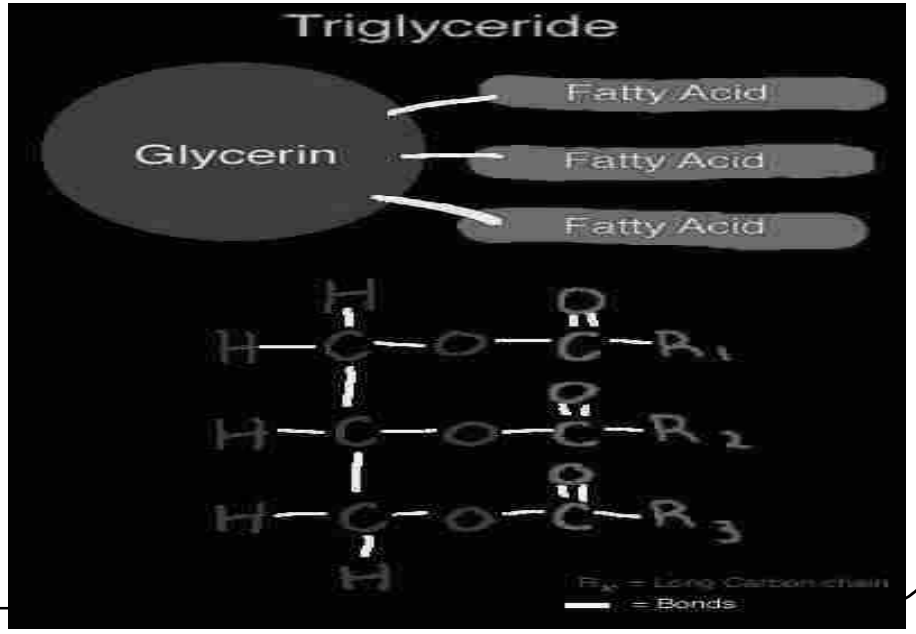
- They are called neutral because they are uncharged due to absence of ionizable groups in it.
- The fat we eat are mostly triglycerides.

- The neutral fats are the most abundant lipids in nature. They constitute about 98% of the lipids of adipose tissue, 30% of plasma or liver lipids, less than 10% of erythrocyte lipids.

- They are esters of glycerol with various fatty acids. Since the 3 hydroxyl groups of glycerol are esterified, the neutral fats are also called "Triglycerides
- Esterification of glycerol with one molecule of fatty acid gives monoglyceride, and that with 2 molecules gives diglyceride.



- **Naturally occurring fats and oils are mixtures of triglycerides.**
- **If all the OH groups are esterified to same fatty acids- It is simple triglyceride. e.g., tripalmitin, triolein**
- **If different fatty acids are esterified – It is known as mixed triglyceride. e.g., stearo-diolein and palmito-oleo-stearin.**



Physical Properties of triglycerides

- Colourless, odourless and tasteless
- Hydrophobic and insoluble in water
- Specific gravity is less than 1.0, consequently all fats floats in water.
- Oils are liquids at 20^0C, they contain higher proportion of unsaturated fatty acids.
- Fats are solid at room temperature and contain saturated long chain fatty acids
- Melting points of fats are usually low.

Essential fatty acids

- Poly unsaturated fatty acids which are essential for health but are not synthesized by body.
- Linoleic acid-vegetable oils ,Linolenic acid,Arachidonic acid
- Functions:
- Required for membrane structure and function
- Arachidonic acid is precursor of prostaglandins
- Formation of Ipoproteins
- Development of Brain and retina
- Deficiency leads to dermatitis phrynoderma or toad skin,Eczema,poor wound healing

Difference between Oils and Fats

- Fats
- Contain higher proportion of long chain saturated fatty acids
- Solid at room temperature
- Generally of animal origin eg.butter fat, pure ghee

- Oil
- Contain higher proportion of short chain or medium chain fatty acids
- Liquid at room temperature
- Plant origin
- Eg.vegetable oils

Mustard oil, sunflower oil etc

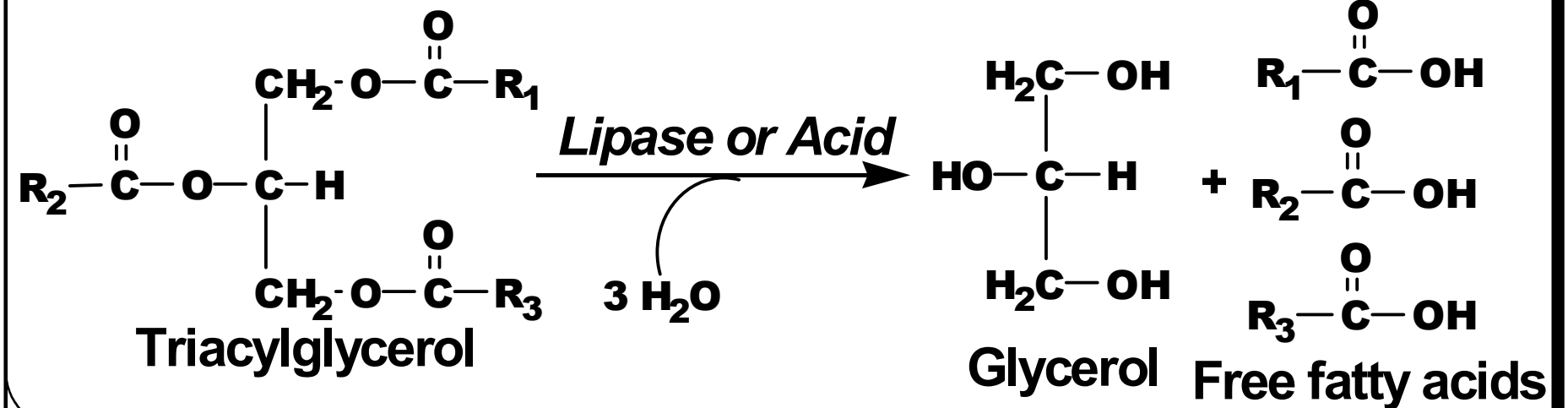
Storage of energy

- Triglycerides are the storage form of energy in adipose tissue
- Normal 70 kg normal person body stores contain about 11 kg of TG = 100,000 Kcal
- When TG stored water molecules are repelled and space requirement is minimal.
- Excess fats in body leads to obesity.

Chemical Properties of fats and oils:

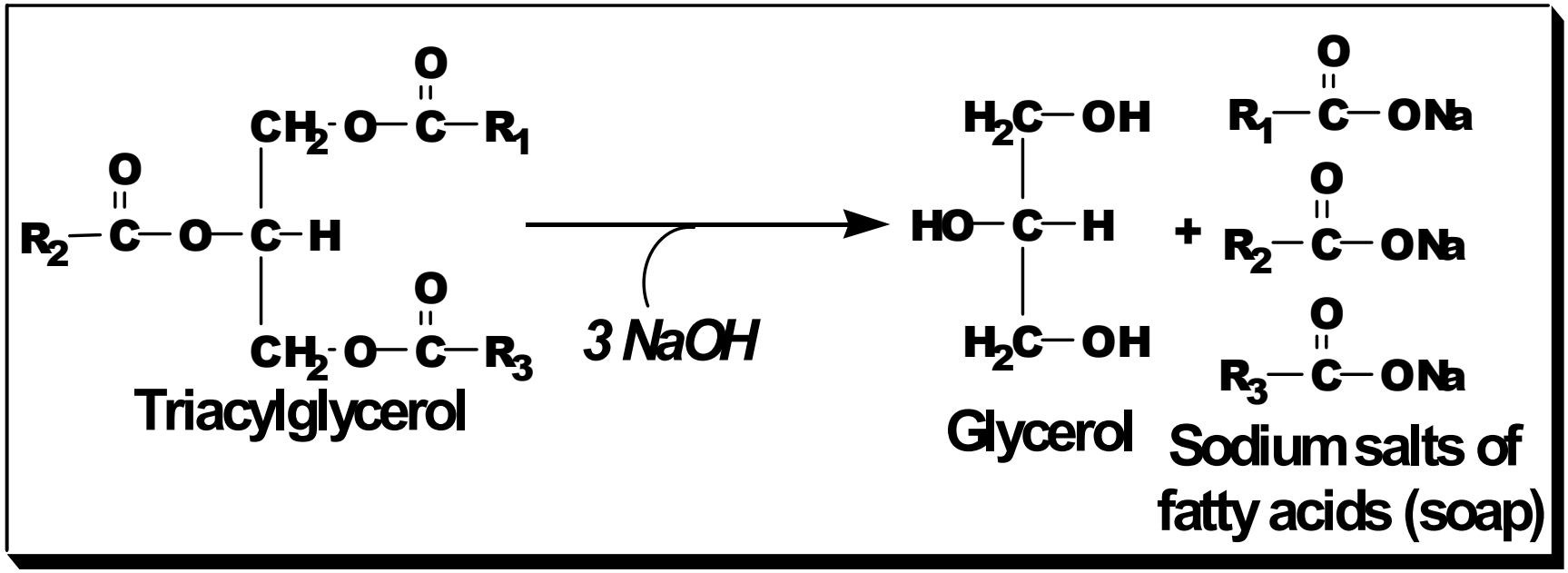
1-Hydrolysis:

- They are hydrolyzed into their constituents (fatty acids and glycerol) by the action of super heated steam, acid, alkali or enzyme (e.g., lipase of pancreas).
- - During their enzymatic and acid hydrolysis glycerol and free fatty acids are produced.
- TG sequentially hydrolysed to diacyl glycerol and mono acylglycerol and finally glycerol plus 3 fatty acids.



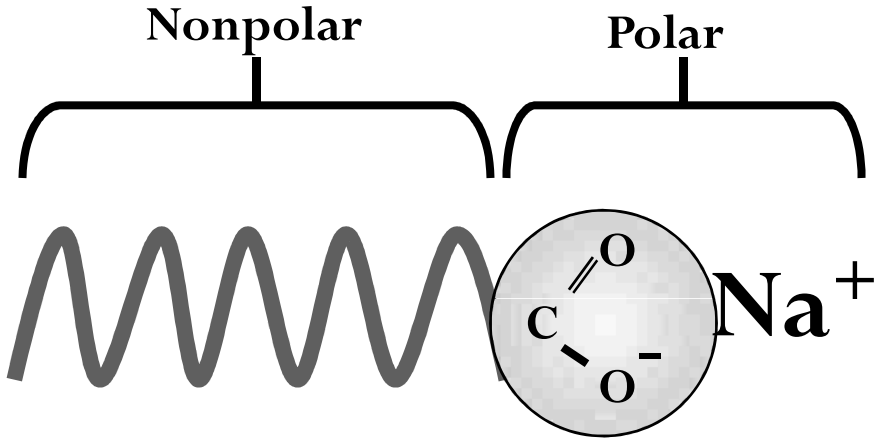
2-Saponification. **Alkaline hydrolysis produces glycerol and salts of fatty acids (soaps).**

- **Soaps cause emulsification of oily material this help easy washing of the fatty materials**

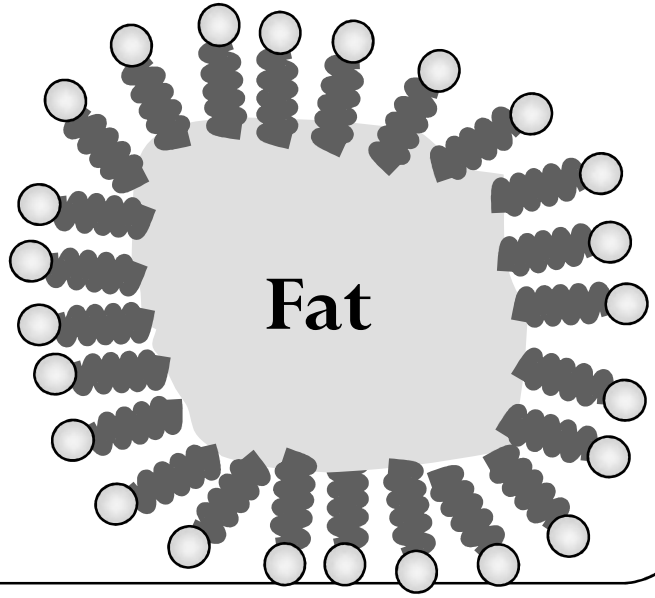


Soap:

Long Chain Fatty Acid Salt



Fatty acid



Fat with soap is water soluble

Soaps

- Are cleansing agents because of their emusifying action.
- Soaps of high molecular weight and high degree of unsaturation are selectively germicides.
- Sodium ricinoleate – detoxifying activity against diphtheria and tetanus toxins
- Unsaponificable residues of natural fats- ketones, hydrocarbons, steroids,high molecular weight alcohols.

Saponification number

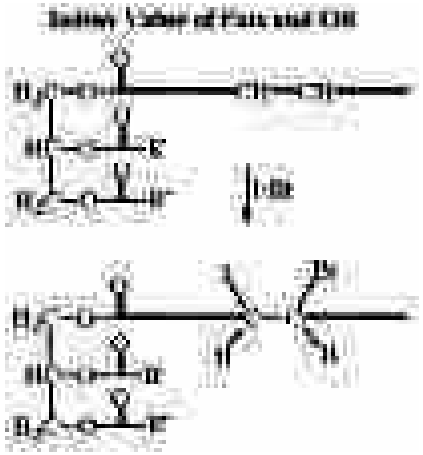
- Number of mg of potassium hydroxide required to saponify one gram of fat
- Fats containing long chain FA,have more mol.wt.and less saponification number
- Indication of molecular weight of fat , is inversely proportional to it.
- Human fat saponification number – 194 – 198
- Butter – 210 – 230
- Coconut oil – 253 - 262

Iodine number

- **Definition:** It is the number of grams of iodine absorbed by 100 grams of fat or oil.
- **Uses:** It is a measure for the degree of unsaturation of the fat, as a natural property for it.
- Unsaturated fatty acids absorb iodine at their double bonds, therefore, as the degree of unsaturation increases iodine number and hence biological value of the fat increase.
- It is used for identification of the type of fat, detection of adulteration and determining the biological value of fat.

IODINE NUMBER

- **butter – 28**
- **Sunflower oil - 130**
- **Palm kernel oil 13 - 17**
- **Coconut oil 8 – 10**
- **Milk fat 26 – 50**
- **Peanut oil (arachis) 83 - 100**
- **Olive oil 79 - 88**



Acids Number

Definition:

- **It is the number of milligrams of KOH required to neutralize the free fatty acids present in one gram of fat.**
- **Acid number is directly proportional to rancidity, and inversely proportional to edibility of oil.**

Uses:

- **It is used for detection of hydrolytic rancidity because it measures the amount of free fatty acids present.**
- **Butter has acid number 0.45,but when rancid its 0.35.**

Rancidity

Definition:

- **It is a physico-chemical change in the natural properties of the fat leading to the development of unpleasant odor or taste or abnormal color particularly on aging after exposure to atmospheric oxygen, light, moisture, bacterial or fungal contamination and/or heat.**

- **Saturated fats resist rancidity more than unsaturated fats that have unsaturated double bonds.**
- **Repeated heating also makes the oil rancid due to polymerization of cyclic**

Hydrolytic rancidity:

- It results from slight hydrolysis of the fat by lipase present in naturally occuring fats & oils.
- From bacterial contamination leading to the liberation of free fatty acids and glycerol at high temperature and moisture.
- Volatile short-chain fatty acids have unpleasant odor.

Oxidative rancidity

- Due to partial oxidation of unsaturated fatty acids with the resultant formation of epoxides and peroxides by free radicals.
- This occurs due to oxidative addition of oxygen at the unsaturated double bond of unsaturated fatty acid of oils.
- In vivo affects integrity of biomembranes, leading to cell death.

Prevention of rancidity

- Many natural fats & oils contain antioxidants eg Vit E which prevent oxidative rancidity.
- PUFA – easily oxidised
- Avoidance of the causes (exposure to light, oxygen, moisture, high temperature and bacteria or fungal contamination). By keeping fats or oils in well-closed containers in cold, dark and dry place (i.e., good storage conditions).

Hazards of Rancid Fats:

1. The products of rancidity are toxic, i.e., causes food poisoning and cancer.
2. Rancidity destroys the fat-soluble vitamins (vitamins A, D, K and E).
3. Rancidity destroys the polyunsaturated essential fatty acids.
4. Rancidity causes economical loss because rancid fat is inedible.
5. Overheating and repeated heating of oils leads to formation of peroxides.

Waxes

- They are esters of higher fatty acids with higher mono hydroxy aliphatic alcohols (eg Cetyl alcohol)
- Have very long straight chain of 60 – 100 carbon atoms
- They can take up water without getting dissolved in it

.

- Used as bases for the preparation of cosmetics, ointments, polishes, lubricants and candles
- In nature, they found on surface of plants and insects
- Eg Lanolin or wool fat, bees wax, spermacetic oil from whale

Compound lipids

Compound Lipids

Definition:

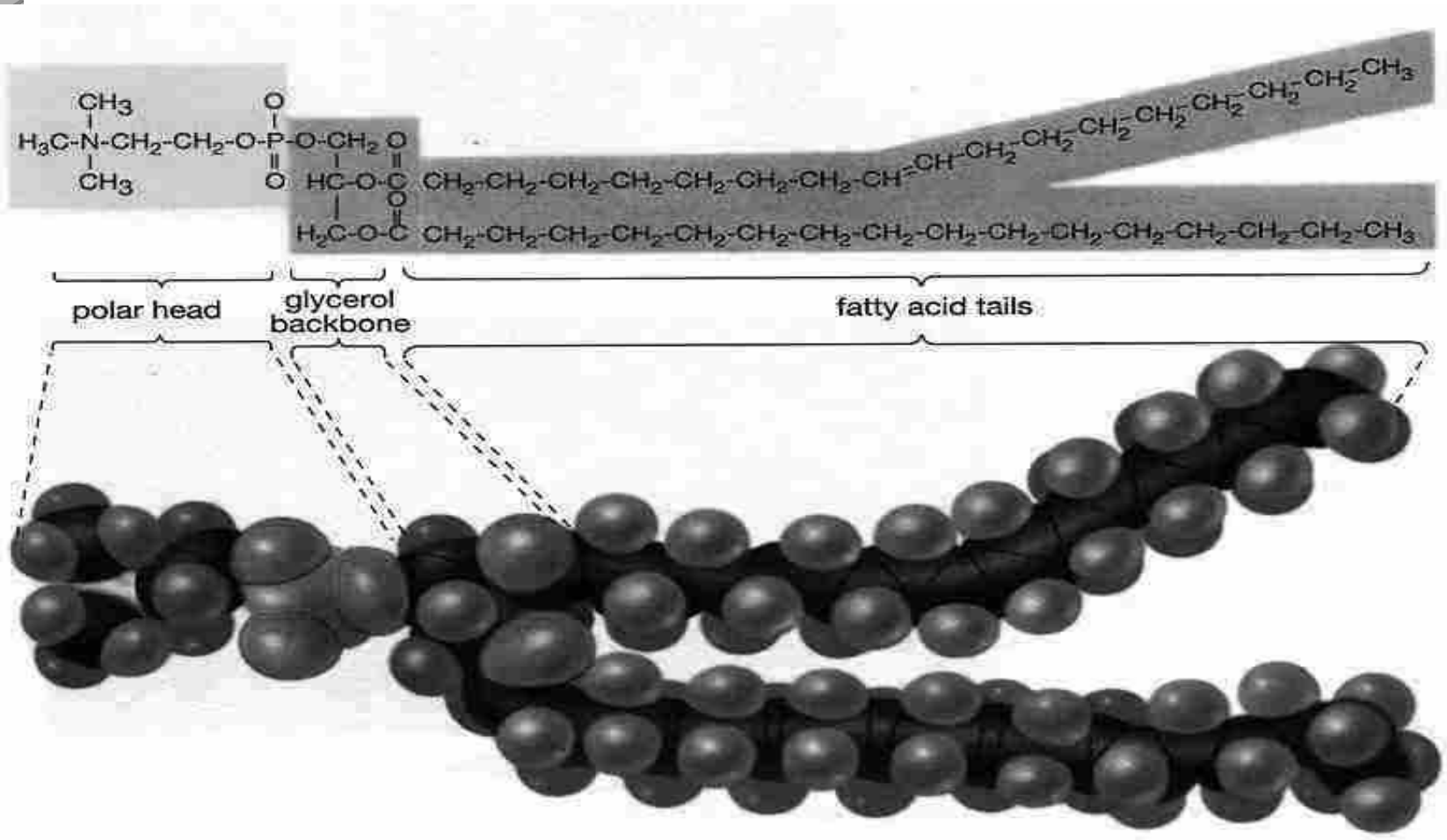
They are lipids that contain additional substances, e.g., sulfur, phosphorus, amino group, carbohydrate, or proteins beside fatty acid and alcohol.

Compound or conjugated lipids are classified into the following types according to the nature of the additional group:

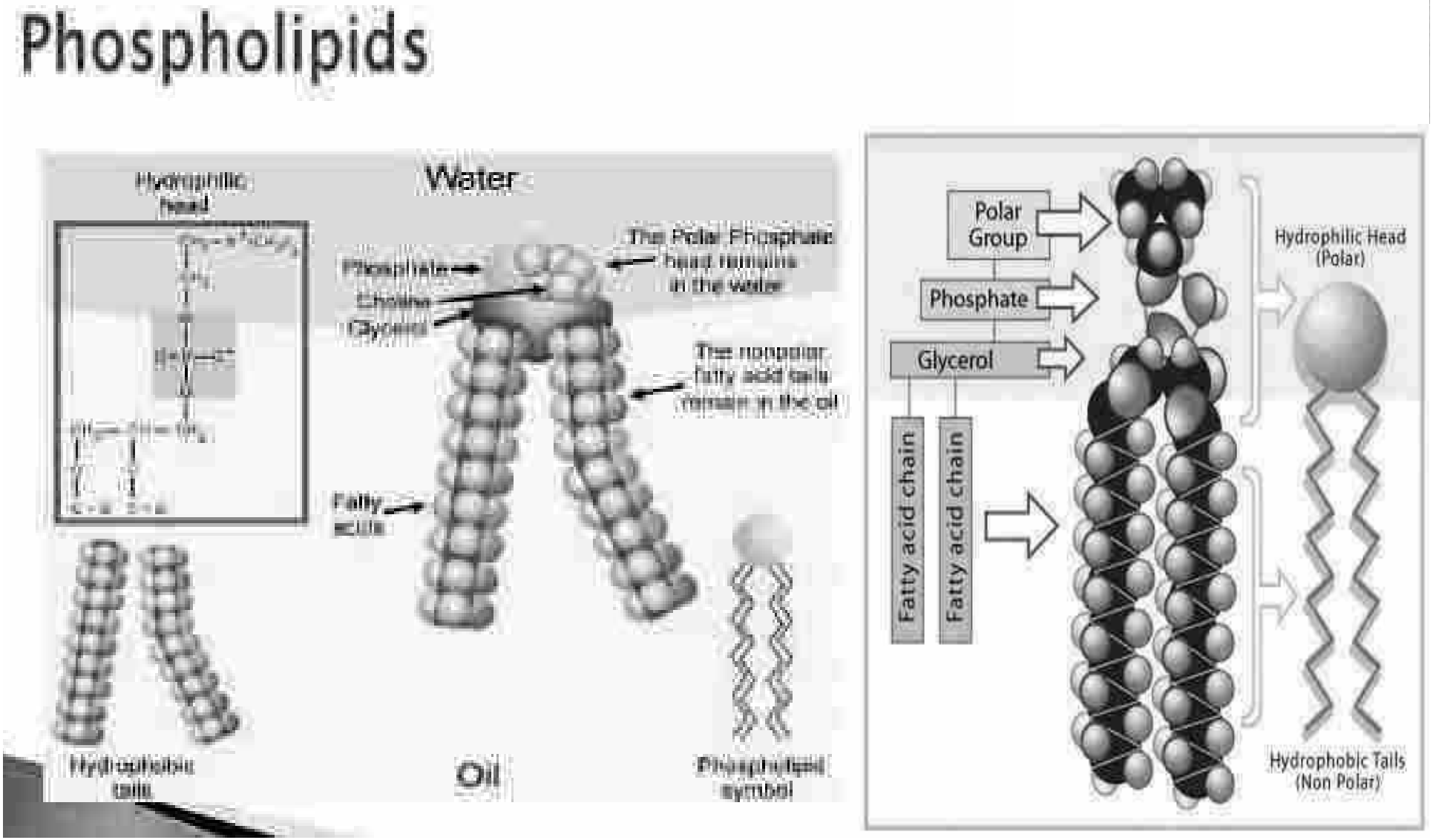
1. **Phospholipids**
2. **Glycolipids.**
3. **Lipoproteins**
4. **Sulfolipids and amino lipids.**

Phospholipids or phosphatides

Definition: Esters of fatty acids with glycerol, which contain phosphoric acid group in their structure and nitrogen base.



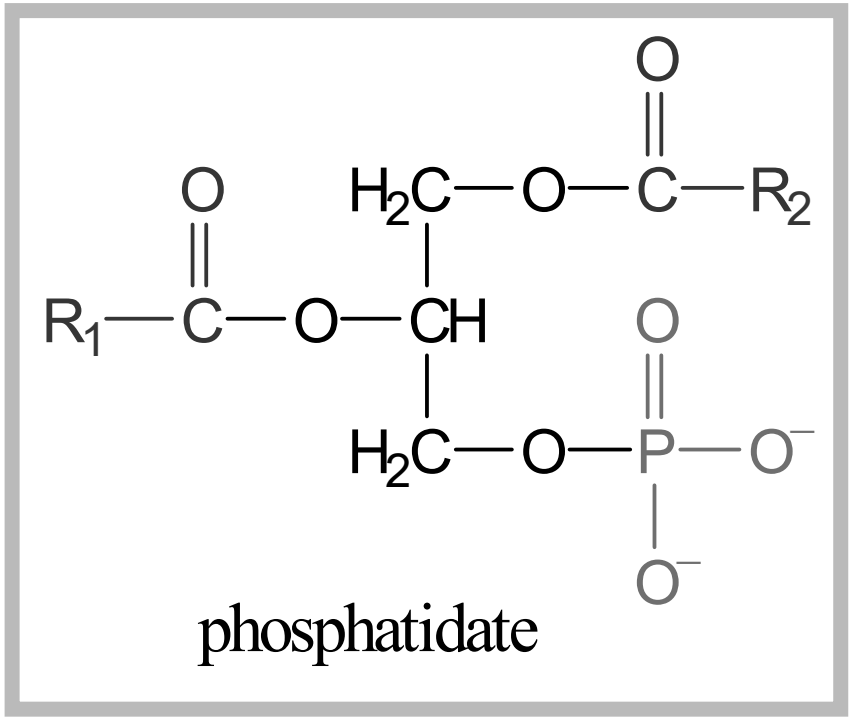
- They are amphipathic molecules containing a polar head & a hydrophobic portion



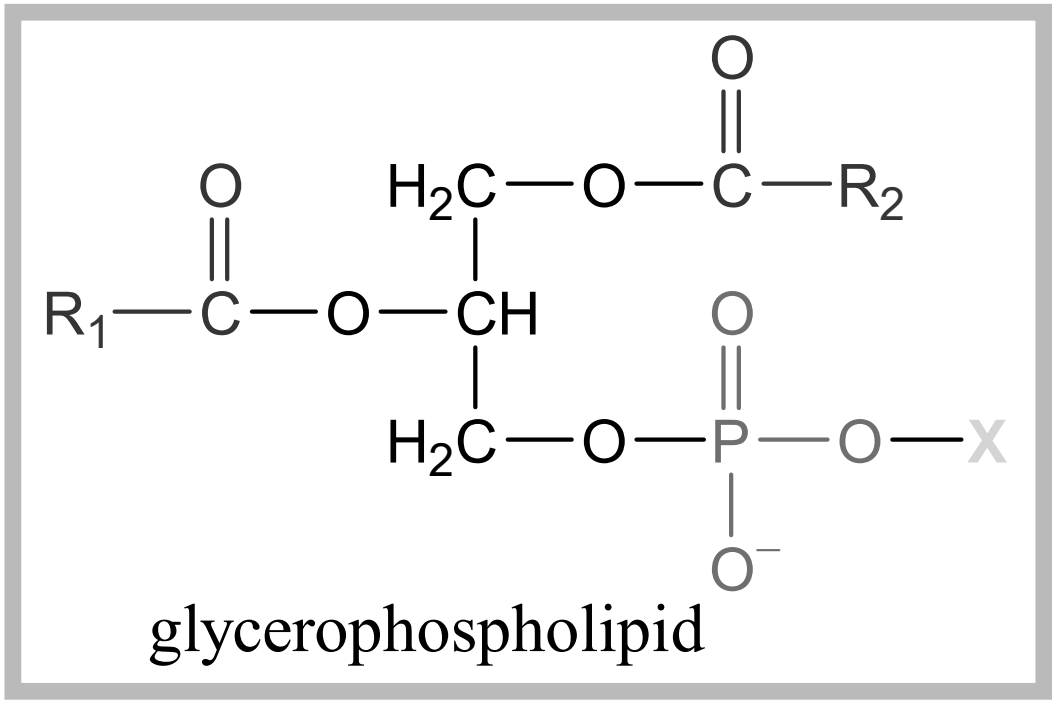
Structure: phospholipids are composed of:

1. **Fatty acids (a saturated and an unsaturated fatty acid).**
2. **Nitrogenous base (choline, serine, threonine, or ethanolamine).**
3. **Phosphoric acid.**
4. **Fatty alcohols (glycerol, inositol or sphingosine).**

- **fatty acids** are esterified to hydroxyls on **C1** & **C2**
- the **C3** hydroxyl is esterified to $\mathbf{P_i}$.



phosphatidate



In most **glycerophospholipids** (phosphoglycerides), **P_i** is in turn esterified to **OH** of a **polar head group** (**X**): e.g., **serine, choline, ethanolamine, glycerol, or inositol**.

- **Classification of Phospholipids are classified into 2 groups according to the type of the alcohol present into two types:**

A-Glycerophospholipids: They are regarded as derivatives of phosphatidic acids that are the simplest type of phospholipids and include:

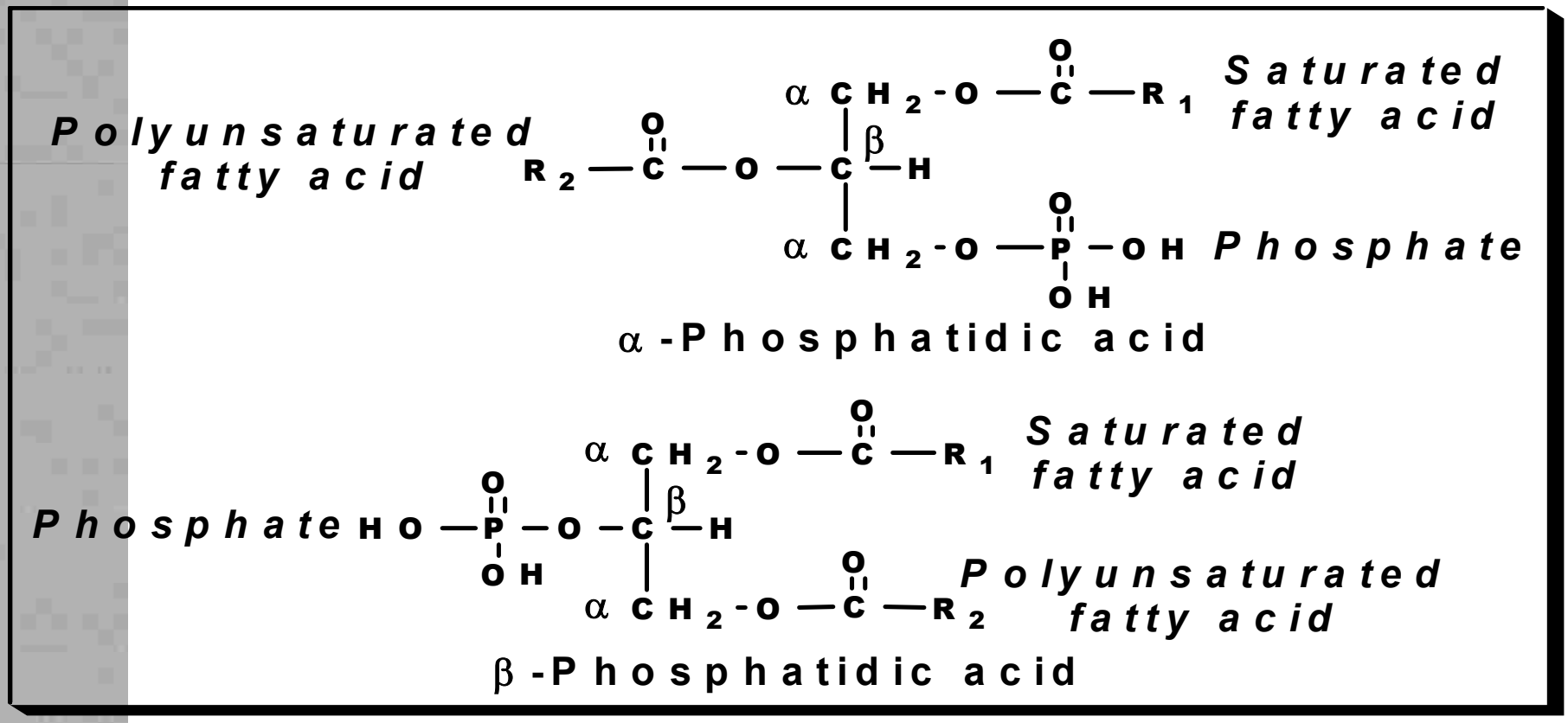
1. **Phosphatidic acids.**
2. **Lecithins**
3. **Cephalins.**
4. **Plasmalogens.**
5. **Inositides.**
6. **Cardiolipin .**
7. **Phosphotidyl serine.**

B-Sphingophospholipids: They contain sphingosine as an alcohol and are named Sphingomyelins.

1-Phosphatidic acids:

They are metabolic intermediates in synthesis of triglycerides and glycerophospholipids in the body and may have function as a second messenger.

They exist in two forms according to the position of the phosphate



Phosphatidic acid:

- Its simplest glycerophospholipid
- Glycerol is esterified by two fatty acids at two carbons and, third carbon carries Phosphoric acid.
- Fatty acid at C2 of glycerol is mostly cis – polyunsaturated ,it tends to curl .This curling prevents tight packing of molecules in membrane ,hence contributes to the membrane flexibility and fluidity.

- **2- Phosphatidyl choline (Lecithins):**

Lecithins are glycerophospholipids that contain choline as a base beside phosphatidic acid.

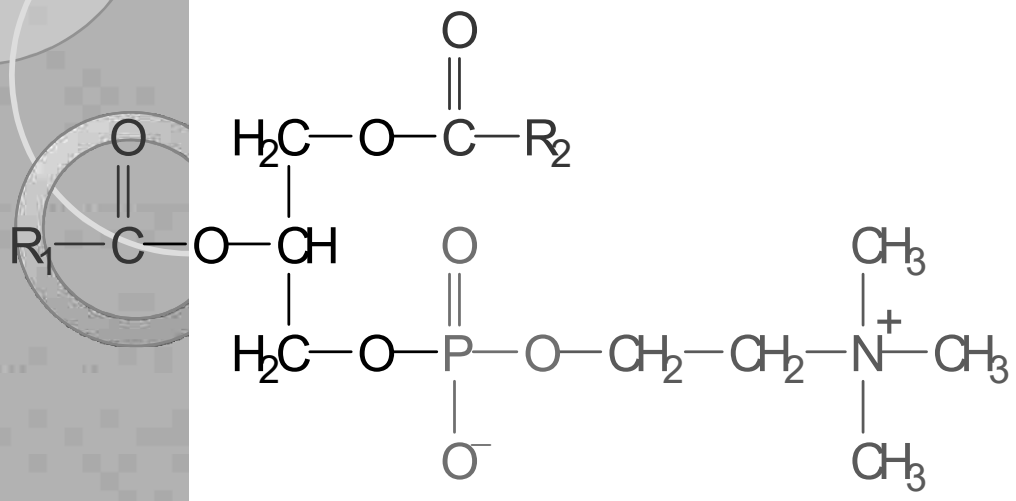
They exist in 2 forms α- and β-lecithins.

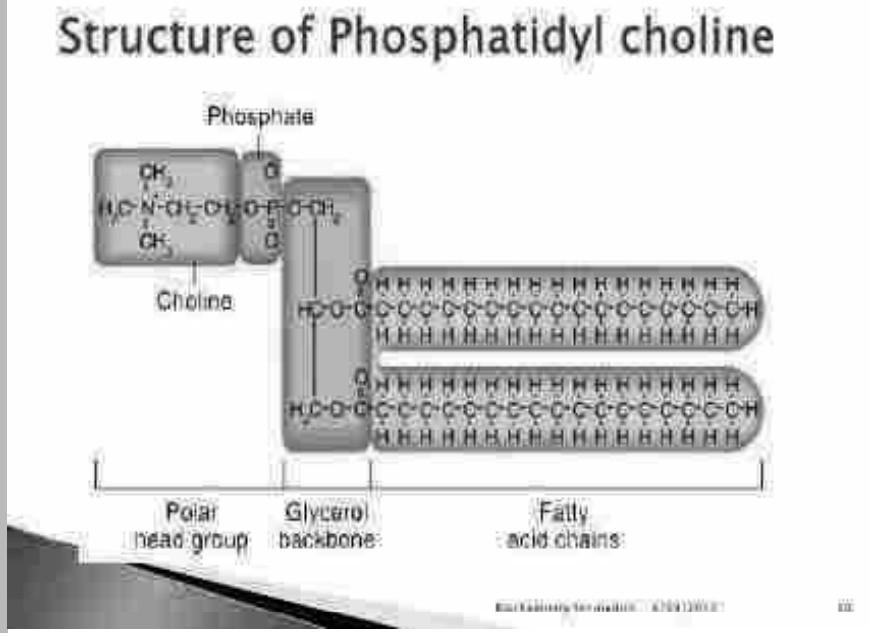
Lecithins are a common cell constituent obtained from brain (α-type), egg yolk (β-type), or liver (both types).

β - carbon usually PUFA molecule.

Most abundant – cell membrane structural function

Lecithins are important in the metabolism of fat by the liver.





Phosphatidylcholine, with choline as polar head group.

Glycerol is connected at C2 or C3 with a polyunsaturated fatty acid, at C1 with a saturated fatty acid, at C3 or C2 by phosphate to which the choline base is connected.

- **Physical properties :**

Waxy ,white substances ,becomes brown when exposed to air

Soluble in ordinary fat solvents except acetone

Decompose when heated

- **Chemical properties :**

Shaken with H2So4 forms phosphatidic acid

Boiled with alkalies , phosphatidic acid further hydrolysed to glycerophosphoric acid & 2 molecules of fatty acids.

Alcoholic hydroxyl group of choline acetylated – acetyl choline is formed.

- **Functions of lecithin**
- **Facilitates combinations with proteins to form lipoproteins of plasma – lipid transport**
- **Constituent of cell membrane**
- **Acetyl choline -** Important in nervous transmission, & as a store of labile methyl groups
- **Esterification of cholesterol**
- **Important lipotropic agent**
- **Acts as lung surfactants.**

Lung surfactant

- Is a complex of dipalmitoyl-lecithin, sphingomyelin and a group of apoproteins called apoprotein A, B, C, and D.
- It is produced by type II alveolar cells and is anchored to the alveolar surface of type II and I cells.
- It lowers alveolar surface tensionand prevents collapse of lung alveoli thus improves gas exchange besides activating macrophages to kill pathogens.
- In premature babies, this surfactant is deficient and they suffer from respiratory distress syndrome.

Acute Pulmonary Respiratory Distress Syndrome (RDS)

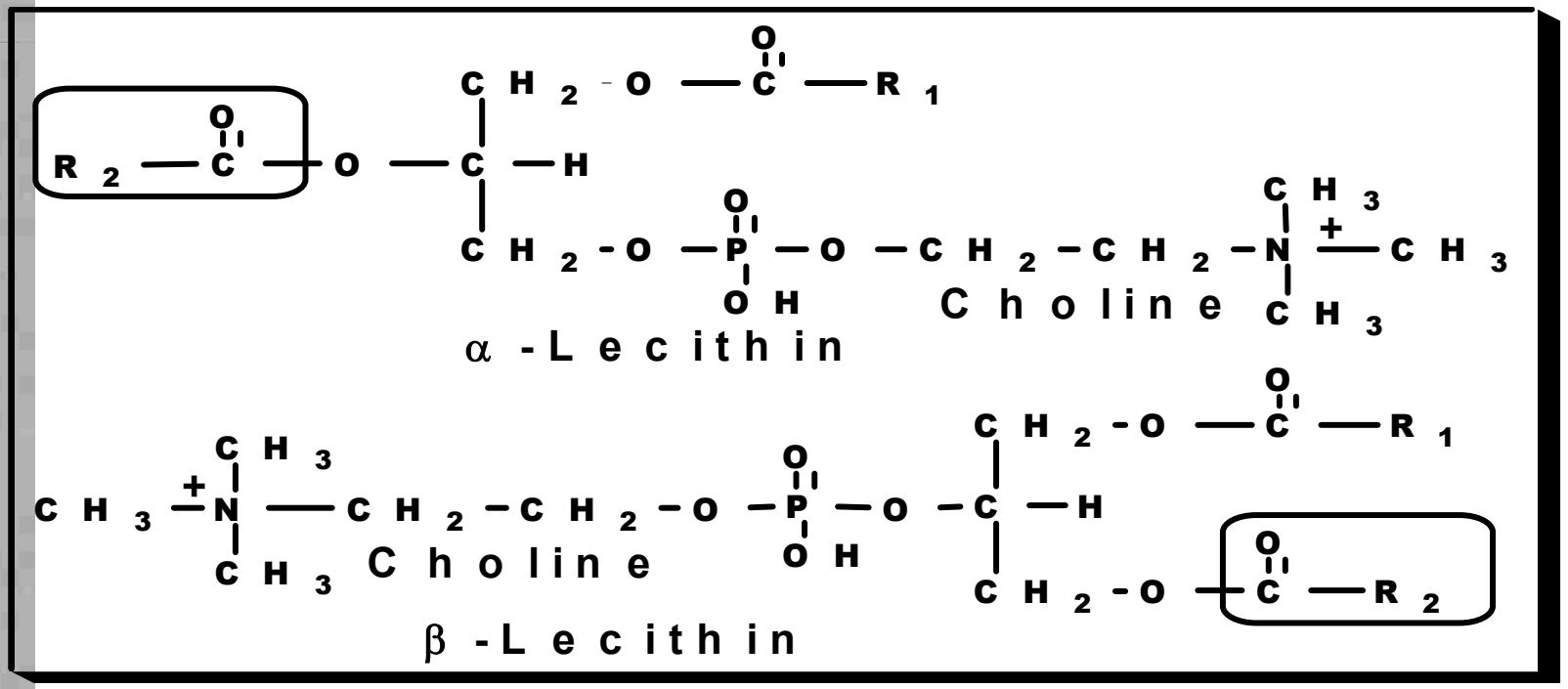
Infants - Insufficient surfactant dipalmitoyl phosphotodylcholine .

Immature infants – not enough type II epithelial cells.

In absence lung tends to collapse – RDS

RDS in adults – Type II epithelial cells are destroyed by immunosuppressive drugs or certain chemotherapeutic agents.

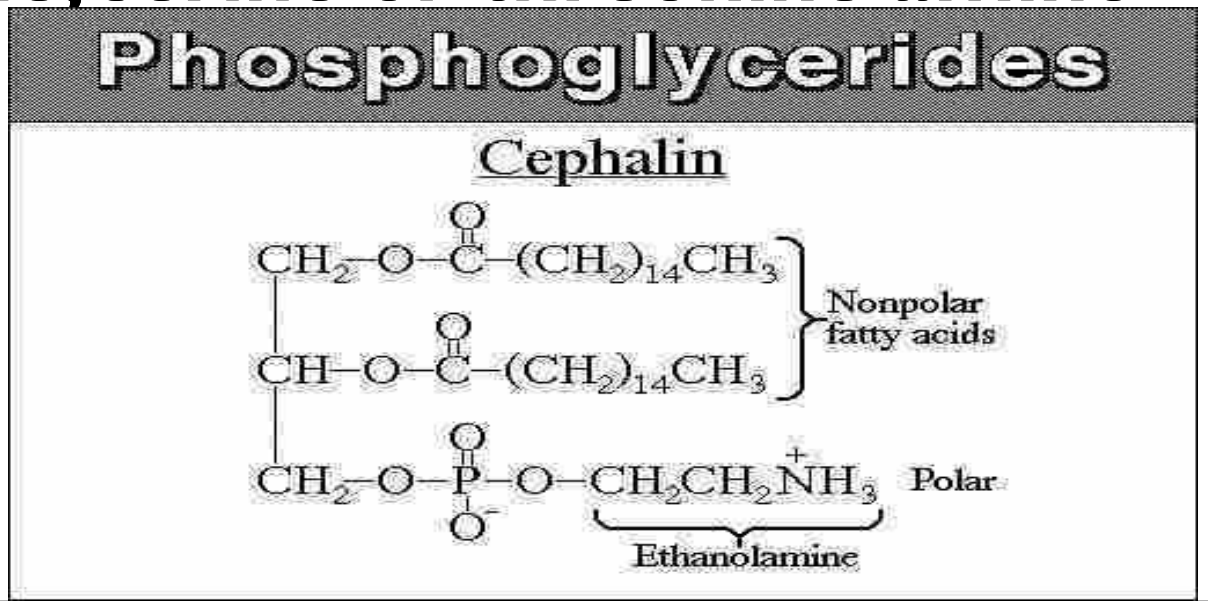
Lysolecithin .It is produced by hydrolysis of phosphoplipid at one of the ester linkage which is hemolytic causes hemolysis of RBCs. Snake venom of number of snakes contains Phospholipases to form lysolecithin. Lysolecithins are intermediates in metabolism of phospholipids.



3-Cephalins (or Kephalins):

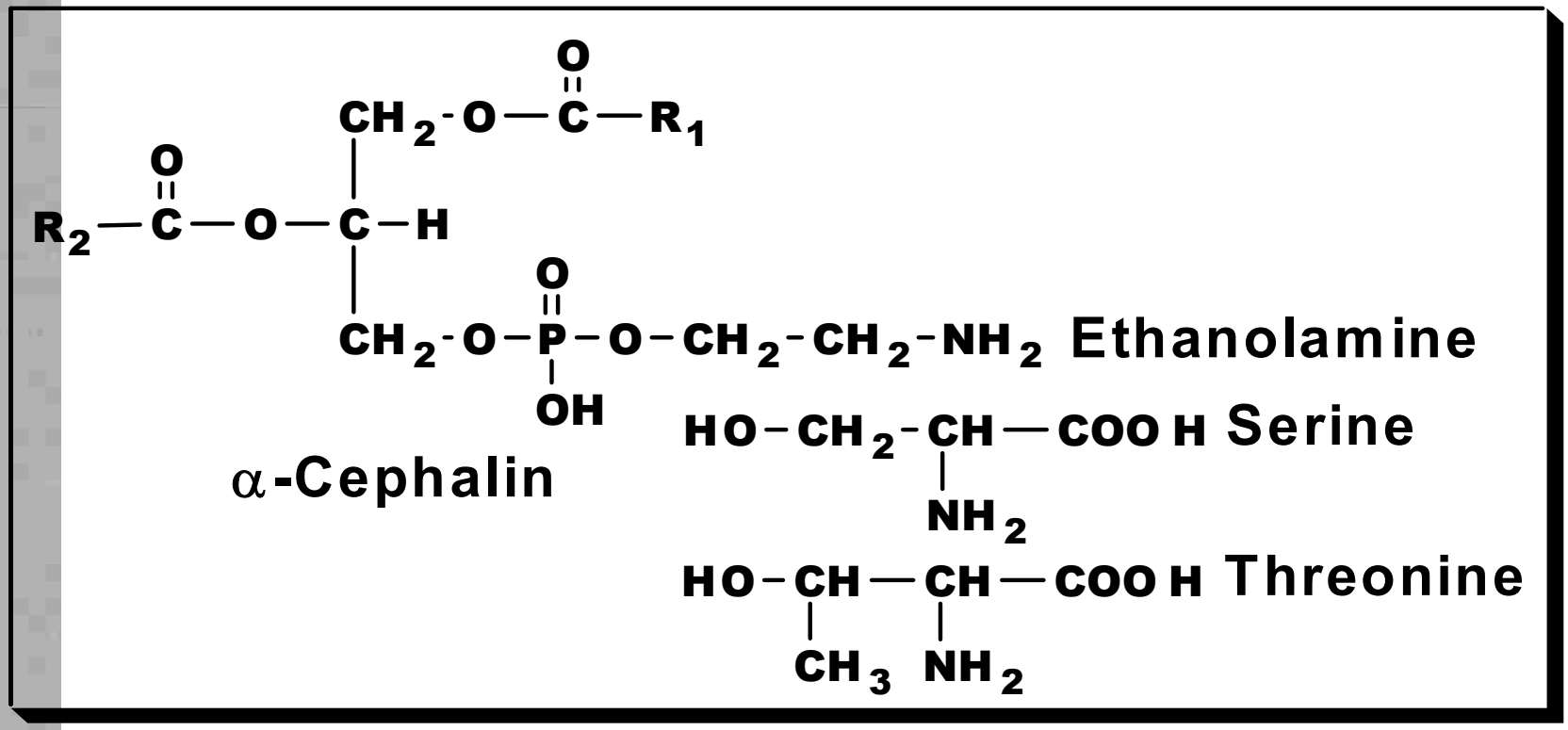
Definition: They are phosphatidyl-ethanolamine or serine. Cephalins occur in association with lecithins in tissues and are isolated from the brain (Kephale = head).

Structure: Cephalins resemble lecithins in structure except that choline is replaced by ethanolamine, serine or threonine amino acids.



- **Certain cephalins are constituents of the complex mixture of phospholipids, cholesterol and fat that constitute the lipid component of the lipoprotein "thromboplastin" which accelerates the clotting of blood by activation of prothrombin to thrombin in presence of calcium ions.**

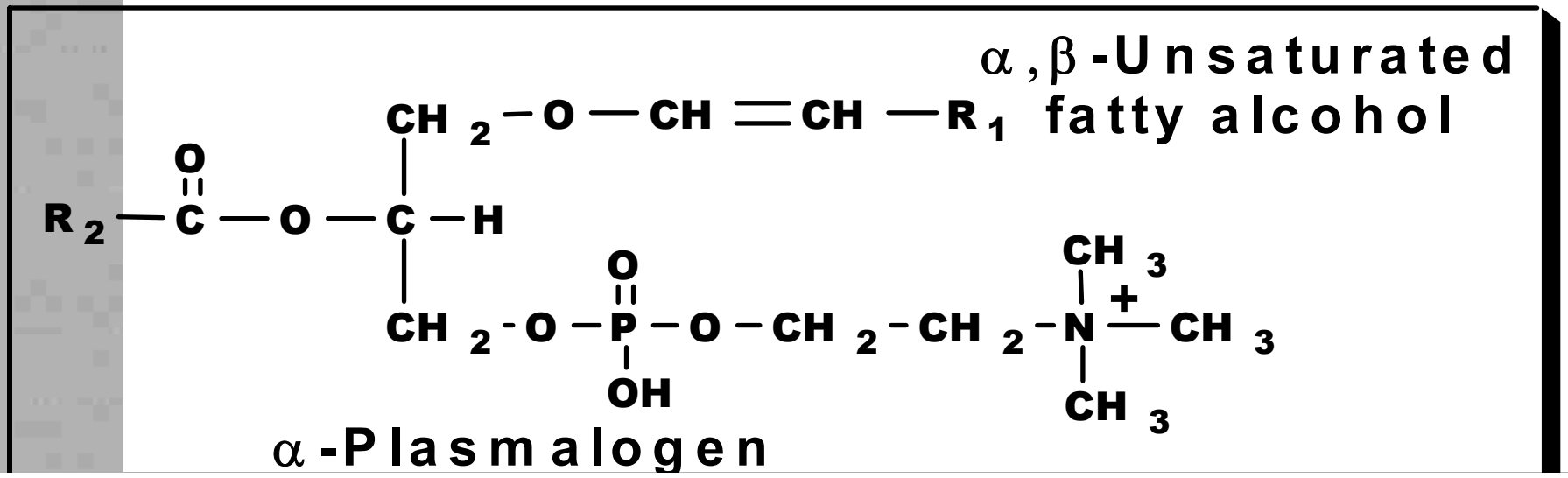
Cephalinase .hydrolyses cephalins to lysocephalins .cephalinase present in snake venoms



4-Plasmalogens:

Structure: Plasmalogens resemble lecithins and cephalins in structure but differ in the presence of α,β-unsaturated fatty alcohol rather than a fatty acid at C1 of the glycerol connected by ether bond.

At C2 there is an unsaturated long-chain fatty acid, however, it may be a very short-chain fatty acid



- Three major classes of plasmalogens
  - Phosphatidalcholines
  - Phosphatidalethanolamines
  - Phosphatidalserines

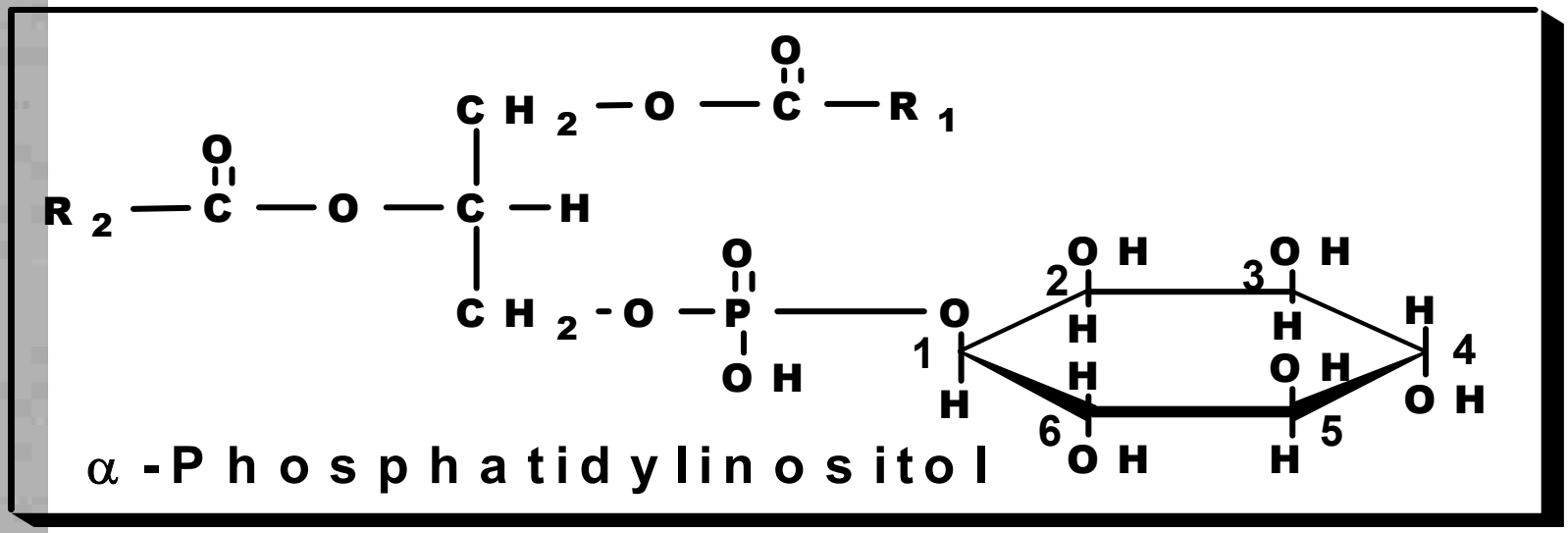
- Found in myelin & in cardiac muscle
- Myelin – large amount of ethanolamine plasmalogen
- Heart muscle - large amount of choline plasmalogen
- Platelet activating factor (PAF) - involved in platelet aggregation and degranulation

5-Inositides:

Definition:

- They are phosphatidyl inositol.

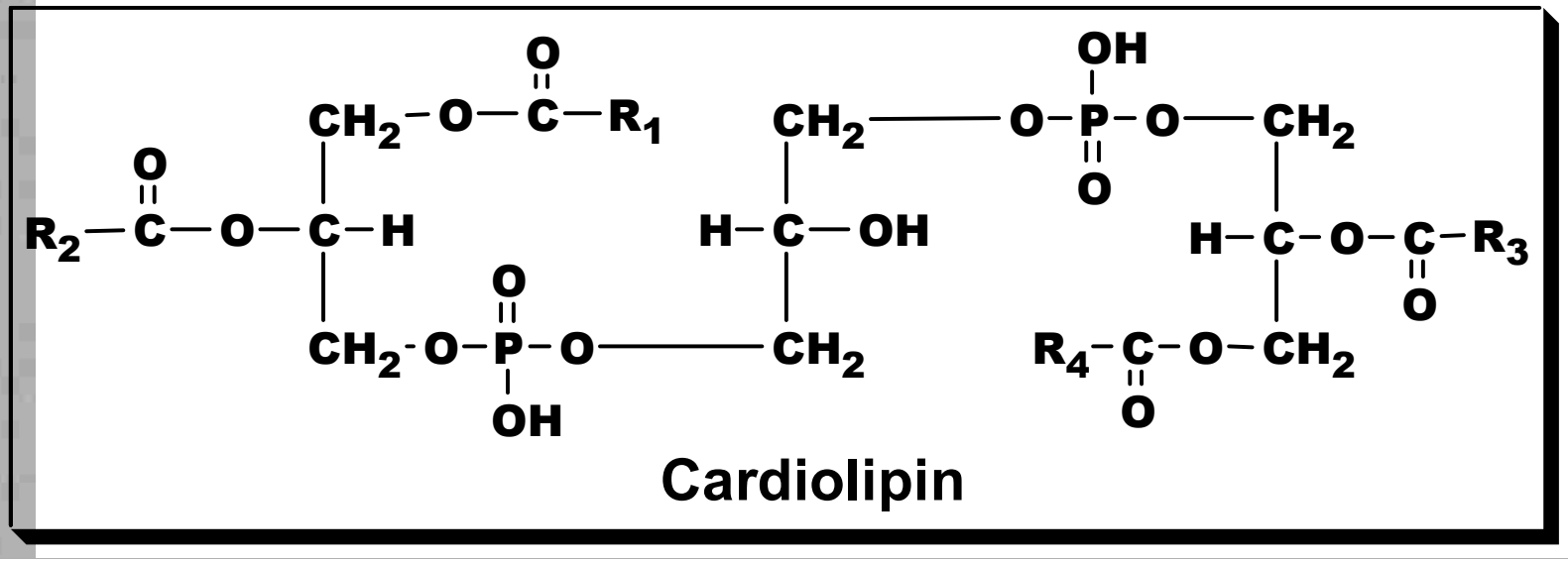
Structure: They are similar to lecithins or cephalins but they have the cyclic sugar alcohol, inositol as the base. They are formed of glycerol, one saturated fatty acid, one unsaturated fatty acid, phosphoric acid and inositol



- Source: **Brain tissues**.
- Function:
- **Phosphatidyl inositol is a major component of cell membrane phospholipids particularly at the inner leaflet of it.**
- **They play a major role** as second messengers during signal transduction for certain hormone..
- **On hydrolysis by phospholipase C,** phosphatidyl-inositol-4,5-diphosphate **produces** diacyl-glycerol and inositol-triphosphate **both act to liberate calcium from its intracellular stores to mediate the hormone effects.**

6-Cardiolipins:

Definition: They are diphosphatidyl-glycerol. They are found in the inner membrane of mitochondria initially isolated from heart muscle (cardio). It is formed of 3 molecules of glycerol, 4 fatty acids and 2 phosphate groups.



Function:

Antigenic - Used in serological diagnosis of autoimmunity diseases

Eg Treponema pallidum, causes syphilis

In mitochondria – optimum function of Electron transport chain

- **B-Sphingophospholipids**

Dervied from alcohol sphingosine

Sphingomyelin – only phospholipid in membrane not derived from glycerol.

Sphingosine – amino alcohol contains long unsaturated hydrocarbon chain.

found in large amounts in brain and nerves and in smaller amounts in lung, spleen, kidney, liver and blood.

Sphingomyelins.

- These contains ;

Sphingosine-long aliphatic amino alcohol

One long chain fatty acid

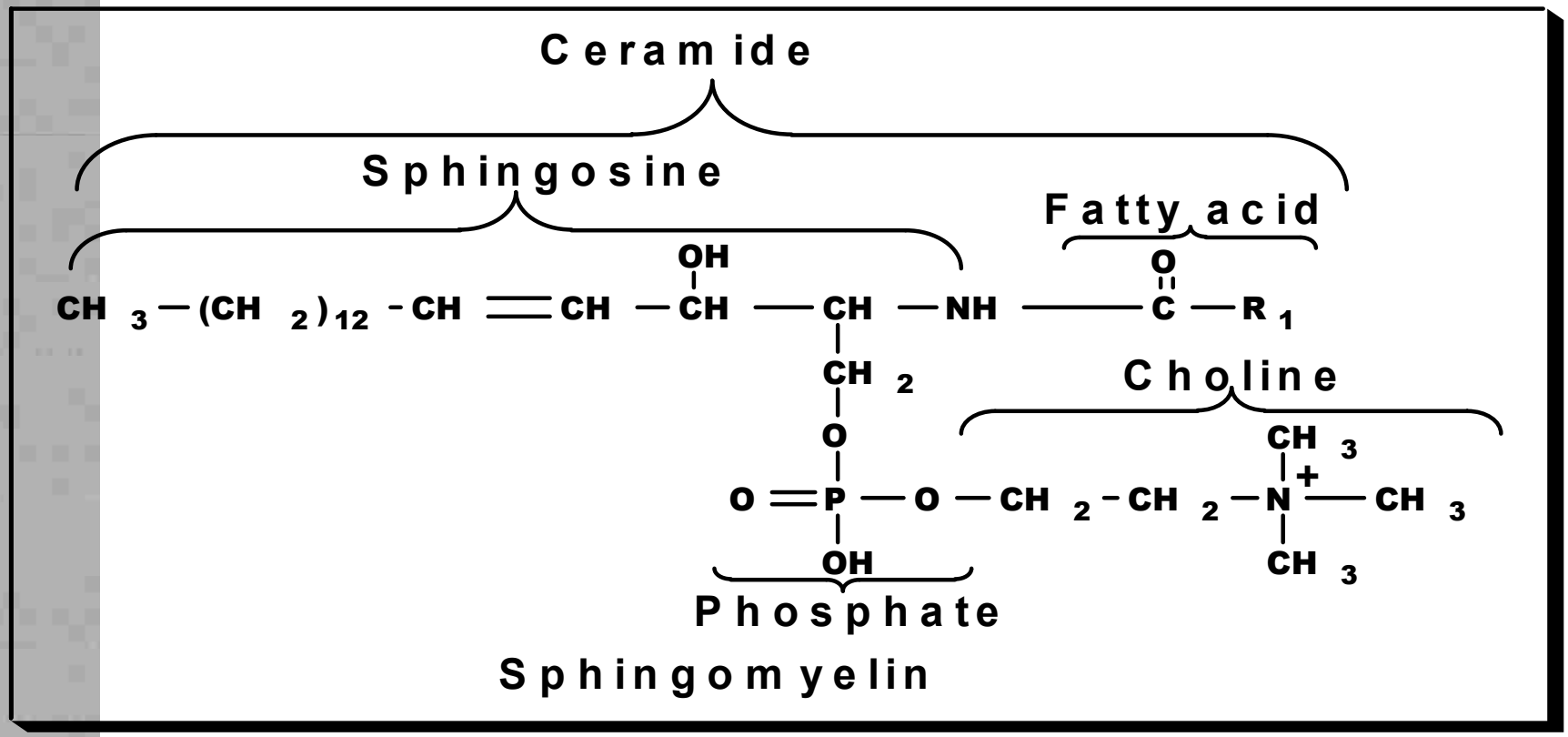
Choline base

Phosphoric acid

- To the amino group of amino alchol sphingosine, one fatty acid is attatched by amide linkage ,this portion is known as ceramide.
- Fatty acids that are present are stearic ,nervonic, lingoceric in brain and nerves

Ceramide **This part of sphingomyelin in which the amino group of sphingosine is attached to the fatty acid by an amide linkage.**

Ceramides have been found in the free state in the spleen, liver and red cells.



Functions of sphingomyelin

- Principal structural lipids of membranes of nerve tissues.
- Sphinomyelin of myelin sheath – contains longer chain fatty acids such as lignoceric and nervonic acids.
- Grey matter of brain – sphingomyelin that contains stearic acid

Niemann Picks Disease

- Rare heriditary fatal diseasae with neuropathic disorders & hepatospleenomegaly.
- Due to abnormal accumulation of SPHINGOMYELINS in liver, spleen and brain.
- Heriditary deficiency of Sphingomyelinase
- Turnover of sphingomyelins is disturbed as they can not be metabolised
- Clinically patient is presented with enlarged liver ,spleen, abdomen,childdren show priogressive mental retardation, majority of them die within 2 years.

Lecithin Sphingomyelin Ratio(L/S)

- L/S Ratio in amniotic fluid is used for the evaluation of fetal lung maturity
- Prior to 34 weeks gestation, lecithin and sphingomyelin concentrations are equal but afterwards there is marked increase in Lecithin concentration.
- A L/S ratio of> 2 or > 5 indicates adequate fetal lung maturity
- Delivery of a premature,low birth weight baby with low L/S ratio (1 or< 1) predisposes the child to respiratory distress syndrome

2-Glycolipids

- They are lipids that contain carbohydrate residues with sphingosine as the alcohol and a very long-chain fatty acid (24 carbon series).
- They are present in cerebral tissue, therefore are called cerebrosides

- **Classification: According to the number and nature of the carbohydrate residue(s) present in the glycolipids the following are**

Cerebrosides. They have one galactose molecule (galactosides).

Sulfatides. They are cerebrosides with sulfate on the sugar (sulfated cerebrosides).

Gangliosides. They have several sugar and sugar amine residues.

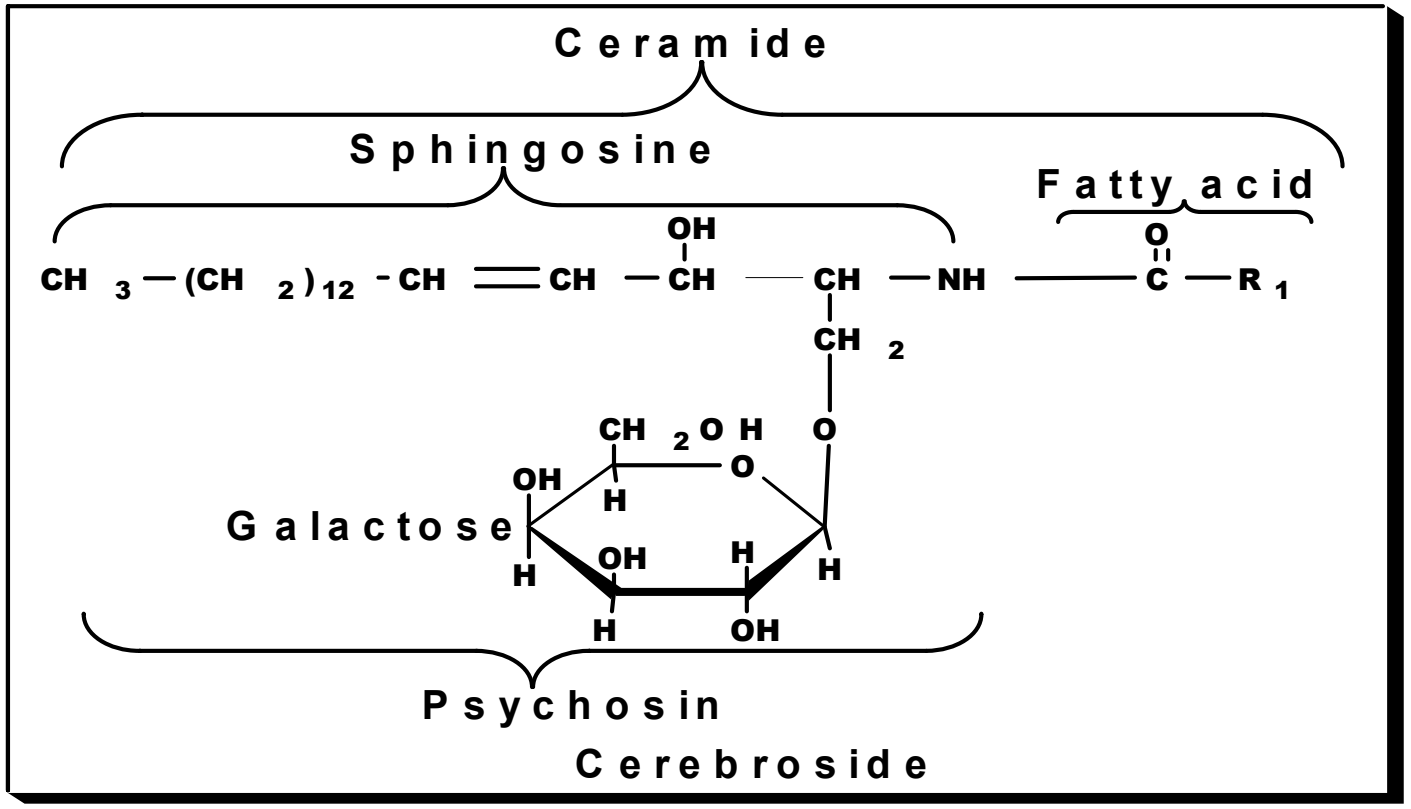
- **1-Cerebrosides:**

Simplest glycolipid – one sugar residue either glucose or galactose linked to ceramide , named glucocerebroside & galactocerebroside repectively.

They occur in myelin sheath of nerves and white matter of the brain tissues and cellular membranes. They are important for nerve conductance

They are non saponifiable,

Structure: They contain sugar, usually β-galactose and may be glucose or lactose, sphingosine and fatty acid, but no phosphoric acid.



Types of cerebrosides

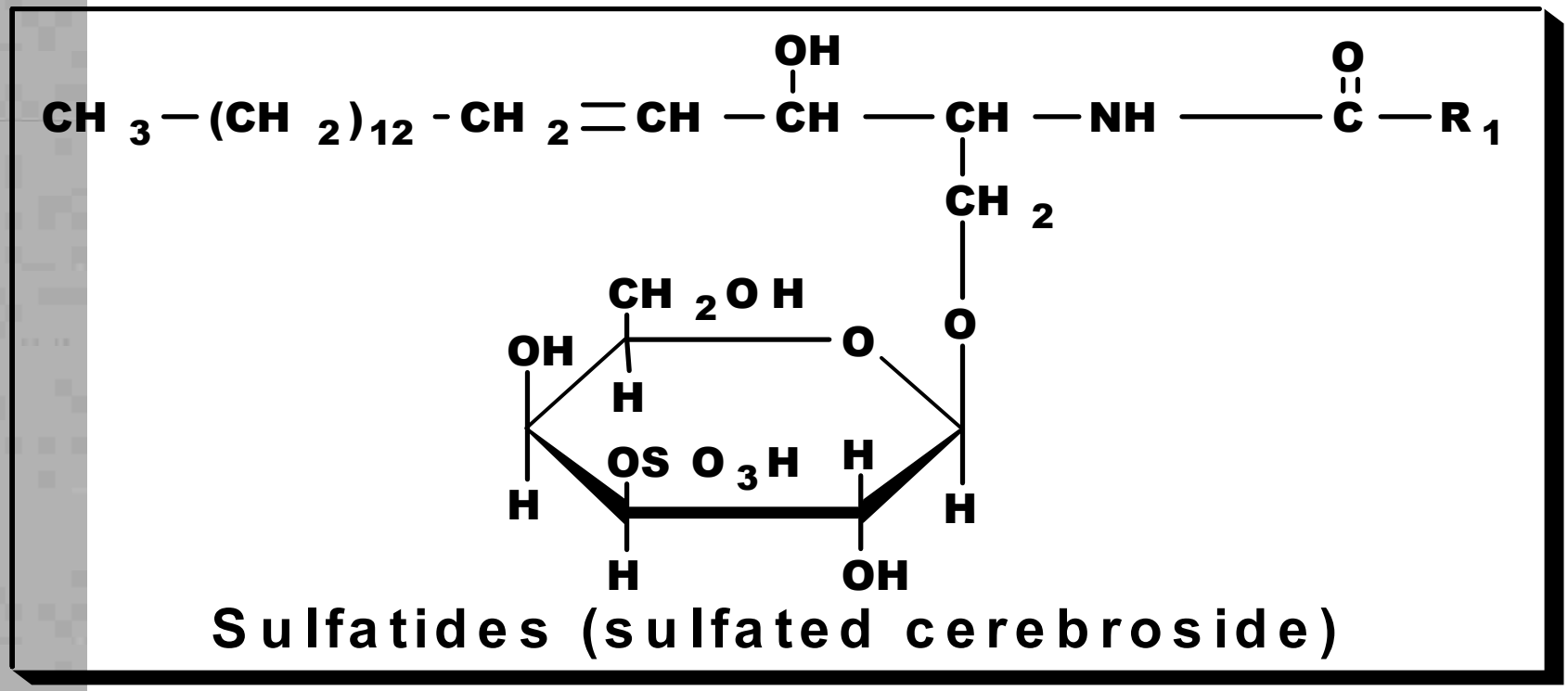
Cerebrosides are differentiated on the basis of kind of fatty acid content in the molecule.

Four types are commonly observed.

- Kerasin – contains Lignoceric acid
- Cerebron –contains cerebronic acid
- Nervon – contains nervonic acid
- Oxynervon – contains hydroxy derivatives of nervonic acid+

2-Sulfatides:

- **They are sulfate esters of kerasin in which the sulfate group is usually attached to the –OH group of C3 or C6 of galactose.**
- **Sulfatides are usually present in the brain, liver, muscles and testes.**



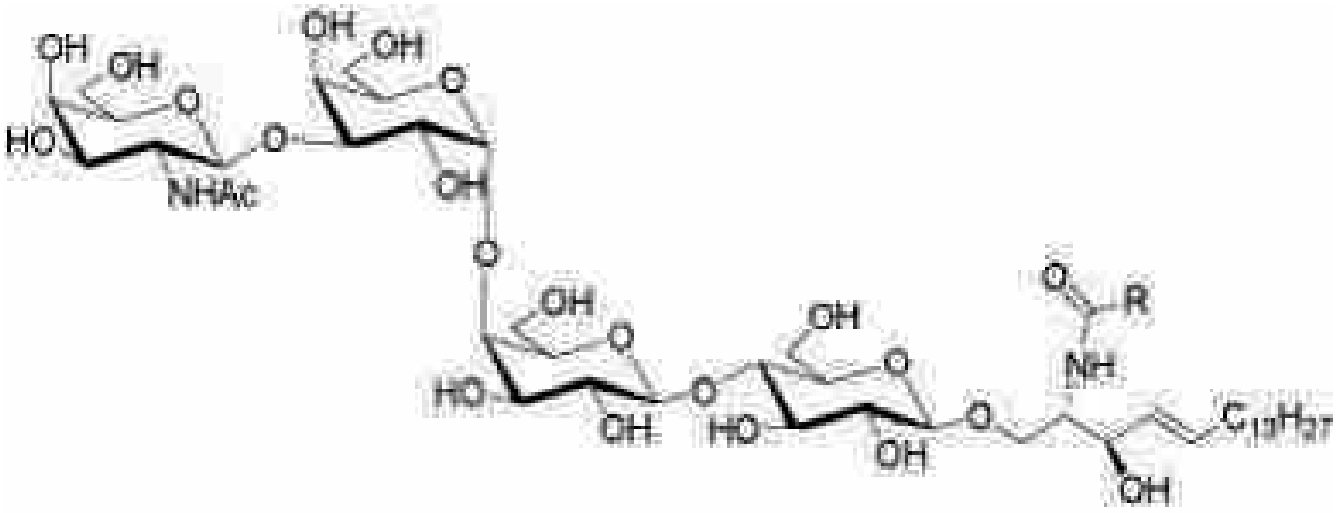
Sulfatides (sulfated cerebroside)

- **3 Globosides:**

Contains 2 or more sugar molecules.

Most often galactose, glucose,or N-acetyl galactosamine attached to ceramide.

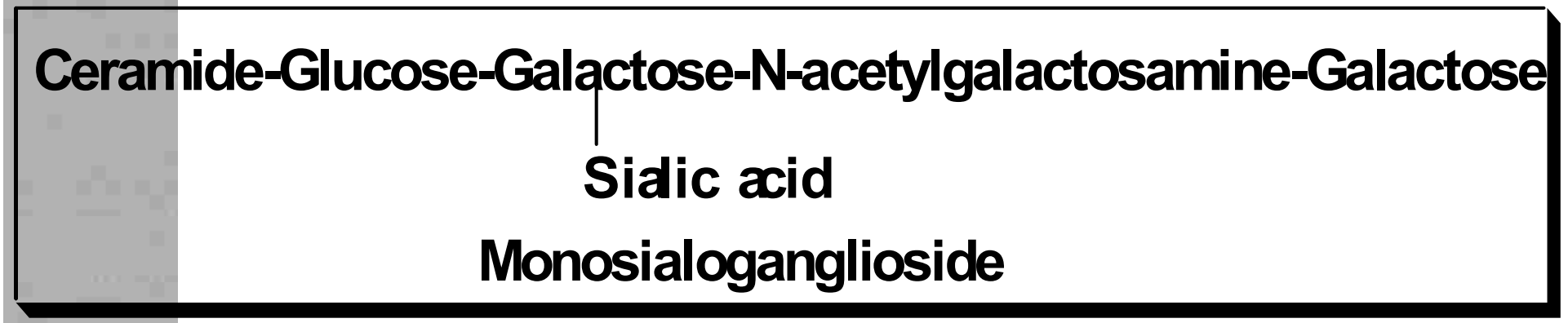
Important constituents of RBC membrane & are the determinants of the A, B, O blood group system.



• 4 Gangliosides:

They are more complex glycolipids,derived from glucocerebroside.

Gangliosides contain sialic acid (N-acetylneuraminic acid) (NANA), ceramide (sphingosine + fatty acid of 18-24 carbon atom length), 3 molecules of hexoses (1 glucose + 2 galactose) and hexosamine.

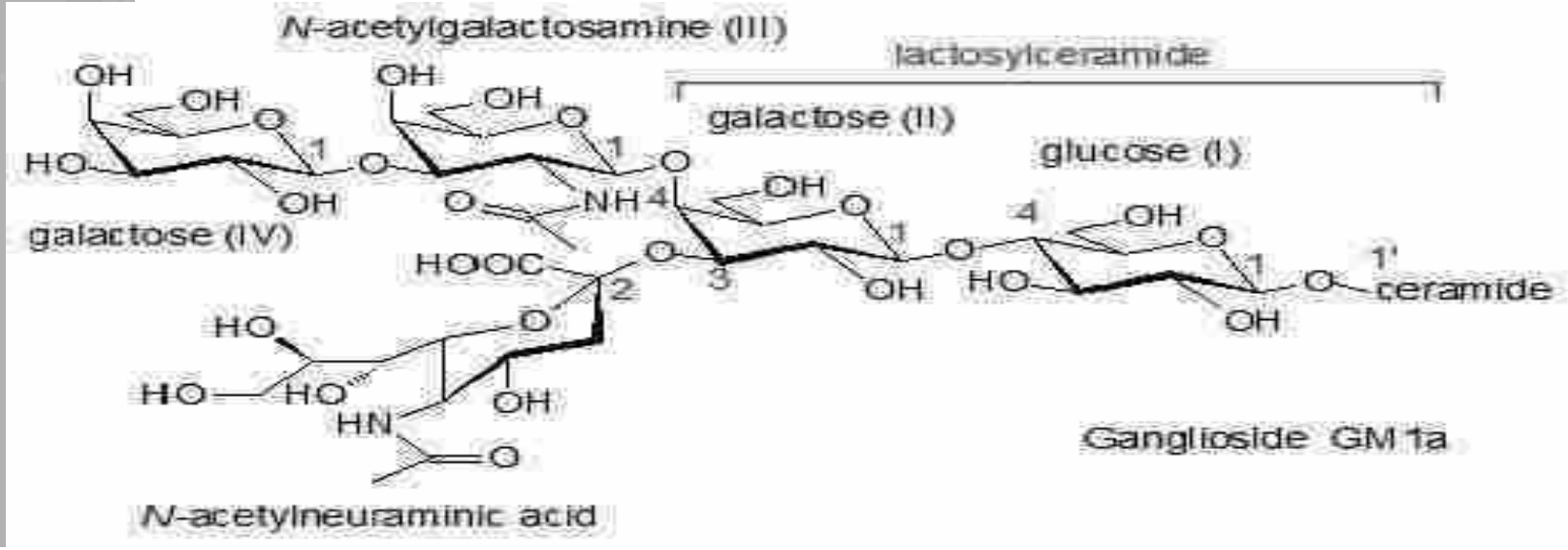


- Involved in special roles in cell membrane receptors for certain polypeptide hormones, for number of drugs and viruses.

Nomenclature & Classification

G based on number of NANA residues present in oligosaccharides.

The subscript M (mono), D (di), T (tri) indicate presence of residues of NANA.



Brain gangliosides contains upto 4 silaic acid residues.

They are GM, GD, GT and GQ.

Important types of GM are :-

GM1, GM2 and GM3

GM3- simplest found in tissues (ceramide+ glu+gal+NANA)

GM1- complex known to be receptor in human intestine for cholera toxin.

Functions.

- Important constituents of nervous tissue, brain & outer leaflet of cell membrane.
- They act as cell surface receptors for various hormones and growth factors
- Play important role in cellular interactions, growth & development.
- They are source of blood group antigen & various embryonic antigens.
- GM1 acts as receptor for cholera toxin in human intestine

Gaucher's disease

- Rare inherited lipid storge disease.
- Deficient enzyme is gluco cerebrosidase
- Cerebrosides gets accumulated in reticoendothelial cells,spleen,liver, bonemarrow brain
- Progressive mental retardation, hepatosplenomegaly,

Lipid storage diseases(Sphingolipidosis)

Disease	Enzyme deficiency	Nature of lipid accumulated	Clinical Symptoms
Tay Szch's Disease	Hexosaminidase A	G_{M2} Ganglioside	Mental retardation, blindness, muscular weakness
Fabry's disease	α-Galactosidase	Globotriaosylceramide	Skin rash, kidney failure (full symptoms only in males; X-linked recessive).
Metachromatic leukodystrophy	Arylsulfatase A	Sulfogalactosylceramide	Mental retardation and Psychologic disturbances in adults, demyelination.

Lipid storage diseases(Sphingolipidosis)- contd.

Disease	Enzyme deficiency	Nature of lipid accumulated	Clinical symptoms
Krabbe's disease	β-Galactosidase	Galactosylceramide	Mental retardation, myelin almost absent.
Gaucher's disease	β -Glycosidase	Glucosyl ceramide	Enlarged liver and spleen, erosion of long bones, mental retardation in infants.
Niemann-Pick disease	Sphingomyelinase	Sphingomyelin	Enlarged liver and spleen, mental retardation, fatal in early life.
Farber's disease	Ceramidase	Ceramide	Hoarseness, dermatitis, skeletal deformation, mental retardation, fatal in early life.

INTRODUCTION & LEGAL PROCEDURES

)

Introduction

Forensic Medicine

Forensis = of forum

Forensic = of courts of law

- Medical aspects of law
- Legal medicine / State medicine

Medical Jurisprudence

Juris = Law ; Prudentia = knowledge

- Legal aspects of medicine

Toxicology

Forensic Medicine - History

4000 BC - **Law medicine problems** - records of India, China, Egypt, Rome,Babylon

3250 BC - **Manu smrihiti** - murder & sex off.

3000 BC - **Vedas** - murder & other offences
Marriage - sister / before puberty x

3000 BC - Chinese **materia medica** - poisons

3000 BC - **Papyrus** of Egypt – sex perversions, marriage, poisoning

2200 BC - Code of **Hammurabi** (Babylon)

7th C BC - Agnivesa **charak samhita** - poisons, privileges of physician

Forensic Med. - History - (Contd..)

572 BC - **Lex Aquilia** of Rome

- Lethality of wounds, incapacity of insane, pregnancy - 300 days

5 - 4th C BC - **Hippocrates** - oath

4th C BC - **Aristotle** - Abortion

4 - 3rd C BC - **Arthshashtra** of kautilaya

- med. neg, murder, sex off, poisons,

4 - 3rd C BC - **Plini** of Rome - superfoetation, suspended animation, death, suicide

200 BC - China - Hashish used in surgery

Forensic Med. - History - (Contd..)

2 - 300 AD - **Sushruta samhita** - FMT, sexual offences, poisoning

13th C AD - China - limited (external) autopsy

1302 - **Bartolomeo De varignana** - 1st autopsy at Bologna (Italy)

1482 - 1st M.L. Journal - Germany

16th C - Penal code - Bishop of Bamberg & Caroline Code - Med. Testimony essential

Forensic Med. - History - (Contd..)

16th C - Autopsy - routine

1602 - 1st book - Fortunato Fedele (Italy)

17th C - Coroner's system - USA

1621-1666 - Paulus Zacchias - 9 vol. M.L. Ques.

1647 - Autopsy - USA

1663 - 1st autopsy, India, Madras - Dr Buckley

1787-1853 - **Orfila** (Germany) - poison detection

1788 - Samuel Farr (Britain) - book

1792 - Andrew Duncan (Britain) - book

Forensic Med. - History - (Contd..)

1823 - TR Beck & JB Beck (USA) - book

1843 - Mc Naghten rule - England

1858 - Sir William Herschel - India-finger prints

1860 - IPC framed

1861 - Cr PC framed

1861 - Police system of investigation - India

1865 - Alfred Swaine Taylor - book

1871 - Coroner - Bombay, Calcutta

1872 - IEA

1877-1947 - Sir Bernard Spilsbury - 26000 autopsies

Forensic Medi. - History - (Contd..)

1883-1969	-	Sir Sydney Smith - book
1920	**-**	**JP Modi** - book
1933	**-**	**MCI** established
1961	**-**	**IAFSc**
1971	**-**	**Instt of For Sc & Criminology**
1972	**-**	**I A F M**
1977	**-**	**1st Medicolegal Institute** - Bhopal
1988	-	Coroner inquest stopped - Calcutta
1995	-	Centre for DNA – FP - Hyderabad
1999	-	Coroner inquest stopped – B'by

Forensic Med. - History - (Contd..)

- **Foreign authors** - Gresham, Gordon, Gradwohl, Gee, Turner, Tedeschi, Taylor, Shapiro, Sydney Smith, Simpson, Mant, Mason, Knight, Krogman, Ludwig, London, Polson, Rosner, Beck, Duncan, Farr & Zacchias
- **Indian authors** - JP Modi, KSN Reddy, A Nandi, JB Mukherjee, CC Mullick, CK Parikh, Nagesh K.G. Rao, VVPillai, K Vij, Guha, Karmarkar, Basu, Subramanyam, Dixit, Dogra, RK Sharma, AC Mohanty & S.K. Singhal

Courts

Definitions

- Court
- Witness
- Evidence
- Summon / subpoena
- Warrant
- Cognisable offence
- Compoundable offence / non
- FIR
- Bail
- Charge sheet
- Summons case
- Warrant case
- I PC 1860
- Cr PC 1861
- I EA 1872
- CPC 1908

Courts - (Contd..)

Courts - Civil, Criminal, Consumer, Coroner

Punishments (53 IPC)

Death, Life imprisonment,

Imprisonment - simple, rigorous, solitary

Fine, Reformatories

Criminal courts - Supreme, High, Distt sessions, Asstt. Sessions, Special, Fast track

Magistrate - Executive, Judicial, Special, Juvenile

Jud. Mag - Cl I - CJM/CMM, SDM, JMFC/MM

- Cl II JM

Courts & their powers - (Contd..)

Powers (29 Cr. P.C.)

- S.C. / H.C. - Any sentence
- DJ. - D.S. to be confirmed (366 Cr PC)
- Asstt DJ – X D.S., Life, > 10 yr.
- CJM / CMM - upto 7 yr, any fine
- SDM / JMFC / MM - upto 3 yr, Rs. 10000
- Cl II JM - upto 1 yr, Rs. 5000
- Magistrate - Power x 2 (> 2 crimes)

Trial of criminal case

Witness

- Gives sworn evidence / testimony before court

1. Simple / ordinary / common
    - Direct / Ist hand knowledge rule
2. Expert / skilled
    - M.O., CA, Finger Print, Writing, Ballistic
        - Opinion & inferences - own / other's obs
        - G.K. /Hypothetical ques on his speciality
        - Can volunteer a statement
    - MO – State facts, ev. not conclusive
        - Simple & expert witness
3. Hostile / adverse / unfavourable (191 IPC)

S.K.S Dhule

Procedure of court

I. Summon
   - Definition (174 IPC & 87 Cr PC)
   - Call books, records / report (91& 349 Cr PC)
   - Subpoena ad testificandum / S. duces tecum
   - Penalty (172-174 IPC, 350 Cr PC)
   - Conduct money (bhatta) – contempt of court
   - Preference

II. Oath / solemn affirmation
   - In name of books / God
   - If religious scruples - solemn affirmation
   - Perjury (193 IPC) - false / doesn't know / doesn't believe to be true

Procedure of court - (Contd..)

III. Recording of Evidence

1. Examination in chief
   - Lawyer of party / DGP /PP
   - To place facts, obs. & opinions
   - No leading questions (141 IEA)
     (If hostile - permitted)
2. Cross examination
   - Opposing / defence counsel
   - To - establish truth
     - extract favourable facts

Recording of evidence

- weaken evidence
- remove stress

2. Cross exam. (Contd..)
   - Most important to establish truth
   - Leading questions permitted
   - No time limit
   - Can challenge expert (152 IEA)
   - Double edged sword

Recording of evidence

3. Re examination
    - Like examination in chief
    - To clear discrepancies / doubts
    - No leading questions, no new matter
    - If new matter – recross
4. Court questions

- Conclusion & discharge
- Can be recalled (311 Cr PC)

Guidelines for medical evidence

- Obey summon, refresh memory, carry records, well dressed, discuss with PP only, not kept waiting, can consult, polite but firm, can request repetition, avoid technicalities, no irritation, avoid approximate, no secrecy, X yes / no, admit ignorance, when shown book, may not apply here, opinion – on observation, stick to report - scientific - non partisan - unbiased, no crossing limits, no quoting, can volunteer, leave after permission.

Inquest

In = in, quasitus = to seek

Judicial / legal enquiry into cause of unnatural death

- If death is natural – body is handed over
- If death is unnatural – enquiry is a must

Examples of unnatural deaths

Types

Medical examiner system (USA, Canada, Japan)

- Carried by medical people at scene
- No judicial powers
- Best inquest in the world

Coroner's inquest

Coroner's inquest (UK, USA, Canada, Aus, Japan, Hong Kong, NZ, S Lanka) (Repealed July 26, 1999)

- Coroner appointed by state
- Is advocate or MM with 5 year exp.
- Rank of class I Judicial Mag.
- Only duty is inquest
- Working limits – city of mumbai

Duties, functions and powers

- Only duty is to carry out inquest
- Views the body in mortuary – death natural or unnatural

Coroner's inquest

- If natural – hands over the body to relatives
- If unnatural – requests the police surgeon to do PM
- In case buried – can order exhumation
- Can summon the witnesses and record evidence
- Can grant conduct money
- Can issue warrant
- Can not impose punishment
- Can appoint a deputy

Coroner's inquest

Court – of enquiry, accused X, Jury, no punishment, for contempt - yes

Procedure

- Coroner's verdict / judgement
- If accused arrested – records to MM
- If perpetrator not arrested – issues warrant
- If accused is not known – open verdict
- Copy to commissioner of police
- Trial by mm

Police inquest (sec. 174 Cr. P.C.)

- Not below HC , known as I.O.
- Commonest inquest in india

Procedure

- On information of dead - notes in DD, informs magistrate & proceeds to site
- With two panchas prepares panchnama (if female, additional female panch)

Panchnama includes -

Police inquest

- Identity – informant, identifier, panch, dead, who last saw him alive
- Place, position, surroundings of dead
- Item near body, clothes & ornaments, condition & pocket contents
- Body & skin, injuries & fractures, natural orifices
- Burnt / buried – whose orders ?
- As per IO & panchas - cause, time, manner of death
- Reason for P.M., signatures of IO & panchas.

Police inquest

- Cause of death established and no foul play – body handed over
- If no cause of death – P.M. / SPOT P.M.
- Records the statements
- Considering statements, PM & complaints-? Crime
- If yes – apprehends criminal, prepares charge sheet, sends to magistrate
- Sec. 202 IPC & 177 IPC

Magistrate's inquest (Sec 176 Cr. P.C.)

By executive magistrate, DM, SDM

Indications

- Police officer suspect
- Gross fallacies
- Buried dead
- Dowry death
- Govt. orders

Coroner's vs magistrate's court

• Only in mumbai	In all dist. & talukas
• Cl I JM	CJM / CMM, JMFC / JMM, Cl II JM
• Only duty inquest	Main duty – trial
• Assisted by jury	No jury
• Court of enquiry	Enquiry & trial
• Accused need not be present	To be
• Can't award punishment	Can
• Contempt of court if in premises	Inside or outside

Coroner's vs police inquest

• By coroner	By PO
• Cl I JM	HC or above
• Only in mumbai	Commonest in india
• In his court	Where body lying
• Can't arrest	Can himself arrest
• Does not inform	To inform ex. mag.
• Can order exhumation	Cannot
• Can issue summons	Cannot
• Records evidence	Cannot

Coroner's vs police inquest

• Can sanction conduct money	Cannot
• Can issue warrant	Cannot
• Can send viscera	Cannot
• Assisted by jury – more reliable	Panchas
• Does not send all for PM	Usually all
• Police surgeon report better	Any MO
• Better judge cause of death	Not
• Best inquest in india	Poor

Medical evidence

- Documents / statements on oath

Types - Written / Documentary

- Medical Certificates
- M.L. reports
- Dying declaration
- Dying deposition (X)

- Oral / parole - direct , indirect
- Circumstantial

Medical certificates

- Issued at patient / relative's request
    - issued by RMP
    - carry identity of patient
    - on observations – false – punishable 197 IPC
    - x blank signed – prescription & certificates
    - retain copy
    - should be complete & detailed
- Sickness, fitness, vaccination, disability, birth

Death certificate

- By RMP - attended death
- Brodrick committee – if attended within 7 days
- 2 Certificates if organ transplantation

Precautions -

- Ensure dead (x apparent death)
- Ensure death - natural
- If natural - Issue, x delay / fee, cause +
- Never without observation, at request
- X Counter signature
- Death in 24 hrs / OT / drugs - MLC

Contents - identity, hospital, Dr., time, place, cause

Imp. – Regd., cremation, claims, property, will

M. L. Reports

- On request of I.O. / Court, eg. Age, inj, poison, alc, burns, rape, impotence, insanity, PM
- Is evidence after Dr. testifies

Precautions

- By RMP
- Based on observations
- Prepared during exam / immediately after
- No adjectives used
- 3 parts - Introduction, observations, opinion

- Item - examined, described, marked, sealed, returned under receipt.

Dying declaration

- Written / verbal statement by one likely to die
- Ideally by Executive magistrate – dowry – or Dr
- Not necessary - sure to die
- M.O. - declares compos mentis
- Encourage pt to write, video, oral
- No relatives / P.O. / oath / leading
- 2 witnesses, ques -ans, vernacular
- At end-exam.pt, readover, place time, signed, sent
- Circumstances - can't write / speak, language, dies
- Importance - 32 & 157 IEA, if > 1

Dying deposition

- Is the deposition by one likely to die to magistrate in presence of lawyers.
- Dr to certify compos mentis
- Any person may be present
- Oath administered
- Cross includes leading questions
- No witnesses required
- Recording as procedure of court
- More important than dying declaration

Dying declaration	*Dying deposition*
• Written / verbal	Oral statement on oath
• By magistrate / PO / MO	By magistrate
• Recorded in presence of 2 disinterested witnesses	In presence of accused & lawyers
• No oath	Oath administered
• Relatives, PO not allowed	May be present
• Leading questions not permitted	During cross examination

Dying declaration	*Dying deposition*
• In question- answer form, in vernacular of pt.	As per the procedure of court
• Recorded as per say of pt.	After cross examination
• Signed by magistrate, pt, Dr & witnesses	By magistrate only
• May be > 1 declaration	Can't be > 1
• Is inferior to deposition	Is superior
• Loses importance if pt survives	Retains full value

Medical evidence (Contd..)

Oral / Parole

- Direct evidence permitted (60 IEA)
- Indirect only if dead / untraceable
- Is superior to written – cross possible
- Written - doesn't need cross - ?

Res Gestae

Circumstantial

struggle, accused's items, hair, cloth blood, suicide note, weapon / poison, bullet, bl gp, finger prints, diseases, poison - purchase - ingestion, disposal.

Med. ev. & Crime detection

- To authenticate / disprove charge
- To prove guilt / innocence–life, liberty of accused
- Court depends for med knowledge
- Neither directly proves / disproves case
- To be within limits of science & truth
- Help police to solve crime
- Collect & send evidence to FSL
- For success in medicolegal field – Subject, law, language, practice, suspicion, observation, imagination, deduction, common sense.

Crime detection - difficulties

- P.O. delays reaching spot – clues lost / decomp.
- P.O. may not touch / undress
- P.O. may miss/ add injuries
- Unreliable panchas
- Hot weather - decomposition
- Disposed - cremated, buried, jungle / water
- Animals / aquatic / insects / birds
- Relatives avoid statement
- Not reported / false charge
- M.O. doesn’t examine properly
- Forensic expert - not called to scene

Crime detection - scene

- Usually body brought to mortuary
- Sometimes spot PM requested
- M.O. can request visit to scene
- Scene - struggle, circumstances, place, manner
- At scene - photographs, sketches, surroundings, body, weapon / articles, foreign body, finger print, reconstruct
- Don't - touch / express opinion

Crime detection - scene - (Contd..)

Disadvantages of not visiting

- X surrounding / circumstances,
- Fresh injuries, tears, stains,
- Rigor broken

- Maintain chain of custody
- Collection of evidence

Likely helpful, stains, finger prints, preserve & label

- Rules for preservation of evidence

be relevant, obtained legally, intact chain of custody, evaluated by qualified.

Medicolegal masquerades

- Decomposition, artefacts, obsure PM, obliterated identity, D/D - rigor, lividity, head injury, scalds
- Difficult Δ - Death, hanging, drowning, sui / homicide, deep bruise, fabricated, rape on married, poison non detection

VISCERA PRESERVATION

General Indications

Living - Poisoning

In dead - Chemical analysis

- Drowning - for diatoms
- Septicemia - C & S
- Virus - in 50% glycerine
- Histopathology - in formaline
- Enzymes - in liquid nitrogen
- DNA fingerprinting - frozen sample

C. A. - Indications

1. I. O. requests
2. M.O. - suspects poisoning
3. Cause of death - X - exclude poisoning
4. Cause of death - Known + evidence of poisoning
5. Decomposed bodies
6. Burns
7. Asphyxial deaths - drowning
8. Traffic accidents - suspicion of alcohol

C. A. - Routine Viscera

Bottle 1 - Whole stomach + 1 mt. Intestine

- Contents - stomach - 100 - 200 ml, intestine - 100 ml.

Bottle 2 - Liver + G.B. - 1/3, children - 1/2

- Spleen - 1/2, children - full

- Kidneys - 1/2, children - both

Bottle 3 - Blood - 100 ml.

CA - additional viscera

- Inj - snake bite - skin + muscle
- Cr. Abortion - ut, Cx, bladder, rectum
- Alcohol – Blood, CSF, lungs, brain, urine
- Gaseous poisoning - brain + lungs
- Cerebral poisoning - brain
- Strychnine poisoning - brain + spinal cord
- Cardiac poisoning - Heart
- Metal poisoning - Bone + B.M. + nails + hair

Additional viscera - collection

1. Blood – vein / heart / abdomen
   - In alcohol – X cavities
   - Bl grouping – may be on filter paper
2. CSF – cisternal puncture / base
3. Urine – catheter sample
4. Bone – 200 – 300 gm, femur shaft 10 – 15 cm
5. Bone marrow – sternum / femur
6. Hair – 10 gm plucked
7. Nails – 20 complete nails pulled
8. Skin & muscle – 1 x 1 x 2 inch

CA - Preservatives

- Gap in dispatch or analysis

1) Viscera - poisoning + carbolic - acids
   - Saturated salt solution (also additional viscera)
   - Acid - Rectified spirit
2) Blood - Gp - Sod. citrate 0.5% + formaline 0.25%
   - Poisoning + alcohol (-) CO, F, oxalic acid
     - 30 mg K oxalate + 10 mg Na F
   - CO - Liquid paraffin
   - F + oxalic acid – 30 mg sod. Citrate/3 ml heparin
3) Urine - salt / spirit / sod citrate / sod benzoate

CA - Procedure

- Proper preservation, packing separately & sealing
- Opened / cut - put in bottles
- Preservative to dip, not full
- Lid greased, cloth, string, tape, sealed, labelled, sealed, viscera box + preservative sample, box closed - locked - sealed - labelled, key + seal sample - envelope - sealed, + PM notes + requisition
- To P.C. under receipt – to CA
- Disposal - after 6 mth.

CA - result interpretation

1(+), 2(-) - not absorbed, X caused death,

$\pm$ P.M. poisoning

1(-), 2(+) - absorbed, ingested > 2 - 6 hr,

$\pm$ other route, cause of death if fatal level

1(+), 2(+) - Partially absorbed

1(-), 2(-) - not poisoning / not detected

FSL - General

Origin - sherlock holmes by Sir Arthur Conan Doyle

- Exam. of physical evidence sent by M.O. & ones recovered from scene
- To link - suspect to victim
  - suspect to scene / crime
- FSL - central / state
- In M.S. - Bombay, Pune, Aurangabad, Nagpur & Nashik

Sections

1. Toxicology - C.A. - viscera / body fluids
   - Blood alcohol / illicit liquor
   - Kerosene - burns / organic solvent - arson
   - Narcotics, stimulants, psychodelic
2. Serology - Species / Blood group
3. Biological - Blood, semen, saliva, stains, hair, wood, vegetable matter, micro organisms
4. Molecular biology - DNA finger printing & HLA typing

Sections - (Contd..)

DNA - Identity - Inj, hit & run, missing, sexual assault, adultery, illegitimacy

5. Physical evidence - Paint, glass, plastic, soil, dust, fibre, metal, tyre marks, tool marks, weapon, cement %
6. Ballistic & explosive - firearms - working, barrel residue, fired shot, range, propellent, wads.
   - Explosives - ingredients (TNT, RDX, Testry), illegal bombs.

Sections - (Contd..)

7. Finger printing
8 Polygraphy & lie detection
9. Document analysis - Handwriting , type writing, forged, currencies, ink analysis, Obliteration / indentation - by videospectral comparator (IR luminescence) or electrostatic detection equipment
10. Photography section

Physical evidence

1. Poisoning / food poisoning
   - viscera, body fluids, food, utensils, clothes, packets, bottles, drugs
2. Assault & murder
   - weapon, blood stains, hair, cigarette butt, clothes, nails
3. Burns
   - clothes, skin, teeth & bones, metallic objects
4. Firearm injuries
   - projectile, case, wad, skin, barrel, weapon

Physical evidence - (Contd..)

5. Hanging / strangulation

   ligature comparison

6. Drowning

   Diatoms

7. Sexual offences

   stains, swabs & smears, vaginal fluid, hair, nails, clothes

8. Vehicular accidents

   tyre marks, blood, grease, mud, tissue, glass, paint, hair, clothes

Physical evidence - (Contd..)

9. Forgery / cheating
   document, currency, typewriter
10. Identification
    finger/foot prints, blood, hair, semen, clothes, bones, teeth, tissues.
11. Disputed paternity / identity
    blood group, DNA finger printing
12. Drug addiction / trafficking
    viscera, fluids, specimen
13. Burglary
    Paint, algae, vegetable matter, glass on tools/ body

VISCERA PRESERVATION

General Indications

Living - Poisoning

In dead - Chemical analysis

- Drowning - for diatoms
- Septicemia - C & S
- Virus - in 50% glycerine
- Histopathology - in formaline
- Enzymes - in liquid nitrogen
- DNA fingerprinting - frozen sample

C. A. - Indications

1. I. O. requests
2. M.O. - suspects poisoning
3. Cause of death - X - exclude poisoning
4. Cause of death - Known + evidence of poisoning
5. Decomposed bodies
6. Burns
7. Asphyxial deaths - drowning
8. Traffic accidents - suspicion of alcohol

C. A. - Routine Viscera

Bottle 1 - Whole stomach + 1 mt. Intestine

- Contents - stomach - 100 - 200 ml, intestine - 100 ml.

Bottle 2 - Liver + G.B. - 1/3, children - 1/2

- Spleen - 1/2, children - full

- Kidneys - 1/2, children - both

Bottle 3 - Blood - 100 ml.

CA - additional viscera

- Inj - snake bite - skin + muscle
- Cr. Abortion - ut, Cx, bladder, rectum
- Alcohol – Blood, CSF, lungs, brain, urine
- Gaseous poisoning - brain + lungs
- Cerebral poisoning - brain
- Strychnine poisoning - brain + spinal cord
- Cardiac poisoning - Heart
- Metal poisoning - Bone + B.M. + nails + hair

Additional viscera - collection

1. Blood – vein / heart / abdomen
   - In alcohol – X cavities
   - Bl grouping – may be on filter paper
2. CSF – cisternal puncture / base
3. Urine – catheter sample
4. Bone – 200 – 300 gm, femur shaft 10 – 15 cm
5. Bone marrow – sternum / femur
6. Hair – 10 gm plucked
7. Nails – 20 complete nails pulled
8. Skin & muscle – 1 x 1 x 2 inch

CA - Preservatives

- Gap in dispatch or analysis

1) Viscera - poisoning + carbolic - acids
   - Saturated salt solution (also additional viscera)
   - Acid - Rectified spirit
2) Blood - Gp - Sod. citrate 0.5% + formaline 0.25%
   - Poisoning + alcohol (-) CO, F, oxalic acid
     - 30 mg K oxalate + 10 mg Na F
   - CO - Liquid paraffin
   - F + oxalic acid – 30 mg sod. Citrate/3 ml heparin
3) Urine - salt / spirit / sod citrate / sod benzoate

CA - Procedure

- Proper preservation, packing separately & sealing
- Opened / cut - put in bottles
- Preservative to dip, not full
- Lid greased, cloth, string, tape, sealed, labelled, sealed, viscera box + preservative sample, box closed - locked - sealed - labelled, key + seal sample - envelope - sealed, + PM notes + requisition
- To P.C. under receipt – to CA
- Disposal - after 6 mth.

CA - result interpretation

1(+), 2(-) - not absorbed, X caused death,

$\pm$ P.M. poisoning

1(-), 2(+) - absorbed, ingested > 2 - 6 hr,

$\pm$ other route, cause of death if fatal level

1(+), 2(+) - Partially absorbed

1(-), 2(-) - not poisoning / not detected

THANK YOU

F S L

General

Origin - sherlock holmes by Sir Arthur Conan Doyle

- Exam. of physical evidence sent by M.O. & ones recovered from scene
- To link - suspect to victim
  - suspect to scene / crime
- FSL - central / state
- In M.S. - Bombay, Pune, Aurangabad, Nagpur & Nashik

Sections

1. Toxicology - C.A. - viscera / body fluids
   - Blood alcohol / illicit liquor
   - Kerosene - burns / organic solvent - arson
   - Narcotics, stimulants, psychodelic
2. Serology - Species / Blood group
3. Biological - Blood, semen, saliva, stains, hair, wood, vegetable matter, micro organisms
4. Molecular biology - DNA finger printing & HLA typing

Sections - (Contd..)

DNA - Identity - Inj, hit & run, missing, sexual assault, adultery, illegitimacy

5. Physical evidence - Paint, glass, plastic, soil, dust, fibre, metal, tyre marks, tool marks, weapon, cement %
6. Ballistic & explosive - firearms - working, barrel residue, fired shot, range, propellent, wads.
   - Explosives - ingredients (TNT, RDX, Testry), illegal bombs.

Sections - (Contd..)

7. Finger printing
8 Polygraphy & lie detection
9. Document analysis - Handwriting , type writing, forged, currencies, ink analysis, Obliteration / indentation - by videospectral comparator (IR luminescence) or electrostatic detection equipment
10. Photography section

Physical evidence

1. Poisoning / food poisoning
   - viscera, body fluids, food, utensils, clothes, packets, bottles, drugs
2. Assault & murder
   - weapon, blood stains, hair, cigarette butt, clothes, nails
3. Burns
   - clothes, skin, teeth & bones, metallic objects
4. Firearm injuries
   - projectile, case, wad, skin, barrel, weapon

Physical evidence - (Contd..)

5. Hanging / strangulation

   ligature comparison

6. Drowning

   Diatoms

7. Sexual offences

   stains, swabs & smears, vaginal fluid, hair, nails, clothes

8. Vehicular accidents

   tyre marks, blood, grease, mud, tissue, glass, paint, hair, clothes

Physical evidence - (Contd..)

9. Forgery / cheating
   document, currency, typewriter
10. Identification
    finger/foot prints, blood, hair, semen, clothes, bones, teeth, tissues.
11. Disputed paternity / identity
    blood group, DNA finger printing
12. Drug addiction / trafficking
    viscera, fluids, specimen
13. Burglary
    Paint, algae, vegetable matter, glass on tools/ body

Analytical techniques

1. Chromatography
   - To separate mixture of substances
   - Chemical moves - solubility & affinity for sorbent
   - Mobile - liquid & stationary phase (sorbent) - solid / gel / liquid

a) TLC

Sample on plate + Al / silica gel

Mobile phase - solvent mixture

View - stain / under U.V.

Analytical techniques - (Contd..)

b) Paper chromatography

Stationary - special grade filter

Rest same as in TLC

c) Column chromatography

- In glass column, absorbents (silica gel, Al, charcoal) at top
- Test material + solvent at top of absorbent
- Absorbed by absorbent

Analytical techniques - (Contd..)

d) Gas chromatography
   - Test material as gas + inert gases N_2 or He
   - Passed through diff absorbing media
     - If solid (Gas - solid chro.)
     - If liquid (Gas - liquid chro.)
   - Detected by electron captor detector / flame ionization detector
   - Graph - chromatogram

e) Pyrolysis gas chromatography

   solid → gas, pyrogram

Analytical techniques - (Contd..)

2) Electrophoresis (Electrochromatography)
   - To identify proteins (serum, bl gp, Ab)
   - On glass slide - starch / agar gel, test material at end
   - Electrodes at ends - proteins move
   - Stain → bands +
3) Paper electrophoresis
   - Whatman 3 paper used
4) Spectroscopy
   - For haemoglobin

Analytical techniques - (Contd..)

5) Spectrophotometry

Estimates colour strength - by light absorbed

a) Colorimeter

using filters, rays absorbed detected

b) UV & I. R. (better)

c) Mass spectrometry

- Sample bombarded with electrons
- Ionic fragments - separated & detected as graph in electro magnetic field

Analytical techniques - (Contd..)

d) Emission - spectrophotometry

on excitation - light spectrum - recorded

e) Atomic absorption spectrophotometry

element vaporised, radiations passed, displaces electrons, energy emitted recorded

6) NAA

- For trace elements, hair, gun powder
- In nuclear reactor - neutron bombarded on atom - gamma rays liberated - counted

S.K.S Dholc

Analytical techniques - (Contd..)

7) Immunoassay

Based on labelled Ag or Ab

EMIT - Enz multiplied immunoassay

ELISA - Enz linked immuno sorbent assay

8) Microscope

a) Comparison microscope

2 microscopes + bridged eyepiece

b) Dark field (ultra) microscope

central stop in condenser - dark background

Analytical techniques - (Contd..)

C) Electron microscope
- Electron beam (not light) forms image
- High magnification & resolution

d) Fluorescence microscope
for fluorochrome stained Ab etc.

e) Polarising microscope
Polarised light produces image

f) Scanning electron microscope
beam of electrons scans & image on flourescent screen of cathode ray tube

g) X-ray microscope
beam of X-ray (not light)

Analytical techniques - (Contd..)

9) DNA finger printing
10) Polygraphy (lie detector)

    Responses of sympathetic stimulation
11) Narcoanalysis (Truth serum drugs)
    - 0.5 mg scopolamine hydrobromide S.C. repeated ½ dose x 20 min
    - Na pentothal (Na amytal) 2.5 -5% I.V. - 1cc/min
    - 0.1 gm Sod. seconal + 15 mg morphine + 0.5 mg scopolamine S.C.
12) Brain mapping

Thank You

TRACE EVIDENCE

Blood & blood stains

General

- Coloured photographs
- Decomposing body - may stain
- Before packing - weapons & clothes dried
- For dry stains - no preservation
- Liquid blood - preserved
- Exam. - FSL, serologist,

Blood stain collection

- On filter paper, dried
- In solvent - 10% KCN / 10% glycerine / weak NH_3 solution
- On porous - dried
- Metallic object - scrapped
- Cloth - dried & preserved

Exam - objects

1. Nature ?

- Preliminary - Physical exam
- Presumptive - Chemical tests
- Confirmatory - M/E, micro chemical
- Most positive - Spectroscopic

2. If blood - human ? - Serological

3. If human - Blood group ?

I Blood- Physical exam (at scene)

1. Colour
2. On coloured clothes
3. Other red stains
4. Time of shedding
   - Clotting
   - Colour
   - Fluorescence
5. Size & shape
6. Direction of shedding
7. Finger / foot print
8. Clothes
9. Body
10. Suspect
11. Suspected weapon
12. Character
    - Infant / adult
    - Male / female
    - Living / dead
    - Arterial / venous
    - Victim / assailant
    - Innocent stain
    - Obscure stain

II Blood - Chemical test

Hb $\rightarrow$ meth Hb - has porphyrin ring -

acts as peroxidase

Salts + H_2 O_2 Peroxidase – coloured base

1. Amidopyrine - Purple
2. Benzidine - Greenish blue
3. Phenolphthalein (Kastle meyer) - Permanganate
4. Guaiacum - Blue
5. Leucomalachite green - Bluish green / peacock blue
6. Ortho - toludine (Kohn) - Blue / green

III Blood - Microscopic

1. M/E - Stain soaked - saline / Vibert's fluid (Nacl + $Hgcl_2$ + H_2o) / Leishman stain
   - RBC - Circular, biconave, non nucleated, 7 micron
2. Takayama haemochromogen crystal test

   on slide - stain + reagent (NaOH + pyridine + sat glucose + H_2O) - warm
   - pink, acicular crystals in clusters
3. Teichmann's haemin crystal test

   on slide - stain + Nacl + glacial acetic acid - warm - brown rhombic crystals

   - single / clusters

IV Blood - Spectroscopy

B	C	D	E	F
Red	Orange	Yellow	Green	Blue

- Oxy Hb - 2 in D & E - Ist half of IInd
- Carboxy Hb - 2 in D & E - $\rightarrow$ Rt.
- Reduced Hb - 1 broad in D & E
- Haemochromogen - 1 in D – E, 1 E - F,- Ist sharp
- Cyn haemochromogen - 2 in D & E - wider
- Meth Hb - 1 C - D, 2 D - E, 1 E - F
- Sulph Hb - 1 C - D, 2 D - E
- Acid haematin - 1 dark near C

V Blood - Serology

For species

- Precipitin test
- Haemagglutination inhibition
- Gel diffusion
- Double diffusion in agar gel
- Precipitation electrophoresis
- Iso enzyme method

VI Blood groups

I Based on red cell antigen

1. ABO - A, B, AB, O
2. MN - M, N, MN
3. Rh - + or -
4. P factor - $P_1 P_1$, $P_1 P_2$, $P_2 P_2$
5. Ii system - All adults have I
6. Kell - K + KK Kk
   - K - kk

Bl. gp. - Red cell Ag

7. Kidd (JK) (a + b -) , (a - b+) , (a + b +) , (a - b-)
8. Duffy (Fy) (a + b -) , (a - b+) , (a + b +) , (a - b-)
9. Diago (Di) (a + b -) , (a - b+) , (a + b +) , (a - b-)
10. Lutheran (Lu) (a + b -) , (a - b+) , (a + b +) , (a - b-)
11. Lewis (Le) (a + b -) , (a - b+) , (a + b +) , (a - b-)
12. Xg - Xg (a +) , xg (a -)

Bl. gp. (contd..)

II Based on white cell Ag

HLA - A, B, C, D

III Based on serum protein

1. Hp - 1 - 1, 1- 2, 2 - 2
2 Gc - 1 - 1, 1- 2, 2 - 2
3. Ag - (x + y-), (x - y+) (x + y+) , (x - y-)
4. Gm - Gm1 in 60% Europeans

   Gm 1, 5 in all Africans
5. Km - 1, 2, 3, blank
6. Lpo - (a + x -) , (a - x+) , (a + x+) , (a - x-)
7. Abnormal Hb - S, C, D, E, barts.

Bl. gp. - enzymes

IV Based on red cell enzymes

1. PGM - 1, 1 -2 , 2
2. AK - 1, 1 -2 , 2
3. ADA - 1, 1 -2 , 2
4. $E_S D$ - 1, 1 -2 , 2
5. GLO - 1- 1, 1 -2 , 2
6. EAP - A, AB, B, AC, BC, C
7. G_6 PD - A, AC , C
8. SCE
9. GPT

Bl. gp. - determination

- If fresh
    - Direct agglutination - known antisera or agglutination of sera - known RBC
- If old -
    - Absorption inhibition
    - Mixed agglutination
    - Absorption elution
- Bl.gp. & heredity
- MLA - Identity , victim / accused , source of blood, disputed paternity / maternity, illegitimacy, transfusion reactions

TRACE EVIDENCE - SEMEN

Stains - clothes, body, bed, scene, seat, floor / grass

+ blood, mucous, pus, faeces

- matted pubic hair

Collection

Cloth - cut & dried

Matted pubic hair - cut & dried

Vagina - 10 ml saline, aspirated

Body - swab

Smooth surface - scrapped

Semen examination

- Physical examination
- Chemical examination
- Microscopic examination
- Biological examination

Semen - physical examinaion

Fresh - viscid, albuminous, yellowish, odour +,

- Cellular - spermatozoa
- Fluid - seminal vesicle - ↑ lecithin, choline
- Prostratic - ↑ acid phosphatase, spermine

One ejaculate - 3 - 5 ml, 60 -120 million / ml, 80% motile, 80% intact, pH - 7.4, coagulates, liquefies in 15 min

Semen - Physical (Contd..)

Stain - stiff , starchy, ↑ yellow, X odour

Improper drying - X stiff

Invisible - Exam UV light - bluish flourescence (choline) (also with other albuminous material)

Stiffness - D/D - starch, pus, sputum, leucorrhoea

Semen - chemical tests

1. Microchemical tests

- Florence test
  - Stain + reagent (2.65 gm I_2 + 1.48 gm KI + 30 ml H_2O) - brown crystals of choline periodide - prismatic / rhombic
  - False + with brain, blood
- Barberio's test
  - Stain + sat. picric acid - yellow crystals of spermine picrate - single / cross / star / feathery

Semen - Chemical (Contd..)

2. Enzyme tests

- Acid phosphatase > 10 KA

Spot test - stain + citrate buffer + disod. Diphenyl PO_4 + 10 min + phenol reagent + $NaHCO_3$ - blue

- stain soaked with moist filter, spray alpha naphthyl PO_4 & fast black K dye - purple

- With Sod carbonate - yellow

Semen - Chemical (Contd..)

2. Enzyme (Contd..)

- Creatinine phosphokinase > 400 units
- Glycoprotein (P30 - PSA) & MHS - 5

3. Chemical test

Stain + Amm. molybdate

- deep yellow due to P.

4. Zn conc > 140 mg / ml

Semen - Microscopic

- Motile spermatozoa - recent intercourse
- If stain - extract - v.dil Hcl / 0.3% acetic acid
- Stain

	H & E	Methylene blue Eosine	Erythrocin Malachite green
Head post 1/3	Red	Red	Red
Rest	Blue	--	Green

Semen - Biological

- Precipitin test
- Blood grouping
- Ratio of testosterone / dihydro testosterone

Proof of semen

- Spermatozoa
- If (-) flourescence, + florence t., ↑ acid phosphatase & CPK
- LDH isoenzymes (Immunoelectrophoresis)
- TLC
- Gel diffusion precipitation
- Gel electro phoresis
- Immuno diffusion
- Iso electric focussing

TRACE EVIDENCE - HAIR

Found - weapon, vehicle, stains, body, clothes

Cases - Murder, assault, veh. acc., rape, unnat sexual offences, ch. metal poisoning, identification

Examination

- Nature - Hair / fibre
- If hair - Human / animal
- If human - Identification
- Evidence of crime
- In dead - cause & time since death

Hair - nature

- Hair - root, shaft, tip
- Hair - cuticle, cortex, medulla
- Cuticle - non pigmented keratin scales
- Cortex - elongated cells, pigment
- Medulla - ±, polyhedral cells in double row

Fibres

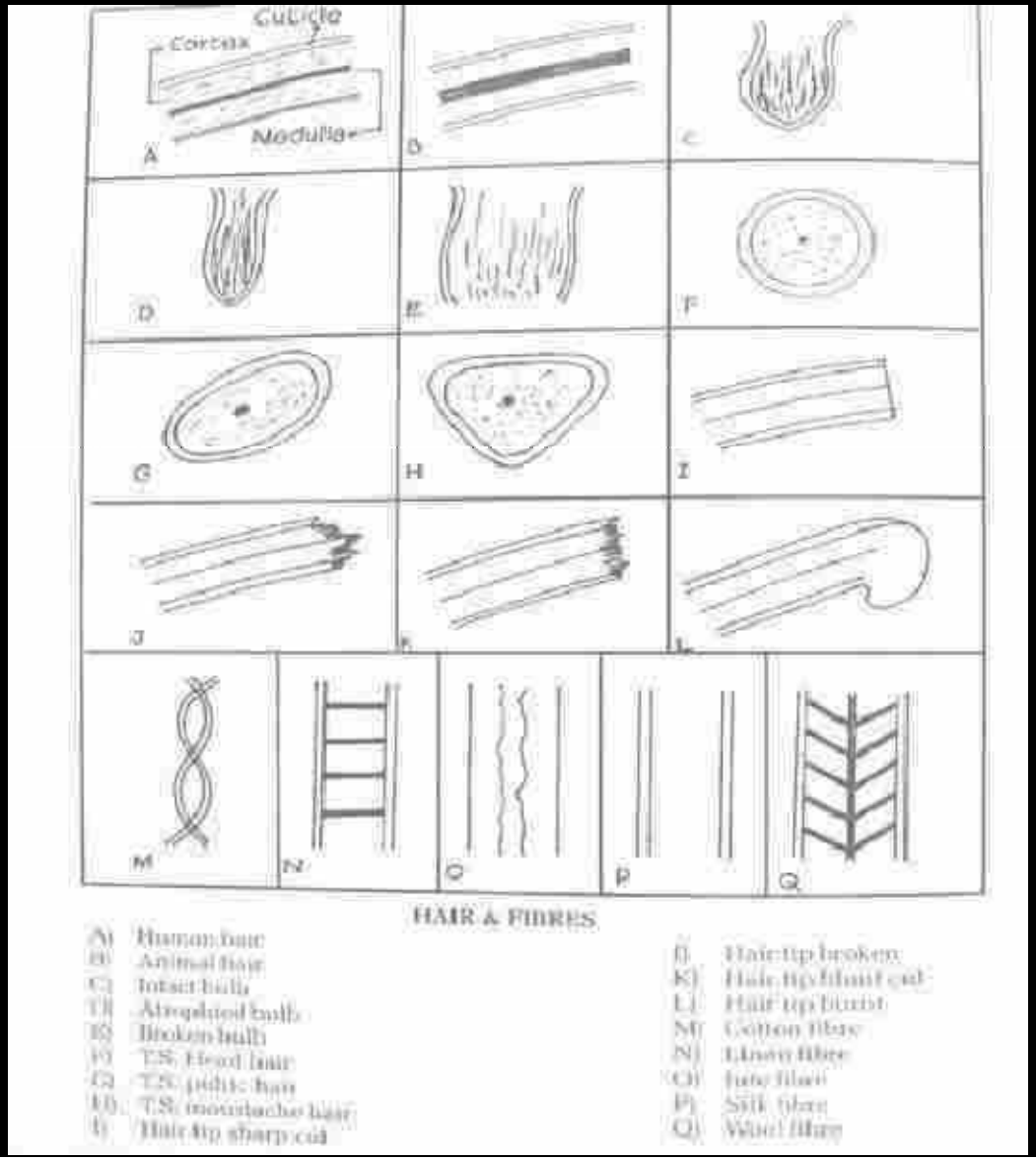
Vegetable - cotton , linen, jute

Animal - silk, wool

- Cold conc. $H_2 SO_4$ - Dissolves silk, cotton
- warm conc. Hcl - Dissolves silk , cotton
- 5% NaOH / KOH - Dissolves animal fibres
- 5% Sod. Nitroprusside - Violet - wool
- Thymol in alc + Hcl + Nacl + boil - red - vegetable fibre

Human	Animal
- Shaft 50 - 150 u	< 25 / > 300
- Scales surround complete	--
- Scales small, flat	Polyhedral
- Cortex = 4-10 medulla	upto twice
- Medulla ±, no pig	+, +
- Med index < 0.3	> 0.5
- Pig - cortex periphery	uniform
- Precipitin t - human	animal

Hair / fibre



HAIR & FIBRES

A) Human hair
B) Animal hair
C) Intact bulb
D) Abraded bulb
E) Broken bulb
F) T.S. Head hair
G) T.S. pubic hair
H) T.S. moustache hair
I) Hair tip sharp cut
J) Hair tip broken
K) Hair tip blunt cut
L) Hair tip bleach
M) Cotton fibre
N) Linen fibre
O) Jute fibre
P) Silk fibre
Q) Wool fibre

Hair - identity

- Race
- Age
- Sex
- Body part
- Occupation
- Curly, waves, dye
- Shades
- Grease / debris
- Changes in disease
- Blood grouping
- NAA - 29 elements
- Zn conc.
- Electrophoresis
- Electrofocussing
- Resist putrefaction

Hair - Crime evidence

- Locard's principle of exchange
- In homicide - grabbed
- In rape - matted pubic hair
- Stains - Blood, semen, mud, saliva
- Injury to hair - Blow, heat / firearm
- Exam of hair tip
- Exam of hair bulb

Hair - in dead, MLA

Cause of death - inj, firearm, burns, metals

Time since death - apparent growth, loosening, buried body

MLA

- Identification - individual
- Identification - nature of crime
- Identification - assailant / victim
- Identification - weapon / vehicle
- Evaluation of scene
- Cause of death
- Time since death

THANK YOU

MALARIA

WHAT YOU NEED TO KNOW

GOALS

- EPIDEMIOLOGY
- UNDERSTANDING THE LIFE CYCLE
- APPROACH TO THE PATIENT
- CLINICAL SYNDROME
- DIAGNOSIS OF FALCIPARUM
- TREATMENT
- PREVENTION

MALARIA - THE FUTURE

- RESISTANCE IS SPREADING
- VACCINE IS A LONG WAY OFF
  - But there have been some recent breakthroughs

MALARIA - THE NUMBERS

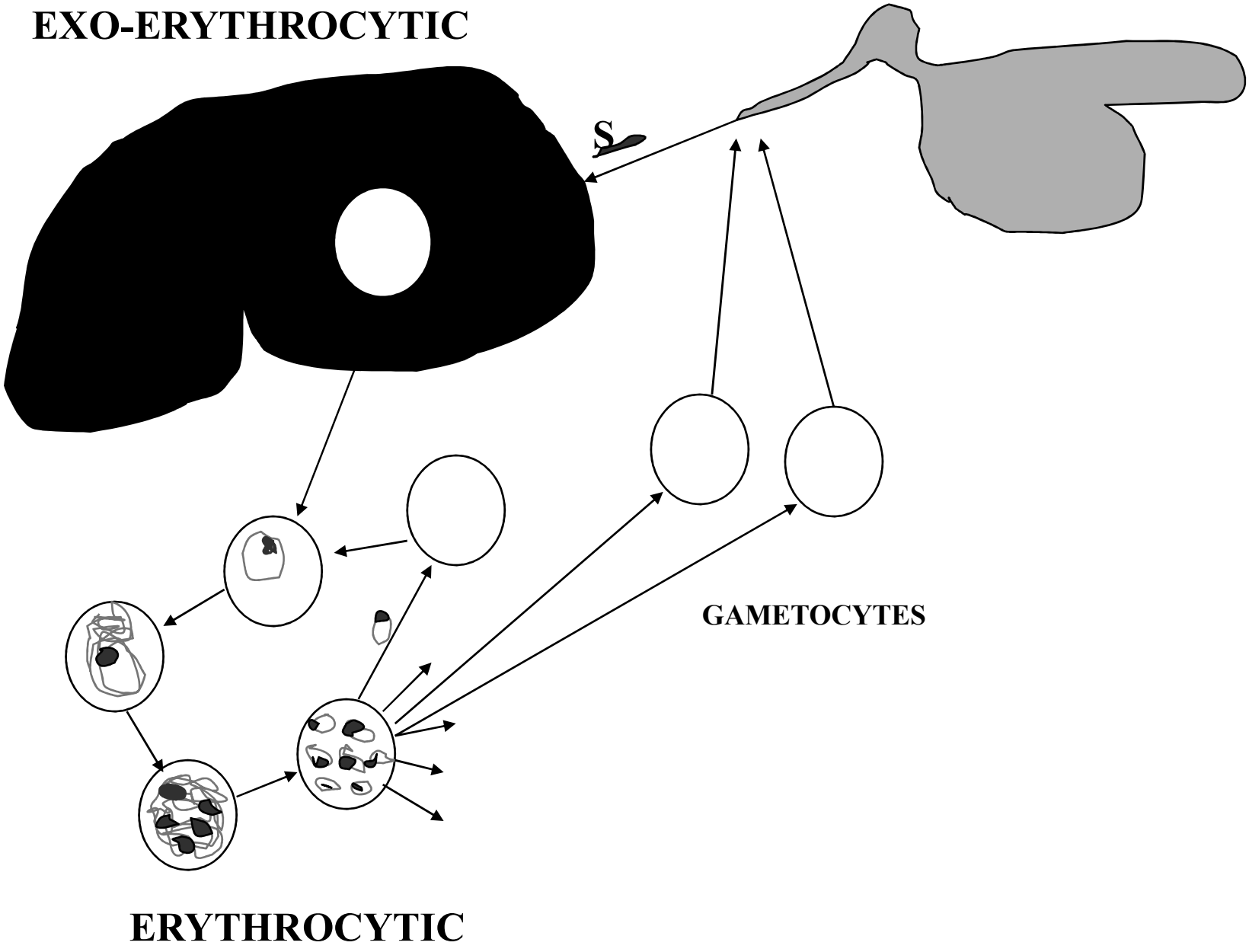
- 200-300 MILLION NEW CASES/YEAR
- 1.5-3 MILLION DEATHS/YEAR

TRANSMISSION

- FEMALE ANOPHELINE MOSQUITO
- TRANSFUSION
- CONGENITAL
- DIRTY NEEDLES
- ORGAN TRANSPLANT
- MALARIA THERAPY

MALARIA HOST RESISTENCE

- DUFFY (-) VIVAX
- HGB S FALCIPARUM
- FETAL HGB FALCIPARUM
- OVALOCYTES FALCIPARUM


EXO-ERYTHROCYTIC
S
GAMETOCYTES
ERYTHROCYTIC

CLINICAL

- THE PRESENTATION IS NONSPECIFIC
  - FEVER
  - RIGORS
  - MYALGIAS
  - HEADACHE
  - 25% HAVE CYCLICAL FEVERS
  - 25% HAVE SOME GI SYMPTOMS

CLINICAL

- NONSPECIFIC EXAMINATION
  - 25% WITH ENLARGED LIVER OR SPLEEN
- NONSPECIFIC LABORATORY
  - 25% WITH ANEMIA
  - 25% WITH LEUCOPENIA
  - 25% WITH THROMBOCYTOPENIA

CLINICAL

- DIFFERENTIAL DIAGNOSIS
  - TYPHOID
  - EARLY MENINGOCOCCUS
  - HFV
  - RICKETTISIAL
  - HEPATITIS
  - INFLUENZA
  - LEPTOSPIROSIS

COMPLICATIONS

- ACUTE
  - CEREBRAL MALARIA
  - DIC
  - ATN
  - ARDS
  - SEVERE HEMOLYSIS
- CHRONIC
  - SPONTANEOUS SPLENIC RUPTURE
  - NEPHROTIC SYNDROME

Species Characteristics

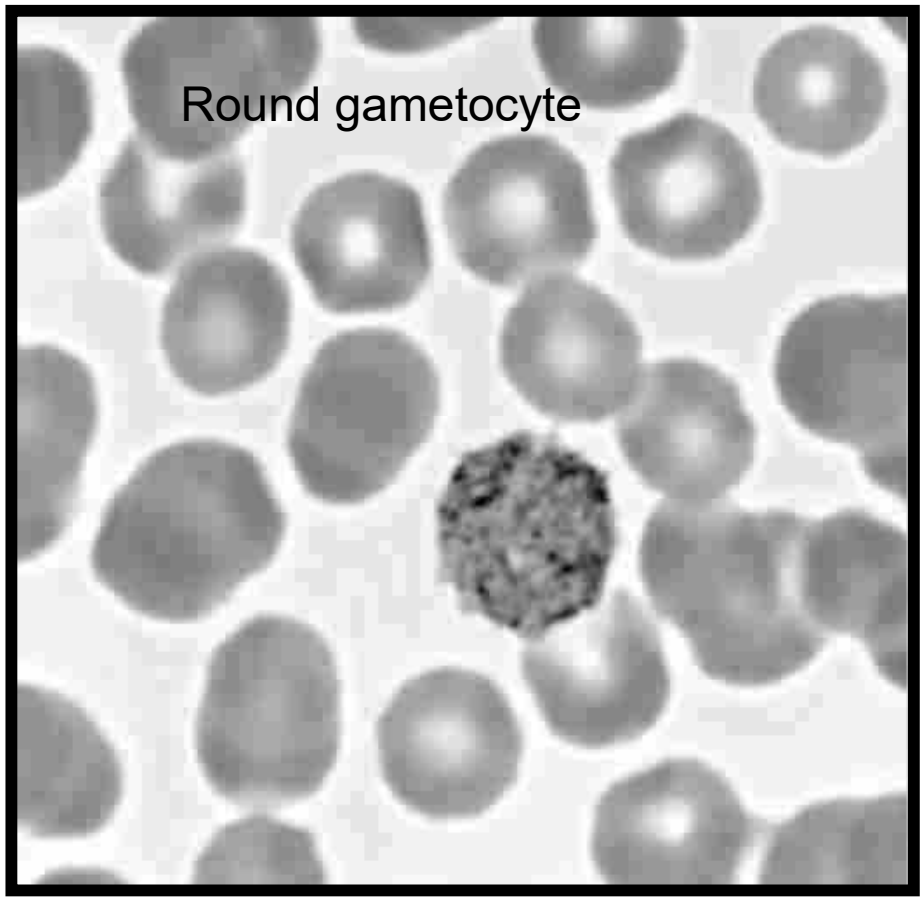
	PV	PO	PM	PF
Periodicity(hrs)	48	50	72	48
Parasites/Ml	20-50	9-30	6-20	50-2000
RBC Age	Young	Young	Old	Any
Hypnozoite	Yes	Yes	No	No
Duration (yrs)	1.5-5	1.5-5	3->50	1-2

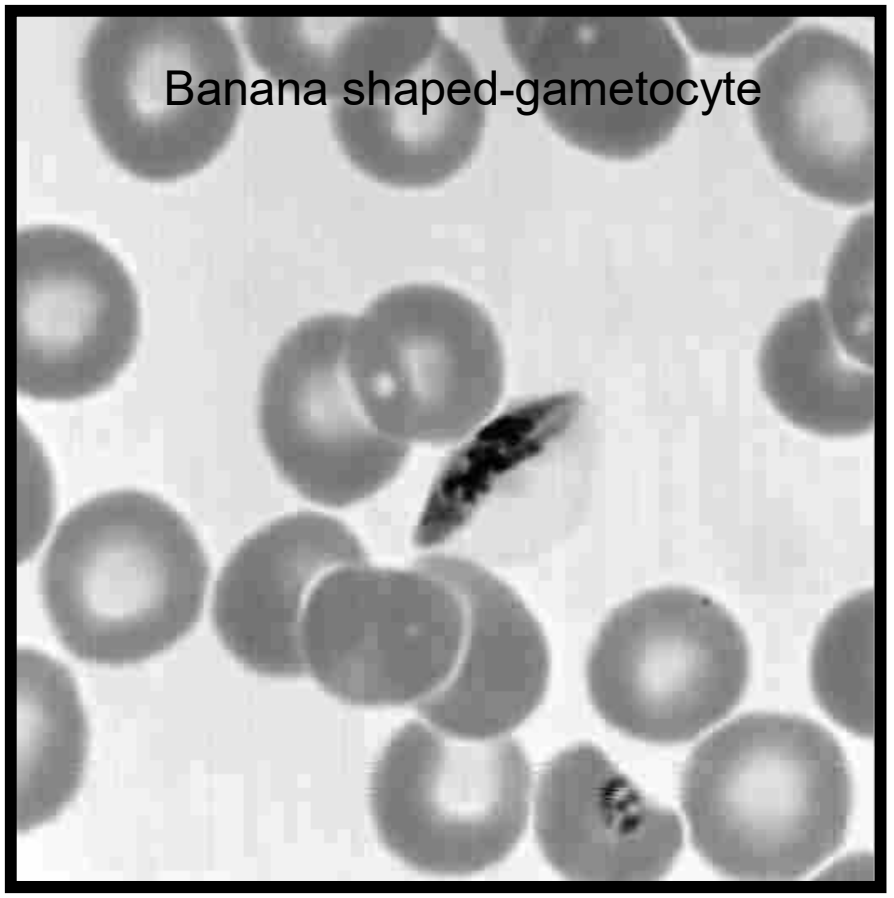
DIAGNOSIS

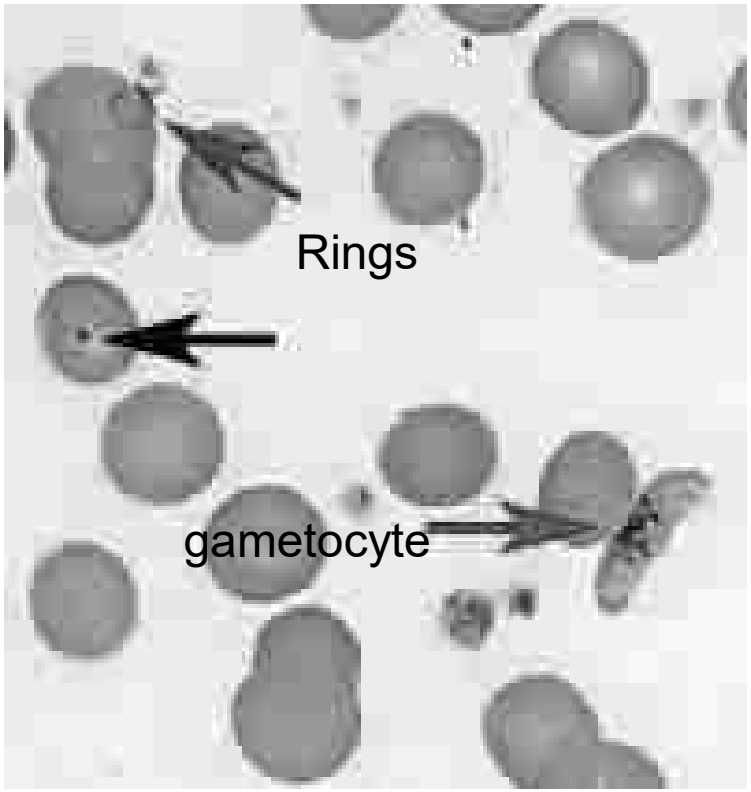
- THINK OF IT AND PROVE IT
- PERIPHERAL SMEAR
  - MYTH: THE TIMING IS NOT IMPORTANT
- SEROLOGY
  - Not very helpful in Botswana
- RAPID TEST

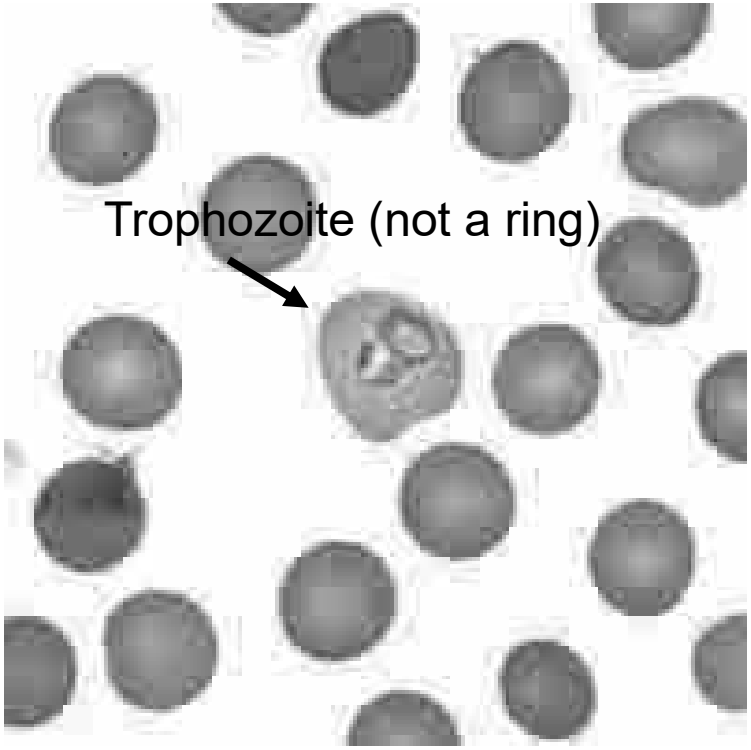
IS IT FALCIPARUM?

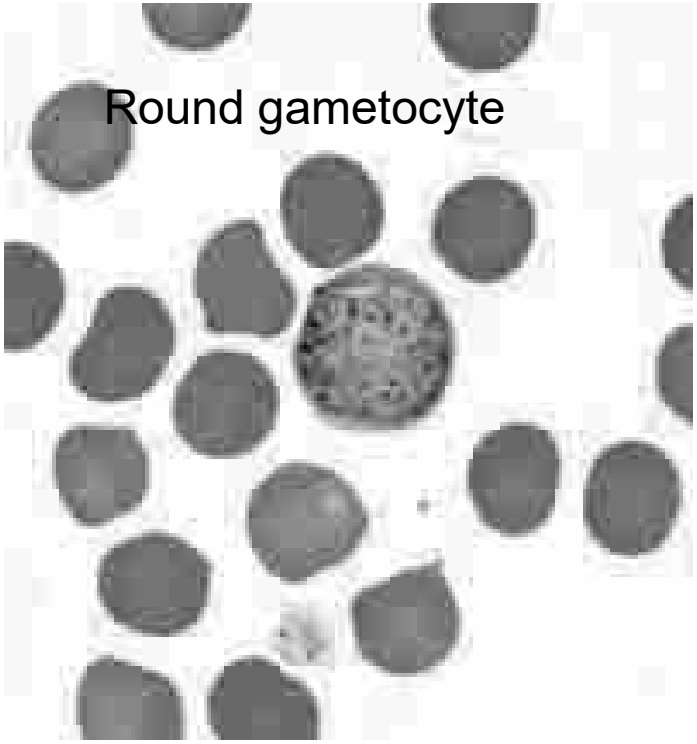
- WHAT DOES THE SMEAR SHOW?
  - >3% PARASITEMIA
  - MONOTONOUS SMALL RINGS
  - NO TROPHOZOITES OR SCHIZONTS
  - BANANA SHAPED GAMETOCYTES
  - MULTIPLY INFECTED CELLS
  - APPLIQUE FORMS
  - CELLS OF ALL SIZES INFECTED

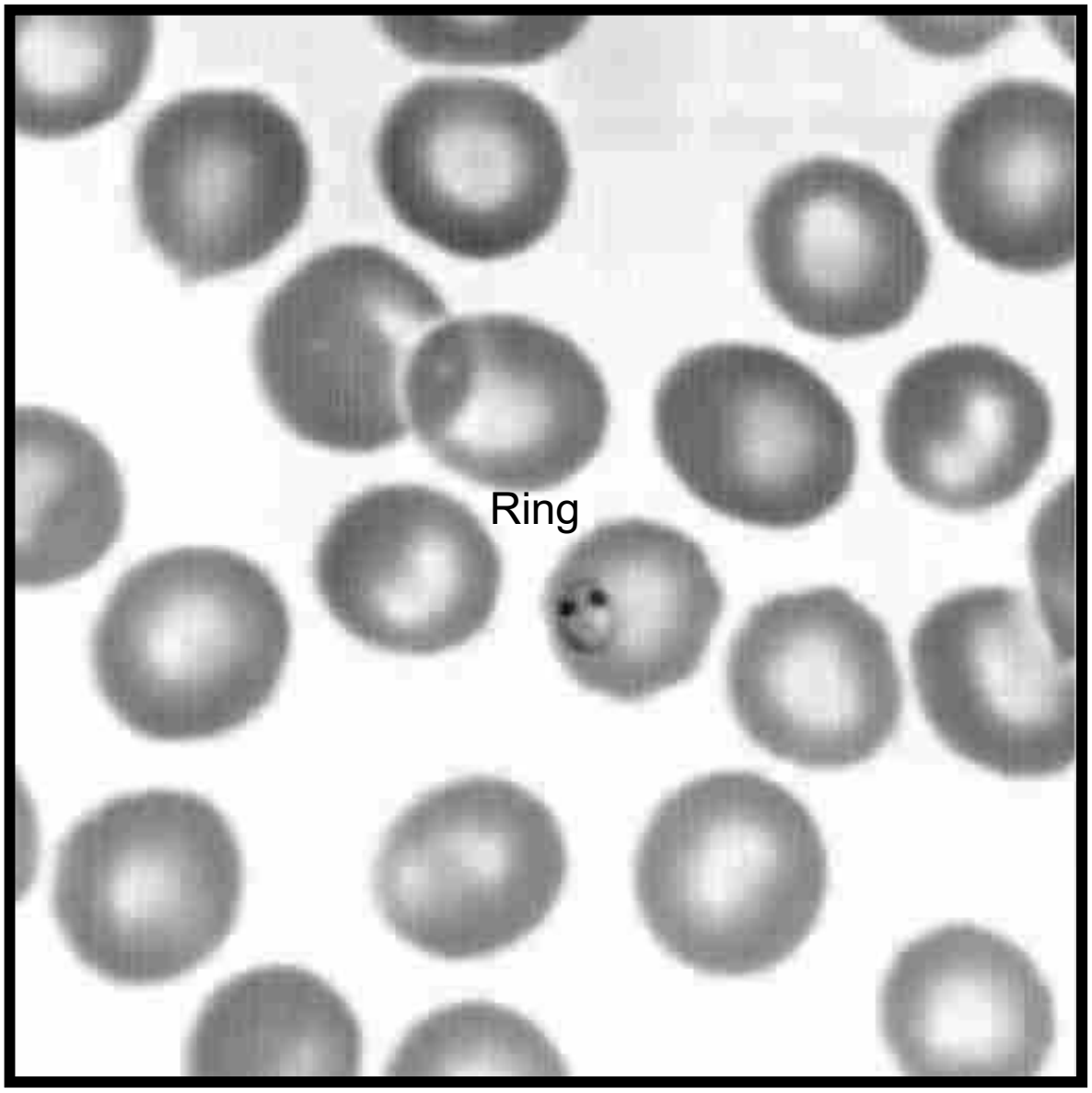

Round gametocyte

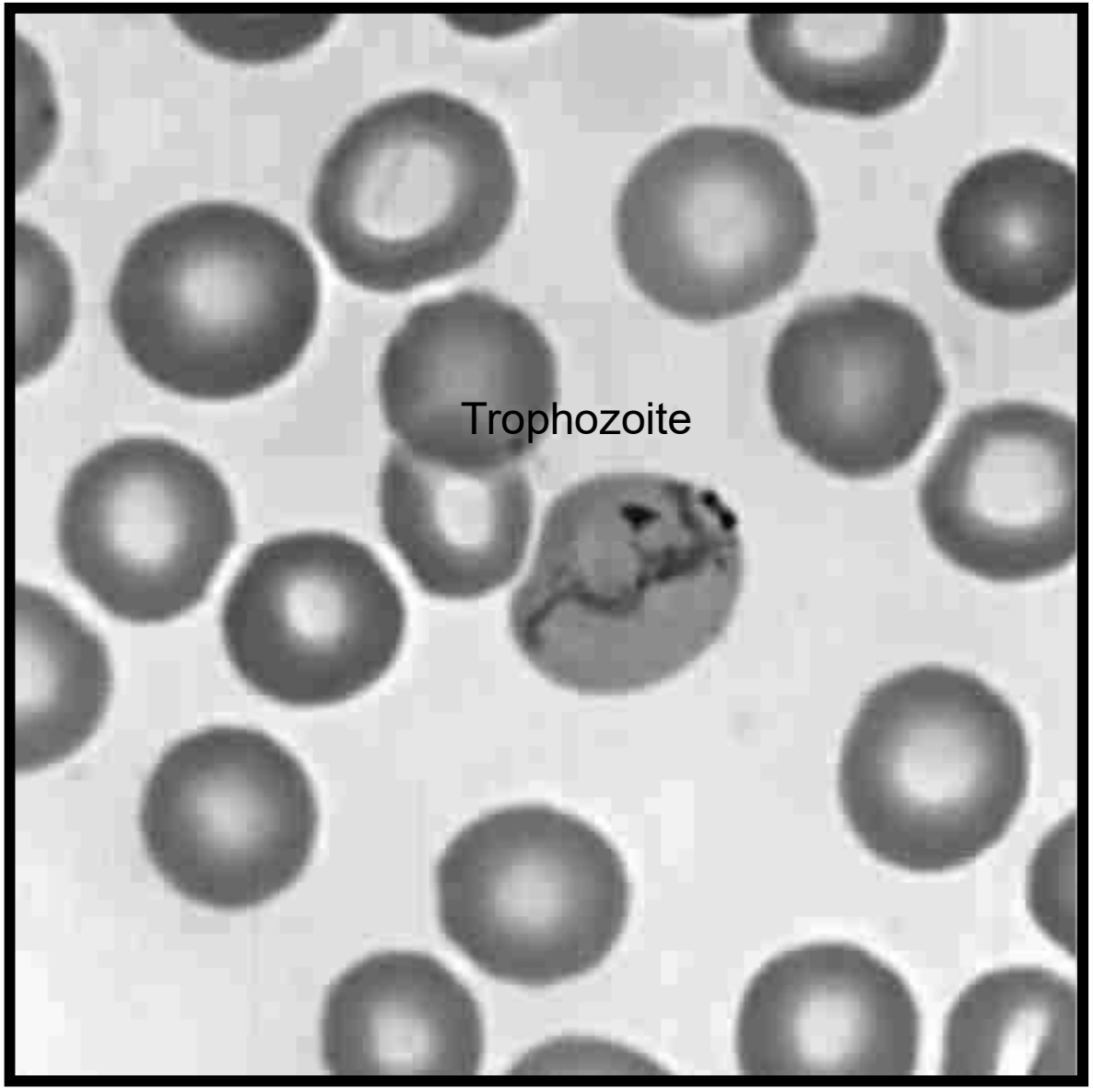

Banana shaped-gametocyte


Rings
gametocyte

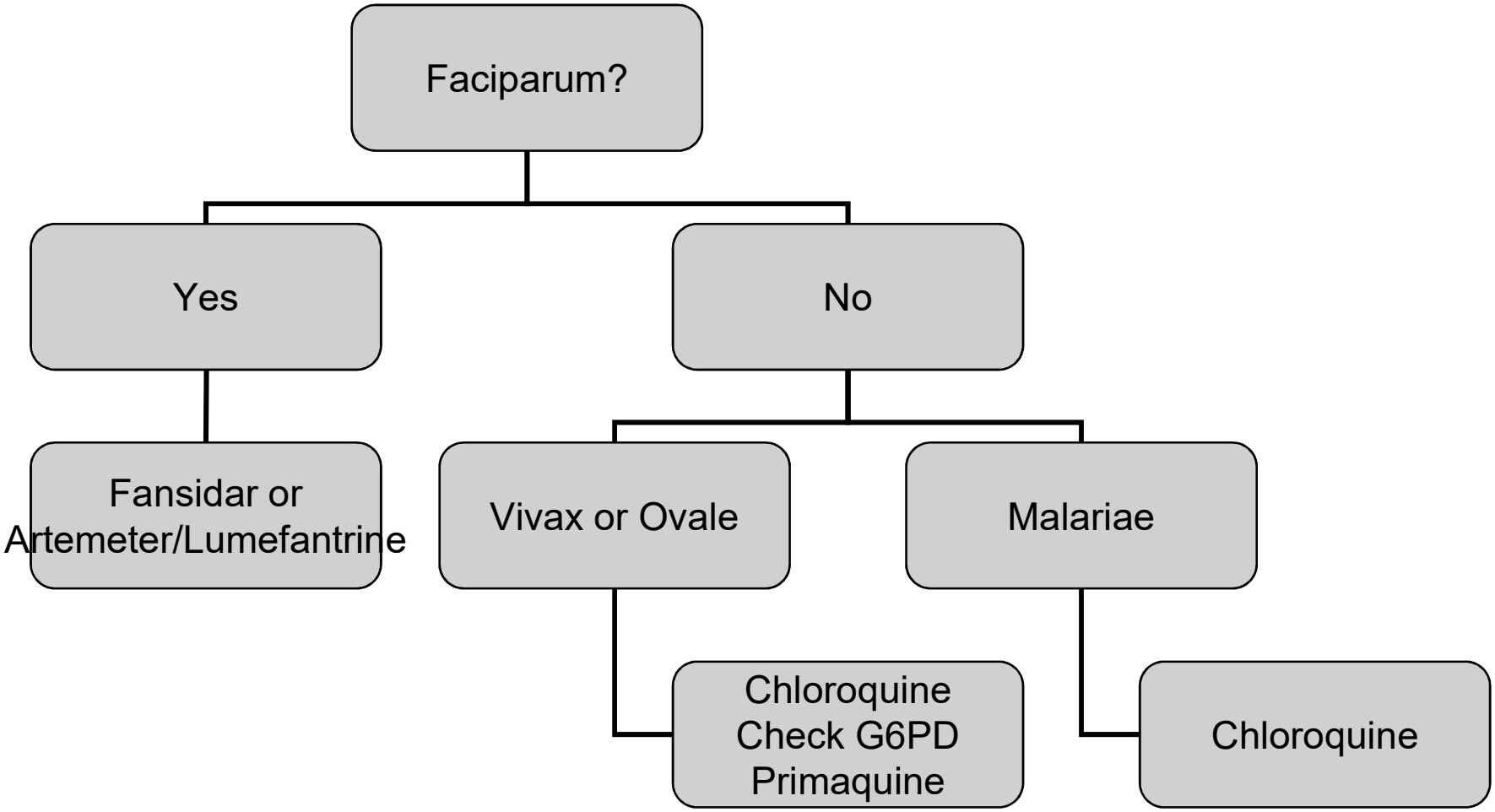

Trophozoite (not a ring)


Round gametocyte


Ring


Trophozoite

Treatment


Faciparum?
Yes
No
Fansidar or
Artemeter/Lumefantrine
Vivax or Ovale
Malariae
Chloroquine
Check G6PD
Primaquine
Chloroquine

TREATMENT 2

- HALOFANTRINE
- MALARONE
  - ATOVAQUONE/PROGUANIL
- TAFENOQUINE
- QUININE based regimens
- CHLOROQUINE/PROGUANIL IS AN *INFERIOR REGIMEN AND SHOULD NOT BE USED*

TREATMENT
OTHER ISSUES

- EXCHANGE TRANSFUSIONS
- STEROIDS
- FE CHELATION
- PREGNANCY

PREVENTION

- PERSONAL PROTECTIVE MEASURES
  - DEET
  - PERMETHRIN
  - SCREENING AND NETTING
- MEDICATIONS
- VACCINE

PREVENTION
MEDICATIONS

- CHLOROQUINE
- MEFLOQUINE
- DOXYCYCLINE
- CARRY FANSIDAR
- CHLOROQUINE/PROGUINIL
- PRIMAQUINE
- MALARONE

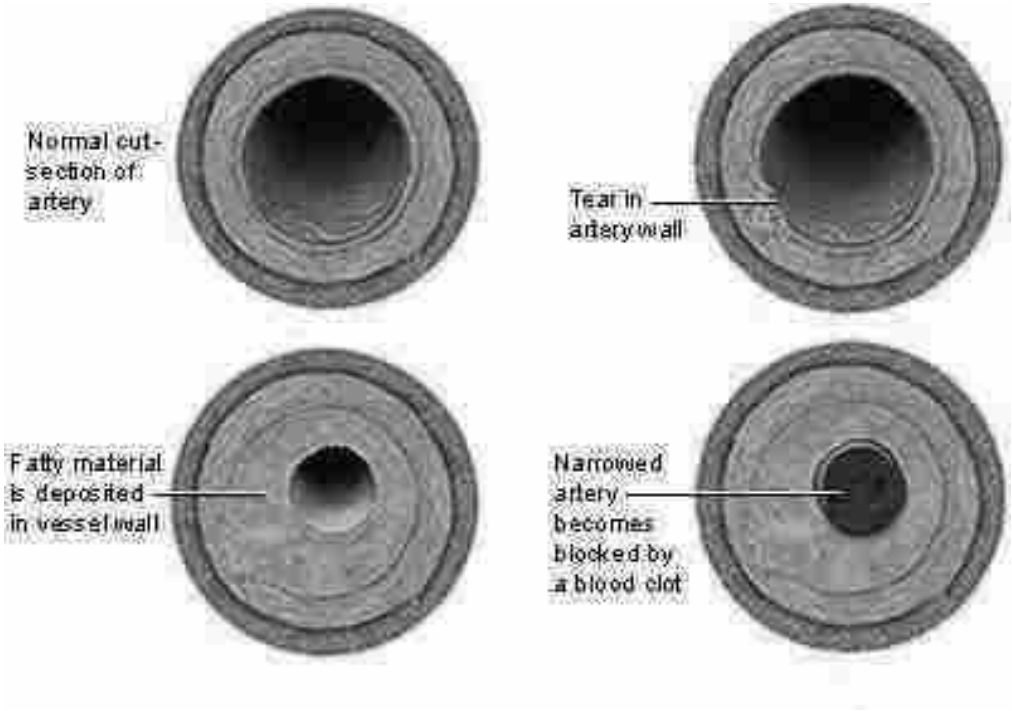
Ischemic Heart Disease

Dr Dilip .R. Patil

H.O.D

DEPT OF MEDICINE

- **<u>Ischemic Heart Disease</u>**
  - Is a condition Typically occurs when there is an imbalance between Myocardial O2 supply & Demand.
  - Most common causes
    - Atherosclerotic disease of coronary vessels.



- Patients with ischemic heart disease fall into **two large groups:**

1) Patients with chronic coronary artery disease (CAD) who most commonly present with **stable angina** & **patients with acute coronary syndromes** (ACSs).

2) & later group composed of **pts with acute MI with ST segment elevation on presentation**.

Ischemic Heart Disease

- **Angina**
  - **Stable**
  - **Unstable**
  - **Prinzmetal's**
- **Myocardial Infarction**
  - **NSTEMI**
  - **STEMI**

Epidemiology

- **IHD causes more deaths and disability.**
- **Incurs greater economic costs** than any other illness in the developed World.
- **IHD is the most common, serious, chronic, life-threatening illness.**
- **Genetic factors, a high-fat & energy-rich diet, smoking, and a sedentary lifestyle.**

Epidemiology

- Obesity, insulin resistance, and type 2 DM are increasing and are powerful risk factors for IHD.
- With urbanization, **elements of the energy-rich Western diet** are being adopted.
- It is **growing among** low-income groups, **but primary prevention has delayed the disease** to later in life in all socioeconomic group.

National Statistics

- About 7.3 million females have a history of heart attack, angina pectoris, or both.
- Women with diabetes and CVD, especially African American and Hispanic, die at a higher rate than men or non-diabetic women
- Since 1984, the number of **CVD deaths for females has exceeded those for males.**
- In 2004, CVD was the cause of death in 459,096 females.

  Females represent 52.8 percent of deaths from CVD.

PATHOPHYSIOLOGY

- DUE TO ETIOLOGICAL FACTORS

- INJURY TO THE ENDOTHELIAL CELL THAT LINING THE ARTERY

- INFLAMMATION AND IMMUNE REACTIONS

- ACCUMULATION OF LIPIDS IN THE INTIMA OF ARTERIAL WALL

- T LYMPHOCYTES AND MONOCYTES THAT BECOMES AS MACROPHAGES INFILTRATE THE AREA TO INGEST THE LIPIDS AND DIE

↓

- PROLIFERATION OF SMOOTH MUSCLE CELLS WITH IN THE VESSEL

↓

- FORMATION OF FIBROUS CAP OVER DEAD FATTY CORE (ATHEROMA)

↓

- PROTRUSION OF ATHEROMA IN TO THE LUMEN OF VESSEL

- NARROWING AND OBSTRUCTION

- IF CAP IS THIN THE LIPID CORE MAY GROW CAUSING IT TO RUPTURE

- HEMORRHAGE INTO PLAQUE ALLOWING THROMBUS TO DEVOLOP

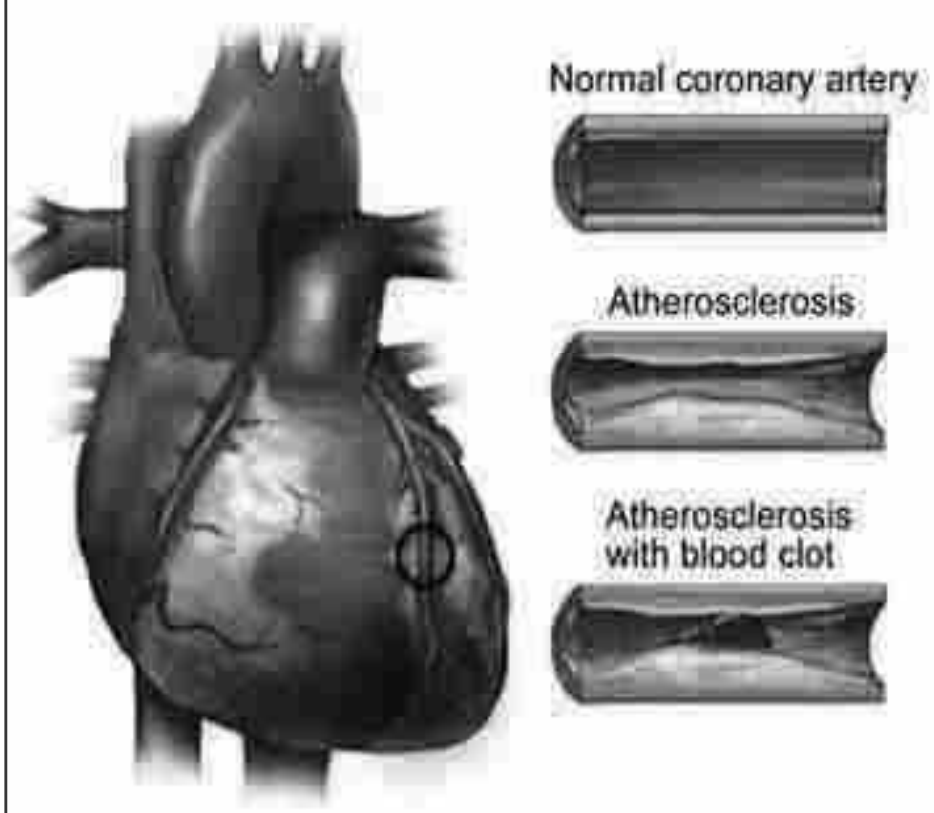
- THROMBUS AND OBSTRUCT THE BLOOD FLOW LEADING TO SUDDEN CARDIAC DEATH OF MYOCARDIAL INFARCTION

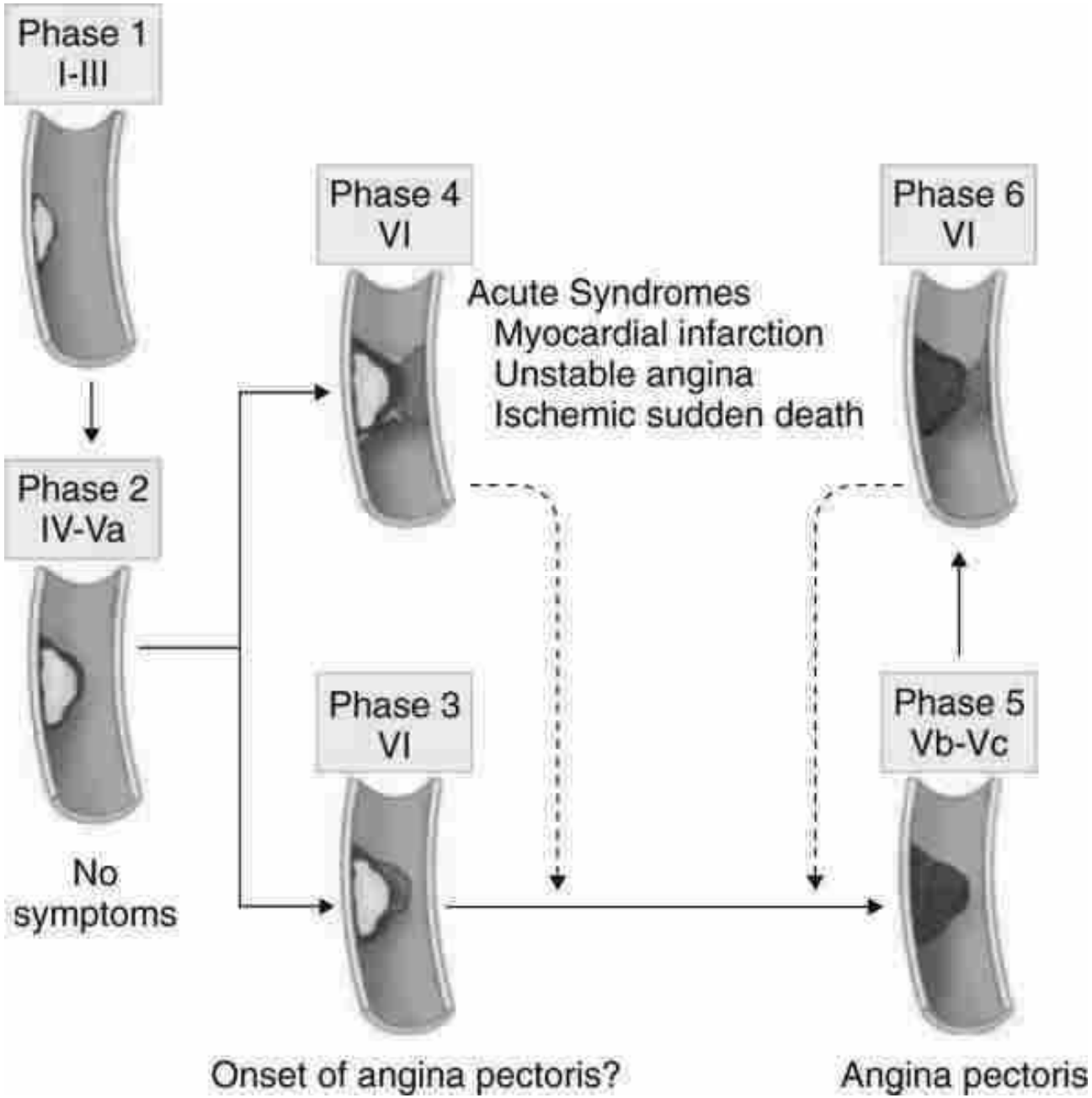
- ANGINA AND OTHER SYMPTOMS

Coronary Atherosclerosis

- **<u>Epicardial Coronary arteries</u>** are the major site of atherosclerosis.
- Segemental narrowing.
- Location of obsrtuction determines the severity.

- **<u>Major risk factors are</u>**
  - High Level of LDL
  - Low Level of HDL
  - Cigarette smoking
  - HTN
  - DM




Phase 1
I-III
Phase 2
IV-Va
No
symptoms
Phase 4
VI
Phase 3
VI
Acute Syndromes
Myocardial infarction
Unstable angina
Ischemic sudden death
Phase 6
VI
Phase 5
Vb-Vc
Onset of angina pectoris?
Angina pectoris

CORONARY HEART DISEASE: CLINICAL MANIFESTATIONS AND PATHOLOGY

Clinical problem	Pathology
Stable angina	Ischaemia due to fixed atheromatous stenosis of one or more coronary arteries
Unstable angina	Ischaemia caused by dynamic obstruction of a coronary artery due to plaque rupture with superimposed thrombosis and spasm
Myocardial infarction	Myocardial necrosis caused by acute occlusion of a coronary artery due to plaque rupture and thrombosis
Heart failure	Myocardial dysfunction due to infarction or ischaemia
Arrhythmia	Altered conduction due to ischaemia or infarction
Sudden death	Ventricular arrhythmia, asystole or massive myocardial infarction

STABLE ANGINA

Stable Angina

- characterized by chest or arm discomfort that may not be described as pain but is reproducibly associated with physical exertion or stress and is relieved within 5–10 minutes by rest and/or sublingual nitroglycerin.

STABLE ANGINA

- Angina pectoris is the symptom complex caused by
  - Transient myocardial ischemia and constitutes a clinical syndrome rather than a diseasE.
- **It may occur whenever there is an**
  - imbalance between myocardial oxygen supply and demand.
- **The most common cause of angina is**
  - Coronary atheroma
- The symptom may also be a manifestation of **other forms of heart disease**, particularly
  - aortic valve disease and hypertrophic cardiomyopathy.

FACTORS INFLUENCING MYOCARDIAL OXYGEN SUPPLY AND DEMAND

*Coronary blood flow occurs **mainly in diastole & some in systole.***

Oxygen demand
Cardiac work 1. Heart rate 2. Blood pressure 3. Myocardial contractility 4. Left ventricular hypertrophy 5. Valve disease, e.g. aortic stenosis

Oxygen supply
Coronary blood flow 1. Duration of diastole Coronary perfusion pressure (aortic diastolic minus coronary sinus or right atrial diastolic pressure) 2. Coronary vasomotor tone 3. Oxygenation Haemoglobin Oxygen saturation

Clinical Features

Clinical Features

- History by far the most important factor.

- **Stable Angina:**
  - Central Chest pain
  - Chest Discomfort
  - Breathlessness (exertion) Relieved on rest.
  - Discomfort when pt starts walking & later it does not return despite of greater Efforts

  (Warm Up Angina).
  - Lasts a few seconds < 10 minutes
  - Relieved by NTG.

Clinical Features

- **History**
  - Typically a pt is a man > 50 yrs or a women >60 yrs.
  - Described as heaviness, Pressure, Squeezing, smothering or choking & rarely frank pain.
  - Also radiate to back, root of neck, teeth, jaw or epigastrium.

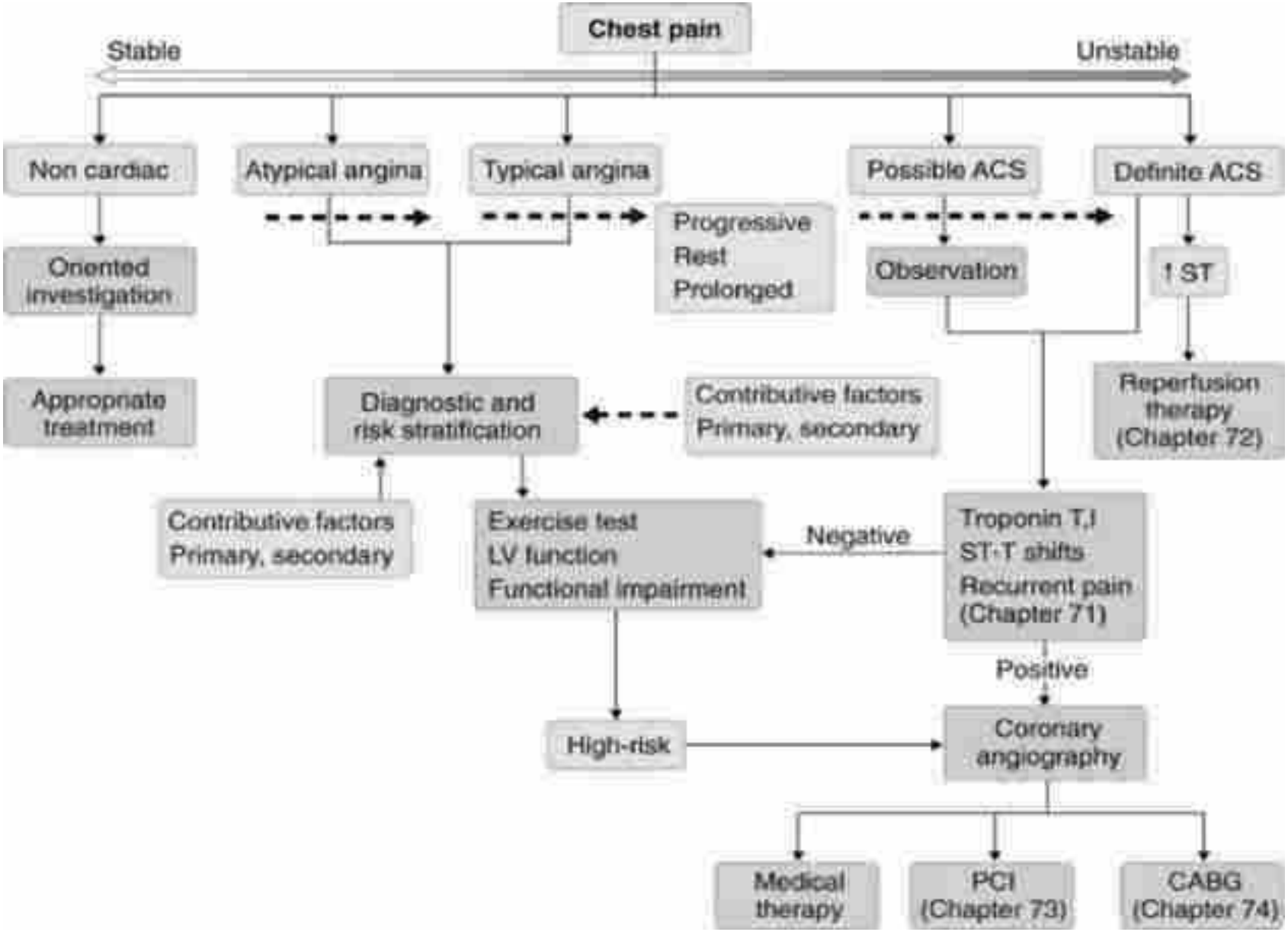
- **LEVINE'S SIGN**
  - He or she typically places hand over sternum, sometimes with Clenched fist, to indicate a squeezing, central, substernal discomfort.

ACTIVITIES PRECIPITATING ANGINA

- **Common**
  - Physical exertion, hurrying or sexual activity.
  - Stress, anger, fright, frustation.
  - Cold exposure.
  - Heavy meals.
  - Intense emotion.

- **Uncommon**
  - Lying flat (decubitus angina).
  - Vivid dreams (nocturnal angina).

Evaluation of Chest pain



Physical Findings

- Physical findings are normal in Stable Angina.
- Search for evidence of atherosclerotic disease at other sites, such as
  - an abdominal aortic aneurysm, carotid arterial bruits, and diminished arterial pulses in the lower extremities.
- **Xanthelasmas** and **xanthomas** .
- **Evidence for peripheral arterial disease**
  - (ankle-brachial index).
- **Examination of the fundi may reveal**
  - An increased light reflex and arteriovenous nicking as evidence of hypertension.

Physical Findings

- **Palpitations due to**
  - Cardiac Enlargement
  - Left ventricular Dyskinesia

- **Auscultation**
  - Arterial Bruits
  - 3rd or 4th heart sound
  - Apical Systolic Murmur in AR due to papillary dysfunction.

- Signs of anemia,
- Thyroid disease, and
- Nicotine stains on the fingertips.

A GUIDE TO RISK STRATIFICATION IN STABLE ANGINA

High risk	**Low risk**
Post-infarct angina	Predictable exertional angina
Poor effort tolerance	Good effort tolerance
Ischemia at low workload	Ischaemia only at high workload
Left main or three-vessel disease	Single-vessel or minor two-vessel disease
Poor LV function	Good LV function
N.B. Patients may fall between these categories.	

Disorders which may cause angina in the absence of coronary atherosclerosis.

Aortic Stenosis.

Aortic Regurgitation.

Pulmonary Hypertension &

Hypertrophic Cardiomyopathy.

Acute Coronary Syndrome

ACS

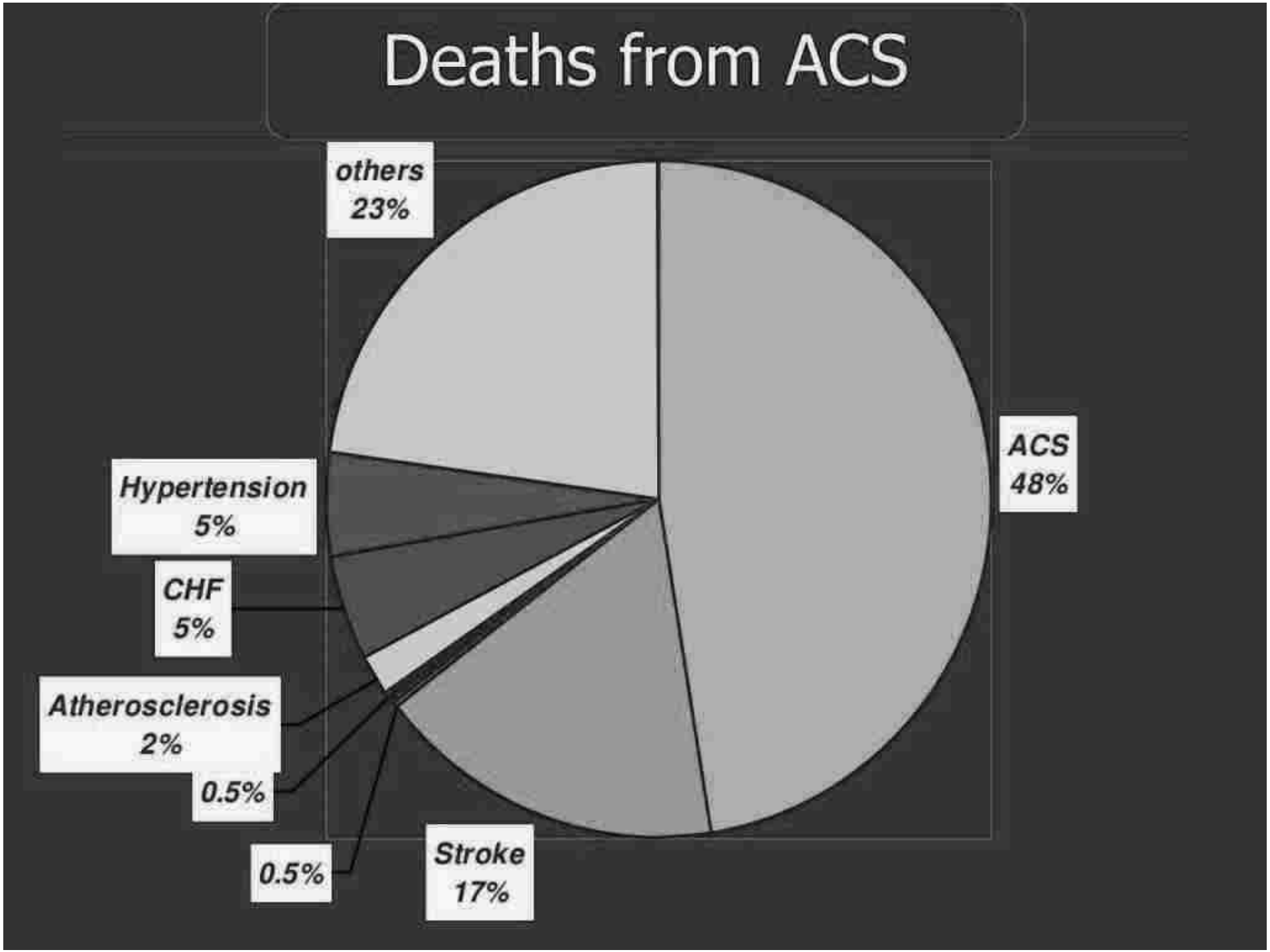
- **Acute coronary syndrome** (ACS) refers to a group of conditions due to decreased blood flow in the coronary arteries such *that part of the heart muscle is unable to function properly or dies*.
  - The most common symptom is **chest pain**,
  - often radiating to the left arm or angle of the jaw,
  - pressure-like in character, and
  - associated with nausea and sweating.

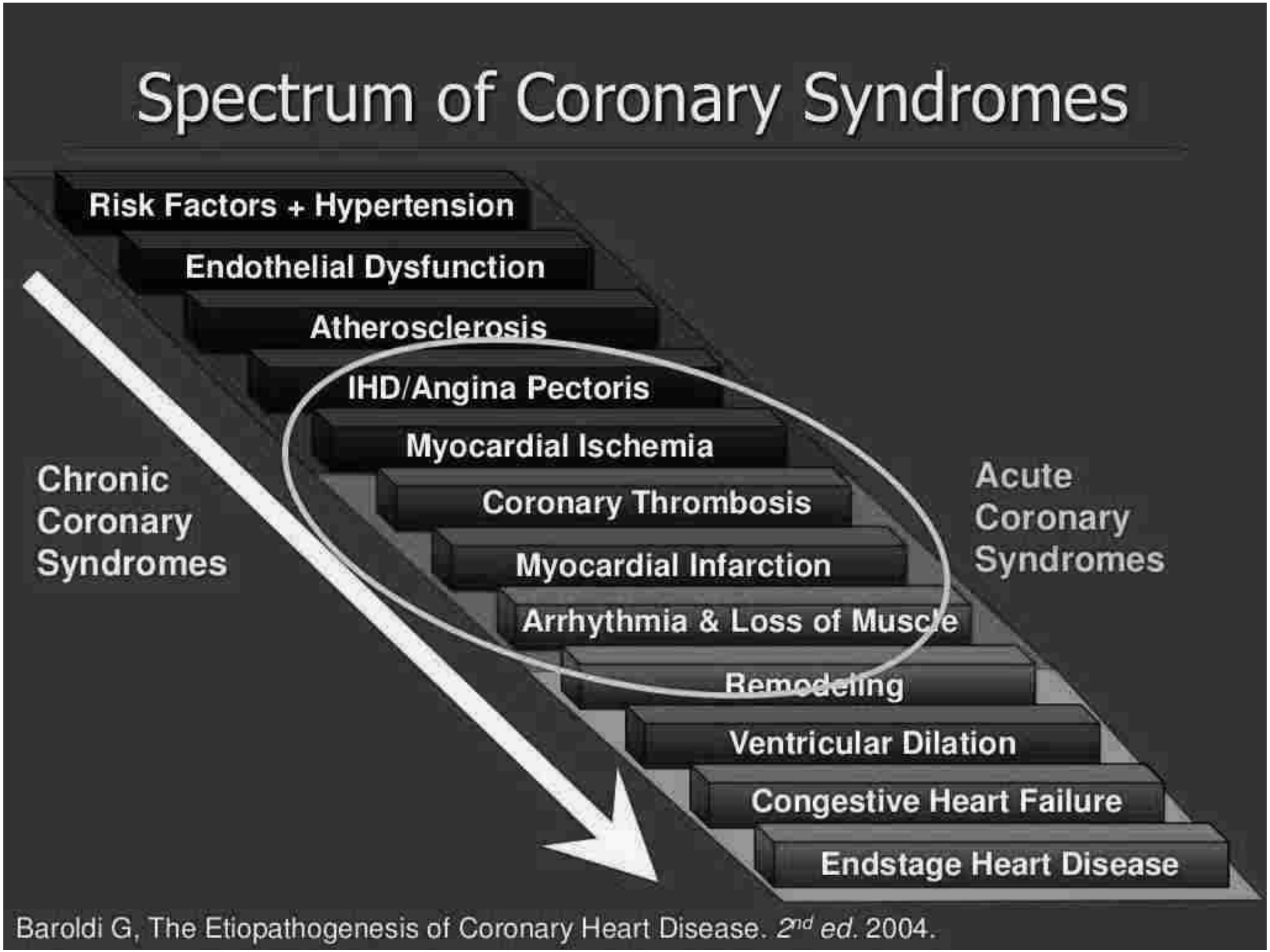
ACS : Definition

- ACC/AHA definition: "a group of clinical syndromes compatible with acute myocardial ischemia"
- Physiologic definition: Plaque rupture leading to thrombus formation, leading to partial or complete blockage of a coronary artery with or without myocyte death.
- Spectrum includes unstable angina (No ST elevation, normal biomarkers), NSTEMI (no ST elevation, abnormal biomarkers), STEMI (ST elevation, abnormal biomarkers)
- Unstable angina includes: angina at rest, new angina, increasing angina
- Anderson JL et al. **ACC/AHA 2007 guidelines for the Management of Patients With Unstable Angina/Non-ST-Elevation Myocardial Infarction**. J AM Coll Cardiology 2007. 50(7): e8

ACS

- Acute coronary syndrome usually occurs as a result of one of three problems:
  - Unstable Angina (38%).
  - STEMI (30%),
  - non ST(25%),


Deaths from ACS
others 23%
ACS 48%
Hypertension 5%
CHF 5%
Atherosclerosis 2%
0.5%
0.5%
Stroke 17%


Spectrum of Coronary Syndromes
Risk Factors + Hypertension
Endothelial Dysfunction
Atherosclerosis
IHD/Angina Pectoris
Myocardial Ischemia
Coronary Thrombosis
Myocardial Infarction
Arrhythmia & Loss of Muscle
Remodeling
Ventricular Dilation
Congestive Heart Failure
Endstage Heart Disease
Chronic Coronary Syndromes
Acute Coronary Syndromes
Baroldi G, The Etiopathogenesis of Coronary Heart Disease. 2nd ed. 2004.

Acute Coronary Syndromes

	Stable Angina	Unstable Angina	STEMI	NSTEMI
Character of pain	Exertional pain	Rest pain	Rest pain	Rest pain
Relievers	Responds to GTN	No GTN effect	No GTN effect	No GTN effect
Enzymes	Normal	Normal	Elevated	Elevated
ECG	Often normal	Often ST depression	ST segment elevation	No ST segment elevation

Risk Stratification to Determine the Likelihood of Acute Coronary Syndrome

Assessment	Findings indicating HIGH likelihood of ACS	Findings indicating INTERMEDIATE likelihood of ACS in absence of high-likelihood findings	Findings indicating LOW likelihood of ACS in absence of high- or intermediate-likelihood findings
History	Chest or left arm pain or discomfort as chief symptom Reproduction of previous documented angina K/c/o of coronary artery disease, including MI	Chest or left arm pain or discomfort as chief symptom Age > 50 years	Probable ischemic symptoms Recent cocaine use
Physical examination	New transient mitral regurgitation, hypotension, diaphoresis, pulmonary edema or rales	Extracardiac vascular disease	Chest discomfort reproduced by palpation
ECG	New or presumably new transient ST-segment deviation (> 0.05 mV) or T-wave inversion (> 0.2 mV) with symptoms	Fixed Q waves Abnormal ST segments or T waves not documented to be new	T-wave flattening or inversion of T waves in leads with dominant R waves Normal ECG
Serum cardiac markers	Elevated cardiac troponin T or I, or elevated CK-MB	Normal	Normal

Causes

- **M/C CAUSE IS**

  rupture of an atherosclerotic lesion within coronary wall with subsequent spasm and thrombus formation
- Coronary artery vasospasm
- Ventricular hypertrophy
- Hypoxia
- Coronary artery emboli
- Cocaine
- Coronary anomalies
- Aortic dissection
- Pediatrics Kawasaki disease,
- Takayasu arteritis
- Increased afterload which increases myocardial demand.

UNSTABLE ANGINA

Unstable Angina

UA is defined as

- **Angina Pectoris or equivalent ischemic discomfort with at least one of three features:**
  - (1) **it occurs at rest** (or with minimal exertion), usually lasting >10 minutes;
  - (2) **it is severe and of new onset** (i.e., within the prior 4–6 weeks); and/or
  - (3) **it occurs with a crescendo pattern** (i.e., distinctly more severe, prolonged, or frequent than previously).

- The diagnosis of NSTEMI is established if
  - a patient with the clinical features of UA develops evidence of myocardial necrosis, as reflected in elevated cardiac biomarkers.

Pathophysiology

- 4 pathophysiologic processes that may contribute to the development of UA/NSTEMI are
  - (1) **plaque rupture or erosion with a superimposed nonocclusive thrombus**
    - NSTEMI may occur with downstream embolization of platelet aggregates and/or atherosclerotic debris.
  - (2) **dynamic obstruction**
    - [e.g., coronary spasm, as in Prinzmetal's variant angina (PVA)].
  - (3)**Progressive mechanical obstruction** &
  - (4) **UA secondary to increased myocardial oxygen demand and/or decreased supply.**
    - More than one of these processes may be involved.

Unstable Angina
RISK STRATIFICATION

	High risk	**Low risk**
Clinical	Post-infarct angina Recurrent pain at rest Heart failure	No history of MI Rapid resolution of symptoms
ECG	Arrhythmia ST depression Transient ST elevation Persistent deep T-wave inversion	Minor or no ECG changes
Biochemistry	Troponin T > 0.1 µg/l	Troponin T < 0.1 µg/l

N.B. There is a 5- to 10-fold difference in risk between the lowest and highest risk groups.

Clinical features of ACS

History & Physical examination

History & Physical examination

- **Hallmark of UA/NSTEMI is**
  - ***Chest pain***
    - Substernal Region (m/c)
    - Epigastrium
    - Radiation to Neck, left shoulder, left arm.

- If pt has **large area of infarction Findings of large STEMI can be seen like**
  - Diaphoresis
  - Cold skin
  - Sinus tachycardia
  - 3^{rd} & 4^{th} heart sound.
  - Basal Rales.
  - Hypotension

Prinzmetal's Variant Angina

- In 1959 Prinzmetal et al. described a syndrome of severe ischemic pain that
  - **occurs at rest** but not usually with exertion &
  - is associated with **transient ST-segment elevation.**

- This syndrome is due to focal spasm of an
  - **epicardial coronary artery, leading to severe myocardial ischemia.**

- The cause of the spasm is not well defined, but it may be related to
  - **hypercontractility of vascular smooth muscle.**

Clinical features

- Patients are generally,
  - **younger**
  - **have fewer coronary risk factors** than patients with UA secondary to coronary atherosclerosis.
  - Cardiac examination is usually unremarkable.

- **The clinical diagnosis made on**
  - **transient ST-segment *elevation with rest* pain.**
- Many patients also exhibit multiple episodes of asymptomatic ST-segment elevation (***silent ischemia***)

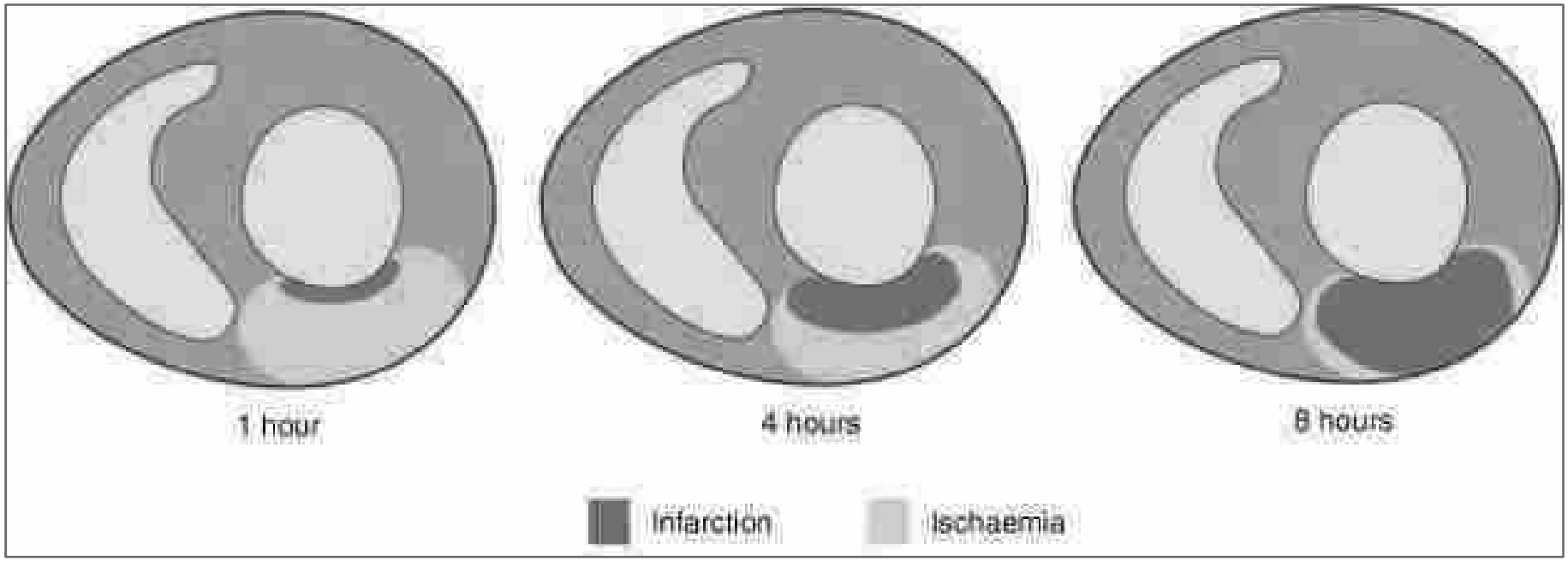
- *Small elevations* ***of troponin may occur in patients with prolonged attacks.***

Clinical & Angiographic

- **Diagnostic hallmark of PVA**
  - Coronary angiography demonstrates transient coronary spasm.
  - spasm usually occurs within 1 cm of the plaque.
  - Focal spasm is most common in the right coronary artery.
- **To Establish the diagnosis**
  - Ergonovine, acetylcholine, other vasoconstrictor medications, and hyperventilation have been used to provoke focal coronary stenosis on angiography.
  - Hyperventilation has also been used to provoke rest angina, ST-segment.
  - **elevation, and spasm on coronary arteriography.**

STEMI

The time course of myocardial infarction.



The relative proportion of ischaemic, infarcting and infarcted tissue slowly changes over a period of 12 hours. In the early stages of myocardial infarction a significant proportion of the myocardium in jeopardy is potentially salvageable.

- Acute myocardial infarction (AMI) is
  - **one of the most common diagnoses in hospitalized patients**
- In the United States,
  - **approximately 650,000 patients experience a new AMI.**
- The early (30-day) mortality rate from AMI is 30%
  - **with more than half of these deaths occurring before the pt reaches the hospital.**
- Mortality rate **after admission** for AMI has declined by 30% over the past two decades,
  - approximately 1 of every 25 patients who survives the initial hospitalization dies in the first year after AMI.
- Mortality is approximately **fourfold** higher in **elderly** pts.

CLINICAL FEATURES OF MYOCARDIAL INFARCTION

Symptoms

- Prolonged cardiac pain (m/c) at
Chest, throat, arms, epigastrium or back
heavy, squeezing, and crushing.

- Anxiety and fear of impending death

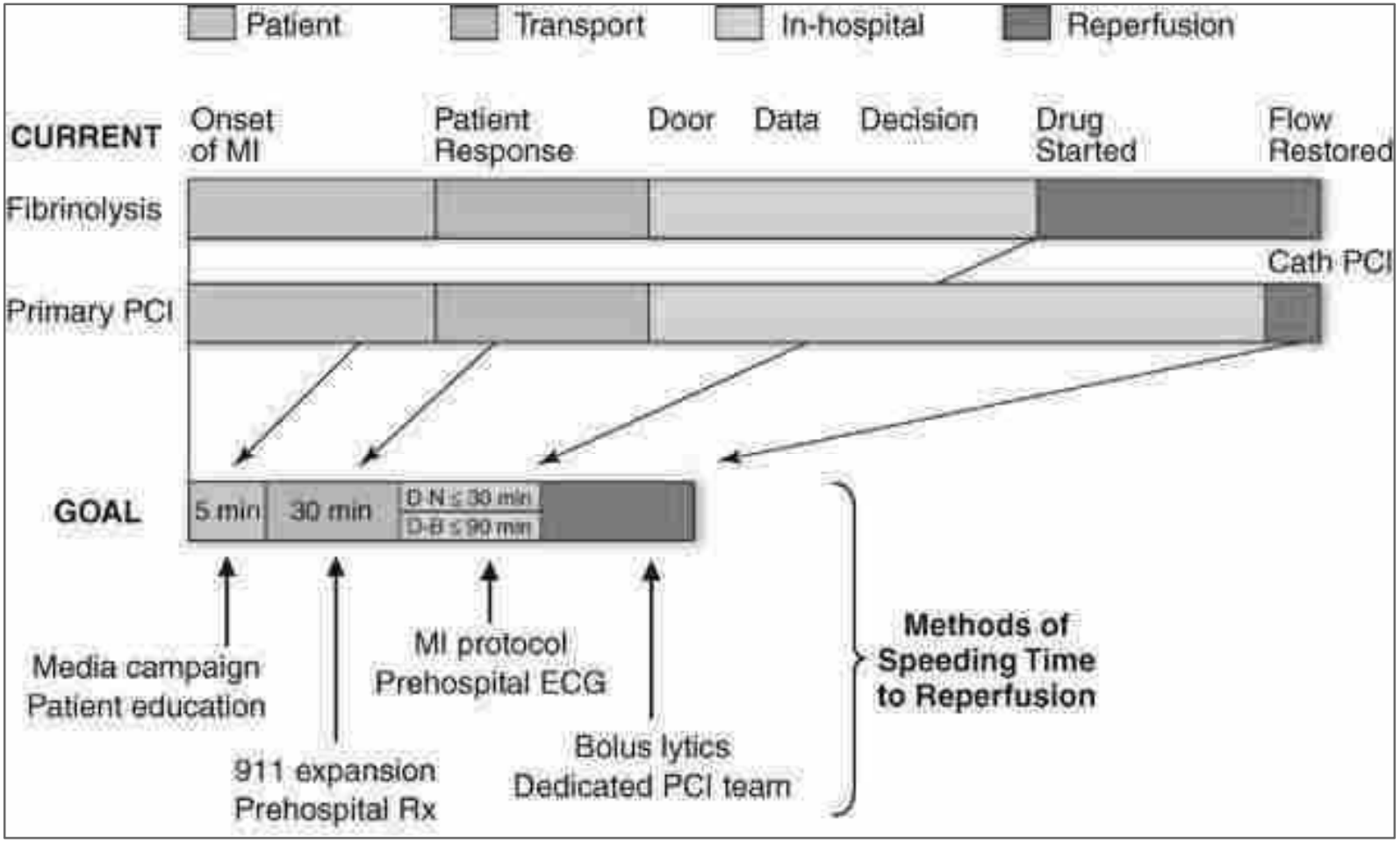
- Nausea and vomiting

- Breathlessness

- Collapse/syncope

Physical signs

- **Signs of sympathetic activation**
  - Pallor, sweating, tachycardia
- **Signs of vagal activation**
  - Vomiting, bradycardia
- **Signs of impaired myocardial function**
  - Hypotension, oliguria, cold peripheries
  - Narrow pulse pressure
  - Raised jugular venous pressure
  - Third heart sound
  - Quiet first heart sound
  - Diffuse apical impulse
  - Lung crepitations
- **Signs of tissue damage**
  - Fever
- **Signs of complications**,
- e.g. mitral regurgitation, pericarditis.



Major components of time delay between onset of symptoms from STEMI and restoration of flow in the infarct-related artery.

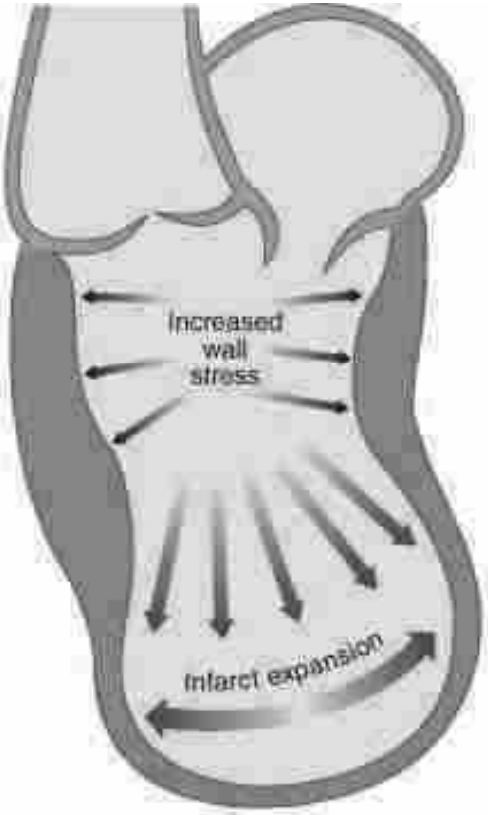
Complications of MI

- Arrhythmias / recurrent Chest Dyscomfort
- Acute circulatory failure
- Pericarditis
- Embolism
- Ischaemia
- Impaired ventricular function, remodelling and ventricular aneurysm.
- Mechanical complications (papillary muscle tear or damage.)

COMMON ARRHYTHMIAS IN ACUTE MYOCARDIAL INFARCTION

- Ventricular fibrillation
- Ventricular tachycardia
- Accelerated idioventricular rhythm
- Ventricular ectopics
- Atrial fibrillation
- Atrial tachycardia
- Sinus bradycardia (particularly after inferior MI)
- Heart block

Impaired ventricular function, remodelling and ventricular aneurysm



Infarct expansion and ventricular remodelling.
Full-thickness myocardial infarction causes thinning and stretching of the infarcted segment (infarct expansion), which leads to increased wall stress with progressive dilatation and hypertrophy of the remaining ventricle (ventricular remodelling).

Differentials diagnosis

- Acute coronary syndrome
- Anxiety
- Aortic stenosis

- Asthma
- Cholecystitis and biliary colic
- COPD

- Aortic Dissection
- Endocarditis
- Esophagitis
- Shock

- Myocarditis
- Pericarditis
- Pulmonary embolism
- Cholethiasis

DIAGNOSIS

Diagnosis of Stable Angina

DIAGNOSIS

1. History collection
2. Physical examination
3. Cardiac Enzymes
4. Electrocardiogram
5. Echocardiograms
6. Stress Tests
7. Nuclear Imaging
8. Angiography

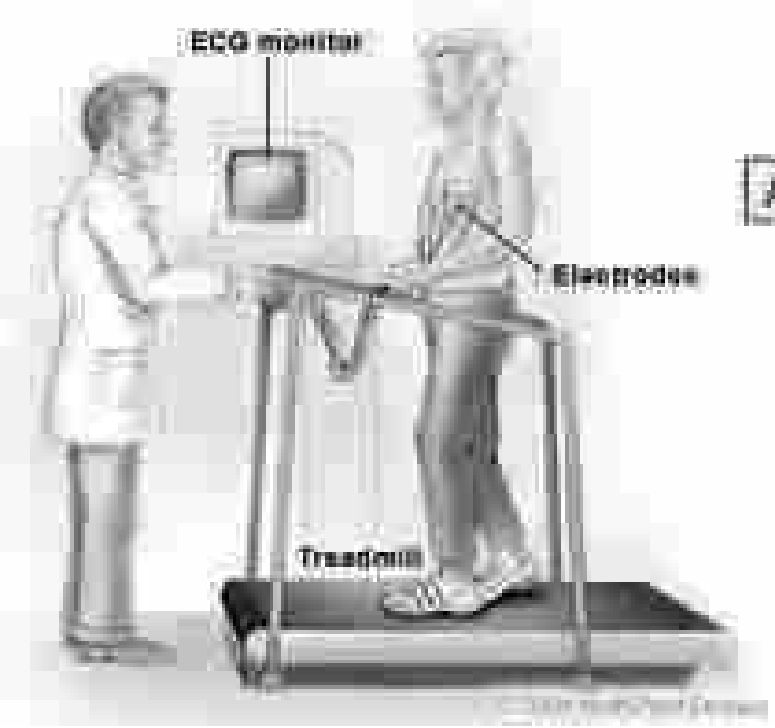
BLOOD Profile

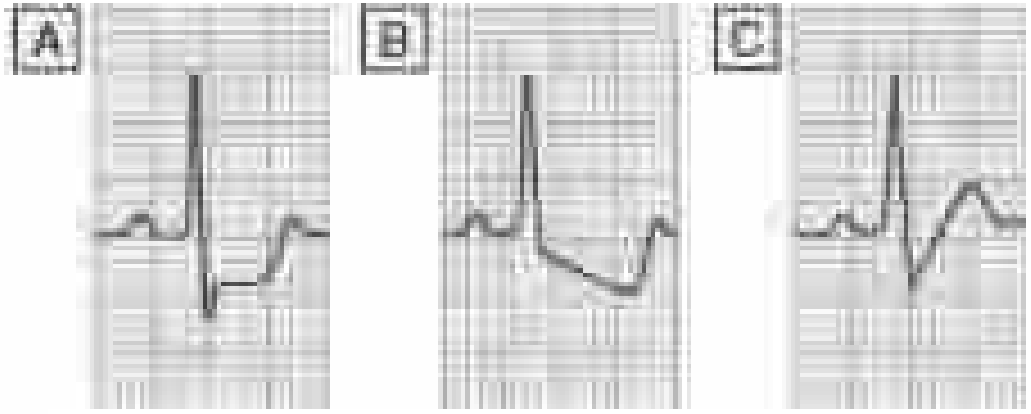
- **CBC – ANEMIA.**
- **Leukocytosis** may be observed within several hours after myocardial injury.
- **Potassium and magnesium levels**
  - should be monitored and corrected.
- **Creatinine levels** must be considered before
  - using contrast dye for coronary angiography.

C-reactive Protein (CRP)

- C- reactive protein is a marker of acute inflammation
- Patients without evidence of myocardial necrosis but with elevated CRP are at increased risk of an event

STRESS TESTS





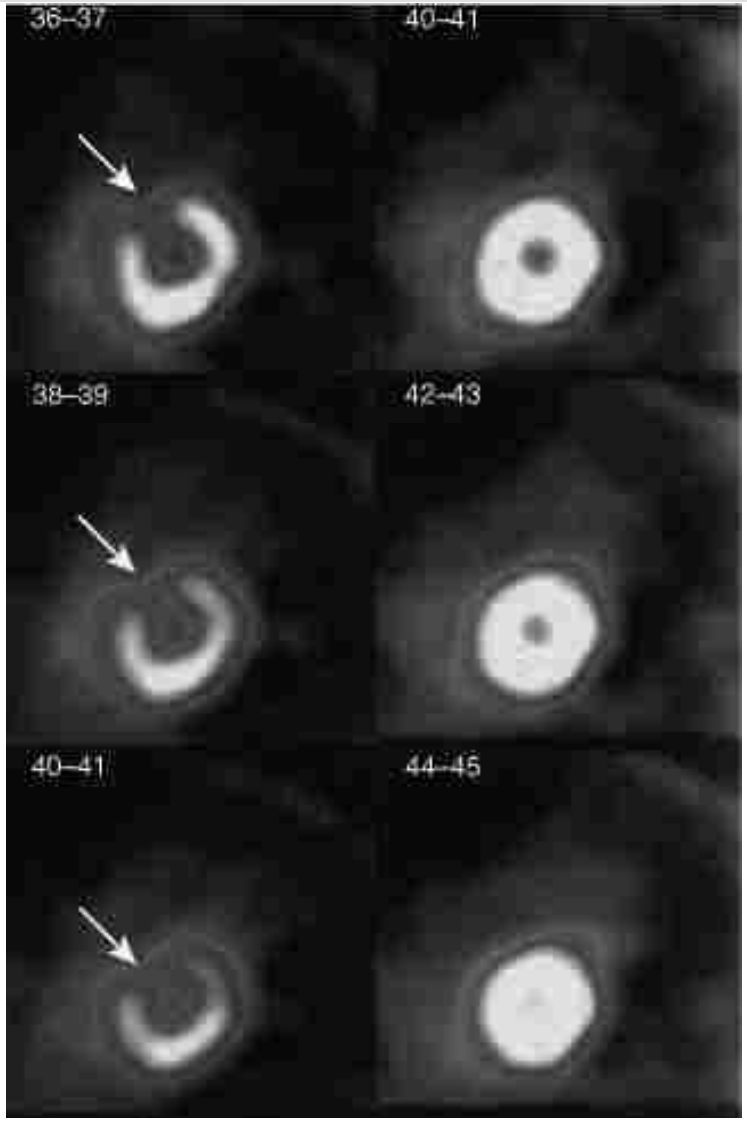
Forms of exercise-induced ST depression.

A) Planar ST depression is usually indicative of myocardial ischaemia.

B) Down-sloping depression also usually indicates myocardial ischaemia**.**

C) Up-sloping depression, however, may be a normal finding.

NUCLEAR CARDIAC IMAGING



A technetium scan showing reversible anterior myocardial ischaemia.
The images are cross-sectional tomograms of the left ventricle.
The resting scans (right) show even uptake of technetium and look like doughnuts; during stress (in this case a dobutamine infusion) there is reduced uptake of technetium, particularly along the anterior wall (arrows), and the scans look like crescents (left).

ANGIOGRAPHY

a) Is the most accurate means by which to examine the coronary arteries.

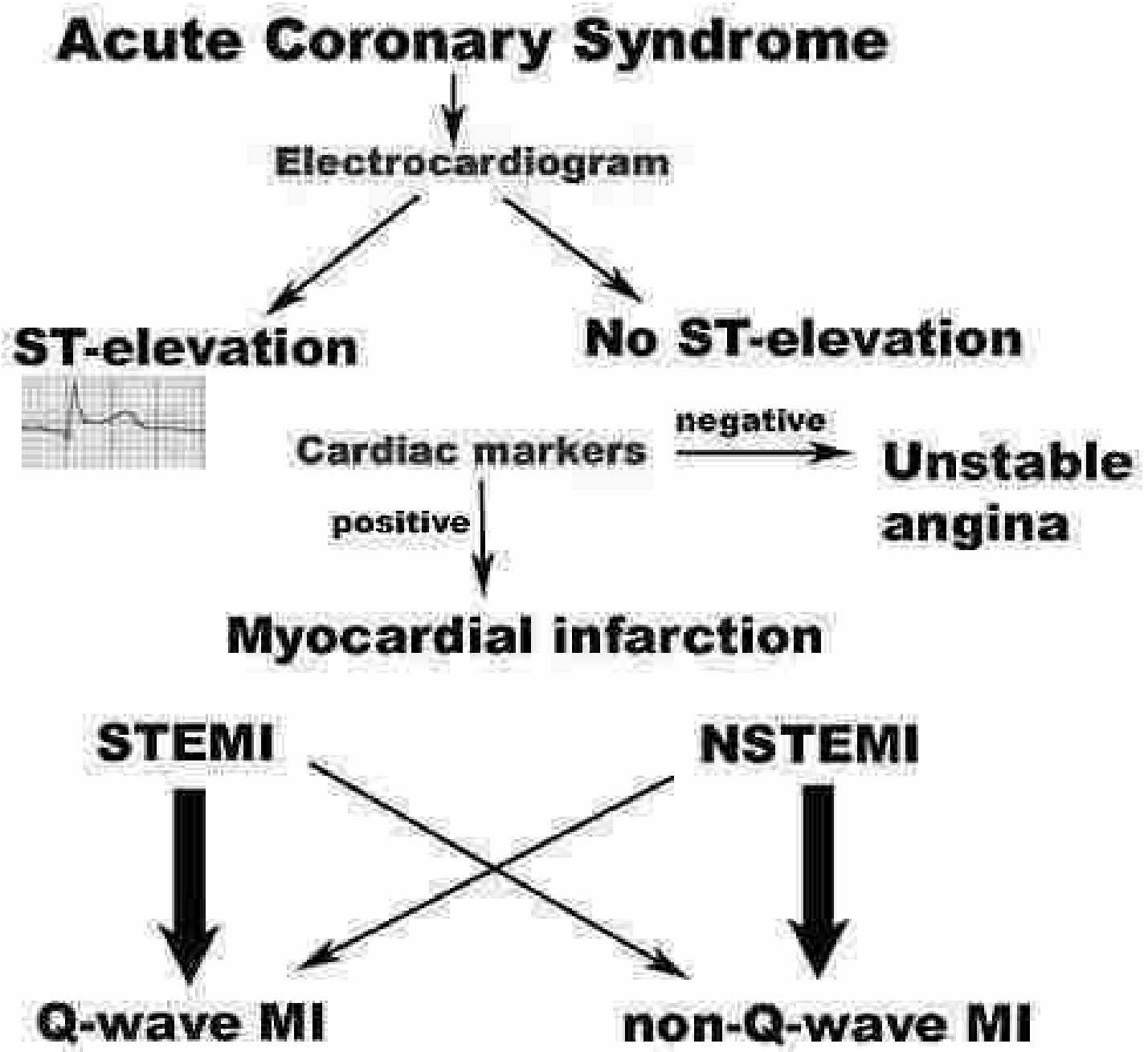
b) It requires a surgical procedure called cardiac catheterization.

c) During the procedure, catheters (small thin plastic tubes) are placed in the artery of the leg or arm, and directed using an x-ray machine to the opening of each of the coronary arteries.

Diagnosis of
Acute Coronary Syndrome

Diagnosis Pathway

1. Recognise or exclude MI using cardiac biomakers.
2. Evaluate for ischemia using serial or continuous ECG.
3. Evaluate for CAD after using provocative stress testing.
4. To exclude obstructive CAD CT- angiography is also used.


Acute Coronary Syndrome
Electrocardiogram
ST-elevation
No ST-elevation
Cardiac markers
negative
Unstable angina
positive
Myocardial infarction
STEMI
NSTEMI
Q-wave MI
non-Q-wave MI

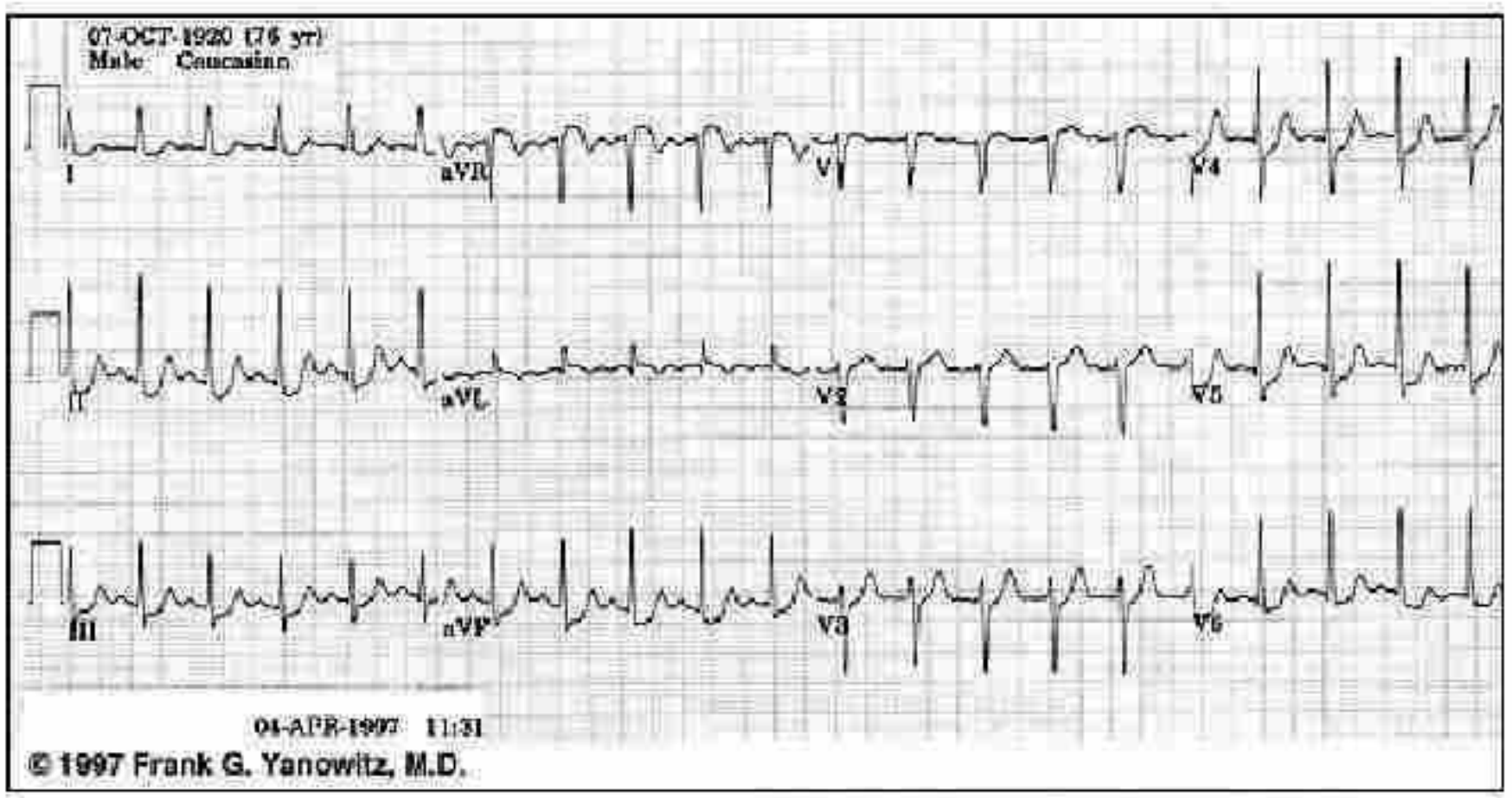
ECG

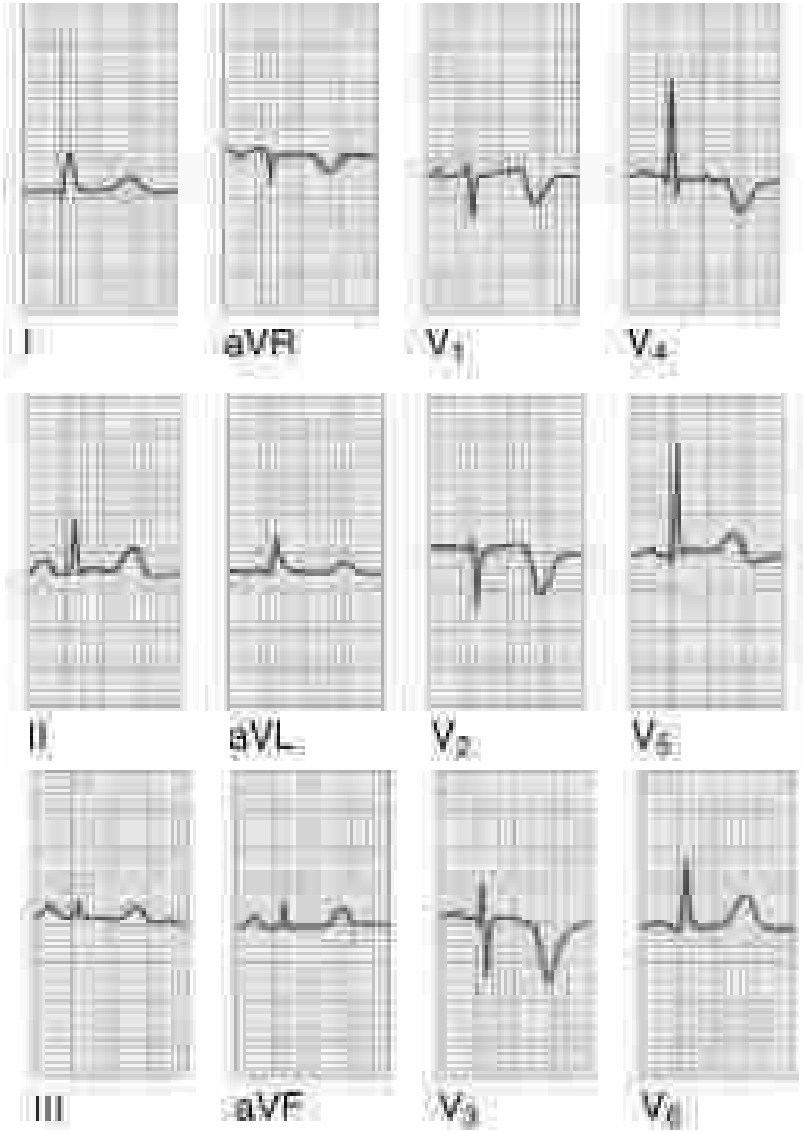
- **In UA**
  - ST-segment depression,
  - transient ST-segement elevation, &
  - T-wave inversion occurs in 30 – 50 % of patients.

- **Important predictor of adverse outcome is**
  - The presence of New ST-segment deviation.

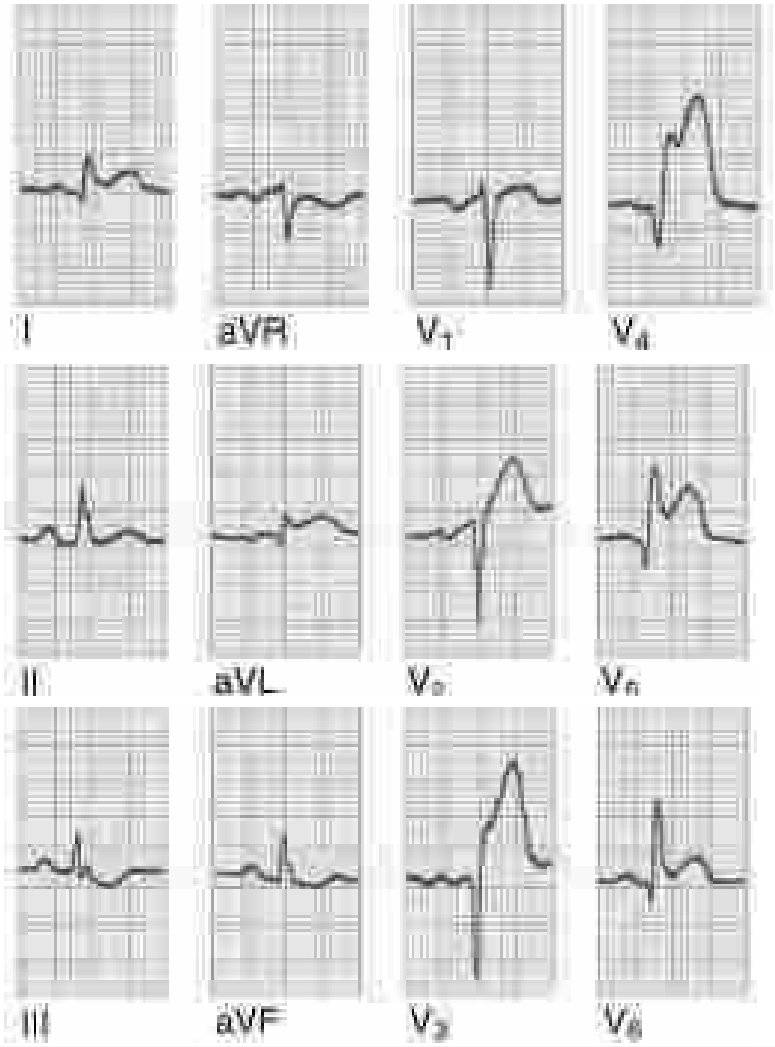
- **T-wave changes are sensitive for ischemia**
  - but less specific
  - unless they are new, deep T-wave inversion (> 0.3 mV)

NSTEMI


07-OCT-1920 (76 yr)
Male Caucasian
I
aVR
V1
V4
II
aVL
V2
V5
III
aVF
V3
V6
04-APR-1997 11:31
© 1997 Frank G. Yanowitz, M.D.

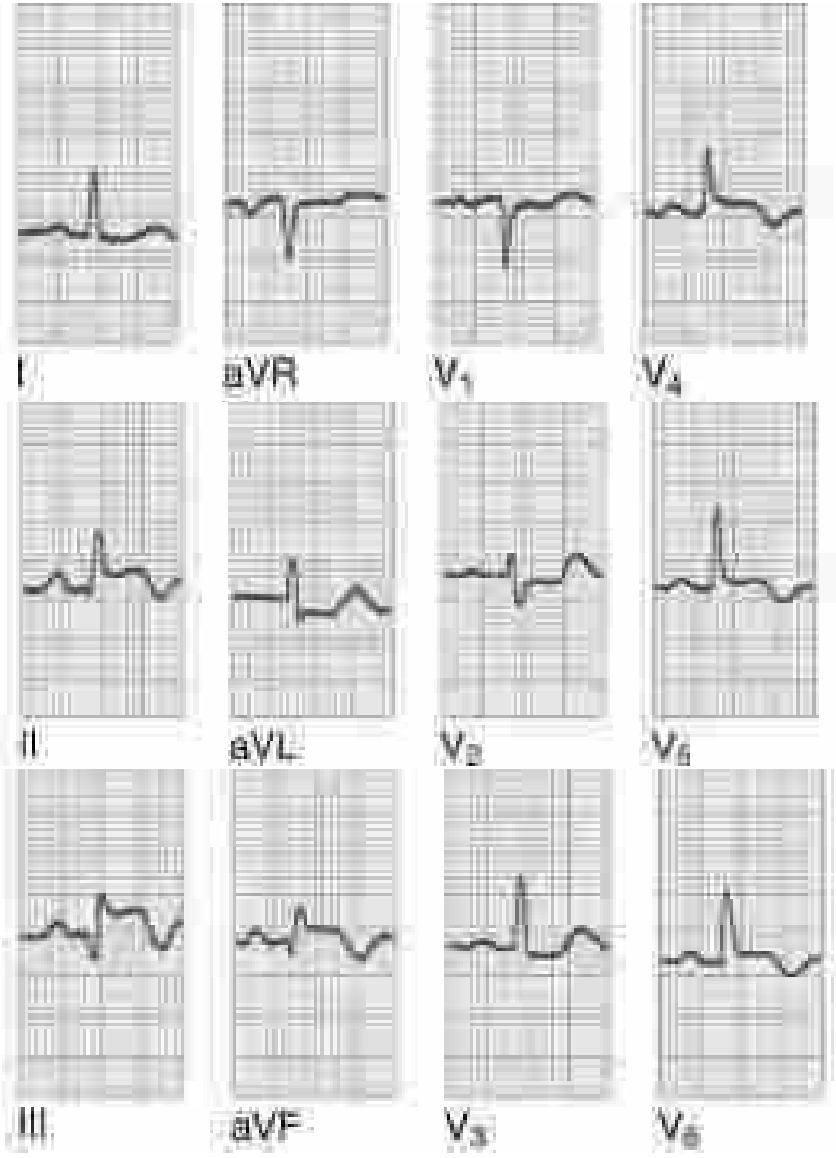


Recent anterior non-ST elevation (partial thickness) infarction. There is deep symmetrical T-wave inversion together with a reduction in the height of the R wave in leads V_1, V_2, V_3 and V_4.

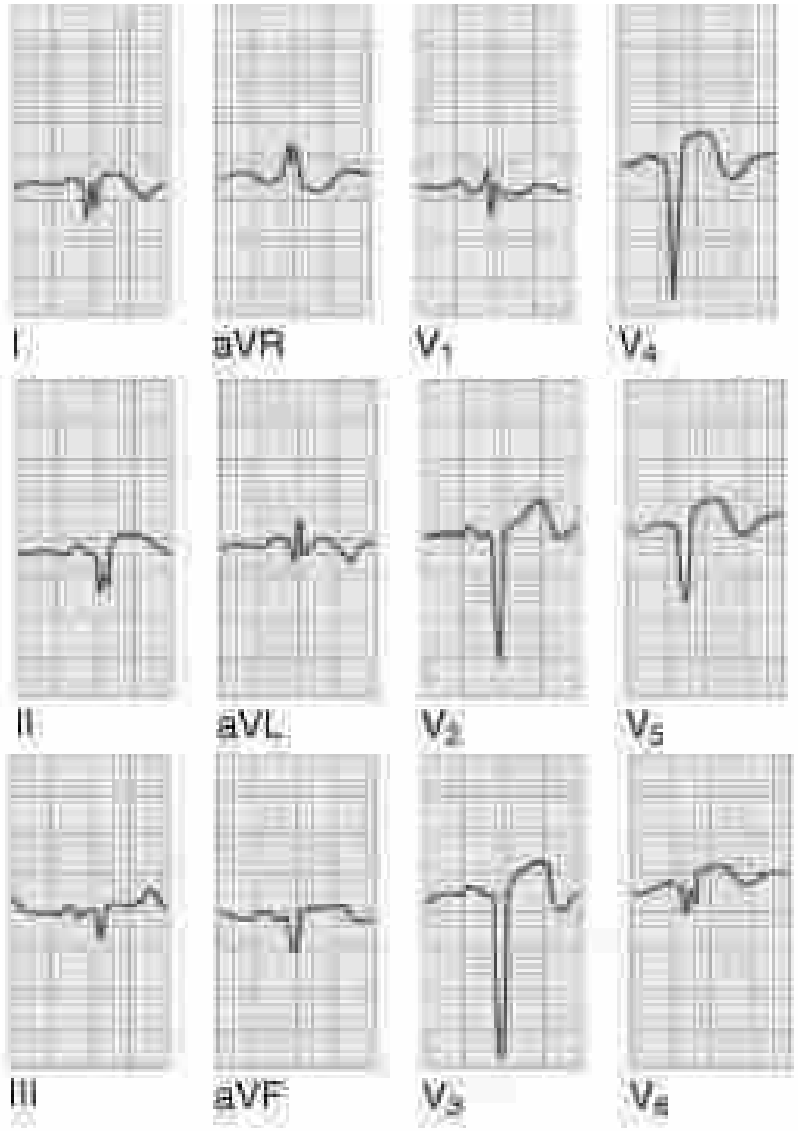


Acute full thickness anterior myocardial infarction.

This ECG was recorded from a 48-year-old man who had developed severe chest pain 6 hours earlier. There is ST elevation in leads I, aVL, V_2, V_3, V_4, V_5 and V_6, and there are Q waves in leads V_3, V_4 and V_5. Anterior infarcts with prominent changes in leads V_2, V_3 and V_4 are sometimes called 'anteroseptal' infarcts, as opposed to 'anterolateral' infarcts in which the ECG changes are predominantly found in V_4, V_5 and V_6.

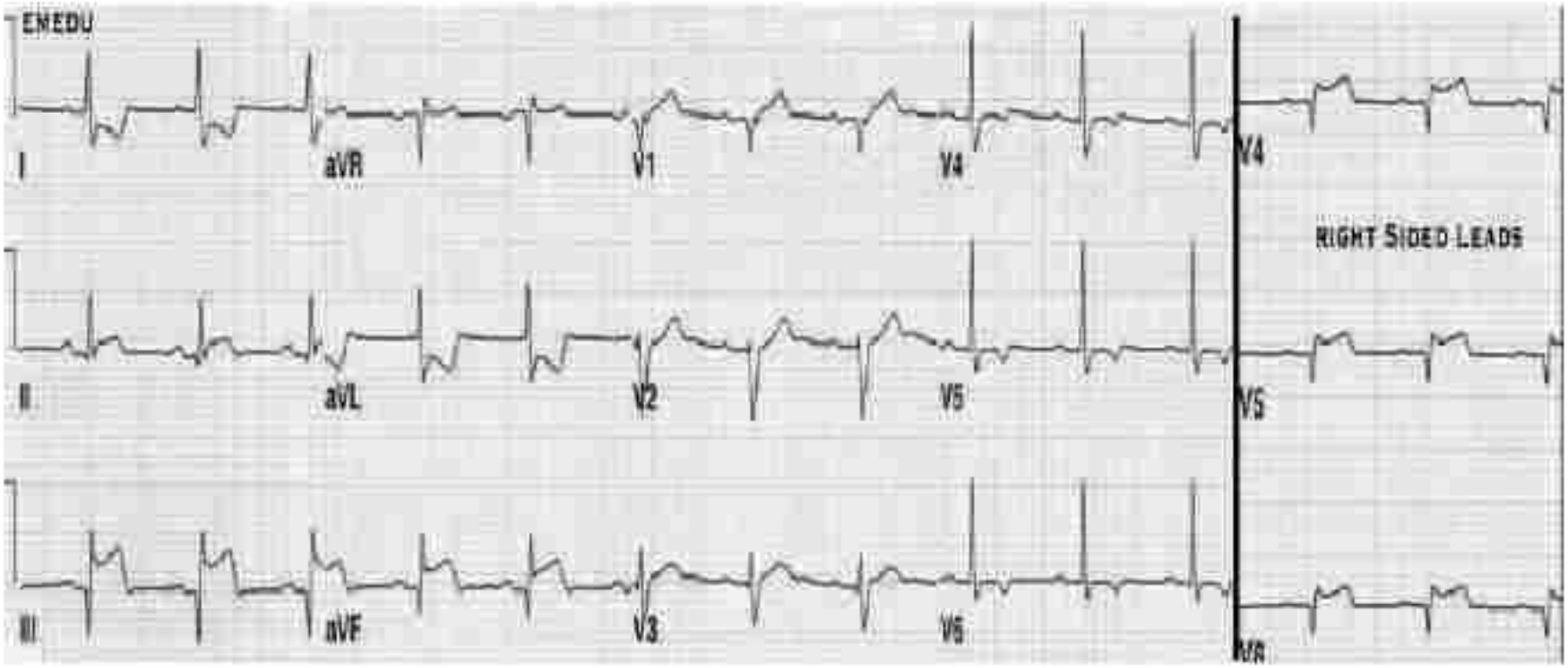


Acute full-thickness inferolateral myocardial infarction.
This ECG was recorded from a 55-year-old woman who had developed severe chest pain 4 hours earlier. There is ST elevation in the inferior leads II, III and aVF and the lateral leads V_4, V_5 and V_6. There is also 'reciprocal' ST depression in leads aVL and V_2.

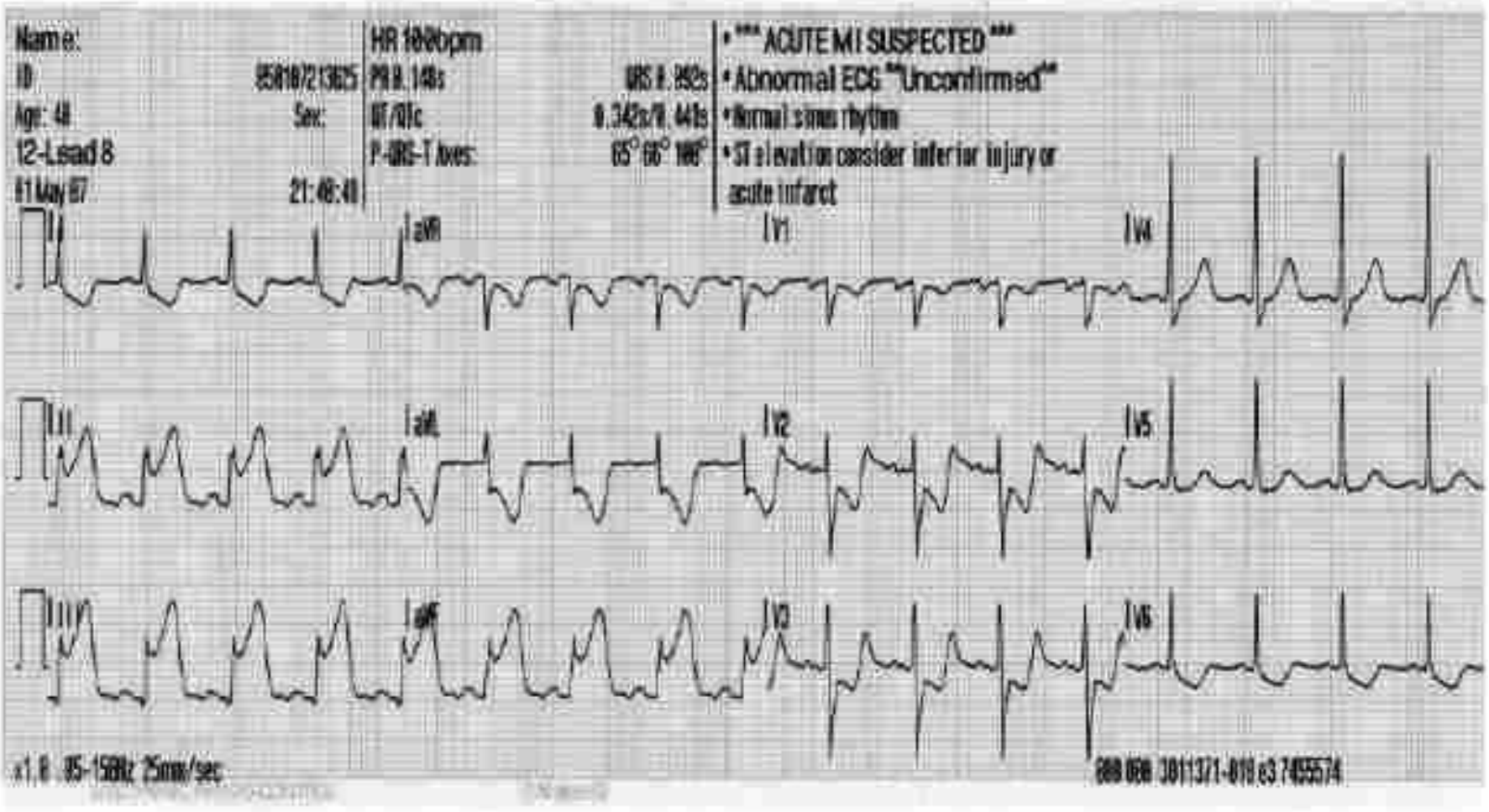


Established anterior and inferior full-thickness infarction. This ECG was recorded from a 70-year-old man who had presented with an acute anterior infarct 2 days earlier and had been treated for an inferior myocardial infarct 11 months before then. There are Q waves in the inferior leads (II, III and aVF) and Q waves with some residual ST elevation in the anterior leads (I and V_2-V_6).

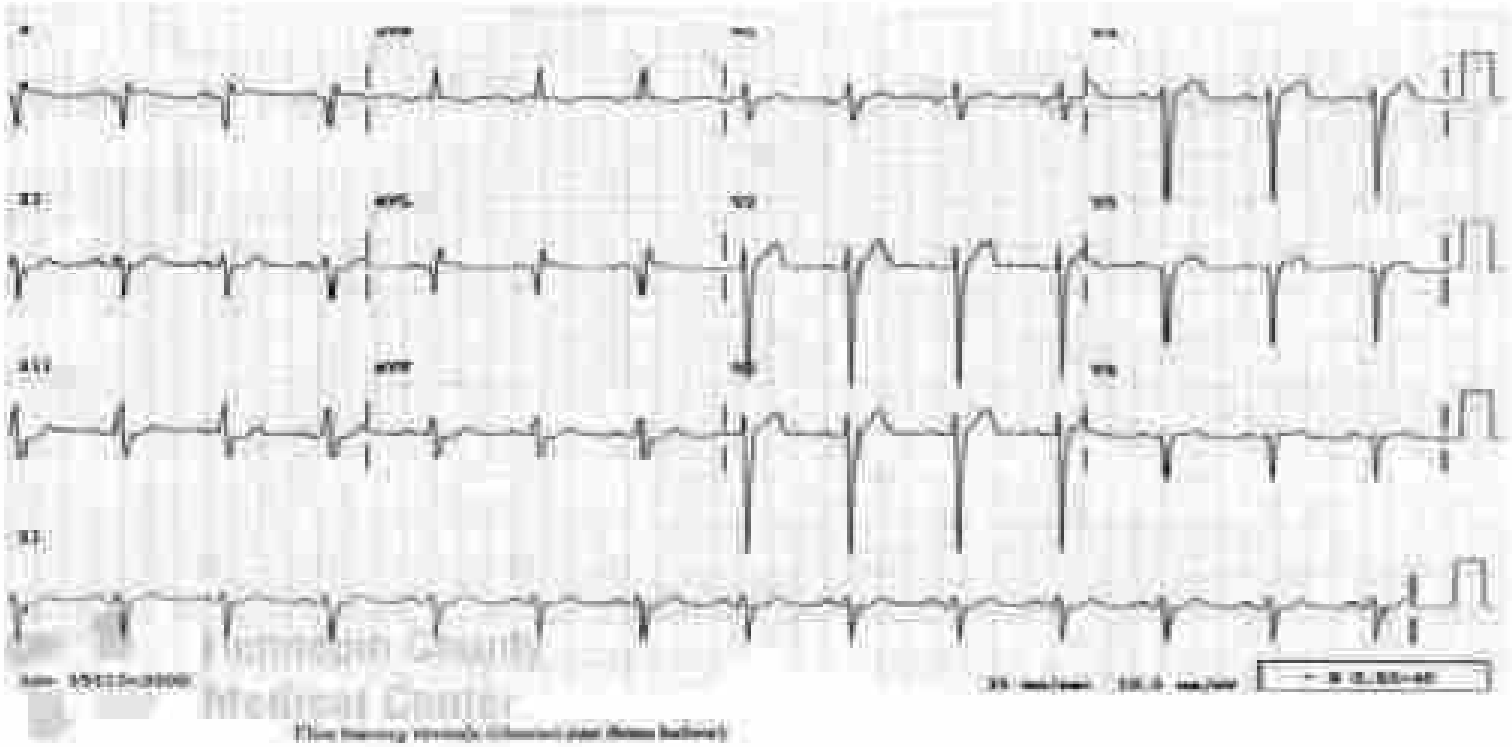
Right Ventricular MI



Inferior MI


Name:
ID
Age: 48
12-Lead 8
01 May 07
Sex:
21:46:48
HR 100bpm
PR 0.14s
QT/QTc
P-QRS-T axes:
QRS 0.09s
0.34s/0.44s
65° 66° 166°
*** ACUTE MI SUSPECTED ***
* Abnormal ECG **Unconfirmed**
* Normal sinus rhythm
* ST elevation consider inferior injury or acute infarct
I
aVR
V1
V4
II
aVL
V2
V5
III
aVF
V3
V6
x1.0 .05-150Hz 25mm/sec

Lateral MI

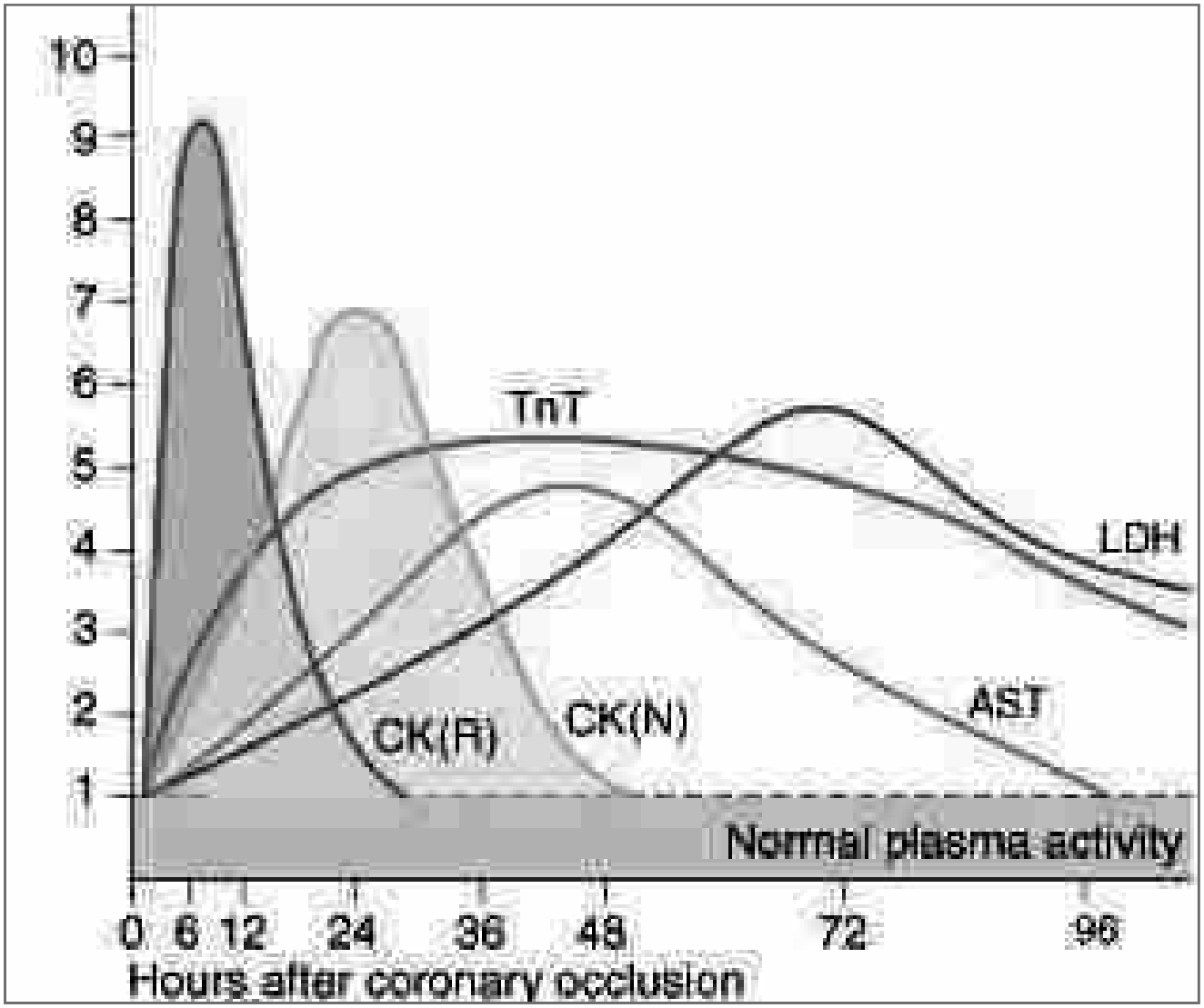


1) Lateral (d)
2) Reversed arm leads
3) Dextrocardia as a part of situs inversus
4) Lateral left and right-sided precordial leads

homedoctor.com

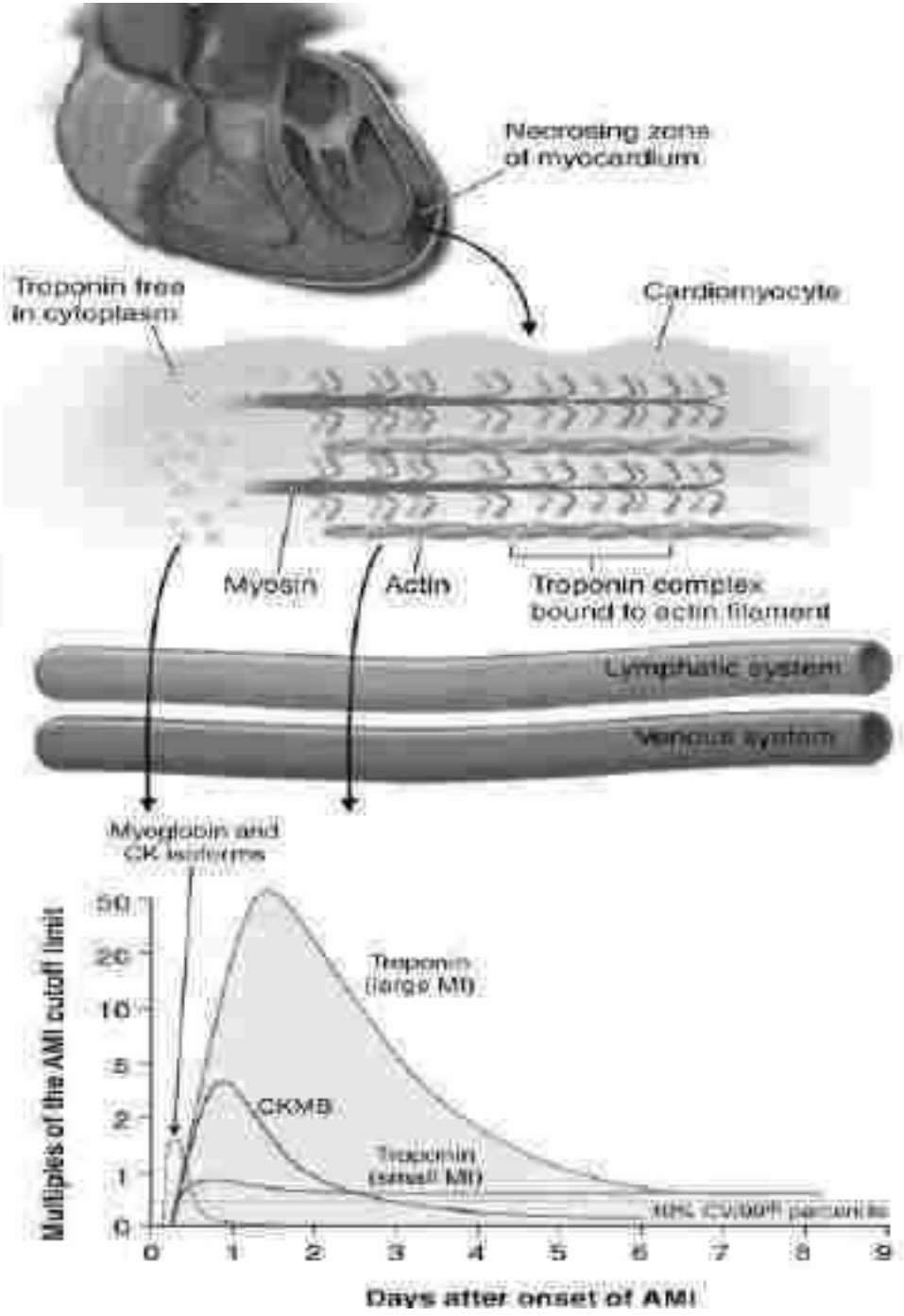
Biomarkers

- **<u>Increased level of CK-MB & Troponin</u>**
  - in patient with UA/STEMI are at increased risk for Death or Recurrent MI.
- **<u>Direct correlation btw</u>**
  - Death & mortality with level of Troponin.
- **<u>Clinical history should also support</u>**
  - UA/STEMI.



Creatine kinase (CK) and troponin T (TnT) are the first to rise, followed by aspartate aminotransferase (AST) and then lactate (hydroxybutyrate) dehydrogenase (LDH). In patients treated with a thrombolytic agent, reperfusion is usually accompanied by a rapid rise in plasma creatine kinase (curve CK (R)) due to a washout effect; if there is no reperfusion, the rise is less rapid but the area under the curve is often greater (curve CK (N)).

Serum Cardiac Biomarkers



- CK starts to rise at 4-6 hours, peaks at about 12 hours and falls to normal within 48-72 hours.

- **CK is also present in**
  - skeletal muscle,
  - sometimes due to an intramuscular injection,
  - vigorous physical exercise or,
  - in old people particularly, a fall.

- **Defibrillation causes significant release of**
  - CK but not CK-MB or troponins.

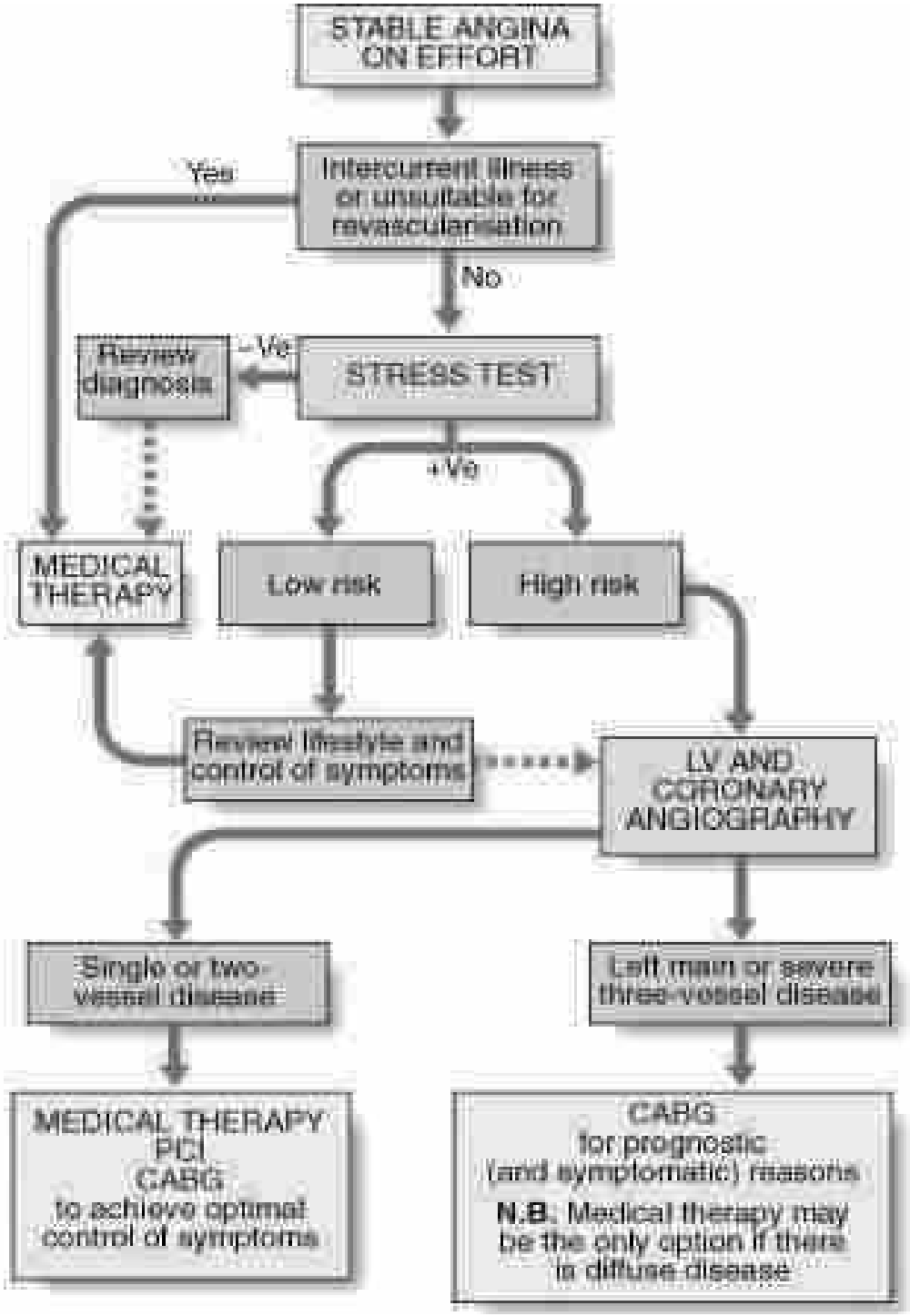
- **Most sensitive markers** of myocardial cell damage are
  - **cardiac troponins T and I**, which are released within 4-6 hours and remain elevated for up to 2 weeks.

American College of Cardiology and the European Society of Cardiology

- **have redefined MI as**
  - 'a typical rise in cardiac troponin T or I, or CK-MB, above the 99th centile for normal, with at least one of the following: ischaemic symptoms, development of pathological Q waves on the ECG, ischaemic ECG changes (ST depression or elevation) or coronary artery intervention (e.g. PCI)'.

- **This definition therefore includes**
  - non-ST segment elevation MIs as well as those that evolve through ST segment elevation and Q wave development.

Treatment of STABLE ANGINA


STABLE ANGINA ON EFFORT
Intercurrent illness or unsuitable for revascularisation
Yes
No
STRESS TEST
-Ve
Review diagnosis
+Ve
MEDICAL THERAPY
Low risk
High risk
Review lifestyle and control of symptoms
LV AND CORONARY ANGIOGRAPHY
Single or two-vessel disease
Left main or severe three-vessel disease
MEDICAL THERAPY
PCI
CABG
to achieve optimal control of symptoms
CABG
for prognostic (and symptomatic) reasons
N.B. Medical therapy may be the only option if there is diffuse disease

ADVICE TO PATIENTS WITH STABLE ANGINA

- Do not smoke
- Aim at ideal body weight
- Take regular exercise (exercise up to, but not beyond, the point of chest discomfort is beneficial and may promote collateral vessels)
- Avoid severe unaccustomed exertion, and vigorous exercise after a heavy meal or in very cold weather
- Take sublingual nitrate before undertaking exertion that may induce angina

Treatment:
Stable Angina Pectoris

(1) explanation of the problem and reassurance about the ability to formulate a treatment plan.

(2) identification and treatment of aggravating condition,

(3) recommendations for adaptation of activity as needed,

(4) treatment of risk factors that will decrease the occurrence of adverse coronary outcomes,

(5) drug therapy for angina, and

(6) consideration of revascularization.

EXPLANATION AND REASSURANCE

- A planned program of rehabilitation can encourage patients,
  - To lose weight,
  - Improve exercise tolerance,
  - Control risk factors with more confidence.

IDENTIFICATION AND TREATMENT OF AGGRAVATING CONDITIONS

- **Obesity**, **HTN**, and **hyperthyroidism** should be treated aggressively.

- **Stop cigarette smoking**
  - (Reduced oxygenation of arterial blood due to decreased Myocardial O2 supply in pulmonary disease or when carboxyhemoglobin.)

- Correction of these abnormalities, if present
  - **may reduce or even eliminate angina pectoris**.

ADAPTATION OF ACTIVITY

- **Physical conditioning usually improves**
  - exercise tolerance of patients with angina
  - psychological benefits.

- **A regular program of isotonic exercise** that is
  - within the limits of the individual patient's threshold for the development of angina pectoris and that does not exceed 80% of the heart rate associated with ischemia on exercise testing .

- **Based on the results of an exercise test**,
  - the number of metabolic equivalent tasks (**METs**)
  - a practical exercise prescription can be formulated to permit daily activities.

Pharmacologic Therapy

- Therapy is aimed in restoring balance btw
  - myocardial oxygen supply and demand.

- **Useful Agents**
  - Nitrates
  - Beta-blockers and
  - Calcium channel blockers.

Table 243–4. Nitroglycerin and Nitrates for Patients with Ischemic Heart Disease

Compound	Route	Dose	Duration of Effect
Nitroglycerin	Sublingual tablets	0.3–0.6 mg up to 1.5 mg	Approximately 10 min
	Spray	0.4 mg as needed	Similar to sublingual tablets
	Ointment	2% 6 x 6 in. 15 x15 cm	Effect up to 7 h
		7.5–40 mg	
	Transdermal	0.2–0.8 mg/h every 12 h	8–12 h during intermittent therapy
	Oral sustained release	2.5–13 mg	4–8 h
	Intravenous	5–200 mcg/min	Tolerance may be seen in 7–8 h
Isosorbide dinitrate	Sublingual	2.5–10 mg	Up to 60 min
	Oral	5–80 mg, 2–3 times daily	Up to 8 h
	Spray	1.25 mg daily	2–3 min
	Chewable	5 mg	2–2 1/2 h
	Oral slow release	40 mg 1–2 daily	Up to 8 h
	Intravenous	1.25–5.0 mg/h	Tolerance in 7–8 h
	Ointment	100 mg/24 h	Not effective
Isosorbide mononitrate	Oral	20 mg twice daily	12–24 h
		60–240 mg once daily	
Pentaerythritol tetranitrate	Sublingual	10 mg as needed	Not known

Table 243–5. Properties of Beta Blockers in Clinical Use for Ischemic Heart Disease

Drugs	Selectivity	Partial Agonist Activity	Usual Dose for Angina
Acebutolol	β1	Yes	200–600 mg twice daily
Atenolol	β1	No	50–200 mg/d
Betaxolol	β1	No	10–20 mg/d
Bisoprolol	β1	No	10 mg/d
Esmolol (intravenous)[a]	β1	No	50–300 mcg/kg/min
Labetalol[b]	None	Yes	200–600 mg twice daily
Metoprolol	β1	No	50–200 mg twice daily
Nadolol	None	No	40–80 mg/day
Nebivolol	β1 (at low doses)	No	5–40 mg/day
Pindolol	None	Yes	2.5–7.5 mg 3 times daily
Propranolol	None	No	80–120 mg twice daily
Timolol	None	No	10 mg twice daily

Note: This list of beta blockers that may be used to treat patients with angina pectoris is arranged alphabetically. The agents for which there is the greatest clinical experience include atenolol, metoprolol, and propranolol. It is preferable to use a sustained-release formulation that may be taken once daily to improve the patient's compliance with the regimen.

[a]Esmolol is an ultra-short-acting beta blocker that is administered as a continuous intravenous infusion. Its rapid offset of action makes esmolol an attractive agent to use in patients with relative contraindications to beta blockade.

[b]Labetolol is a combined alpha and beta blocker.

Table 243–6. Calcium Channel Blockers in Clinical Use for Ischemic Heart Disease

Drugs	Usual Dose	Duration of Action	Side Effects
Dihydropyridines			
Amlodipine	5–10 mg qd	Long	Headache, edema
Felodipine	5–10 mg qd	Long	Headache, edema
Isradipine	2.5–10 mg bid	Medium	Headache, fatigue
Nicardipine	20–40 mg tid	Short	Headache, dizziness, flushing, edema
Nifedipine	Immediate release:* 30–90 mg daily orally Slow release: 30–180 mg orally	Short	Hypotension, dizziness, flushing, nausea, constipation, edema
Nisoldipine	20–40 mg qd	Short	Similar to nifedipine
Nondihydropyridines			
Diltiazem	Immediate release: 30–80 mg 4 times daily Slow release: 120–320 mg qd	Short Long	Hypotension, dizziness, flushing, bradycardia, edema
Verapamil	Immediate release: 80–160 mg tid Slow release: 120–480 mg qd	Short Long	Hypotension, myocardial depression, heart failure, edema, bradycardia

Drugs

- **<u>Primary coronary vasospasm:</u>**
  - no treatment with beta blockers, it could increase coronary constriction
- **Nitrates and CCB are preferred.**
- **<u>Concomitant hypertension:</u>**
  - BB or CCB are useful in treatment
- **<u>Ischemic Heart Disease & Atrial Fibrillation:</u>**
  - treatment with BB, verapamil or Cardizem can slow ventricular rate

Combination Therapy

- If patients do not respond to initial antianginal therapy
  - a drug dosage increase is recommended unless side effects occur.

- **<u>Combination therapy:</u>**
  - successful use of lower dosages of each agent while minimizing individual drug side effects.

Combination Therapy Include:

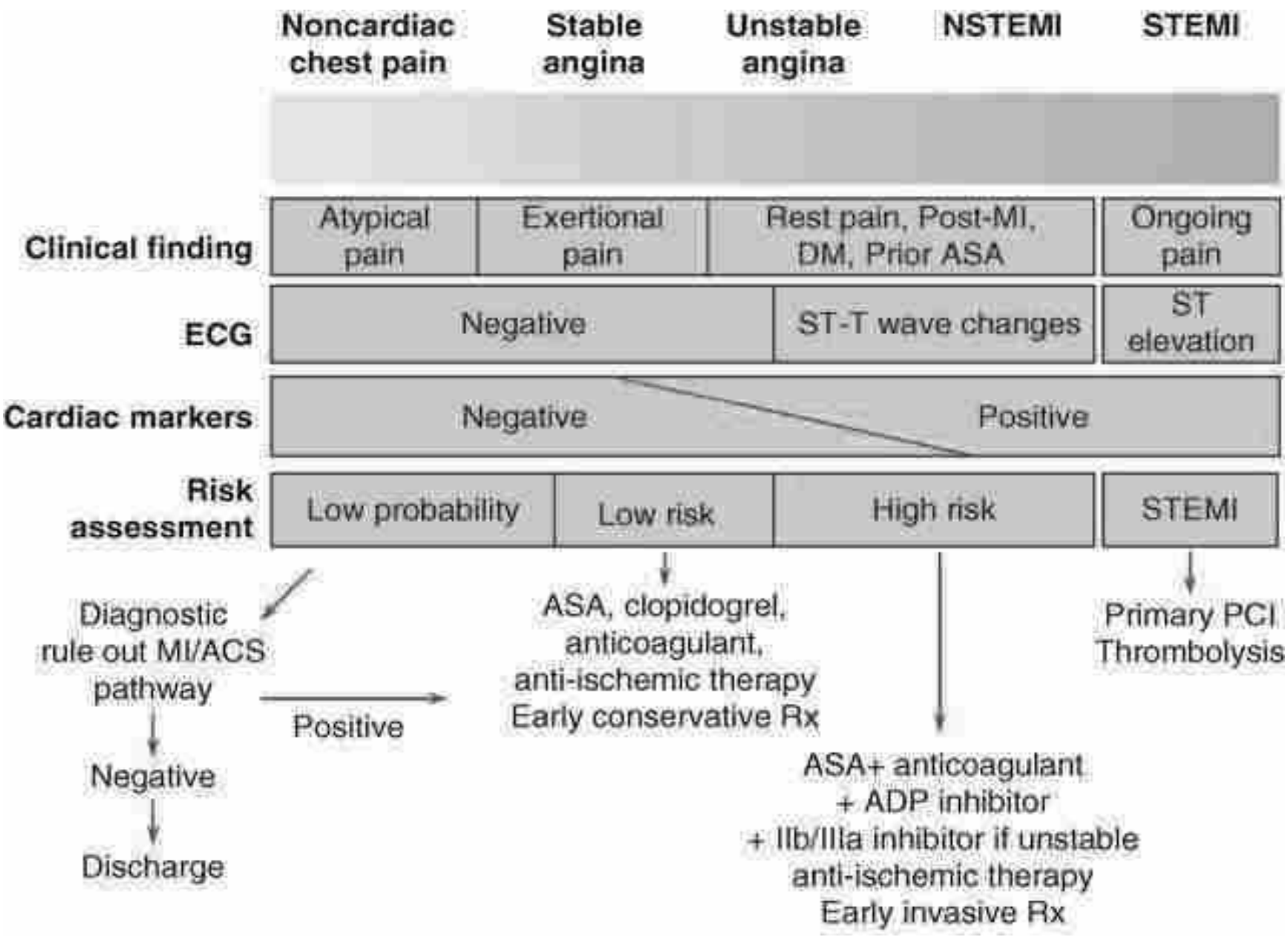
- **Nitrate** and **beta blocker.**
- **Nitrate** and **verapamil** or **cardizem** for similar reasons
- Long acting **dihydropyridine calcium channel blocker & beta blocker.**
- A dihydropyridine and nitrate is often not tolerated without concomitant beta blockade
    - because of marked vasodilatation with resultant head ache and increased heart rate

Combinations

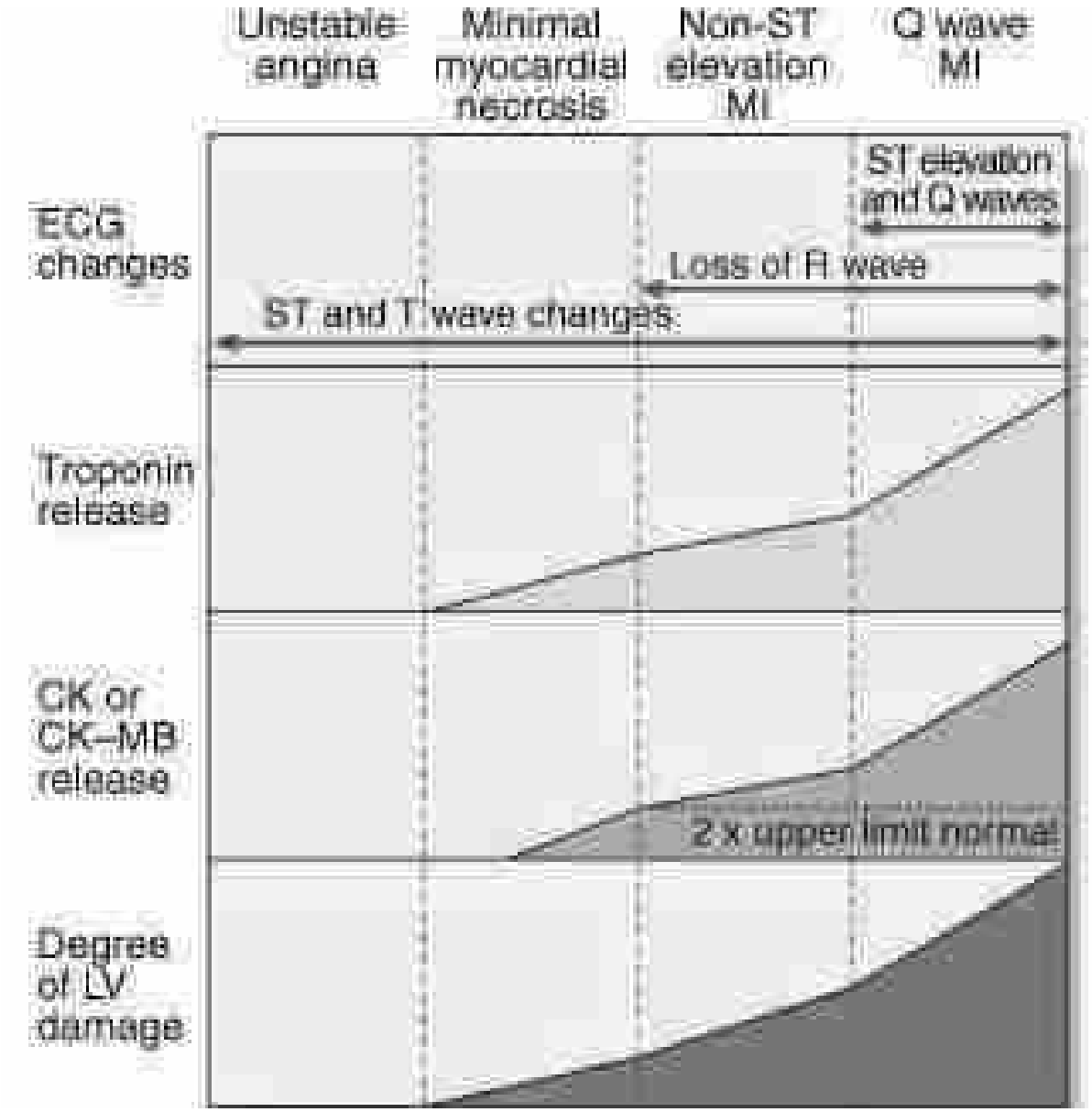
Beta blockers

- **<u>should be combined only very cautiously with</u>**
  - Verapamil or Cardizem,
  - because of potential **of excessive bradycardia or CHF in pts with LV dysfunction.**

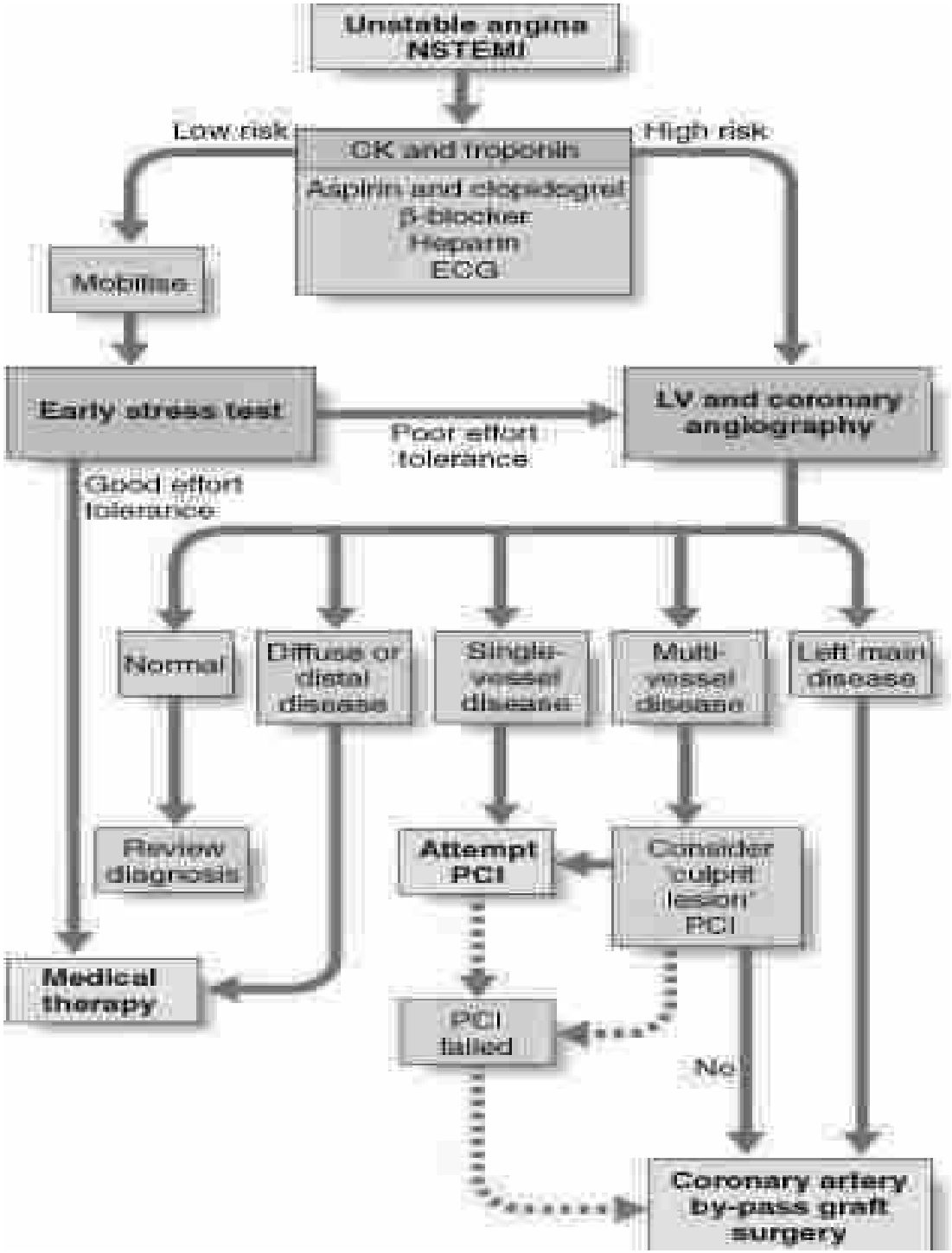
Algorithm for risk stratification and treatment of patients with suspected coronary artery disease.



Treatment of UNSTABLE ANGINA



The spectrum of acute coronary syndromes.
The relation between ECG changes, biochemical markers of damage and the extent of myocardial necrosis. (CK = creatine kinase)


Unstable angina
NSTEMI
Low risk
CK and troponin
Aspirin and clopidogrel
β-blocker
Heparin
ECG
High risk
Mobilise
Early stress test
Poor effort tolerance
LV and coronary angiography
Good effort tolerance
Normal
Diffuse or distal disease
Single-vessel disease
Multi-vessel disease
Left main disease
Review diagnosis
Attempt PCI
Consider 'culprit lesion' PCI
Medical therapy
PCI failed
No
Coronary artery by-pass graft surgery

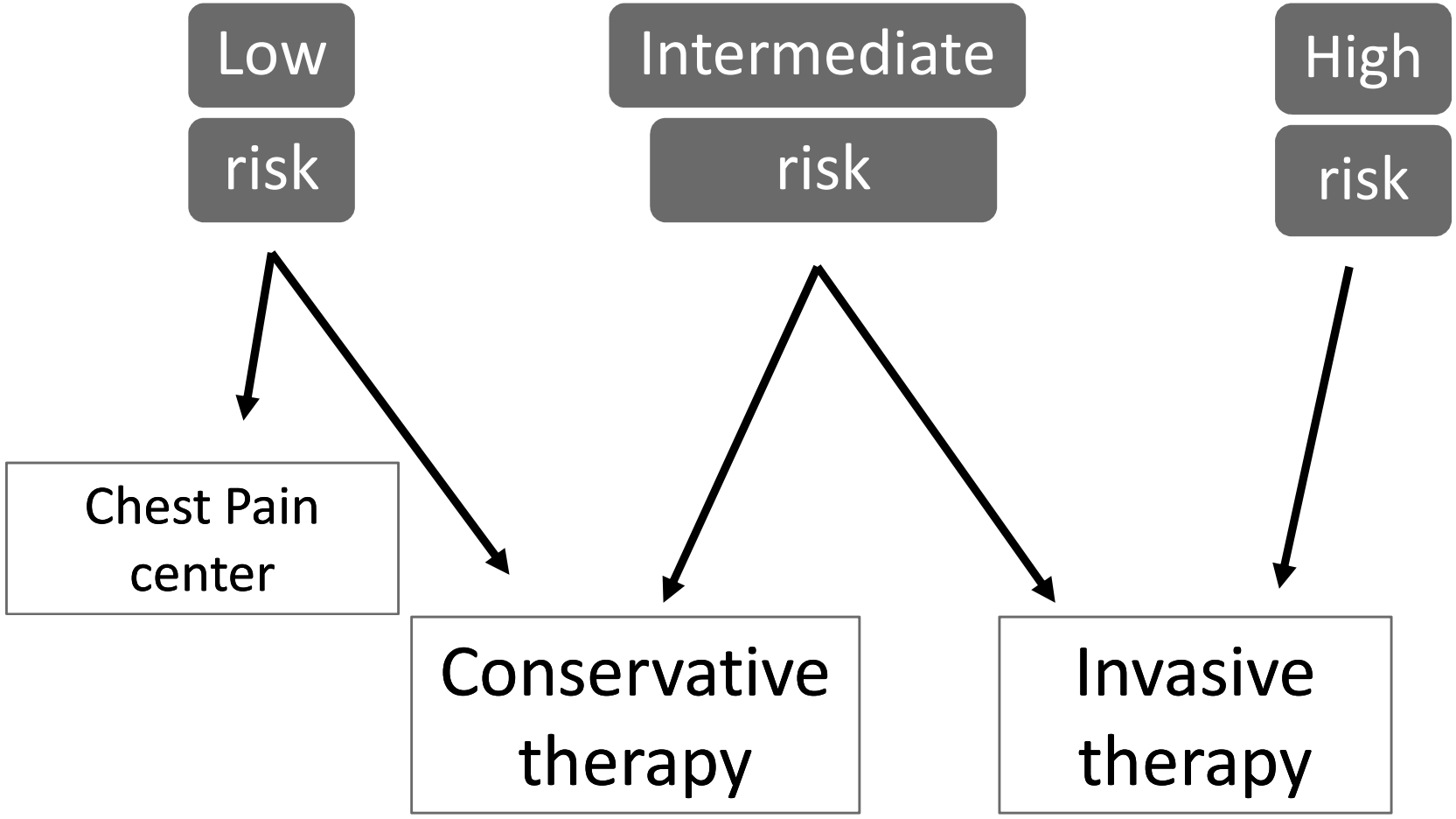
Treatment of
ACUTE CORONARY SYNDROME

Golden Hours

- Maximum damage to heart muscle
- Maximum efficacy of treatment seen
- Survival is best if thrombolysis is given within this period

"Time is muscle and muscle is time"

"Each minute of delay in the first 3 hours confers 10 lost days of survival"


Low
risk
Intermediate
risk
High
risk
Chest Pain
center
Conservative
therapy
Invasive
therapy

Management of ACS

- Good IV access
- Supplemental O2
- Aspirin 300mg ; consider Clopidogrel 300mg too.
- Nitrates 1-2 tabs SL.
- Morphine 5-10mg IV.
- Beta blocker, eg Atenolol 5mg IV (unless Asthma or LVF).
- Restore coronary perfusion : PCI or thrombolysis in STEMI .
- Heparin (LMW) in NSTEMI and U.angina.
- Call cardiology fellow! …….. CCU.

Subsequent management

- Bed rest 48hrs, continuous ECG monitoring.
- Daily Ex.
- Prophylaxis againest thromboembolism until fully mobile (consider warfarin for 3 months if large Ant.MI).
- Aspirin eg 75mg to decrease vascular events.
- Long term B blockers.
- Starts statin.
- ACE inhibitors in all pts ...stop if EF normal.
- Address modifiable risk factors eg smoking.
- If uncomplicated discharge after 5-7 days.

Medical treatment

- **Bed Rest**
- **Continuous ECG monitoring**
  - for ST seg elevation & cardiac arrhythmia.
- **Biomaker study of necrosis**
  - At baseline
  - 4 – 6 hours
  - 12 – 24 hours

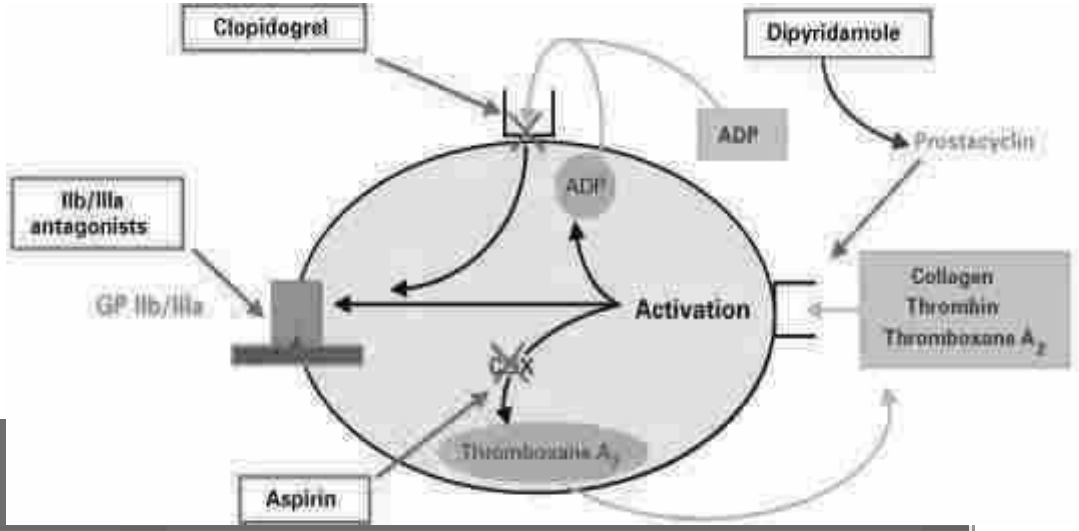
Anti – Ischemic Treatment

- To provide relief and prevention of recurrent chest pain.
  - **Bed rest**
  - **Nitrates**
    - Sublingual or buccal spray 0.3 to 0.6 mg
    - Max 3 dosage given 5 mins apart.
    - If pain persist intravenous NTG should be given 5 to 10 ug/min.
    - **ABSOLUTE CONTRAINDICATIONS**
      - Hypotension
      - Use of Sildenafil
  - **Beta Blockers**

Medical therapy

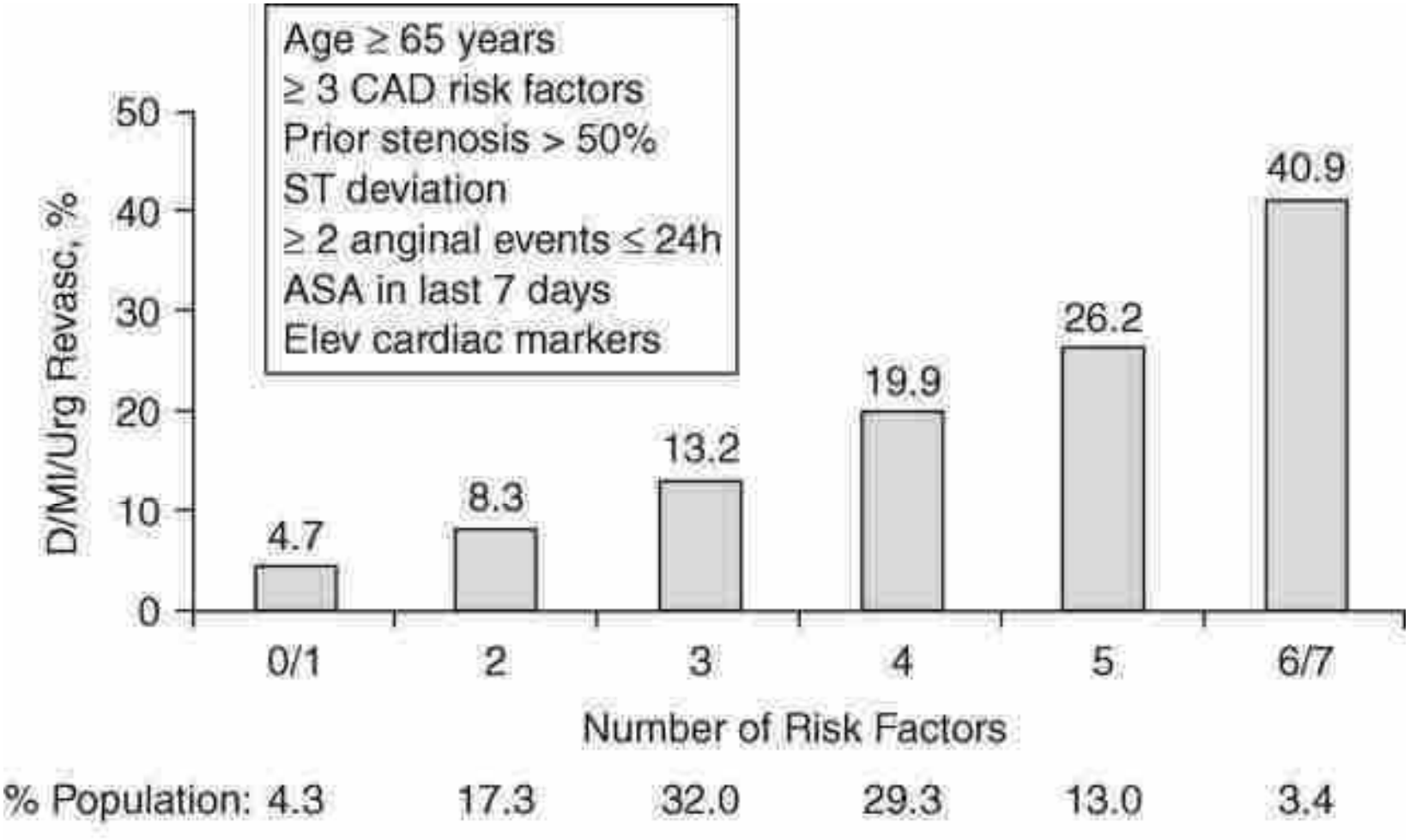
Drug Category	Clinical Condition	When to Avoid[a]	Dosage
Beta blockers[b]	Unstable angina	PR interval (ECG) >0.24 s 2° or 3° atrioventricular block Heart rate <60 beats/min Systolic pressure <90 mmHg Shock Left ventricular failure Severe reactive airway disease	Metoprolol 25–50 mg by mouth every 6 h If needed, and no heart failure, 5-mg increments by slow (over 1–2 min) IV administration

Drug Category	Clinical Condition	When to Avoid[R]	Dosage
Calcium channel blockers	Patients whose symptoms are not relieved by adequate doses of nitrates and beta blockers, or in patients unable to tolerate adequate doses of one or both of these agents, or in patients with variant angina	Pulmonary edema Evidence of left ventricular dysfunction (for diltiazem or verapamil)	Dependent on specific agent
Morphine sulfate	Patients whose symptoms are not relieved after three serial sublingual nitroglycerin tablets or whose symptoms recur with adequate anti-ischemic therapy	Hypotension Respiratory depression Confusion Obtundation	2–5 mg IV dose May be repeated every 5–30 min as needed to relieve symptoms and maintain patient comfort



Oral Antiplatelet Therapy	
Aspirin	Initial dose of 162–325 mg nonenteric formulation followed by 75–162 mg/d of an enteric or a nonenteric formulation
Clopidogrel	Loading dose of 300-600 mg followed by 75 mg/d
Prasugrel	Pre-PCI: Loading dose 60 mg followed by 10 mg/d

Intravenous Antiplatelet Therapy	
Abciximab	0.25 mg/kg bolus followed by infusion of 0.125 g/kg per min (maximum 10 g/min) for 12 to 24 h
Eptifibatide	180 g/kg bolus followed by infusion of 2.0 g/kg per min for 72 to 96 h
Tirofiban	0.4 g/kg per min for 30 min followed by infusion of 0.1 g/kg per min for 48 to 96 h



The TIMI Risk Score for UA/NSTEMI, a simple but comprehensive clinical risk stratification score to identify increasing risk of death, myocardial infarction, or urgent revascularization to day 14. CAD, coronary artery disease; ASA, aspirin.

Heparins*	
Unfractionated Heparin (UFH)	Bolus 60–70 U/kg (maximum 5000 U) IV followed by infusion of 12–15 U/kg per h (initial maximum 1000 U/h) titrated to a PTT 50–70 s
Enoxaparin	1 mg/kg SC every 12 h; the first dose may be preceded by a 30-mg IV bolus; renal adjustment to 1 mg/kg once daily if creatine Cl < 30 cc/min
Fondaparinux	2.5 mg SC qd
Bivalirudin	Initial bolus intravenous bolus of 0.1 mg/kg and an infusion of 0.25 mg/kg per hour. Before PCI, an additional intravenous bolus of 0.5 mg/kg was administered, and the infusion was increased to 1.75 mg/kg per hour.

Thrombolysis

Criteria:

- ST elevation; >1mm in 2 or more limb leads or >2mm in 2 or more chest leads.
- Newly developed LBBB.
- Posterior changes deep ST depression and tall R waves in V1 toV3.

Contraindications;

- Internal bleeding,
- recent surgery and severe HTN.
- eg Streptokinase.

RELATIVE CONTRAINDICATIONS TO THROMBOLYTIC THERAPY

- Active internal bleeding
- Previous subarachnoid or intracerebral haemorrhage
- Uncontrolled hypertension
- Recent surgery (within 1 month)
- Recent trauma (including traumatic resuscitation)
- High probability of active peptic ulcer
- Pregnancy

DYSLIPIDEMIA

- **aiming for**
  - long-term relief from angina,
  - reduced need for revascularization, &
  - reduction in myocardial infarction and death.

- A powerful treatment effect of
  - **statins** on atherosclerosis,
  - IHD, and
  - outcomes is seen regardless of the pretreatment.

- **Fibrates or niacin** can be used to
  - raise HDL cholesterol and lower triglycerides

Treatment of
ACUTE MI (STEMI)

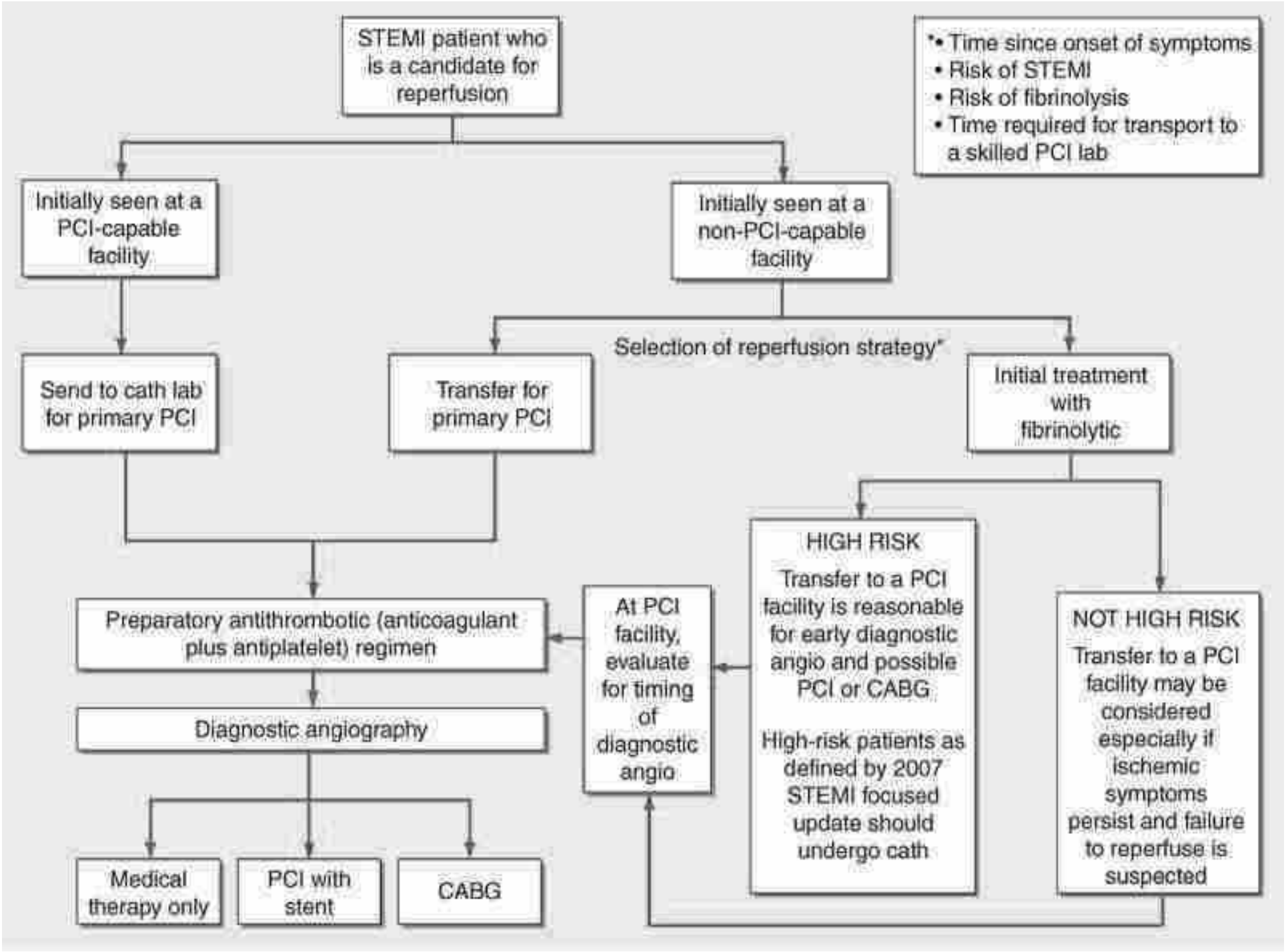
EARLY MANAGEMENT OF ACUTE MYOCARDIAL INFARCTION

Provide facilities for defibrillation

- **Immediate measures High-flow oxygen**
- I.v. access
- ECG monitoring
- 12-lead ECG
- I.v. analgesia (opiates) and antiemetic
- Aspirin 300 mg

- **Reperfusion**
- Primary PCI or thrombolysis

- **Detect and manage acute complications**
- Arrhythmias
- Ischaemia
- Heart failure


STEMI patient who is a candidate for reperfusion
• Time since onset of symptoms
• Risk of STEMI
• Risk of fibrinolysis
• Time required for transport to a skilled PCI lab
Initially seen at a PCI-capable facility
Initially seen at a non-PCI-capable facility
Selection of reperfusion strategy*
Send to cath lab for primary PCI
Transfer for primary PCI
Initial treatment with fibrinolytic
HIGH RISK
Transfer to a PCI facility is reasonable for early diagnostic angio and possible PCI or CABG
High-risk patients as defined by 2007 STEMI focused update should undergo cath
NOT HIGH RISK
Transfer to a PCI facility may be considered especially if ischemic symptoms persist and failure to reperfuse is suspected
At PCI facility, evaluate for timing of diagnostic angio
Preparatory antithrombotic (anticoagulant plus antiplatelet) regimen
Diagnostic angiography
Medical therapy only
PCI with stent
CABG

Class I Recommendations for use of Early Invasive strategy

Recurrent angina at rest / low level activity despite of t/t
Elevated TnT or TnI
New ST-segment depression
Associated CHF symptoms, rales, MR
+ve Stress test
EF < 0.40
Decreased BP
Sustained VT
PCI < 6 months prior to CABG

SURGICAL INTERVENTION

ANGIOPLASTY

STENTS

CORONARY ARTERY BYPASS GRAFTING (CABG)

PTCA

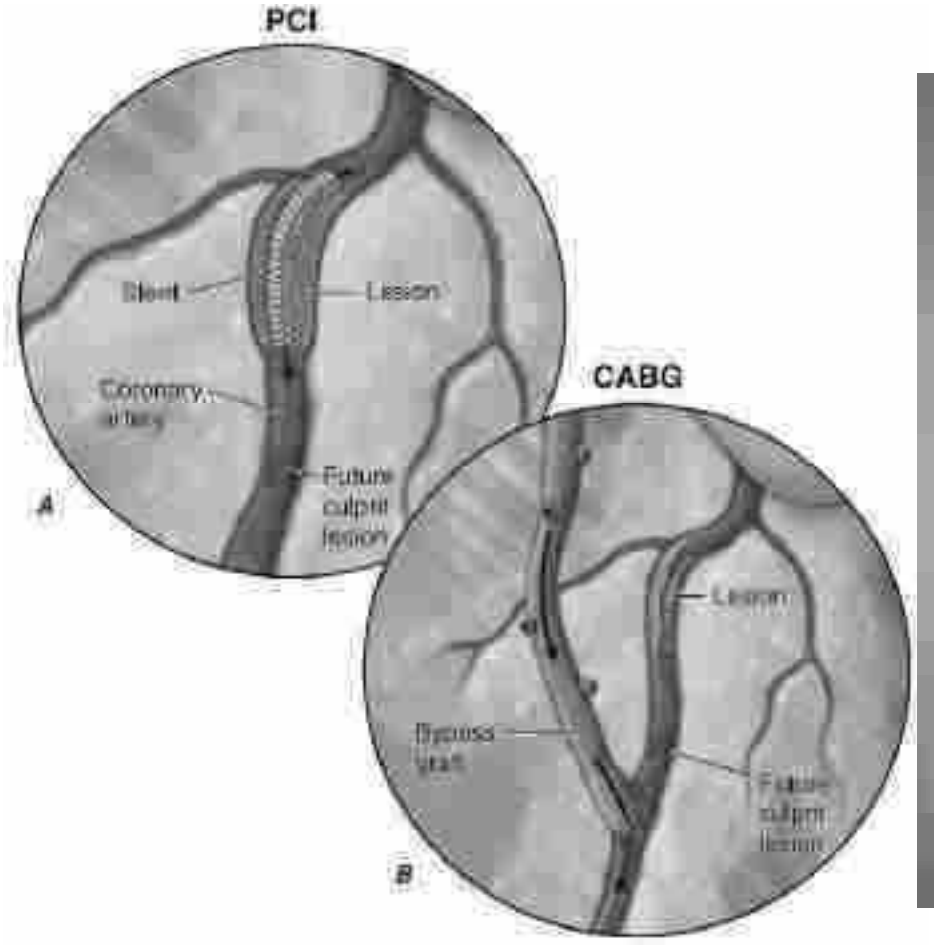
Cardiac Catheterization Procedures

Before & After

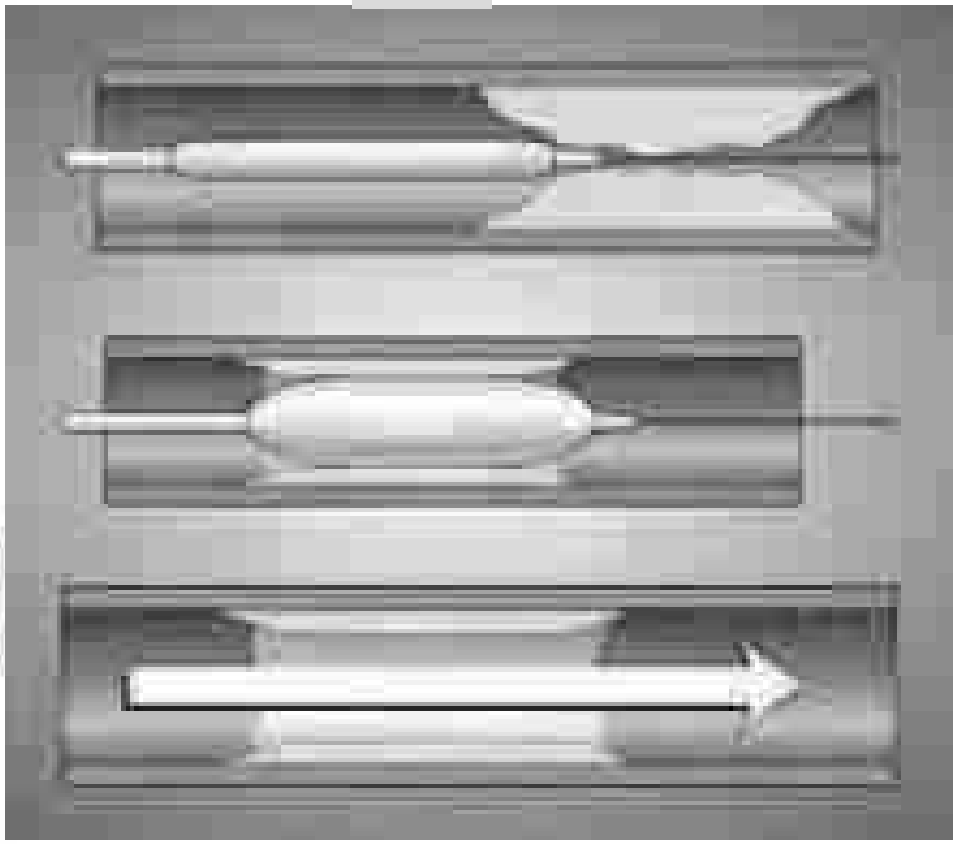
PCI PROCEDURAL REFINEMENTS: STENTS

Expandable metal mesh tubes that buttresses the dilated segment, limit restenosis.
Drug eluting stents: further reduce cellular proliferation in response to the injury of dilatation.

CORONARY ARTERY BYPASS GRAFTING (CABG) & PTCA

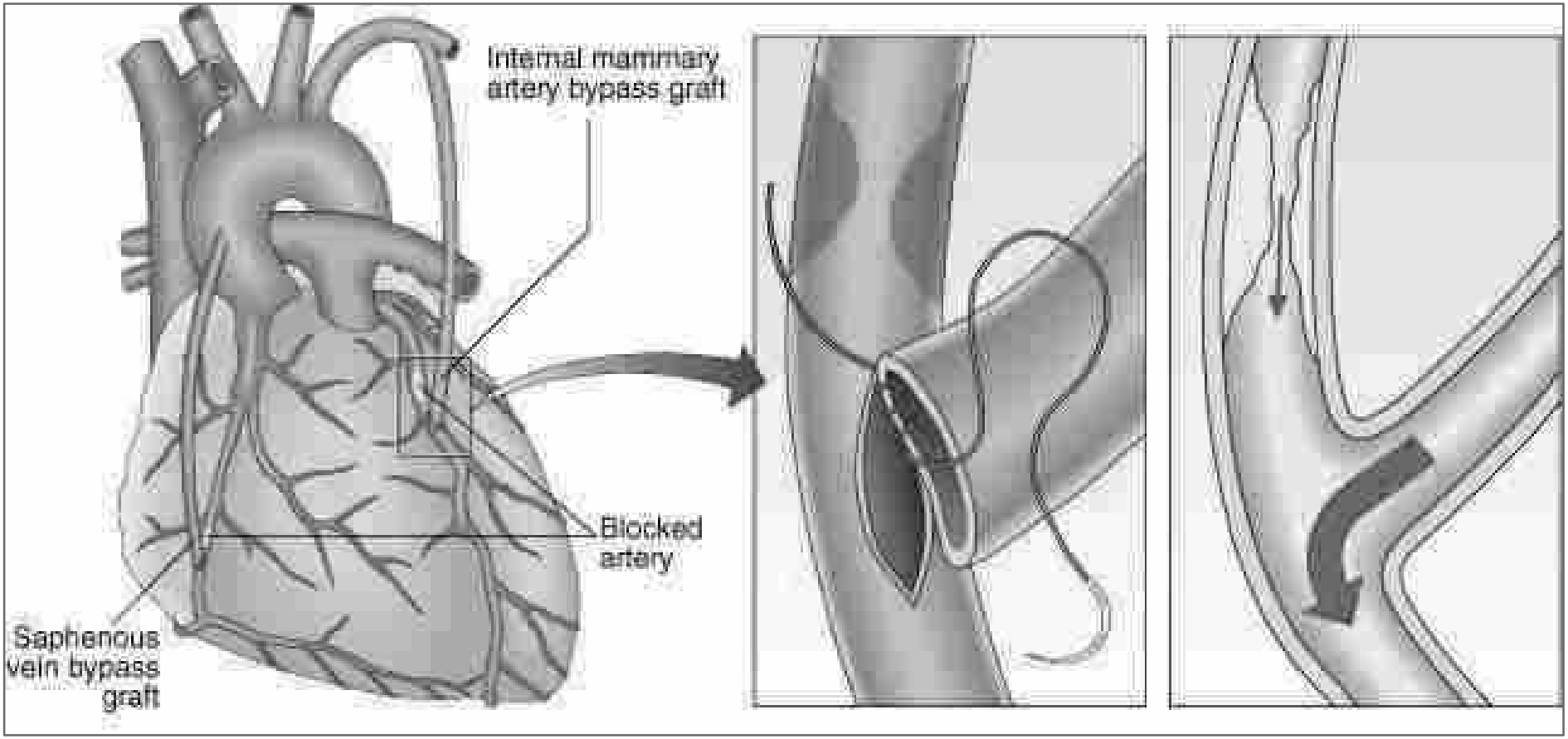


PTCA

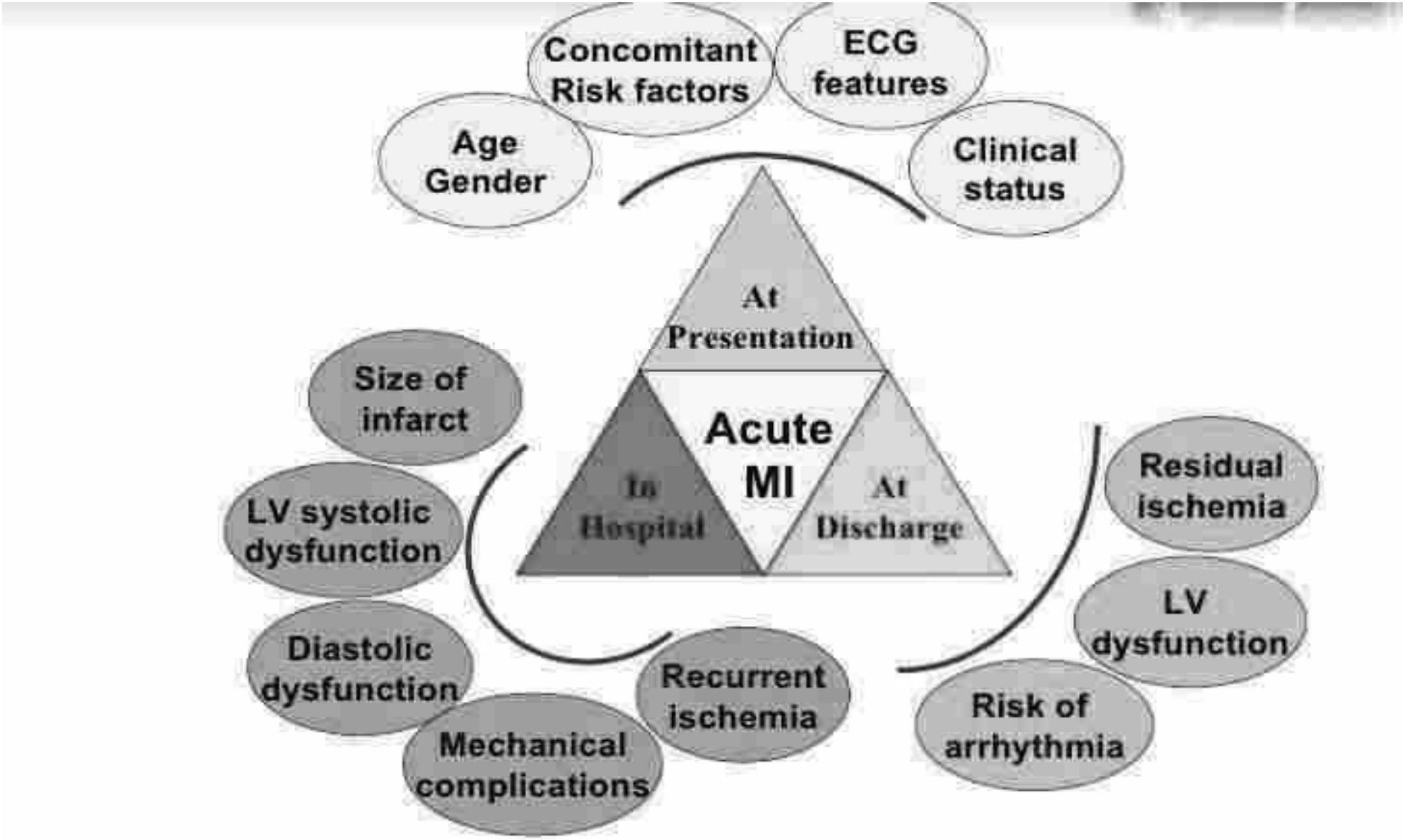


Difference in the approach to the lesion with percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG).

CORONARY ARTERY BYPASS GRAFTING (CABG)



Parameters influencing Prognosis



Long Term Management

- Risk factor modification
- Long Term Treatment
  - Beta blockers (anti-ischemic; reduce triggers for MI)
  - Statins (long term plaque stabilization)
  - ACE Inhibitors (long term plaque stabilization)
  - Anti platelet therapy (Aspirin + Clopidogrel for at least 9-12 months)

Secondary Prevention

- **Disease**
  - HTN, DM, HLP
- **Behavioral**
  - smoking, diet, physical activity, weight
- **Cognitive**
  - Education, cardiac rehab program

Secondary Prevention disease management

- **Blood Pressure**
  - Goals < 140/90 or <130/80 in DM /CKD
  - Maximize use of beta-blockers & ACE-I
- **Lipids**
  - LDL < 100 (70) ; TG < 200
  - Maximize use of statins; consider fibrates/niacin first line for TG>500; consider omega-3 fatty acids
- **Diabetes**
  - A1c < 7%

Secondary prevention behavioral intervention

- **Smoking cessation**
  - Cessation-class, meds, counseling
- **Physical Activity**
  - Goal 30 - 60 minutes daily
  - Risk assessment prior to initiation
- **Diet**
  - DASH diet, fiber, omega-3 fatty acids
  - <7% total calories from saturated fats

THANK YOU!

Acute Rheumatic Fever

DR.DILIP. R. PATIL

M.B.B.S (I.G.M.C.NAGPUR)
M.D (I.G.M.C.NAGPUR)
B.A (Sociology)
M.A (Political Science)
M.A. (History)
LL.B. (N.M.U Jalgaon)
N.E.T. (UGC New Delhi)
S.E.T. (Maharashtra State)

Prof & H.O.D.
Dept. of Medicine
A.C.P.M. Medical College,
Dhule

Acute Rheumatic Fever

- **Definition**
  - It is a multisystem disease resulting from an autoimmune reaction to infection with group A Streptoccous.
  - RHD is most common.
  - Other systemic manifestations resolve completely.

Global Consideration

- Acute Rheumatic Fever (ARF) & RHD are diseases of *poverty.*

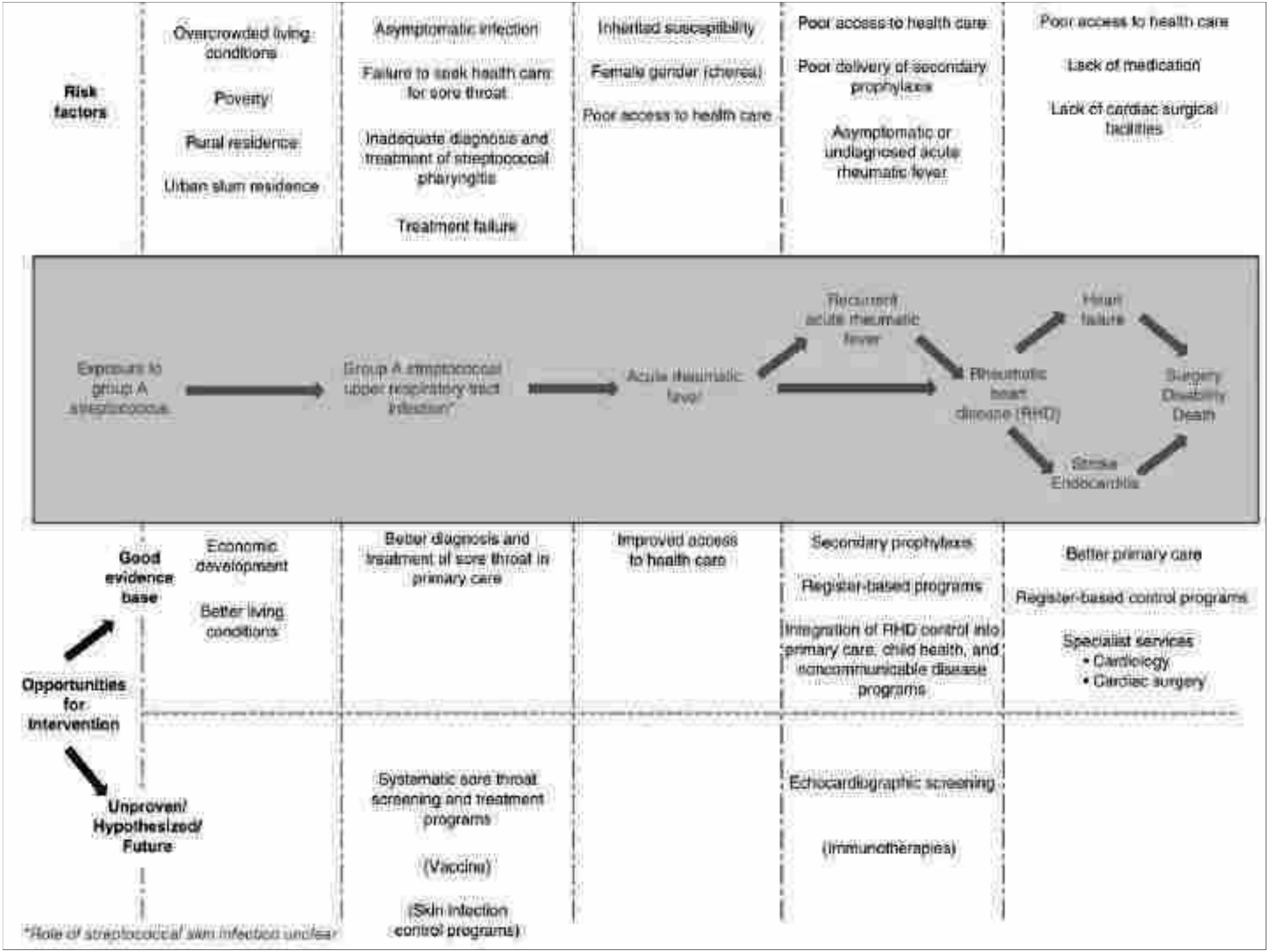
- Incidence ↓ in industrialized nations due to –
  - Improved living conditions
  - Less crowding & better hygiene - ↓ transmission
  - Antibiotics & improved medical care

- Where as in Developing Countries –
  - RHD is M.C cause of heart disease in children
  - It is major cause of mortality & morbidity in adults
  - Persistence of incidence is due to
    - Lack of resources & capacity
    - NO register based RHD control programme
    - ↑ing population & crowding

Epidemiology

- Acute Rheumatic Fever is mainly disease of children age 5 – 14 yrs.
- Initial episodes are less common in older adolescents & young adults.
- Recurrent episodes are relatively common in adolescents & young adults.
- Prevalence of RHD peaks btw 25 & 40 yrs.
- ARF – incidence M = F
- RHD – incidence F = 2 x M

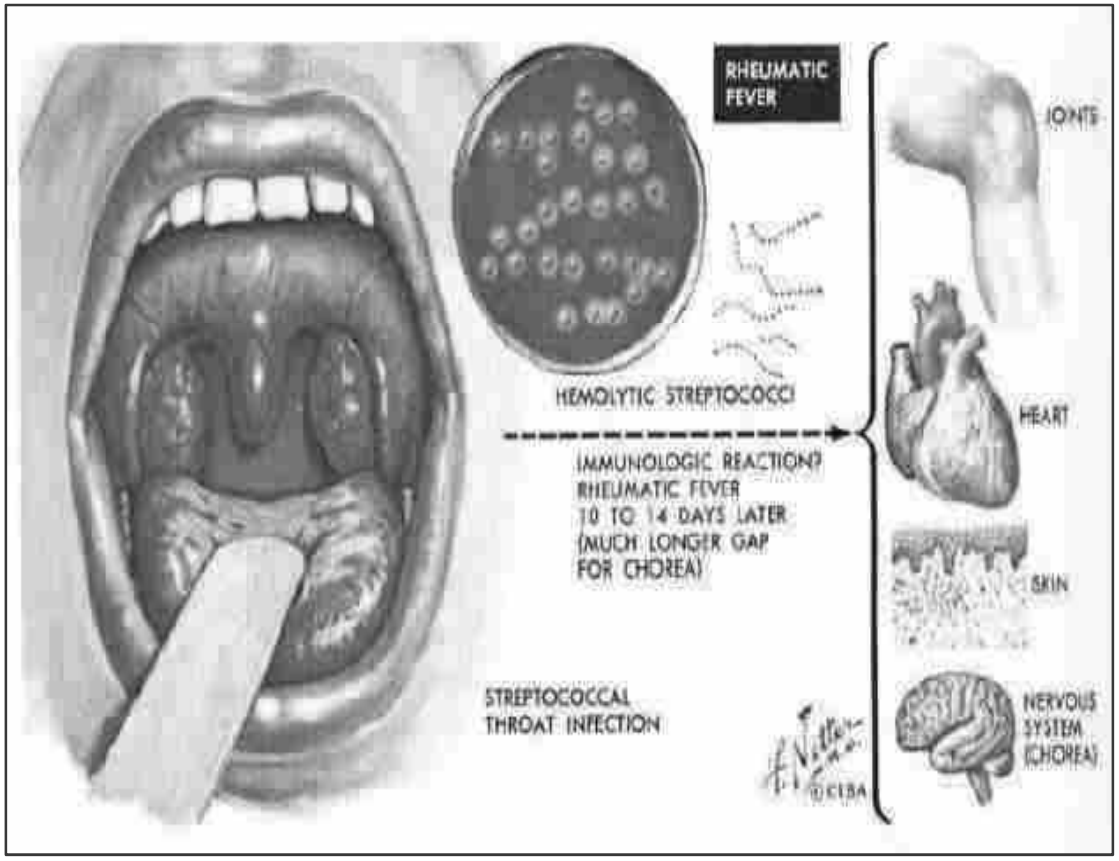
Pathogenetic pathway for Acute Rheumatic Fever & RHD


Risk factors
Overcrowded living conditions
Poverty
Rural residence
Urban slum residence
Asymptomatic infection
Failure to seek health care for sore throat
Inadequate diagnosis and treatment of streptococcal pharyngitis
Treatment failure
Inherited susceptibility
Female gender (chorea)
Poor access to health care
Poor access to health care
Poor delivery of secondary prophylaxis
Asymptomatic or undiagnosed acute rheumatic fever
Poor access to health care
Lack of medication
Lack of cardiac surgical facilities
Exposure to group A streptococcus
Group A streptococcal upper respiratory tract infection*
Acute rheumatic fever
Recurrent acute rheumatic fever
Rheumatic heart disease (RHD)
Heart failure
Surgery
Disability
Death
Stroke
Endocarditis
Opportunities for intervention
Good evidence base
Economic development
Better living conditions
Better diagnosis and treatment of sore throat in primary care
Improved access to health care
Secondary prophylaxis
Register-based programs
Integration of RHD control into primary care, child health, and noncommunicable disease programs
Better primary care
Register-based control programs
Specialist services
• Cardiology
• Cardiac surgery
Unproven/ Hypothesized/ Future
Systematic sore throat screening and treatment programs
(Vaccine)
(Skin infection control programs)
Echocardiographic screening
(Immunotherapies)
*Role of streptococcal skin infection unclear

Pathogenesis

- It is studied under 3 headings –

  1. Organism factors
  2. Host factors
  3. immune Response



Organism Factors

- It is exclusively caused by infection of upper respiratory tract with group A streptococci.
- In high incidence regions certain M- serotypes are associated with ARF.
- It is now thought that any strain of group A Streptococci has potential to cause ARF.
- Skin infections & group C & G streptococci has doubtful role.

Host factors

- Susceptibility to ARF is an inherited characteristic, with –
  - 44% concordance in monozygotic twins
  - 12% in dizygotic twins
  - Heritability at 60%
- ↑ susceptibility with –
  - HLA-DR7 & HLA-DR4
- Protection with –
  - HLA-DR5, HLA-DR6, HLA-DR51, HLA-DR52, HLA-DQ
- ↑ susceptibility with –
  - Polymorphisms with TNF α locus
  - Toll-like receptors
  - High circulating mannose binding lectin

Immune response

- Based on concept of molecular mimicry.
- Immune response targeted at streptococcal antigens on M protein & N- acetyl glucosamine of group A streptococcal carbohydrate also recognizes human tissues.
- Alternative hypothesis that initial damage is due to
  - streptococcal invasion of epithelial surfaces
  - Binding of M protein to type IV collagen RHD allowing it to become immunogenic

Immune response

Cross reactive antibodies bind to endothelial cells of heart valve

→ Activation of adhesion molecule VCAM 1

↓

Cross reactive antibodies bind to endothelial cells of heart valve

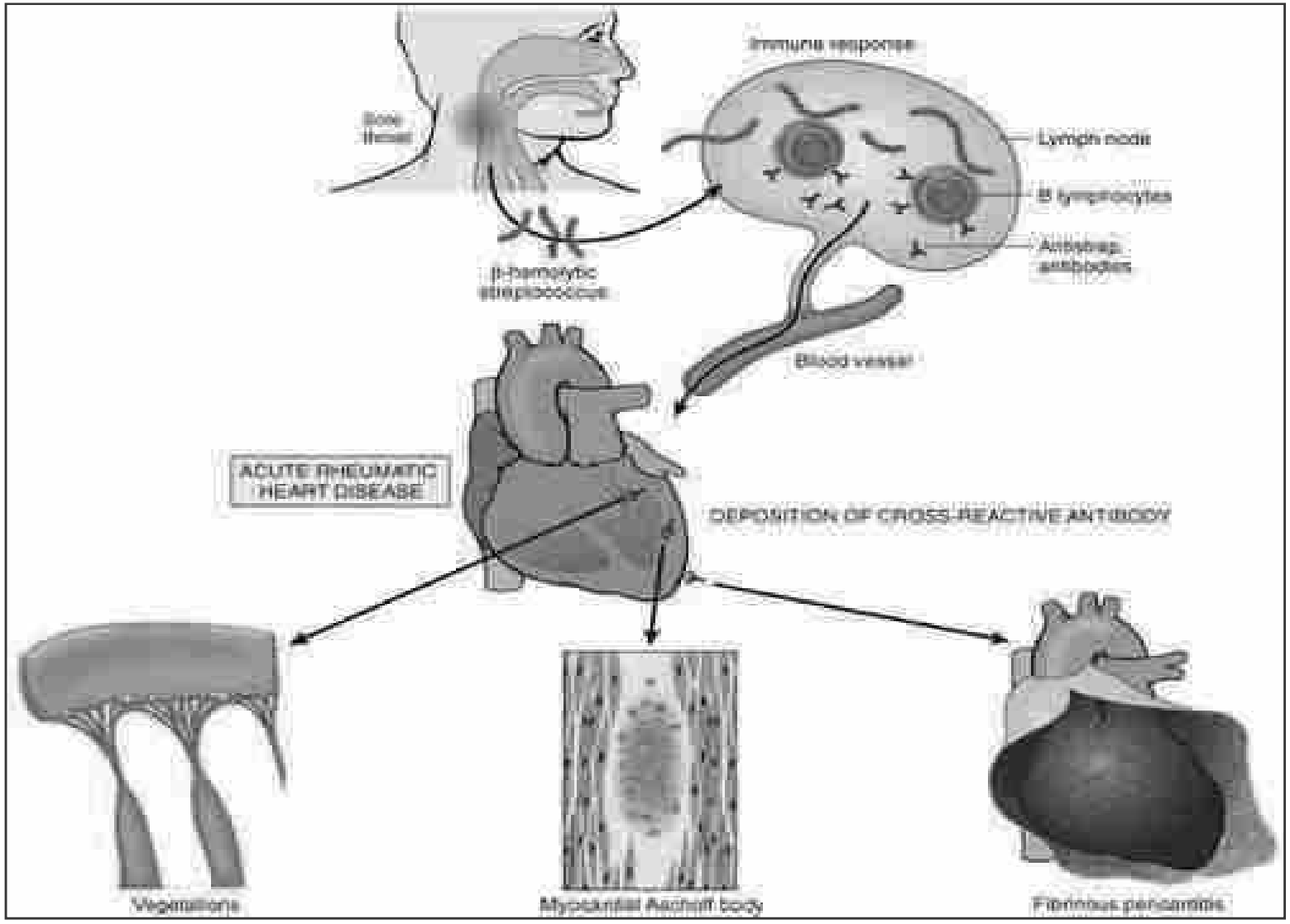
→ Recruitment of activated lymphocytes & lysis of endothelial cells in presence of compliment

↓

Release of peptides Laminin, Keratin, Tropomysin which activates cross reactive T Cells

→ Amplification of damage & causing epitope spreading

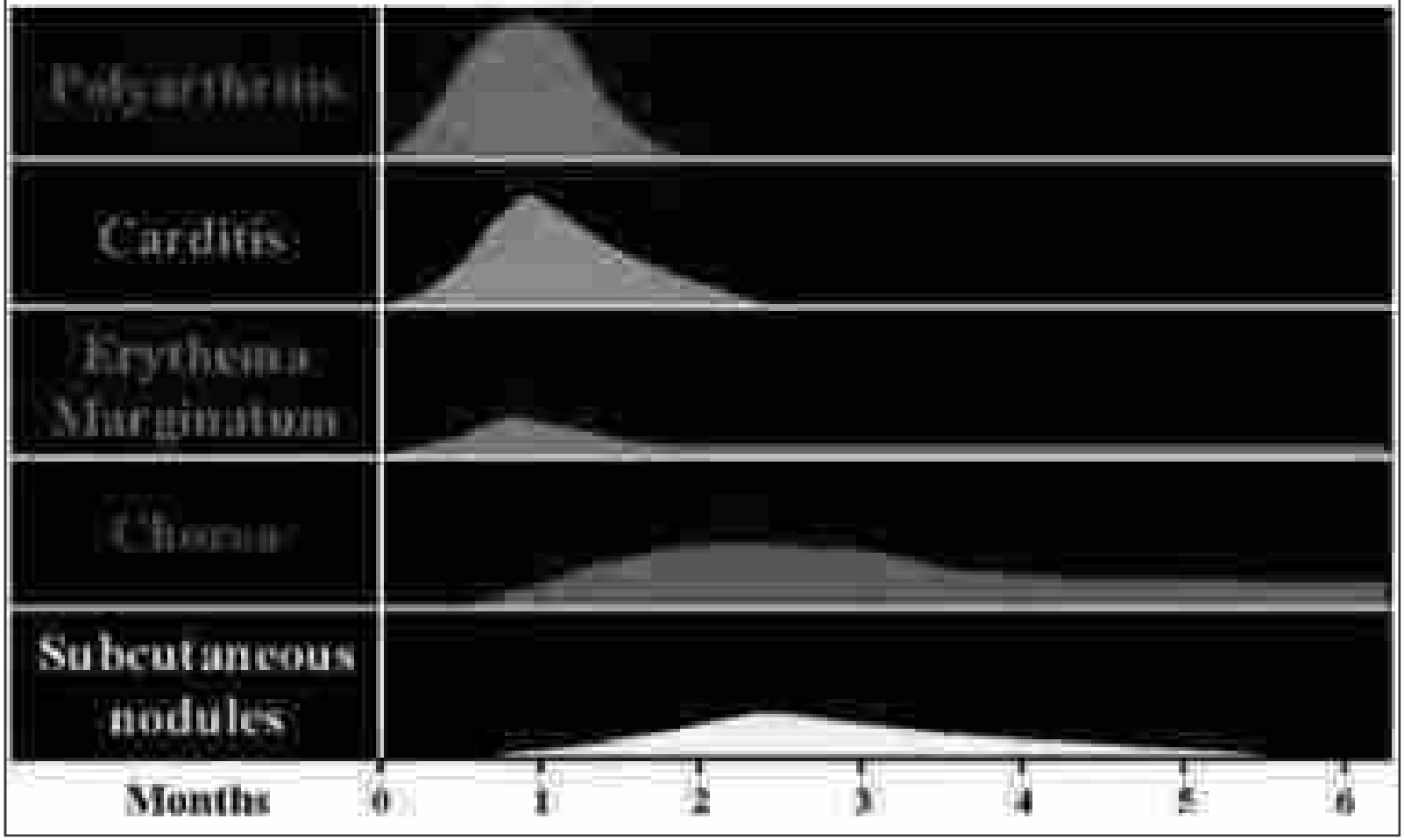
Clinical features



Clinical features

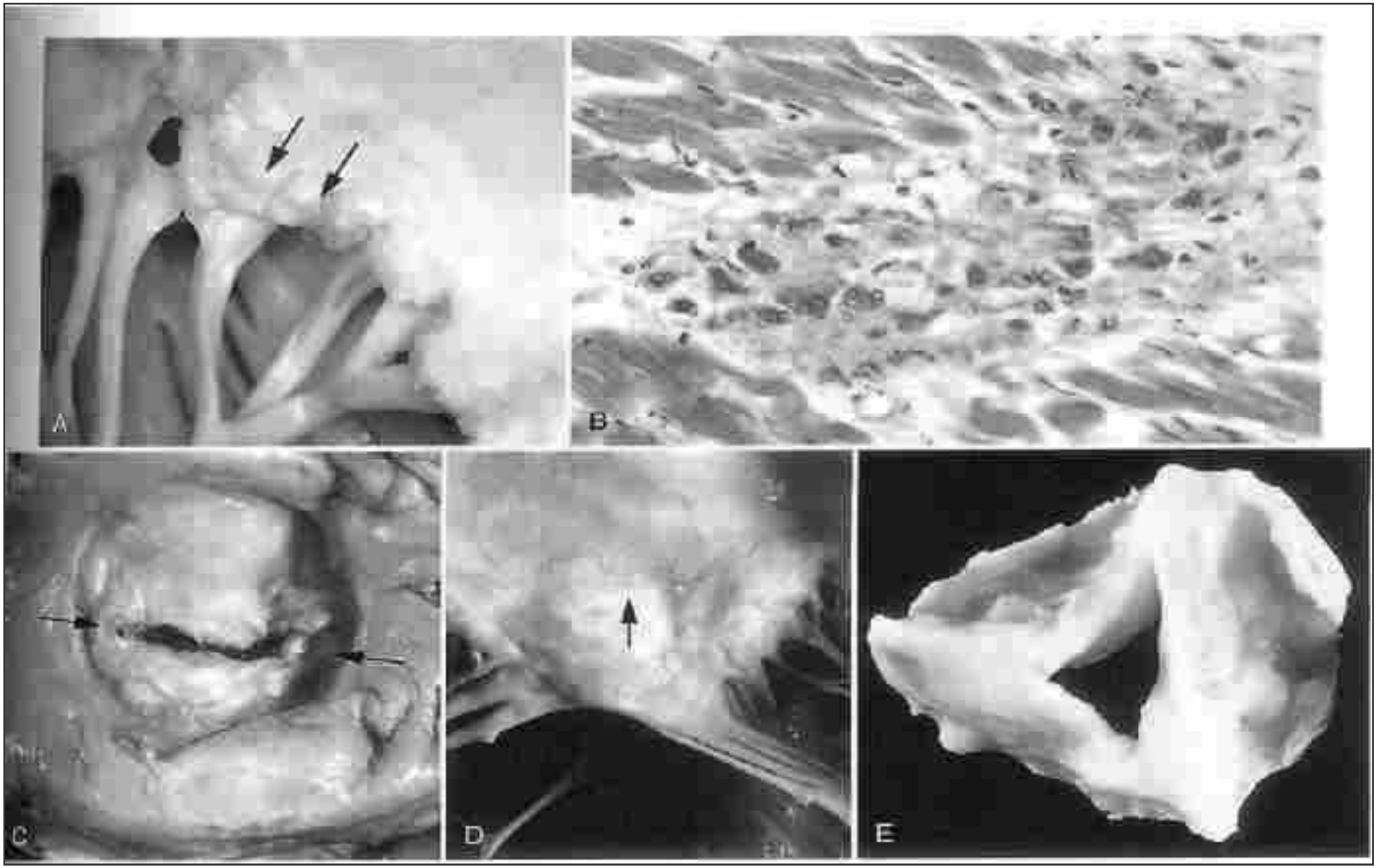
- Latent period – 1 -5 weeks btw group A streptococcus infection & appearance of clinical features of ARF.
- Exception are Chorea & indolent Carditis which follow latent period upto 6 months.
- Commonly subclinical, can only be confirmed using streptococcal antibody testing.
- Most common clinical features are –
  - Polyarthritis in 60-75% cases
  - Carditis in 50-60% cases
  - Prevalence of chorea <2-30% cases
  - Erythema marginatum & subcutaneous nodules <5% cases

Clinical Manifestations of Acute Rheumatic Fever



Heart involvement

- 60% of patients with ARF progress to RHD.
- Can affect Endocardium, Pericardium or Myocardium.
- Hallmark of rheumatic carditis – Valvular damage.
- Mitral valve –
  - Almost always affected
  - Sometimes along with aortic valve
  - Isolated aortic valve involvement is rare
- Pulmonary or tricuspid valves
  - Damaged secondary to ↑ pulmonary pressure due to left sided valvular disease.
- Early valvular damage leads to Regurgitation.
- Valvular stenosis occur due to recurrent episodes, leaflet thickening, scarring & calcification.
- Myocarditis can affect conduction pathways leading to
  - Prolong PR interval & soft 1st heart sound.


A
B
C
D
E

Heart involvement

- Diseased are asymptomatic for many years.
- Symptoms appear when progress to cardiac failure.
- Cases remain undiagnosed in early ages due to poor resource settings.
- Auscultation – poorly sensitive & specific for RHD diagnosis in asymptomatic cases.
- Such cases easily picked up by 2d echocardiographic screening and managed by secondary prophylaxis.

WORLD HEART FEDERATION CRITERIA FOR ECHOCARDIOGRAPHIC DIAGNOSIS OF RHEUMATIC HEART DISEASE (RHD) IN INDIVIDUALS <20 YEARS OF AGE[a]

Definite RHD (either A, B, C, or D):

(A) Pathologic MR and at least two morphologic features of RHD of the mitral valve

(B) MS mean gradient ≥4 mmHg (note: congenital MV anomalies must be excluded)

(C) Pathologic AR and at least two morphologic features of RHD of the AV (note: bicuspid AV and dilated aortic root must be excluded)

(D) Borderline disease of both the MV and AV

Borderline RHD (either A, B, or C):

(A) At least two morphologic features of RHD of the MV without pathologic MR or MS

(B) Pathologic MR

(C) Pathologic AR

Normal Echocardiographic Findings (all of A, B, C, and D):

(A) MR that does not meet all four Doppler criteria (physiologic MR)

(B) AR that does not meet all four Doppler criteria (physiologic AR)

(C) An isolated morphologic feature of RHD of the MV (e.g., valvular thickening), without any associated pathologic stenosis or regurgitation

(D) Morphologic feature of RHD of the AV (e.g., valvular thickening), without any associated pathologic stenosis or regurgitation

Definitions of Pathologic Regurgitation and Morphologic Features of RHD:

Pathologic MR: All of the following: seen in two views; in at least one view, jet length 2 cm; peak velocity ≥3 m/s; pansystolic jet in at least one envelope

Pathologic AR: All of the following: seen in two views; in at least one view, jet length ≥1 cm; peak velocity ≥3 m/s; pandiastolic jet in at least one envelope

Morphologic features of RHD in MV: anterior MV leaflet thickening ≥3 mm (age specific); chordal thickening; restricted leaflet motion; excessive leaflet tip motion during systole

Morphologic features of RHD in AV: irregular or focal thickening; coaptation defect; restricted leaflet motion; prolapse

Joint involvement

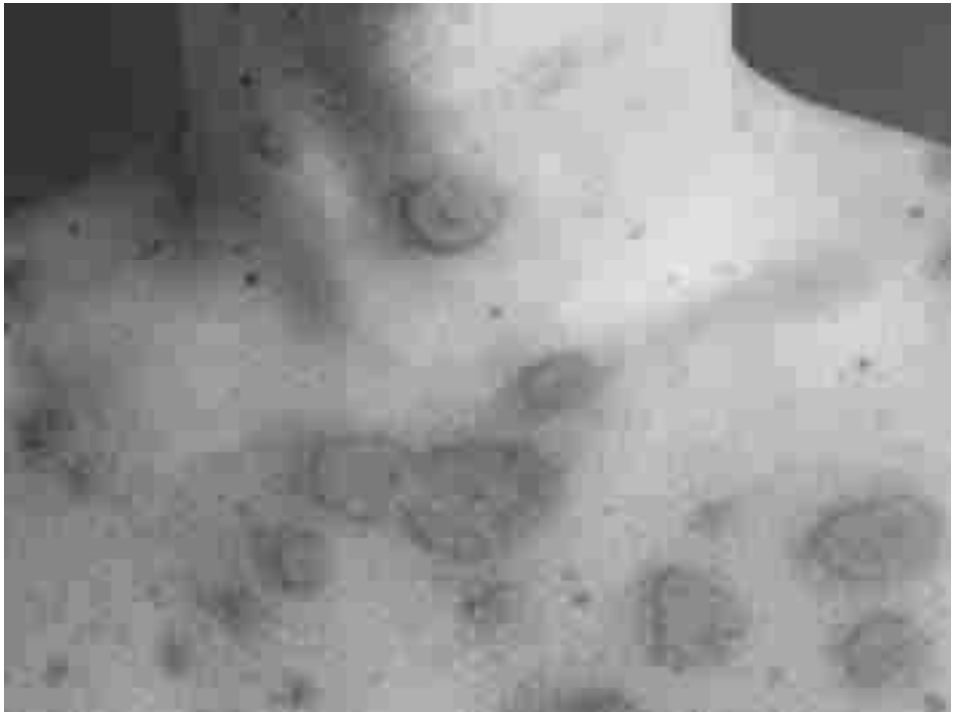
- M.C form is Arthritis with following characteristic –
  - Objective evidence of inflammation
  - Polyarthritis i.e. more than 1 joint involvement
  - Typically Migratory
  - Almost always involve large joints –
    - M.C. knees, ankles, hips & elbows
  - Asymmetric joint involvement
  - Pain is severe
  - Highly responsive to salicylates & other NSAIDs
  - If pain persists for more than 1-2 day after starting NSAIDs is unlikely due to ARF
- Arthralgia without objective joint inflammation affects large joints in migratory pattern.
- Aseptic monoarthritis is presenting feature in some.

Chorea

- *Sydenham's chorea* commonly occurs in absence of other manifestation.
- Follows prolonged latent period.
- Found mainly in females.
- Choreiform movements affects particularly Head (darting movements of tongue) & upper limbs.
- May be generalized or restricted to one side of body i.e. Hemi chorea.
- In severe cases person is unable to perform daily activities.
- Often associated emotional lability or obsessive compulsive traits lasting longer than choreiform movements.

Skin manifestation

- Classic rash is *Erythema Marginatum*
  - Begins as pink macules that clear centrally
  - Leaves serpiginous spreading edge
- Rash is evanescent, appearing & disappearing.
- Occurs usually on trunk, sometimes on limbs, never on face.



Skin manifestation

- Subcutaneous nodules
  - Painless, small (0.5-2cm)
  - Mobile lumps beneath the skin overlying bony prominences of
    - Hands, feet, elbow, occiput, vertebrae
  - Delayed manifestations, appearing 2-3 weeks after onset of disease.
  - Commonly associated with carditis

Other features

- Fever
  - High grade fever is a rule (≥39^{o}C).
  - Lower grade temperature elevation are not uncommon.
- ↑ acute phase reactants.
- Evidence of preceding group A streptococcal infection –
  - ↑ ASO.
  - Positive throat culture.
  - Recent scarlet fever.
  - Rapid antigen test.

Confirming the Diagnosis

2002–2003 WORLD HEALTH ORGANIZATION CRITERIA FOR THE DIAGNOSIS OF RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE (BASED ON THE 1992 REVISED JONES CRITERIA)

Diagnostic Categories	Criteria
Primary episode of rheumatic fever[a]	Two major or one major and two minor manifestations plus evidence of preceding group A streptococcal infection
Recurrent attack of rheumatic fever in a patient without established rheumatic heart disease	Two major or one major and two minor manifestations plus evidence of preceding group A streptococcal infection
Recurrent attack of rheumatic fever in a patient with established rheumatic heart disease[b]	Two minor manifestations plus evidence of preceding group A streptococcal infection[c]
Rheumatic chorea Insidious onset rheumatic carditis[b]	Other major manifestations or evidence of group A streptococcal infection not required
Chronic valve lesions of rheumatic heart disease (patients presenting for the first time with pure mitral stenosis or mixed mitral valve disease and/or aortic valve disease)[d]	Do not require any other criteria to be diagnosed as having rheumatic heart disease

Major manifestations	Carditis Polyarthritis Chorea Erythema marginatum Subcutaneous nodules
Minor manifestations	Clinical: fever, polyarthralgia Laboratory: elevated erythrocyte sedimentation rate or leukocyte count[e] Electrocardiogram: prolonged P-R interval
Supporting evidence of a preceding streptococcal infection within the last 45 days	Elevated or rising anti-streptolysin O or other streptococcal antibody, *or* A positive throat culture, *or* Rapid antigen test for group A streptococcus, *or* Recent scarlet fever[e]

Rheumatic Fever: Criteria

knowmedge

Mnemonic: "JONES CAFE PAL"

Major Criteria

J	Joint Involvement
O	**O** looks like a heart = myocarditis
N	Nodules, subcutaneous
E	Erythema marginatum
S	Sydenham chorea

Minor Criteria

C	CRP Increased
A	Arthralgia
F	Fever
E	Elevated ESR
P	Prolonged PR Interval
A	Anamnesis of Rheumatism
L	Leukocytosis

Diagnosis

Throat cultures growing GABHS
OR
Elevated anti-streptolysin O titers

+

2 Major criteria

OR

1 Major criterion and 2 Minor criteria

Recommended tests in cases of possible ARF

Recommended for All Cases
White blood cell count
Erythrocyte sedimentation rate
C-reactive protein
Blood cultures if febrile
Electrocardiogram (if prolonged P-R interval or other rhythm abnormality, repeat in 2 weeks and again at 2 months if still abnormal)
Chest x-ray if clinical or echocardiographic evidence of carditis
Echocardiogram (consider repeating after 1 month if negative)
Throat swab (preferably before giving antibiotics)—culture for group A streptococcus
Antistreptococcal serology: both anti-streptolysin O and anti-DNase B titers, if available (repeat 10–14 days later if first test not confirmatory)
Tests for Alternative Diagnoses, Depending on Clinical Features
Repeated blood cultures if possible endocarditis
Joint aspirate (microscopy and culture) for possible septic arthritis
Copper, ceruloplasmin, antinuclear antibody, drug screen for choreiform movements
Serology and autoimmune markers for arboviral, autoimmune, or reactive arthritis

Prevention

Primary prevention

- Elimination of major risk factors.
- Primary prophylaxis –
  - Timely & complete t/t of streptococcal sore throat.
- In poor-resources settings
  - All cases with sore throat be treated with penicillin.
  - Clinical algorithm be used to identify patients with higher likelihood of streptococcal pharyngitis.

Secondary prevention

- Very important as patients with ARF are at dramatically high risk of developing further episode of ARF after group A streptococcal infection.
- Such patients should receive long term penicillin prophylaxis to prevent recurrences.
- Benzathine penicillin G
  - In dose of 1.2 million units or 600,000 units if ≤27kg .
  - To be given every 4 weeks.
- Orally penicillin V (250mg) BD can be given but is less effective.

AMERICAN HEART ASSOCIATION RECOMMENDATIONS FOR DURATION OF SECONDARY PROPHYLAXIS[a]

Duration of secondary prophylaxis depends on –

- Duration since last episode of ARF
- Age
- Severity of RHD

Category of Patient	Duration of Prophylaxis
Rheumatic fever without carditis	For 5 years after the last attack or 21 years of age (whichever is longer)
Rheumatic fever with carditis but no residual valvular disease	For 10 years after the last attack, or 21 years of age (whichever is longer)
Rheumatic fever with persistent valvular disease, evident clinically or on echocardiography	For 10 years after the last attack, or 40 years of age (whichever is longer); sometimes lifelong prophylaxis

Treatment

Treatment

- **Antibiotics** :
  - Penicillin is drug of choice
  - can be given orally as
    - Phenoxymethyl penicillin 500mg BD (250mg BD if ≤27kg)
    - Amoxicillin 50mg/kg (max. 1gm) daily for 10 days
  - Or as Single dose of 1.2 million units (600,000 units for children ≤27kg) IM – Benzathine Penicillin G

- **Salicylates & NSAIDs :**
  - For arthritis, arthralgia, fever
  - Aspirin is drug of choice
    - at a dose 50-60 mg/kg / day upto max. 80-100 mg/kg/day in 4-5 divided doses
    - Monitoring to be done for salicylate toxicity
  - Naproxen 10-20 mg/kg /day is suitable alternative to aspirin

Treatment

- **Glucocorticoids:**
  - Prednisone or prednisolone is recommended at a dose of 1-2 mg/kg /day (max. 80mg) usually for few days or upto max. 3 weeks.
- **Chorea:**
  - Medication to control movements do not alter duration or outcome of chorea.
  - Carbamazepine or sod. Valproate is preferred to haloperidol.
  - Corticosteroids are effective & lead to more rapid symptom reduction.
- **Bed rest:**
  - In cases of arthritis , arthralgia & pt. with heart failure.
- **Management of Heart Failure:**

Prognosis

- Untreated ARF lasts on avg. 12 weeks.
- With t/t pts, Can be discharged with 1-2 wks.
- Inflammatory markers monitored every 1-2wks.
- 2D Echo to be done after 1 month to determine progression of carditis if any.
- Ensue long term clinical follow-up & adherence to secondary prophylaxis.
- Pts. & there families should be educated about disease & importance of secondary prophylaxis.

Rheumatic Heart Disease
THANK YOU
Don't Forget me!

Approach to Renal Diseases

Symptomatology & Syndrome

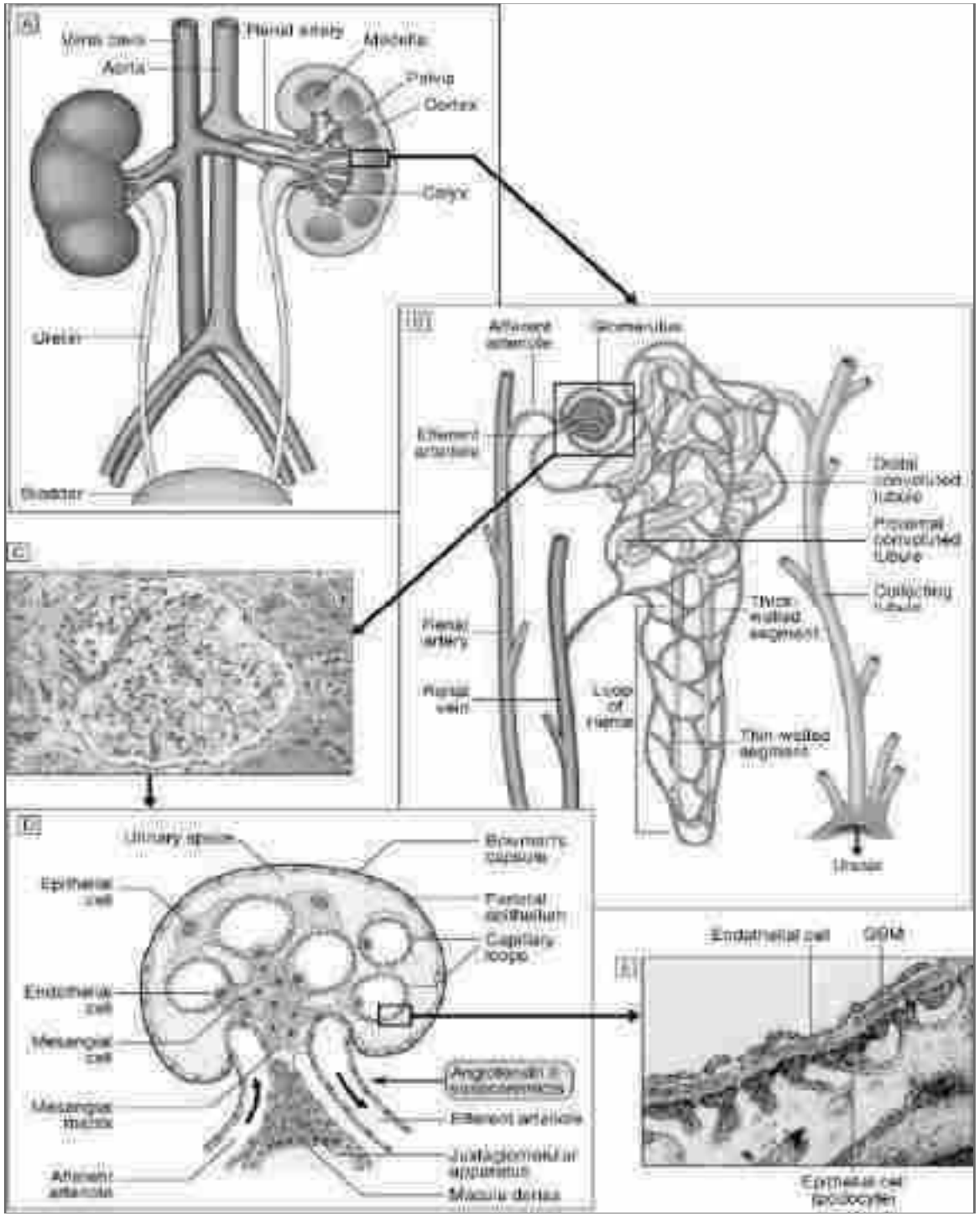
- Azotaemia
- Anasarca
- Proteinuria
- Haematuria
- Electrolyte imbalance
- pH Imbalance
- Sediments in Urine
- Renal Biopsy
- Pyrexia
- Pain
- ↑BP
- ↓BP
- Anorexia
- Polycythemia
- Cachexia

- Glomerulonephritis
- Nephrotic Syndrome
- ARF
- CRF
- Proteinuria
- Haematuria
- UTI
- Pyelonephritis
- Nephrolithiasis
- Obstructive Uropathy
- RTA
- Asymptomatic Abnormalities
- ↑BP
- ↓BP
- Neurogenic Bladder

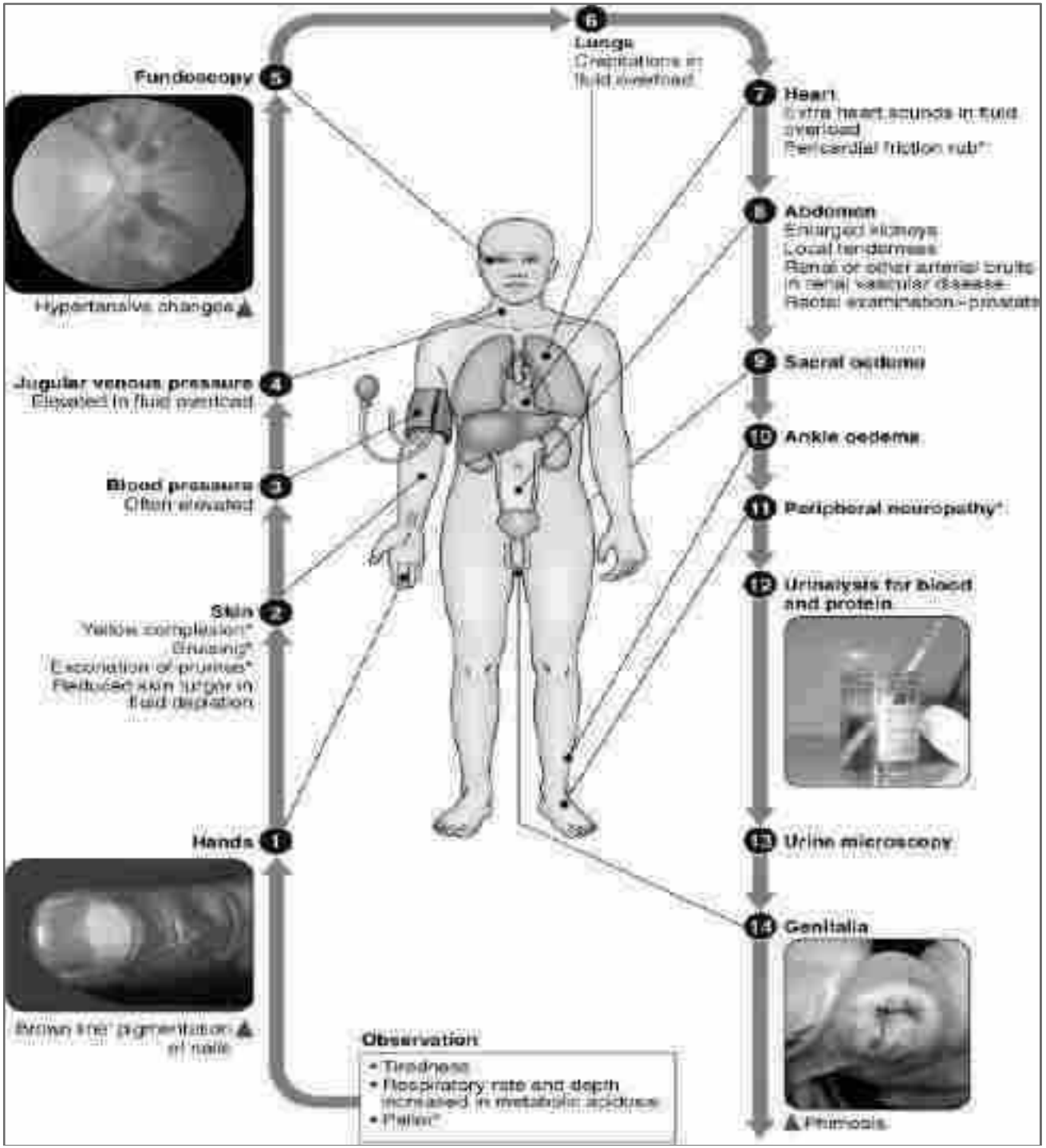
↓or ↑Na+
↓ or ↑K+
↓or ↑Ca+
↓or ↑Po4-
↓ or ↑Mg+

Metabolic Acidosis
Metabolic alkalosis
Respiratory Acidosis (Acute/Chronic)
Respiratory Alkalosis (Acute/Chronic)
Mixed ABG analysis
Compensatory ABG analysis

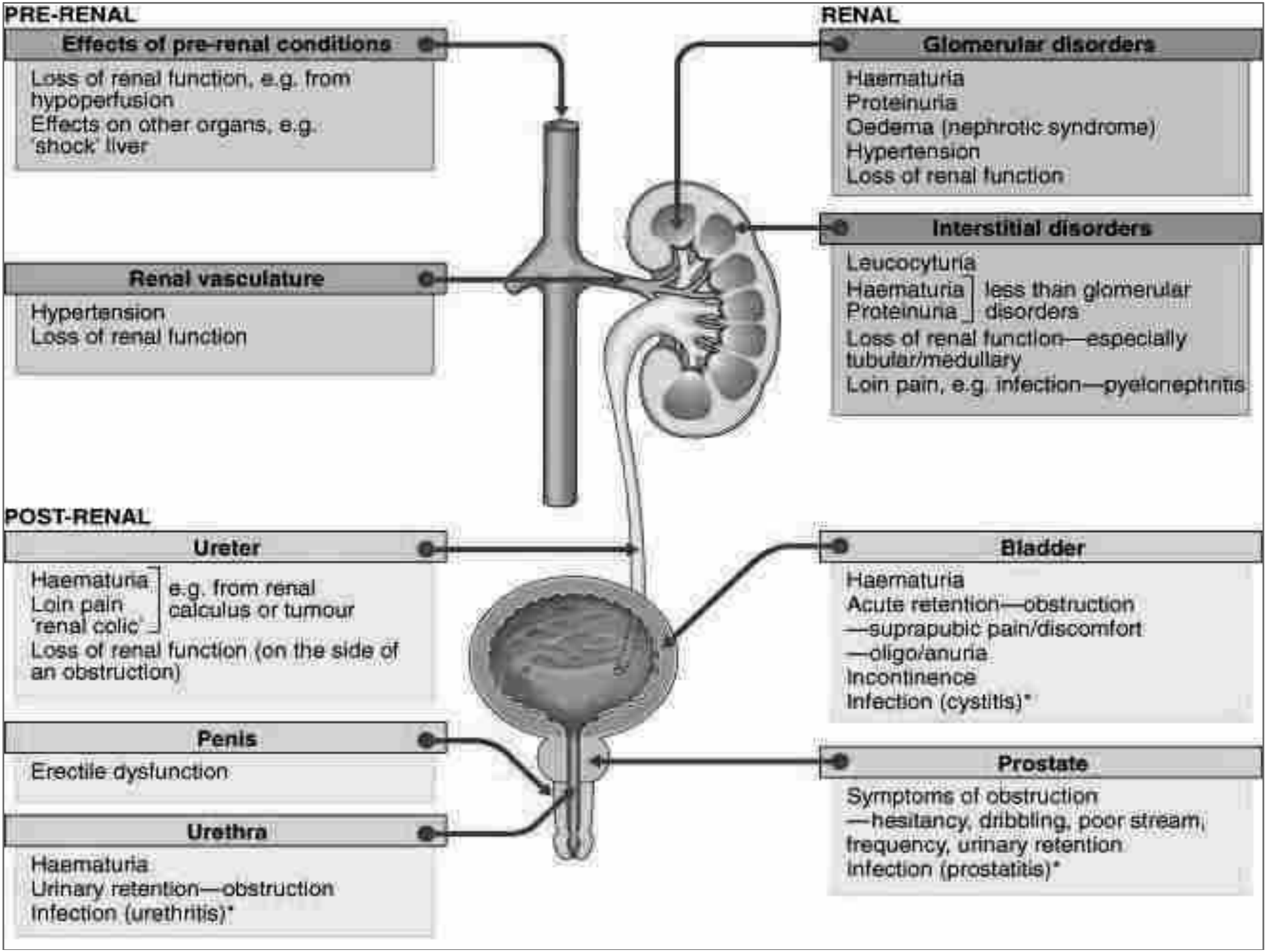
Anatomy of the kidneys



CLINICAL EXAMINATION OF THE KIDNEY AND URINARY TRACT

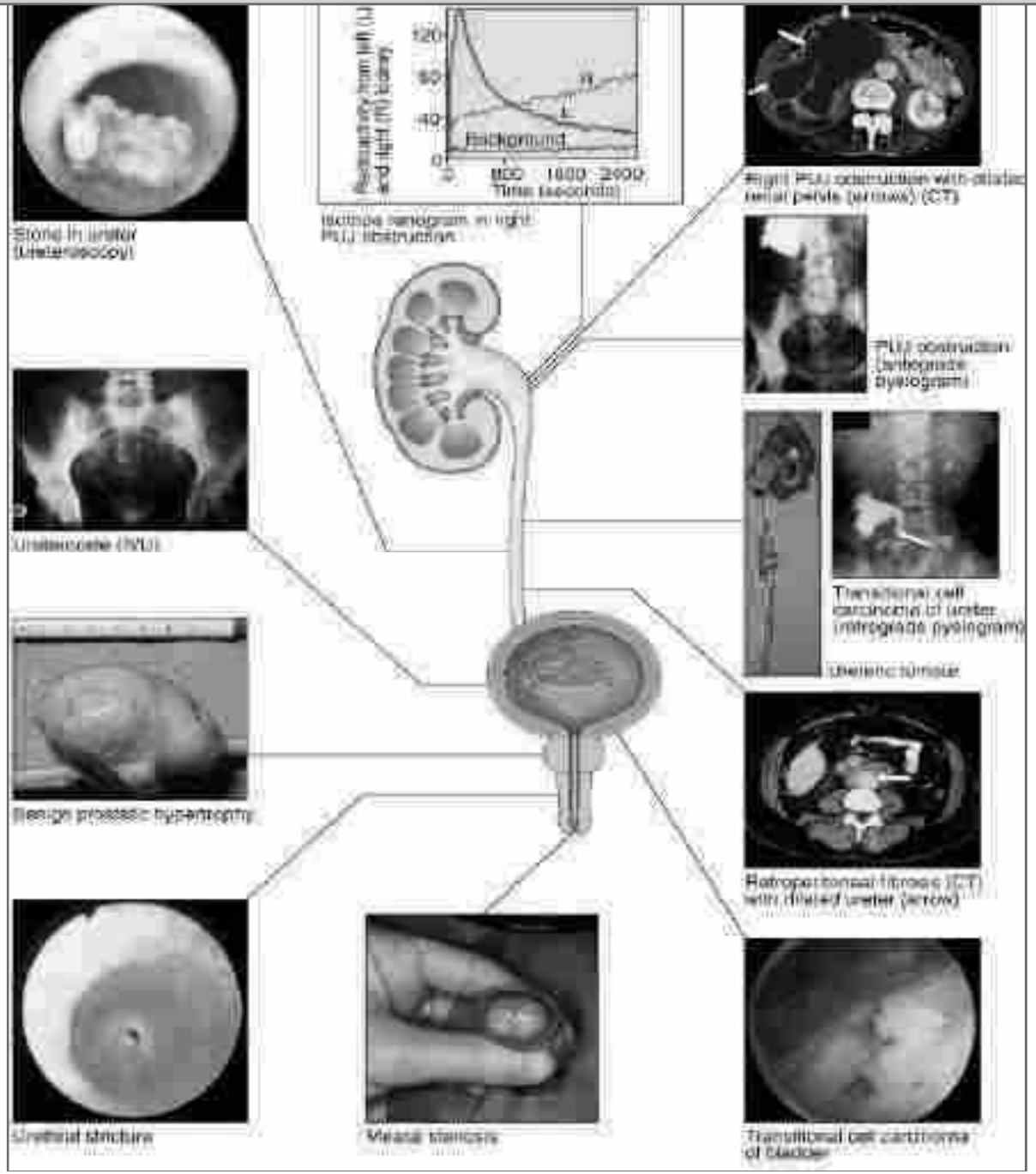


Classification and manifestations of renal and urinary tract disease


PRE-RENAL
Effects of pre-renal conditions
Loss of renal function, e.g. from hypoperfusion
Effects on other organs, e.g. 'shock' liver
Renal vasculature
Hypertension
Loss of renal function
RENAL
Glomerular disorders
Haematuria
Proteinuria
Oedema (nephrotic syndrome)
Hypertension
Loss of renal function
Interstitial disorders
Leucocyturia
Haematuria
Proteinuria
less than glomerular disorders
Loss of renal function—especially tubular/medullary
Loin pain, e.g. infection—pyelonephritis
POST-RENAL
Ureter
Haematuria
Loin pain
'renal colic'
e.g. from renal calculus or tumour
Loss of renal function (on the side of an obstruction)
Penis
Erectile dysfunction
Urethra
Haematuria
Urinary retention—obstruction
Infection (urethritis)*
Bladder
Haematuria
Acute retention—obstruction
—suprapubic pain/discomfort
—oligo/anuria
Incontinence
Infection (cystitis)*
Prostate
Symptoms of obstruction
—hesitancy, dribbling, poor stream, frequency, urinary retention
Infection (prostatitis)*

Urinary tract obstruction

Urinary tract obstruction. Some common causes and their locations. (PUJ = pelviureteric junction)



MAJOR SYNDROMES

SYNDROME	IMPORTANT CLUES	COMMON FINDINGS
Acute or Rapid progressive renal failure	Anuria, Oliguria, ↓GFR	HTN/ Hematuria/ Casts/ Proteinuria/ Pyuria/ Edema
Acute Nephritis	Hematuria/ RBC cast/ Azotemia/ Oliguria/ Edema/ HTN	Proteinuria/ Pyuria/ Ciculatory Congestion
CRF	Azotemia > 3months Signs of Uremia/ Prolonged Symptoms/ ↓ Kidney size/ Broad Cast	Proteinuria/ Casts/ Polyuria/ Nocturia/ Edema/ HTN/ Electrolyte disturbance.
Nephrotic Syndrome	Proteinuria > 3.5g/ 24h Hypoalbuminemia/ Edema/ Hyperlipidemia	Casts/ Lipiduria/ Hypercoagulable state.
UTI/ Pyelonephritis	Bacteriuria/ Other Infection in Urine/ Pyuria/ Leucocyte Casts/ Frequency/ Urgency/ Bladder Tenderness/ Flank Tenderness	Hematuria/ Mild Azotemia/ Mild Proteinuria/ Fever.

SYNDROME	IMPORTANT CLUES	COMMON FINDINGS
Renal Tubular Defect	Electrolyte Disturbance Polyuria/ Nocturia/ Renal Calcification/ Large Kidney/ Renal Transport Defect	Hematuria/ Tubular Proteinuria < 1g/24h urine Enuresis
HTN	Systolic/ Diastolic Hypertension	Proteinuria/ Casts/ Azotemia
Nephtolithiasis	Previous history of stone or removal/ Renal Colic	Hematuria/ Pyuria/ Frequency/ urgency
Urinary Tract Obstruction	Azotemia/ oliguria/ Anuria/ Urinary Retention/ Slowing of urinary stream/ Large Prostate/ Large Kidney/ Flank Tenderness/ Full bladder after Voiding.	Hematuria Pyuria Enuresis Dysuria

Azotemia

Azotemia

- ***Definition***
  - Retention of nitrogenous waste products

- **classification**

1) Pre renal
   - Due to reduced renal perfusion
   - 40-80% cases of ARF
   - reversible if approriate treatment
2) Renal
3) Post renal

Assessment of GFR

- *GFR is the primary metric of kidney "function" & is direct measurement of Radioactive isotopes which are nither reabsorbed or secreted throughout the tubule .*

- **Method of measurement**:
  - 1. inulin
  - 2.iothalamate

- **Plasma Creatinine** is the widely used Marker for GFR.

- Symptomatic Uremia develops in pt's when GFR < 15ml/min. (severe renal insufficiency)

Glomerular filtration rate (GFR) i

$$GFR = \frac{\text{Urine Concentration} \times \text{Urine Flow}}{\text{Plasma Concentration}}$$

Creatinine Clearance C_{Cr}

$$C_{Cr} = \frac{U_{Cr} \times \dot{V}}{P_{Cr}}$$

Estimated creatinine clearance rate (eC_{Cr}) using Cockcroft-Gault formula

$$eC_{Cr} = \frac{(140 - \text{Age}) \times \text{Mass (in kilograms)} \times [0.85 \; if \; Female]}{72 \times \text{Serum Creatinine (in mg/dL)}}$$

Estimated GFR (eGFR) using Modification of Diet in Renal Disease (MDRD) formula

$$\text{eGFR} = 186 \times \text{Serum Creatinine}^{\,-1.154} \times \text{Age}^{\,-0.203} \times [1.210 \; if \; Black] \times [0.742 \; if \; Female]$$

$$\text{eGFR} = 141 \times \min(\text{SCr}/\text{k},1)^{a} \times \max(\text{SCr}/\text{k},1)^{-1.209} \times 0.993^{Age} \times [1.018 \; if \; Female] \times [1.159 \; if \; Black]$$

Renal Function Tests

RFT	
Creatinine Clearance Test **Inulin Clearance Test**	Assess GFR
Water Deprivation Test	Assess Distal Tubular Function
(PAH) Para-aminohippurate Clearance	Assess Renal Blood Flow/ Renal Plasma Flow

Causes of Pre Renal Failure

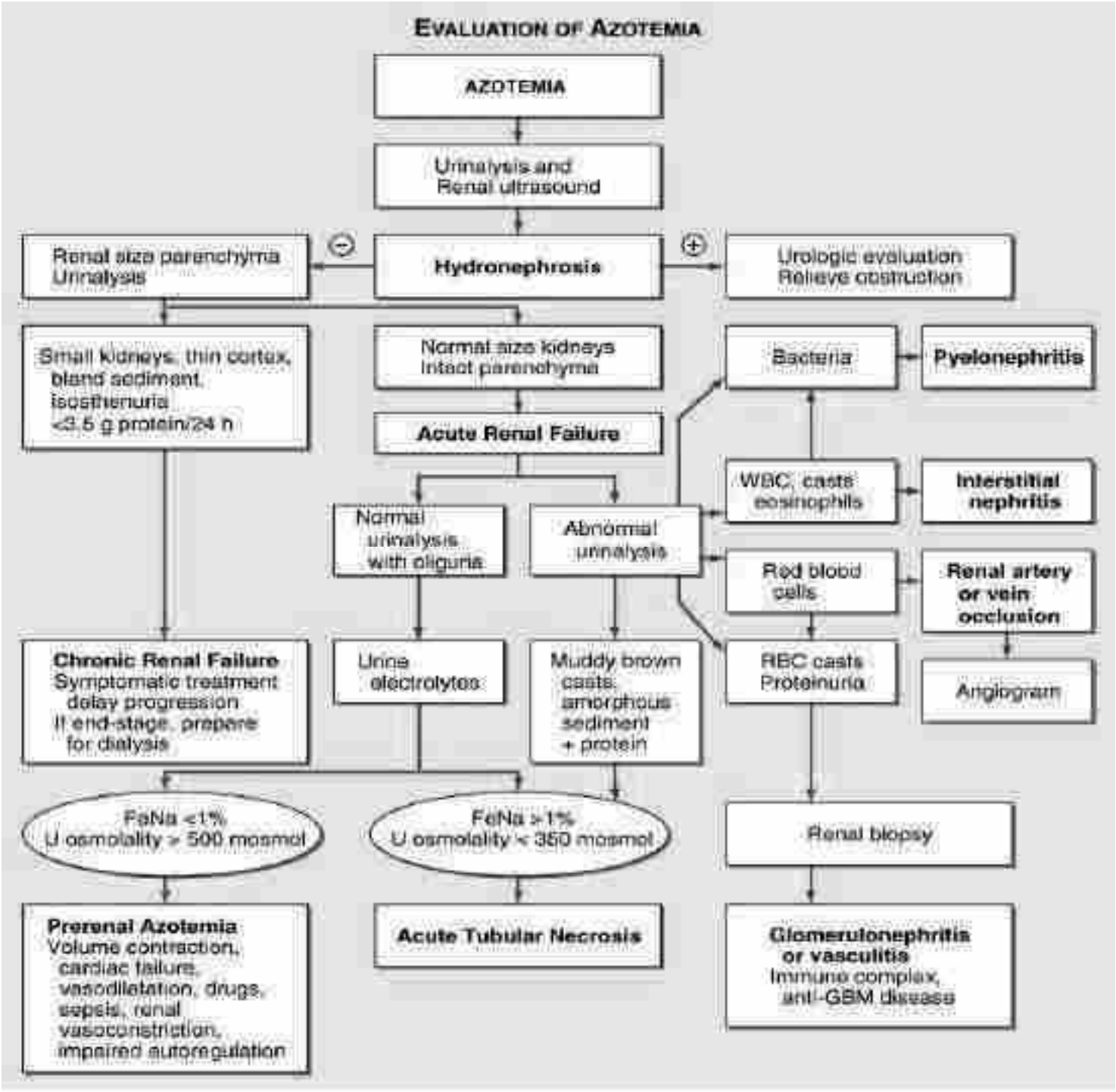
Decreased circulating blood volume	Volume sequestration	Decreased effective arterial volume	Decreased cardiac output
GI hemmorrhage	Pancreatitis	Cardiogenic shock	Sepsis
Burns	Peritonitis	Septic shock	Severe heart failure
Diarrhea	rhabdomyolysis		Hepatorenal syndrome
Diuretic			NSAIDS / Drugs

Postrenal azotemia

- Urinary tract obstruction <5% of ARF but reversible

- Causes :
  1. obstruction at urethra or bladder outlet
  2. Bilateral ureteral obstruction
  3. Unilateral obstruction with single functioning kidney

- Diagnosis :
  - ureteral or renal pelvic dilatation on renal usg


EVALUATION OF AZOTEMIA
AZOTEMIA
Urinalysis and Renal ultrasound
Renal size parenchyma Urinalysis
Hydronephrosis
Urologic evaluation Relieve obstruction
Small kidneys, thin cortex, bland sediment, isosthenuria <3.5 g protein/24 h
Normal size kidneys Intact parenchyma
Acute Renal Failure
Bacteria
Pyelonephritis
WBC, casts eosinophils
Interstitial nephritis
Normal urinalysis with oliguria
Abnormal urinalysis
Red blood cells
Renal artery or vein occlusion
Angiogram
Chronic Renal Failure Symptomatic treatment delay progression If end-stage, prepare for dialysis
Urine electrolytes
Muddy brown casts, amorphous sediment + protein
RBC casts Proteinuria
FeNa <1% U osmolality > 500 mosmol
FeNa >1% U osmolality < 350 mosmol
Renal biopsy
Prerenal Azotemia Volume contraction, cardiac failure, vasodilatation, drugs, sepsis, renal vasoconstriction, impaired autoregulation
Acute Tubular Necrosis
Glomerulonephritis or vasculitis Immune complex, anti-GBM disease

Intrinsic renal disease

- Involve
  - large renal vessels,
  - intrarenal microvasculature and
  - glomeruli or tubulo-interstitium.
- Ischemic & toxic ATN :
  - 90% cases.
- Structural tubule injury.

Causes

Ischemic ATN	Nephrotoxic ATN	Tubulointerstitial	Other
•Major surgery •Trauma •Severe hypovolemia •Overwhelming sepsis •Extensive burns	•Intrarenal vasoconstriction •Direct tubule toxicity •Tubule obstruction	•Drug induced interstitial nephritis •Severe infection •SLE Sarcoidosis •Lymphoma •Leukemia	•Occlusion of large vessels •Atheroemboli •Aortic dissection •Vasculitis

Haematuria

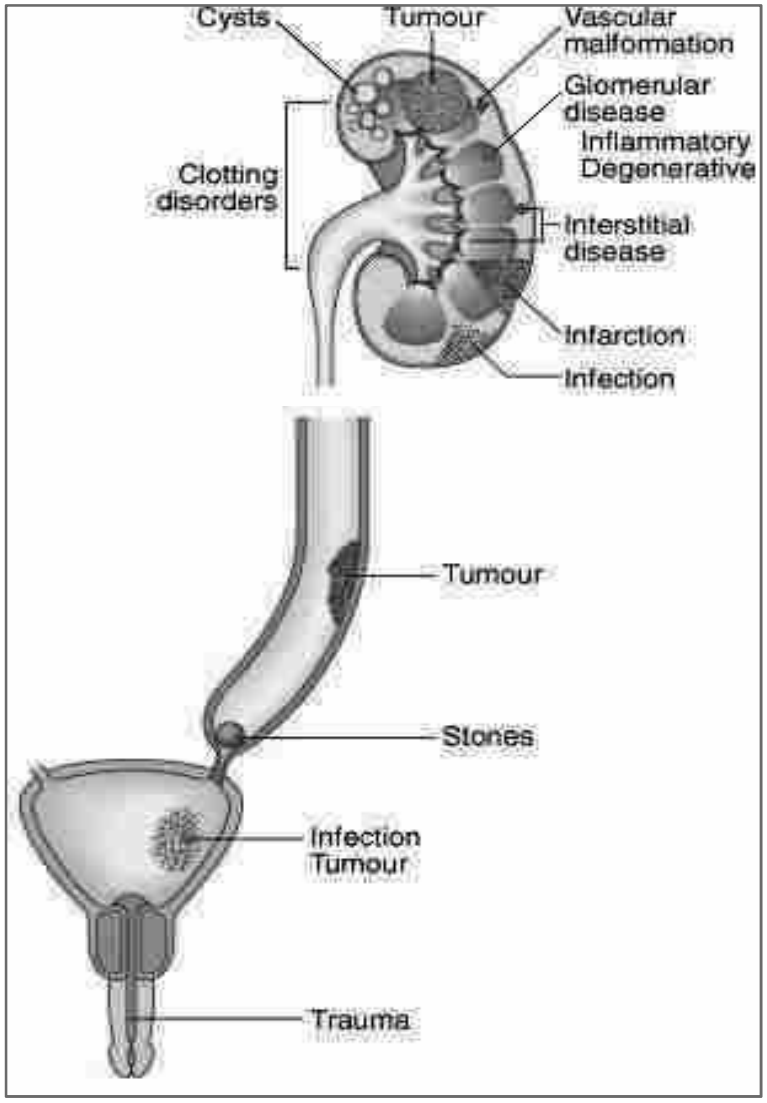
HEMATURIA

- **Defination** –
  - Two to Five RBCs per high power field (HPF) and
  - Can be detected by dipstick.

- **Persistent or Significant Hematuria** –
  - >3 RBCs/HPF on three urinalysis, a single uinalysis with >100 RBCs, or gross hematuria.

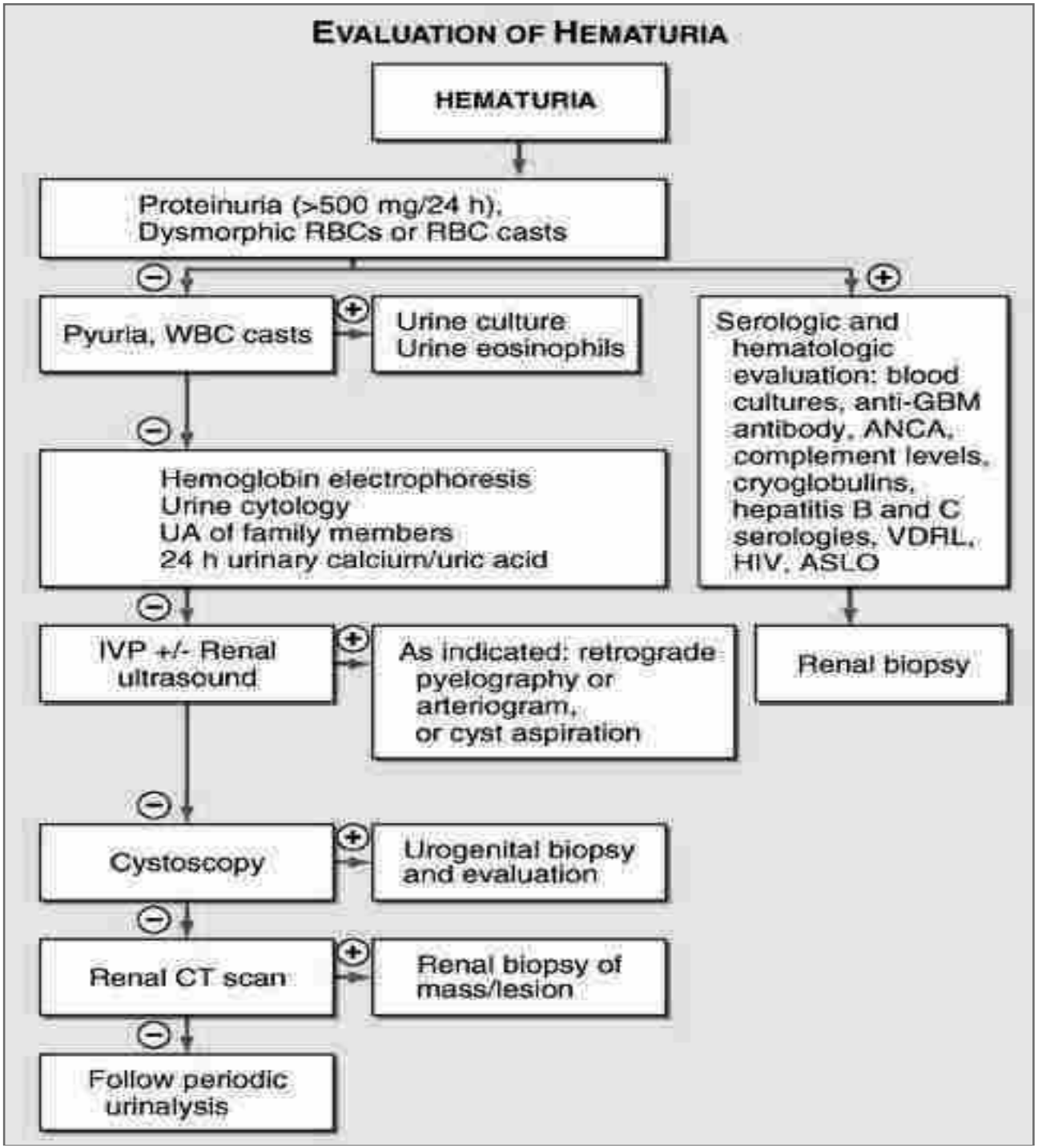
- **Bleeding from the Urinary Tract**
  - Isolated hematuria without poteinuria, other cells or casts.

Causes



Isolated Hematuria	Stones Neoplasms Tuberculosis Trauma Prostatitis
Single Urinalysis With Hematuria	Menstruation Viral illness Allergy Exercise Mild trauma
Isolated Glomerular Hematuria	IgA nephropathy Hereditary nephritis Thin Basement Membrane Disease

- **Glomerulonephritis**
  - Hematuria with Dysmorphic RBCs, RBC cast, Protein excretion >500 mg/d is diagnostic.
- **Infection**
  - Hematuria with Bacteriuria and Pyuria is typical.
- **Gross Hematuria**
  - Acute Cystitis or Urethritis in woman can cause.
- **Unexplained Isolated Hematuria**
  - Hypercalciuria and Hyperuricosuria
- **Urogenital Neoplasms**
  - Isolated Painless hematuria with Nondysmorphic RBCs


EVALUATION OF HEMATURIA
HEMATURIA
Proteinuria (>500 mg/24 h), Dysmorphic RBCs or RBC casts
Pyuria, WBC casts
Urine culture
Urine eosinophils
Serologic and hematologic evaluation: blood cultures, anti-GBM antibody, ANCA, complement levels, cryoglobulins, hepatitis B and C serologies, VDRL, HIV, ASLO
Hemoglobin electrophoresis
Urine cytology
UA of family members
24 h urinary calcium/uric acid
IVP +/- Renal ultrasound
As indicated: retrograde pyelography or arteriogram, or cyst aspiration
Renal biopsy
Cystoscopy
Urogenital biopsy and evaluation
Renal CT scan
Renal biopsy of mass/lesion
Follow periodic urinalysis

Oliguria & Anuria

Oliguria & anuria

- **Definition**
  - Oliguria - 24hr urine output <400ml.
  - Anuria - complete absence of function <100ml.

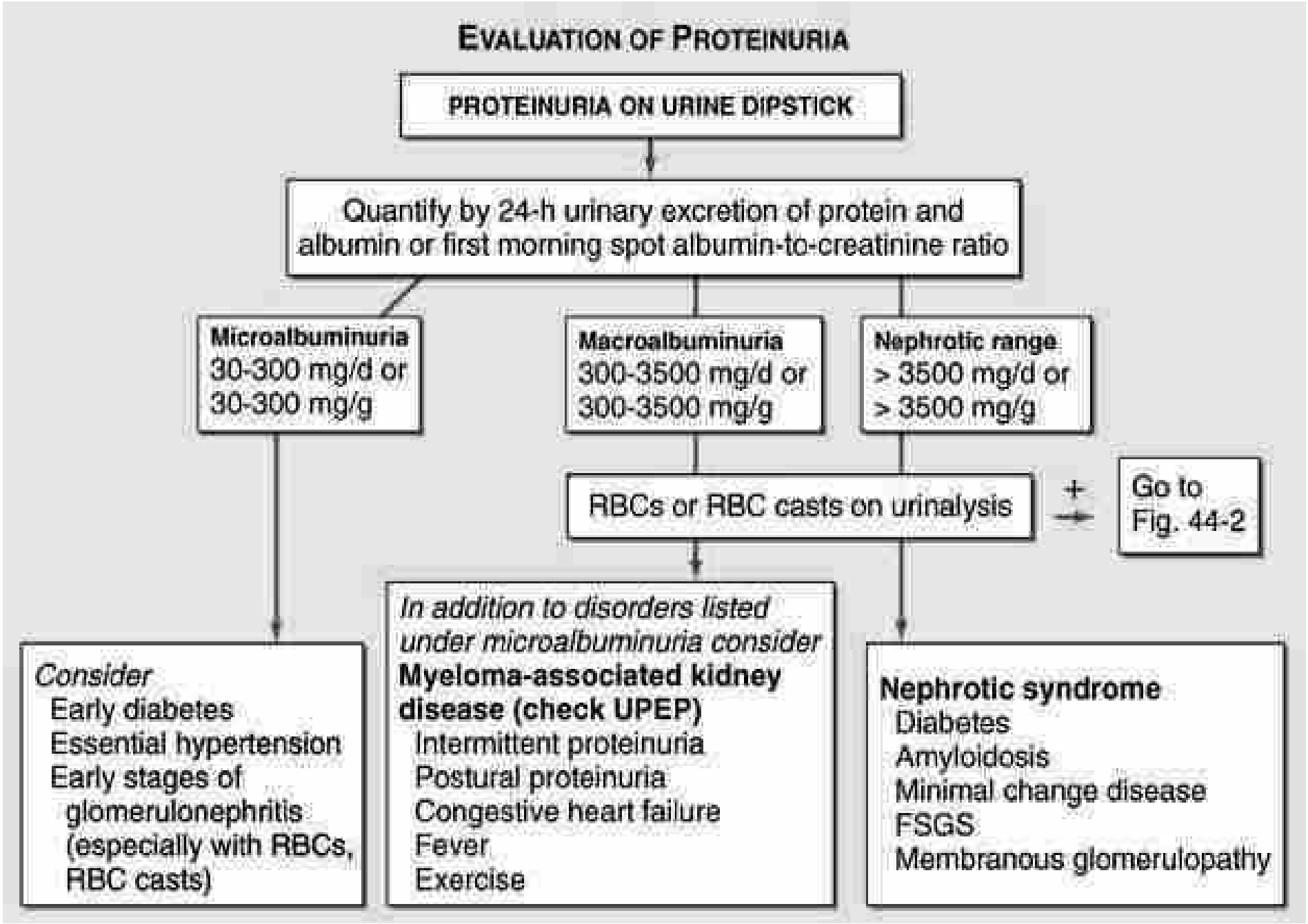
- **Anuria causes**
  - Total urinary obstruction.
  - Total renal artery or vein occlusion.
  - Shock.
  - Cortical necrosis.
  - ATN.
  - RPGN.

Proteinuria

Proteinuria

- **<u>Dipstick measurement :</u>**
  - Detect only albumin.
  - False positive results at ph > 7, concentrated urine, blood contamination.

- **<u>Albumin to creatinine ratio</u>**
  - 24 hr albumin excretion rate.

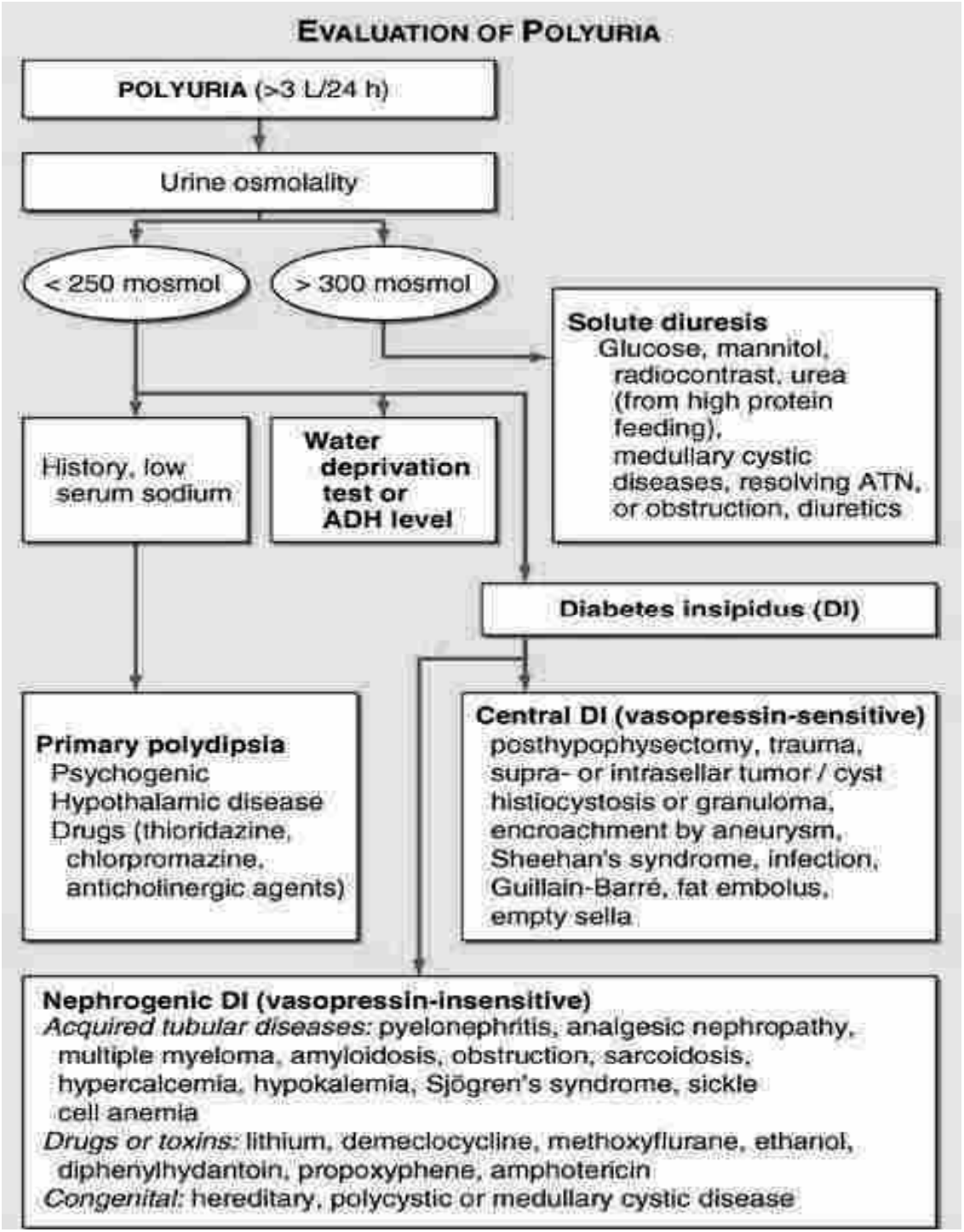

EVALUATION OF PROTEINURIA
PROTEINURIA ON URINE DIPSTICK
Quantify by 24-h urinary excretion of protein and albumin or first morning spot albumin-to-creatinine ratio
Microalbuminuria
30-300 mg/d or
30-300 mg/g
Macroalbuminuria
300-3500 mg/d or
300-3500 mg/g
Nephrotic range
> 3500 mg/d or
> 3500 mg/g
RBCs or RBC casts on urinalysis
+
−
Go to Fig. 44-2
Consider
Early diabetes
Essential hypertension
Early stages of glomerulonephritis (especially with RBCs, RBC casts)
In addition to disorders listed under microalbuminuria consider
Myeloma-associated kidney disease (check UPEP)
Intermittent proteinuria
Postural proteinuria
Congestive heart failure
Fever
Exercise
Nephrotic syndrome
Diabetes
Amyloidosis
Minimal change disease
FSGS
Membranous glomerulopathy

Polyuria

Polyuria

- Urine output > 3L/day
- **Causes** :
  - Excretion of nonabsorbable solutes such as glucose
  - Excretion of water d/t defect in ADH production or renal responsiveness.

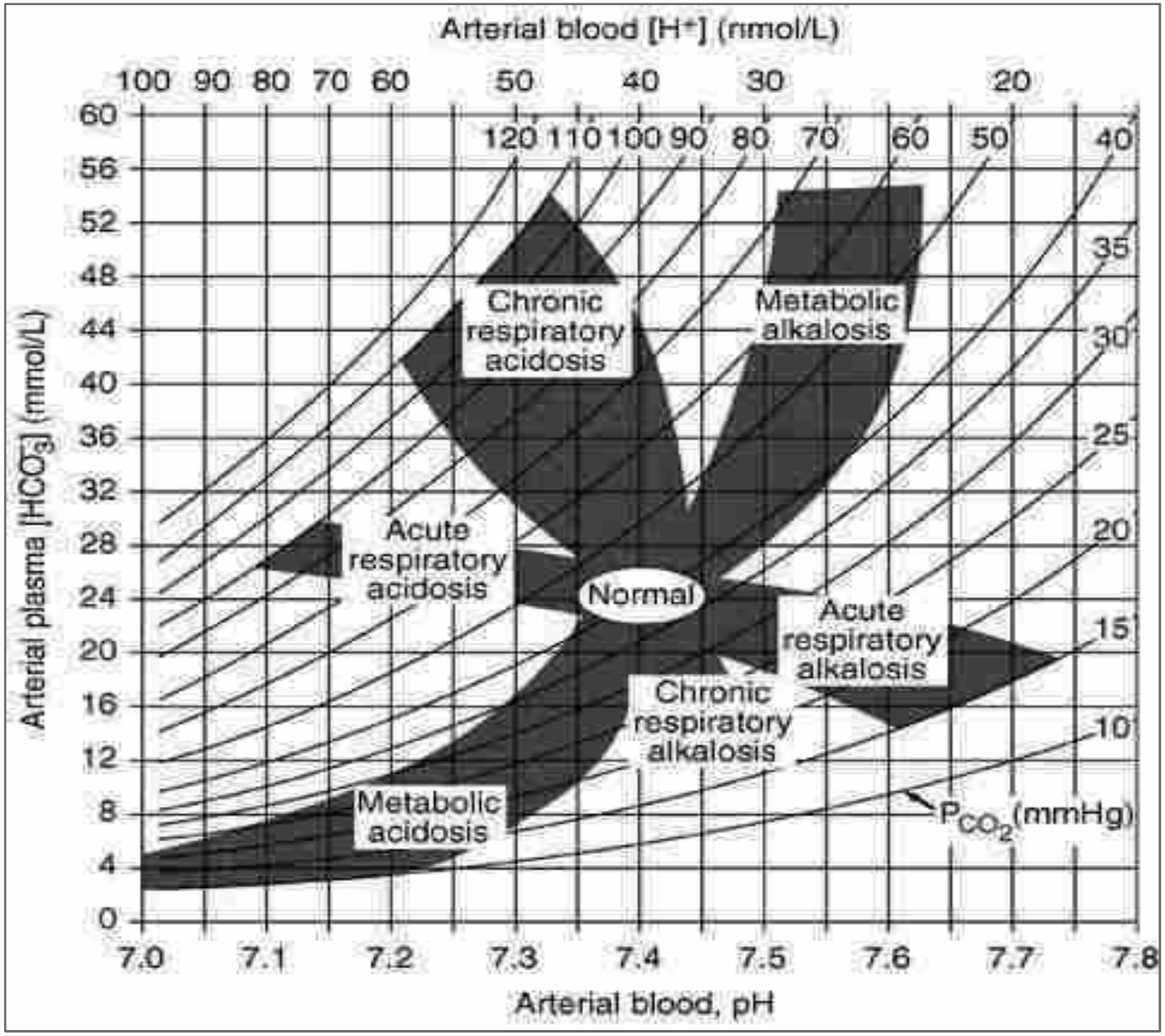
- **Investigation**:
  - urine osmolality.
  - Plasma vasopressin level best to distinguish between central & nephrogenic DI.
  - Water deprivation test +exogenous vasopressin to r/o primary polydipsia from DI.


EVALUATION OF POLYURIA
POLYURIA (>3 L/24 h)
Urine osmolality
< 250 mosmol
> 300 mosmol
Solute diuresis
Glucose, mannitol, radiocontrast, urea (from high protein feeding), medullary cystic diseases, resolving ATN, or obstruction, diuretics
History, low serum sodium
Water deprivation test or ADH level
Diabetes insipidus (DI)
Primary polydipsia
Psychogenic
Hypothalamic disease
Drugs (thioridazine, chlorpromazine, anticholinergic agents)
Central DI (vasopressin-sensitive)
posthypophysectomy, trauma, supra- or intrasellar tumor / cyst histiocystosis or granuloma, encroachment by aneurysm, Sheehan's syndrome, infection, Guillain-Barré, fat embolus, empty sella
Nephrogenic DI (vasopressin-insensitive)
Acquired tubular diseases: pyelonephritis, analgesic nephropathy, multiple myeloma, amyloidosis, obstruction, sarcoidosis, hypercalcemia, hypokalemia, Sjögren's syndrome, sickle cell anemia
Drugs or toxins: lithium, demeclocycline, methoxyflurane, ethanol, diphenylhydantoin, propoxyphene, amphotericin
Congenital: hereditary, polycystic or medullary cystic disease

Pyuria

- Isolated Pyuria is unusual.
- Presence of bacteria suggests infection.
- Presence of WBC cast with bacteria indicative of Pyelonephritis.
- Pyuria with WBCs or WBC cast seen in
  - Acute Glomerulonephitis.
  - Tubulointerstitial processes as
    - Interstitial Nephritis
    - Transplant Rejection.

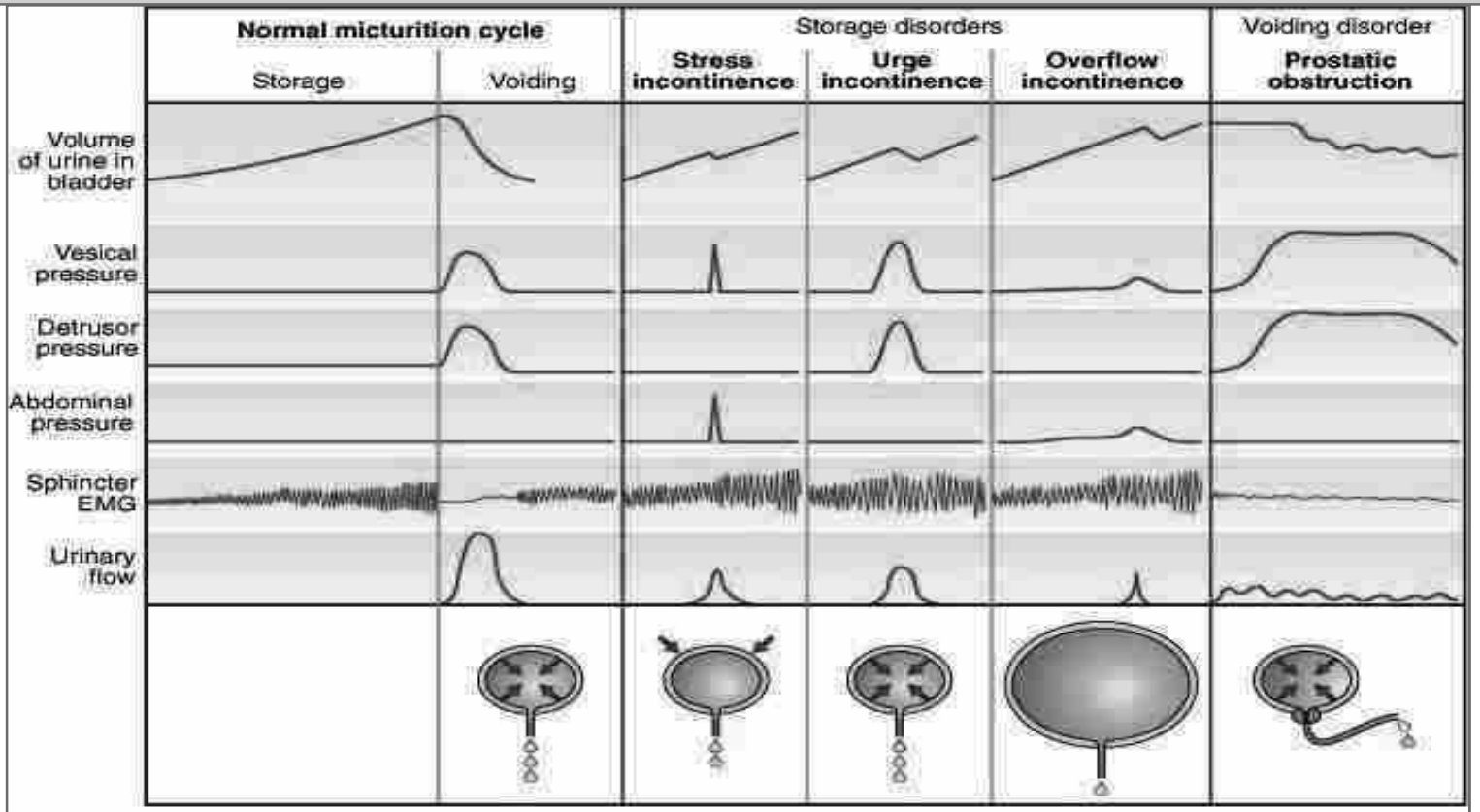
pH Imbalance



Acid-Base nomogram. Shown are the 90% confidence limits(range of values) of normal respiratory and metabolic compensationsfor primary acid-base disturbances

Urinary incontinence

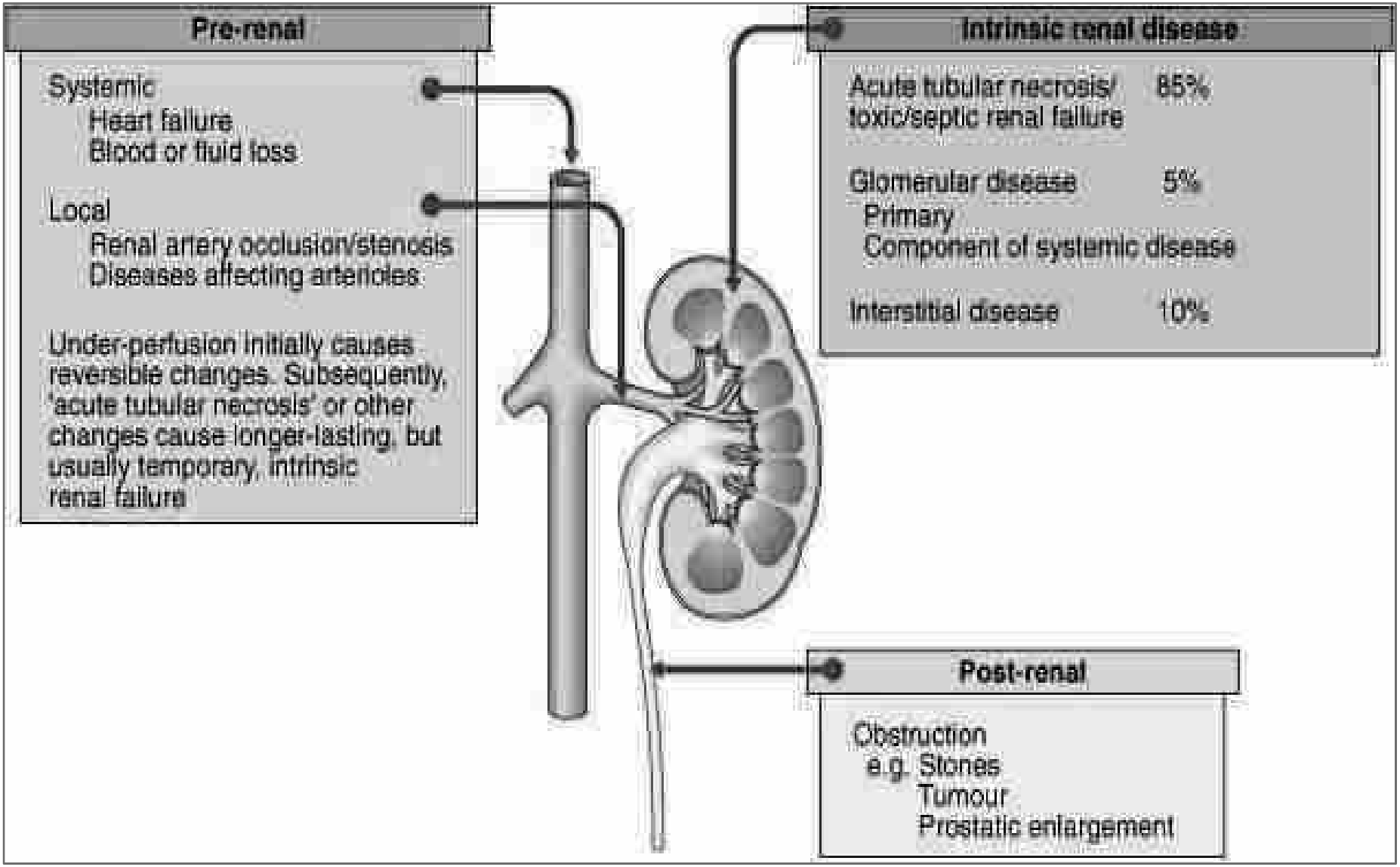
Urodynamic abnormalities in patients with urinary incontinence. (EMG = electromyogram



Normal micturition cycle	Storage	Minimal pressure rise in bladder as it fills. No detrusor activity, sphincter muscle tone increased during filling
	Voiding	Sphincter relaxes, detrusor contracts and vesical pressure increases. Good urine flow until bladder empty
Stress incontinence		As for normal but during cough there is passive increase in the vesical pressure (no active detrusor contraction). This vesical pressure is greater than urethral pressure transiently with resultant urine leakage
Urge incontinence		As for normal but during filling there is abnormal detrusor contraction; this results in a raised vesical pressure, and if this is greater than the urethral pressure, leakage occurs
Overflow incontinence		Bladder is very full. No detrusor contractions but minimal increases in abdominal and vesical pressure result in urine leakage (as in stress incontinence)
Prostatic obstruction		This shows only the voiding phase; the storage phase is the same as the normal. During voiding there is a strong detrusor contraction resulting in high vesical pressure. Despite this high pressure there is poor flow because of the obstruction in the prostatic urethra. Flow is typically wavy as the prostatic obstruction is somewhat elastic and varies throughout the void

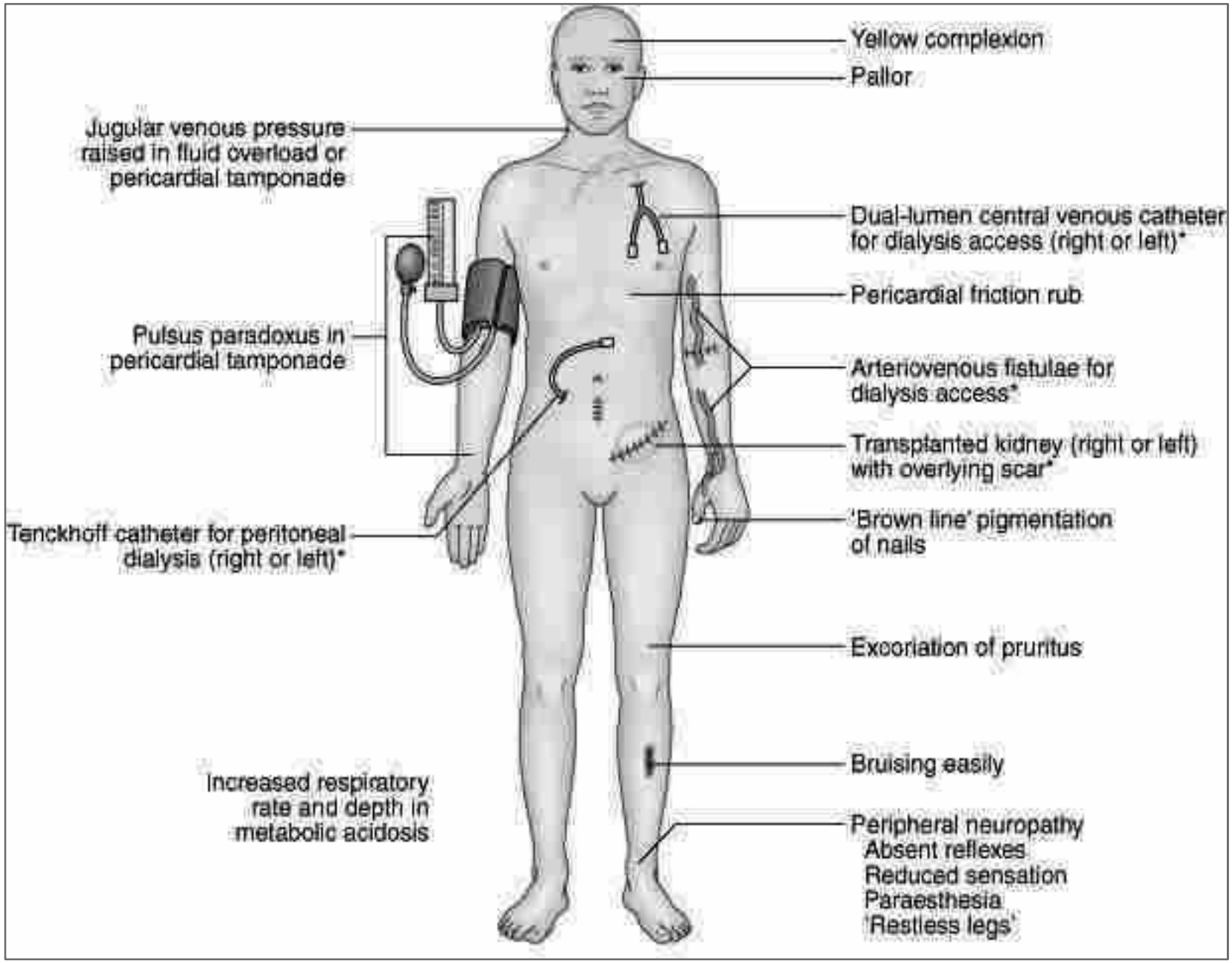
ARF

Acute renal failure



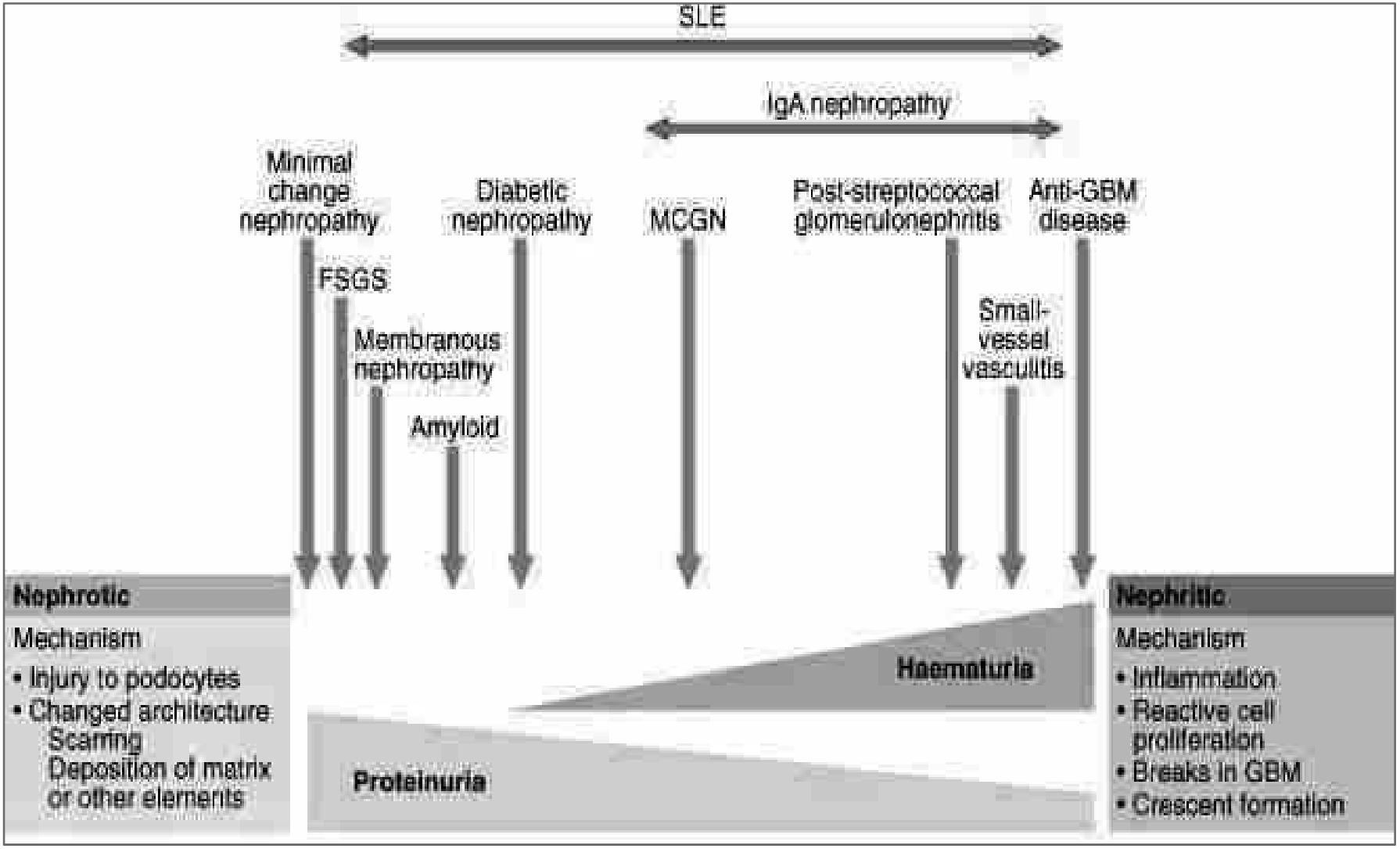
CRF

Physical signs in chronic renal failure. (* Features of renal replacement therapy)



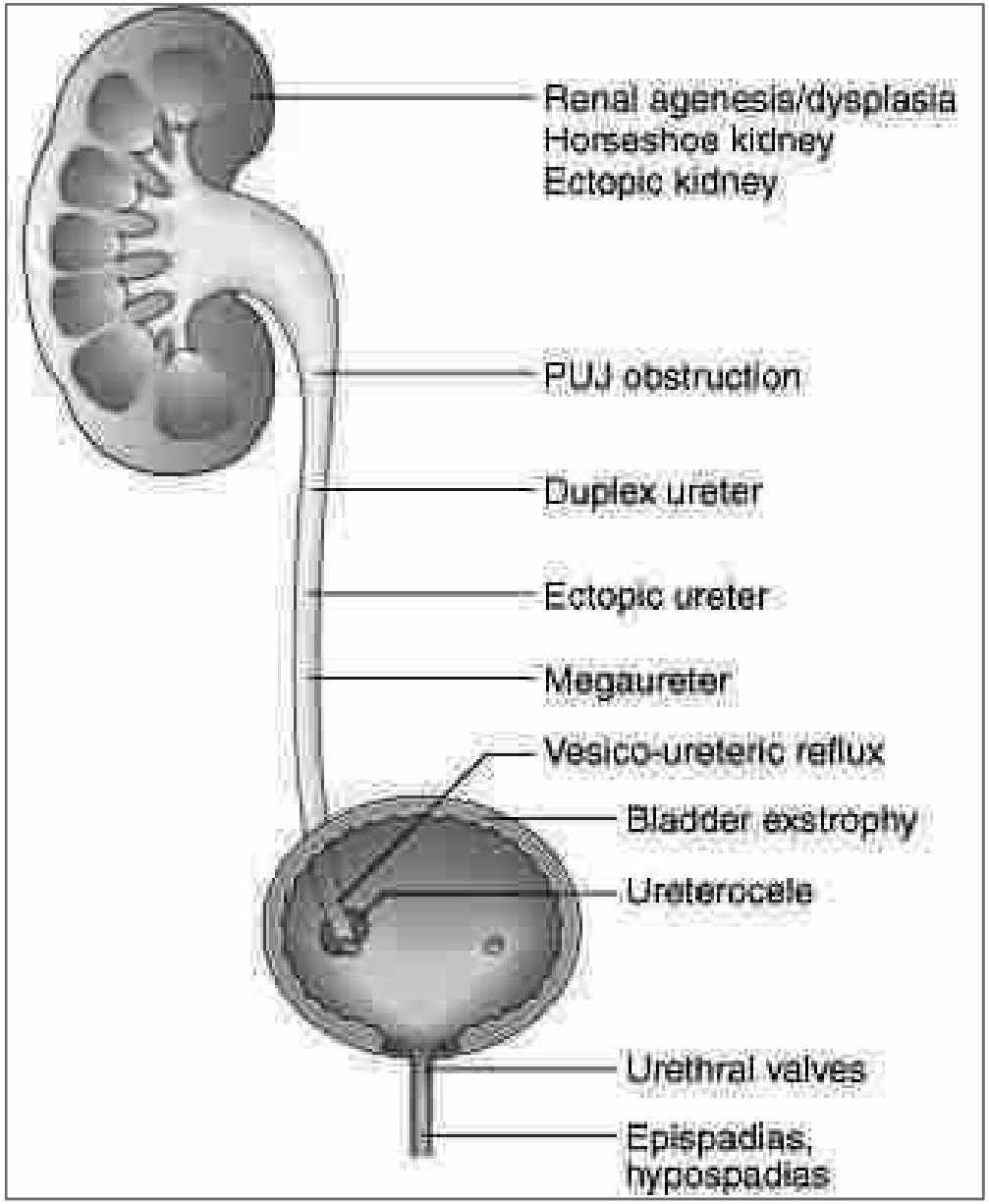
Spectrum of glomerular diseases

Spectrum of glomerular diseases



Congenital abnormalities of the urinary tract

Congenital abnormalities of the urinary tract



Investigations

Investigations

- **Urine** :
  - Proteinuria/ hematuria/ Pyuria/ WBC casts / Urine Culture/
- CBC Profile
- Peripheral Blood Smear for Anemia
- **USG**
- **Intravenous Pyelography**

- **Intravenous urography (IVU)**
- **DMSA isotope renogram**.

- **Renal biopsy**
- **Coagulation Profile/ Lipid Level/TSH in Nephrotic Syndrome**
- Allergic interstitial nephritis:
  - eosinophiluria

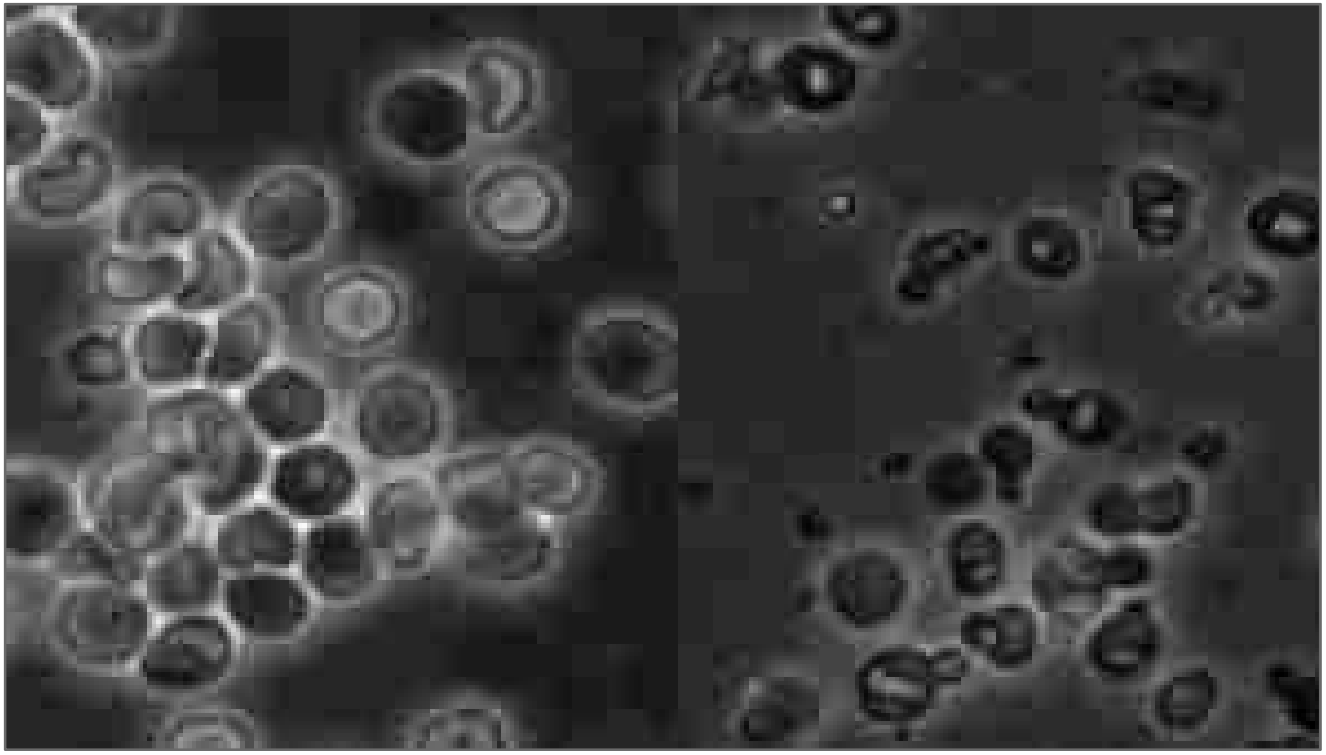
Urine Cast

- *Urine cast are formed when proteins & other organic matter solidify in the Renal Tubule.*
  - Indicative of Disease of Nephron.
  - Cast may contain
    - Proteins (Hyaline)
    - Erythrocytes (RBC)
    - Leucocytes (WBC)
    - Epethelial Cells.

Clinical significance of Urine Cast

Hyaline Cast	Normal Constitute Tom Horsfall Protein Loop of Henle Excreted as Hyaline Cast
RBC cast	Suggest Glomerular Disease
WBC Cast	Interstitial Nephritis Interstitial Injury
WBC Cast with Bacteria	indicates Pyelonephritis
BROAD cast	CRF Interstitial Fibrosis & Dilatation of tubules
Pigmented Muddy Brown Granular Cast	Ischemic or Nephrotoxic Drugs

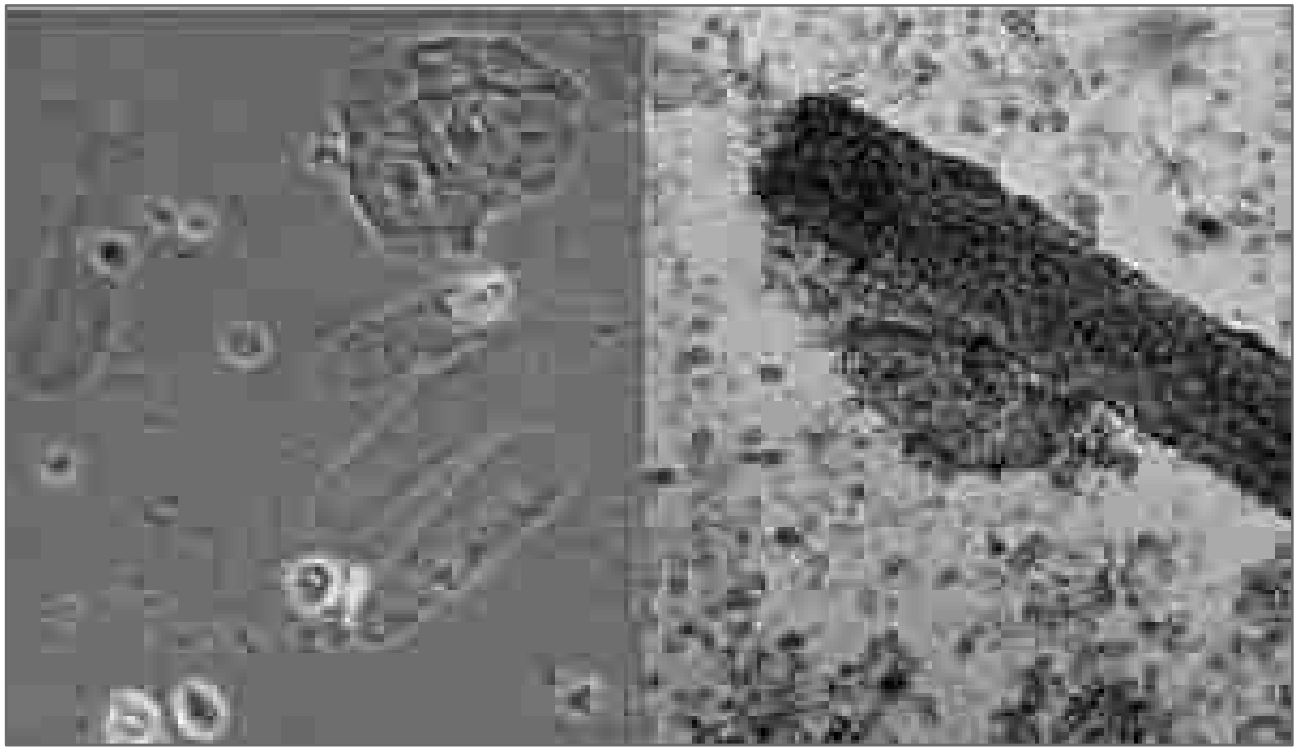
URINE MICROSCOPY



red blood cells (× 400) **glomerular bleeding** with many dysmorphic forms including acanthocytes (**teardrop forms**),

red blood cells showing on the right and on the **left bleeding from lower in the urinary tract.**

URINE MICROSCOPY

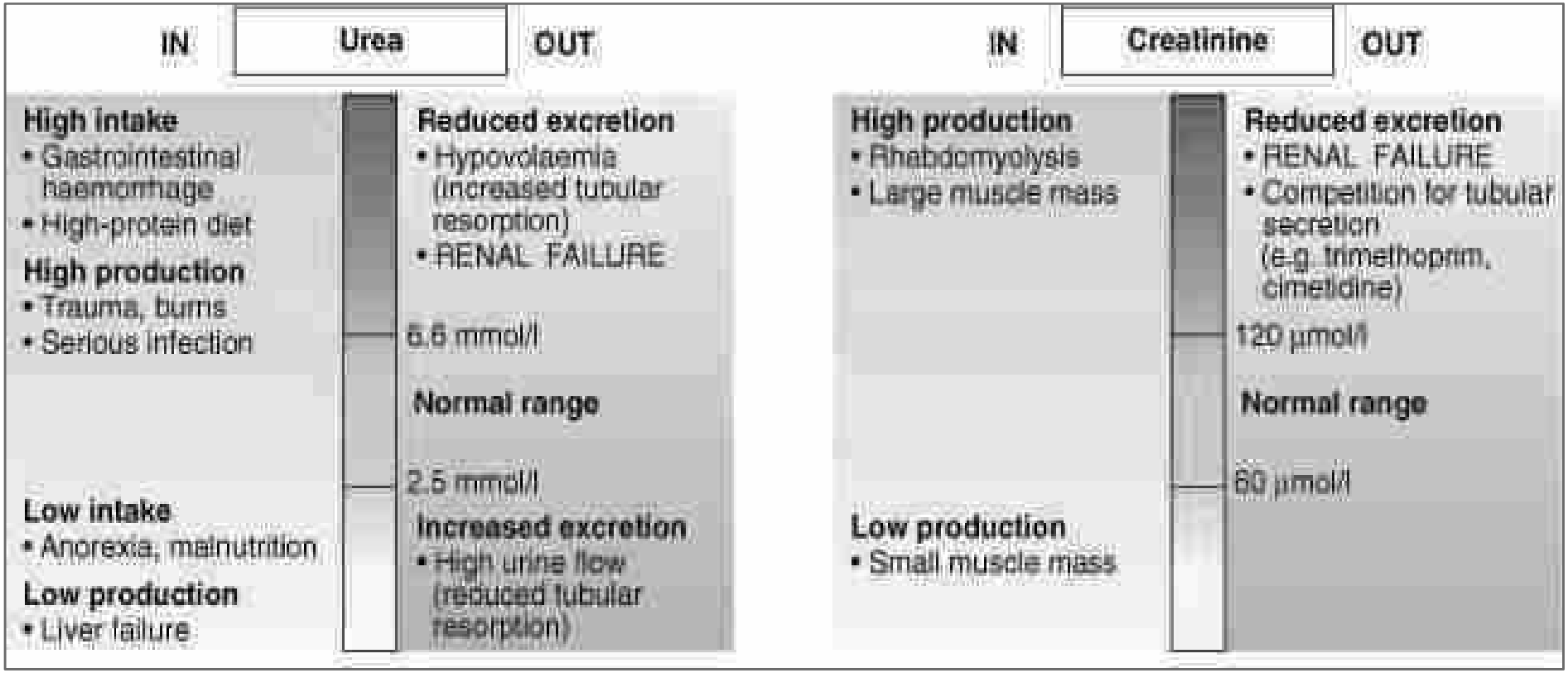


phase contrast images show **hyaline casts**, a normal feature of urine (× 160).

numerous **red cells** and a **large red cell cast** in acute glomerular inflammation (× 100, not phase contrast).

TESTS OF FUNCTION

Factors affecting blood levels of urea and creatinine.

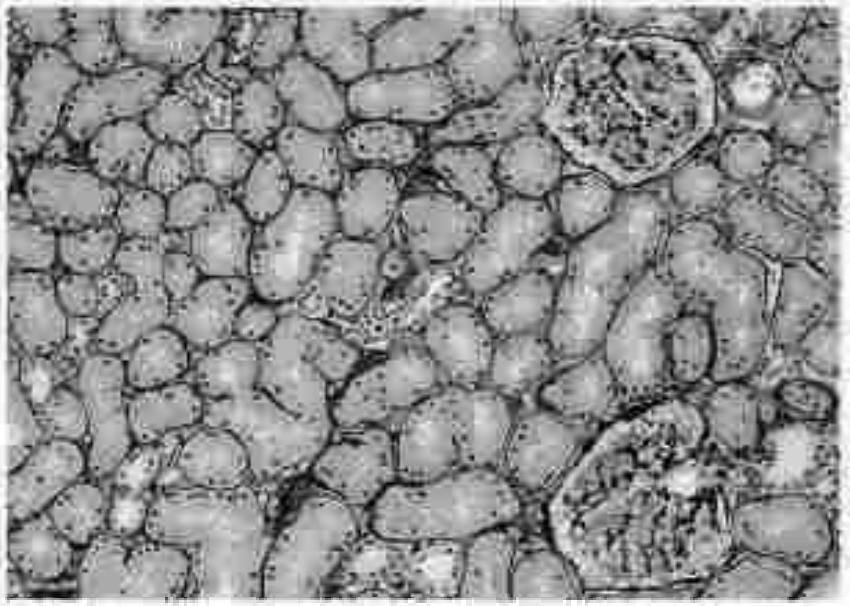


INTERPRETATION OF DIPSTICK-POSITIVE HAEMATURIA

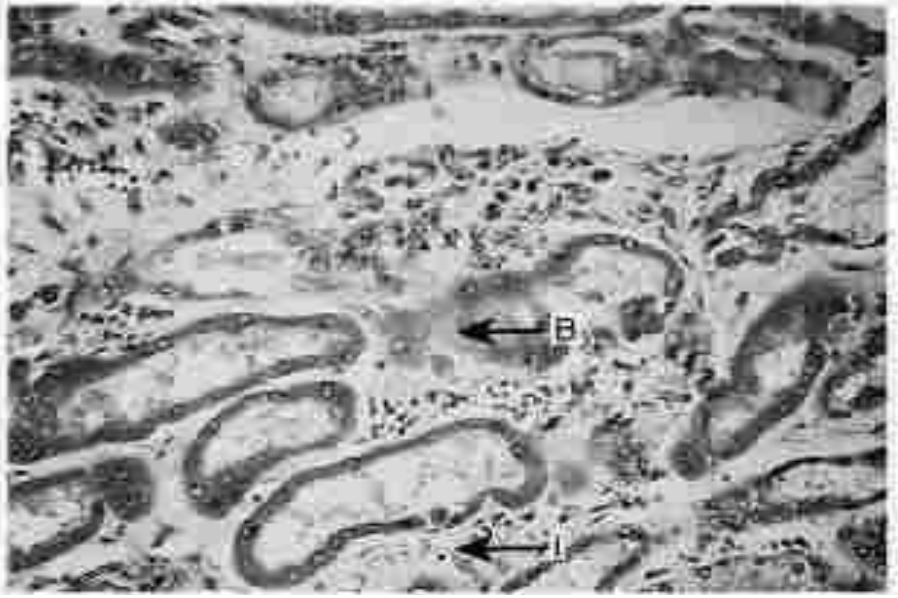
Dipstick test positive	Urine microscopy	Suggested cause
Haematuria	White blood cells	Infection
-	Abnormal epithelial cells	Tumour
-	Red cell casts	Glomerular bleeding*
-	Dysmorphic erythrocytes (phase contrast microscopy)	
Haemoglobinuria	No red cells	Intravascular haemolysis
Myoglobinuria	No red cells	Rhabdomyolysis

Histopath studies of glomerular disease

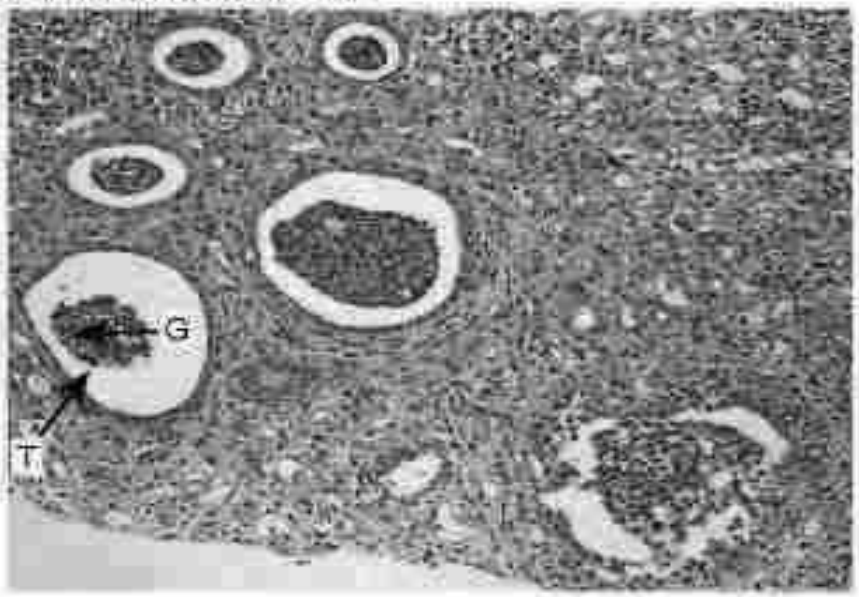
A Normal tubular histology



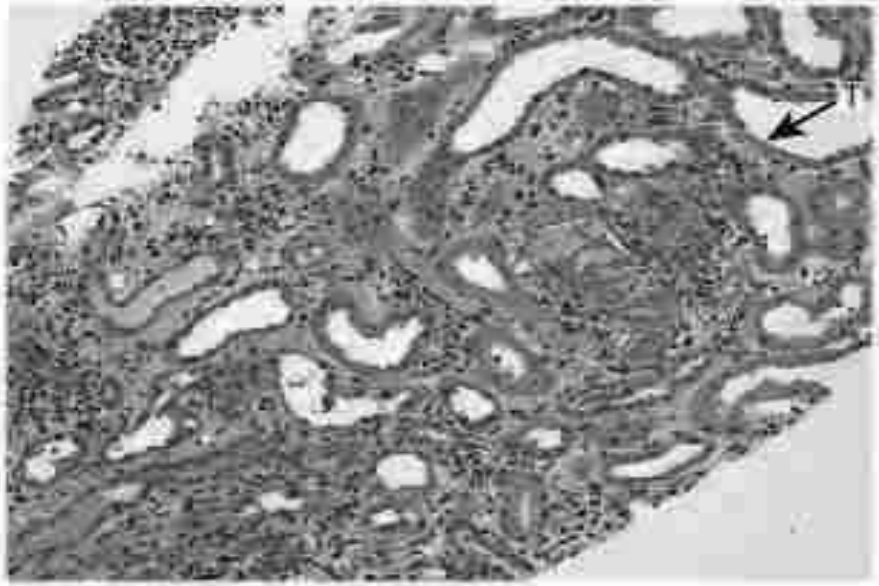
B Acute tubular necrosis



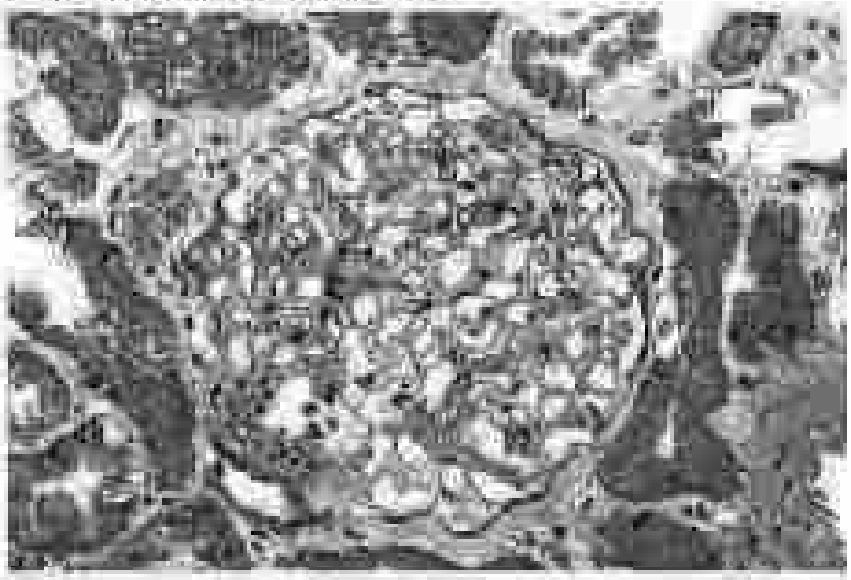
C Acute pyelonephritis



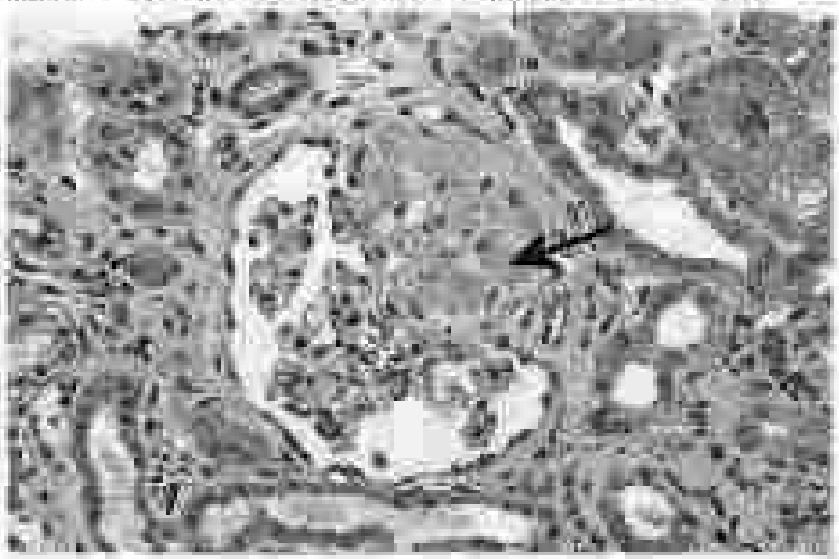
D Acute interstitial nephritis



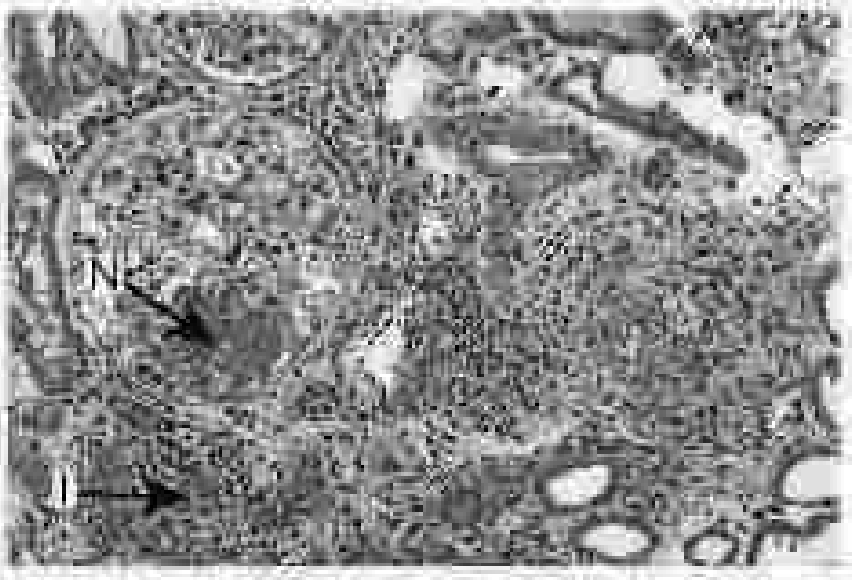
A Normal glomerulus



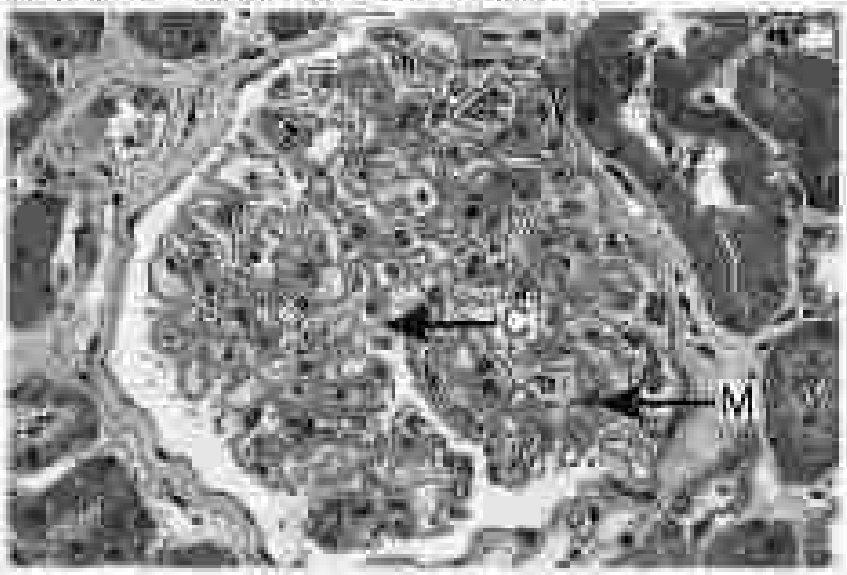
B Focal segmental glomerulosclerosis



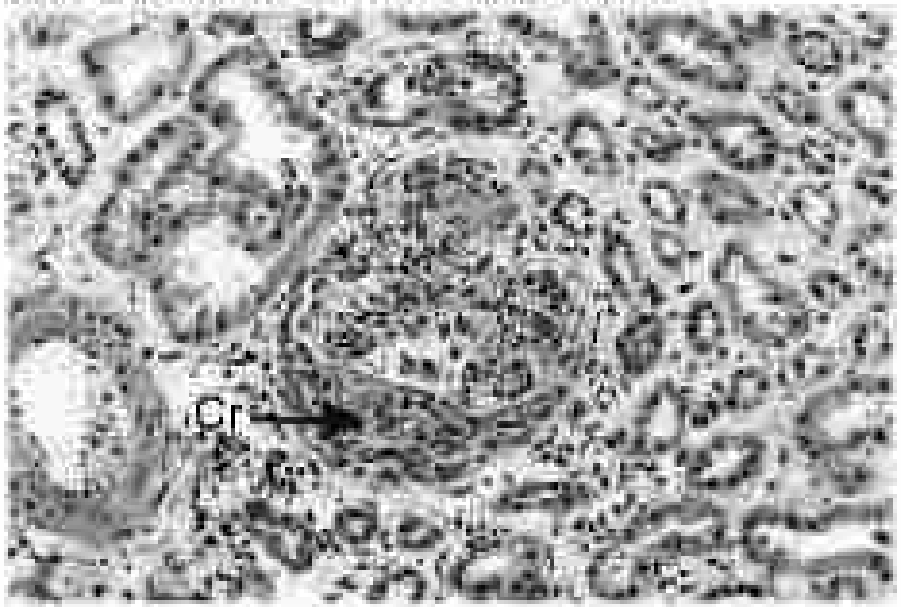
C Focal necrotising glomerulonephritis



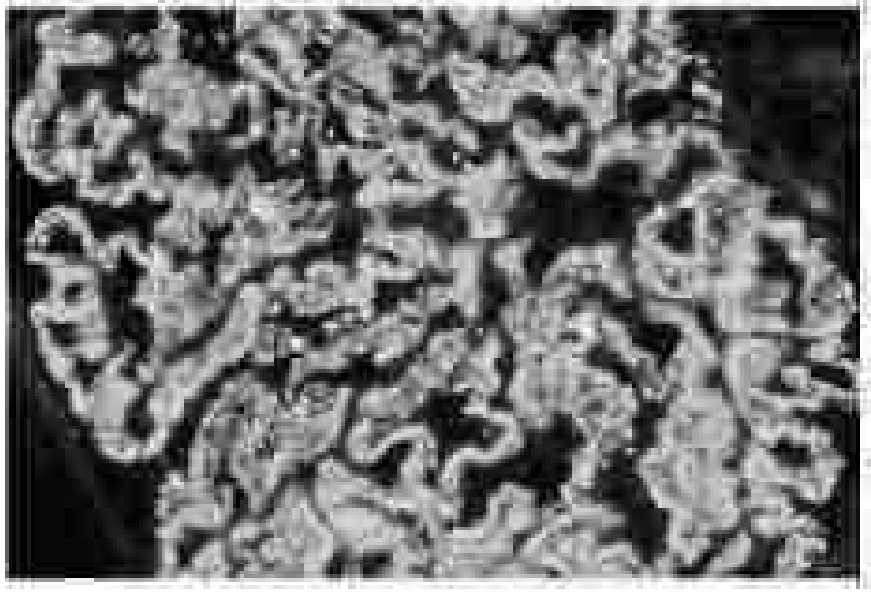
D Membranous nephropathy



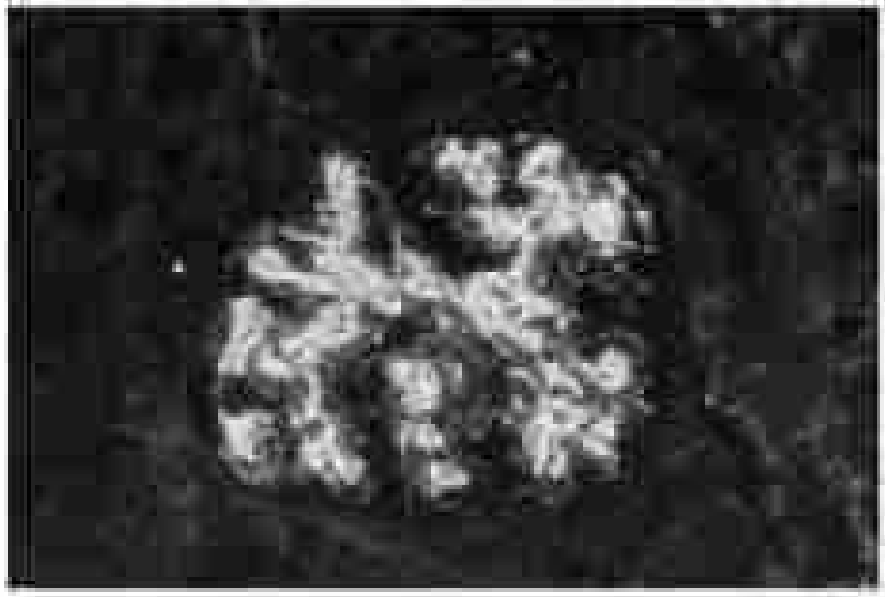
E Crescentic glomerulonephritis



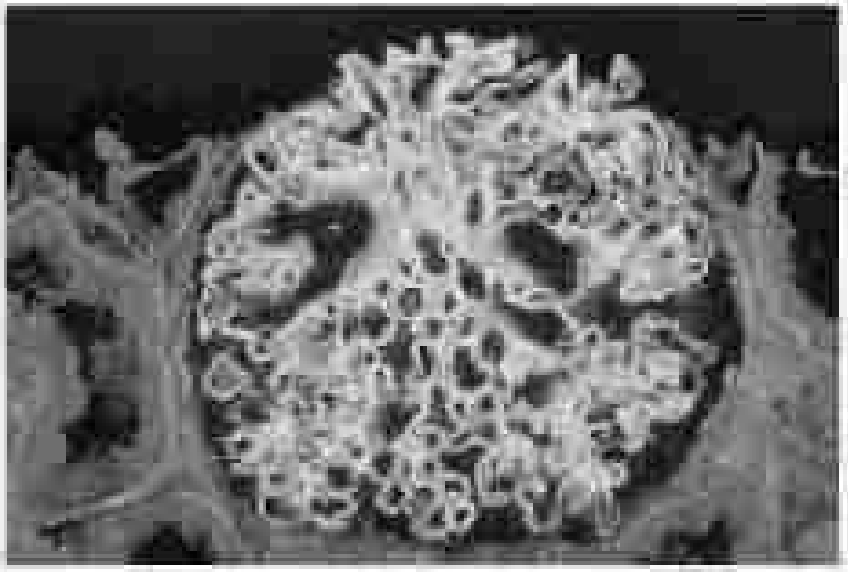
F Granular IgG–membranous nephropathy



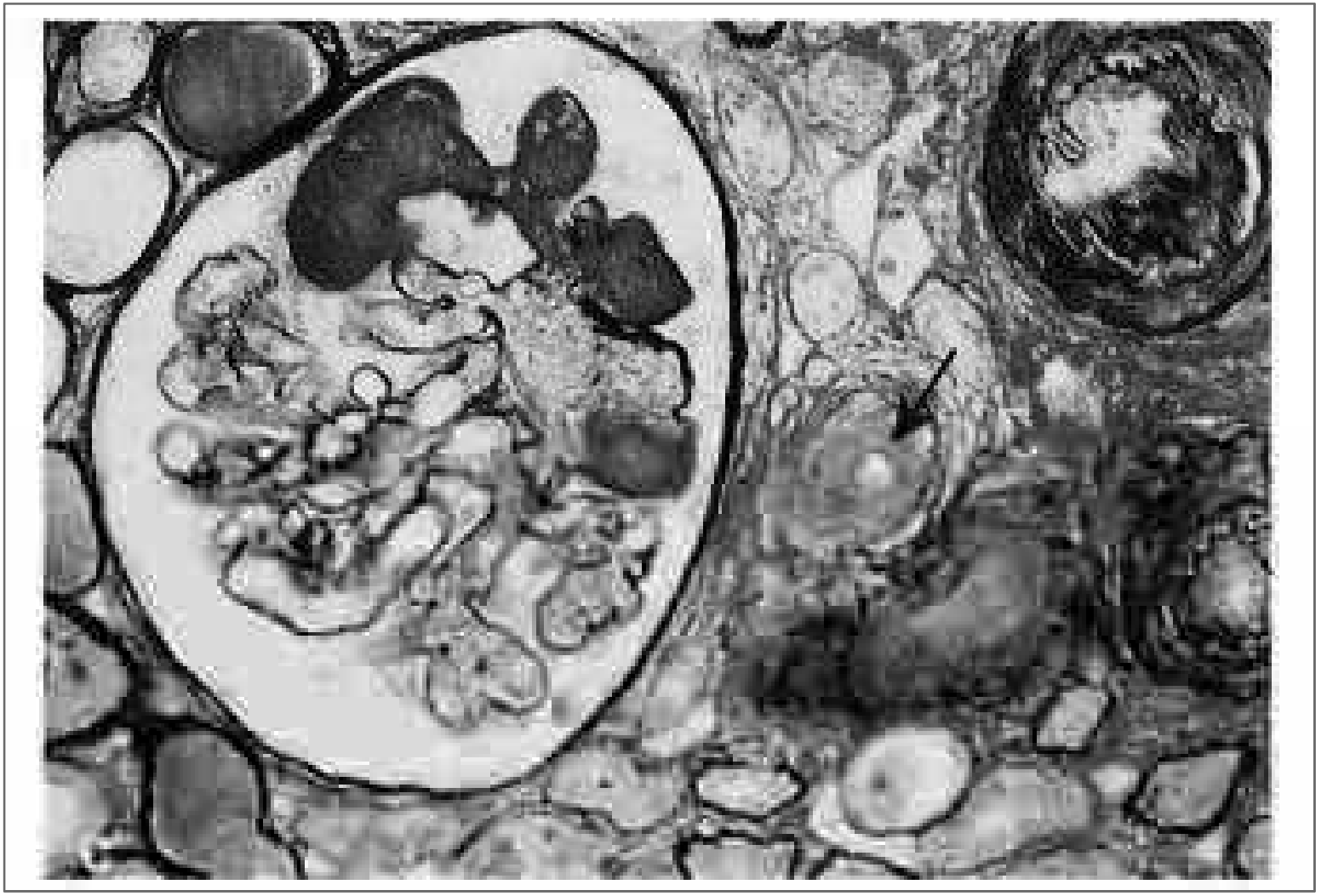
G Mesangial IgA– IgAnephropathy



H Linear IgG–anti-GBM disease

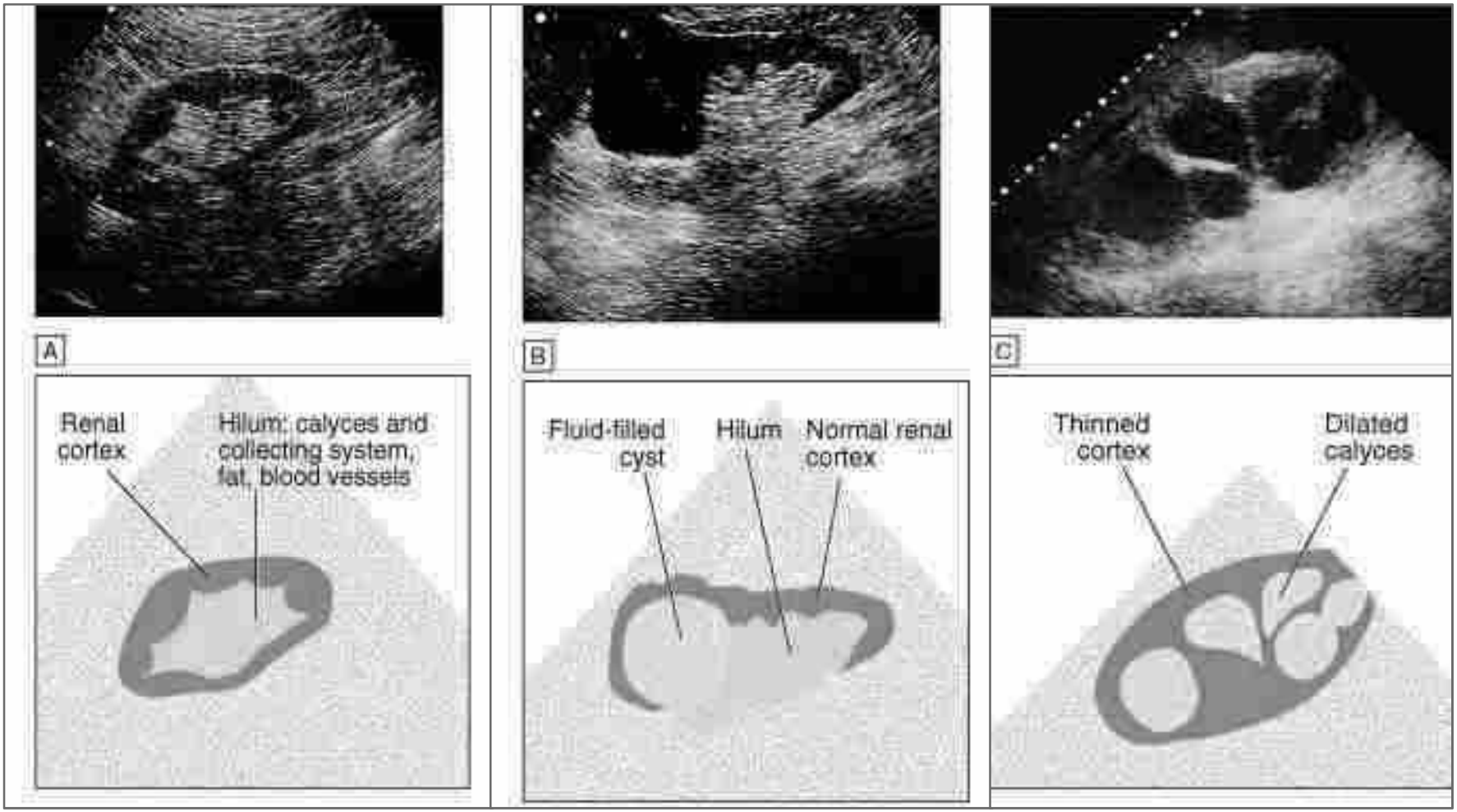


Glomerular capillary thrombosis in malignant hypertension



LAB findings in ARF

INDEX	Prerenal azotemia	Oliguric ARF
BUN/P creat ratio	>20:1	10-15:1
Urine Na meq/l	<20	>40
Urine osmolality	>500	<350
Fractional excretion of Na+	<1%	>2%
Urine /plasma creat	>40	<20
Urinalysis	None or granular/hyaline	Muddy brown



A. Normal kidney. The normal cortex is less echo-dense (blacker) than the adjacent liver.

B. A simple cyst occupies the upper pole of an otherwise normal kidney.

C. The renal pelvis and calyces are dilated by a chronic obstruction to urinary outflow. The thinness and increased density of the remaining renal cortex indicate chronicity

Intravenous urography (IVU)

Nephrogram phase at 1 minute.

Collecting system at 5 minutes.

Retrograde pyelography.

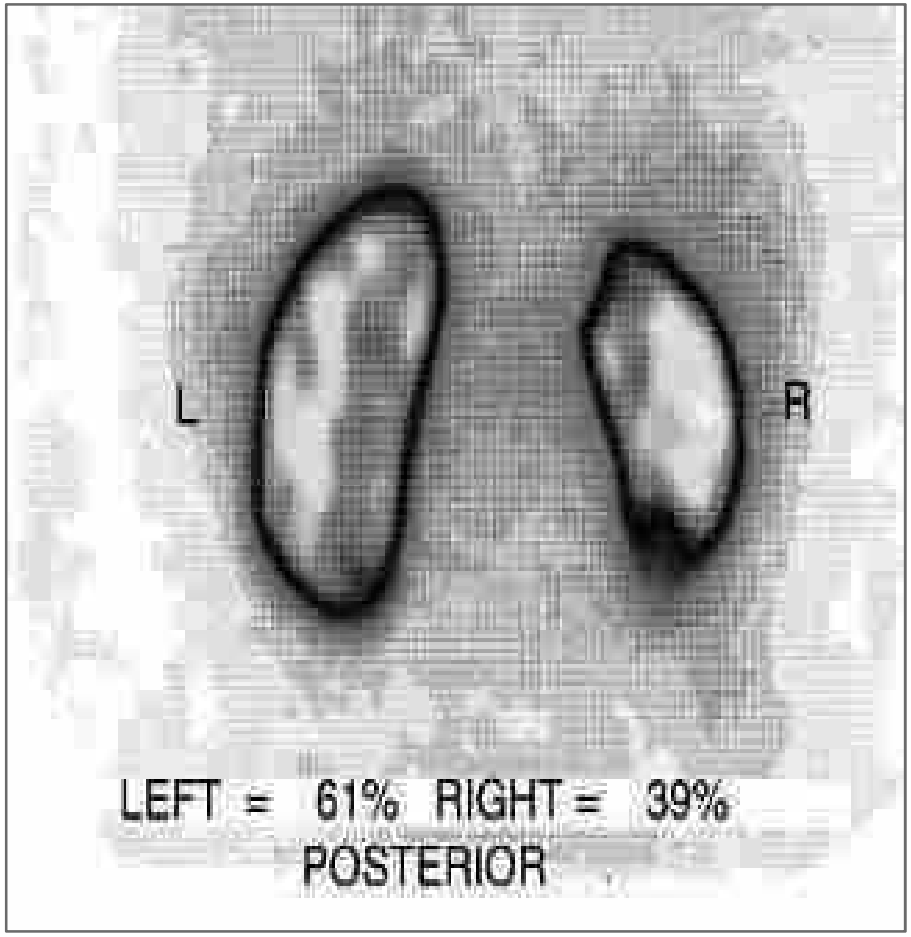
A catheter has been passed into the left renal pelvis at cystoscopy. The anemone-like calyces are sharp-edged and normal.

Bilateral staghorn calculi

The intravenous pyelogram demonstrates that, while some dye is being excreted by the right kidney, there is little function on the left.

Renal artery stenosis

DMSA isotope renogram



DMSA isotope renogram. A posterior view is shown of a normal left kidney and a small right kidney (with evidence of cortical scarring at upper and lower poles) which contributes only 39% of total renal function.

Renal biopsy

- **<u>Indications –</u>**
  - ARF not adequately explained
  - CRF with normal sized kidneys
  - Nephrotic sndrome or glomerular proteinuria in adults
  - Nephrotic syn in children wit atypical features or not responding to treatment
  - Isolated hematuria or preoteinuria with renal characteristics

Renal biopsy

- **Contraindications**:
  - Disordered coagulation or thrombocytopenia
  - Uncontrolled HTN
  - Kidneys<60% of predicted size
  - Solitary kidney except transplant.

- **Complications**:
  - Mild pain
  - Bleeding into urine minor
  - Bleeding around kidney , occ massive requiring angiography or surgery
  - Arteriovenous fistula rarely clinical significant

THANK YOU

EPILEPSY

Dr. Dilip R. Patil
Prof. and HOD General Medicine
ACPM Medical College, Dhule

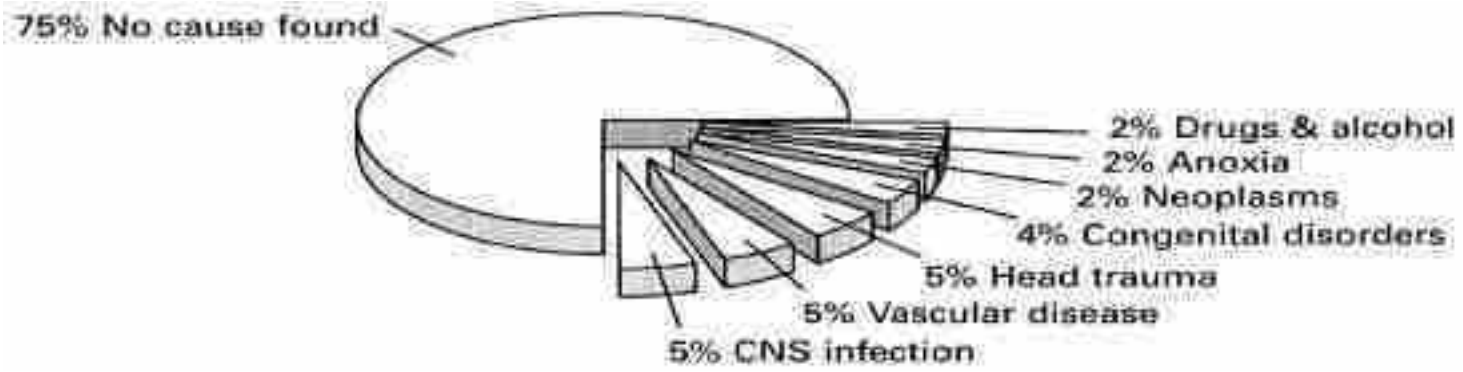
Epilepsy and Seizures

- Definition of seizures and epilepsy
- Epidemiology
- Classification of seizures
- Examples
  - Focal motor seizures
  - Complex partial seizures
    - Temporal lobe epilepsy and frontal lobe epilepsy
  - Generalized seizures

DEFINITIONS

- A *seizure* is a paroxysmal event due to abnormal excessive or synchronous activity in the brain.

- *Epilepsy* describes a condition in which a person has recurrent seizures due to a chronic, underlying process.
- This definition implies that a person with a single episode of seizure due to correctable or avoidable circumstances does not necessarily have epilepsy



Definition

- A chronic neurologic disorder manifesting by repeated epileptic seizures (attacks or fits) which result from paroxysmal uncontrolled discharges of neurons within the central nervous system (grey matter disease).
- The clinical manifestations range from a major motor convulsion to a brief period of lack of awareness. The stereotyped and uncontrollable nature of the attacks is characteristic of epilepsy.

What are Seizures?

- Clinical Definition of Seizures
  - “Paroxysmal episodes of brain dysfunction manifested by stereotyped alteration in behavior”
    - Clinical manifestations of a seizure based on anatomy of the brain that is seizing
      - Symptoms: sensory, motor, autonomic with or without loss of consciousness
  - Epilepsy is recurrent and unprovoked seizures

What are Seizures?

- Cellular Definition
  - **Excessive** or **oversynchronized** discharges of cortical neurons
    - Primarily grey matter!!!!!!
  - Ineffective recruitment of inhibitory neurons together with excessive neuronal excitation
    - GABA receptor mediates inhibition responsible for normal termination of a seizure.
    - NMDA (Glutamate) receptor activation required for propagation of seizure activity

Epidemiology

- Approximately 1% population (3 million epilepsy cases in US).
- Second most common neurological disease
- Comparable prevalence in men vs. women

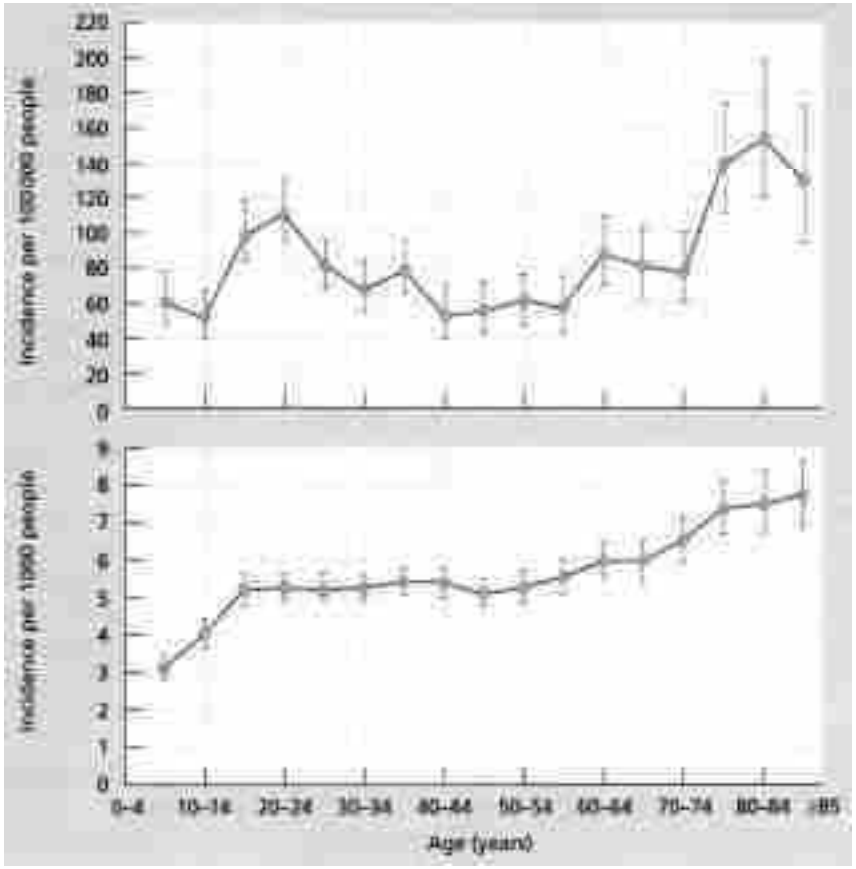
Begley CE et al. Epilepsia 2000;41:342-351
MMWR Weekly. November 11, 1994/43(44);810-811,817-818
Sander JW. Cur Opin neurol 2003;16:165-170

Epidemiology and course

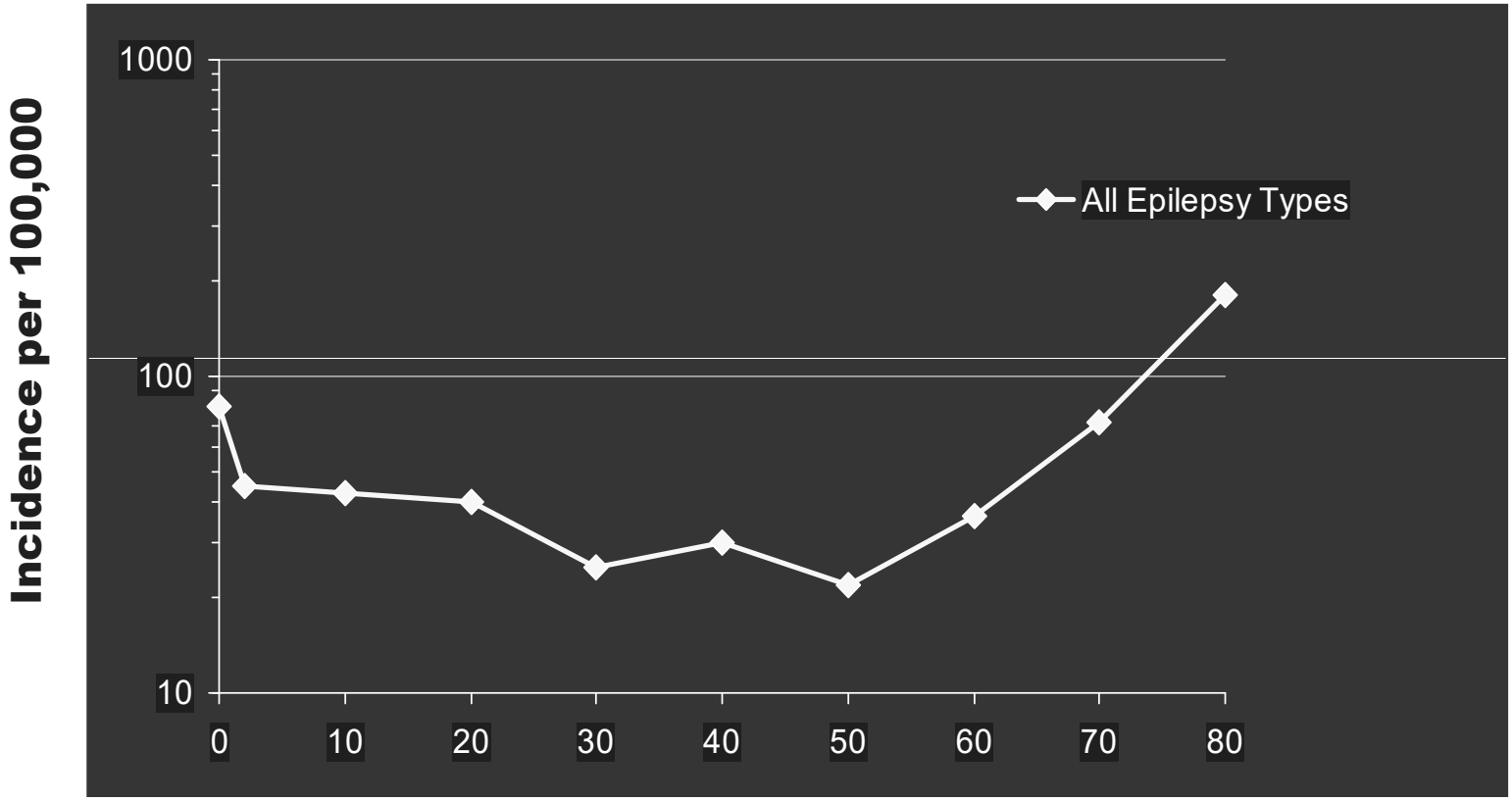
- 5% of the population suffer a single sz at some time
- 0.5-1% of the population have recurrent sz = EPILEPSY
- 70% = well controlled with drugs (prolonged remissions); 30% epilepsy at least partially resistant to drug treatments = INTRACTABLE EPILEPSY.

Epidemiology and course

- Epilepsy usually presents in childhood or adolescence but may occur for the first time at any age.



Epilepsy Incidence Rates by Age*



*Data from Rochester, MN (1975-84)
Hauser WA et al. *Epilepsia*. 1991;32:429-445.

Seizure Terms

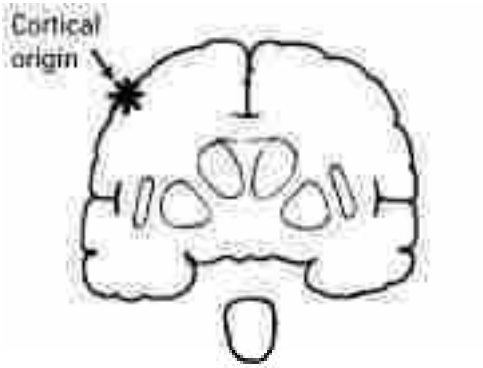
- Ictal=seizure
- Post Ictal=after the seizure
- Aura=sensation seconds before seizure occurs
- Automatisms=nonsensical movements that pts do during a seizure.
- Convulsions=shake
- Tonic=posturing
- Clonic=repetitive, forceful rhythmic movements
- Complex=lose consciousness
- Simple=don't lose consciousness
- Partial=involving part of the brain
- Generalized=involving whole brain
- Grand mal and petite mal="street terms" for convulsive and non-convulsive seizure respectively

CLASSIFICATION

- **Focal seizures**
  - Motor
  - Sensory
  - Autonomic
  - Cognitive
- **Generalized Seizures**
  - Absence seizures
  - Tonic seizures
  - Clonic seizures
  - Tonic-Clonic seizures
  - Atonic seizures
  - Myoclonic seizures
- **Focal, generalised or unclear**
  - Epileptic spasms

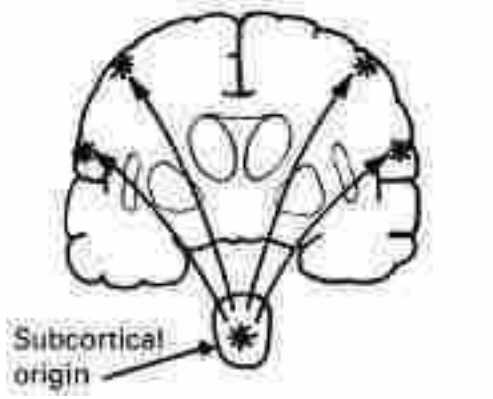
Epilepsy - Classification

- Focal seizures – *account for 80% of adult epilepsies*
  - Simple partial seizures
  - Complex partial seizures
  - Partial seizures secondarilly generalised



- Generalised seizures

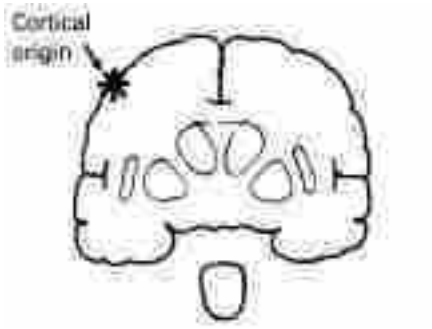
- Unclassified seizures



Focal (partial) seizures

Simple partial seizures

- Motor, sensory, vegetative or psychic symptomatology
- Typically consciousness is preserved

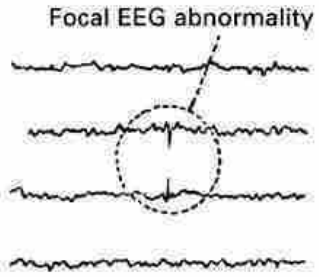


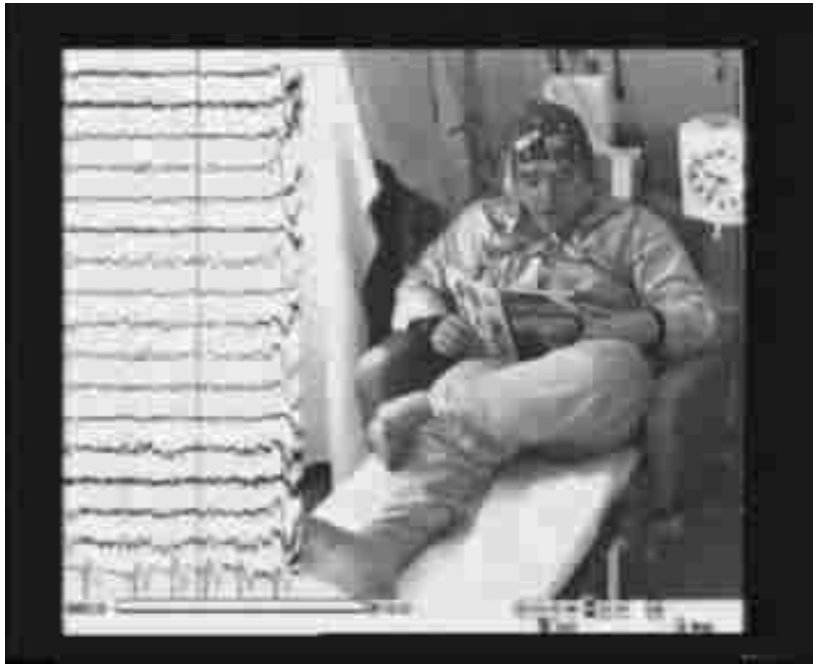
Focal (partial) seizures

- Simple partial seizures

  Motor, sensory, vegetative or psychic symptomatology

  Typically consciousness is preserved



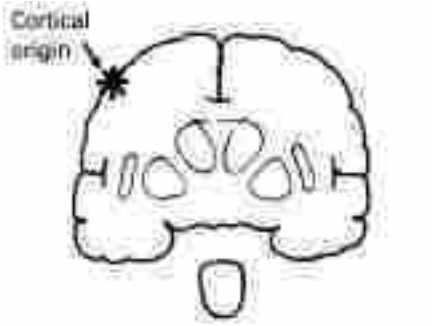


Focal (partial) seizures

- Simple partial seizures

  Motor, sensory, vegetative or psychic symptomatology

  Typically consciousness is preserved

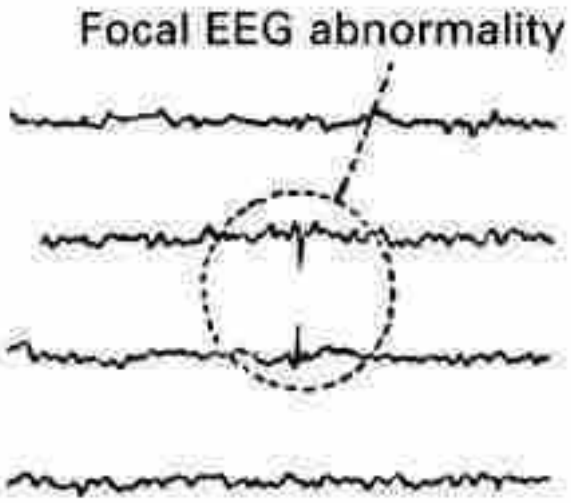


Focal (partial) seizures

- Complex partial seizures (= psychomotor seizures)

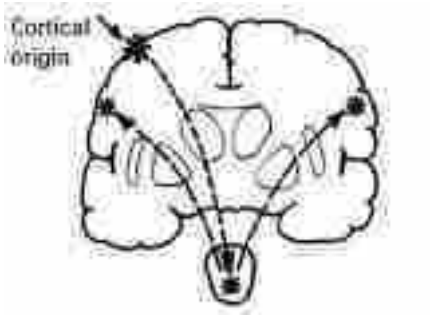
  Initial subjective feeling (aura), loss of consciousness, abnormal behavior (perioral and hand automatisms)

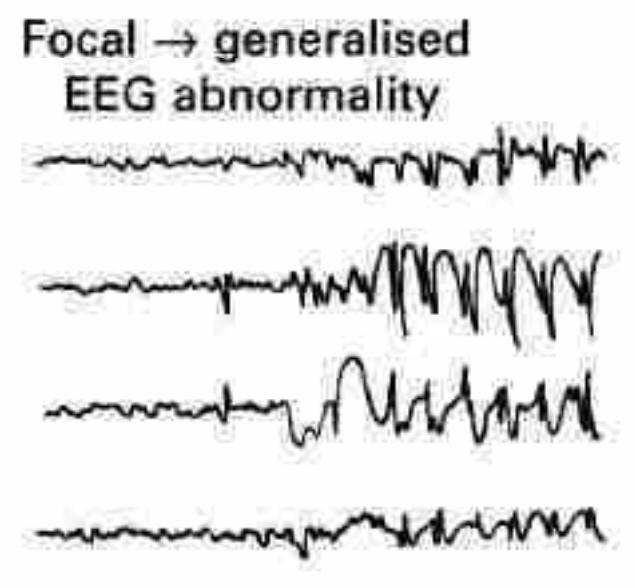
Usually originates in TL



Focal (partial) seizures

- Partial seizures evolving to tonic/clonic convulsions – secondary generalised tonic/clonic seizures (sGTCS)

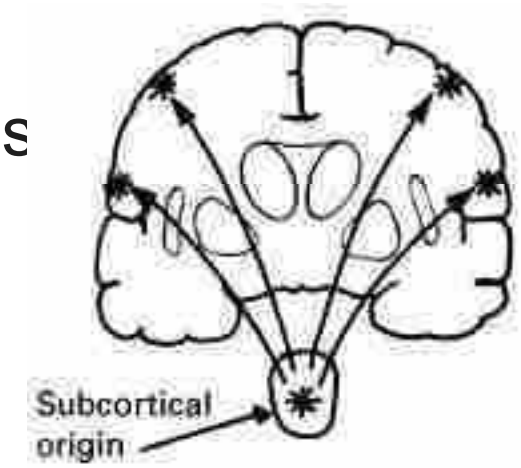


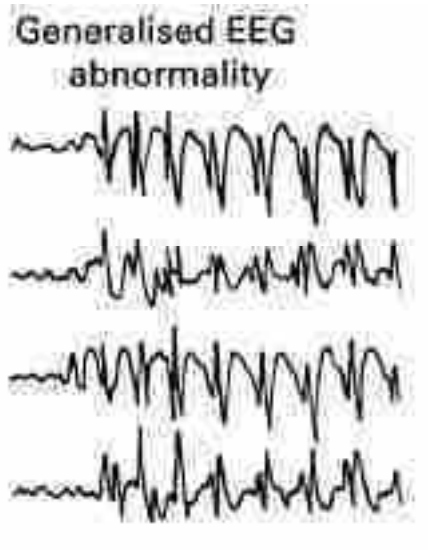


Generalized seizures

(convulsive or non-convulsive)

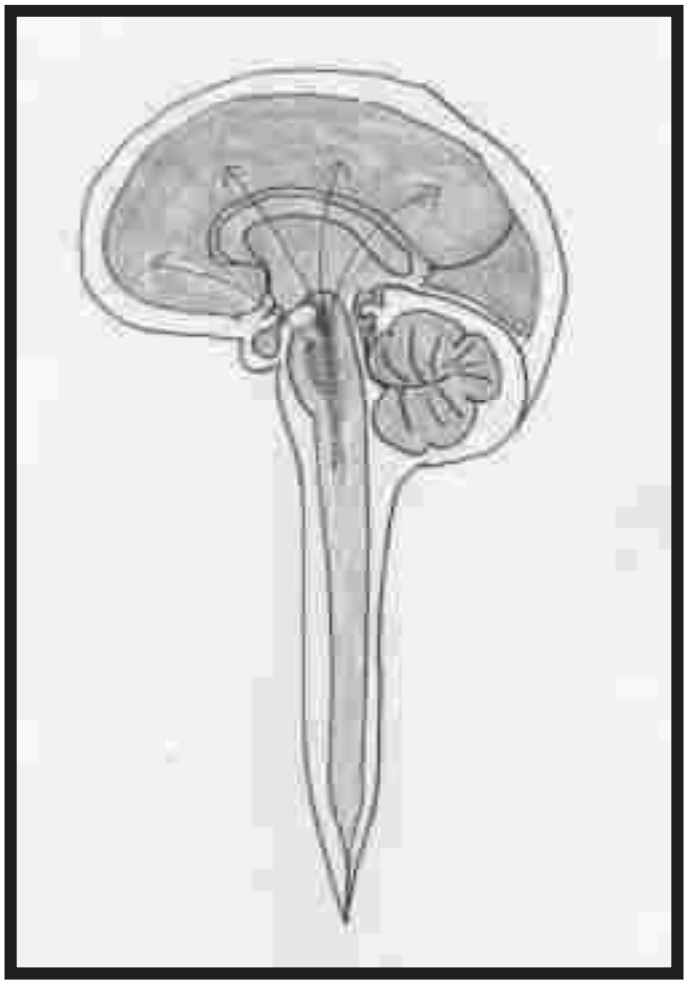
- Absences
- Myoclonic seizures
- Clonic seizures
- Tonic seizures
- Atonic seizures





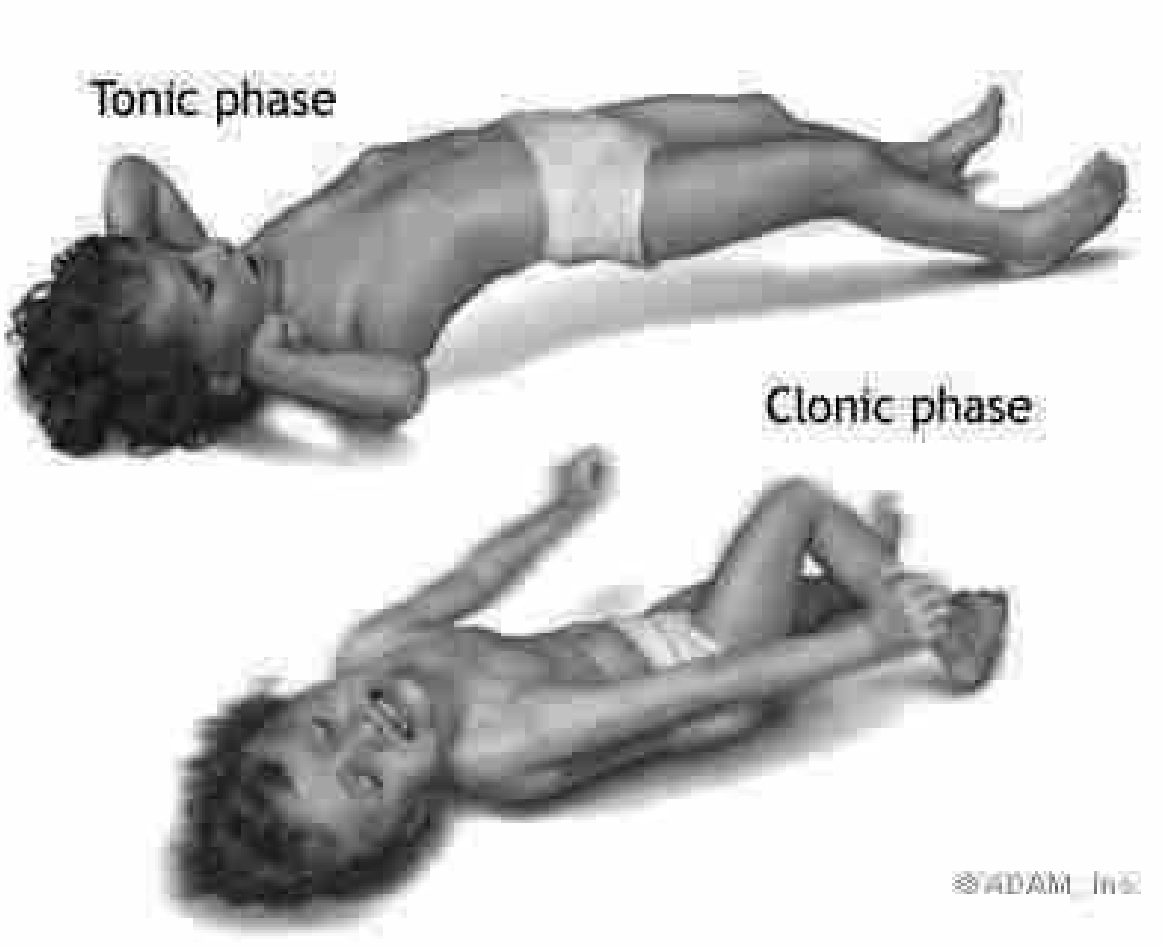
Generalized Seizures

- <u>Consciousness</u>
  - 2 Systems Involved
    - **Both** Cerebral Hemispheres
    - Reticular Activating System
      - various neurotransmitters



Tonic and Clonic

(Most often, these alternate)



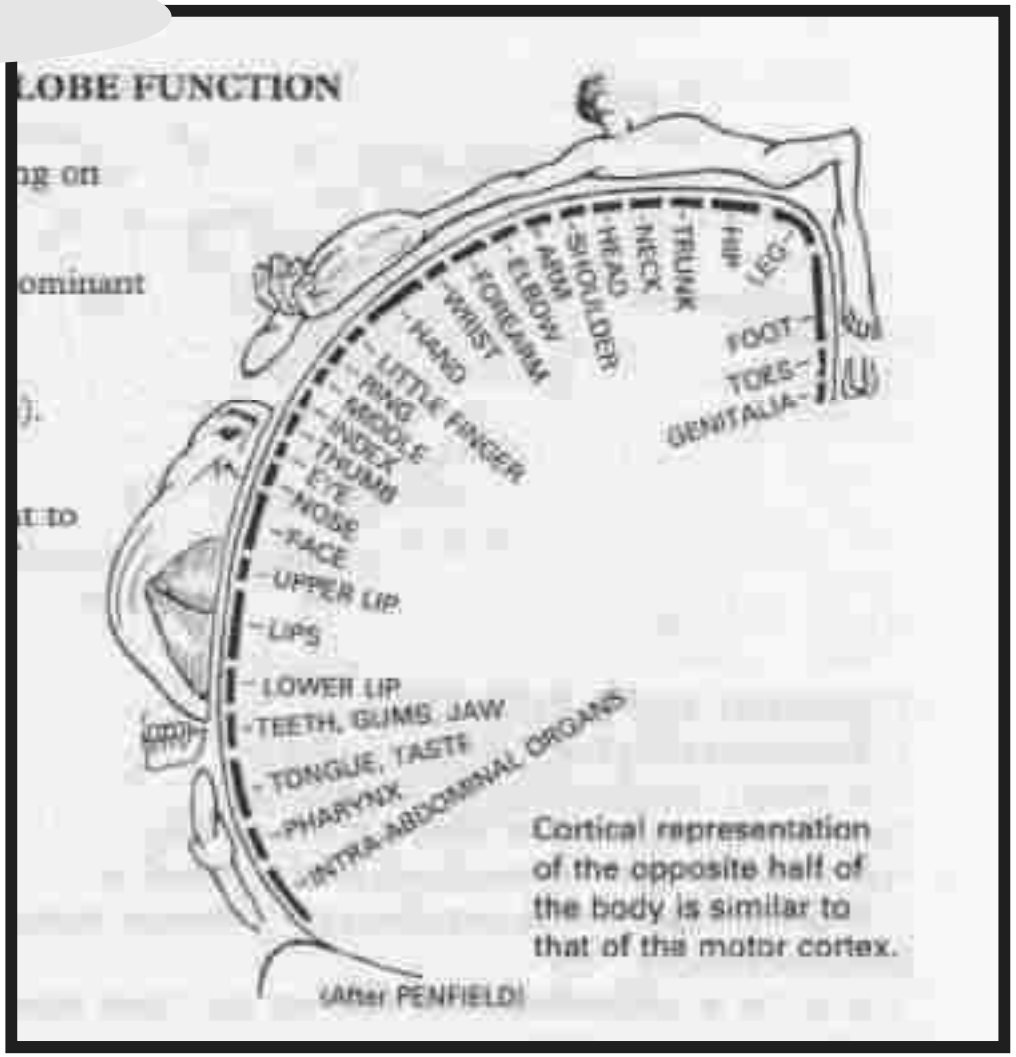
Generalized seizures

- Absences
- Myoclonic seizures
- Clonic seizures
- Tonic seizures
- Atonic seizures

Classification of Seizures

- Partial Seizures (focal onset)
  - Simple Partial Seizure (**without impaired consciousness**)
    - Motor Symptoms (aka focal motor seizure)
      - Involves motor strip.
      - Patients will have abnml movements of an extremity.
      - Jacksonian March-spread or “march” of seizure over whole motor
      - Todd’s paralysis-post ictal weakness of extremity that was seizing
    - Somatosensory symptoms
      - Involves sensory strip, temporal lobe (hearing and smell), occip (visual)
    - Autonomic symptoms
      - Involves temporal lobe (tachycardia, pallor, flushing, sweating)
    - Psychological symptoms
      - Involve frontal or temporal lobe (limbic system). Pts appear agitated

Partial Seizures
Homunculus



Neurology and Neurosurgery Illustrated. Lindsay, Kenneth, Bone Ian, 3rd edition. Churchill Livingstone, 1999.London

Classification of Seizures

- Partial Seizures
  - Complex Partial Seizures (**loss of consciousness**)
    - Simple partial onset followed by impaired consciousness
    - Many times will progress to a generalized seizure
    - Frequently seen in adult onset epilepsy
    - Temporal lobe epilepsy usually involves complex partial seizures

Classification of Seizures

- Unclassified Seizures….
  - Pseudoseizures-Are clinical manifestations anatomically impossible?
    - Can be conversion or malingering
      - Many pts have history of abuse
    - Commonly seen when observers around
    - Episodes involve “disorganized” movements that are not clearly tonic-clonic
      - Pelvic thrusting
      - Violent behavior directed towards certain people
    - VideoEEG can be helpful in diagnosing

CAUSES

- Neonates (< 1 month)
  - Perinatal hypoxia amd ischaemia
  - Intracranial hemorrhage and trauma
  - Acute CNS infection
  - Hypogylemia, hypocalcemia, hypomagnesemia, pyridoxine deficiency
  - Drug withdrawal
  - Developmental disorders
  - Genetic disorders

CAUSES

- Infants and children (> 1 month to < 12 yrs)
  - Febrile seizures
  - Genetic disorders
    - Metabolic
    - Degenrative
    - Primary epilepsy syndromes
  - CNS infection
  - Developmental disorders
  - Trauma
  - Idiopathic

CAUSES

- Adolescents (12 – 18 years)
  - Trauma
  - Genetic disorders
  - Infection
  - Brain tumor
  - Ilicit drug use
  - Idiopathic
- Young adults (18 – 35 years)
  - Trauma
  - Alcohol withdrawal
  - Ilicit drug use
  - Brain tumor
  - Idiopathic

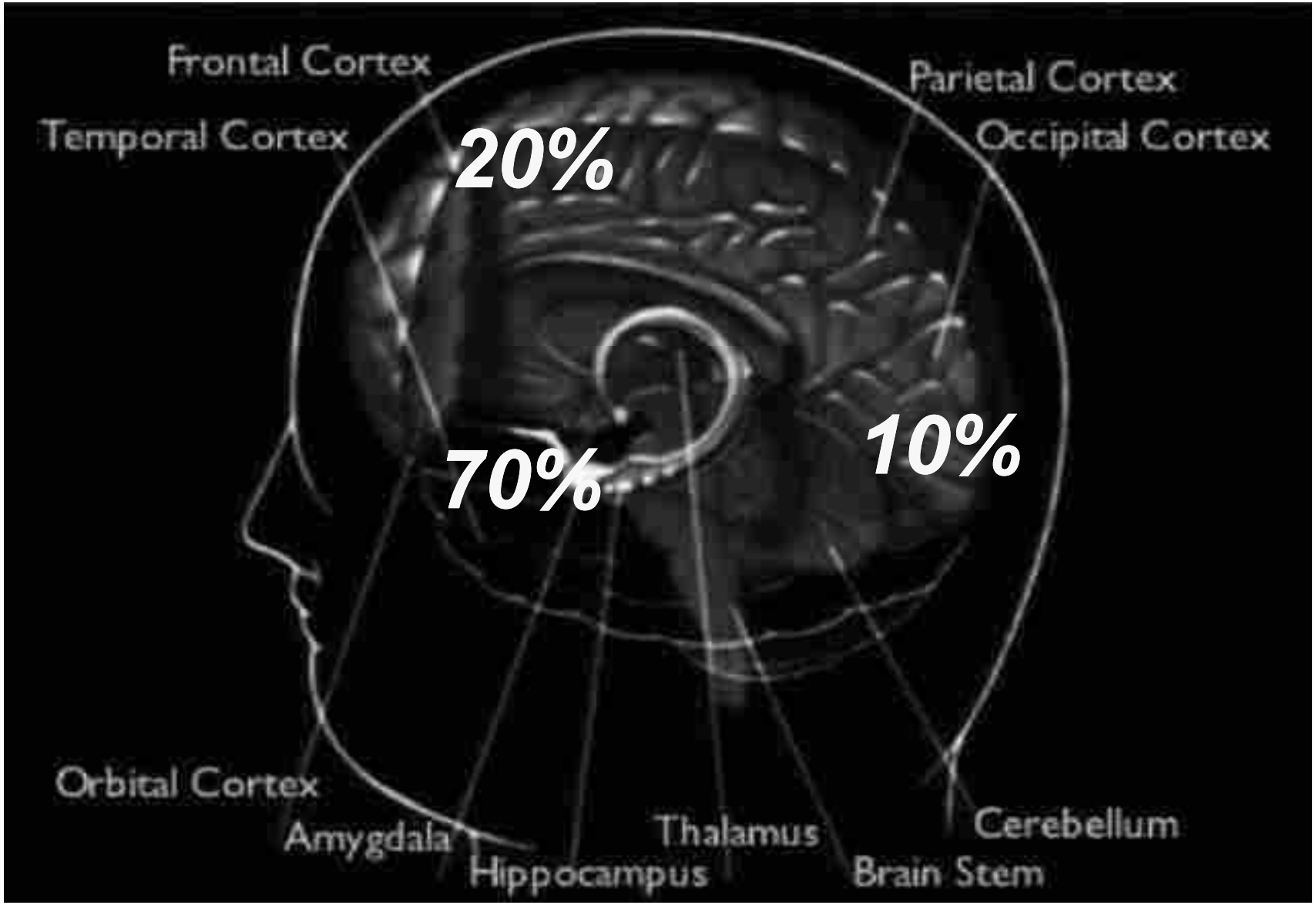
CAUSES

- Older adults (> 35yrs)
  - Cerebrovascular disease
  - Brain tumor
  - Alcohol withdrawal
  - Metabolic disorders
    - Uraemia
    - Hepatic failure
    - Electrolyte abnormalities
    - Hypoglycemia
    - Hyperglycemia
  - Alzheimer’s disease and other degenrative disease
  - Idiopathic

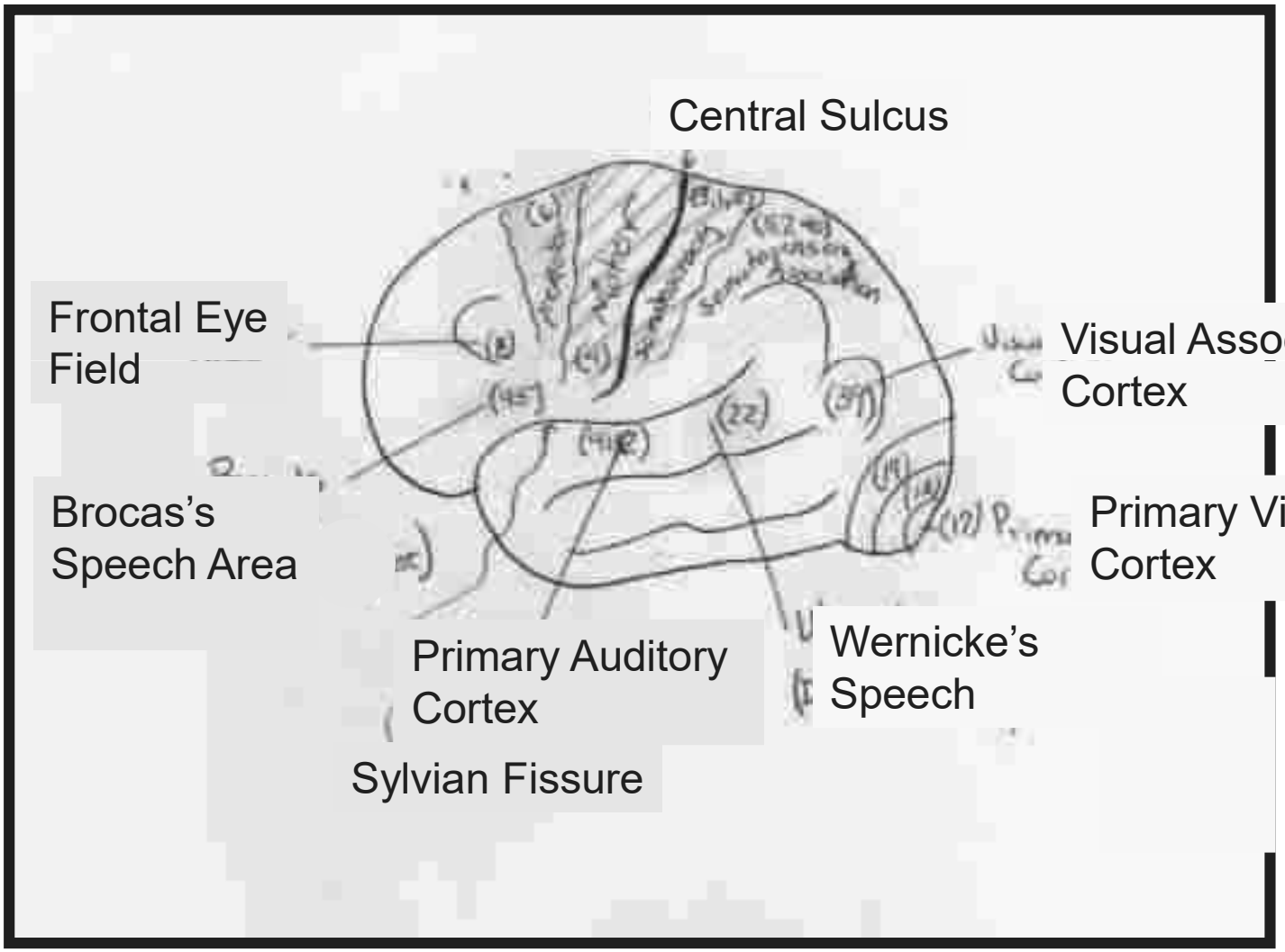
SEIZURE PHENOTYPES

THINK OF ANATOMY!!

Localization of Seizure



Cortex


Central Sulcus
Frontal Eye Field
Visual Assoc. Cortex
Brocas's Speech Area
Primary Visual Cortex
Primary Auditory Cortex
Wernicke's Speech
Sylvian Fissure

Frontal Lobe

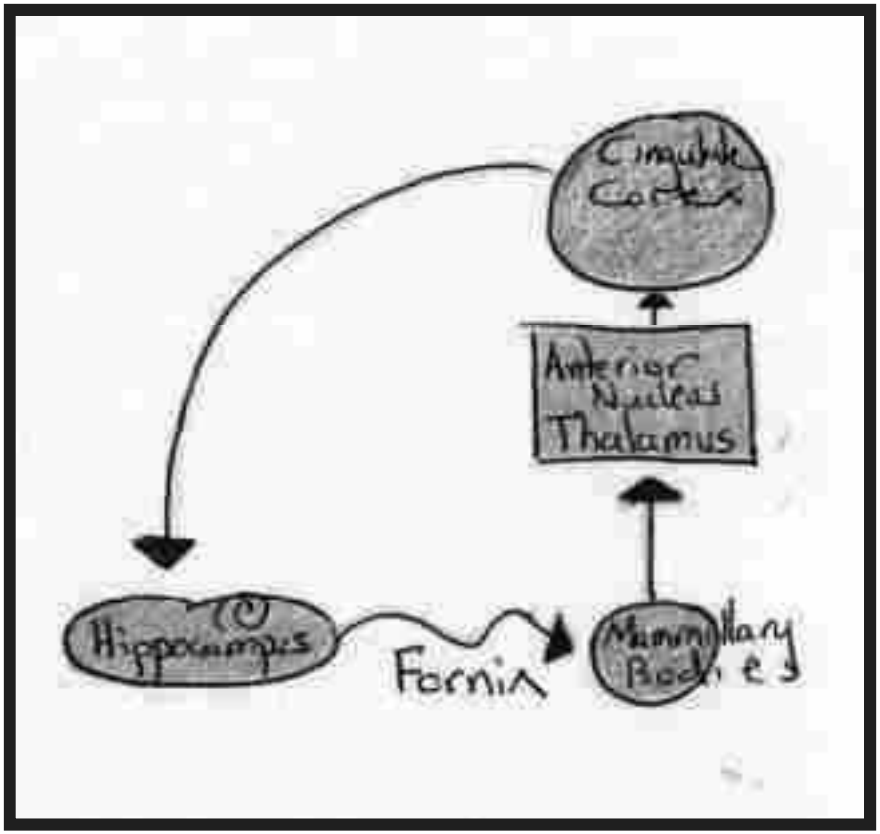
- Frontal Eye Field (Brodman's 8)
  - Lesion: deviation of **eyes to ipsilateral side**
  - Sz: ***overstimulation->eyes to contralateral side***
- Prefrontal Cortex (Brodman's 9-12,46,47)
  - Lesion: deficits in concentration, judgment and behavior
  - Sz: agitation, odd behavior
- Broca's Speech Area (Brodman's 44,45)
  - Lesion/Sz: expressive nonfluent aphasias
- Primary Motor Cortex (Brodman's 4)
  - Lesion: contralateral spastic paresis
  - Sz: contralateral posturing/convulsions

Temporal Lobe

- Hippocampal Cortex
  - Bilateral lesions: inability to process short term to long term memory
  - Sz: chronic seizures lead to deficits in short term memory
- Wernicke's Speech area (Brodman's 22)
  - Lesion/Sz: loss of receptive speech, fluent aphasia
- Anterior Temporal Lobe
  - Bilateral lesions: "Kluver-Bucy syndrome" of visual agnosia, oral tendencies, hyperphagia, hypersexuality, docility
  - Sz: pts "freeze" and might have oral automatisms
- Primary Auditory (Brodman's 41, 42)
  - Bilateral lesion: cortical deafness
  - Sz: auditory hallucinations
- Olfactory Bulb (Brodman's 34)
  - Lesion: ipsilateral anosmia
  - Sz: olfactory and gustatory hallucinations

Limbic System

- Means "*border*" in Latin
  - Border between cortex and hypothalamus
  - Involved with emotional behavior
    - Fear/anger/sexual behavior
    - Short term memory
  - Includes:
    - Hippocampus
    - Fornix
    - Mammilary bodies
    - Ant. Nucleus thalamus
    - Cingulate cortex
  - Seizures involving the hippocampus can have clinical symptoms of poor short term memory and abnormal mood

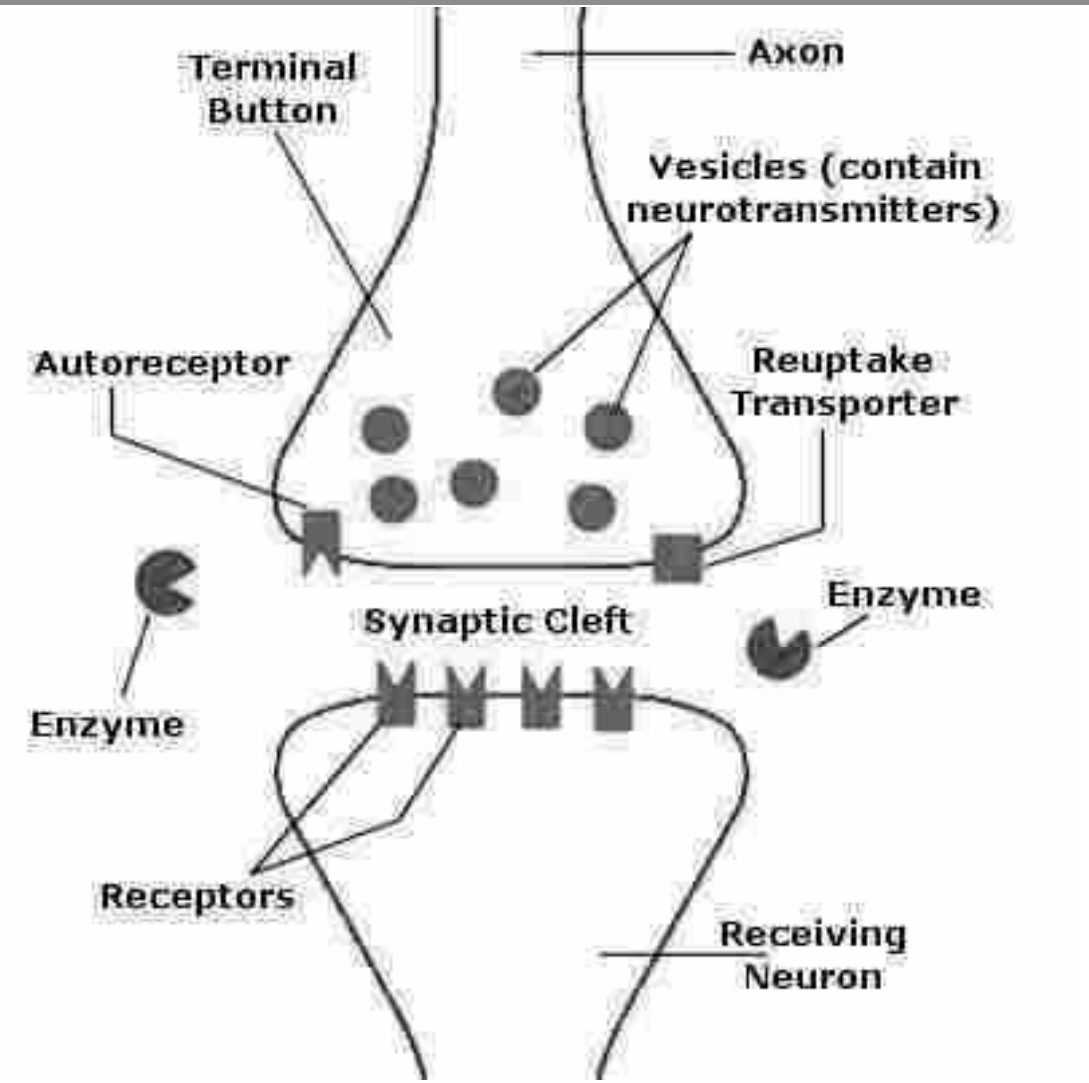


Parietal and Occipital Lobe

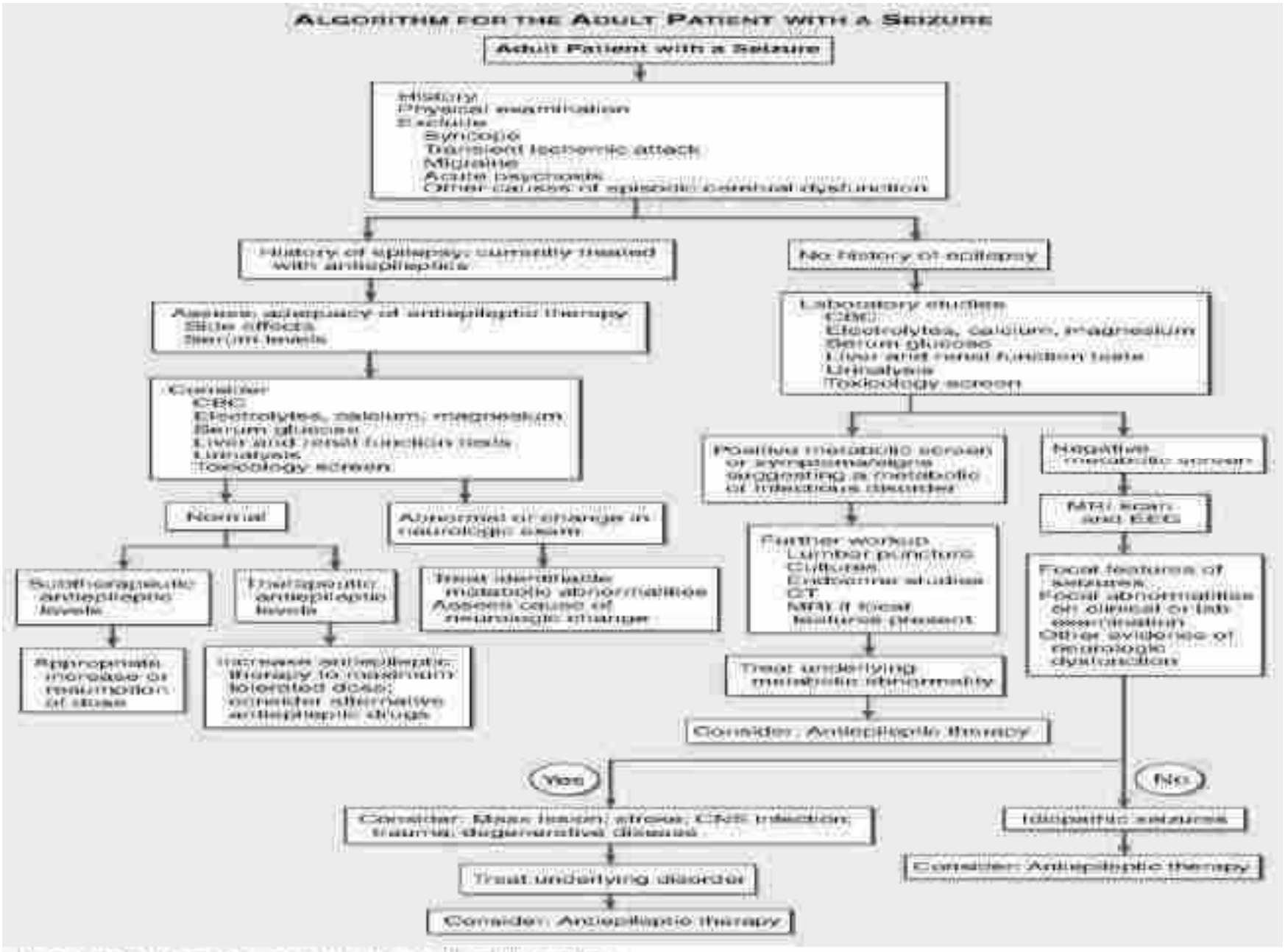
- Primary Sensory Cortex (Brodman's 3,1,2)
  - Lesion: contralateral hemihypestheisa and astereognosis
  - Sz: contralateral sensory symptoms ie: tingling, heat
- Occipital lobe (Brodman's 17)
  - Lesion: contralateral hemianopsia with macular sparing
  - Sz: flashing or colored lights in contralateral visual field

Pathogenesis

- The 19th century neurologist Hughlings Jackson suggested “a sudden excessive disorderly discharge of cerebral neurons“ as the causation of epileptic seizures.
- Recent studies in animal models of focal epilepsy suggest a central role for the excitatory neurotransmiter glutamate (increased in epi) and inhibitory gamma amino butyric acid (GABA) (decreased)



- Signals are electrochemical
  - Neurotransmitters can be excitatory or inhibitory


ALGORITHM FOR THE ADULT PATIENT WITH A SEIZURE
Adult Patient with a Seizure
History
Physical examination
Exclude
Syncope
Transient ischemic attack
Migraine
Acute psychosis
Other causes of episodic cerebral dysfunction
History of epilepsy; currently treated with antiepileptics
No history of epilepsy
Assess adequacy of antiepileptic therapy
Side effects
Serum levels
Consider
CBC
Electrolytes, calcium, magnesium
Serum glucose
Liver and renal function tests
Urinalysis
Toxicology screen
Laboratory studies
CBC
Electrolytes, calcium, magnesium
Serum glucose
Liver and renal function tests
Urinalysis
Toxicology screen
Normal
Abnormal or change in neurologic exam
Positive metabolic screen or symptoms/signs suggesting a metabolic or infectious disorder
Negative metabolic screen
MRI scan and EEG
Subtherapeutic antiepileptic levels
Therapeutic antiepileptic levels
Treat identifiable metabolic abnormalities
Assess cause of neurologic change
Further workup
Lumbar puncture
Cultures
Endocrine studies
CT
MRI if focal features present
Focal features of seizures
Focal abnormalities on clinical or lab examination
Other evidence of neurologic dysfunction
Appropriate management of stress
Increase antiepileptic therapy to maximum tolerated dose; consider alternative antiepileptic drugs
Treat underlying metabolic abnormality
Consider Antiepileptic therapy
Yes
No
Consider: Mass lesion; stroke; CNS infection; trauma; degenerative disease
Idiopathic seizures
Treat underlying disorder
Consider Antiepileptic therapy
Consider: Antiepileptic therapy

Seizure Precipitants

Low (less often high) blood glucose

Low sodium

Low calcium

Low magnesium

Stimulant or other proconvulsant toxicity (i.e., cocaine)

Sedative (i.e., valium or alcohol) withdrawal

Severe sleep deprivation

Epilepsy – Investigation

- The concern of the clinician is that epilepsy may be symptomatic of a treatable cerebral lesion.
- **Routine investigation:** Haematology, biochemistry (electrolytes, urea and calcium), chest X-ray, electroencephalogram (EEG).

  Neuroimaging (CT/MRI) should be performed in all persons aged 25 or more presenting with first seizure and in those pts. with focal epilepsy irrespective of age.
- **Specialised neurophysiological investigations:** Sleep deprived EEG, video-EEG monitoring.
- **Advanced investigations** (in pts. with intractable focal epilepsy where surgery is considered): Neuropsychology, Semiinvasive or invasive EEG recordings, MR Spectroscopy, Positron emission tomography (PET) and ictal Single photon emission computed tomography (SPECT)

EEG Abnormalities

- Background abnormalities
  - -Significant asymmetries and/or degree of slowing inappropriate for clinical state
- Transient abnormalities associated with seizures
  - -Spikes (< 70 m sec)
  - -Sharp waves (~70 – 200 msec)
  - -Spike-wave complexes
- May be focal, lateralized or generalized

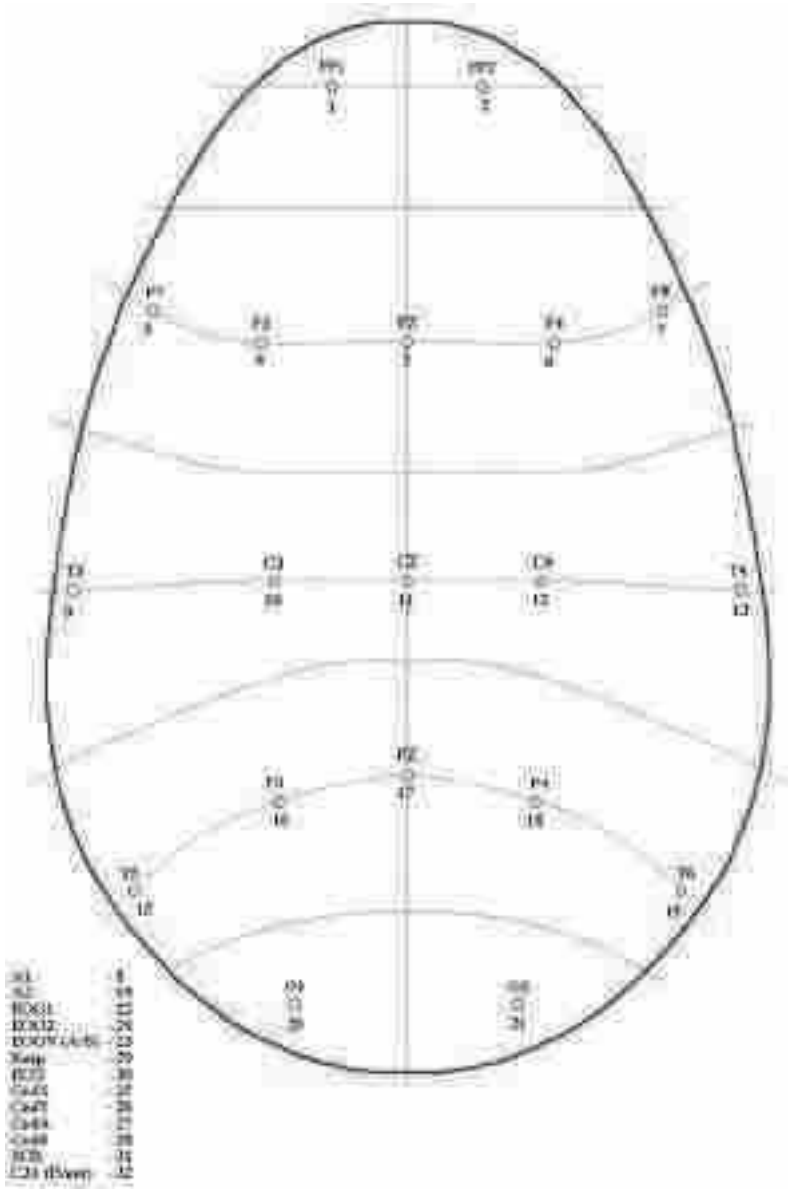
EEG Abnormalities

Epilepsy
Differential Diagnosis

The following should be considered in the diff. dg. of epilepsy:

- Syncope attacks (when pt. is standing; results from global reduction of cerebral blood flow; prodromal pallor, nausea, sweating; jerks!)
- Cardiac arrythmias (e.g. Adams-Stokes attacks). Prolonged arrest of cardiac rate will progressively lead to loss of consciousness – jerks!
- Migraine (the slow evolution of focal hemisensory or hemimotor symptomas in complicated migraine contrasts with more rapid “spread“ of such manifestation in SPS. Basilar migraine may lead to loss of consciousness!
- Hypoglycemia – seizures or intermittent behavioral disturbances may occur.
- Narcolepsy – inappropriate sudden sleep episodes
- Panic attacks
- PSEUDOSEIZURES – psychosomatic and personality disorders

EEG Montage



EEG Depicting Seizure

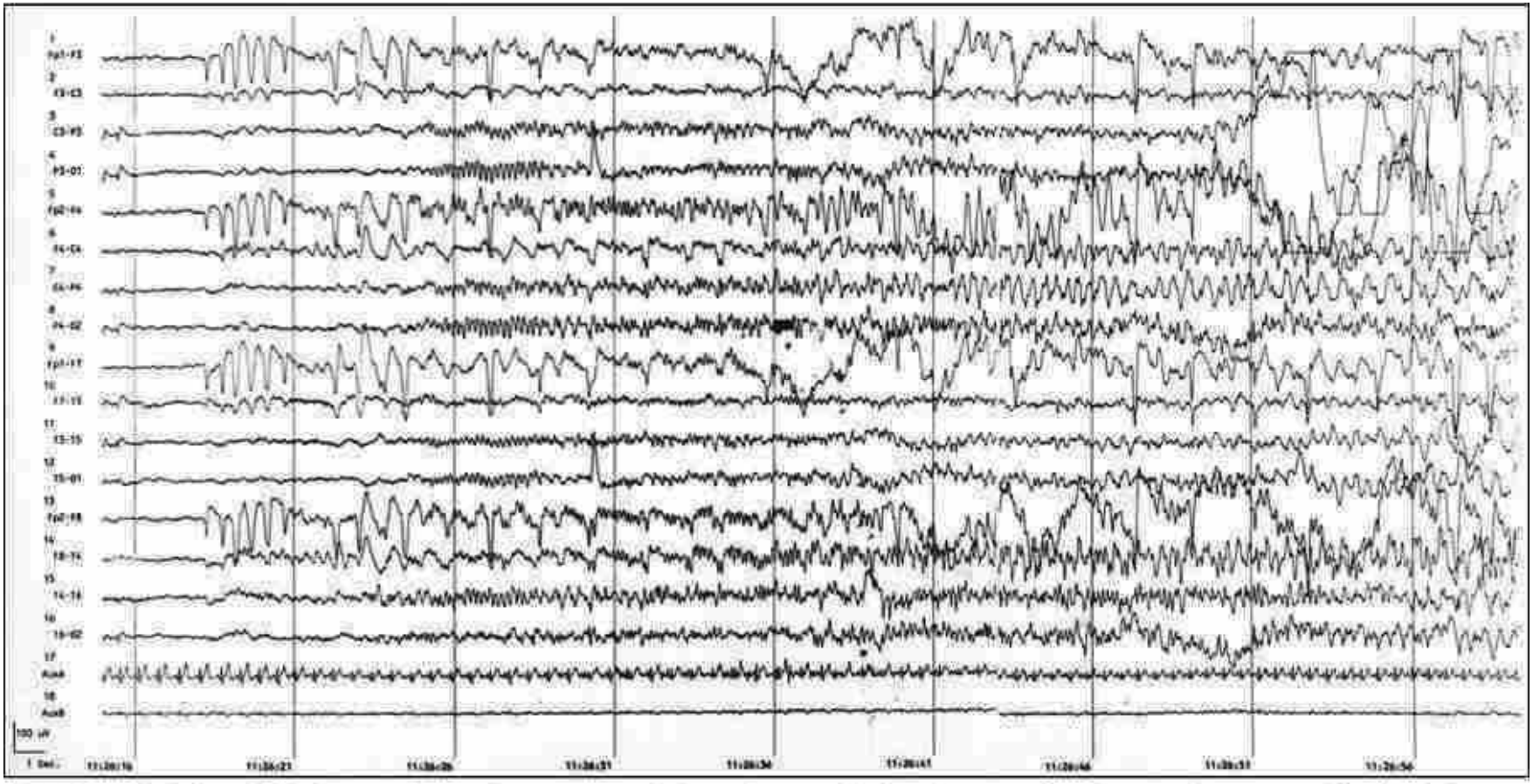


Fig 1. Patient 3. Scalp ictal EEG showing a bitemporal seizure onset, with electrical activity of higher voltage over the right temporal region, contra-lateral to the lesion on MRI.

Evaluation for Surgery- Neuroimaging

MRI

-hippocampal volumetrics

greater than ~0.5cc difference increases chances for seizure remission

-1.5 mm coronal cuts with sequences sensitive to gray-white differentiation and to gliosis

-inversion recovery/high resonance for cortical dysplasia

PET

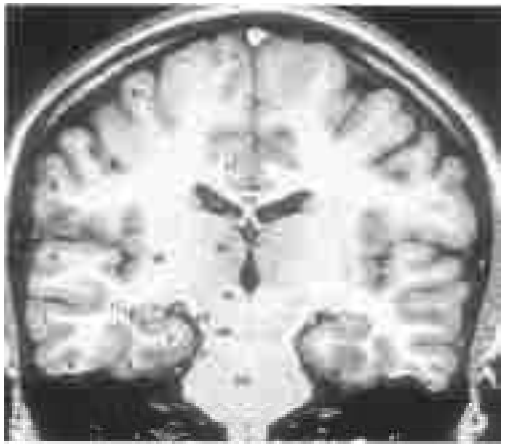
Ictal/interictal SPECT

MR Spectroscopy

Decreased NAA (due to neuronal loss)

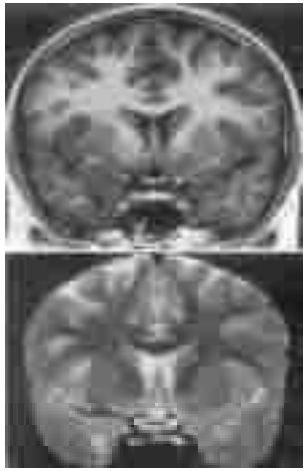
Normal to high Cho and Creatine (represents astrocytosis)

Epilepsy Surgery- Neuroimaging



Hippocampal atrophy in temporal lobe epilepsy

Ganglioglioma



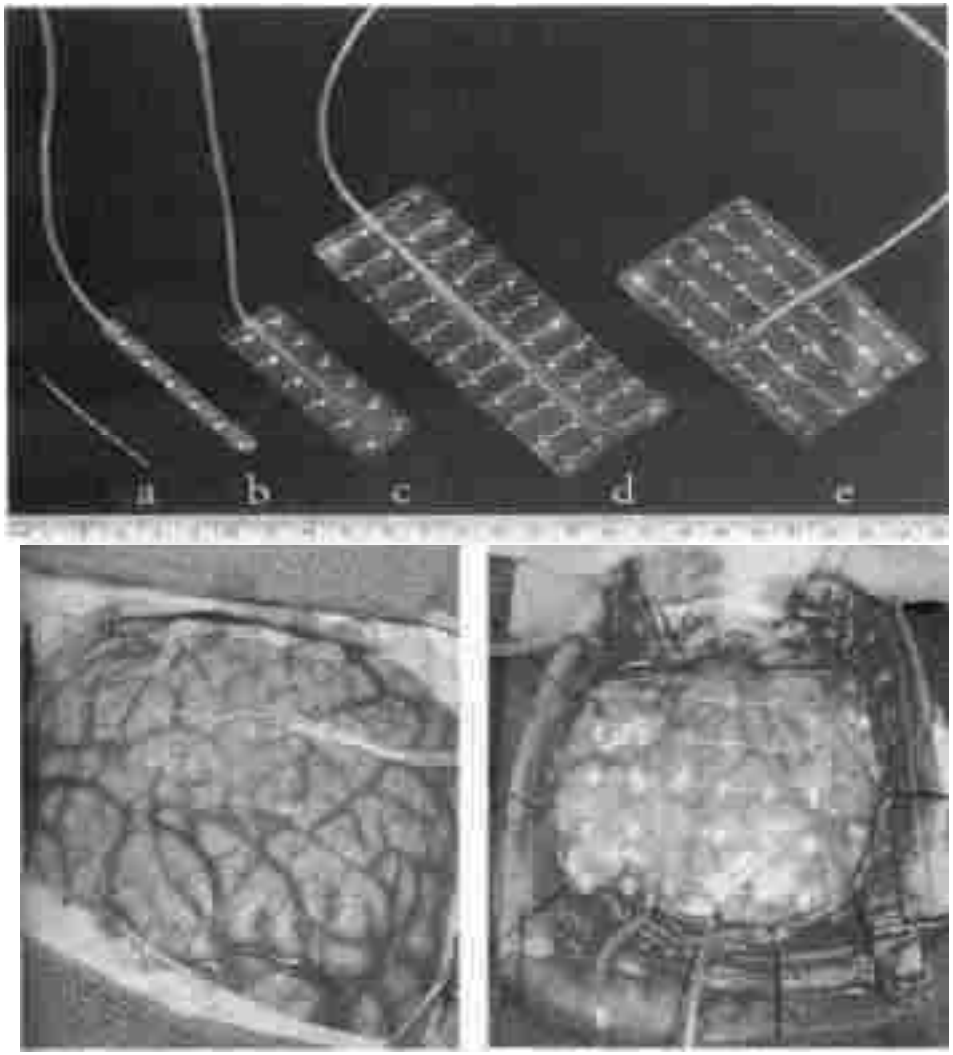
Dysembryoplastic Neuroepithelial Tumor

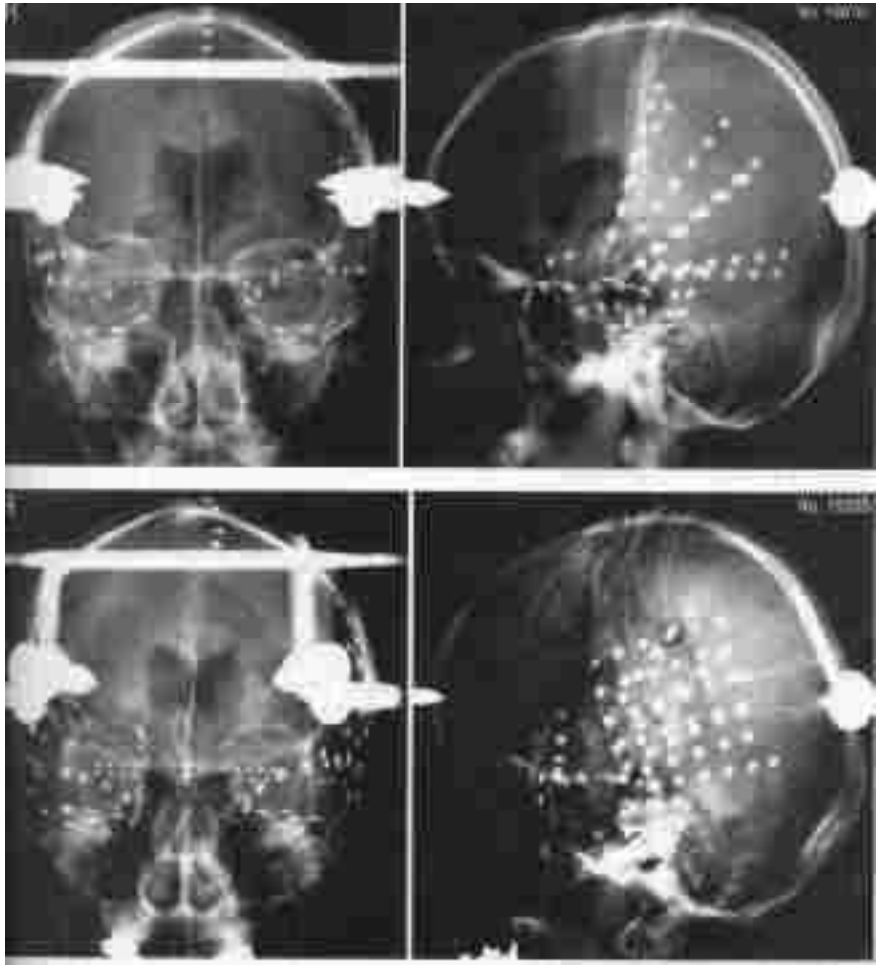
Cortical Dysplasia

AVM

Cavernous Angioma

Evaluation for Surgery- Subdural Grid Electrodes





SEIZURE MANAGEMENT

Seizure Treatment

- **Acute Management**
  - 90% of seizures stop without treatment in under 5 minutes!!!
  - Can give ativan or valium for seizures >5min
  - Monitor ABCDs, avoid injury and aspiration
- **Long Term Management**
  - Anti-epileptic medications
  - Surgery-Best for temporal lobe epilepsy
  - VNS (vagal nerve stimulator)

Anti Epileptic Medication

- **Older Agents**
  - Phenobarbital (Mysoline)
  - Valproic Acid
  - Phenytoin Sodium
  - Ethosuximide
  - Carbamazepine
- **Newer Agents**
  - Lamotrigine
  - Topiramate
  - Gabapentin
  - Levetiracetam
  - Zonisamide
  - Oxcarbazepine
  - Lacosamide
  - Rufinamide

Other Treatment Options

- Ketogenic diet in children
- Surgery
  - Removal of epileptic focus
  - Mostly for patients with temporal lobe seizures
    - Possibility of a 70% chance of cure!!
- VNS (Vagal nerve stimulator)
  - Current given to vagus nerve with theory of decreasing seizures over time

Selection of Anti Epileptic Drug

- Generalised Onset Tonic Clonic
  - FIRST LINE
    - Valproic Acid
    - Lamotrigine
    - Topiramate
  - Alternatives
    - Zonisamide
    - Phenytoin
    - Carbamazepine
    - Oxcarbamazepine
    - Phenobarbital

Selection of Anti Epileptic Drug

- Focal Seizures
  - First Line
    - Lamotrigine
    - Carbamazepine
    - Oxcarbamazepine
    - Phenytoin
    - Levetiracetam
  - Alternatives
    - Topiramate
    - Zonisamide
    - Valproic acid
    - Gabapentin
    - Phenobarbital

Selection of Anti Epileptic Drug

- Absence seizures
  - First line
    - Valproic acid
    - Ethosuximide
  - Alternatives
    - Lamotrigine
    - Clonazepam

Selection of Anti Epileptic Drug

- Atypical absence, myoclonic, atonic
  - First Line
    - Valproic acid
    - Lamotrigine
    - Topiramate
  - Alternatives
    - Clonazepam
    - Felbamate

Summary of Serious and Non-serious Adverse Events of the Newer AEDs

AED	Serious Adverse Events	Nonserious Adverse Events
Gabapentin	None	Weight gain, peripheral edema, behavioral changes
Lamotrigine	Rash, including Stevens Johnson and toxic epidermal necrolysis (increased risk for children, also more common with concomitant valproate use and reduced with slow titration); hypersensitivity reactions, including risk of hepatic and renal failure, DIC, and arthritis	Tics and insomnia
Levetiracetam	None	Irritability/behavior change
Oxcarbazepine	Hyponatremia (more common in elderly), rash	None
Tiagabine	Stupor or spike wave stupor	Weakness
Topiramate	Nephrolithiasis, open angle glaucoma, hypohidrosis (predominantly children)	Metabolic acidosis, weight loss, language dysfunction
Zonisamide	Rash, renal calculi, hypohidrosis (predominantly children)	Irritability, photosensitivity, weight loss

Epilepsy - Treatment

- The majority of pts respond to drug therapy (anticonvulsants). In intractable cases surgery may be necessary. The treatment target is seizure-freedom and improvement in quality of life!
- Basic rules for drug treatment: Drug treatment should be simple, preferably using one anticonvulsant (monotherapy). "Start low, increase slow". Polytherapy is to be avoided especially as drug interactions occur between major anticonvulsants.
- The commonest drugs used in clinical practice are:
  Carbamazepine, Sodium valproate, Phenytoin (first line drugs)
  Lamotrigine, Topiramate, Levetiracetam, Pregabaline (new AEDs)

Epilepsy – Treatment (cont.)

- If pt is seizure-free for three years, withdrawal of pharmacotherapy should be considered. Withdrawal should be carried out only if pt is satisfied that a further attack would not ruin employment etc. (e.g. driving licence). It should be performed very carefully and slowly! 20% of pts will suffer a further sz within 2 yrs.
- The risk of teratogenicity is well known (~5%), especially with valproates, but withdrawing drug therapy in pregnancy is more risky than continuation. Epileptic females must be aware of this problem and thorough family planning should be recommended. Over 90% of pregnant women with epilepsy will deliver a normal child.

Guideline for Management of the Adult Seizing Patient[1]

- General Management:
- Call for help
- Assess and secure the airway
  - Do not place anything in the mouth except when to suction
    - DO NOT try to force suction/airway through clenched teeth
  - When the patient stops convulsing, place patient in lateral decubitus.
  - Begin supplemental oxygen
- Assess safety of the patient
  - Ensure lights in room are on
  - Remove any object within reach of patient that could cause injury
  - Loosen clothing
  - Side rails should be up if patient is in bed
  - Do not try to “hold the patient down”
- Obtain vital signs including pulse oximetry and stat accucheck
- Ask an associate to **notify the primary service**
  - If concerned for overdose or withdrawal, contact toxicology service (pager #19708) and consider 100 mg pyridoxine IV (alternative management may be necessary)
  - If glucose is < 70 mg/dl (or if accucheck unobtainable) administer amp D50.
    - Note: Ideally,100mg thiamine IVPB should be given prior to, or soon after, glucose
- Check CBC, BMP, Mg, Phos, urine tox. If patient on AEDs draw levels

Guideline for Management of the Adult Seizing Patient[1]

- Initial Assessment:
- Observation of the seizing patient is critical for diagnosis and management!
- Note time of the seizure and its length
- Assess:
  - Mental status/level of consciousness-*can patient follow commands?*
  - Language function-*can patient speak? Understand when spoken to? Is speech appropriate?*
  - Motor activity-*does the patient have motor automatisms (picking, rhythmic movements), tonic (rigid), and/or clonic (jerking) movements? What part of body is involved in the movements? (face, arm, leg?) Are movements unilateral or bilateral? Is there head deviation, eye deviation? And to which side?*

Guideline for Management of the Adult Seizing Patient[1]

- ***At: 3-5 minutes-*** *Prolonged Seizure*
- *Note: Most seizures cease without any medical intervention within 1-3 minutes*
- General Management:
- **Consult neurology** (pager #91516)
- Continue to monitor vital signs and assess airway (Q5 minutes).
- Continue to ensure patient safety
- Consider activating the rapid response team (if pt. is admitted)

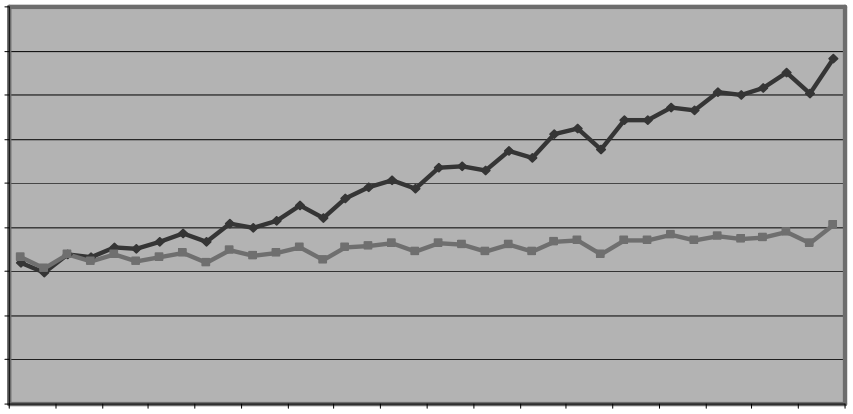
- **At: 10 minutes -** if seizures persist, patient is in *Status Epilepticus (S.E.)*
- Arrange transfer to the ICU
- Refer to LUMC Guidelines for the Acute Management of Status Epilepticus and Seizure Clustering in Adults and Children

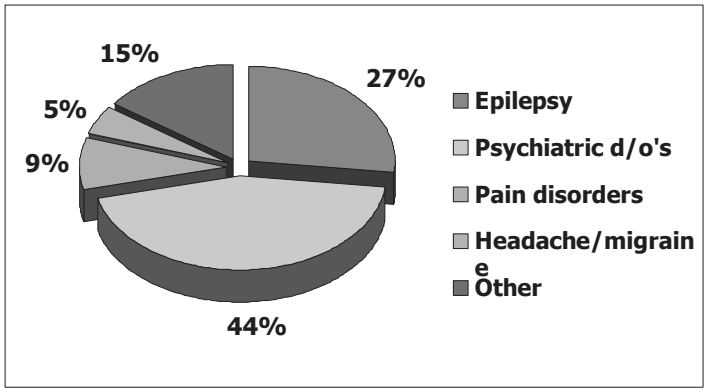
Guideline for Management of the Adult Seizing Patient[1]

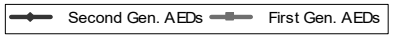
- Medical Treatment (directed by primary service or neurology):
- First line agent:
  - **Lorazepam (Ativan) 1-2mg IV one time.**
    - Can be repeated up to a dose of 0.1mg/kg IV at 2mg/min.
    - If IV access unavailable, consider IM administration.
    - Monitor respiratory status
    - Re-assess patient every 2 minutes for continued seizure activity. If seizures persist, consider a second line agent.
- Second line agents (select one of the following):
  - **Phenytoin (Dilantin) 20mg/kg IV at 50mg/min**
    - An additional 5-10mg/kg can be given
  - **Fosphenytoin (Cerebyx) 18-20mg/kg PE at 150mg/minute**
    - An additional 5-10mg/kg PE can be given
    - Neurology approval required for the use of Fosphenytoin
    - If IV access is unavailable, consider IM administration.
  - **Newer agents are occasionally used at this stage:**
    - Valproate (Depakote)
    - Levetiracetam (Keppra)

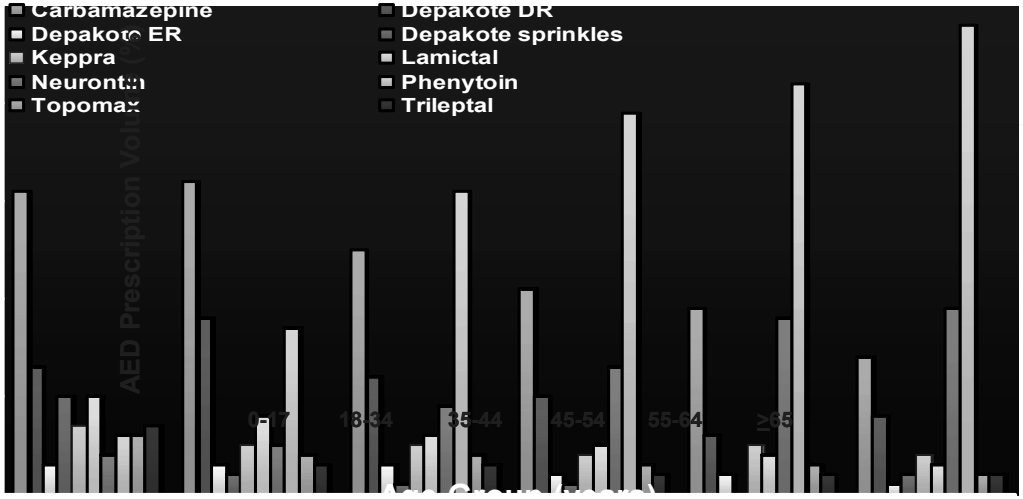
Rational Use of AEDs: All Prescriptions

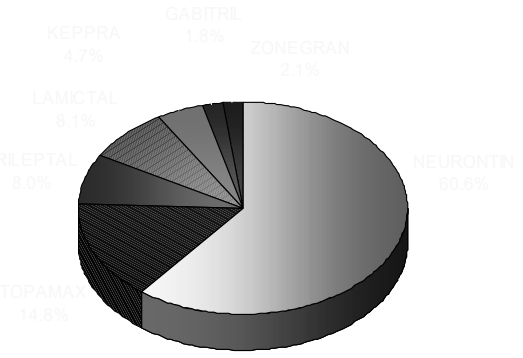
Market Dynamics for All Indications and Epilepsy











Treatment/Evaluation Sequence for Pharmacoresistent Epilepsy

1st Monotherapy AED Trial

2nd Monotherapy AED Trial

3rd Monotherapy/Polytherapy AED Trial

Strongly consider videoEEG Monitoring

Non-epileptic

Epilepsy

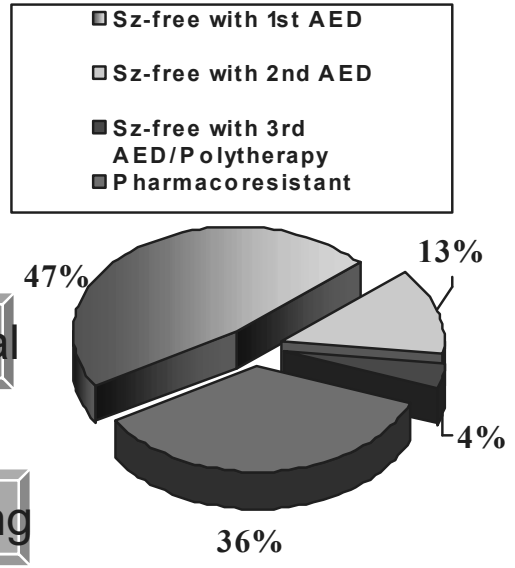
Psychogenic, migraine, syncope, sleep disorders, movement disorder's, etc.

Epilepsy Surgery/VNS Therapy/ Neuropace Evaluation

Polytherapy AED Trials

Resective Surgery

Stimulator Therapy



Kwan P, Brodie MJ. *NEJM*;342:314-319.

Epilepsy Surgery

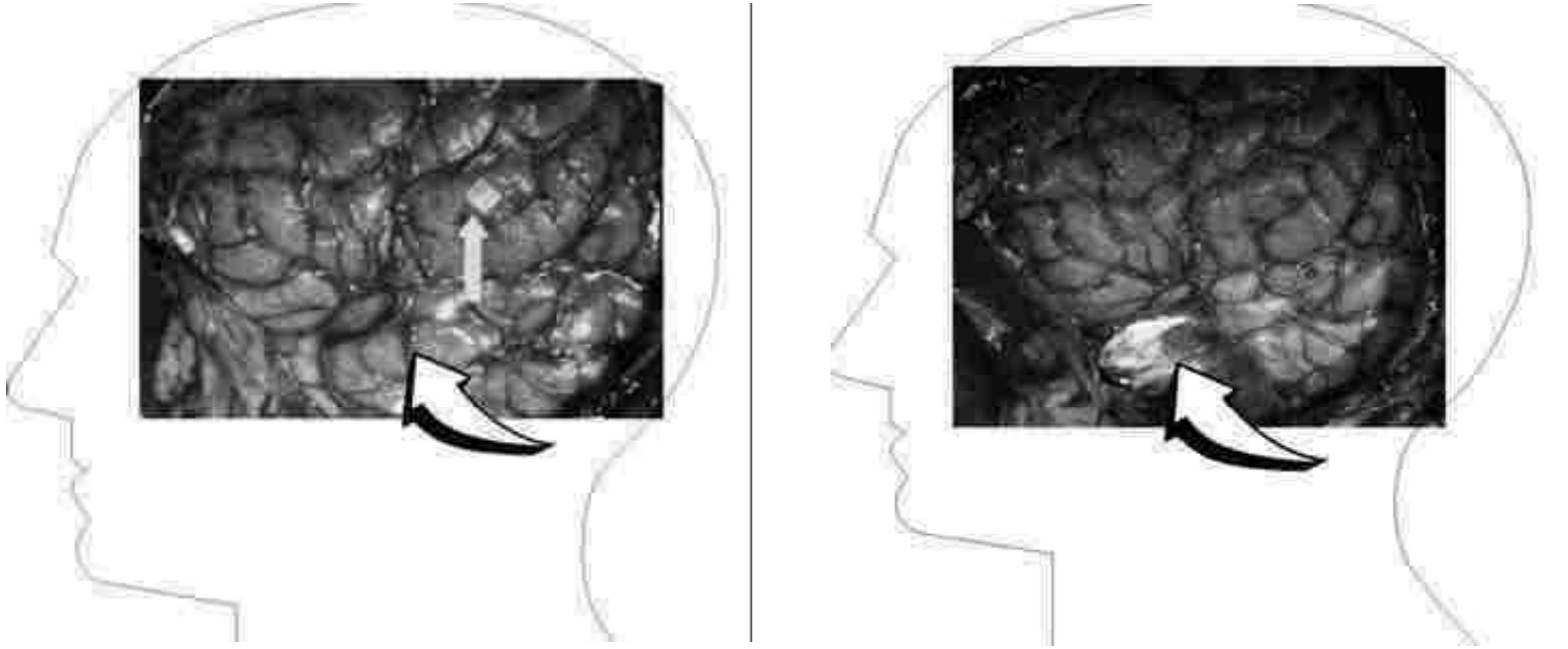
Factors influencing decision

- Likelihood seizures are due to epilepsy
- Likelihood surgery will help
- Ability to identify focus of seizures
- Other treatments attempted, and seizures couldn’t be treated with 2-3 medications
- Benefits vs risks

Epilepsy – Surgical Treatment

- A proportion of the pts with intractable epilepsy will benefit from surgery.
- Epilepsy surgery procedures: Curative (removal of epileptic focus) and palliative (seizure-related risk decrease and improvement of the QOL)
- Curative (resective) procedures: Anteromesial temporal resection, selective amygdalohippocampectomy, extensive lesionectomy, cortical resection, hemispherectomy.
- Palliative procedures: Corpus callosotomy and Vagal nerve stimulation (VNS).

Left Anterior Temporal Loectomy



Vagus Nerve Stimulation

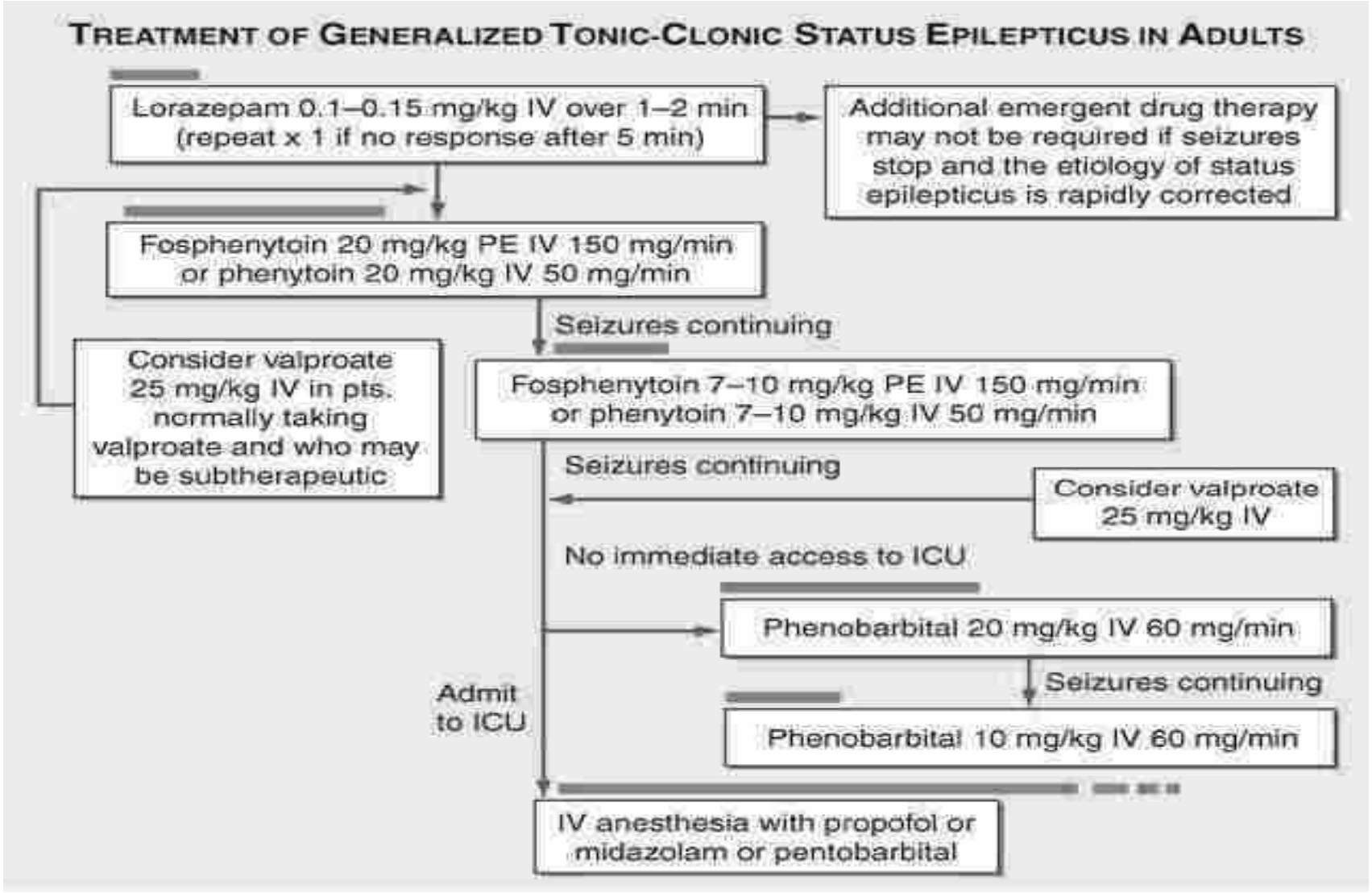
- Device is implanted to control seizures by delivering electrical stimulation to the vagus nerve in the neck, which relays impulses to widespread areas of the brain
- Used to treat partial seizures when medication does not work

Courtesy of Cyberonics Inc.

STATUS EPILEPTICUS

Status Epilepticus

- A Neurological EMERGENCY!!!!
- Definition
  - A seizure that lasts greater than 30 minutes!
  - Greater than 2 seizures in a row and patient does not regain consciousness in between
- Etiology
  - New onset seizures, infection, trauma, SAH, Stroke, Drugs (legal and illegal), toxin, mets. Non-compliance with seizure meds



Source: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 18th Edition: www.accessmedicine.com

Copyright © The McGraw-Hill Companies, Inc. All rights reserved.

Status Epilepticus

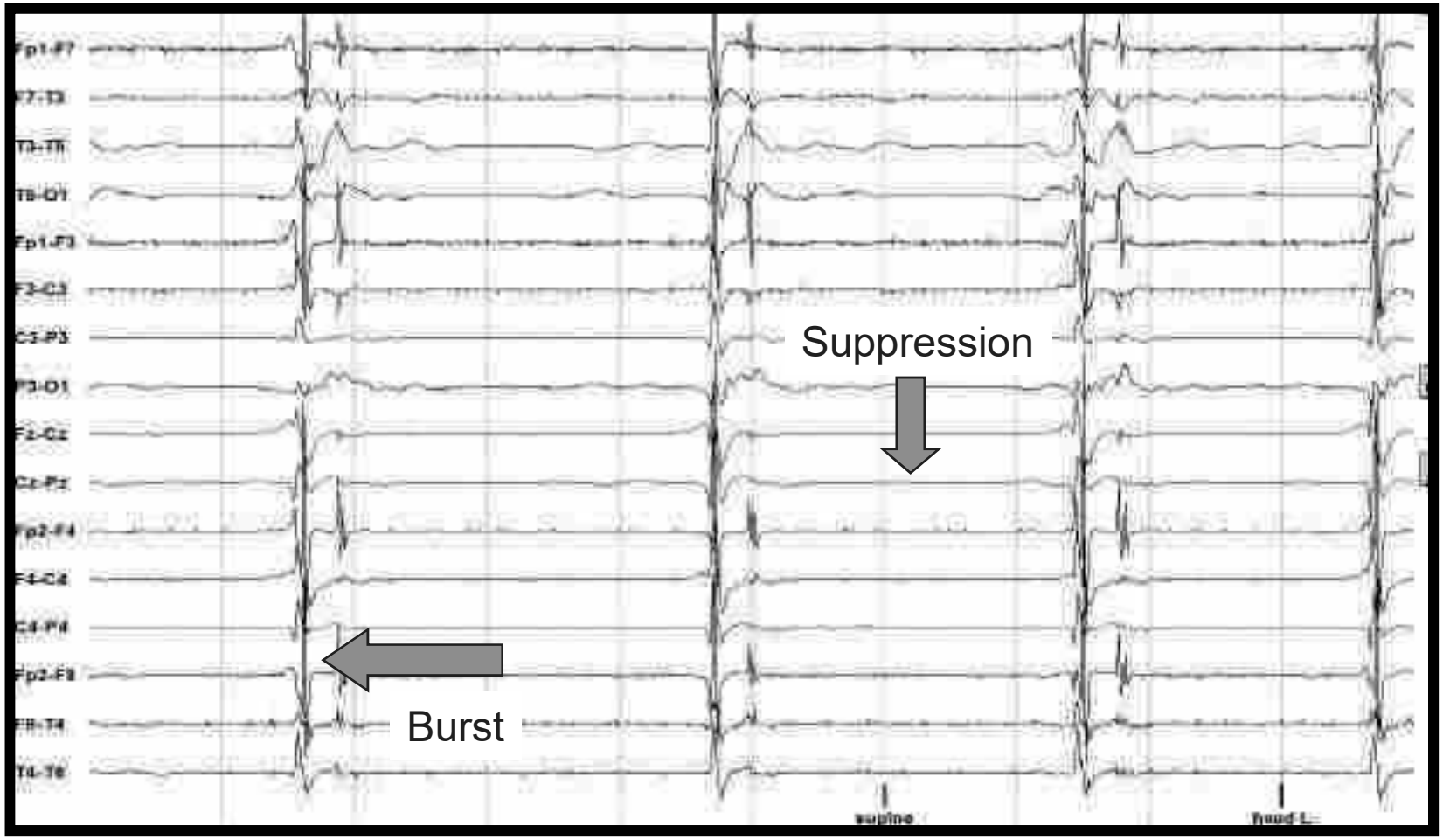
- Initial Treatment
  - ABCD!!!!!
  - Lorezepam 2mg IV q2min x4
    - Or… diazepam 5mg IV x2
  - Phenytoin (Dilantin) 15-18mg/kg IV
    - Infuse slow, no greater than 50mg/min
  - Evaluate for etiology
    - Draw CBC, LFTs, head CT, Mg, Ca, drug screen
    - Consider LP

Status Epilepticus

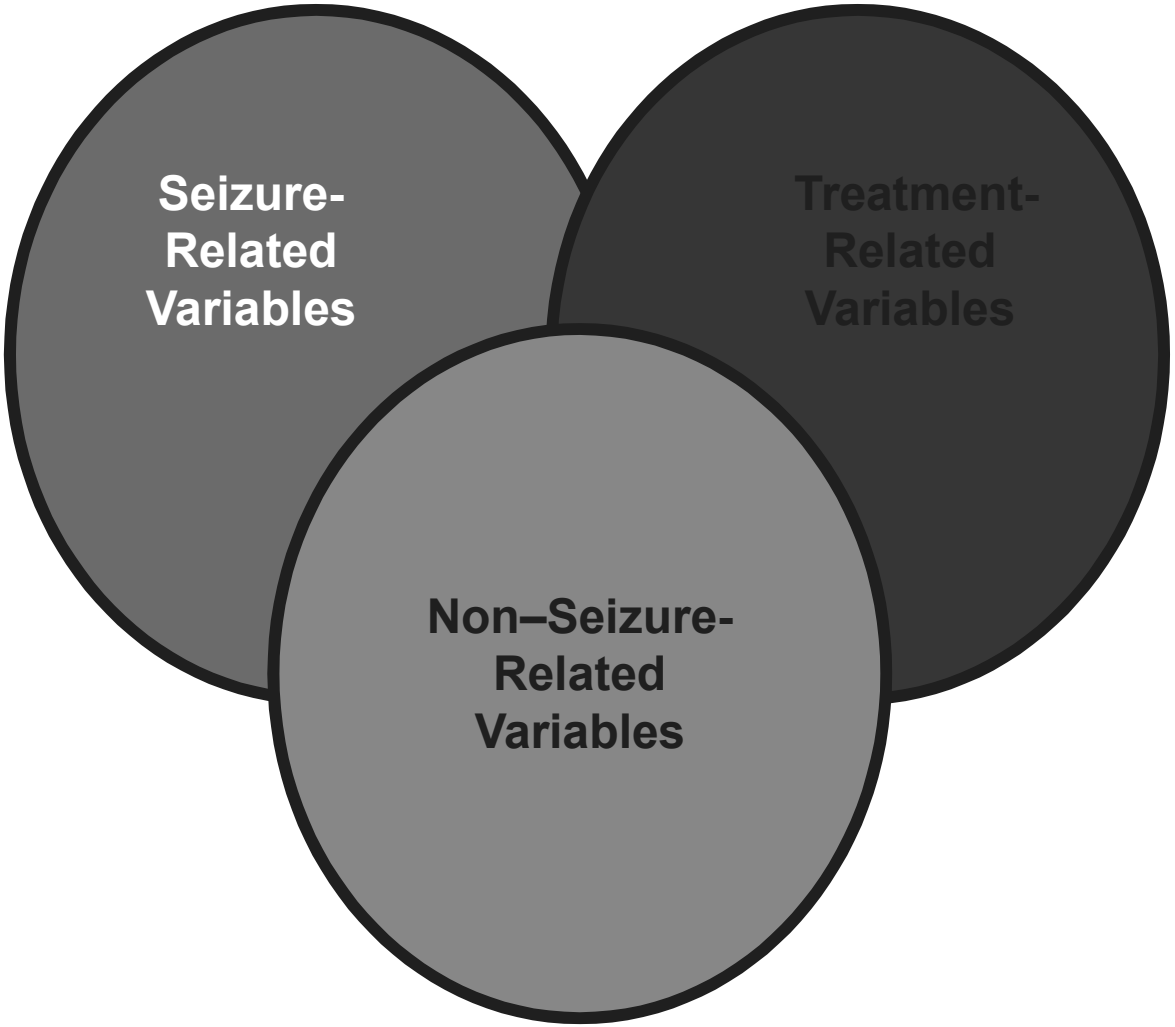
- Second line treatment (if initial fails)
  - Phenobarbital-20mg/kg IV
    - Watch for respiratory depression
  - Depakote-10-15mg/kg IV
- Third line treatment
  - Warrants an ICU admission/Intubation
  - Pentobarbital
    - 5mg/kg IV load and then titrate to burst suppression
  - Midazolam
    - 0.2mg/kg IV load and then titrate to burst suppression

Status Epilepticus

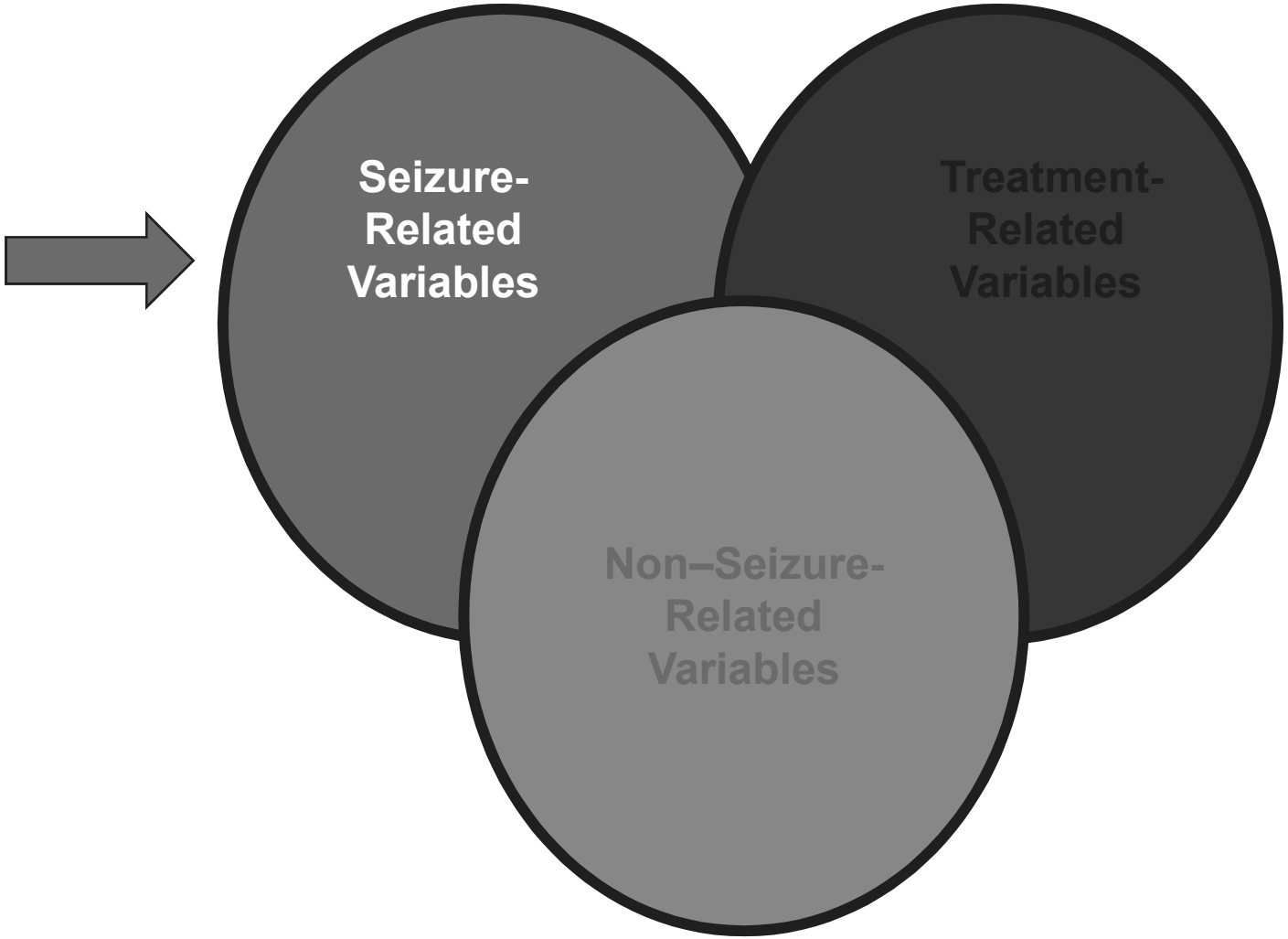
- Titrate IV agents to burst suppression



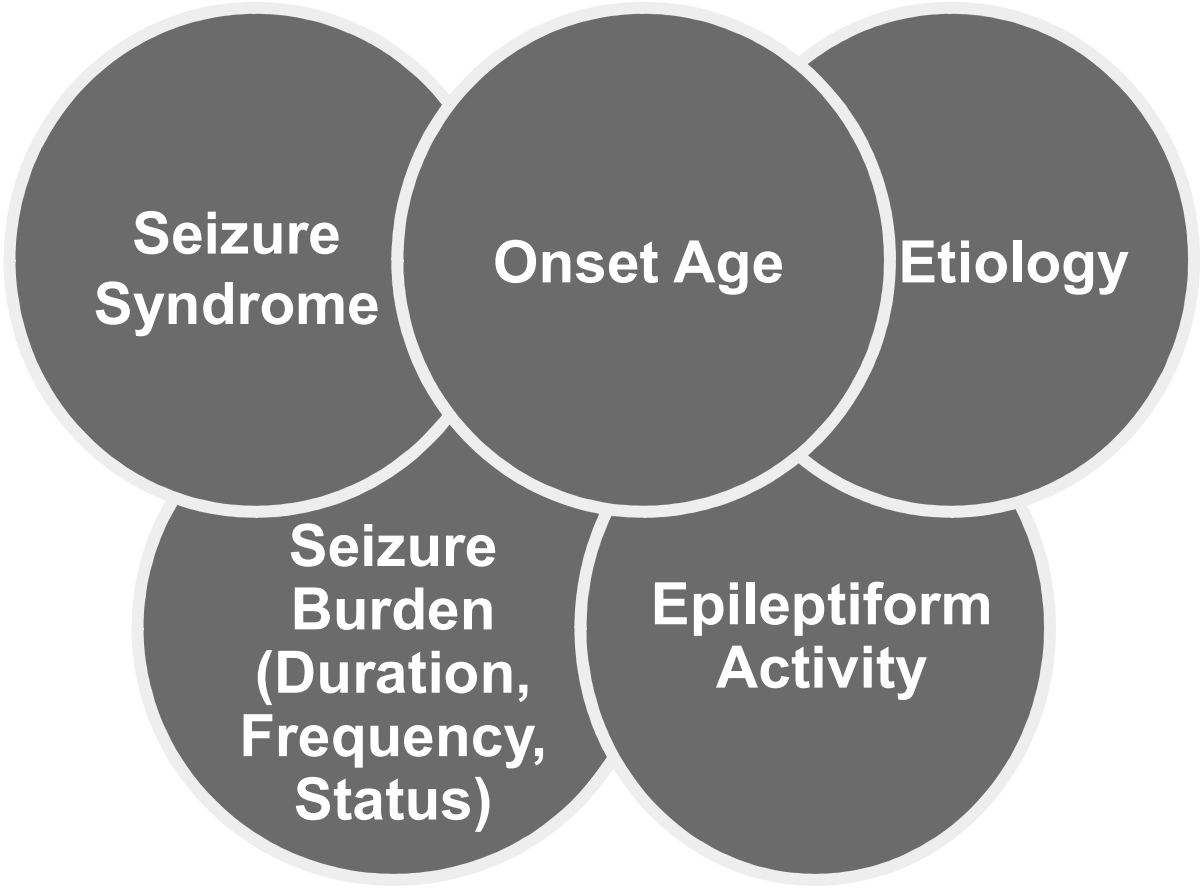
Factors Affecting Adaptation in Epilepsy



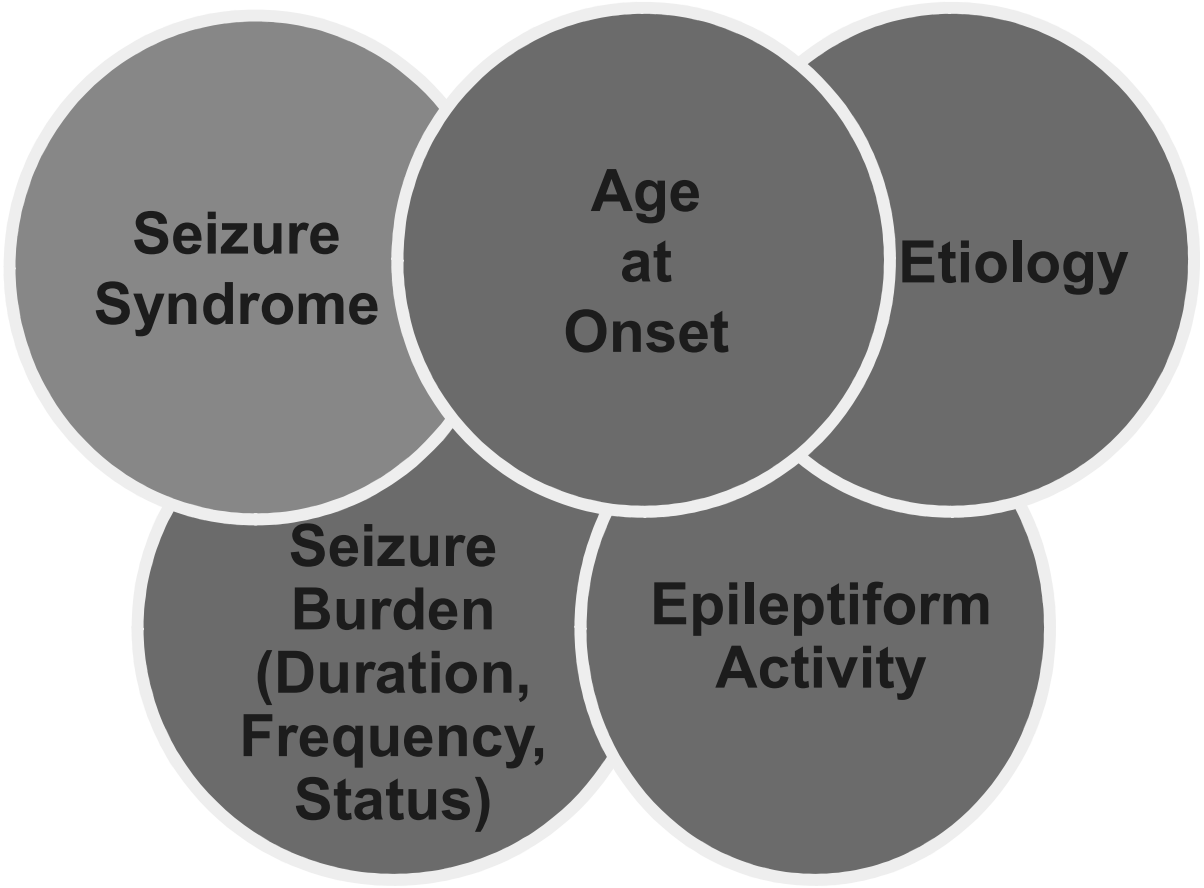
Factors Affecting Cognitive Function in Epilepsy



What Seizure Related Factors May Affect Cognition in Epilepsy?



Seizure-Related Variables That May Affect Cognition and Behavior



Epileptic Syndrome

- Some epilepsy syndromes are known to be associated with more adverse cognitive consequences than others.
  - Idiopathic Benign syndromes—e.g., BECTS (Rolandic), absence
  - Adverse syndromes—e.g., Lennox-Gastaut
  - Variable syndromes—Localization related epilepsies

Idiopathic Syndromes

	Deficit	Outcome
JME	Mild executive deficits	Presumed favorable
Generalized with absence or GTCS	Mild attentional deficits	Unknown
Centrotemporal spikes benign	Mild Heterogeneous	Mostly favorable (interictal abn)
Occipital epilepsy	Mild Heterogeneous	Unknown

Elger et al. (2004)

Adverse Syndromes

	Deficit	Outcome
CSWDS	Variable (diffuse or executive)	Variable - duration dependent
Landau Kleffner	Auditory agnosia, Expressive Language	Variable – early onset worse
West Syndrome	Retardation, regression	Poor, retardation
Lennox-Gastaut	Retardation, decline	Poor, retardation worse early onset

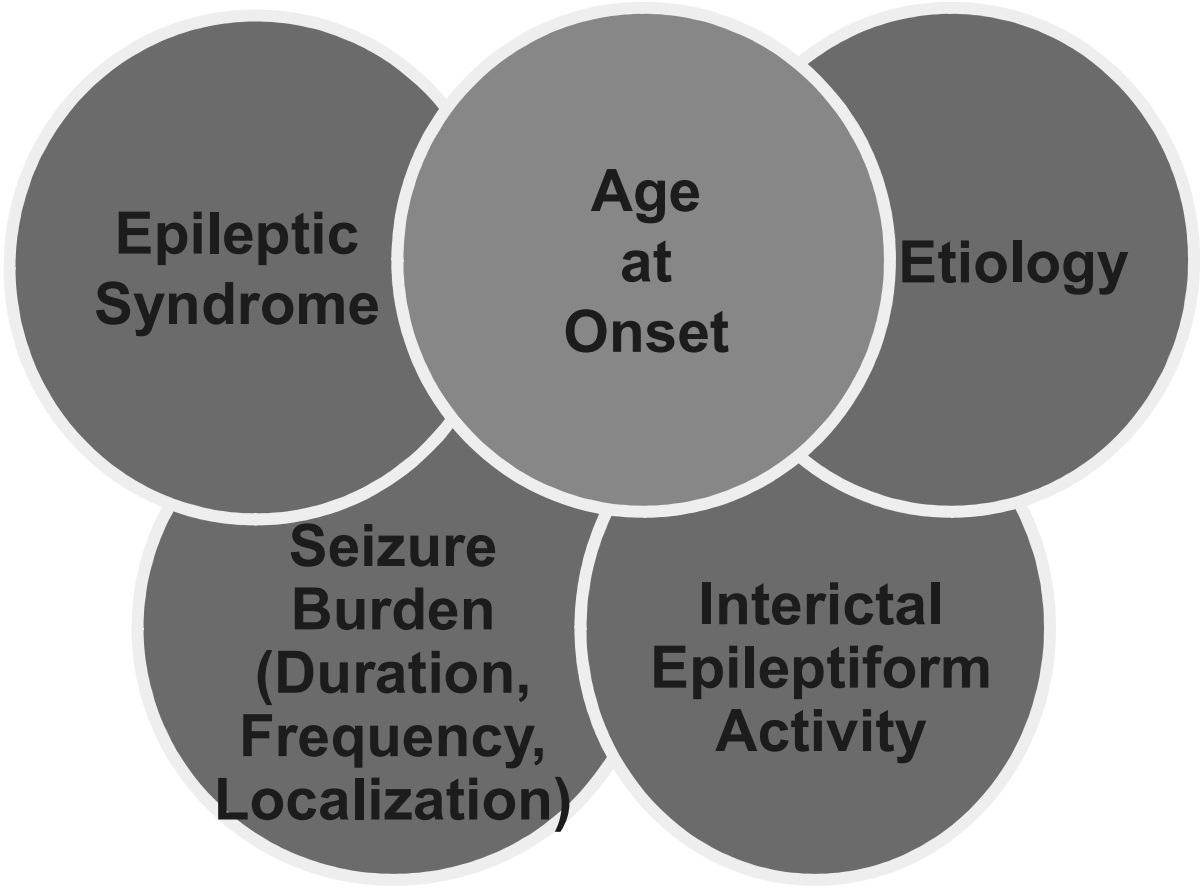
Elger et al. (2004)

Localization Related Syndromes

	Deficit	Outcome
Frontal	Executive function, attention, speed	Unknown
Temporal	Material-specific memory (executive 2º gen), naming, achievement	Very slow deterioration
Parietal Occipital	Unknown (variable)	Unknown (heterogeneous)

Elger et al. (2004)

Seizure-Related Variables That May Affect Cognition and Behavior

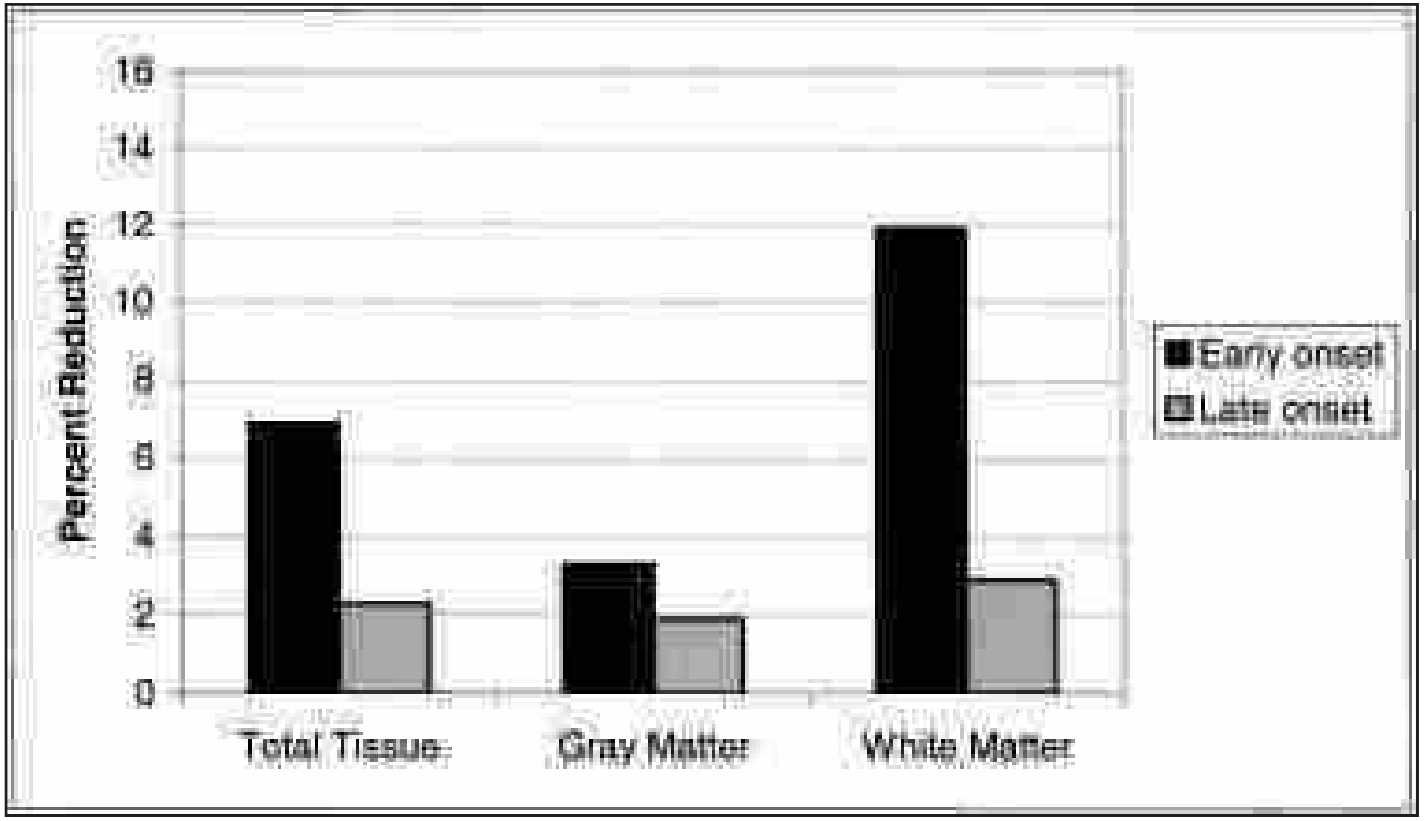


Adults with Childhood Seizure Onset

- Less Education
- Decreased rates of employment
- Lower rates of marriage
- Poorer physical health
- Increased incidence of psychiatric disorders

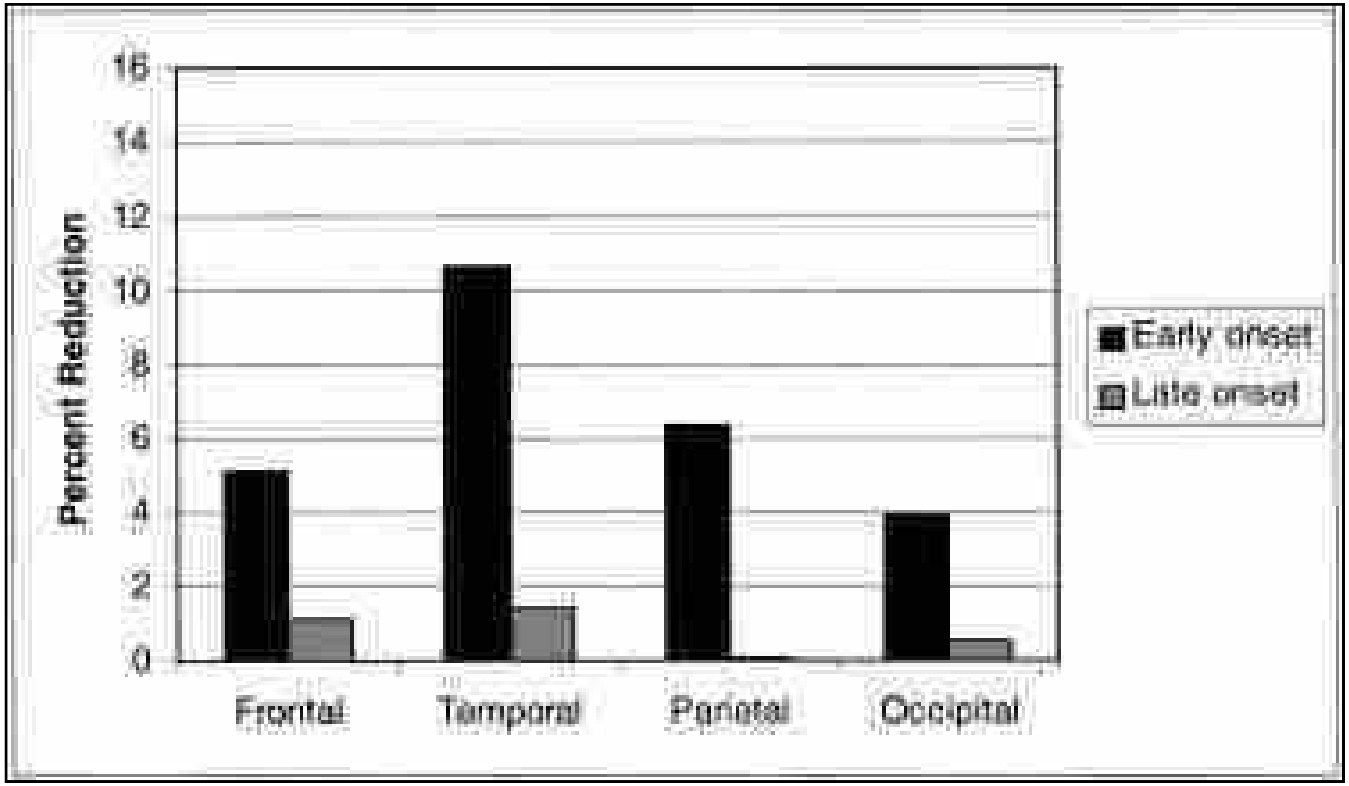
Jalava et al., 1997a,b,c, Sillanpää (1998)

Total and Segmented Volumes
(7.8 years vs. 23.3 years)



Hermann et al, *Epilepsia* 2002;43:1062-71

Total Lobar White Matter



Hermann et al, *Epilepsia* 2002;43:1062-71

Cause or Effect?

- Does white matter volume abnormality reflect neurodevelopmental abnormality associated with early insult to developing brain?
- Does early lesion affect subsequent normal development of white matter connectivity?

Age of Onset and Neuropsychological Outcome

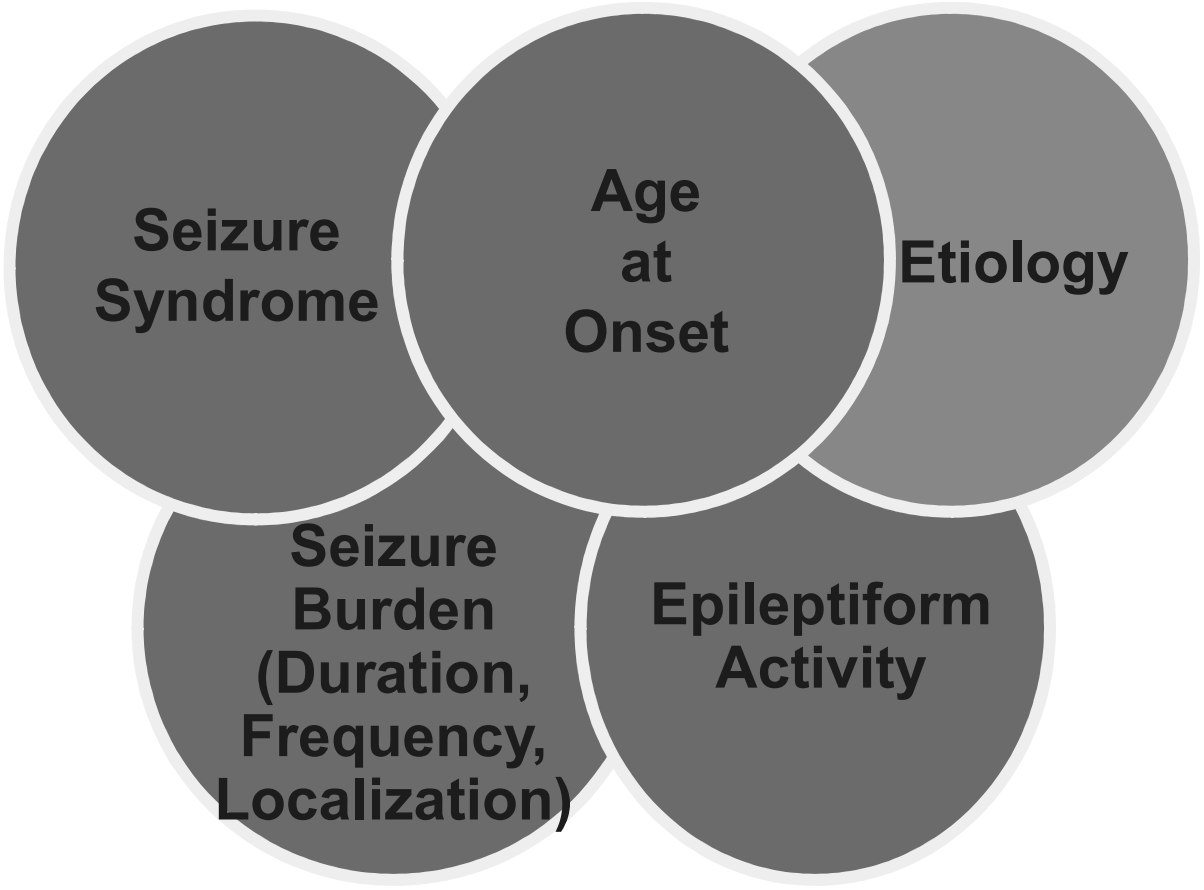
	Early (7.8 yr)	*Late (23.3 yr)*	*Healthy Controls*
N	62	37	16
FSIQ	90*	100	107
Naming	47	52	55
Verbal Mem	44	51	52
NV Mem	46	55	62
WCST PE	13	8	8

Hermann et al, *Epilepsia* 2002;43:1062-71

Childhood TLE Onset

- Generalized cognitive compromise
- Reduction in cerebral volume, particularly white matter (~6-12%)
- Cerebral volume reduction not limited to temporal lobe
- Less focal impairment (e.g., memory)
- Less surgical risk
- Greater likelihood of functional reorganization (e.g., bilateral language, pathologic left handedness)

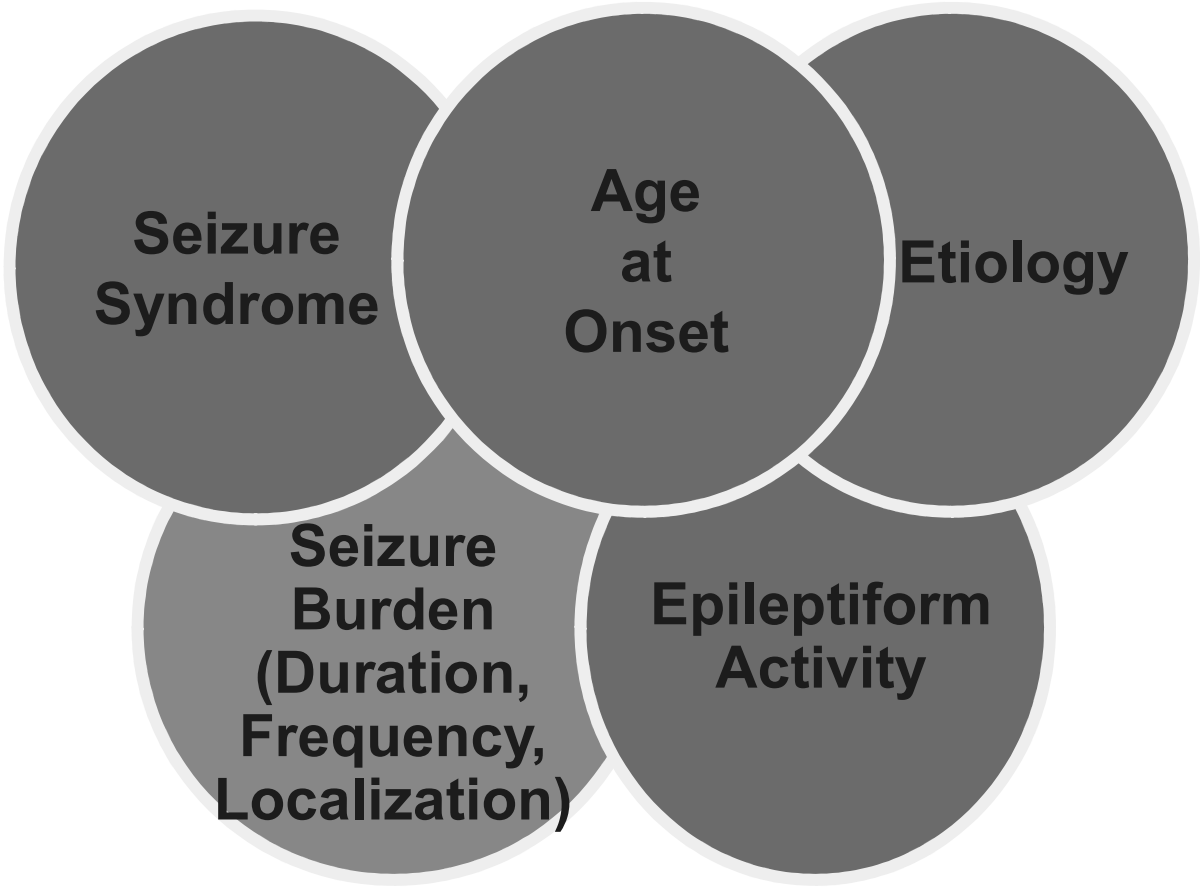
Seizure-Related Variables That May Affect Cognition and Behavior



Etiology

- Individuals with known causes for their epilepsy (e.g., head injuries, brain infections) typically have more detectable cognitive difficulties than those with no known etiology

Seizure-Related Variables That May Affect Cognition and Behavior



Seizure Burden

- Individuals with poorly controlled and severe seizures often have more detectable cognitive consequences than individuals with well-controlled and/or minor seizures

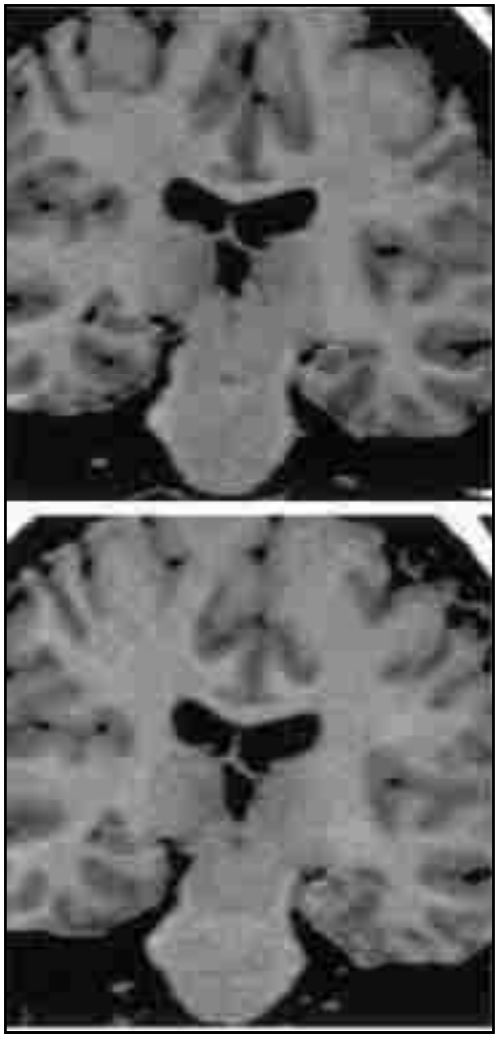
Cumulative Seizure Effects? (is epilepsy progressive?)

- Structural Imaging vs Behavior
- Cognitive and behavioral impairments present prior to treatment
- Newly diagnosed L TLE patients have verbal memory impairment

Äikiä, *Epilepsy & Behavior* (2001)
Äikiä , *Epilepsy Research* 1995;22:157-164

Progressive Hippocampal Sclerosis

- Progressive hippocampal atrophy occurred only in patients with TLE and continuing seizures
- n=12 unilateral TLE
- Repeat MRI=2.5-5.2 yr



Fuerst et al, *Ann Neurol* 2003;53:413-6

Neuropsychological Effects of Poorly Controlled Seizures

- 20 longitudinal studies in children-adults
- 12/20 reported relationship/decline
- 5/20 mixed results
- 3/20 no relationship

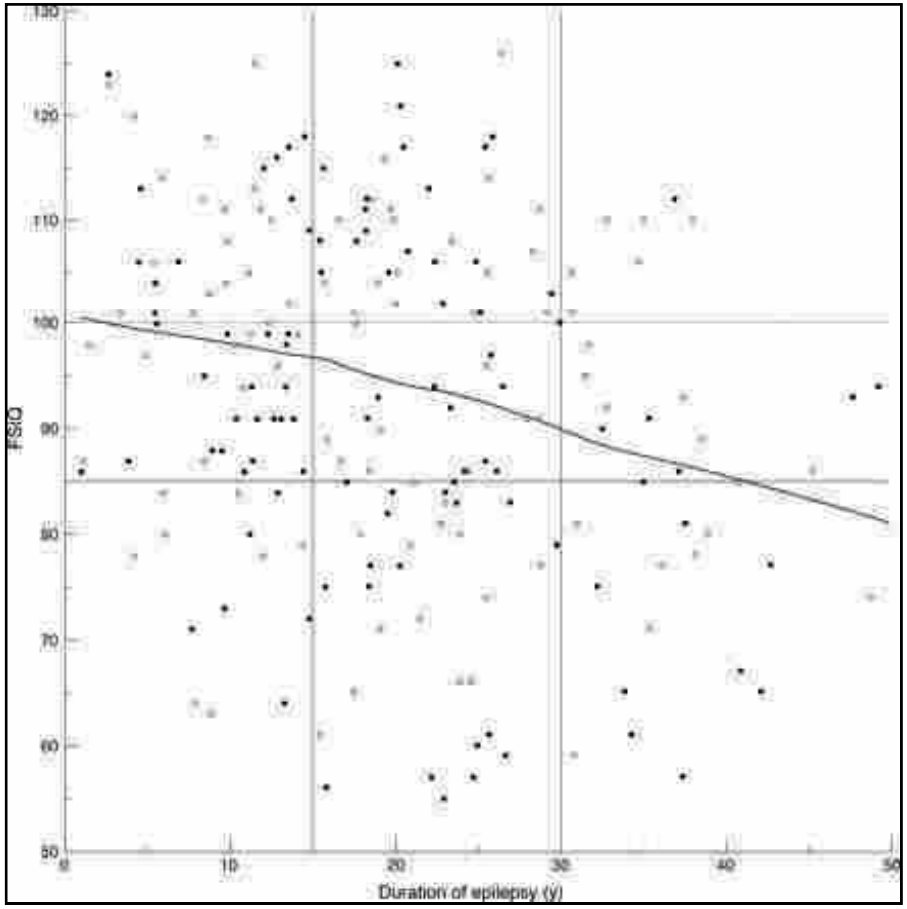
Dodrill, *Epilepsy & Behavior* (2004)

Neuropsychological Effects of Seizures

- Decreased scores with higher number of seizures
- IQ lower with increased seizure frequency
- Greater performance “improvement” in controls than patients
- Losses seen beyond “memory”

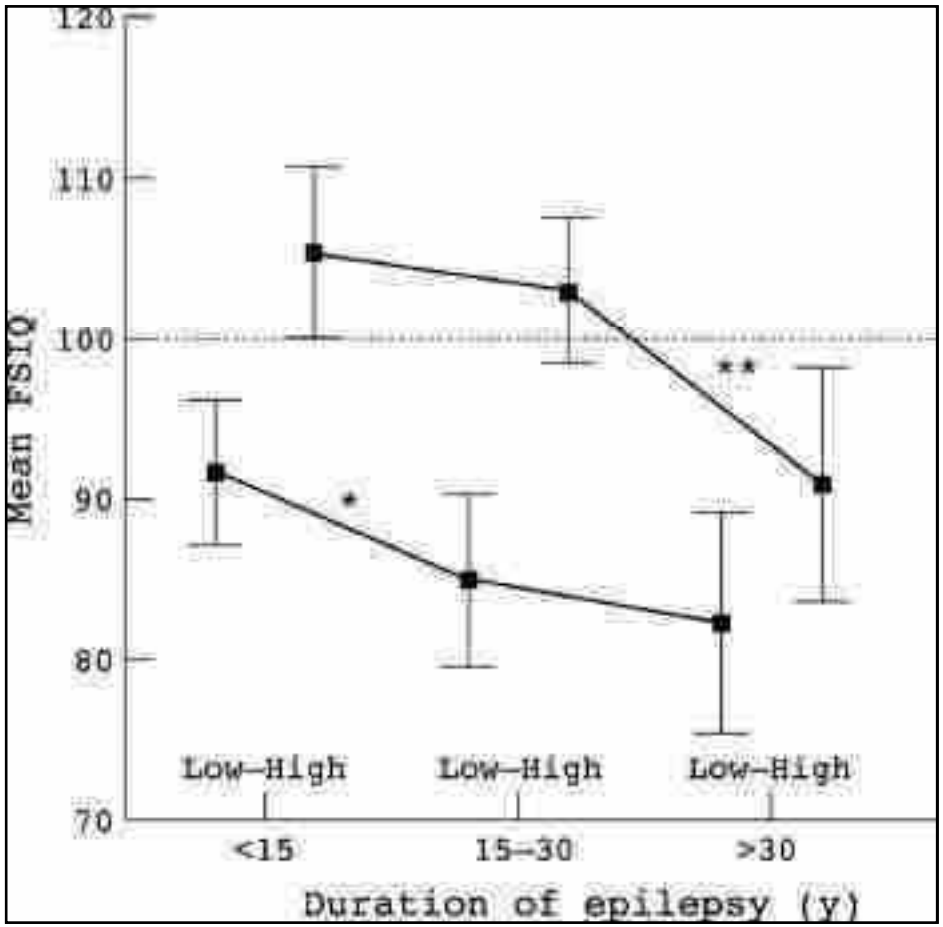
Dodrill, *Epilepsy & Behavior* (2004)

Cross-sectional TLE Neuropsychological Outcome



Jokeit et al, *JNNP* 1999;67:44-50

Educational Attainment and Seizure Duration



Jokeit et al, *JNNP* 1999;67:44-50

How do Seizures/Epilepsy Affect You?

- Seizures may restrict driving, working, and social opportunities and also affect self-esteem. But remember, you can influence how epilepsy will affect you.
- Most people's epileptic seizures can be controlled. Some people end their seizures with the first medication they try. Others will need to partner with their Neuro-Oncologist or epileptologist to find the right dosage and combination of medications, especially as brain tumors can change geometry and location.

Take Control of Managing Seizures

- Understand your epilepsy, including your seizure type
- Make sure to talk to your Neuro-Oncologist or epileptologist about all available treatment options
- Fill out a medical history and seizure calendar, so you can become involved in your medical care
- Learn how to communicate well with your neurologist and his or her staff

Take Control of Managing Seizures

- Bring your epilepsy medications or a detailed printed list to each Neuro-Oncology visit Take your medication regularly.
- Linking this to a routine may be helpful (when you brush your teeth, prepare for bed, etc.).
- There are also devices that can help like a watch that beeps when your dose is due or special blister packaging that is pre-dosed Get enough sleep- lack of it can bring on epileptic seizures

Common Myths

- Epilepsy is contagious
- People may swallow their tongue during a seizure
- Putting things in people's mouths (like wallets or a stick) while seizing is a good idea
- Ambulance should be called immediately
  - If the seizure lasts longer than 5 minutes, it is their first seizure, they request an ambulance, or have multiple seizures in a row

Summary

- In most instances, there is a zero-tolerance policy for seizure control but there are exceptions
- Most commonly, we have to strike a balance between seizure control and toxicity of medications
- Seizures are often a frightening experience which in turn can make them tricky to discern from emotional repercussions such as panic, abnormal movements such as from spasticity, etc. As usual, knowledge gives us power to gain control over even the toughest situations

Paracelsus (1493-1541)

"All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy."

THANK YOU

Introduction and History

Learning objectives

At the end of the session, the students will be able to know:

- Clinical Microbiology – Terminologies
- Classification of Microorganisms
- Brief History

Medical Microbiology

Deals with the study of microorganisms such as:

- Bacteria
- Viruses
- Parasites
- Fungi

Clinical Microbiology

Concerned with - **prevention, diagnosis and treatment** of infectious diseases. It includes :

- General Microbiology
- Immunology
- Hospital infection control
- Systemic Microbiology (Infectious diseases)

Clinical Microbiology (Cont..)

Branch	Study of
General Microbiology	General properties of microorganisms –taxonomy, etc
Immunology	Immune system and immunological mechanisms of diseases
Hospital infection control	Control measures to prevent the transmission of HAIs
Systemic Microbiology (Infectious diseases)	Microorganisms that infect various organ systems

Clinical Microbiology (Cont..)

Systemic Microbiology (Infectious diseases) - classified into various clinical syndromes:

- Blood stream and cardiovascular system infections
- Gastrointestinal and hepatobiliary system infections
- Skin, Soft tissue and musculoskeletal system infections
- Central nervous system infections
- Respiratory tract infections
- Urinary tract infections
- Genital tract infections
- Infections of eye, ear and other organs

CLASSIFICATION OF MICROORGANISMS

Characteristics		Prokaryotes	Eukaryotes
Major groups		Bacteria, blue green algae	Fungi, parasites, other algae, plants and animals
Nucleus		Diffuse	Well defined
	Nuclear membrane	Absent	Present
	Nucleolus	Absent	Present
	Ribonucleoprotein	Absent	Present
	Cell division	Binary fission	Mitosis, meiosis
	Chromosome	One, circular	Many, linear

CLASSIFICATION OF MICROORGANISMS (Cont..)

Characteristics	Prokaryotes	Eukaryotes
Extrachromosomal DNA	Found in plasmid	Found in mitochondria
Cell membrane	Does not contain sterols except in Mycoplasma	Contain sterols
Cellular organelles like mitochondria, etc.	Absent (except ribosome)	Present
Ribosome	70S	80S
Site of respiration	Mesosome	Mitochondria
Pinocytosis	Absent	Present

HISTORY

Antonie Philips van Leeuwenhoek (1676)

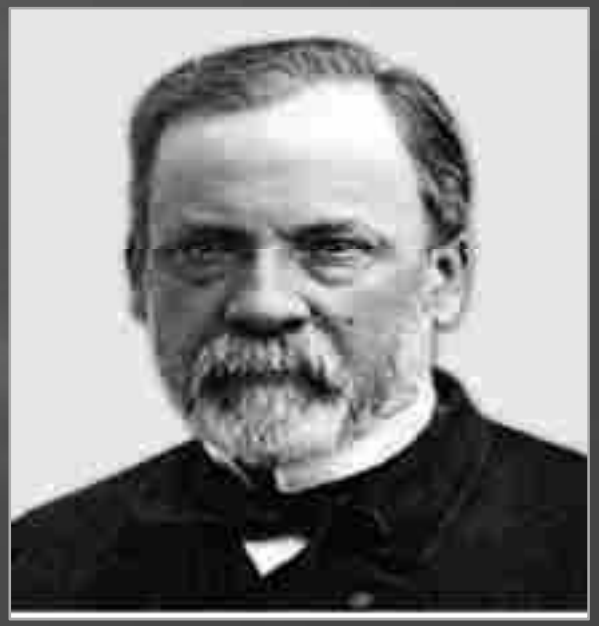
- First scientist to observe bacteria and other microorganisms - using **single-lens microscope**.
- Named - small organisms as ***'Little animalcules'***

Edward Jenner (1796)

- Developed first vaccine - **smallpox vaccine.**
- Used cowpox virus (*Variolae vaccinae*) to immunize children against smallpox.
- Hence the term - **‘vaccine’** was derived.

Louis Pasteur (1822–1895)

- **Father of microbiology**.
- Professor of chemistry in France.



Louis Pasteur (1822–1895) (Cont..)

Contributions:

- Proposed - **principles of fermentation.**
- Introduced - **sterilization techniques.**
- Described - **pasteurization of milk.**
- **Vaccine development** against - anthrax, fowl cholera and rabies.

Louis Pasteur (1822–1895) (Cont..)

Contributions (Cont..):

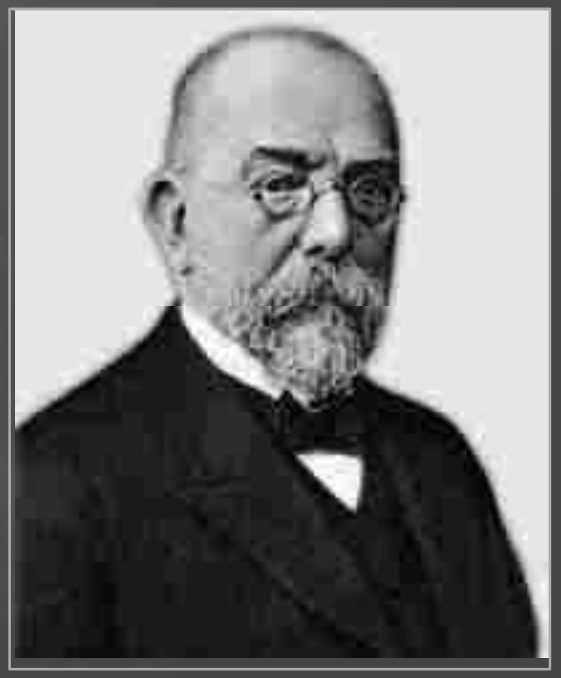
- Disproved - theory of spontaneous generation and postulated - '**germ theory of disease'**
- **Liquid media concept –** for growth of microorganisms.
- Founder of the Pasteur Institute, Paris.

Joseph Lister (1867)

- **Father of antiseptic surgery**.
- Observed that postoperative infections were reduced by using **diluted carbolic acid** to sterilize the instruments and to clean wounds during surgery

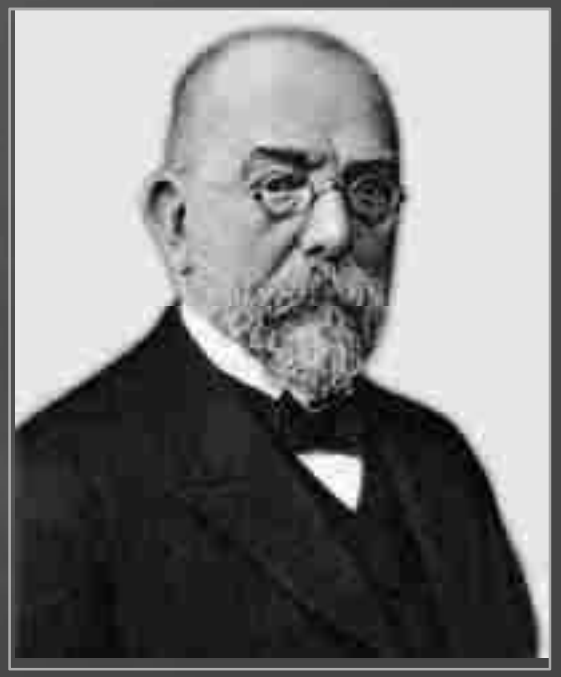
Robert Koch (1843–1910)

- German general practitioner.
- First to use **agar** (solidifying agent) for preparation of solid media
- Introduced concept of **pure culture.**



Robert Koch (1843–1910) (Cont..)

- Described - **hanging drop method** for testing motility.
- Discovered **anthrax bacilli**, **tubercle bacilli** and **cholera bacilli**.
- Introduced **staining techniques** using aniline dye.



Essentials of Medical Microbiology by Apurba S Sastry © Jaypee Brothers Medical Publishers

Robert Koch (1843–1910) (Cont..)

Koch's phenomenon:

- Guinea pigs previously infected with tubercle bacillus developed a **hypersensitivity reaction** when injected with tubercle bacilli or its protein.

Robert Koch (1843–1910) (Cont..)

Koch's postulates:

1. Microorganism - **constantly associated** with the lesions of the disease.
2. **Isolate** the organism in pure culture.
3. **Same disease** must result - when isolated microorganism is inoculated into a suitable laboratory animal.
4. Possible to **re-isolate** the organism in pure culture.

Robert Koch (1843–1910) (Cont..)

- An additional **fifth** criterion was introduced subsequently - states that **antibody** to the causative organism should be demonstrable in the patient's serum.

Robert Koch (1843–1910) (Cont..)

Exceptions to Koch's postulates:

- *Mycobacterium leprae* and *Treponema pallidum* - cannot be grown *in vitro*; but - maintained in experimental animals
- *Neisseria gonorrhoeae* - no animal model, but - grown *in vitro*.

Paul Ehrlich (1854–1915)

- **Father of chemotherapy**
- 1st to report - **acid-fast nature** of tubercle bacillus.
- Proposed - toxin-antitoxin interaction - called **Ehrlich phenomenon.**

Paul Ehrlich (1854–1915) (Cont..)

- Proposed - '**side chain theory** for antibody production'.
- **Chemotherapy**- Discovered **salvarsan** - an arsenical compound (also called as the 'magic bullet') - first effective medicinal treatment for syphilis.

Paul Ehrlich (1854–1915) (Cont..)

- Bacteria ***'Ehrlichia'*** - named after him.
- Nobel prize (1908) in Physiology or Medicine –**Immunology**
- Founder and first director of - Paul Ehrlich Institute, Germany.

Other Important Contributors

Scientist	Contributions
Hans Christian Gram (1884)	Developed - 'Gram stain'
Ernst Ruska (1931)	Founder of electron microscope
Alexander Fleming (1929)	Discovered penicillin
Goodpasture	Described - viral culture technique in chick embryo
Barbara McClintock	Described - mobile genetic elements in bacteria - transposons
Walter Gilbert and Frederick Sanger (1977)	DNA sequencing
Karry B Mullis (1993)	Discovered PCR and was awarded noble prize

Microorganisms named after discoverer

Discoverer	Organism
Ogston	*Staphylococcus aureus*
Neisser	*Neisseria gonorrhoeae*
Weichselbaum	*Neisseria meningitidis*
Loeffler	*Corynebacterium diphtheriae*
Frenkel	*Streptococcus pneumoniae*
Bruce	*Brucella melitensis*
Kitasato	*Clostridium tetani*

Microorganisms named after discoverer

Discoverer	Organism
Hansen	*Mycobacterium leprae*
Yersin & Kitasato	*Yersinia pestis*
Schaudinn & Hoffman	*Treponema pallidum*
Daniel Carrion	*Bartonella bacilliformis*
d'Herelle	Bacteriophages
W.H. Welch	*Clostridium perfringens*
Anthony Epstein and Yvonne Barr	Epstein-Barr virus

Bacteria named after the discoverers

Common name	Scientific name
Kleb-Loeffler bacillus	*Corynebacterium diphtheriae*
Preisz Nocard bacillus	*Corynebacterium pseudotuberculosis*
Koch Week bacillus	*Haemophilus aegyptius*
Pfeiffer's bacillus	*Haemophilus influenzae*
Whitmore bacillus	*Burkholderia pseudomallei*
Battey bacillus	*Mycobacterium intracellulare*
Johne's bacillus	*Mycobacterium paratuberculosis*
Eaton's agent	*Mycoplasma pneumoniae*
Gaffky Eberth bacillus	*Salmonella Typhi*

Nobel laureates – For contributions in microbiology

Nobel laureate	Year	Research
Emil A von Behring	1901	Development of antitoxin against diphtheria
Sir Ronald Ross	1902	Life cycle of malarial parasite in mosquitoes
Robert Koch	1905	Discovery of the causative agent of tuberculosis
Charles LA Laveran	1907	Discovery of malarial parasite in unstained preparation of blood
Paul Ehrlich and Elie Metchnikoff	1908	Discovered selective theory of antibody formation
Charles Richet	1913	Discovered anaphylaxis
Jules Bordet	1919	Discovered complement and developed complement fixation test

Nobel laureates – For contributions in microbiology

Nobel laureate	Year	Research
Karl Landsteiner	1930	Described ABO blood group
Sir Alexander Fleming	1945	Discovery of penicillin
F Enders, FC Robbins, TH Weller	1954	Cultivation of polio viruses in tissue culture
J Lederberg and EL Tatum	1958	Discovery of conjugation in bacteria
Sir M Burnet and Sir PB Medawar	1960	Postulated immunological tolerance
Watson and Crick	1962	Discovered double helix structure of DNA

Nobel laureates – For contributions in microbiology

Nobel laureate	Year	Research
Peyton Rous	1966	Discovered viral oncogenesis
Holley, Khurana and Nirenberg	1968	Discovered genetic code
BS Blumberg	1976	Discovered Australia antigen (HBsAg)
Rosalyn Yallow	1977	Developed radioimmunoassay
B Benacerraf, F Dausset and G Snell	1980	Discovered HLA antigen
Barbara McClintoch	1983	Discovered mobile genetic elements (transposon)
Georges Kohler	1984	Developed hybridoma technology for monoclonal antibodies

Nobel laureates – For contributions in microbiology

Nobel laureate	Year	Research
Niels Jerne	1984	Postulated idiotype network hypothesis (Jerne hypothesis)
S Tonegawa	1987	Elucidated the nature of antibody diversity
Kary B Mullis	1993	Invented polymerase chain reaction
Stanley B Prusiner	1997	Described Prions
J Robin Warren and Barry J Marshal	2005	Discovery of *Helicobacter pylori* and its role in peptic ulcer disease
Luc Montagnier and F Barre-Sinoussi	2008	Discovery of human immunodeficiency virus (HIV)

Nobel laureates – For contributions in microbiology

Nobel laureate	Year	Research
Harald zur Hausen	2008	Human papilloma viruses causing cervical cancer
Bruce A Beutler and Jules A Hoffmann	2011	For their discoveries concerning the activation of innate immunity
Ralph M Steinman	2011	For his discovery of dendritic cell and its role in adaptive immunity
Sir John B. Gurdon and Shinya Yamanaka	2012	'Mature cells can be reprogrammed to become pluripotent"
William C Campbell	2015	Anti-parasitic effect of ivermectin in filariasis
Youyou Tu	2015	Artemisinin, a novel drug used for malaria

Questions:

Q1. Correct sentence regarding prokaryotes?

a. Nucleolus is present

b. Cell membrane does not contain sterols except in *Mycoplasma*

c. Cellular organelles like mitochondria are present

d. Extrachromosomal DNA is found in mitochondria

Questions (Cont..) :

Q2. Who is the Father of Microbiology?

a. Antonie Philips van Leeuwenhoek

b. Edward Jenner

c. Louis Pasteur

d. Joseph Lister

Questions (Cont..) :

Q3. Match the correct common name with the scientific name of bacteria?

a. Eaton's agent - *Mycobacterium paratuberculosis*

b. Battey bacillus - *Burkholderia pseudomallei*

c. Koch Week bacillus - *Corynebacterium pseudotuberculosis*

d. Kleb-Loeffler bacillus - *Corynebacterium diphtheriae*

MICROSCOPY

Essentials of
MEDICAL MICROBIOLOGY

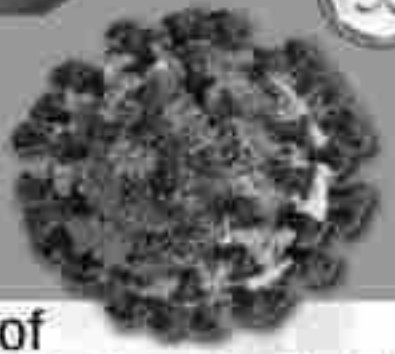
THIRD EDITION

Essentials of MEDICAL MICROBIOLOGY

Apurba S Sastry
Sandhya Bhat

JAYPEE BROTHERS

THIRD EDITION



Essentials of MEDICAL MICROBIOLOGY

As per the Competency Based Medical Education Curriculum (MCI)

Apurba S Sastry
Sandhya Bhat

Forewords
Pallab Ray
Sujatha Sistla

Learning objectives

At the end of the session, the students will be able to know:

- Types and properties of microscope
- Principle and uses of light microscope, dark field microscope, phase contrast microscope, fluorescence microscope and electron microscope

INTRODUCTION

Size:

- **Bacteria-** 0.2–1.5 μm in diameter; 3–5 μm in length
- **Viruses-** 20–300 nm in diameter
- **Parasites-** Protozoans measured in μm; helminths - few mm to meters.
- **Fungi-** Grow as hyphae - thread-like structures 2–10 μm in diameter and up to several cm in length

Different types of Microscopes

- Bright-field or light microscope
- Dark field (or dark ground) microscope
- Phase contrast microscope
- Fluorescence microscope
- Electron microscope.

PROPERTIES OF A MICROSCOPE

1. Good resolution: ability to produce separate images of closely placed objects - distinguished as two separate entities.

- Refractive Index – Oil>Air (oil enhances the resolution power)

Object	Resolution power
Human eye	200 μm
Light microscope	0.2 μm
Electron microscope	0.5 nm

PROPERTIES OF A MICROSCOPE (Cont..)

2. Good contrast:

- Improved by staining the specimen.
- When stains bind to the cells, the contrast is increased

PROPERTIES OF A MICROSCOPE (Cont..)

3. **Good magnification:** Achieved by use of lenses.

- Ocular lens - magnification power of 10x
- Objective lens-scanning (4x), low power (10x), high power (40x) and oil immersion (100x)
- Total magnification = magnification of objective lens * ocular lens.

BRIGHT-FIELD OR LIGHT MICROSCOPE

BRIGHT-FIELD OR LIGHT MICROSCOPE

- Forms a dark image against a brighter background.

Structure

- The parts are divided into three groups:

Mechanical Parts

Magnifying Parts

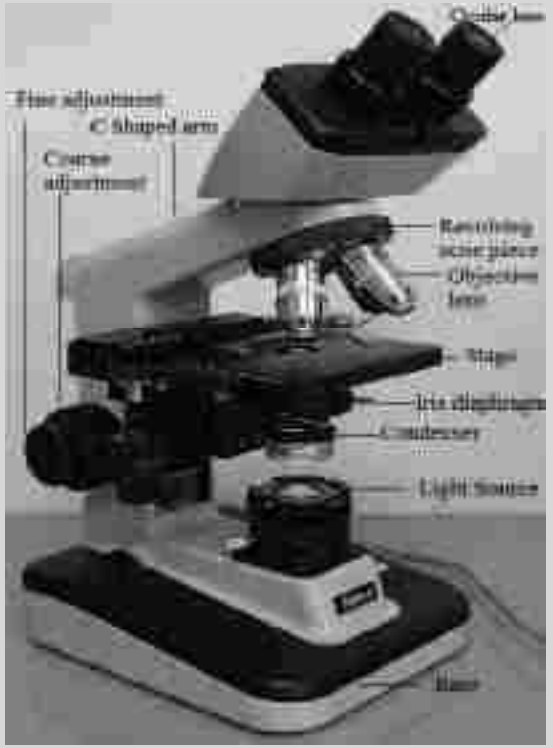
Illuminating Parts

Mechanical Parts

- **Base:** Holds various parts of the microscope
- **C-shaped arm:** Holds the microscope, connects - ocular lens to objective lens
- **Mechanical stage:** Hold the slides.

Magnifying Parts

- Ocular lens
- Objective lens



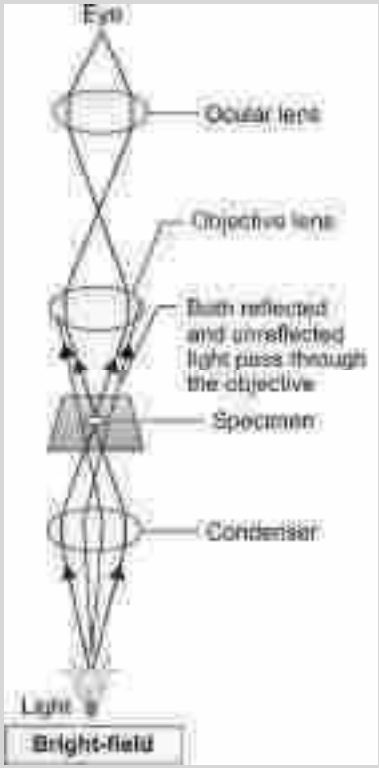
Illuminating Parts

- **Condenser:** Focuses light on the slide (beneath the stage)
- **Iris diaphragm:** Controls the light that passes through the condenser
- **Light source:** mirror or an electric bulb
- **Fine and coarse adjustment knobs:** Sharpen the image.

Working Principle

Rays from the light source pass through the iris and fall on the specimen

Light rays are gathered by the objective and a magnified image is formed

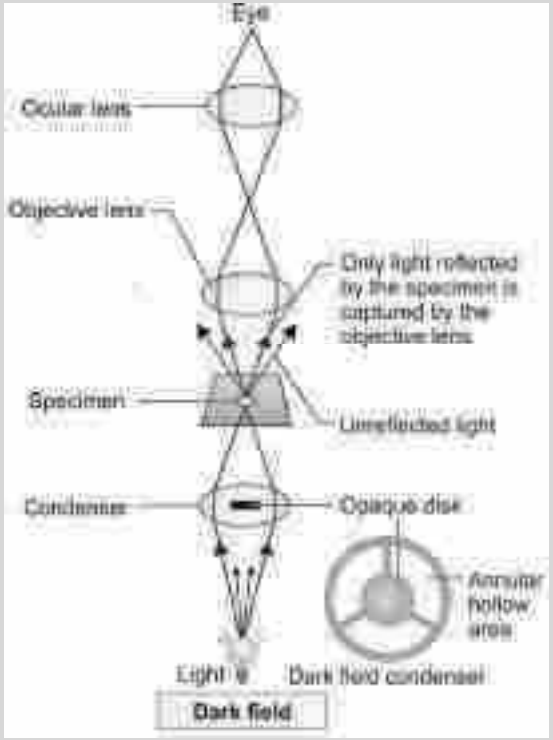


DARK FIELD MICROSCOPE

DARK FIELD MICROSCOPE

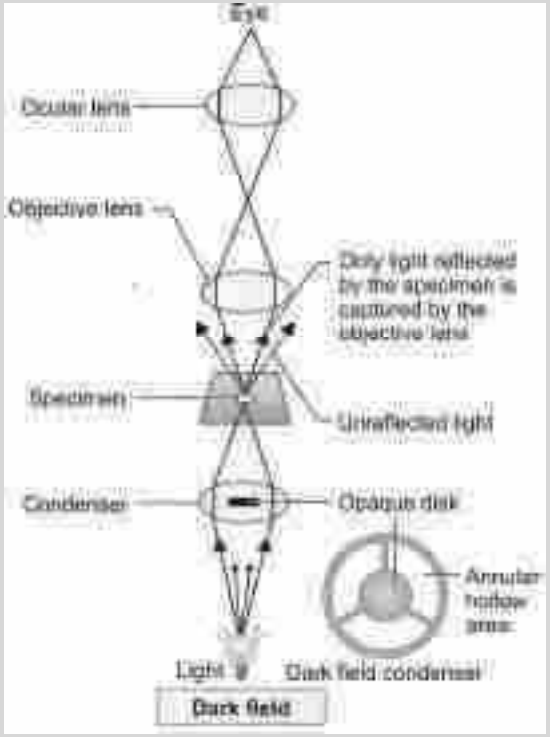
Working principle: Object appears bright against a dark background - by use of a special **dark field condenser**.

- **Central opaque area** (blocks light from entering the objective lens)
- **Peripheral annular hollow area** (allows the light to pass through and focus on the specimen obliquely)



DARK FIELD MICROSCOPE

- Light - reflected by specimen enters the objective lens
- Unreflected light does not enter the objective.
- So the specimen is bright; and the background appears dark.



Applications

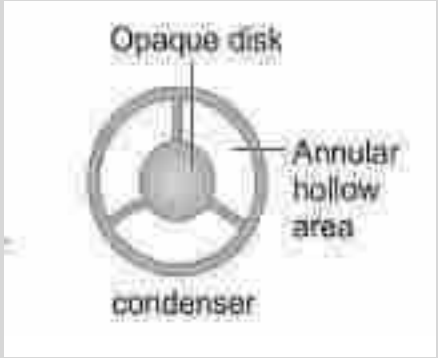
- Used to identify the living, unstained cells and thin bacteria like spirochetes which cannot be visualized by light microscopy.

PHASE CONTRAST MICROSCOPE

PHASE CONTRAST MICROSCOPE

- Contrast is enhanced.
- Visualizes the unstained living cells by creating **difference in contrast** between cells and water.

Working principle



- Condenser is similar to dark field microscope.

Cone of light passes through a cell

some light rays are bent due to variations in density and refractive index within the specimen and are retarded by 1/4th of a wavelength

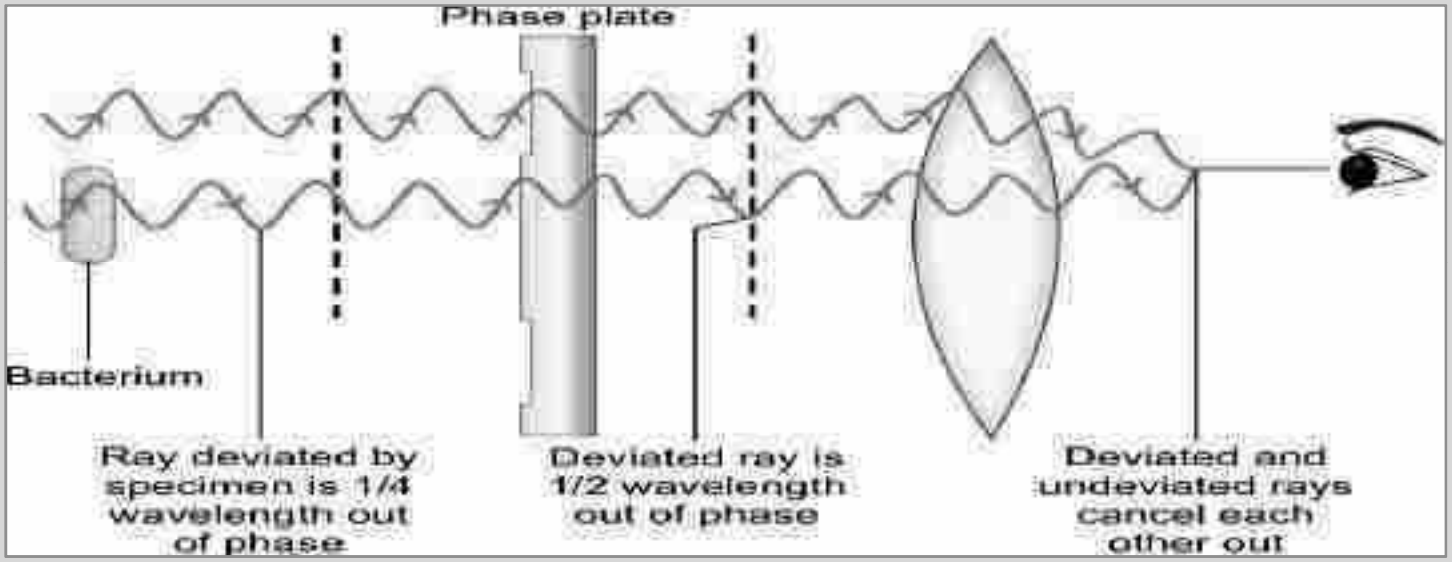
Working principle (Cont..)

Undeviated light rays strike a **phase ring**

Deviated rays miss the ring and pass through the rest of the plate

Background is bright, while the unstained object appears dark

Working principle (Cont..)



The light rays go through → condenser → specimen (e.g. bacteria) → phase ring → objective lens → ocular lens.

Applications

For studying:

- Microbial motility
- Determining the shape of living cells
- Detecting microbial internal cellular components

FLUORESCENCE MICROSCOPE

FLUORESCENCE MICROSCOPE

Working principle:

Fluorescent dyes - exposed to UV rays, become excited and fluoresce

i.e. they convert short wavelength rays into longer wavelengths

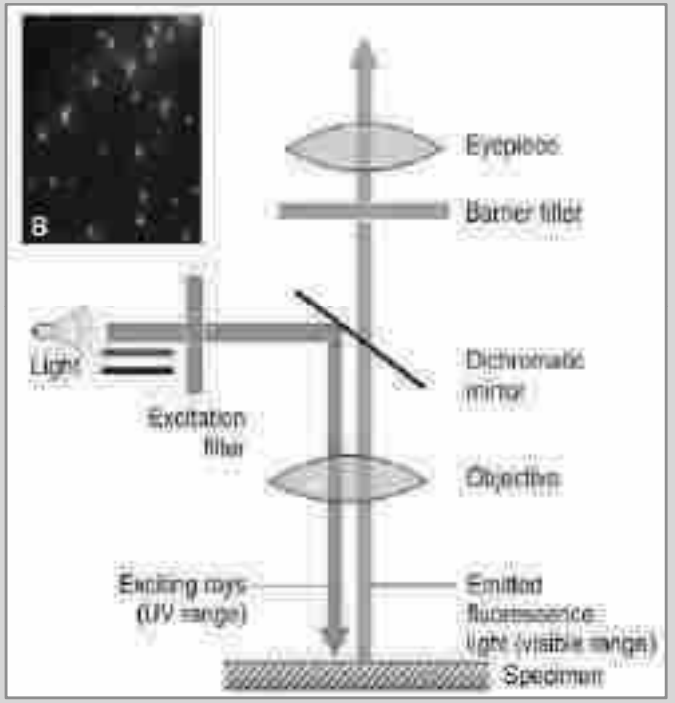
Mercury lamp emits rays that pass through an excitation filter

Working principle (Cont..)

Excitation filter allows only short wavelength UV light to pass through

Exciting rays get reflected by a dichromatic mirror - fall on the specimen stained by fluorescent dye.

Barrier filter – removes remaining UV light or blue and violet light



Applications

Epifluorescence microscope: Simplest form of fluorescence microscope. Has following applications:

- Auto fluorescence (eg. *Cyclospora)*
- Microbes coated with fluorescent dye (eg. Acridine orange dye)
- Immunofluorescence

Applications (Cont..)

Confocal microscope: Advanced design, uses optical sectioning to get better resolution of the fluorescent image.

ELECTRON MICROSCOPE

ELECTRON MICROSCOPE

- Invented by German physicist - **Ernst Ruska** in 1931.
- **Source of illumination** - Accelerated electrons
- Wavelength of electrons 100,000 times shorter than visible light photons - better resolving power than a light microscope.

ELECTRON MICROSCOPE (Cont..)

It is of two types:

- Transmission electron microscope (TEM, most common type)
- Scanning electron microscope (SEM)

Differences between light microscope and electron microscope

Features	Light microscope	Electron microscope
Highest practical magnification	About 1,000–1,500	Over 100,000
Best resolution	0.2 μm	0.5 nm
Radiation source	Visible light	Electron beam
Medium of travel	Air	High vacuum
Specimen mount	Glass slide	Metal grid (usually copper)
Type of lens	Glass	Electromagnet

TEM – Specimen Preperation

- Very thin specimens (20–100 nm thickness) - suitable for EM.
- Steps to prepare thin specimen:

1. **Fixation:** Cells are fixed by using glutaraldehyde or osmium tetroxide for stabilization
2. **Dehydration:** Specimen is then dehydrated with acetone or ethanol.

Transmission Electron Microscope (Cont..)

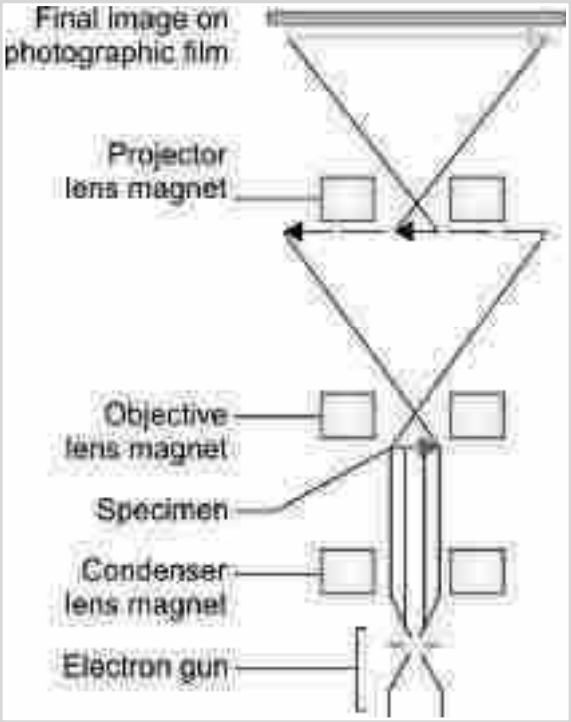
3. Embedding: Specimen is embedded in plastic polymer.

4. Slicing: Specimen is then cut into thin slices by an ultramicrotome knife.

Such thin slices of the specimen are mounted on a metal slide (copper).

Electron Pathway

- Electrons - generated by electron gun-travel in high speed.
- Medium of travel - fully vacuum path - because in air path - electrons get deflected by collisions with air molecules.



Applications and Modifications of EM

- **Virus detection**
- The **contrast of EM** - increased by —

  (i) Negative staining with heavy metals (phosphotungstic acid)

  (ii) Shadowing.
- **Freeze-etching technique** - alternative method for specimen preparation.

Scanning Electron Microscope

- Examine the **surfaces of microorganisms** in detail.
- Resolution of 7 nm or less.
- Produces image from electrons emitted by object’s surface.

ATOMIC FORCE MICROSCOPY

ATOMIC FORCE MICROSCOPY

- Advanced microscope - uses scanning probe technology to study the **cellular structure**.
- Has a resolution power in fraction of nanometer.

Questions:

Q1. Electrons are used as a source of illumination in?

a. Light microscope

b. Phase contrast microscope

c. Electron microscope

d. Fluorescence microscope

Questions (Cont..) :

Q2. Scanning probe technology is used in ?

a. Light microscope

b. Phase contrast microscope

c. Electron microscope

d. Atomic force microscope

Questions (Cont..) :

Q3. Microscope used for studying microbial motility?

a. Light microscope

b. Phase contrast microscope

c. Electron microscope

d. Atomic force microscope

Questions (Cont..) :

Q4.Resolution power of microscope is improved by using?

a. Oil

b. Stain

c. Lenses

d. Condenser

GENERAL BACTERIOLOGY: LABORATORY DIAGNOSIS OF BACTERIAL INFECTIONS

Learning objectives

At the end of the session, the students will be able to understand:

- Specimen Collection
- Direct Detection, Staining Techniques
- Culture, Identification and AST
- Serology
- Molecular Methods

LABORATORY DIAGNOSIS OF BACTERIAL INFECTIONS

Useful for the following purposes:

- Identification
- Treatment
- Surveillance purpose
- For outbreak investigation
- To start PEP (post-exposure prophylaxis)
- To initiate appropriate infection control measures

LABORATORY DIAGNOSIS OF BACTERIAL INFECTIONS (Cont..)

Comprises of several steps

- Specimen collection
- Direct detection
- Culture
- Identification and Antimicrobial susceptibility test
- Serology
- Molecular methods

SPECIMEN COLLECTION

Types of infections and various specimens collected

Type of infections	Specimens collected
Blood stream infection, sepsis, endocarditis	Paired blood culture specimens • Collected aseptically by two-step disinfection of skin; first with alcohol followed by chlorhexidine • 8-10mL of blood (for adults) collected in blood culture bottles
Infectious diseases requiring serology	• Blood (2 mL/investigation) • Collected by minimal asepsis (one-step skin disinfection with alcohol) • Collected in vacutainer
Diarrheal diseases	Stool (mucus flakes), rectal swab
Meningitis	Cerebrospinal fluid (CSF)

Types of infections and various specimens collected (Cont..)

Type of infections	Specimens collected
Infections of other sterile body area	Sterile body fluids; e.g. pleural fluid, synovial fluid, peritoneal fluid
Skin and soft tissue infection	Pus or exudate, wound swabs, aspirates from abscess and tissue bits
Anaerobic culture	Aspirates, tissue specimens, blood and sterile body fluids, bone marrow (swabs, sputum not satisfactory)
Upper respiratory tract infection	Throat swab with membrane over the tonsil, nasopharyngeal swab, per-nasal swab
Lower respiratory tract infection	Sputum, endotracheal aspirate, bronchoalveolar lavage (BAL), protected specimen brush (PSB) and lung biopsy
Pulmonary tuberculosis	• Sputum- early morning and spot • Collected in well-ventilated area • Gastric aspirate for infants

Types of infections and various specimens collected (Cont..)

Type of infections	Specimens collected
Urinary tract infections	Midstream urine Suprapubic aspirated urine Catheterized patient: collected from the catheter tube, not from urobag
Genital specimens	Urethral swab, cervical swab- for urethritis Exudate from genital ulcers
Eye infections	Conjunctival swabs Corneal scrapings Aqueous or vitreous fluid
Ear infections	Swabs from outer ear Aspirate from inner ear

General Principles

Following principles should be followed while collecting the specimen:

- **Standard precautions**
- **Before antibiotics start**
- **Contamination** with indigenous flora should be avoided
- **Swabs** - convenient but considered inferior to tissue, aspirate and body fluids

General Principles (Cont..)

- **Container:** sterile, tightly sealed, leak proof, wide-mouth
- **Labeling:** All specimens - labelled with name, age, gender, treating physician, diagnosis, etc.
- **Rejection:** Specimens contaminated or improperly labelled may be rejected
- **Anaerobic culture** - proper anaerobic collection containers with media should be used.

Specimen Transport

Most specimens - transport time should not exceed **two hours**.

However, there are some exceptions.

- **Immediate transport** (<15 minutes) - CSF and body fluids, ocular specimens, tissue specimens, suprapubic aspirate and bone specimen.
- **Urine (midstream)** - added with preservative (boric acid) - transported within 2 hours.

Specimen Transport (Cont..)

- **Stool culture -** transported within 1 hour.
- **Rectal swabs** - up to 24 hours is acceptable.
- **For anaerobic culture** - Robertson's cooked meat broth or any specialized anaerobic transport system.

Specimen Storage before Processing

Most specimens - stored **at room temperature - up to 24 hours**.

There are some exceptions:

- **Blood cultures -** incubated at 37°C immediately up on receipt.
- **Sterile body fluids** - immediately plated up on receipt - incubated at 37°C.
- **Corneal scrapping** - plated at bed-side.

Specimen Storage before Processing (Cont..)

- **Stool culture** - stored up to 72 hours at 4°C
- **Urine (mid-stream and from catheter), lower respiratory tract specimen, gastric biopsy (for *Helicobacter pylori*)** - stored up to 24 hours

DIRECT DETECTION

STAINING TECHNIQUES

- Structural details of bacteria cannot be seen under light microscope due to lack of contrast.
- It is necessary to use staining methods to produce color contrast and thereby increase the visibility.
- Before staining - smears are fixed - so they will not be displaced during the staining process.

STAINING TECHNIQUES (Cont..)

- Fixation - protects the internal structures of cells.
- Done by two methods.

    1. **Heat fixation:** Gentle flame heating an air-dried film of bacteria.

    2. **Methanol fixation**—used for blood smears.

Common staining techniques

- **Simple stain:** Basic dyes (methylene blue or basic fuchsin) are used to provide the color contrast, but imparts the same color to all the bacteria in a smear.
- **Negative staining:** A drop of bacterial suspension is mixed with dyes (India ink or nigrosin). The background gets stained black where as unstained bacterial/yeast capsule stand out in contrast.

Common staining techniques (Cont..)

- **Impregnation methods:** Bacterial cells and structures are thickened by impregnation of silver salts on their surface to make them visible, e.g. for demonstration of bacterial flagella and spirochetes.

Common staining techniques (Cont..)

- **Differential stain:** Two stains are used - impart different colors to different bacteria. Most commonly used differential stains are:
    1. Gram stain: Differentiates bacteria into gram-positive and gram-negative groups.
    2. Acid-fast stain: Differentiates bacteria into acid fast and non acid-fast groups.
    3. Albert stain: Differentiates bacteria having metachromatic granules from other bacteria that do not have them.

Gram Stain

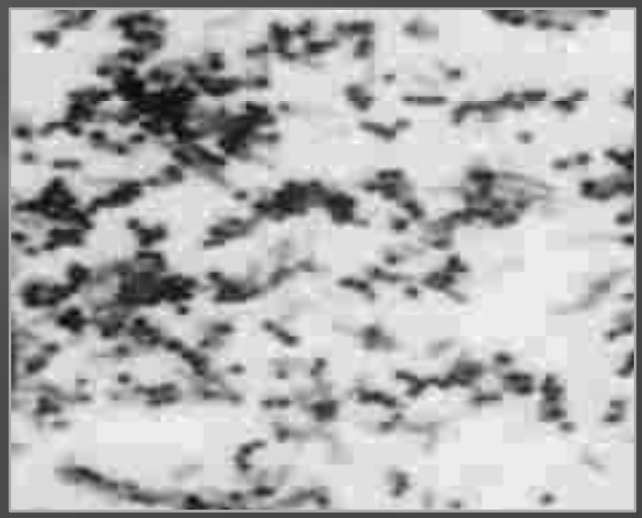
- Originally developed by Hans Christian Gram (1884).
- Gram stain still remains the most widely used test in diagnostic bacteriology.

Principle and procedure of Gram staining

Interpretation of Gram Stain

- Gram-positive bacteria resist decolorization and retain the color of primary stain i.e. violet.
- Gram-negative bacteria are decolorized and, therefore, take counterstain and appear pink.

Gram staining demonstrating violet-colored gram-positive cocci in clusters and pink colored gram-negative bacilli in scattered arrangement



Principle of Gram Staining

pH theory:

- Cytoplasm of gram-positive bacteria - more acidic -can retain the basic dye (e.g. crystal violet) for longer time.
- **Iodine** serves as mordant - combines with the primary stain to form a dye-iodine complex - gets retained inside the cell

Principle of Gram Staining (Cont..)

Cell wall theory:

- Gram-positive cell wall - thick peptidoglycan layer (50–100 layers thick).
- Acts as permeability barrier preventing loss of crystal violet.
- Alcohol is thought to shrink the pores of the thick peptidoglycan.

Principle of Gram Staining (Cont..)

Cell wall theory *(Cont..)*:

- Gram-negative cell wall - more permeable thus allowing the out flow of crystal violet easily.
- Thin peptidoglycan layer in gram-negative cell wall - is not tightly cross linked
- Presence of lipopolysaccharide layer in the cell wall of gram-negative bacteria - gets disrupted easily by the decolorizer

Modifications of Gram Staining

Modifications	Explanation
Kopeloff and Beerman's	Primary stain - methyl violet Counter stain - basic fuchsin
Jensen's	Absolute alcohol as decolorizer and neutral red as counter stain. Useful for meningococci and gonococci
Brown and Brenn	Used for Actinomycetes.

Uses of Gram Stain

- To differentiate bacteria into gram-positive and gram negative
- Identification of Gram staining from bacterial culture helps in further identification of bacteria
- Initiation of empirical treatment with broad spectrum antibiotics - started early before the culture report is available.
- Early presumptive identification of fastidious organisms - *Haemophilus.*

Uses of Gram Stain

- Gram stain gives a preliminary clue in anaerobic culture (*Clostridium)*
- Gram stain is useful for staining certain fungi such as *Candida* and *Cryptococcus* (appear gram-positive)
- Helps in screening the quality of the sputum specimen before processing it for culture.

Acid-fast Stain

- Discovered by Paul Ehrlich and subsequently modified by Ziehl and Neelsen.
- Acid fastness - due to presence of mycolic acid in the cell wall.

Ziehl-Neelsen Technique (Hot Method)

Smear preparation and heat fixation:

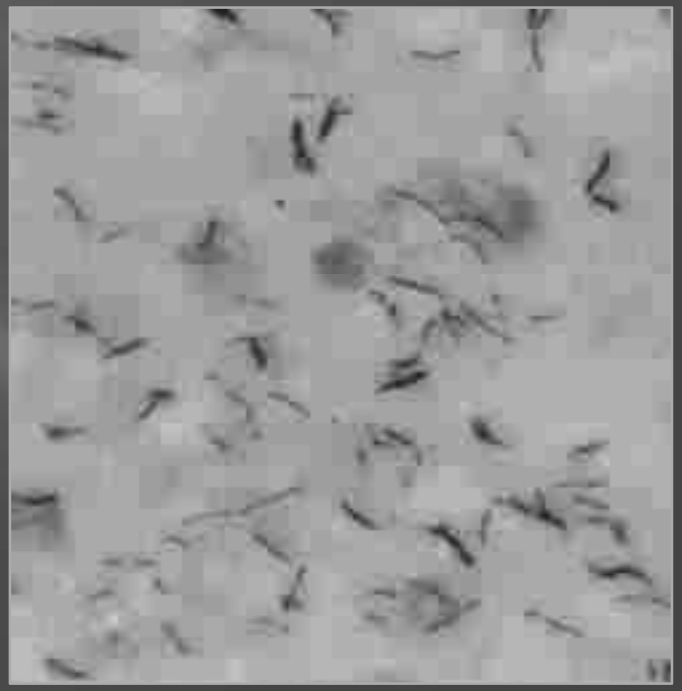
- Smear = 2 × 3 cm in size from the yellow purulent portion of the sputum.
- Smear - neither be too thick nor too thin - When placed over a printed matter, the print should be readable through the smear.
- The smear is air dried for 15–30 minutes and then heat fixed by passing over the flame 3–5 times for 3–4 seconds.

Procedure of Ziehl-neelsen technique (Hot Method)

- **Step 1 (primary stain) -** Carbol fuchsin (1%) for 5 minutes. Intermittent heating is done by flaming until the vapours rise.
- **Step 2 (decolorization) -** 25% sulfuric acid for 2–4 minutes.
- **Step 3 (counter staining) -** 0.1% methylene blue slide for 30 seconds.

Interpretation

- *Mycobacterium tuberculosis* *appears as long slender,* straight or slightly curved and beaded, red colored acid fast bacillus.



Modifications of Acid-Fast Staining

- Cold method (Kinyoun's method): Intermittent heating is not required.
- Acid-alcohol can be used as decolorizer alternatively.
- Malachite green can be used as counter stain.
- Concentration of sulfuric acid - vary depending on the acid-fastness of the structure to be demonstrated.

Acid-fast organisms/structures and percentage of sulfuric acid suitable for staining

Acid fast organisms /structures	Sulphuric acid (%) needed for decolorization
Mycobacterium tuberculosis	25%
Mycobacterium leprae	5%
Nocardia	1%
Acid fast parasites such as *Cryptosporidium , Cyclospora, Cystoisosopra, Microsporidia*	1%
Bacterial spore	0.25-0.5%

Albert Stain

- Used to demonstrate - metachromatic granules of *Corynebacterium diphtheriae*.

Procedure

- Heat fixation → Smear is covered with Albert I (Albert's stain) for 5 minutes → drain the solution → Albert II (iodine solution) is added for 1 minute → Slide is washed with water

Composition

Composition of Albert stain includes:

- Albert I: Comprises of toluidine blue , malachite green, glacial acetic acid , alcohol (95% ethanol), and distilled water
- Albert II: Contains iodine in potassium iodide

Interpretation

- *Corynebacterium diphtheriae* appears as green colored bacilli arranged in Chinese letter or cuneiform pattern, with bluish black metachromatic granules at polar ends.
- Differentiated from diphtheroids- do not show granules and are arranged in palisade pattern.

OTHER METHODS OF DIRECT DETECTION

Antigen Detection

Various immunological methods such as latex agglutination test, immunochromatographic test are available which detect antigens in clinical specimens.

Examples:

- Detection of capsular antigen of pneumococci, meningococci, *H. influenzae* in CSF specimen, etc.

Molecular Diagnosis

- Bacterial DNA or RNA can be directly detected in the clinical specimens by various molecular methods such as polymerase chain reaction (PCR).

CULTURE, IDENTIFICATION AND AST

CULTURE MEDIA

- Liquid or solid substance - contains nutrients to support the growth, and survival of microorganisms.

Constituents of Culture Media

Constituent	Explanation
Water and Electrolytes	Sodium chloride
Peptone	Mixture of partially digested proteins - obtained from various sources - heart muscle, casein or fibrin, or soya.
Agar	Used for solidifying the culture media ***Source:*** Prepared from - cell wall of seaweeds and available commercially in powder form ***Preparation****:* Agar powder is dissolved in water and subjected to sterilization by autoclave. When the temperature of the molten agar comes down to 45°C, it is poured into the Petri dishes and then allowed to set for 20 minutes. ***Concentration****:* Solid agar preparation - 1-2% Semisolid agar- 0.5% Solid agar to inhibit Proteus swarming- 6%

Constituents of Culture Media (Cont..)

Constituent	Explanation
Meat extract	Highly concentrated meat stock, usually made from beef.
Yeast extract	(Prepared from Baker's yeast) and **malt extract** (contains maltose)
Blood and serum	Important components of enriched media; provide extra nutrition to fastidious bacteria. 5–10% of sheep blood is used. Alternatively, horse, ox or human blood can also be used.

Types of Culture Media

Based on consistency, culture media are grouped into:

- Liquid (or broth) media
- semisolid media
- solid media

Types of Culture Media

Based on the growth detection, culture media are classified as:

Conventional culture media:

Prepared from nutrients - aqueous extract of meat, peptone.

- Simple/basal media
- Enriched media
- Enrichment broth
- Selective media
- Differential media
- Transport media
- Anaerobic media.

Automated culture media:

Mainly available for blood and sterile body fluid culture.

Simple/Basal Media

Contain minimum ingredients that support the growth of non-fastidious bacteria.

- **Peptone water:** Contains peptone (1%) + NaCl (0.5%) + water
- **Nutrient broth:** Peptone water + meat extract (1%).
- **Nutrient agar:** Nutrient broth + 2% agar
- **Semisolid medium:** Concentration of agar - reduced to 0.2–0.5 %.

Uses of Basal Media

- Testing the non-fastidiousness of bacteria.
- Serve as base for the preparation of many other media.
- Nutrient broth - used for studying the bacterial growth curve.
- Nutrient agar - preferred medium for:
  - Performing the biochemical tests, such as oxidase, catalase, etc.
  - Study the colony morphology
  - Pigment demonstration.

Enriched Media

- Basal medium added with additional nutrients - blood, serum or egg
- In addition to non-fastidious organisms, support the growth of fastidious nutritionally exacting bacteria.

Enriched Media (Cont..)

Blood agar –

- Prepared by adding 5-10% of sheep blood to the molten nutrient agar at 45^0C.
- Tests the hemolytic property of the bacteria, which may be either- i) partial or α (green) hemolysis and ii) complete or β hemolysis

Enriched Media (Cont..)

Chocolate agar:

- Heated blood agar, prepared by adding 5 -10% of sheep blood to the molten nutrient agar at 70°C.
- More nutritious than blood agar - supports certain highly fastidious bacteria - *Haemophilus influenzae* that does not

Enriched Media (Cont..)

Loeffler's serum slope: Contains serum - used for isolation of *Corynebacterium diphtheriae*

Blood culture media: Used for isolating microorganisms from blood - available as conventional or automated blood culture media.

Enrichment Broth

- Liquid media added with inhibitory agents which selectively allow certain organism to grow and inhibit others.
- Important for isolation of pathogens from clinical specimens which also contain normal flora (e.g. stool and sputum specimen).

Enrichment Broth (Cont..)

Examples :

- Tetrathionate broth—Used for *Salmonella* Typhi
- Gram-negative broth—Used for *Shigella*
- Selenite F broth—Used for *Shigella*
- Alkaline peptone water (APW)—Used for *Vibrio cholerae*.

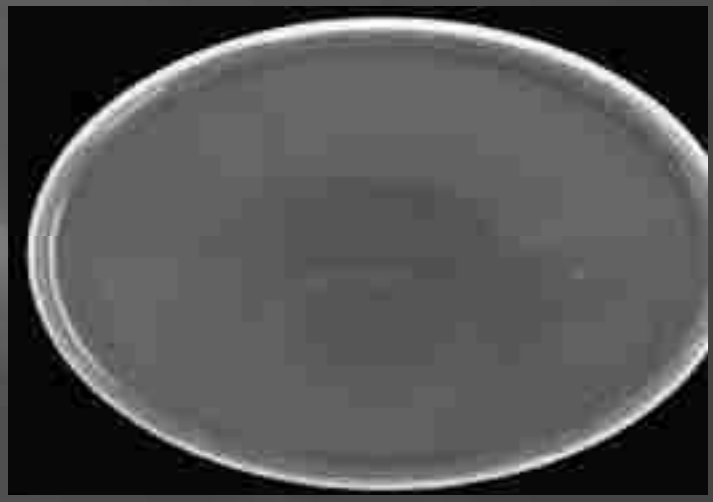
Selective Media

Solid media containing inhibitory substances - inhibit the normal flora present in the specimen and allow the pathogens to grow.

Media	Used for isolation of
Lowenstein Jensen (LJ) medium	*Mycobacterium tuberculosis*
Thiosulphate Citrate Bile salt Sucrose (TCBS)	*Vibrio* species
DCA (Deoxycholate Citrate Agar)	*Salmonella* and *Shigella* from stool
XLD (Xylose Lysine Deoxycholate) agar	*Salmonella* and *Shigella* from stool
Potassium tellurite agar (PTA)	*Corynebacterium diphtheriae*

Selective Media (Cont..)

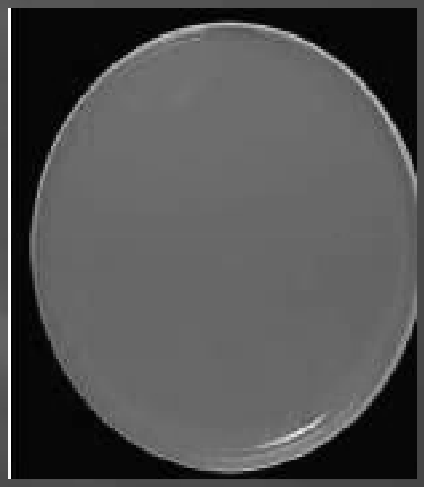
Lowenstein–Jensen medium



TCBS agar

Selective Media (Cont..)

DCA (Deoxycholate Citrate Agar)



XLD (Xylose Lysine Deoxycholate) agar

Transport Media

- Used for the transport of the clinical specimens suspected to contain delicate organism or when delay is expected while transporting the specimens from the site of collection to the laboratory
- Bacteria do not multiply in the transport media - they only remain viable.

Transport Media (Cont..)

Organism	Transport media
Neisseria	Amies medium, Stuart's medium
Vibrio cholerae	VR (Venkatraman-Ramakrishnan) medium Autoclaved sea water Cary Blair medium
Shigella, Salmonella	Buffered glycerol saline Cary Blair medium

Differential Media

- Differentiate between two groups of bacteria - by using an indicator.

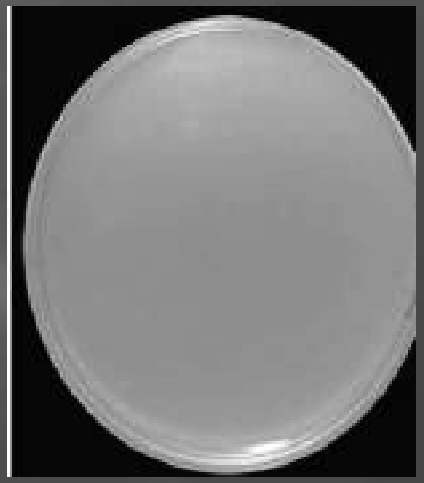
Differential media	Features
MacConkey agar	➢ Differential and low selective medium - used for the isolation of enteric GNB. ➢ Differentiates organisms into LF (pink colonies, e.g. *Escherichia coli*) and NLF or (colorless colonies, e.g. *Shigella*). ➢ Composition- Peptone, lactose, agar, neutral red (indicator) and taurocholate

Differential Media (Cont..)

Differential media	Features
CLED agar (cysteine lactose electrolyte-deficient agar)	➢ Capable of differentiating between LF and NLF. ➢ Used as an alternative to combination of blood agar and MacConkey agar, for the processing of urine specimens

Differential Media (Cont..)

MacConkey agar



CLED agar

Anaerobic Culture Media

- Contain reducing substances which take up oxygen and create lower redox potential - permit the growth of obligate anaerobes, such as *Clostridium*

Media	Features
Robertson's cooked meat (RCM) broth	➢ Contains chopped meat particles (beef heart), which provide glutathione (a sulfhydryl group containing reducing substance) and unsaturated fatty acids. ➢ Widely used ➢ Used for maintenance of stock cultures.

Anaerobic Culture Media (Cont..)

Other anaerobic media include:

- Thioglycollate broth
- Anaerobic blood agar
- BHIS agar (Brain-heart infusion agar) with supplements (vitamin K and hemin)
- Neomycin blood agar
- Egg yolk agar
- Phenyl ethyl agar
- *Bacteroides* bile esculin agar (BBE agar).

Blood Culture Media

- Recovery of bacteria from blood is difficult - they are usually present in lesser quantity and many of the blood pathogens are fastidious.
- Therefore, enriched media - used for isolating microorganisms from blood.
- Available either as conventional or automated media

Conventional Blood Culture Media

Two types:

- Monophasic medium: Contains brain–heart infusion (BHI) broth.
- Biphasic medium: Liquid phase containing BHI broth and a solid agar slope made up of BHI agar

Disadvantages of conventional media

Subcultures are made manually - performed less-frequently (once a day) as it is cumbersome.

Broth	Disadvantages
Monophasic BHI broth	➢ Subcultures are made, periodically for 1 week. ➢ Higher risk of contamination - due to opening of the cap of the bottle every time when subcultures are made.
Biphasic BHI broth	➢ Subcultures - made just by tilting the bottles so that the broth runs over the agar slope. ➢ Lower risk of contamination as it obviates the opening of the cap of the bottle

Automated Blood Culture Techniques

- **Continuous automated monitoring:** Blood culture bottles - periodically monitored for the microbial growth - every 10 minutes by the instrument.
- **Composition:** Tryptic soy broth and/or brain heart infusion broth + polymeric resin beads - adsorb and neutralize the antimicrobials present in blood specimen.

Automated Blood Culture Techniques (Cont..)

- **Specimens:** Used for culture of blood, bone marrow and sterile body fluids.
- **More sensitive:** Higher yield of positive cultures from clinical specimens
- **Rapid:** Less time than conventional methods
- **Less labor intensive:** fully-automated

Automated Systems

BacT/ALERT 3D:

- Principle - colorimetric detection of growth.
- When bacteria multiply - produce CO2 - increases the pH - changes the color of a blue-green sensor present at the bottom of the bottle to yellow

Automated Systems (Cont..)

BacT/ALERT VIRTUO (bioMerieux): Advanced form of BacT/ALERT – has several advantages, such as:

- Automatic loading and unloading of bottles,
- Faster detection of growth,
- Determine volume of blood present in bottle

Automated Systems (Cont..)

BACTEC (BD Diagnostics)

- Principle - fluorometric detection of growth.
- Uses an oxygen-sensitive fluorescent dye present in the medium.

Disadvantages of automated systems

- High cost of the instrument and culture bottles,
- Inability to observe the colony morphology as liquid medium is used.

CULTURE METHODS

CULTURE METHODS

- Involve inoculating the specimen on to appropriate culture media, followed by incubating the culture plates in appropriate conditions.

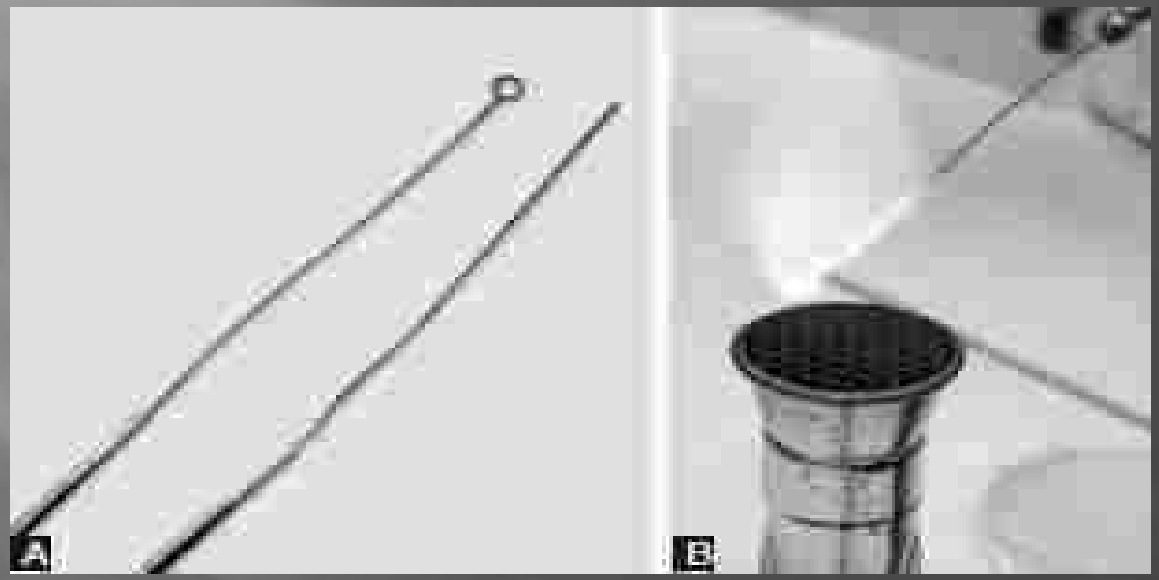
Selection of Media

Specimens	Recommended culture media
Pus, wound swab, aspirates, and tissue bits	Blood agar + MacConkey agar
Sterile body fluids	Blood agar, plus MacConkey agar, plus chocolate agar OR Automated blood culture bottles
Blood	Blood culture bottles (Conventional or automated)
Urine	Blood agar plus MacConkey agar CLED agar can be used alternatively
Stool	Selenite-F broth plus MacConkey agar plus DCA and/or XLD agar (if cholera is suspected- add TCBS agar)
Respiratory specimens	Blood agar, plus MacConkey agar, plus chocolate agar

Inoculation of the Specimens

- Carried out by bacteriological loops made up of platinum or nichrome wire.
- Inoculating loop - heated in the Bunsen flame - then made cool waiting for 10 seconds.
- Carried out in biological safety cabinet and wearing appropriate personal protective equipment.

Inoculation of the Specimens *(Cont..)*



A. Bacteriological loop and straight wire **B.** Flaming the loop (red hot)

Biosafety Cabinet (BSC)

- Enclosed, ventilated laboratory workstation - protect the laboratory personnel while working.
- Air is blown into the cabinet away from the worker and exhausted outside through a duct lined with HEPA filters.

Inoculation Methods

Two types:

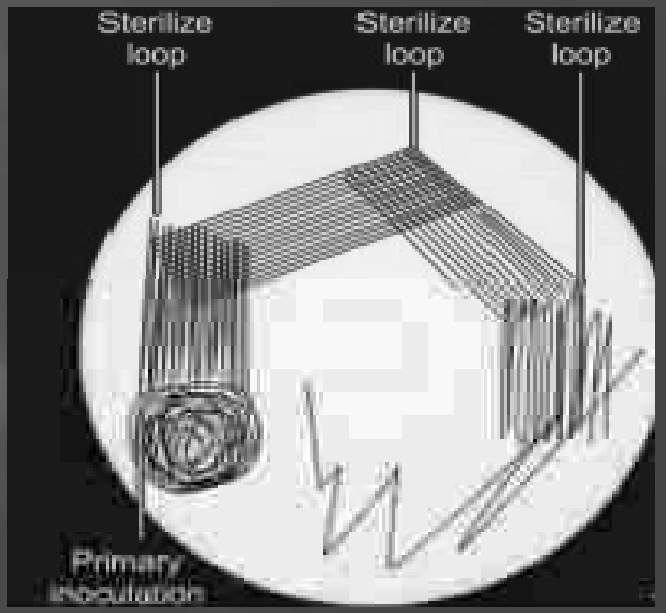
- Methods used for inoculating clinical specimens on to the culture media
- Methods used for inoculating colonies on to various media for further processing.

Streak Culture

- Most common inoculation method
- Used for - inoculation of the specimens on to the solid media.
- Used for obtaining individual isolated colonies from a mixed culture of bacteria.

Streak Culture (Cont..)

Streaking: Loopful of specimen - smeared onto solid media to form round-shaped primary inoculum - spread over the culture plate by streaking parallel lines to form the secondary, tertiary inoculum and finally a feathery tail end



Streak Culture (Cont..)

Intermittent heating: Loop is flamed and cooled in between the different set of streaks to get isolated colonies on the final streaks.

Liquid Culture

- Used for culture of specimens - blood or body fluids - inoculated by directly adding the specimen in to the liquid medium or with the help of a syringe or pipette.

Liquid Culture (Cont..)

Bacterial growth - detected by observing turbidity in the medium. Some aerobic bacteria form surface pellicles.

Liquid Culture (Cont..)

Uses:

- Blood, or body fluids culture,
- Automated culture for mycobacteria
- Water analysis

Liquid Culture (Cont..)

Advantages: Preferable for culture of:

- Specimens containing small quantity of bacteria
- Specimens containing antibiotics and other antibacterial substances - get neutralized by dilution in the medium
- Large yields of bacteria are required

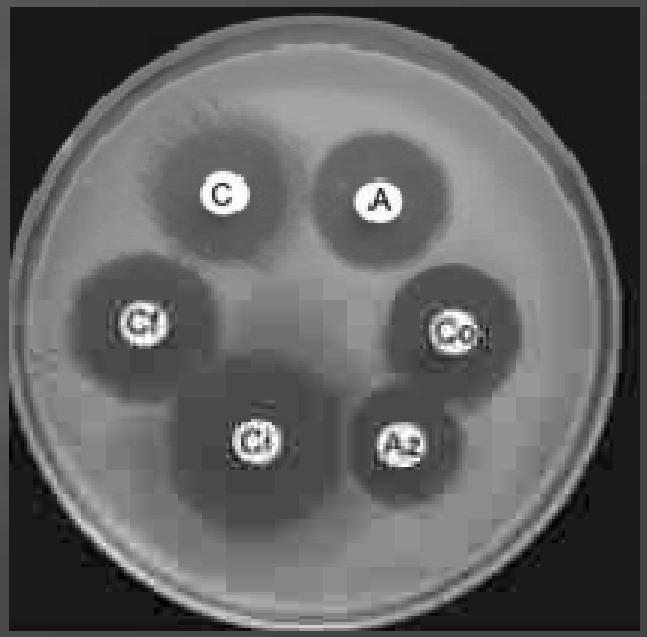
Liquid Culture (Cont..)

Disadvantages:

- Do not provide a pure culture from a mixed inoculum
- No visible colonies - does not given

Lawn or Carpet Culture

- Useful to carry out antimicrobial susceptibility testing (AST) by disk diffusion method.
- Uniform lawn of bacterial growth is obtained - by either swabbing or flooding with a bacterial broth onto the culture plate.



Pour Plate Technique

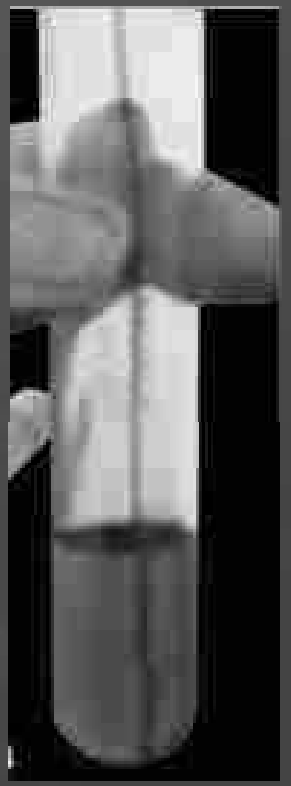
- Seldom used for quantifying the bacterial load present in the specimens - urine or blood.
- Serial dilutions of the specimen - added on to the molten agar.
- After being cooled and solidified - Petri dishes are incubated - colony count is estimated.

Stroke Culture

- Carried out on agar slopes or slants by streaking the straight wire in a zigzag fashion.
- Used for biochemical test such as urease test.

Stab Culture

- Made by stabbing the semisolid agar butt by a straight wire. It is
- Used for motility testing using mannitol motility medium.



Incubatory Conditions

- Most pathogenic bacteria - aerobes or facultative anaerobes - grow best at 37°C.
- Inoculated culture plates - incubated at 37°C aerobically for overnight in an incubator.

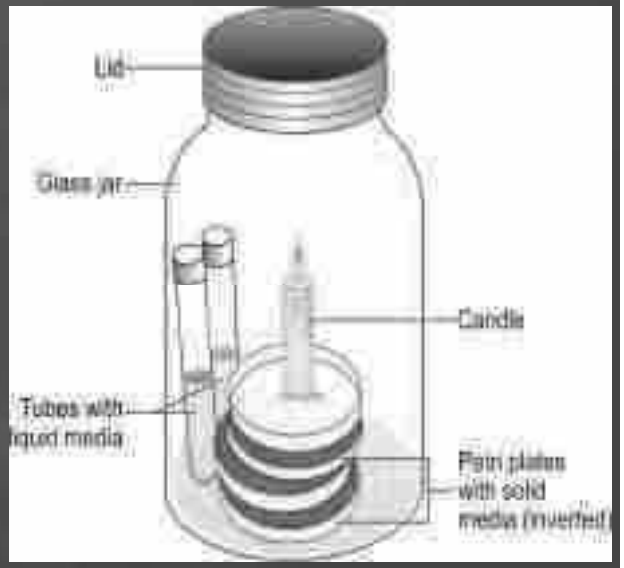
Bacteriological Incubator

- Device used to incubate culture plates, biochemical tests and AST plates.
- Incubator maintains optimal temperature.
- Some incubators are specially designed to maintain other conditions

Other Incubatory Conditions

For capnophilic bacteria: Candle jar is used

- Inoculated media - placed inside a jar with a lighted candle - burning candle reduces oxygen to a point where the flame goes off.
- Provides an atmosphere of approximately 3–5% CO_2 .
- Useful for capnophilic bacteria - *Brucella, Streptococcus*, pneumococcus and gonococcus.



Other Incubatory Conditions (Cont..)

For microaerophilic bacteria - *Campylobacter* and *Helicobacter* - require 5% oxygen for optimum growth

For obligate anaerobes - anaerobic culture methods are used

Anaerobic Culture Methods

- Obligate anaerobic bacteria - grow only in the absence of oxygen.
- Anaerobic culture methods includes:
  - *Evacuation and Replacement*
  - *Absorption of Oxygen by Chemical Methods*
  - *Anaerobic Glove box and Anaerobic Work Station*
  - *Reducing Agents*
  - *Pre-reduced Anaerobically Sterilized (PRAS)*

Evacuation and Replacement

- Involves evacuation of the air from jar and replacement with inert gas like hydrogen followed by removal of the residual oxygen by use of a catalyst. It is
- Carried out either by:
  - **Manual method** – using McIntosh and Filde's anaerobic
  - **Automated system (Anoxomat)**

Evacuation and Replacement (Cont..)

- **Manual method-** McIntosh and Filde's anaerobic jar - most popular method for creating anaerobiosis in the past, now not in use.

Evacuation and Replacement (Cont..)

- **Automated system (Anoxomat)**: Automatically evacuates air - replaces by hydrogen gas from a cylinder.
- Catalyst - sachet containing aluminum pellets coated with palladium.

Absorption of Oxygen by Chemical Methods

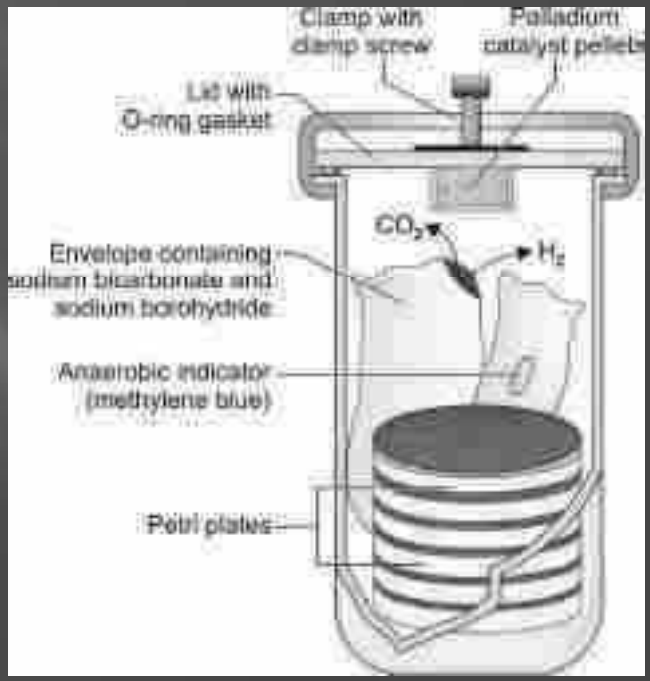
GasPak system (BD diagnostics):

- Principle - oxygen is removed by chemical reactions.
- Uses - sachet containing sodium bicarbonate and sodium borohydride - react chemically in presence of water - produce hydrogen and CO_2 gas
- Traces of oxygen - removed by same catalyst used for Anoxomat.

Absorption of Oxygen by Chemical Methods (Cont..)

GasPak system (BD diagnostics) (Cont..):

- Traces of oxygen - removed by same catalyst used for Anoxomat.



Absorption of Oxygen by Chemical Methods (Cont..)

GasPak system (BD diagnostics) (Cont..):

- **Indicator of anaerobiosis:**
  - **Chemical indicator:** Reduced methylene blue - remains colorless in anaerobic conditions - turns blue on exposure to oxygen.
  - **Biological indicator –** *Pseudomonas* - absence of growth indicates perfect anaerobiosis.

Absorption of Oxygen by Chemical Methods (Cont..)

GENbag (bioMérieux):

- Consists of an airtight transparent bag with a generator sachet - rapidly produces carbon dioxide and creates anaerobic environment.

Anaerobic Glove box and Anaerobic Work Station

- Provide facility for easy processing, incubation and examination of the specimens without exposure to oxygen

Reducing Agents

- Oxygen in culture media - reduced by various reducing agents - glucose, thioglycollate, cooked meat pieces, cysteine and ascorbic acid.
- **Robertson cooked meat broth** - most widely employed anaerobic culture
- medium - uses chopped meat particles (beef heart) as reducing agent.

Pre-reduced Anaerobically Sterilized (PRAS)

- Prepared entirely under oxygen-free conditions from initial sterilization to packaging in sealed foil packets.

Colony Morphology

- After overnight incubation - culture media are removed from the incubator - examined under bright illumination.
- Appearance of bacterial colony on culture medium - characteristic for many organisms - helps in their preliminary identification.

Colony Morphology (Cont..)

- Size - in millimetres e.g. pin head size is characteristic of staphylococcal colony & pin point size is characteristic of streptococcal colony.
- Shape - circular or irregular
- Consistency - dry, moist or mucoid
- Density - Opaque, translucent or transparent
- Hemolysis on blood agar

Colony Morphology (Cont..)

Color of the colony:

- Colored due to - certain properties of the media or organisms.
- Eg - Pink colonies produced by lactose fermenters on MacConkey agar and black colonies by *Corynebacterium diphtheriae* on potassium tellurite agar.
- May also be due to pigment production by the bacteria.

Colony Morphology (Cont..)

Pigment production: Bacteria may produce two types of pigments.

- **Diffusible pigments**
- **Non-diffusible pigments**

Colony Morphology (Cont..)

Hemolysis on Blood Agar

- Partial or α hemolysis
- Complete or β hemolysis
- No hemolysis (γ hemolysis, a misnom

Culture Smear and Motility Testing

- Colonies grown on the culture media - subjected to Gram staining and motility testing by hanging drop method.

CULTURE IDENTIFICATION

CULTURE IDENTIFICATION

Identification of bacteria from culture is made either by:

- Conventional biochemical tests or
- Automated identification systems.

Biochemical Identification

Based on the type of colony morphology, Gram- staining appearance - appropriate biochemical tests are employed.

- **Initially -** catalase and oxidase tests are done.

Biochemical Identification (Cont..)

- **For gram-negative bacilli -** Common biochemical tests done routinely – 'ICUT'
  - Indole test
  - Citrate utilization test
  - Urea hydrolysis test
  - Triple sugar iron test (TSI).

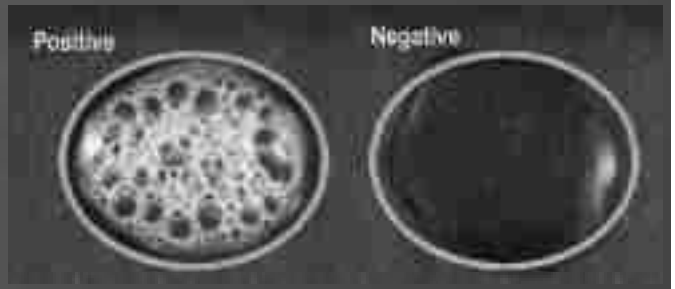
Biochemical Identification (Cont..)

- **For gram-positive cocci:**
  - Coagulase test (for *Staphylococcus aureus*)
  - CAMP (Christie-Atkins-Munch-Petersen) test - group B *Streptococcus.*
  - Bile esculin hydrolysis test (for *Enterococcus*)
  - Inulin fermentation (for pneumococcus)
  - Bile solubility test (for pneumococcus)

Biochemical Identification (Cont..)

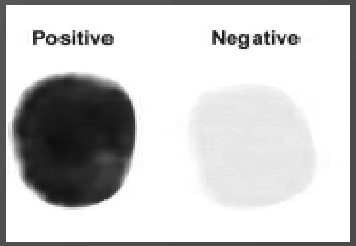
- Antimicrobial susceptibility tests - for bacterial identification:
  - Optochin susceptibility test - pneumococcus (sensitive)
  - Bacitracin susceptibility test - differentiate group A (sensitive) from group B (resistant) *Streptococcus*.

Catalase Test



- When a drop of hydrogen peroxide (3% H_2O_2) - added to a colony of any catalase producing bacteria - effervescence or bubbles appear due to breakdown of H_2O_2 by catalase to produce oxygen.
- Differentiate - *Staphylococcus* (positive) from *Streptococcus* (negative).

Oxidase Tests



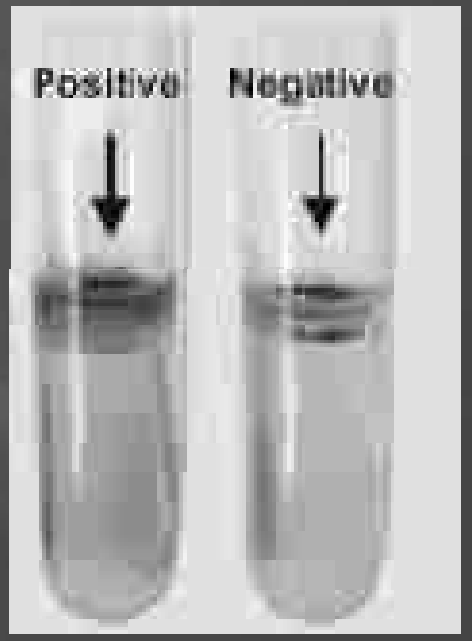
- Detects the presence of cytochrome oxidase enzyme in bacteria which catalyses the oxidation of reduced cytochrome by atmospheric oxygen.
- Oxidase positive (deep purple)- *Pseudomonas , Vibrio, Neisseria, Bacillus* etc.
- Oxidase negative (no colour change) - members of family Enterobacteriaceae, *Acinetobacter,* etc.

Indole Test

- Detects the ability of certain bacteria to produce enzyme tryptophanase - breaks down amino acid tryptophan present in the medium into indole.
- **Indole positive** - Red colored ring is formed near the surface of the broth. Eg: *Escherichia coli, Proteus vulgaris, Vibrio cholerae*, etc.

Indole Test

- **Indole negative** - Yellow colored ring is formed near the surface of the broth, e.g. *Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas, Salmonella,* etc.

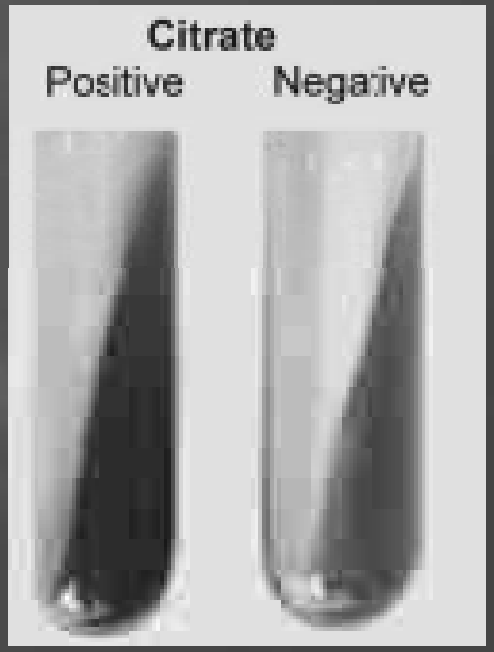


Citrate Utilization Test

- Detects the ability of a few bacteria to utilize citrate as the **sole source of carbon** for their growth, with production of alkaline metabolic products.
- Performed on **Simmon's citrate medium** - Citrate utilizing bacteria produce growth and a color change, i.e. original green color changes to blue

Citrate Utilization Test (Cont..)

- Citrate test - positive for *Klebsiella pneumoniae, Citrobacter, Enterobacter,* etc.
- Negative for *Escherichia coli, Shigella,* etc.

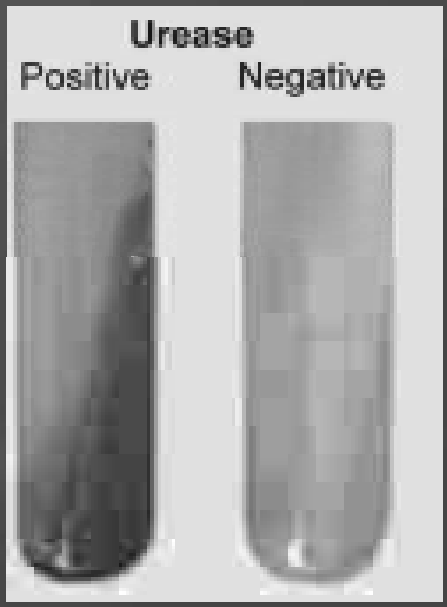


Urea Hydrolysis Test

- Urease producing bacteria - split urea present in the medium to produce ammonia - makes the medium alkaline.
- Test - done on **Christensen's urea medium** - contains phenol red indicator - changes to pink color in alkaline medium

Urea Hydrolysis Test (Cont..)

- **Urease test is positive for -** *Klebsiella pneumoniae, Proteus* species, *Helicobacter pylori, Brucella,* etc.
- **Urease test is negative for -** *Escherichia coli, Shigella, Salmonella,* etc.



Triple Sugar Iron (TSI) Agar Test

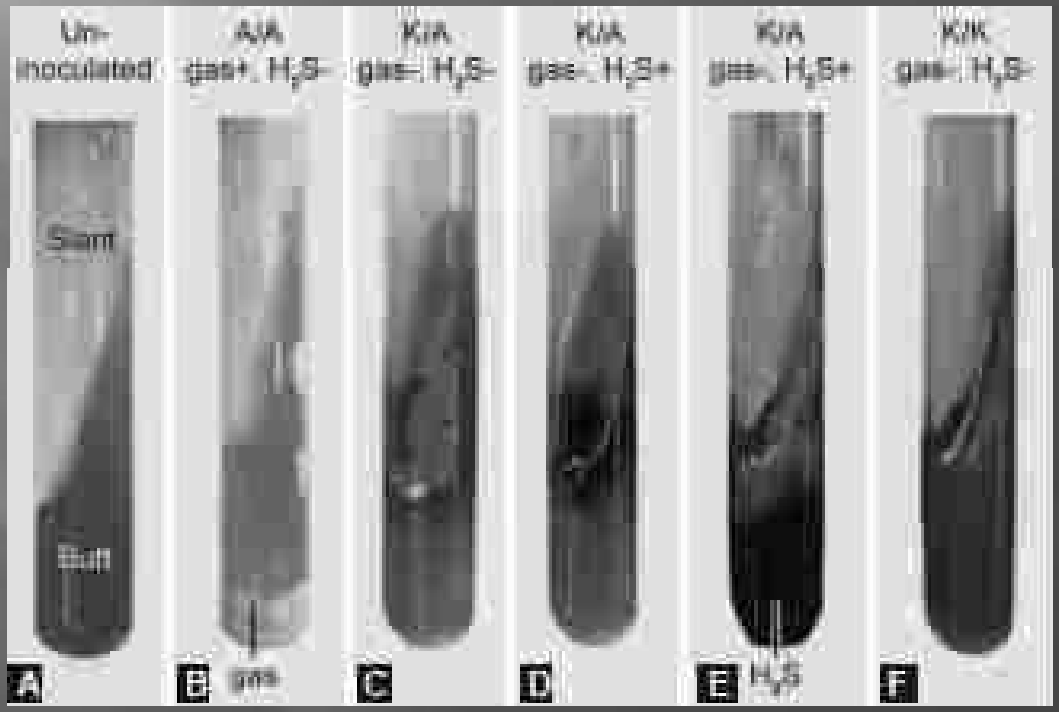
- Contains three sugars - glucose, sucrose and lactose in the ratio of 1:10:10 parts.
- Uninoculated TSI medium - red in color
- After inoculation - the medium is incubated at 37°C for 18–24 hours.

Triple Sugar Iron (TSI) Agar Test (Cont..)

Interpretation: detects three properties of bacteria, such as

- Fermentation of sugars to produce acid
- Fermentation of sugars to produce gas
- Production of H2 S.

Triple Sugar Iron (TSI) Agar Test (Cont..)



Triple Sugar Iron (TSI) Agar Test (Cont..)

Reactions in TSI	Examples
Acidic slant/acidic butt	**≥2 sugars fermented -** (1) glucose, (2) lactose or/and sucrose
A/A, gas produced, no H2S (Fig B)	*Escherichia coli, Klebsiella pneumoniae*
Alkaline slant/acidic butt	**Only glucose-fermenter group**
K/A, no gas, no H2S (Fig C)	*Shigella*
K/A, no gas, H2S produced (small amount) (Fig D)	*Salmonella* Typhi
K/A, no gas, H2S produced (abundant) (Fig E)	*Proteus vulgaris*
K/A, gas produced, H2S produced (abundant)	*Salmonella* Paratyphi B
K/A, gas produced, no H2S	*Salmonella* Paratyphi A
Alkaline slant/alkaline butt	**Non-fermenters group**
K/K, no gas, no H2S (Fig F)	*Pseudomonas, Acinetobacter*

Automated Systems for Bacterial Identification

Advantages

- Produce faster result
- Identify wide range of organisms with accuracy, which are otherwise difficult to identify (e.g. anaerobes) through conventional biochemical tests.

Automated Systems for Bacterial Identification (Cont..)

- MALDI–TOF (Matrix-assisted laser desorption/ionization time-of-flight)
- VITEK 2 (bioMérieux) - automated identification and antimicrobial susceptibility test.
- Phoenix (BD Diagnostics) - automated identification and antimicrobial susceptibility test
- MicroScanWalkAway system (Beckman Coulter) - automated identification and antimicrobial susceptibility test.

MALDI-TOF

- Can identify bacteria, fungi, and mycobacteria with a turnaround time of few minutes and with absolute accuracy.
- Two systems are commercially available: VITEK MS (bioMérieux) and Biotyper system (Bruker).

MALDI-TOF (Cont..)

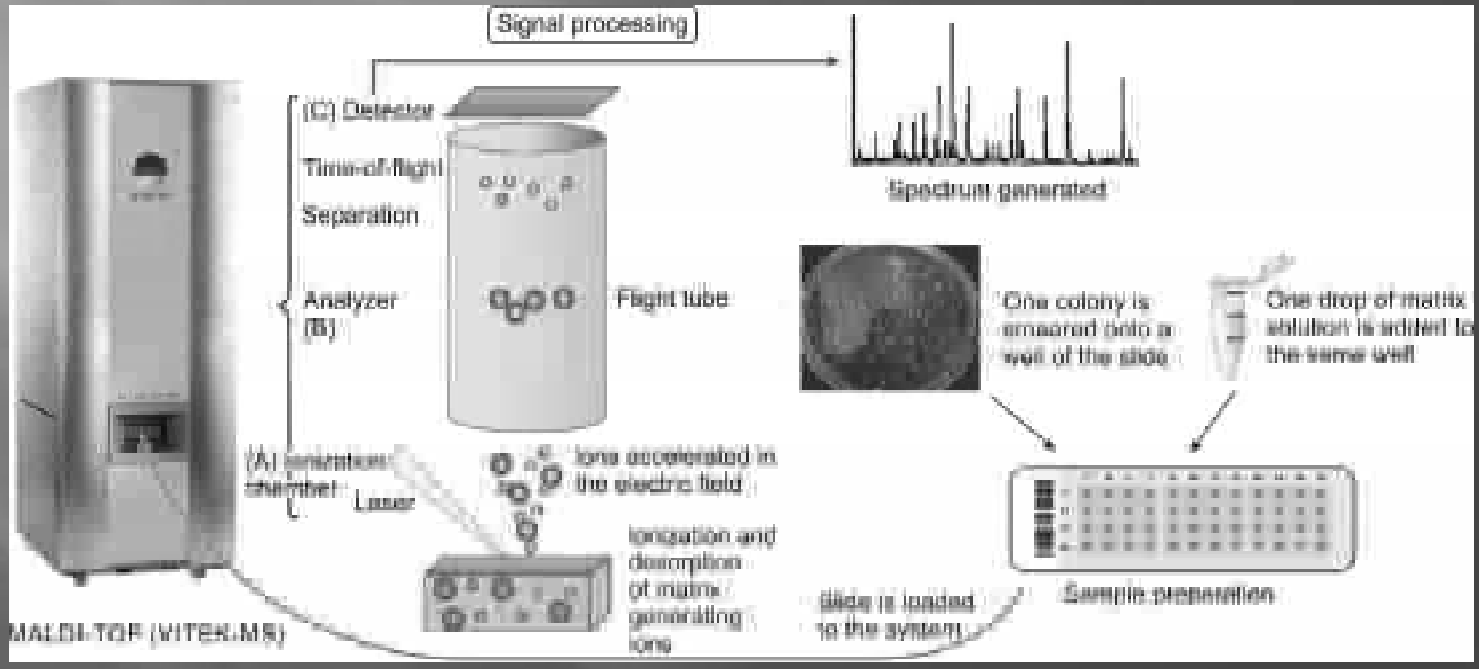
Principle

- Examines the pattern of ribosomal proteins present in the organism.
- **Sample preparation:** The colony of an organism - smeared onto a well of the slide and one drop of matrix solution (composed of cyano-hydroxy-cinnamic acid) - added to the same well and mixed - slide is loaded in the system.

MALDI-TOF (Cont..)

- **Steps after loading:** three steps occurring in three chambers of the system.
  - 1. Ionization chamber
  - 2. Analyzer
  - 3. Detector

MALDI-TOF (Cont..)



VITEK 2 automated system

- Perform both identification and antimicrobial susceptibility testing (AST) of bacteria and yeast.
- Principle of VITEK for identification: Uses colorimetric reagent card containing 64 wells; each well contains an individual test substrate.
- Result of identification - usually available within 4–6 hours.

VITEK 2 automated system (Cont..)

ANTIMICROBIAL SUSCEPTIBILITY TEST

ANTIMICROBIAL SUSCEPTIBILITY TEST

- Bacteria exhibit great strain variations in susceptibility to antimicrobial agents - AST plays a vital role to guide the clinician for tailoring the empirical antibiotic therapy to pathogen-directed therapy.
- Performed only for pathogenic bacteria isolated from the specimen, and not for the commensal bacteria.
- Classified into phenotypic and genotypic methods.

ANTIMICROBIAL SUSCEPTIBILITY TEST (Cont..)

Phenotypic methods -

- Disk Diffusion Method- e.g. Kirby–Bauer's disk diffusion (DD) test
- Dilution tests: Broth Dilution and agar dilution Method
- Epsilometer or E-test
- Automated AST-e.g. Vitek, Phoenix and Microscan systems

Genotypic methods -

- PCR detecting drug resistant genes

Procedure (Colony disk diffusion)

- Antibiotic disks - impregnated on to a suitable medium lawn cultured with the test isolate.

Antibiotic disks:

- Antibiotic disks - commercially or prepared in-house.
- Sterile filter paper disks - 6 mm diameter are impregnated with standard quantity of antibiotic solution.

Procedure (Colony disk diffusion) (Cont..)

Medium:

- Mueller–Hinton agar (MHA) - standard medium used for AST.
- *S. pyogenes* and *S. pneumoniae* - Mueller–Hinton blood agar (MHBA) containing 5% of sheep blood is used.

Procedure (Colony disk diffusion) (Cont..)

Inoculum preparation:

- Directly suspending the colony in the normal saline or
- Inoculating into suitable broth and incubating at 37°C for 2 hours

Turbidity:

- Adjusted to 0.5 McFarland opacity standard - equivalent to approximately 1.5 × 108 cfu/mL of bacteria.

Procedure (Colony disk diffusion) (Cont..)

Lawn culture:

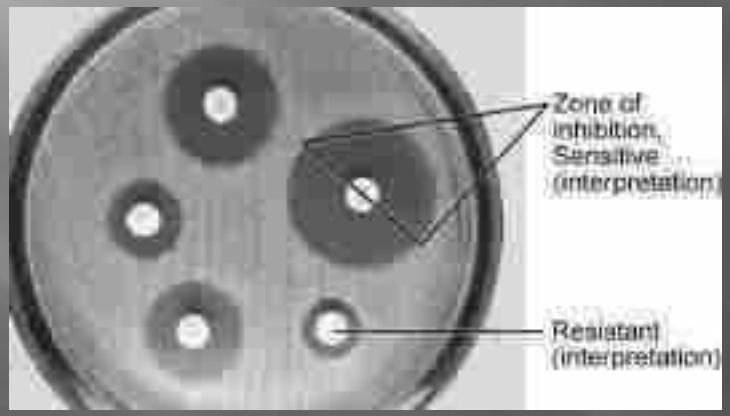
- Broth - inoculated on to the medium by spreading with sterile swabs

Disks impregnation:

- Disks - placed atleast 24 mm (center to center) apart on the MHA plate.
- Maximum up to 6 disks can be applied on a 100 mm plate.

Interpretation

- Susceptibility to the drug - determined by the zone of inhibition of bacterial growth around the disk - measured by Vernier caliper.



concentrations and interpretation of disk diffusion test (as per CLSI

Antimicrobial agents	Disk strength (μg)	Zone diameter break points (mm)			MIC breakpoints (μg/ml)		
		Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Ceftazidime	30	≥ 21	18-20	≤ 17	≤ 4	8	≥ 16
Ceftriaxone	30	≥ 23	20-22	≤ 19	≤ 1	2	≥ 4
Ciprofloxacin	5	≥ 26	22-25	≤ 21	≤ 0.25	0.5	≥ 1
Cefoxitin (*S.aureus*)	30	≥ 22	-	≤ 21	≤ 4	-	≥ 8
Levofloxacin (*S.aureus*)	5	≥ 19	16 18	≤ 15	≤ 1	2	≥ 4
Cotrimoxazole (*S.aureus*)	1.25/23.75	≥ 16	11-15	≤ 10	≤ 2	-	≥ 4

Direct Disk Diffusion Test

- Performed when results are required urgently and single pathogenic bacterium is suspected in the specimen (for positively flagged blood culture bottle, sterile fluid or urine).
- Specimen - directly inoculated - on to the surface of an agar plate and the antibiotic disks are applied.

Direct Disk Diffusion Test (Cont..)

- Results of the direct-DD test - verified by performing AST from the isolate separately.

Dilution Tests

- Antimicrobial agent - serially diluted - each dilution is tested with the test organism for ASTand the MIC is calculated.
- MIC (minimum inhibitory concentration) - lowest concentration of an antimicrobial agent that will inhibit the visible growth of microorganism after overnight incubation.

Dilution Tests (Cont..)

- Depending upon whether the dilutions of the antimicrobial agent are made in agar or broth, there are two types of dilution tests.
  - *Broth Dilution Method*
  - *Agar Dilution Method*

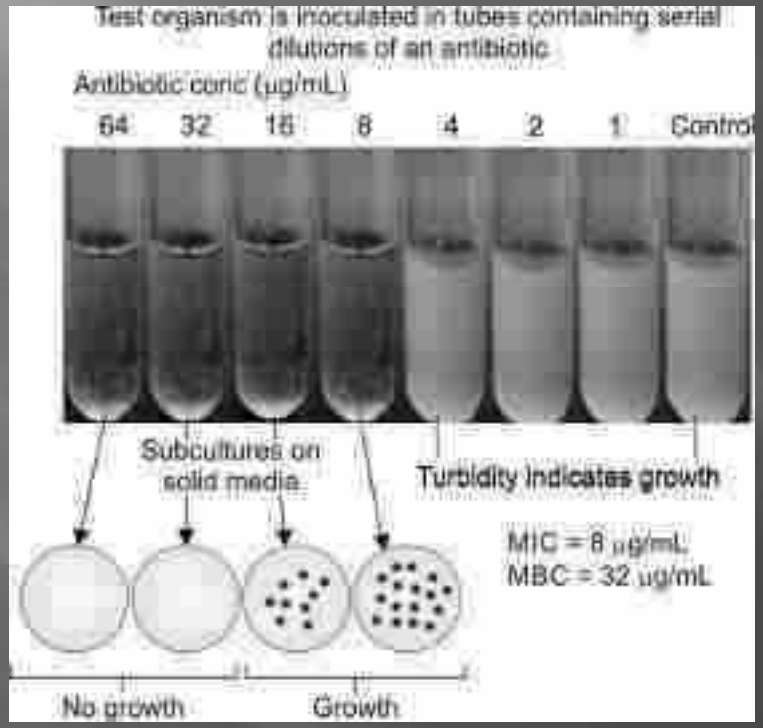
Broth Dilution Method

- Two types:
  - Macro broth dilution (performed in tubes)
  - Micro broth dilution (performed in microtiter plate).

Broth Dilution Method (Cont..)

- Serial dilutions of the antimicrobial agent in Mueller Hinton broth are taken in tubes - each tube is inoculated with a fixed amount of suspension of the test organism.
- MIC - determined by - lowest concentration of the drug at which there is no visible growth, i.e. broth appears clear

Broth Dilution Method (Cont..)



Broth Dilution Method (Cont..)

- **Minimum bactericidal concentration (MBC) -** obtained by subculturing from each tube (showing no growth) onto a nutrient agar plate without any antimicrobial agent.
- Tube containing the lowest concentration of the drug that fails to show growth, on subculture, is the MBC of the drug for that test strain.

Agar Dilution Method

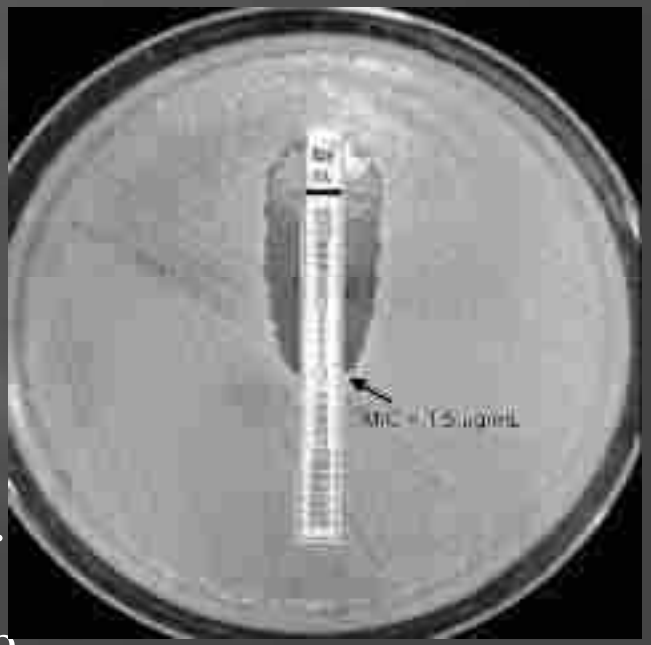
- Serial dilutions of the drug - prepared in molten agar and poured into petri dishes.
- Test strain is spot inoculated.

Advantage:

- Several strains can be tested at the same time by using the same plate
- Directly measures the MBC

Epsilometer or E-test

- Uses an absorbent strip containing predefined gradient (serial dilution) of antibiotic concentration immobilized along its length.
- Applied to a lawn inoculum of a bacterium.
- Antibiotic concentration at which the ellipse edge intersects the strip -MIC value.



Automated Antimicrobial Susceptibility Tests

- VITEK 2 identification and antimicrobial sensitivity system
- Phoenix System (Becton Dickinson)
- Micro Scan Walk Away system.

VITEK 2 Automated System for AST

- **Principle -** microbroth dilution.
- Uses reagent card containing 64 wells - contain doubling dilution of antimicrobial agents. The
- Organism suspension - added to the wells (Figure 3.3.23 and Table 3.3.7)
- Cards are incubated in the system at 35.5 ±1°C.

VITEK 2 Automated System for AST (Cont..)

- **Reading -** taken once in every 15 minutes.
- **Results -** available within 8-10 hours for gram negative bacilli and 16-18 hours for gram-positive cocci.

Role of MIC

- **To select the most appropriate antibiotic -** Lower is the MIC, better is the therapeutic efficacy
- Better guided - calculating the **therapeutic index** - ratio of susceptibility breakpoint divided by the MIC of the test isolate.
- **MIC-guided therapy**

Interpretation of AST

Terminology	Definition
Susceptible (S)	Antibiotic is clinically effective when used in standard therapeutic dose.
Intermediate (I)	Antibiotic is not clinically effective when used in standard dose; but may be active when used in increased dose.
Susceptible dose dependent (SDD)	Antibiotic will be clinically active only if given in increased dose.
Resistant (R)	Antibiotic is NOT clinically effective when used in either standard dose or increased dose.

Choice of Antibiotics to be Included in Panel

- Clinically indicated
- Organism isolated and local resistance pattern
- Intrinsic resistance - excluded from the panel
- Antimicrobial agent - oral and parenteral - included
- Locally available antibiotic
- Site of action - tested which are active in the site.
- Predicting susceptibility

Selective or Cascade Reporting

- Clinical microbiology laboratory - test for the full antibiotic panel, but - should report only in a selective manner.
- If all antimicrobial agents are susceptible - only first-line agents are reported - second-line and restricted antimicrobials are supressed.

Selective or Cascade Reporting (Cont..)

Enterobacteriaceae	Antimicrobial agent used in VITEK
First line	Ampicillin
First line	Amoxicillin- clavulanic acid
First line	Ciprofloxacin
Second line	Cefoperazone-sulbactam
Second line	Piperacillin- tazobactam
Restricted	Meropenem
Restricted	Colistin

SEROLOGY

SEROLOGY

Detection of either antigen or antibody in the serum of the patient, by various immunological assays such as—

- Precipitation
- Agglutination
- ELISA
- Rapid test.

MOLECULAR METHODS

MOLECULAR METHODS

- Polymerase chain reaction (PCR)
- Real-time polymerase chain reaction (rt-PCR)
- Loop mediated isothermal amplification (LAMP)
- Automated PCR such as Biofire FilmArray
- Automated real-time PCR such as cartridge based nucleic acid amplification test (CBNAAT)

Polymerase Chain Reaction (PCR)

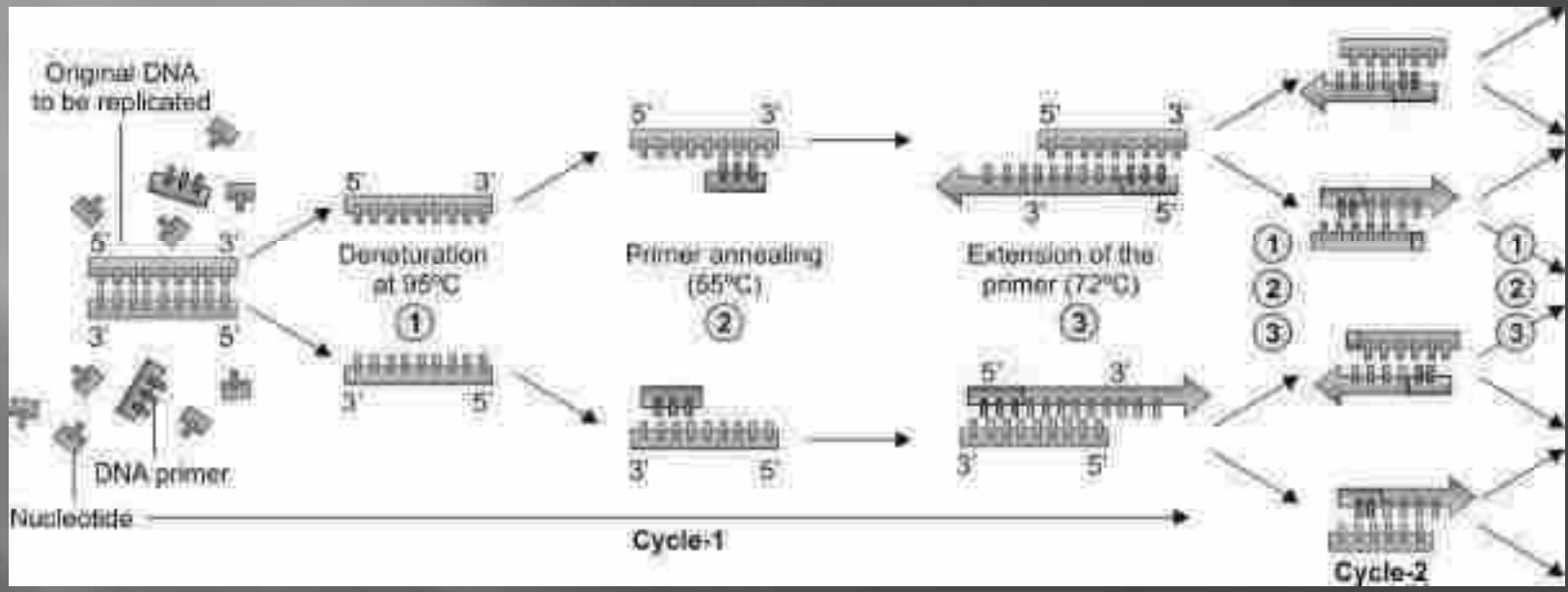
- Technology in molecular biology used to amplify a single or few copies of a piece of DNA to generate millions of copies of DNA.
- Developed by Kary B Mullis

Polymerase Chain Reaction (PCR) (Cont..)

Principle : three basic steps

1. DNA extraction from the organism
2. Amplification of extracted DNA
3. Gel electrophoresis of amplified product:

Polymerase Chain Reaction (PCR) (Cont..)



Modifications of PCR

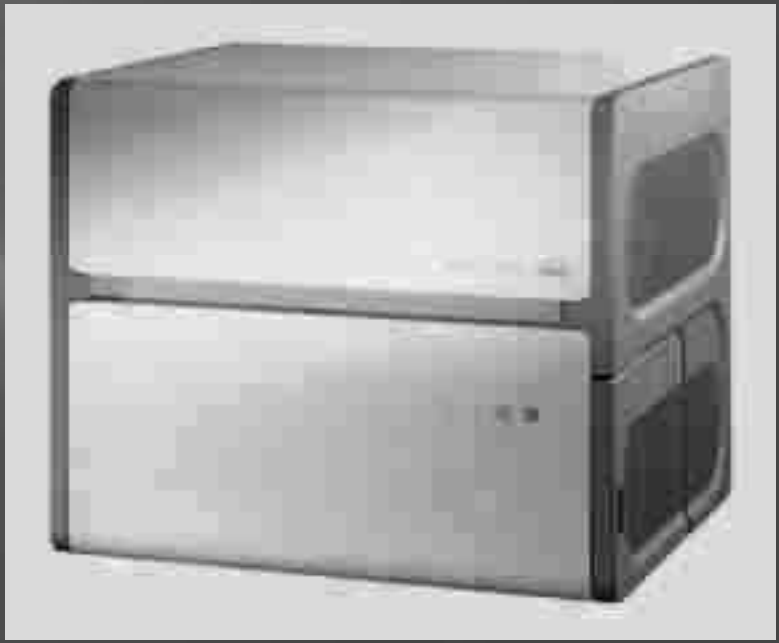
1. Reverse transcriptase PCR (RT-PCR)
2. Nested PCR
3. Multiplex PCR

Biofire FilmArray

- Multiplex nested PCR system
- Four panels are available such as respiratory, gastrointestinal, meningitis-encephalitis and blood culture identification panels

Real-time PCR (rt-PCR)

- PCR technology, which is used to amplify and simultaneously detect or quantify a targeted DNA molecule on real-time basis.



Loop Mediated Isothermal Amplification (LAMP)

- Isothermal nucleic acid amplification technique.
- Amplification is carried out at a constant temperature of 60–65° (in contrast to alternating temperature cycles in PCR)

Nucleic Acid Probes

- Radiolabeled or fluorescent labelled pieces of single stranded DNA or RNA - used for the detection of homologous nucleic acid by hybridization.
- Two types of nucleic acid probes—DNA probes and RNA probes.

MICROBIAL TYPING

MICROBIAL TYPING

Refers to characterization of an organism beyond its species level.

Applications:

- Used to determine the relatedness between different microbial strains of the same species.
- Investigate outbreaks
- Determine the source and routes of infections
- Trace cross-infection
- Differentiate virulent strains from avirulent strains of same species

MICROBIAL TYPING (Cont..)

Classification:

- Phenotypic methods
- Genotypic methods.

Characteristics of Typing Methods

- Typeability
- Reproducibility
- Discriminative power
- Practicality

Phenotypic Methods

Bacteriophage typing: Strains of an organism - further differentiated into subspecies level based on their susceptibility to bacteriophages.

Bacteriocin typing: Bacteriocin - antibiotic like proteinaceous substance - based on the ability of a strain to produce particular bacteriocin which inhibits the growth of a set of selected indicator strains.

Phenotypic Methods (Cont..)

Biotyping: Refers to intra species classification based on different biochemical properties of the organism.

Antibiogram typing: Classifies the organism into different groups based on their resistance pattern to different antimicrobials.

Serotyping: Typing method based on the antigenic property of an organism

Genotypic Methods

- *Restricted Fragment Length Polymorphism (RFLP)*
- *Ribotyping*
- *Pulse Field Gel Electrophoresis (PFGE)*
- *Amplified Fragment Length Polymorphism (AFLP)*
- *Sequencing-based Methods*

Questions:

- Q1. Agar concentration required to prepare semisolid media:

a. *0.1%*

b. *0.5%*

c. *2%*

d. *6%*

Questions:

- Q2. Type of specimen collected for URTI:

a. *Blood*

b. Throat swab

c. Sputum

d. *BAL*

Questions:

- Q3. Sulfuric acid (%) needed for decolorization of *M. tuberculosis*:

a. 25%

b. 5%

c. 1%

d. 0.5%

Questions:

- Q4. Examples of enriched media are all except:

a. Blood agar

b. Loeffler's serum slope

c. Blood culture media

d. MacConkey agar

Questions:

- Q5. Automated blood culture bottles contains:

a. Selenite F broth

b. Tryptic soy broth

c. Peptone water broth

d. BHI broth

Questions:

- Q6. Which of the following is not an equipment for automated blood culture:

a. BacT/ALERT

b. VITEK2

c. BACTEC

d. VersaTREK

Questions:

- Q7. BacT/ALERT - principle is based on::

a. Calorimetric detection

b. Fluorescent detection

c. Detection of change of pressure

d. All the above

Questions:

- Q8. Three basic steps of PCR cycle involves all, except:

a. Denaturation

b. Amplification

c. Extension

d. Gel documentation

A- Scan and B- Scan

A – scan :

-Amplitude modulation scan performed with a single ultrasound source.

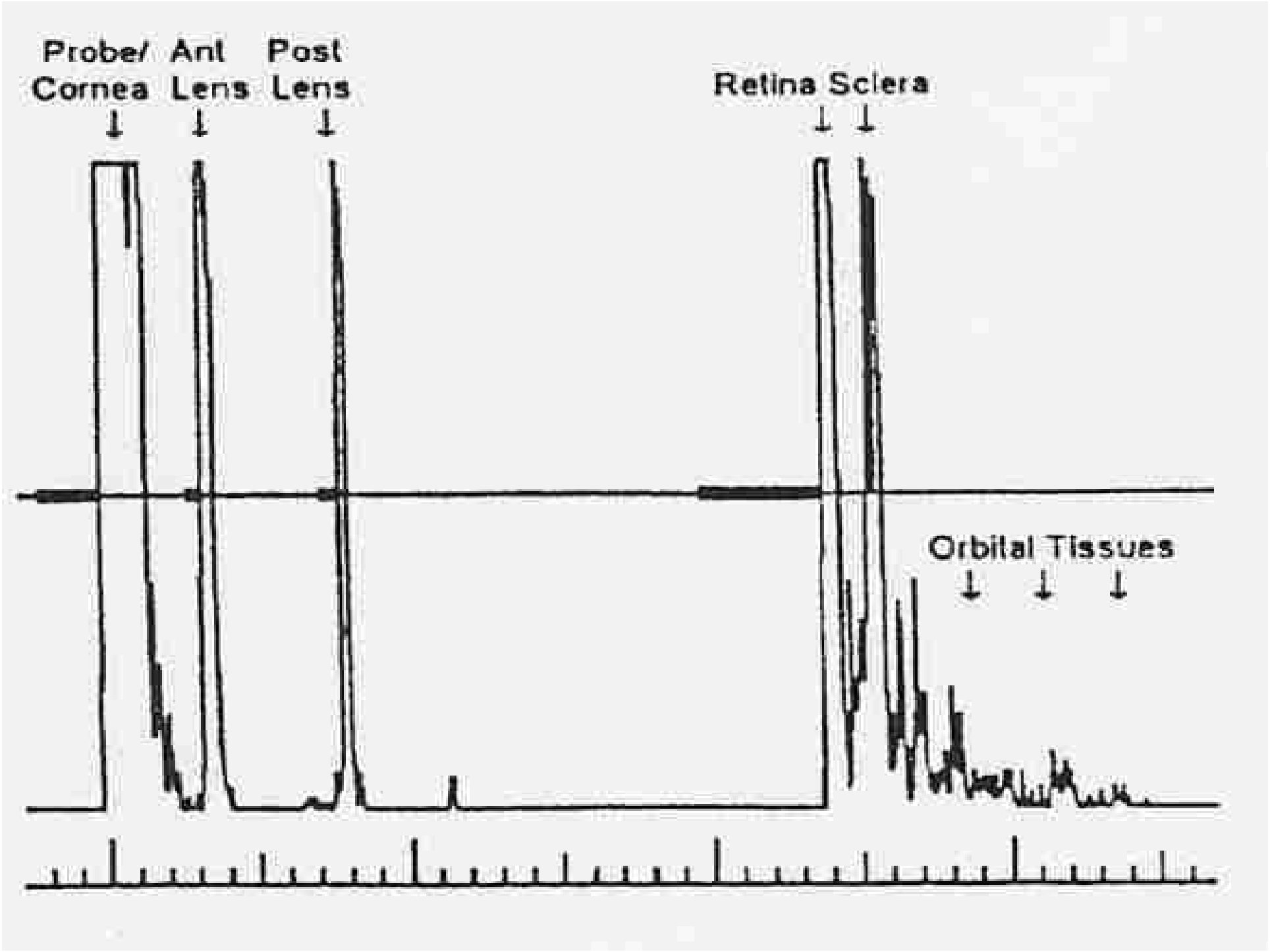
-Noninvasive procedure

-Produces one dimentional time amplitude evaluation in the form of vertical spikes along baseline.

-in A – Scan 10MHz frequency used

-Emits high frequency radar like waves that are reflected by ocular structures and converted into electrical impulse.

Allows excellent resolution of smaller particles.


Probe/ Cornea
Ant Lens
Post Lens
Retina Sclera
Orbital Tissues

Uses :

-To determine axial length of eye for calculation of IOL power- biometric scan- biometric A scan

- diagnostic A scan- differentiates tissues within eye. Commonly tumors. Echoes produced by malignant melanoma are different from dat produced by benign hemangioma

-Anterior chamber depth

-Lens thickness

-To determine size, type n ultrasound characteristics of masses in eye

-one thin parallel beam emitted from probe tip at freq of 10MHz .

-Sound beam strike each interface (junction between two media of different densities n velocities), produces echo.

-Echo bouncing back into probe tip and converted by biometer to spikes arising from baseline.

-Greater difference in two media of each interface , stronger echo and higher spike.

-In area of vitreous floaters, post viterous detachment – weak echo, shorter spike.

A – scan measures two parameters of echo :

-Amplitude and pulse transit time.
-Transit time is correlated to reflector depth or distance.
-Distance = velocity into time

- In area of normal viterous , non cataractous lens , media with identical density n velocity. Thus no echo produced , echo goes to baseline.

- cataractous lens – beam strikes the different densities within lens, produces multiple spikes within central lens area.

-As in cataractous lens, greater the density of structure thro which sound passes, greater amount of absorption

-thus , lens absorbs sound , retinal spikes quality reduced.

Height of spike affected by :

- Differences in densities

- Angle of incidence , which is determine by probe orientation to visual axis

- Shape of interface

- Smoothness of interface

- Density of structure

Probe – held so that emitted sound beam strikes to retina in perpendicular manner, and receives echoes back into probe tip.

-If probe held in nonparallel manner, part of echo diverted away from probe tip and not received by machine.
This increases angle of incidence

-Greater angle of incidence – weaker signal , shorter the spike amplitude

Irregularity of surface of interface: macular pathology

-Reflection n refraction of waves away from tip.
-If macular surface is convex as in macular edema , pig epithelial detachment- part of echo reflected away from tip.

B- Scan :

-Performed with a vector probe or a linear probe

-In vector probe, one main source of ultrasound oscillates back and forth to produce a sweep of ultrasound.

-In linear probe, multiple sources of ultrasound are aligned in grid to cover a specific area.

-Amount of reflected sound is seen as a dot of light.

-The more sound reflected, the brighter the dot

- B- scan two dimentional ultrasonography provides topographic information of size, shape, n quality of lesion and its relationship to other structures.

-Three dimentional imaging is also available n can be used to measure tumor volume and enhance localization of radioactive plaque over tumor.

-Addition of doppler facility allows the evaluation of blood flow.

The frequency of transducer determines which part of globe or orbit is examined.

1. Low frequency transducers : useful in detecting orbital pathology

2. Moderate frequency (7-10 MHz) : used to examine the globe.

main indications :

1. Evaluation of eyes with opaque media for retinal detachment
2. Evaluation of post intraocular tumors
3. Detection of calcification in retinoblastoma and optic disc drusen.

3. High frequency (30- 50 MHz) :

-Allows high defination imaging of anterior segment but only to a depth of 5mm

-Particular value in evaluation of congenital corneal opacification.

Technique of B- scan USG.

1. Technique :

- Anaesthetic drops instilled and pt placed in supine position
- Examiner sit behind the pt’s head, hold probe with dominant hand.
- Methylcellulose or ophthalmic gel is placed on tip of probe as coupling agent
- Vertical scanning is performed with marker oriented superiorly
- Horizontal scanning performed with marker pointing towards nose.
- Eye examined with pt looking straight ahead , up, down, lt n right.

-For each position, horizontal and vertical scan can be performed.

-Examiner then moves the probe in opposite direction to movement of eye.

Dynamic scan :
-Performed by moving the eye but not the probe.

Gain :

-Adjusts the amplification of echo signal , similar to volume control of radio.

-Higher gain increases the sensitivity of instrument in displaying weak echoes , vitreous opacity

-Lower gain- allows display of only stronger echoes such as retina, sclera, also to improve the resolution

Uses : useful when direct visualization of intraocular structures is difficult.

-Lid problems- severe lid edema , total tarsorraphy

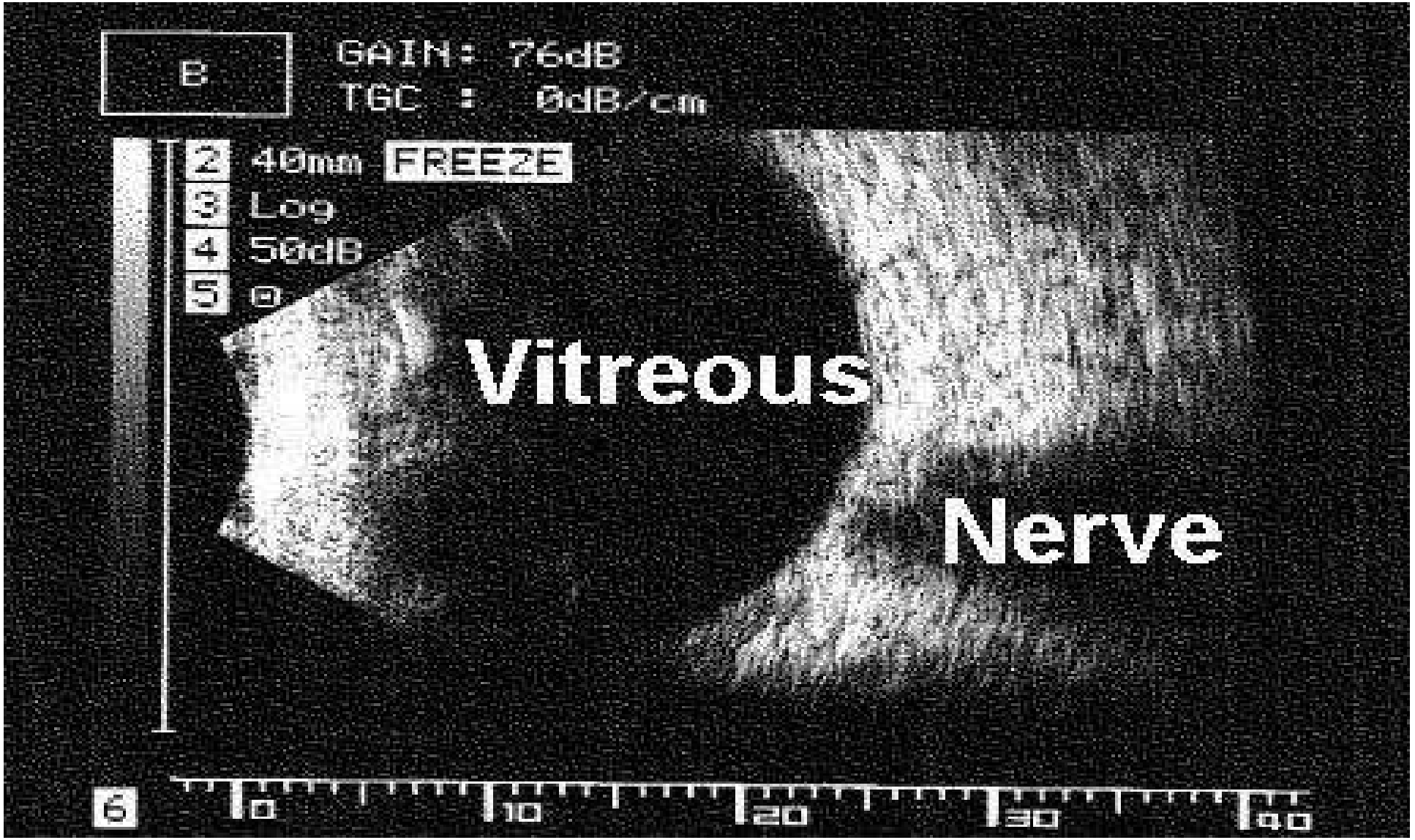
- keratoprostheis

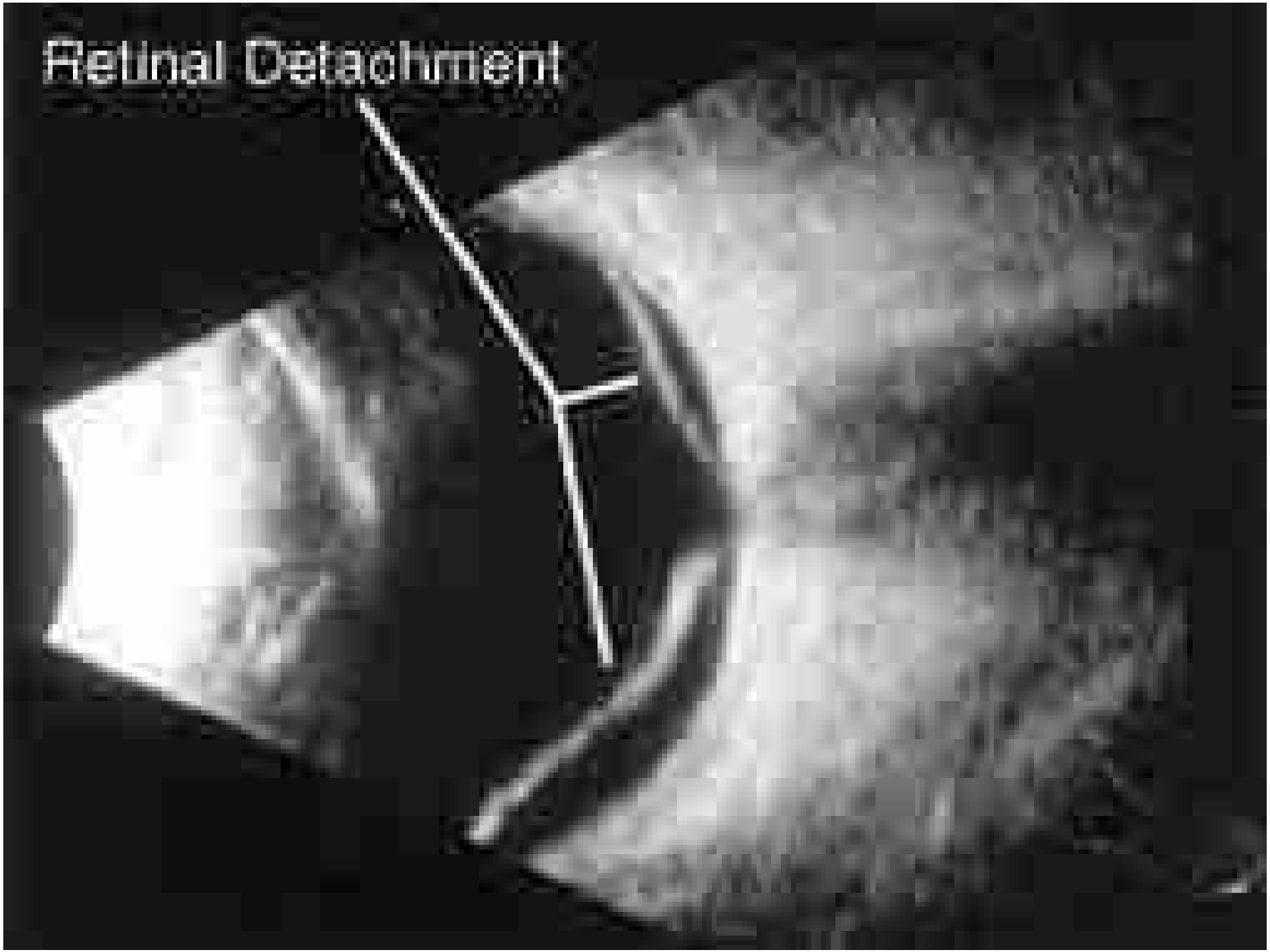
- corneal opacities, scar

-Hyphema, hypopyon, dense cataract

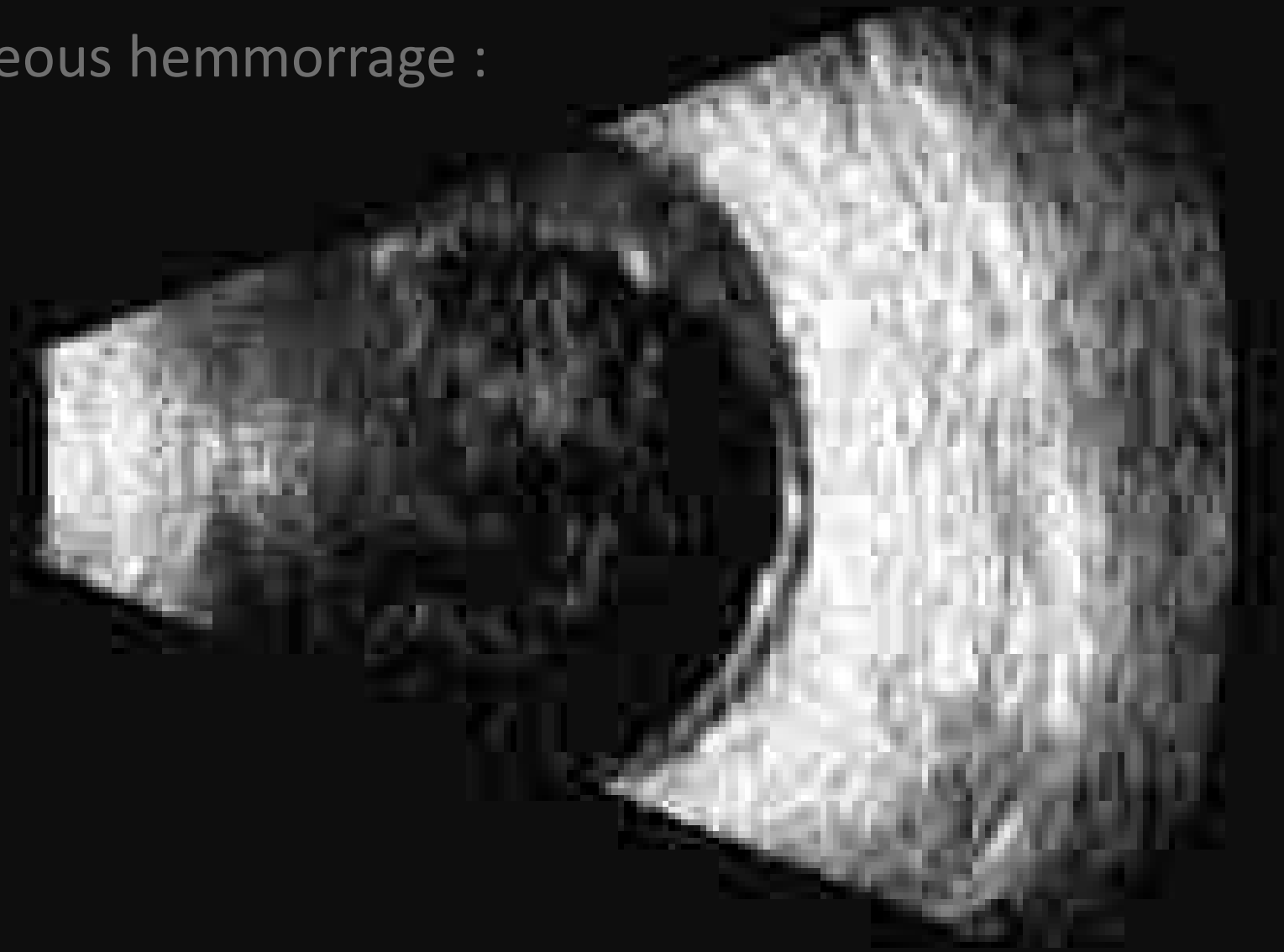
-Vitreous hemorrhage, inflammatory debris

B- SCAN

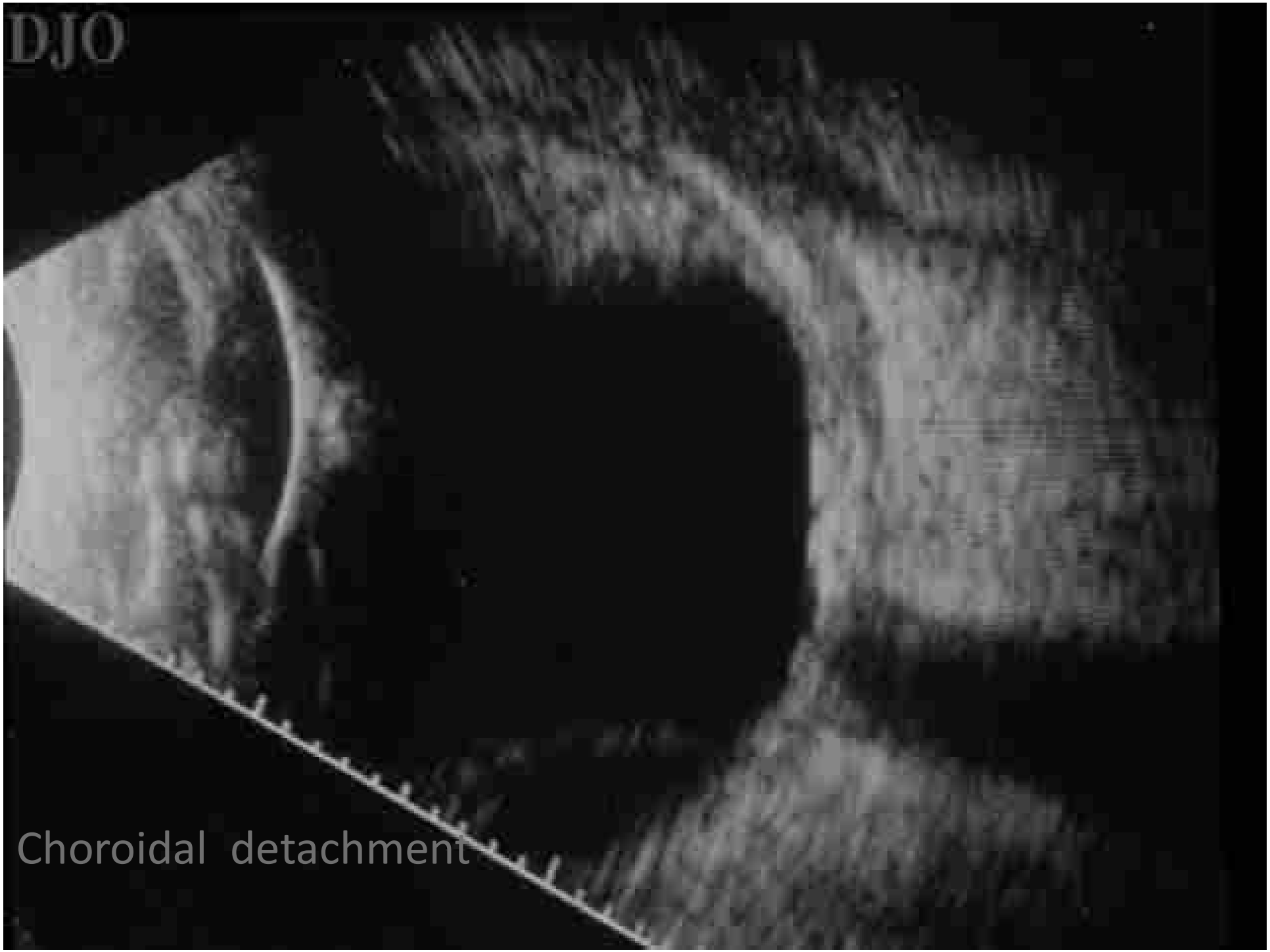

B
GAIN: 76dB
TGC : 0dB/cm
2 40mm FREEZE
3 Log
4 50dB
5 0
Vitreous
Nerve
6
0
10
20
30
40


Retinal Detachment

Vitreous hemmorrage :

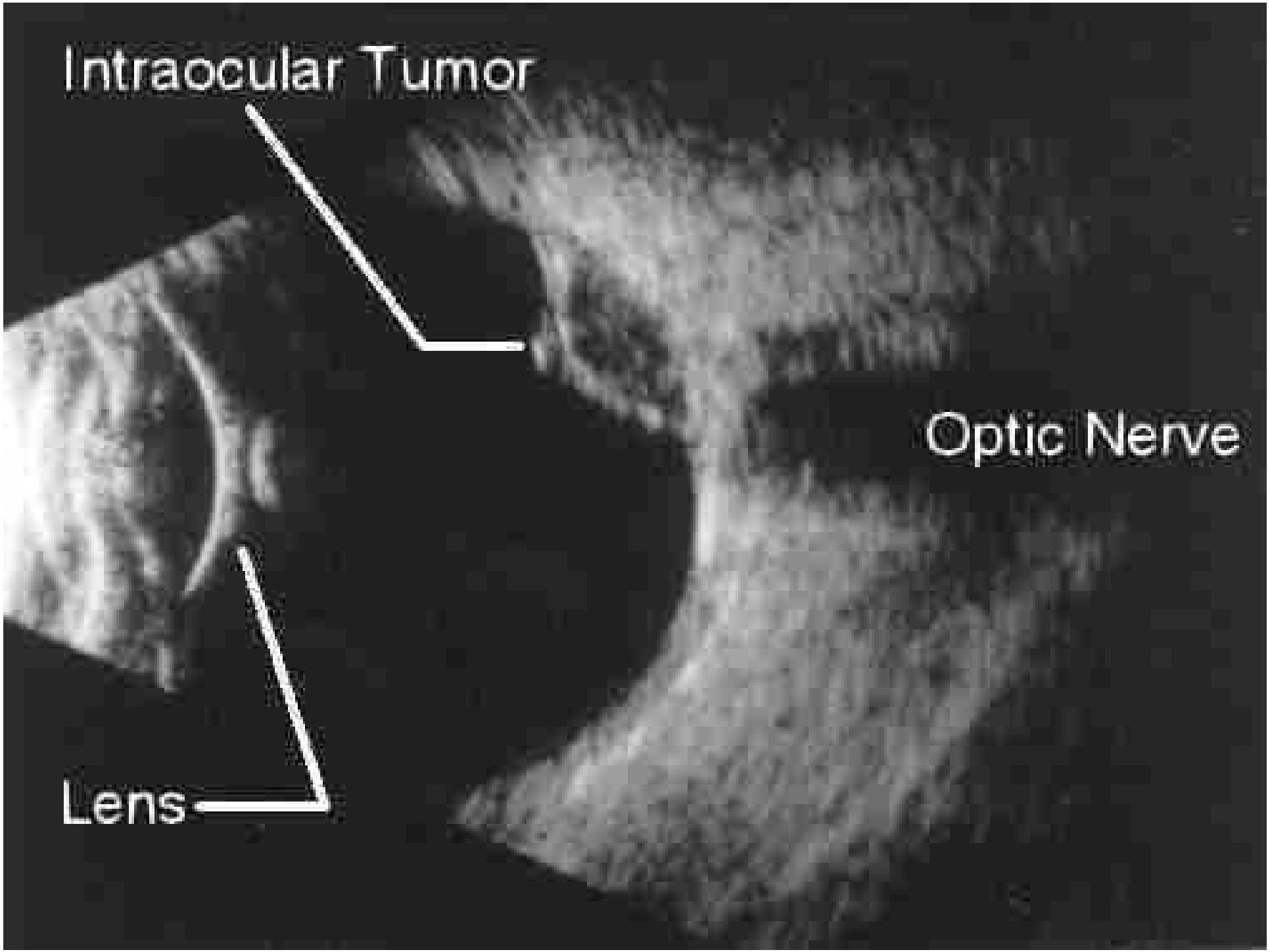


posterior
vitreous
detachment
choroidal detachment
vitreous
blood



Choroidal detachment

Choroidal melanoma
©ECN


Intraocular Tumor
Optic Nerve
Lens

Thank you

Acanthamoeba

Acanthamoeba :

-Member of phylum sarcomastigophora. (1)
-Free lying amoeba
-Motile, replicating
-Also k/a amphizoic amoebae
-Cause infection of eye, skin, CNS.

TWO FORMS:

-Tropozoite
-Cyst

5 protozoan species (2)

-A. Castelleni
-A. Culbertsoni
-A. Polyphaga
-A. Hatchetti
-A. Rhysodes

Acanthamoeba keratitis

-Increasing incidence, difficulty in diagnosis, unsatisfactory treatment. (3)

Modes of infection : (4)

1. contact lens wearers- home made salines

2. Non cantact lens related- mild trauma with vegetable matter, salt water diving, trauma with organic matter.

3. Opportunistic infection- herpetic keratitis, bact keratitis, neuroparalytic keratitis, bullous keratopathy

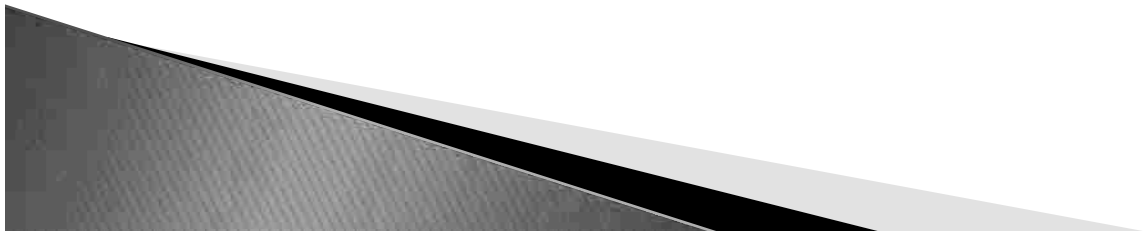
Clinical features

Symptoms: (5) very severe pain, watering, photophobia, bhepharospasm , blurred vision

Signs:

1. Initial lesions:

- Limbitis
- Radial keratoneuritis
- Epitheliopathy (6)



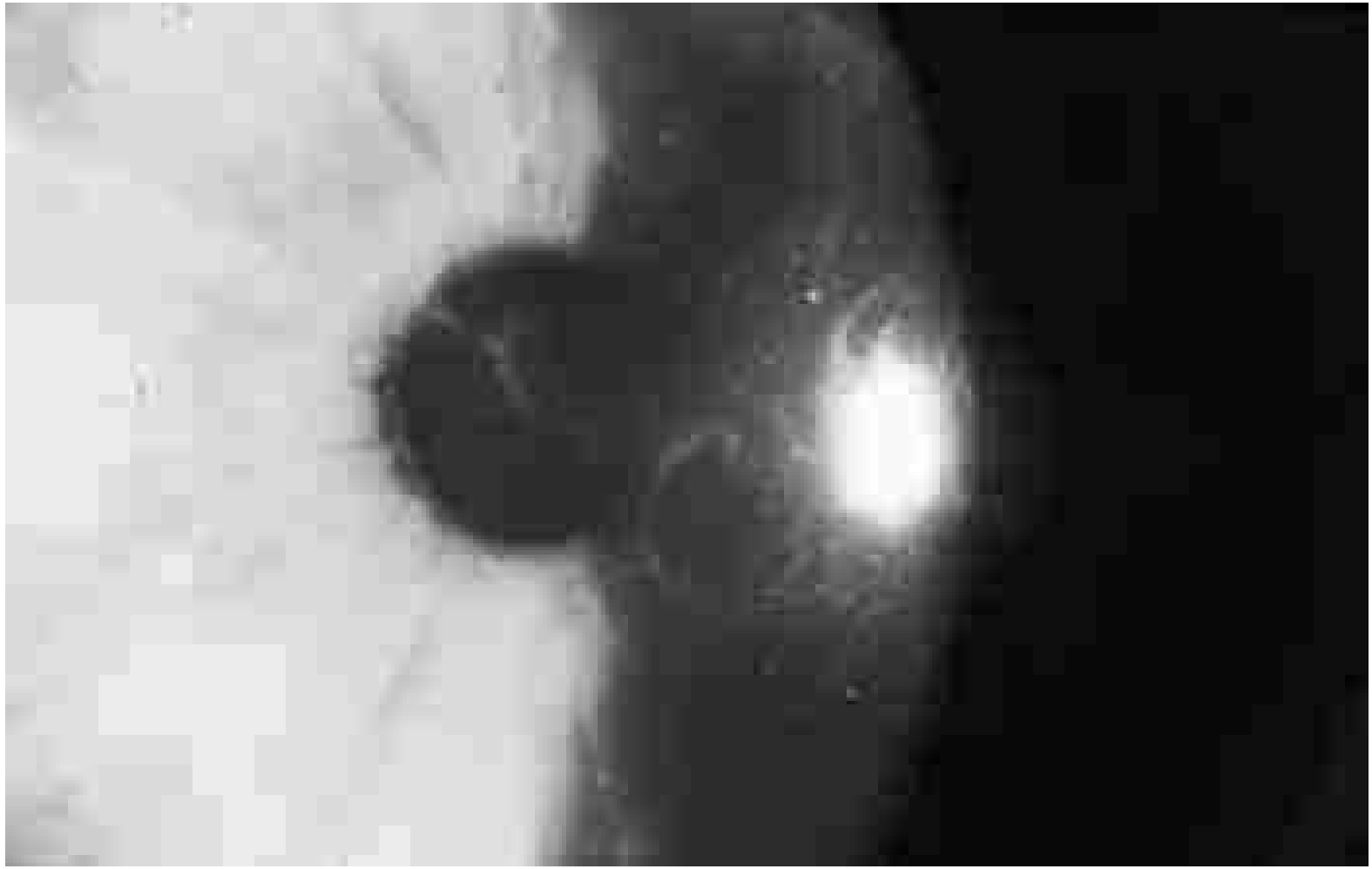
2.Advanced cases :

-Stromal infiltrates
-Ring infiltrates
-Hypopyon

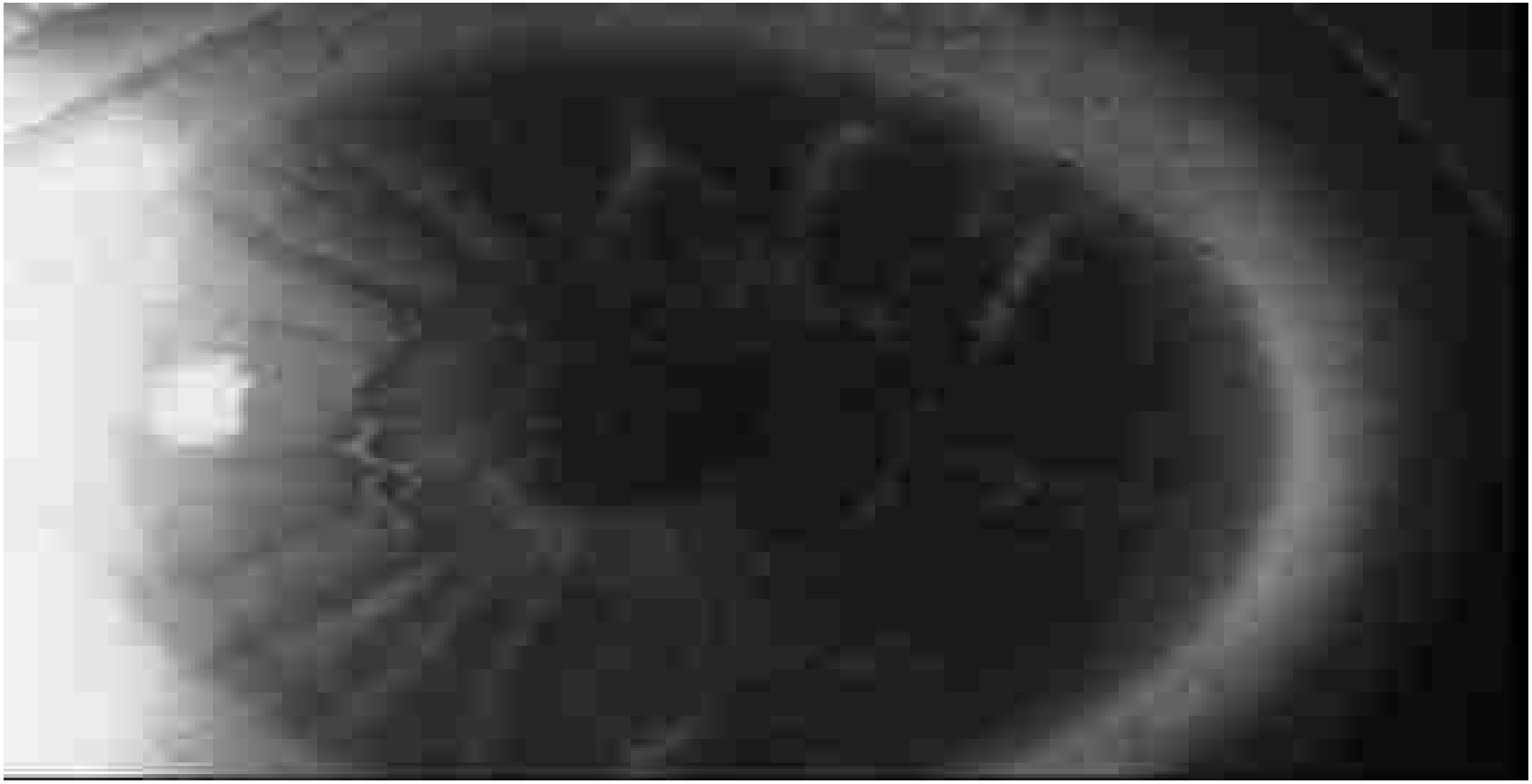
3.Severe cases:

-Ring abscess
-stromal necrosis, opacification
-Corneal melting

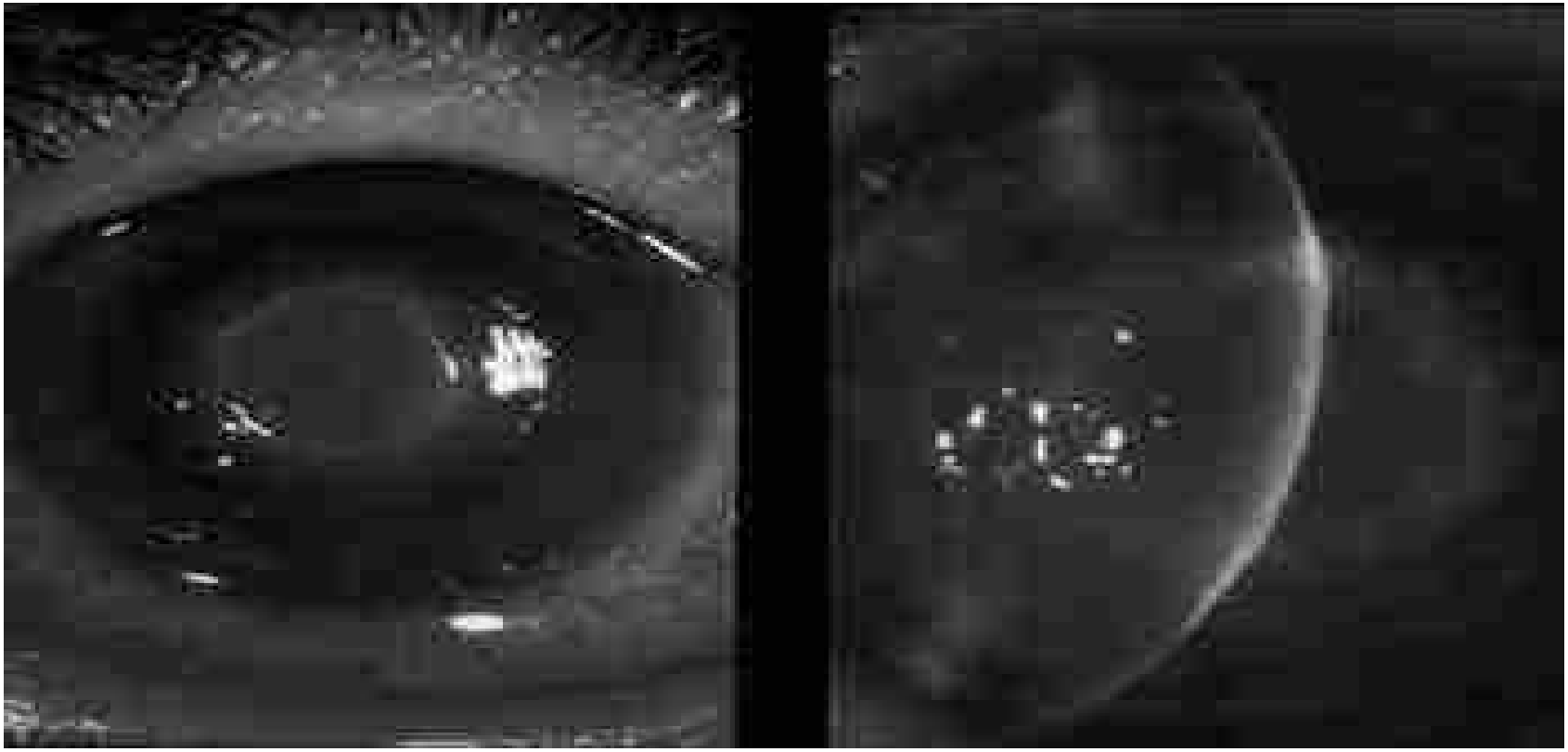
Dendritic epitheliopathy



Radial keratoneuritis:

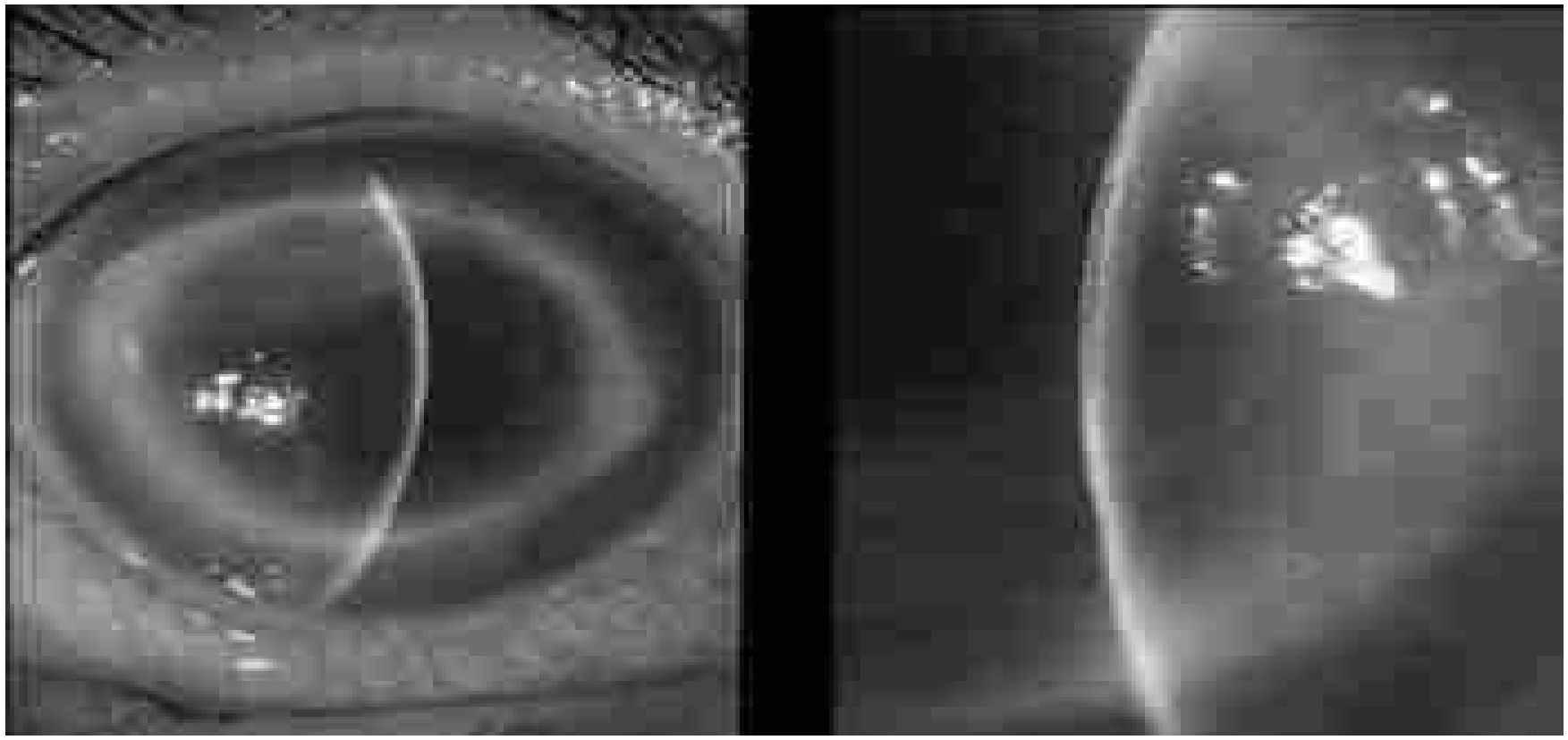


Stromal infiltration



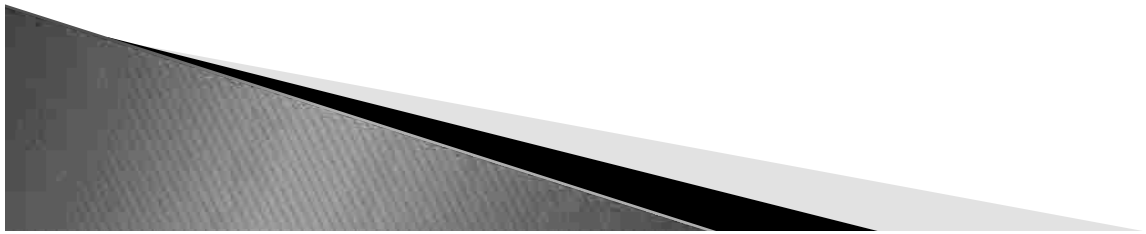
Early phase of ring infiltrate

Advanced ring infiltrate



Pathophysiology :

Histopathology: (7)



Diagnosis:

Timely diagnosis is imp.

1.Clinical : early stage : missed (8)

2. Laboratory diagnosis:

-Trophozoites : do not stain

-Cyst : stain by gram , geimsa, H n E, PAS Stain, calcifluor white stain, wrights trichrome, methamine silver stain

-Lactophenol cotton blue stained

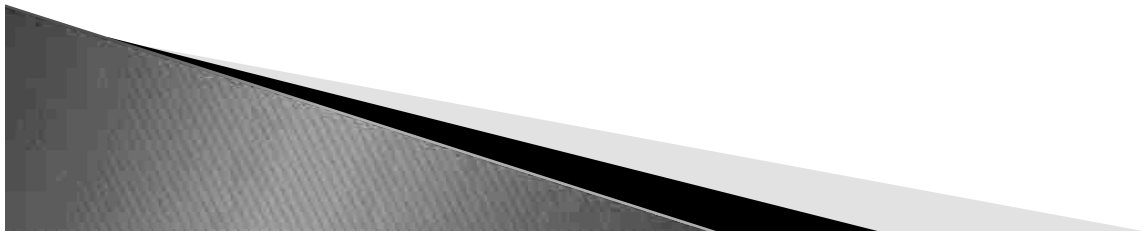
-Sample for staining (9)

2. cultures:

-at room temp on non nutrient agar, with gram negative bact.
-30% pt's- culture negative (10)
-KOH mount

3. other:

-Immunohistochemistry
-PCR
-Confocal microscopy
-Corneal biopsy (11)



Treatment :

Outcome of t/t much better if started within 4 wks of symptoms

1. Debridement: (12)

2. Topical amoebicides:

PHMB and chlorhexidine digluconate 0.02%

Diamidines : 1.brolene (propamide isethionate) – altered or inactivate nucleoprotein 0.1%

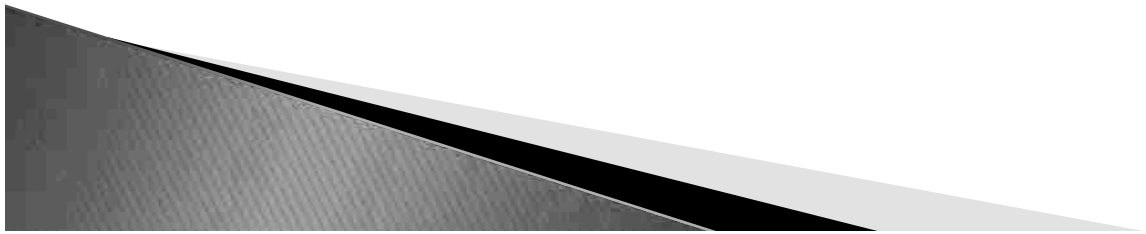
2. Pentamidine (13)

Aminoglycocides : neomycin n paromycin

3. Antifungal agents:

Orally : Ketoconazole, itraconazole
Topically: miconazole, cotrimazole

4. Topical steroids :



MULTIDRUG THERAPY : (14)

For early epithelial lesions: 3-4 mnths
Stromal lesions : 6-12 mnths

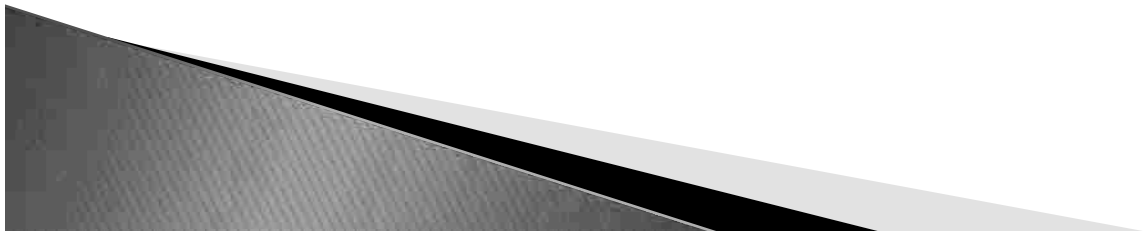
5. Prophylactic therapy

6. Pain management: (15)

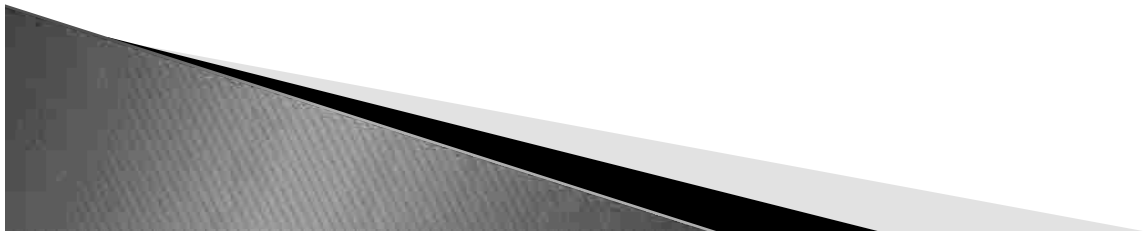
-oral NSAID

7. Surgery: (16)

1. Leibowitz 2nd edition, 719
2. Leibowitz 2nd edition 720
3.Khurana 5th edition,107
4.Khurana 107
5, 6.Kanski, 7th edition 194, leibowitz, parson's 21st edition 203.
7. Leibowitz 721
8. parson's 21st edition 203
9. Leibowitz 723
10,11,12Kanski 7th edition 194
13. Kanski 7th edition 194, leibowitz, khurana 5th edition 108
14. leibowitz, 724 khurana 108
15. Kanski 196, leibowitz
16.leibowitz, kanski 196.



Thank you



Amblyopia

Definition :-

“ Amblyopia is unilateral or bilateral decrease in BCVA caused by form vision deprivation or abnormal binocular interaction,for which there is no identifiable pathology of eye or visual pathway.

Most common cause of visual defect in children

Most amblyopia is unilateral

.

Cause of amblyopia :-

1. **Straight eye amblyopia** –

a. With high hypermetropia in the amblyopic eye - anisometropia amblyopia.
b. With equal refractive error in the both eyes.
c. With no significant refractive error.

2. **Amblyopia with squint :-**

a. With central fixation.
b. With or without fixative refractive error in the amblyopic deviating eye .

3. **With eccentic fixation**

- **Types of amblyopia :-**

1. Strabismic amblyopia.
2. Anisometropic amblyopia.
3. Ametropic amblyopia.
4. Stimulus deprivation amblyopia.
5. Meridional amblyopia.

Strabismus amblyopia

- m/c form of amblyopia.
- Develops in consistently deviating eye of a child with strabismus.
- Constant , non- deviating heteropias (typically eso-deviation) are likely to cause significant amblyopia.
- Cause from competitive or inhibitory interaction b/w neuron carrying the non - fusiable inputs from 2 eye. Which leads to domination of cortical vision centers by fixating eye & chronically reduced responsiveness to input by non fixating eye.

- Strabismus amblyopia is characterized by better grating and pattern acuity compared to snellen's acuity.
- Neutral density filter effect and eccentic fixation are commonly observed .

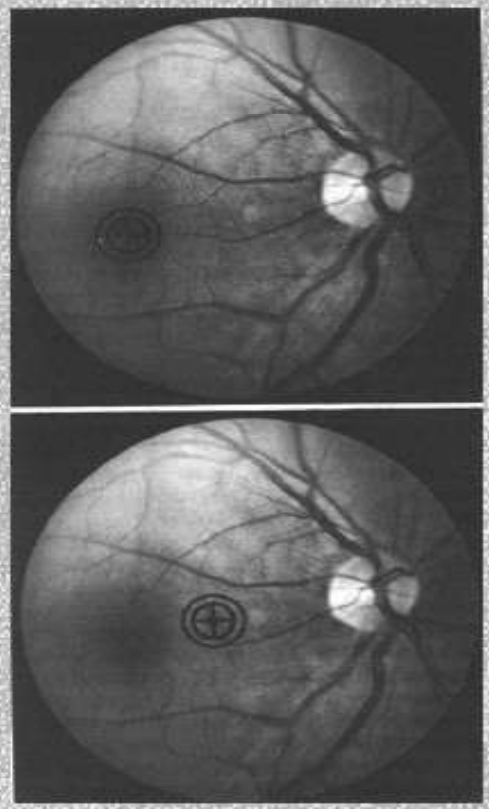
Eccentric fixation:-

- The consistent use of a non-foveal region of the retina for monocular viewing by an amblyopia.
- Visuscopy :- use for detect minor degree of eccentric fixation.

Visuoscopy

central fixation >

nasal eccentric fixation >



- **Classification of fixation :-**

1) central fixation (foveolar).

2) Eccentric fixation (non- foveolar).

- ✓ para- foveal within 2° of the fovea.

3) no fixation (erratic). –

no definite area of fixation around the true fovea.

- ✓ paramacular :- b/w 2° to 4° away from true fovea.
- ✓ Centrocaecal :- > 4° b/w the macula & optic disc.
- ✓ Paracentral :- around optic disc.

Anisometropic amblyopia

- Known as suppression amblyopia , straight eye amblyopia .
- 2nd m/c amblyopia.
- Develops when unequal refractive error in the both eyes causes the image on 1 retina to be chronically defocused.
- Active inhibition of the fovea in anisometropic amblyopia eliminate the sensory interference caused by focused and defocused images originating from fixation point (abnormal binocular interaction

- Mild degree of hyperopic or astigmatism anisometropia (1 -2 D) – MILD AMBLYOPIA.
- Mild myopic anisometropia (less than -3D) – No AMBLYOPIA.
- Unilateral high myopia (-6 D) – SEVERE AMBLYOPIA

Ametropic amblyopia

- Results from large, approximately equal, uncorrected , bilateral refractive errors.
- Mechanism – effect of blurred retinal images alone.
- Commonly due to high hyperopia or astigmatism.
- At least 5 D of hyperopia or 2.50 D of astigmatism (meridional amblyopia) is required.
- Also seen in cases of high myopia (usually >5 D)

Stimulus deprivation amblyopia

- Amblyopia ex anopsia or disuse amblyopia.
- Least common but most damaging.
- Cause when the visual axis is obstructed.
- m/c cause is congenital cataract or early acquired cataract , but corneal opacity and vitreuos hemorrhage may also implicated.

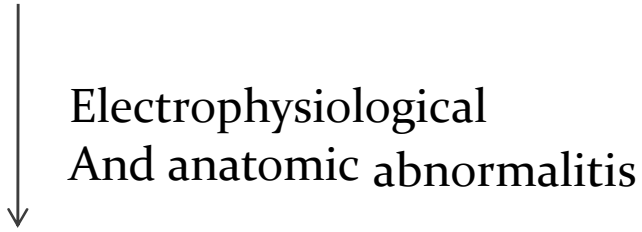
In children:

- Less than 6 yrs – sever amblyopia.
- After 6 yrs – less harmful .
- Occlusion amblyopia – type of deprivational amblyopia caused by excessive therapeutic patching.

Patho-physiology

- Explained by hubel and wiesel.
- At 2- 4th weeks, a period of dramatic developmental plasticity occur during which the developing functional and anatomic organization of the visual system is markedly influence by visual experience ,both uniocular and binocular.

- Concept of critical period – when the developing visual system is sensitive to abnormal input caused by stimulus deprivation, strabismus or significant refractive errors.



- 1) A developmental defect of spatial visual processing occurring in the visual pathway.
  2) Poor transmission from the fovea, optic nerve to the Striate Cortex of the affected eye.
  3) LGB & Striate cortex develop abnormally.
  4) Ganglion cells in foveal area are affected; Shrinkage of LGB Nucleus & Striate cortical fibres in the amblyopic eye.
  5) Loss of binocularly driven cells in LGB & Striate Cortex

Clinical features

- Decrease in Binocular Visual Acuity –

-.

- Two line difference between amblyopic and normal eye

-Crowding – Improved visual acuity when tested with isolated letters rather than lines or groups

Neutral density filter effect –

- With reduced illumination, acuity of an amblyopic eye declines less sharply than that of an normal eye. This mean that amblyopic eye can act under mesopic condition.

- Fixation patterns – central, eccentric or no fixation
- Contrast sensitivity - reduced
- Visual fields – largest acuity losses in esotropic amblyopia occur in the temporal field.
- Accomodation – defective
- RAPD – occasionally.

Assessment of Visual Acuity

- **Preverbal children –**

1. CSM method –

- C – location of corneal light reflex under monocular conditions
- Eccentric fixation (uncentral {UC}) – implies VA of 6/60 or lesser
- S – steadiness of fixation on target (monocular)
- M – maintain alignment first with one eye, then with the other , as opposite eye is uncovered. Maintenance of fixation is evaluated under binocular conditions.

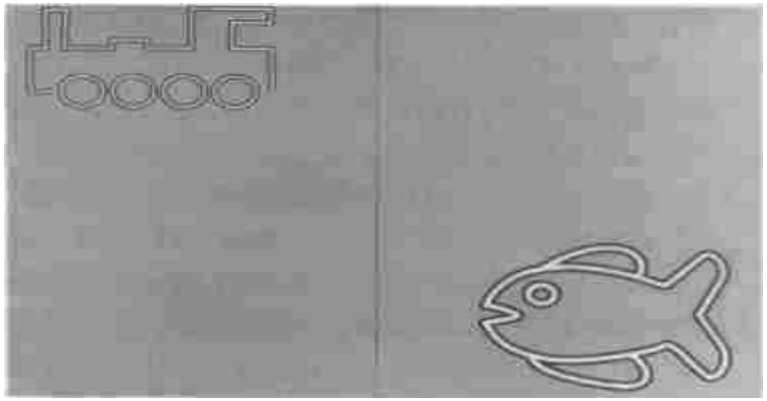
- Inability to maintain fixation with either eye with the opposite eye un-covered is presumptive evidence of a difference in acuity b/w the eyes.
- Preverbal /nonverbal patients with strong fixation in one eye should be suspected of having amblyopia in other eye.

- Test for maintain fixation with either eye under binocular viewing conditions.
- Strong fixation prefrence for 1 eye indiacates amblyopia in non-preferred eye.

Rotation test :-

- It is a gross qualitative test .
- examinar hold child facing him rotate at 360°.
- If vision is normal , eye will deviate in direction of rotate under influence of vestibulo –ocular response. Eye flick back to primary position to produce rotational nystagmus.
- **Rotation stops –**
- nystagmus observed in opp . Direction in 1-2 sec and should then cease due to suppression of post- rotatory nystagmus by fixation.
- If vision severely impaired – post rotation nystagmus does not stop because vestibulo-ocular response is not blocked by visual feedback

- **Preferential looking –**
- Grating acuity often exceeds snellen acuity in amblyopia.
- Teller Acuity Cards – tend to overestimate acuity in strabismic amblyopia.



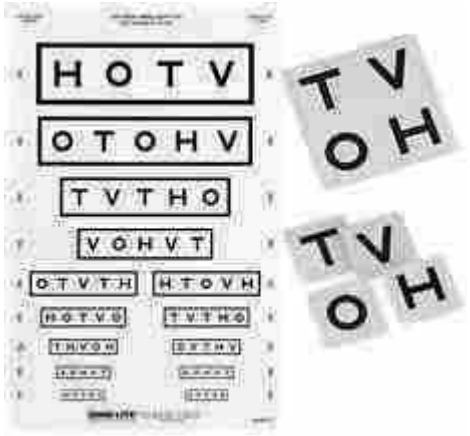
Cardiff acuity cards

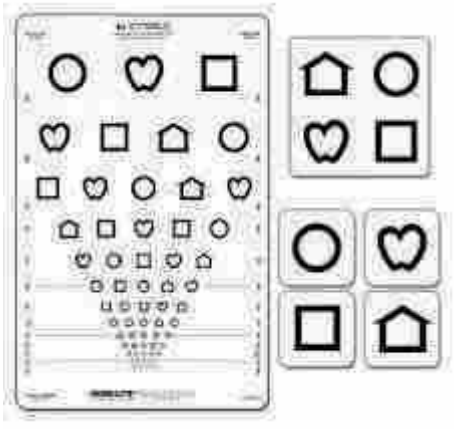
- **pattern visual evoked potential :-**

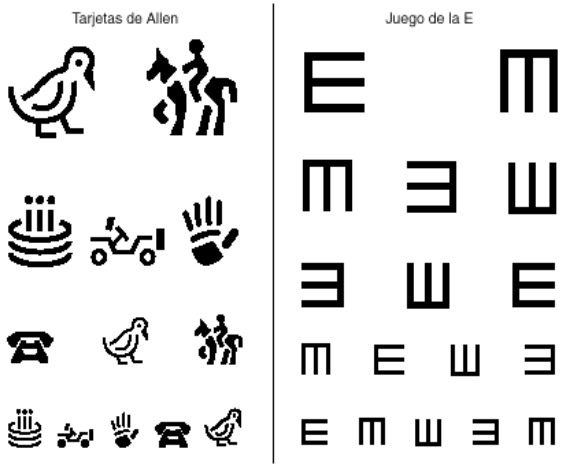
  In amblyopic eye, reduction in amplitude and a normal or slight prolonged latency .

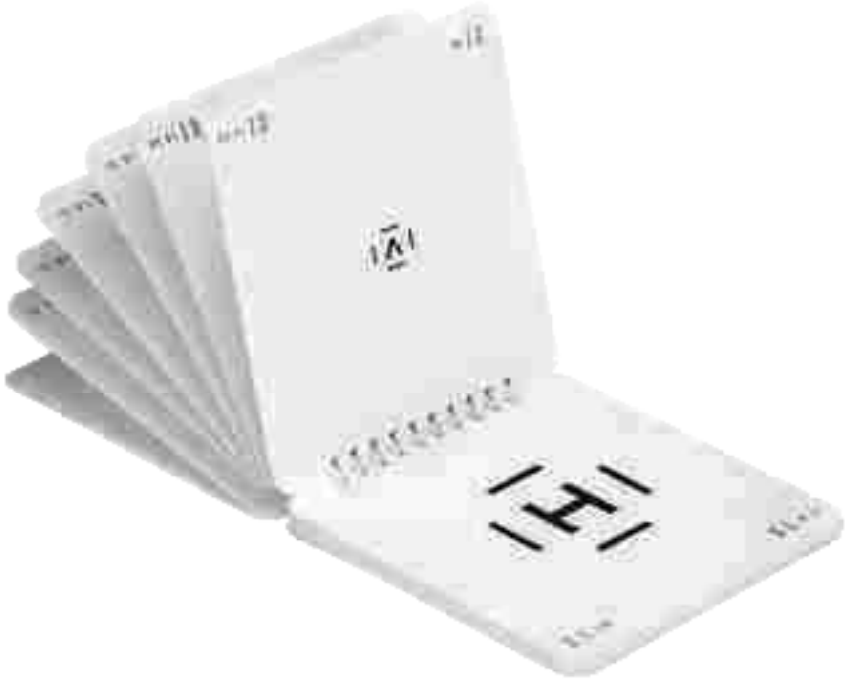
Testing in verbal children

- **Preliterate children –**
- - HOTV, Illiterate E test, Lea symbols, Allen pictures
- - Contour bars must be used wherever possible to reduce overestimation of VA that may occur with isolated optotypes

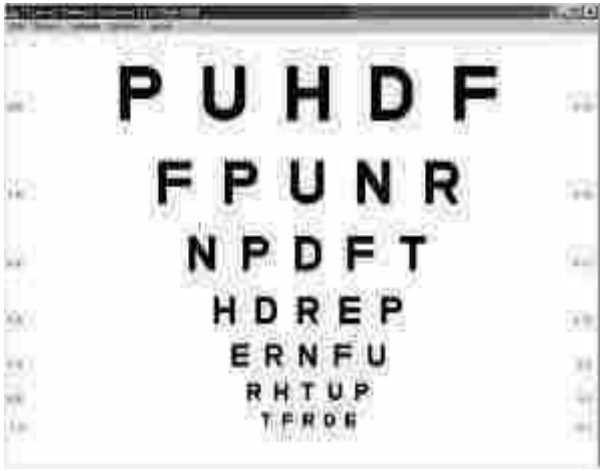






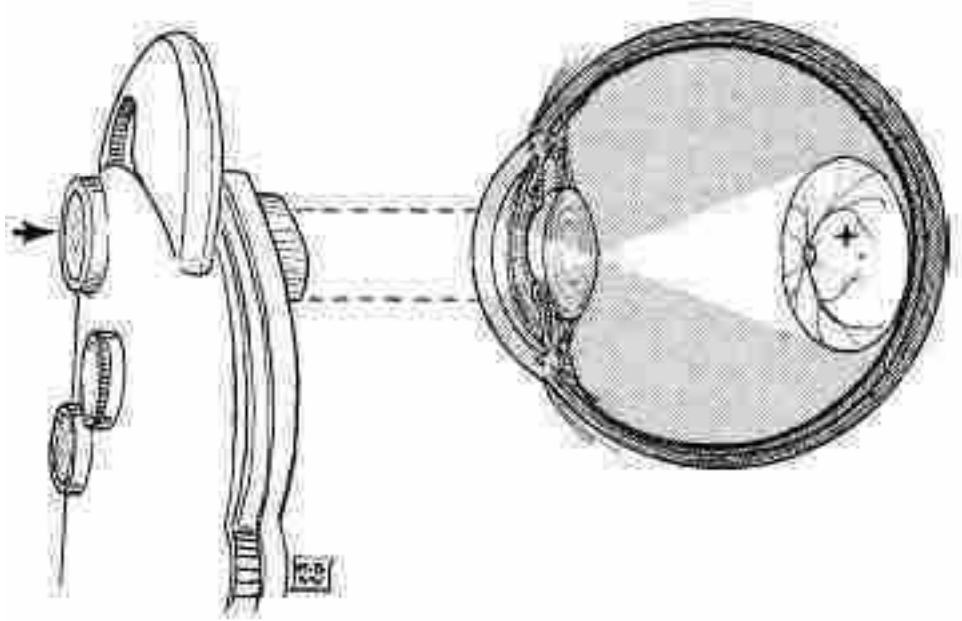


- **Litrate children** :-
- Snellen's chart , keeler logMAR chart.
- Amblyopia can only be accurately diagnosed using a crowded test requiring target recognition .
- logMAR chart is best to measure against which improvement with amblyopia therapy can be assessed.



Other tests :

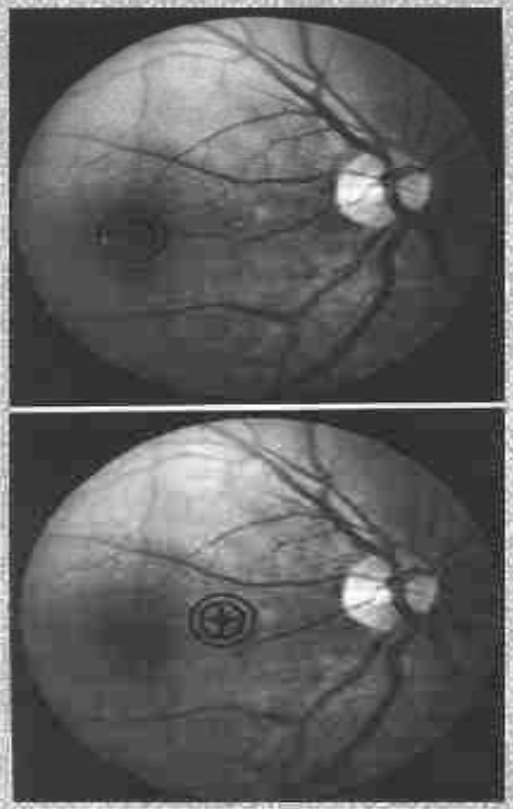
- - Visuscope – Type of direct ophthalmoscope that projects a focused image onto the retina, if pt has central fixation, pt will refixate to place the image on the fovea.
- In cases of eccentric fixation,pt will show wandering, unsteady fixation.



Visuoscopy

central fixation >

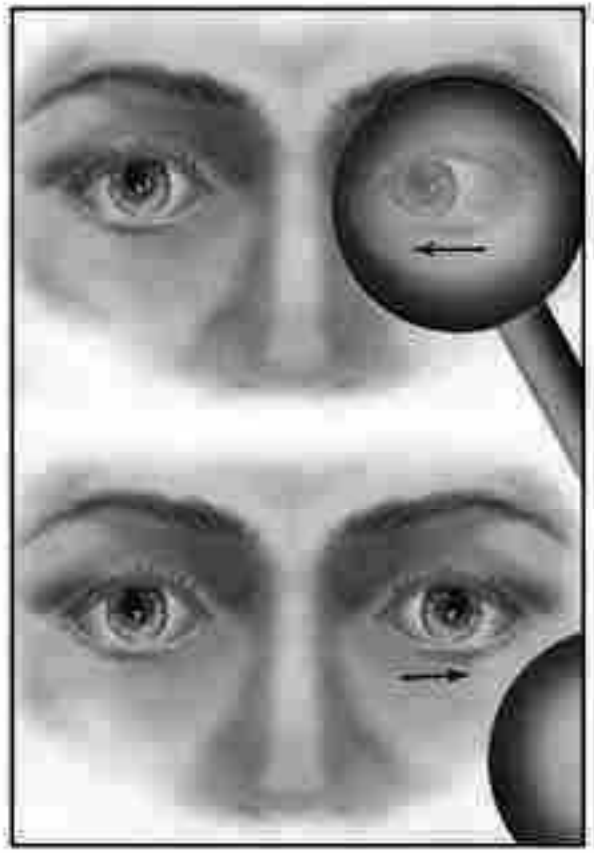
nasal eccentric fixation >



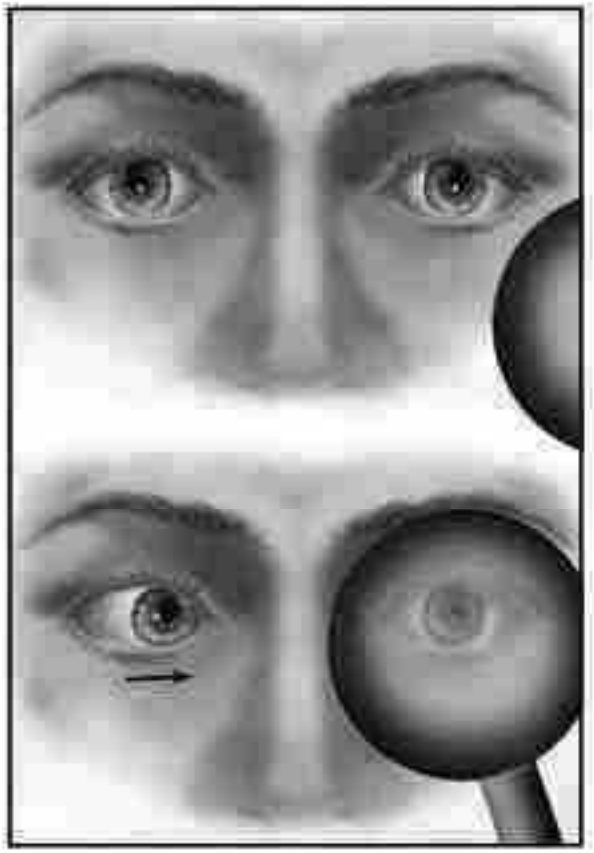
Test for ocular alignment

- Cover test:-

1) Cover-uncover test – detect a heterotropia.
2) Alternate cover test – detect a heterophoria.
3) Prism cover test – measure the angle of deviation on near or distance fixation and in any gaze.
4) Maddox wing – dissociate eye for near fixation and measure heterophoria.
5) Maddox rod- measure heterophoria.



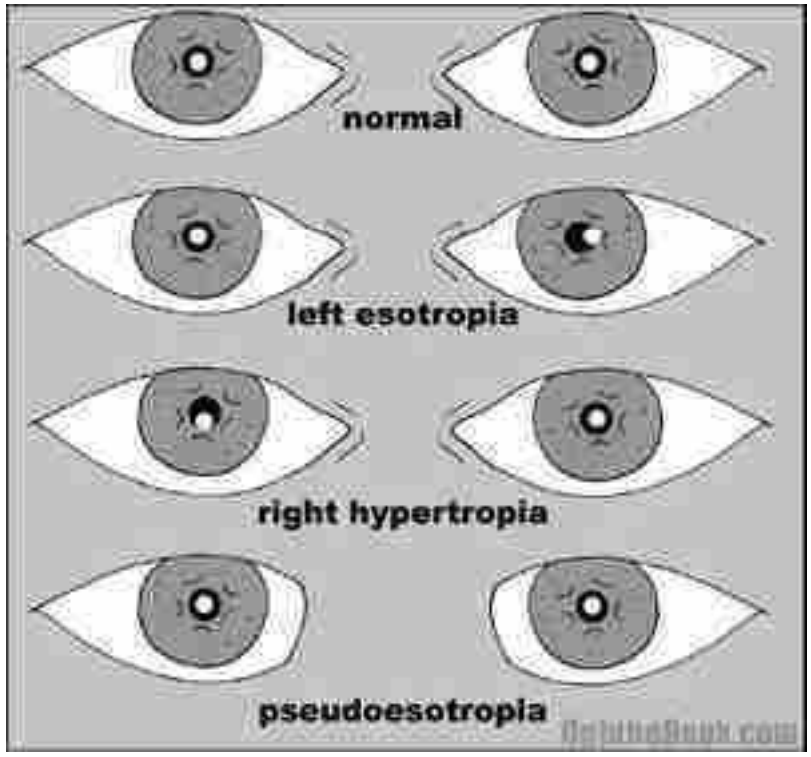
Left covered eye is weaker.
(Left exophoria)



Right uncovered eye is weaker.
(Right esophoria)

Corneal reflex tests –

- **Hirschberg test-**
- Test gives rough objective estimate of the angle of a manifest strabismus .
- Used in young or uncooperative patients or fixation in the deviating eye is poor.
- 1 mm of decentration of the corneal light reflection responds to approx 7° or 15Δ of ocular deviation of visual axis.


normal
left esotropia
right hypertropia
pseudoesotropia

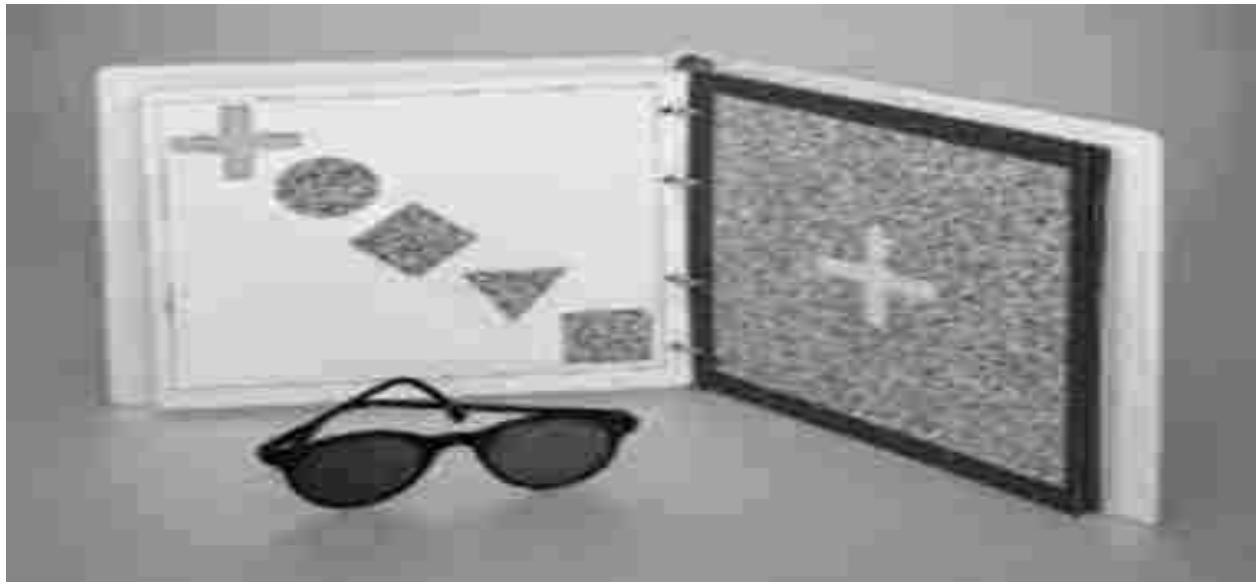
- **- Krimsky test**-
- Placement of prism in front of the fixating eye until the corneal light reflection are symmetrical.
- This test reduces the problem of parallax .

Test for stereopsis

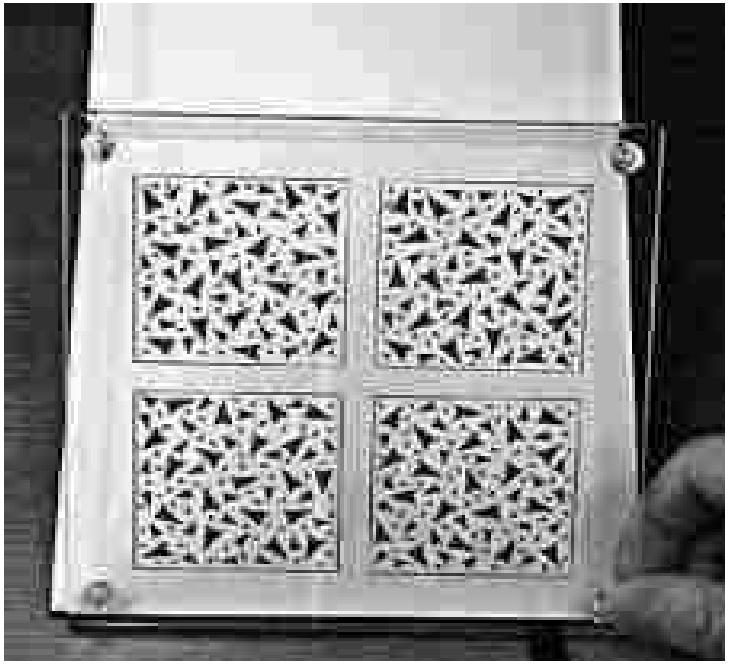
- Stereopsis is measured in seconds of arc (1° = 60 min of arc . 1 min = 60 sec).
- It is useful to remember normal spatial v/a is 1 min and normal steroacuity is 60 sec (which equal to 1 min).
- Lower the value the better the acuity.
- Other test :- TNO , Frisby , lang , titmus.

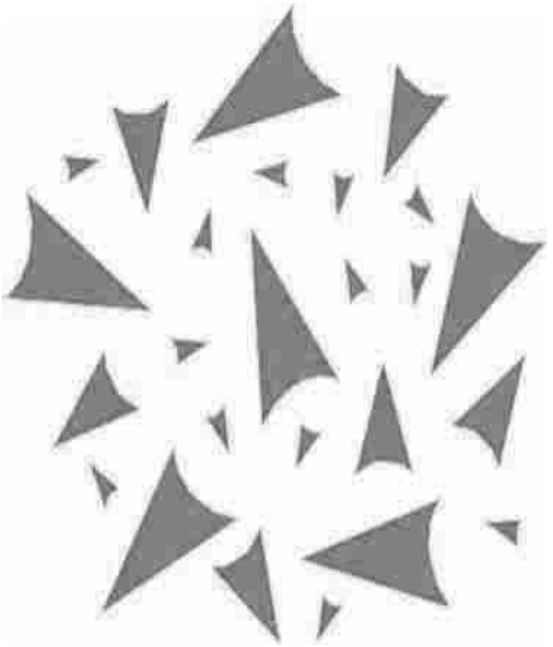
TNO TEST –

- It provides true positive measurement of stereopsis than the titmus , but can give false negative errors when fusion is poor.
- Disparities measure from range from 480 to 15 seconds arc tested at 40 cm.



- Frisby test :-
- Disparities measure range from 600 to 15 seconds of arc.





Lang test:-

- Used to assess stereopsis in very young children and babies , who reach out to touch the pictures.
- The degree of disparity is quite gross , ranging 200-1200 seconds of arc at 40 cm.

- Titmus test;-
- It consist of 3-D Polaroid vectograph consisting of two plates viewed through Polaroid spectacles..
- 1) fly – test for gross stereopsis (3000 seconds of arc). Useful for young children.
- 2) circle :- test fine depth perception.
- 3)animals:- the degree of disparity range from 400 to 100 seconds of arc.

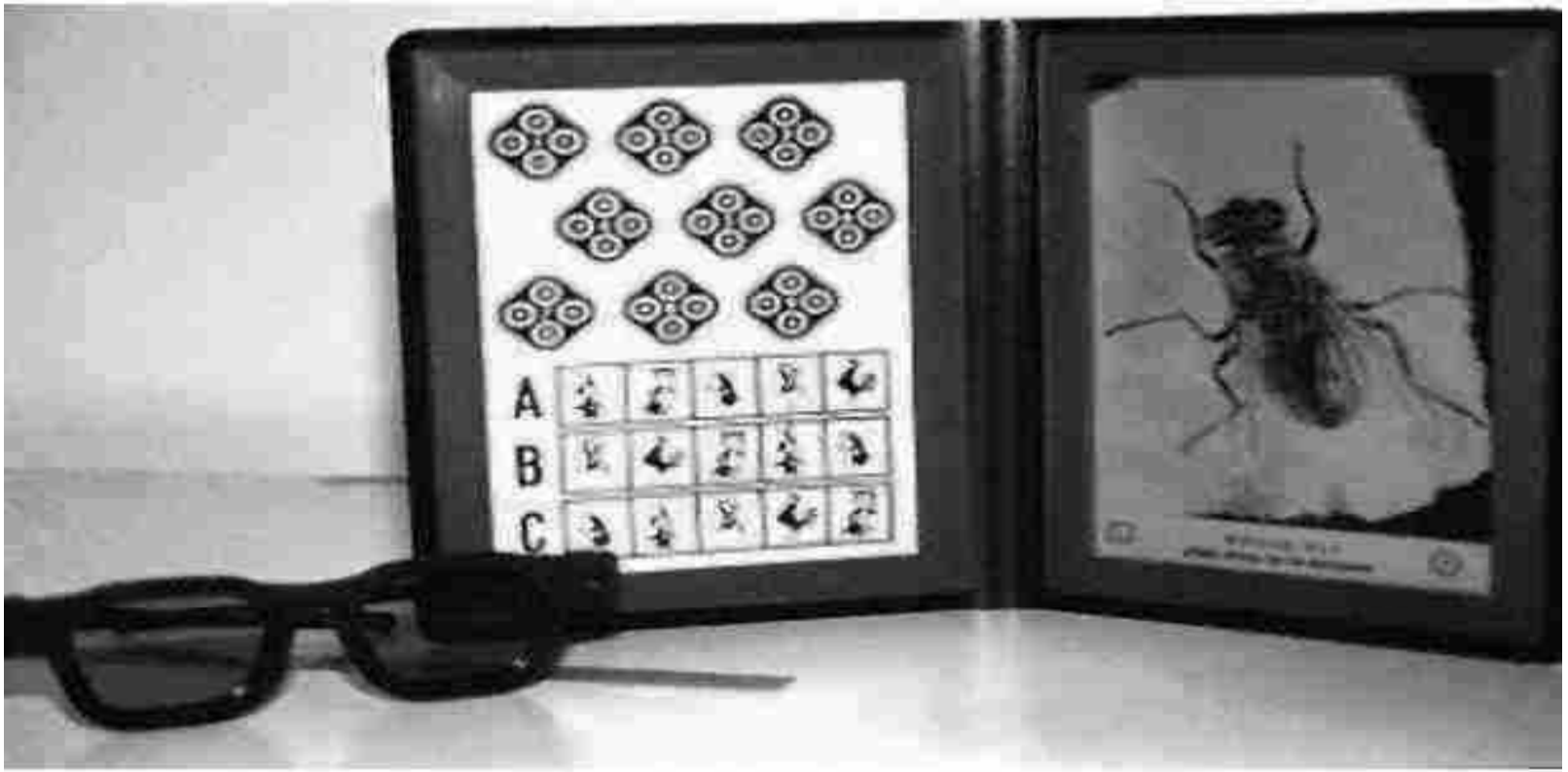


Figure 8. Titmus Fly Stereotest

- **Cyclopegic refraction :-**
- Detailed fundoscopy is mandatory in the context of strabismus , to exclude any underlying ocular pathology such as retinoblastoma or optic disc hypoplegia.

- **Cyclopegic used :-**

-Cyclopentolate-

0.5% under 6 months.

1 % above 6 months

Duration of action –

Maximum cyclopegia within 30 mins.

Recovery of accommodation within 2-3 hours.

Resolution of mydriasis within 24 hours.

- **Atropine –**
- Used in high hypermetropia or heavily pigmented irides.
- 0.5 % under age of 12 months.
- 1 % above 12 months.
- Duration of action :

maximum cyclopegia at 3 hours.'

Recovery of accommodation start after 3 days.

Resolution after 10 days.

Treatment

- Treatment of amblyopia involves following steps:-
- 1) eliminate any obstacles to vision , such as cataract.
- 2) correct any significant error.
- 3) force use of the poorer eye by limiting use of the better eye.

Cataract removal

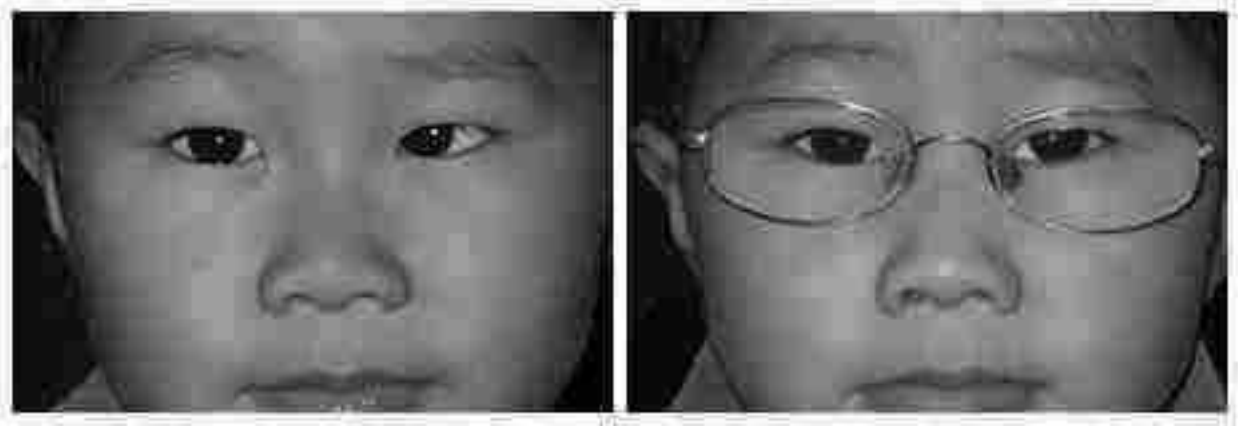
- Removal of congenital lens opacity- first 4 -6 week of life.
- If symmetrical b/l cases- interval b/w 1st & 2nd eye should not be not more than 1-2 weeks.
- Developing severe traumatic cataract in children less than 6 yrs removed within few weeks of injury.

Refractive correction:-

- Refractive correction should be done with cyclopagia for amblyopic eyes
- Cyclopagic drugs used for refractive correction

1. Cyclopentolate
2. Atropine

- It is important to check patient with strabismus at least every year and more frequently in younger children if acuity is reduced



When To Prescribe

- Most children are mildly hypermetropic(1-3D)

1. Hypermetropia –

- 4D of hypermetropia should not be corrected in a child without a squint unless they are having problem with near vision
- With degree of hypermetropia greater than this 2/3rd correction is usually given
- Esotropia full cycloplegic correction should be prescribed even under the age of 2 years

2. Astigmatism :-

- A cylinder of 1.5 diaptore or more should be prescribed especially in cases of anisometropia after the age of 18 months.

3. Myopia :-

- Correction depends on age of child
- Under age of 2 years -5D or more of myopia should be corrected
- 2-4 years of age -3D should be corrected
- Older children should have correction of even low myopia to allow clear distance image

4. Anisometropia:-

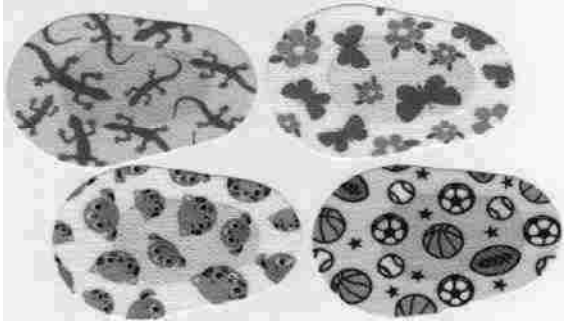
- After the age of 3 years the full difference in refraction between the eyes should be prescribed if it more then 1D

- • Occlusion therapy
- – Gold Standard
- – Dominant eye occluded which forces fixation to amblyopic eye

- Types of occlusion
- - Total occlusion-
- inverse occlusion – amblyopia with eccentric fixation.
- Continual – rarely used , day and night time patching without period of non-occlusion.
- Full time -occlusion during waking hours.
- - Part time occlusion-occlusion for 2-6 hours/day.

  moderate to severe amblyopia at least 6 hrs/day.

- Occlusion devices
- • Patches.



• Micropore tape with soft tissue paper

• Spectacle patch / frost glass

• Doyne's occluder

- • Opaque contact lens

- Occlusion in squinting eye with eccentric fixation:-

1. Inverse occlusion :-

- After 2yr occlusion of sound eye is contraindicated in amblyopes with eccentric fixation because they reinforce anomalous fixation behavior .
- Amblyopic eye should be occluded to break up abnormal fixation behavior this is INVERSE OCCLUSION
- It is use for initial phase and then switch to sound eye occlusion.

2. red filter treatment:-

- Apply Red filter on glass before amblyopic eye.
- Red light entering in amblyopic eye is ineffective in stimulating the retinal area of eccentric fixation.
- Red filter motivate the child to use fovea and inhibits use of eccentric –fixation area.
- Once it became central ,red filter removed and occlusion of sound eye is continued till v/a of amblyopic eye improves

Prisms :

- Use base in or base out prism before amblyopic eye with occlusion of sound eye to break eccentric fixation.
- Base-out prism put before fixating eye in convergent squint to align the deviated amblyopic eye with eccentric fixation.
- This method combined with penalization.

Penalization:-

- Alternate method Due to difficulties encountered in children with occulsion .
- Atropine 1% ointment in fixating sound eye.
- Along with atropinization of sound eye a miotic drop instilled at bedtime in hypermetropic children.
- It is not superior to occlusion.
- It is used for moderate amblyopia.

CAM(cambridge stimulator) TREATMENT

- Slowly rotating high contrast square wave grating of different spatial frequencies
- Principle – rotating gratings provide specific stimulation for cortical neurons
- Use of non linear targets and grey discs instead of wave gratings –equally effective
- the visual improvement was found to be better in emmetropes and hypermetropes than those in strabismus amblyopia

- THANK YOU

ANATOMY OF FOURTH NERVE

Trochlear nerve :

- Fourth cranial nerve entirely motor in function

- Supplies only superior oblique muscle of eyeball

It differs from other cranial nerve in being

1. The only cranial nerve to arise from the dorsal aspect of brain (midbrain)

2. Only cranial nerve to cross completely on other side (4^{th} nerve nucleus innervates contralateral sup oblique muscle)

3. Longest and thinnest of all cranial nerve

Functional components :

1. Somatic efferent :

- Concerned with movement of eyeball thro superior oblique muscle

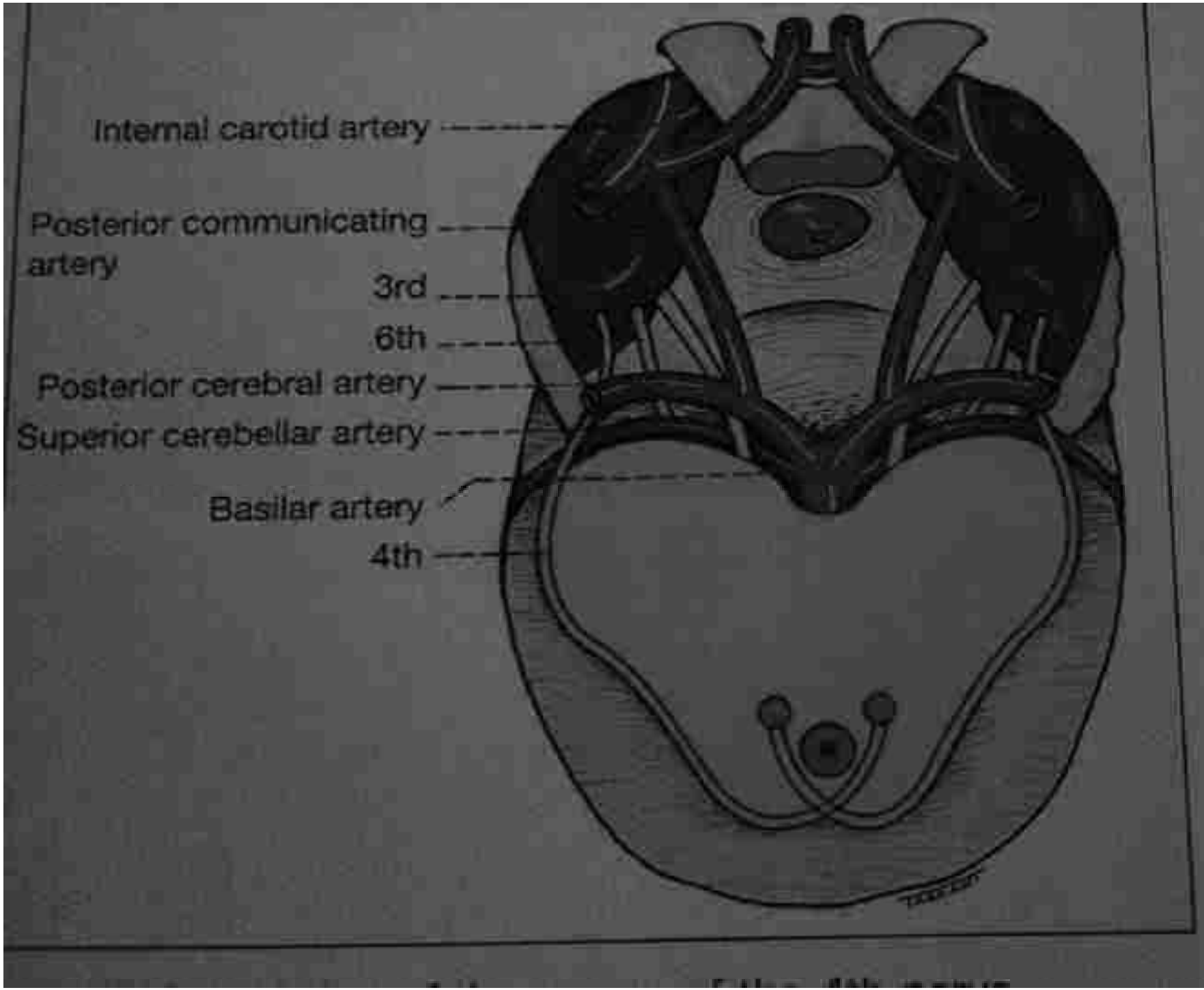
2. General somatic afferent :

- Carries propioceptive impulses from superior oblique muscle

- These impulses relayed to the mesencephalic nucleus of trigeminal nerve

Nucleus :

- Trochlear nucleus situated ventral to sylvian aqueduct
- at the level of inferior colliculus
- caudal to and continuous with third nerve nucleus complex
- closely related to medial longitudinal bundle.


Internal carotid artery
Posterior communicating artery
3rd
6th
Posterior cerebral artery
Superior cerebellar artery
Basilar artery
4th

connections of nucleus :

1. Cerebral cortex :

- Motor cortex (precentral gyrus) thro corticonuclear tracts
- Visual cortex
- Frontal eye fields

2. Nuclei of 3rd, 6 and 8th cranial nerves thro medial longitudinal bundle

3. Superior colliculus thro predorsal bundle

4. Vertical and torsional gaze

5. Cerebellum thro vestibular nuclei

Course and distribution :

1. Fascicular part
2. Precavernous part
3. Intracavernous part
4. Intraorbital part

Fascicular part :

-Consists of efferent fibres which after leaving nucleus , pass posteriorly around aqueduct

-And decussate completely in the anterior medullary velum

Precavernous part :

-Trochlear nerve trunk leaves brainstem just below the inferior colliculus , on dorsal aspectt of midbrain

-Winds around the superior cerebellar peduncle and cerebral peduncle just above pons

-It runs beneath the free edge of tentorium

-Passes between post cerebral and superior cerebellar artery and appear lateral to cerebral peduncle

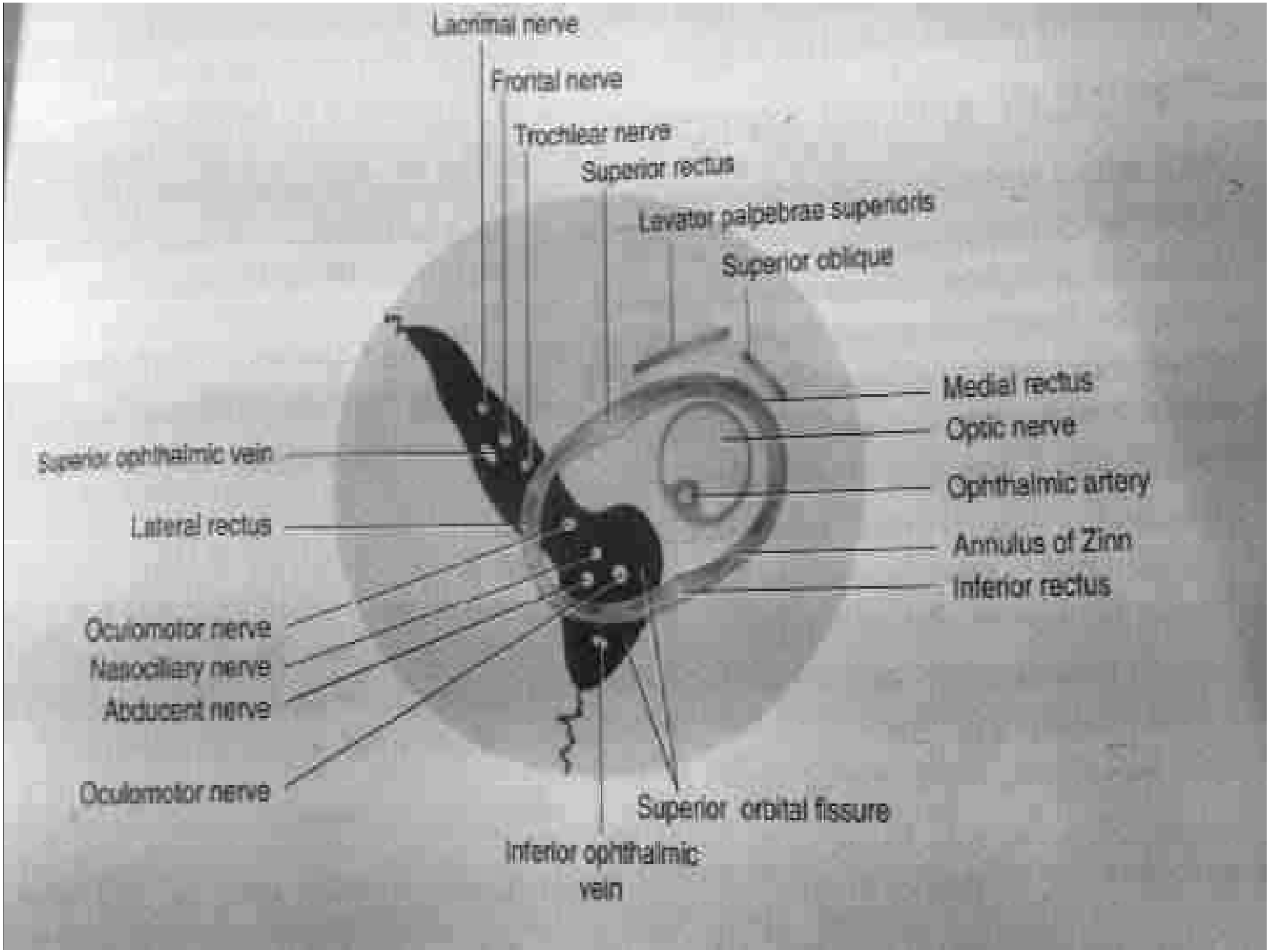
-Then pierces dura n enters the cavernous sinus..

Intracavernous part :

-In cavernous sinus , nerve runs forward in lateral wall lying below the occulomotor nerve and the first division of fifth cranial nerve

-In anterior part of cavernous sinus , crosses over third nerve and leaves sinus to pass thro lateral part of superior orbital fissure.

-In sup orbital fissure it lies superolateral to annulus of zinn and medial to frontal nerve.


Lacrimal nerve
Frontal nerve
Trochlear nerve
Superior rectus
Levator palpebrae superioris
Superior oblique
Medial rectus
Optic nerve
Ophthalmic artery
Annulus of Zinn
Inferior rectus
Superior ophthalmic vein
Lateral rectus
Oculomotor nerve
Nasociliary nerve
Abducent nerve
Oculomotor nerve
Superior orbital fissure
Inferior ophthalmic vein

Intraorbital part :

- After entering orbit , nerve passes medially above origin of LPS and ends by supplying the superior oblique muscles

-Number of fibres in intraorbital part are greater than its intracranial part

-These extra fibres carrying propioceptive impulses from superior oblique muscle leave trochlear nerve to join ophthalmic div of 5^{th} nerve.

- fibres relay in mesecephalic nucleus of 5^{th} nerve

Applied aspects

4^{th} nerve palsy :

"Acute onset of vertical diplopia in absence of ptosis, combined with characteristic head posture strongly suggests 4^{th} nerve palsy "

- Features of nuclear, fascicular, n peripheral 4^{th} nerve palsies are clinically identical expect nuclear palsy produce contralateral superior oblique weakness.

Fourth nerve palsy :

1. Hyperdeviation

- Becomes more obvious when head tilted towards ipsilateral shoulder.
  (Bielschowsky's head tilt test)

2. Ocular movements :

- Depression limited in adduction
- Extorsion is also limited
- Normal depression and elevation.

3. Diplopia :

- homonymous vertical n torsional diplopia , on looking downwards

4. Abnormal head posture :

- To avoid diplopia, head takes posture towards the action of superior oblique muscle.

- Chin depressed and head is tilted towards the oppo shoulder

Bilateral involvement :

- Should always be suspected until proved otherwise

- Right hypertropia in left gaze , left hypertropia in right gaze

- Greater than 10 degree of cyclodeviation on double maddox rod test

- V pattern esotropia

- bilaterally positive bielschowsky test

Special tests :

1. Parks three step test :

First step :

- Assess which eye is hypertropic in primary position

- Consider Left eye is hypertropic.- caused by one of following four muscles

- Weakness of depressors of left eye – sup oblique or inferior rectus

- weakness of elevators of right eye – infe oblique or superior rectus

Step two :

- Determine whether left hypertropia is greater in right gaze or left gaze

- increase on right gaze – left superior oblique or right superior rectus

- increase on left gaze - right inferior oblique or left inferior rectus

Step three :

- The beilschowsky head tilt test

- pt fixating a straight ahead target at 3 mtr.

- head tilted to right and then to left

- increase of left hypertropia on left head tilt – left superior oblique paralysis

- increase of right hypertropia on left head tilt – right inferior rectus weakness.

Double maddox rod test :

-Red and green maddox rods with cylinders vertical , are placed in front of either eye.

- each eye will perceive horizontal line of light

- in cyclodeviation , line perceived by paretic eye – tilted and distinct from that of other eye

- one madox rod then rotated till fusion of line is achieved

-Amount of rotation is measured

- in U/L – less 10 degree and in B/L more than 20 degree of cyclodeviation

Causes of 4th nerve paralysis :

1. Congenital : 40 %

2. Trauma : 34%

- Causes bilateral trochlear palsy due to impact in area of ant medullary velum, where two nerves decussate.

3. Idiopathic :

4. Vascular n neurological causes : aneurysm and tumors rare.

- Ocular myasthenia gravis may as isolated u/l sup oblique paralysis.

Thank you

ANATOMY OF SIXTH NERVE

Abducent nerve :

- small, entirely motor nerve
- supplies lateral rectus muscle of eyeball

Functional components :

1. Somatic efferent :
- Lateral movement of eye

2. General somatic afferent :
- Propioceptive impulses from lateral rectus muscle
- These impulses reach mesencephalic nucleus of trigeminal nerve

Nucleus :

- Abducent nucleus situated in lower part of pons, close to midline, beneath floor of fourth ventricle.

- Closely related to fasciculus of facial nerve

- two types of multipolar cells – large n small

- Large cells : gives fibres of abducent nerve
- Small cells : relay in oculomotor nucleus via medial longitudinal fasciculus n form para-abducent nucleus

-It lies in line with nuclei of 4^{th} and 3^{rd} nerves above and hypoglossal nerve below.

Connections of nucleus :

1. Cerebral nucleus
- Motor cortex
- Visual cortex
- Frontal cortex

2. Nuclei of 3^{rd}, 4^{th}, n 8^{th} nerve thro medial longitudinal bundle

3. Pretectal nucleus

4. Horizontal gaze centre

5. Cerebellum thro vestibular nuclei

Course and distribution :

1. Fascicular part :

- Efferent fibres start from nucleus , pass forward trasversing the medial leminiscus and pyramidal tract

- Then emerge from junction of pons n medulla just lateral to pyramidal prominence by 7-8 rootlets

- Rootlets join to form one nerve

2. Basilar part :

- Runs forward , upwards and laterally thro cisterna pontis between pons and occipital bone

-Nerve runs upwards on back of petrous temporal bone near its apex

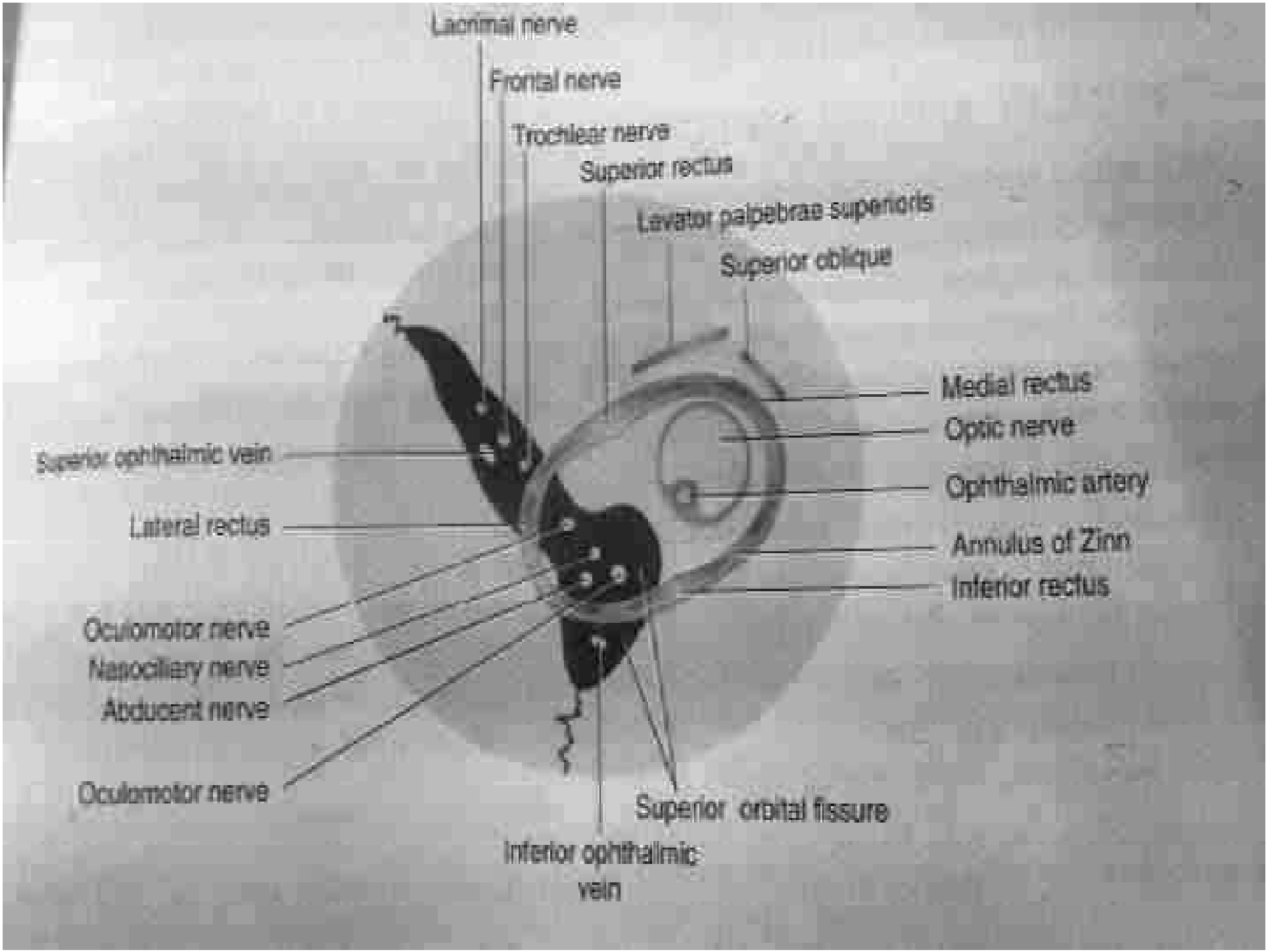
- at upper border of petrous bone , nerve bends forward at right angle under petrosphenoidal ligament and enters cavernous sinus at lateral to dorsum sellae and sup to apex of petrous temporal bone.

3. Intracavernous part :

- In cavernous sinus , nerve runs horizontally forwards and ocupying position below n lateral to internal carotid artery.

- Nerve leaves cavernous sinus , enter orbit thro middle part of superior orbital fissure within annulus of zinn.

- In superior orbital fissure, abducent nerve lies inferolateral to occulomotor and nasociliary nerve.


Lacrimal nerve
Frontal nerve
Trochlear nerve
Superior rectus
Levator palpebrae superioris
Superior oblique
Medial rectus
Optic nerve
Ophthalmic artery
Annulus of Zinn
Inferior rectus
Superior ophthalmic vein
Lateral rectus
Oculomotor nerve
Nasociliary nerve
Abducent nerve
Oculomotor nerve
Superior orbital fissure
Inferior ophthalmic vein

4. Intraorbital part :

- In orbit , nerve runs forwards , divides into 3-4 branches , enters ocular surface of lateral rectus muscle in its middle part.

Applied aspects

Sixth nerve palsy :

1. Deviation :
- In primary position, eyeball converged due to unopposed action of medial rectus muscle

2. Ocular movements :
- Limited abduction

3. Diplopia :
- Uncrossed horizontal diplopia , worse towards action of paralysed muscle.

4. Head posture :
- Face turned towards the action of paralysed muscle to minimize diplopia

Lesions at various levels :

1. Supranuclear lesions :

- Loss of conjugate movements of eyeball

2. Nuclear lesions :

- Isolated sixth nerve palsy

Lesion in n around 6^{th} nerve nucleus causes

- Ipsilateral 6^{th} nerve palsy
- Ipsilateral 7^{th} nerve palsy of upper motor neuron due to involvement of facial fasciculus
- Loss of conjugate movement to same side – involvement of horizontal gaze centre in PPRF.- pontine paramedian reticular formation.

3.Fascicular lesion :

foville’s syndrome : lesion of dorsal pons

- Ipsilateral 6th nerve pasy
- ipsilateral facial nerve palsy
- Facial analgesia from involvement of fifth nerve
- deafness

Millard gubler syndrome :
Lesion of ventral pons involvement of fasciculus as passes thro pyramidal tracts

- Ipsi 6th nerve palsy
- contralateral hemiplegia
- signs of dorsal pontine lesion

4. Lesion of basilar part :

Causes :- acoustic neuroma- first symptom hearing loss
first sign is diminised corneal sensations

- nasopharyngeal tumor
- fracture of base of skull

Gradenigo syndrome :
-Ipsi 6th nerve palsy
- deafness
- neuralgia in first division of trigeminal nerve
- facial weakness

5. Lesions of intracavernous part :

-6th nerve runs thro middle of cavernous sinus , most prone to damage than other cranial nerves

- causes :
-Aneurysms
- meningioma
- carotid cavernous fistulae
- granulomatous inflammation
- vascular causes : diabetes, hypertension

- In intracavernous part- 6th nerve joined by sympathetic branch from paracarotid plexus . Thus 6th nerve palsy a/w horner’s syndrome

6. Lesion of intraorbital part :

-Not so common

- causes : orbital apex syndrome and superior orbital fissure syndrome.

Thank you

Introduction To Pathology

The Discipline Of Pathology

CBME PA-1.1

What Is Pathology

- Pathology literally is the study (logos) of suffering (pathos)
- Pathology is the scientific study of disease
- Pathology is the foundation of medical science & practice
- Pathology is a bridging discipline devoted to the study of the structure & functional changes in cells, tissues & organs that underlie diseases

- Pathology attempts to explore the “ whys” and “ wherefores” of the signs and symptoms of diseases
- Pathology much of it has a visible expressions _ Radiographs

  _CT-scans

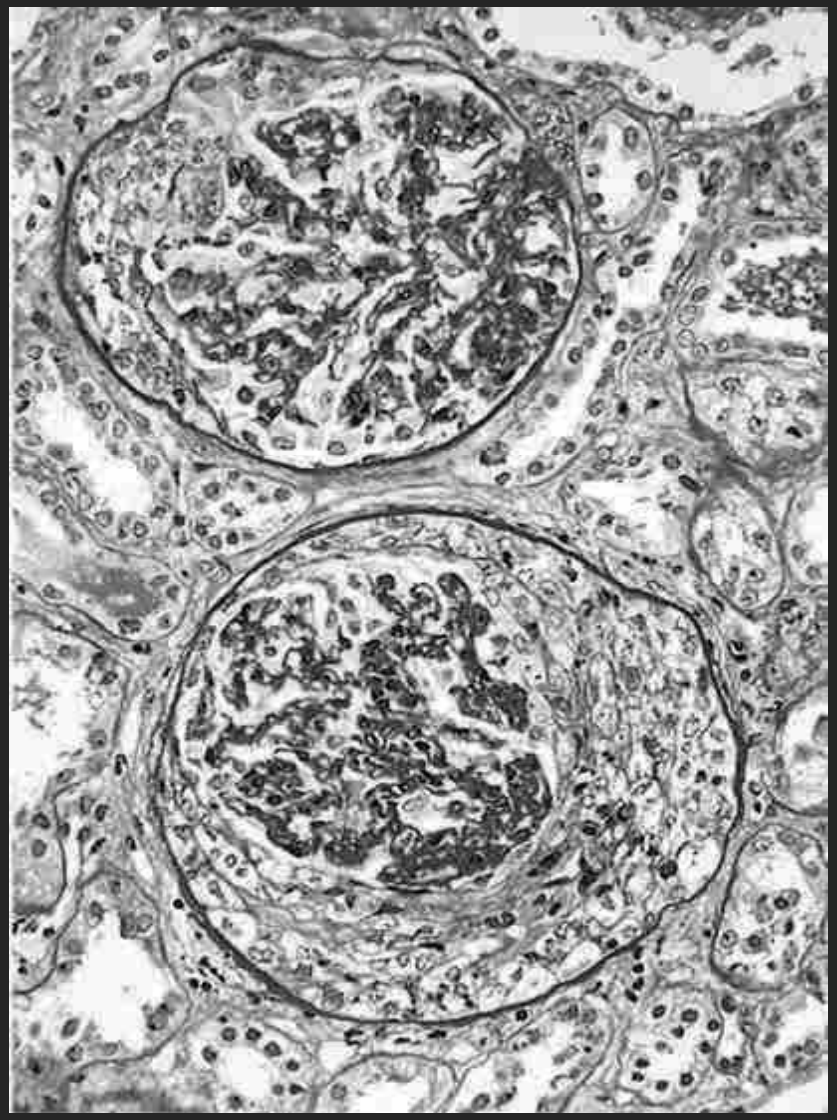
  _MRI (magnetic resonance imaging)

  _Ultrasound

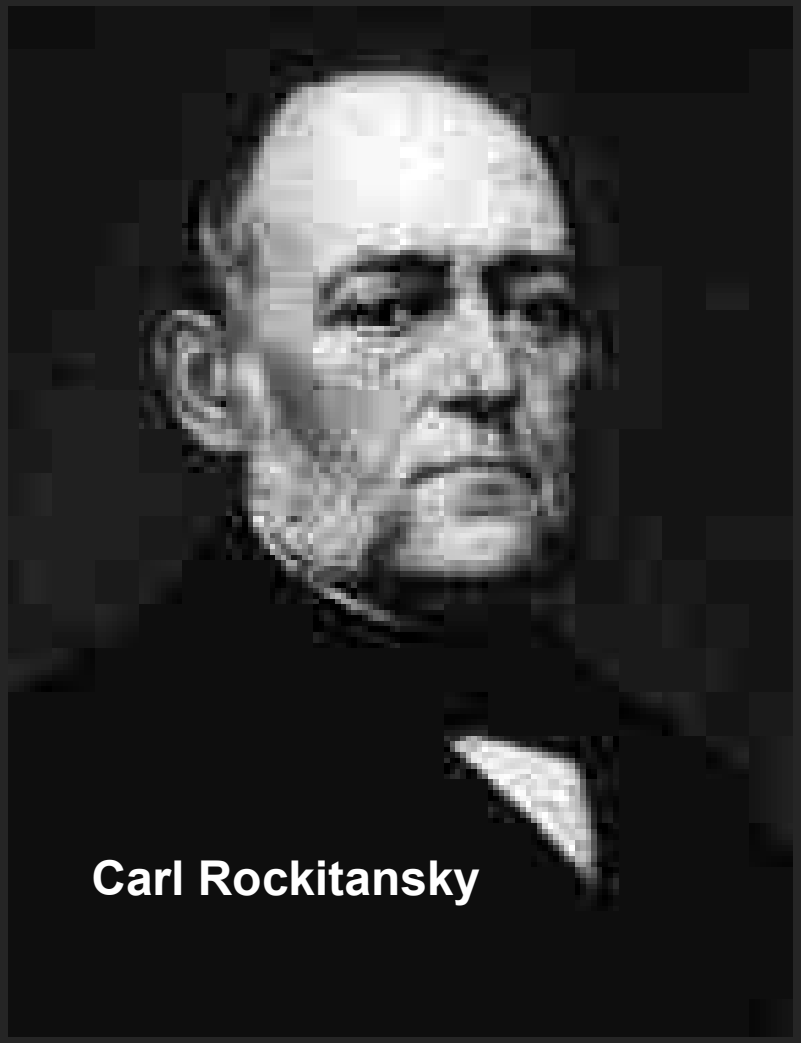
  _Clear drawings

  MOLECULAR abnormalities

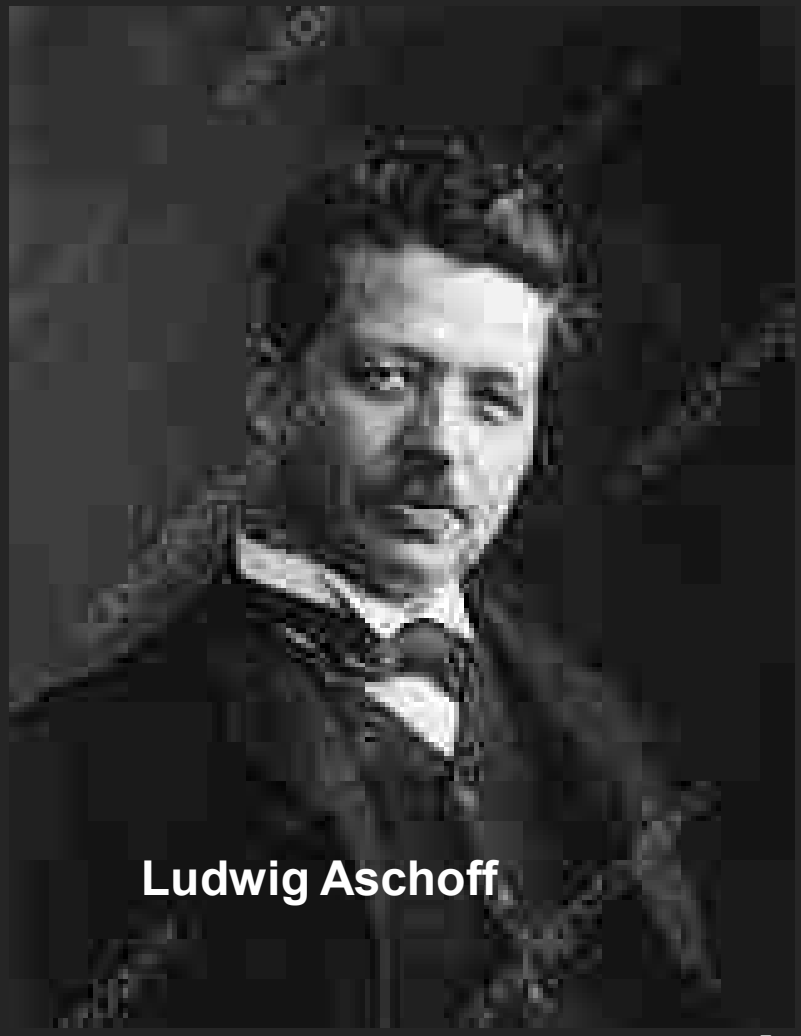
Pathology explores " whys" and " wherefores" of the signs and symptoms of diseases



History Of Pathology-
Autopsies performed scientifically



Carl Rockitansky

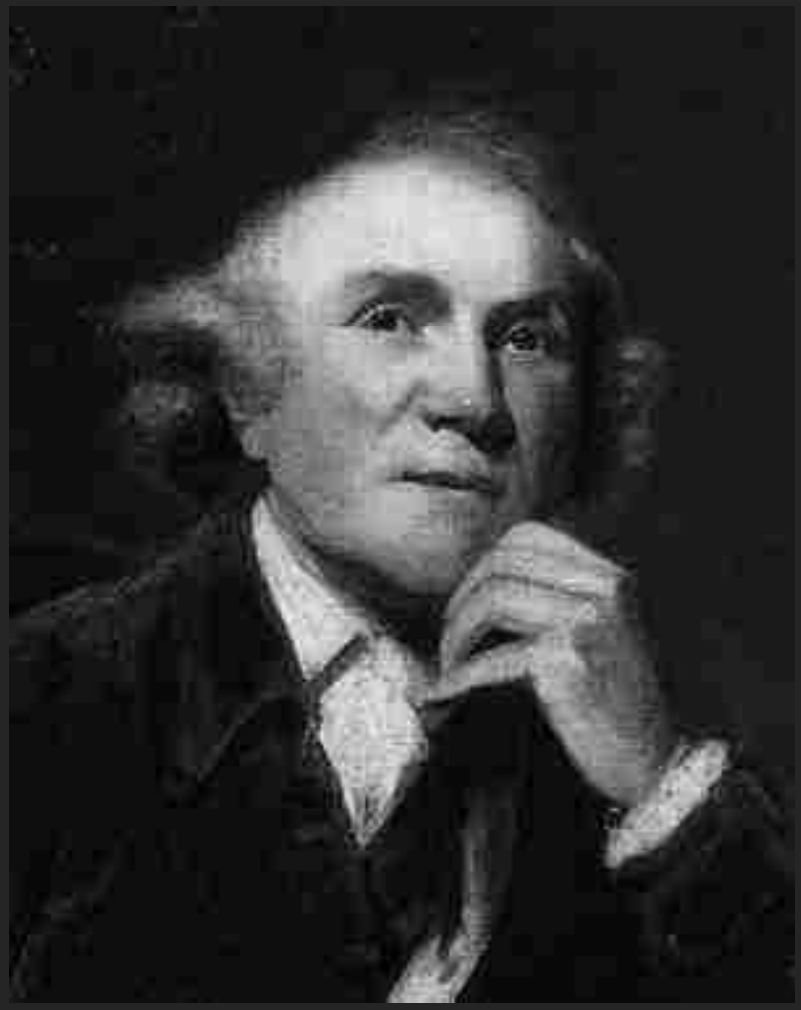


Ludwig Aschoff

History Of Pathology

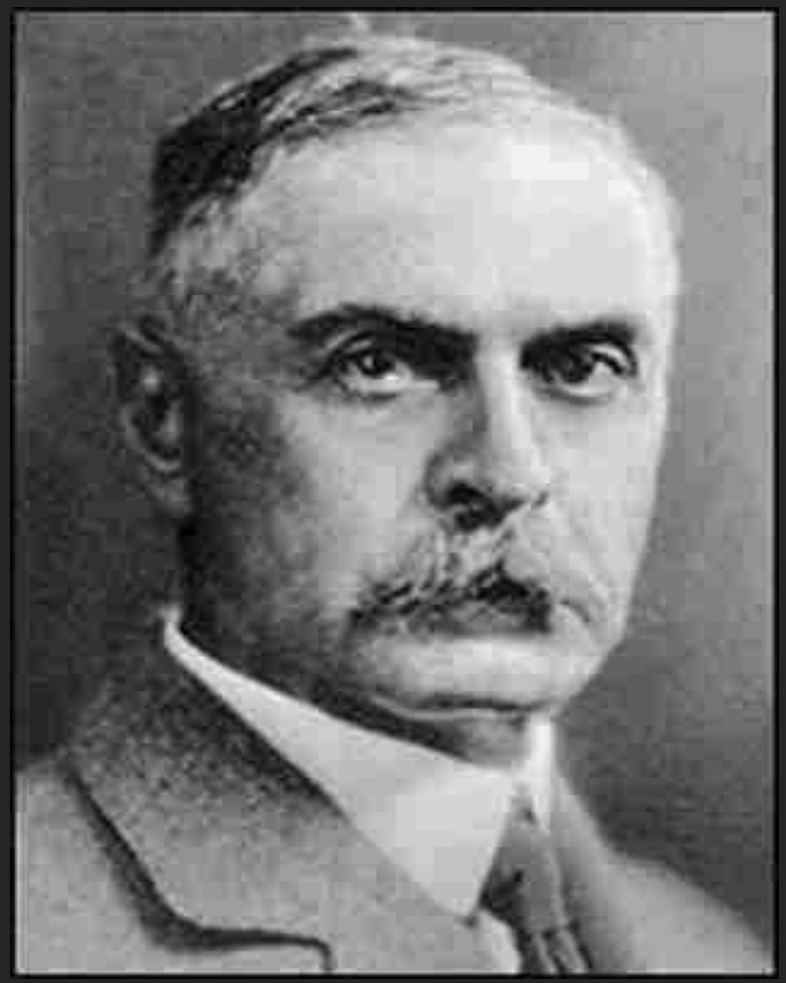
Rudolf Virchow (1821-1902)
CELLULAR PATHOLOGY

John Hunter (1728-1793)
Pathology museum



History Of Pathology

Karl Landsteiner (1863-1943)



George Papanicolaou (1883-1962)

Subdivisions of Pathology

- Histopathology: examination of tissues
- Cytopathology: examination of isolated cells
- Haematology: examination of blood and components
- Chemical pathology : analysis in tissues & fluids
- Forensic pathology: pathology for legal purposes
- Toxicology: effects of known or suspected poisons

Techniques Of pathology

- Light Microscopy- the structure of tissues & cells in health & disease
- Histochemistry- is the study of the chemistry of tissues
- Immunohistochemistry- employ antibodies
- (immunoglobulins with antigen specificity) to visualize substances in tissues sections or cell preparations

- Electron Microscopy- study of disorders at an organelle level, & to the demonstration of viruses in tissue samples
- Biochemical techniques-fluid & electrolyte homeostasis, serum enzyme assays e.g.raised levels of cardiac enzymes in the blood
- Haematological techniques- in the diagnosis & study of blood disorders

- **Molecular Pathology-many advances come from the relatively new science of molecular pathology**

  Defects in the chemical structure of molecules arising from errors in the genome, using IN SITU HYBRIDISATION e.g. chromosomal molecule, alterations in the genome governing the control of cell & tissue growth

General & Systemic Pathology

- **General Pathology: is our current understanding of the causative mechanisms and characteristics of the major categories of disease (congenital, acquired, degenerations, inflammation & tumors)**

  - **General Pathology is the foundation of knowledge that has to be laid down**

- **Systemic Pathology is our current knowledge of specific diseases as they affect individual organs or systems**

- **Oropharyngeal , GIT, liver, spleen**
- **RS, CVS, GUT, CNS, endocrine**

Pathology learning

Text book

Didactic lectures

Tutorials

Demonstrations(gross & microscopy)

Post – mortem teaching

Problem- based learning

Practical hands on training

E learning

Specific Learning Objectives

- Definition of the pathology
- Nomenclature and Nosology
- Incidence & epidemiology (agent, host, environment)
- Etiology (causes) & Pathogenesis (mechanisms)
- Classification & clinical features
- Morphology- Gross & microscopy
- Sequelae, complications & prognosis
- Diagnosis & Investigations
- Clinico- pathological correlation

Diagnostic Pathology

- **Histopathologic examination-**

1)Biopsies are samples of tissue removed from a patient for diagnostic purposes (needle biopsy, endoscopic biopsy and incisional biopsy)

2)Resections specimens are the whole or part of an organ removed for a previously diagnosed condition.

3) Autopsy- cause of death, audits and education.

Cytology- Examination of dispersed cells

- Exfoliative cytology: cells shed from, or scraped or brushed off ,an epithelial surface
- Fluid cytology :cells withdrawn with the fluid in which they are suspended
- Washings: cells flushed out of an organ using an irrigating fluid
- Fine –needle aspiration cytology: cells sucked out of a solid tissue using a thin needle attached to a syringe.

Cell Injury

- Functional and morphological effects of variety of stresses due to aetiological agents
- Resulting in changes of internal & external environment.

Cellular responses to stress vary & depends upon

1. Host factors: type of cell & tissue involved
2. Factors pertaining to injurious agent : extent & type of cell injury.

Causes of cell injury

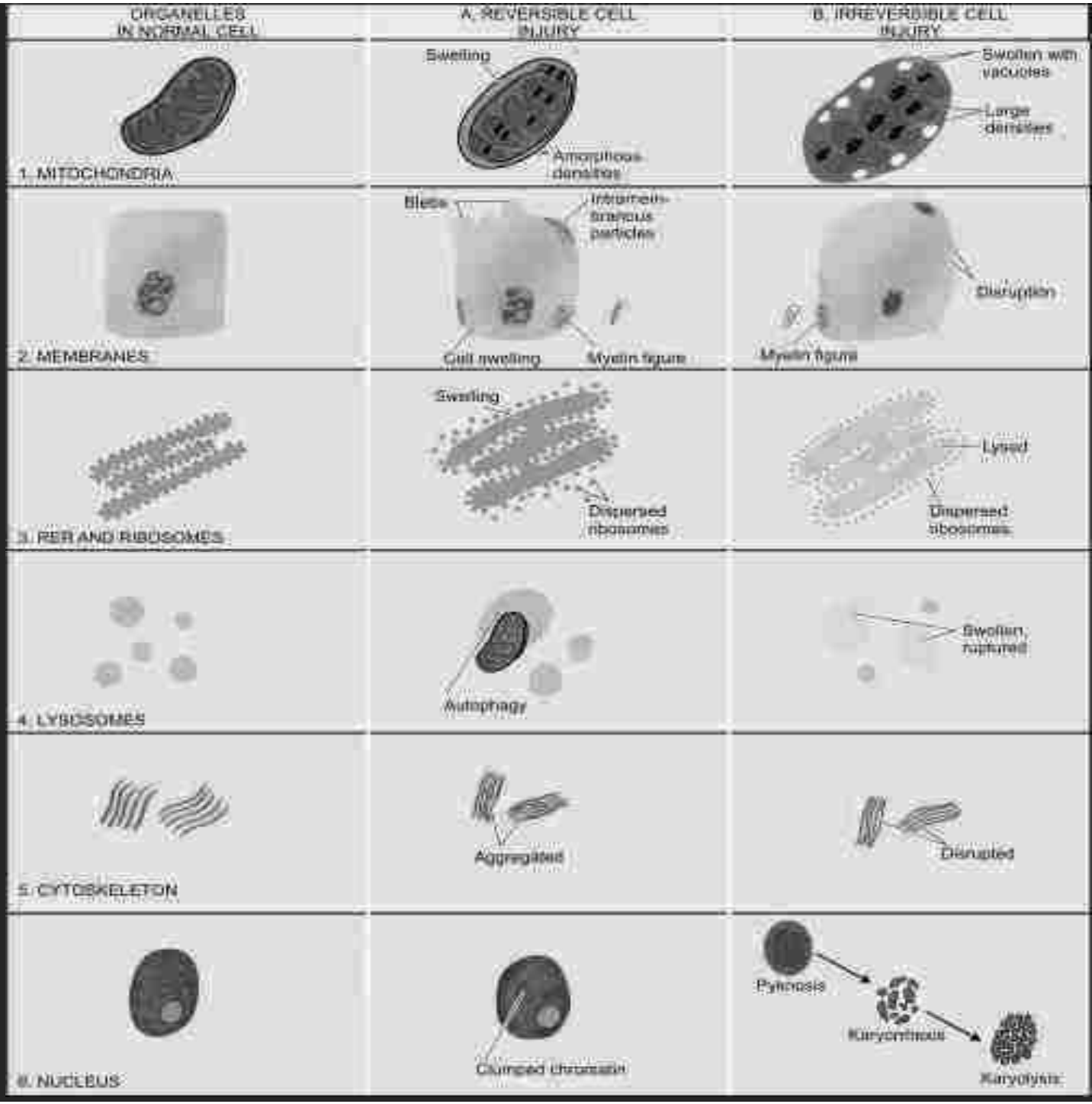
A. Genetic causes

B. Acquired causes –

- Hypoxia- anoxic, stagnant, histotoxic
- Ischemia- local (embolus),systemic (cardiac)
- Physical agents- trauma, heat, cold, pressure, electrical
- Chemical agents- acids, alkalis, escharotics,poisons
- Alcohol & Drugs- abnormal metabolites

Causes of cell injury

- Microbial agents-bacteria, viruses, fungi, parasites
- Immunological agents-hypersensitivity, autoimmunity
- Nutritional derangements- deficiency/ excess malnutrition
- Psychological factors- stress, strain, anxiety, depression, schizophrenia
- Iatrogenic


ORGANELLES IN NORMAL CELL
A. REVERSIBLE CELL INJURY
B. IRREVERSIBLE CELL INJURY
1. MITOCHONDRIA
Swelling
Amorphous densities
Swollen with vacuoles
Large densities
2. MEMBRANES
Blebs
Intramembranous particles
Cell swelling
Myelin figure
Disruption
Myelin figure
3. RER AND RIBOSOMES
Swelling
Dispersed ribosomes
Lysed
Dispersed ribosomes
4. LYSOSOMES
Autophagy
Swollen, ruptured
5. CYTOSKELETON
Aggregated
Disrupted
6. NUCLEUS
Clumped chromatin
Pyknosis
Karyorrhexis
Karyolysis

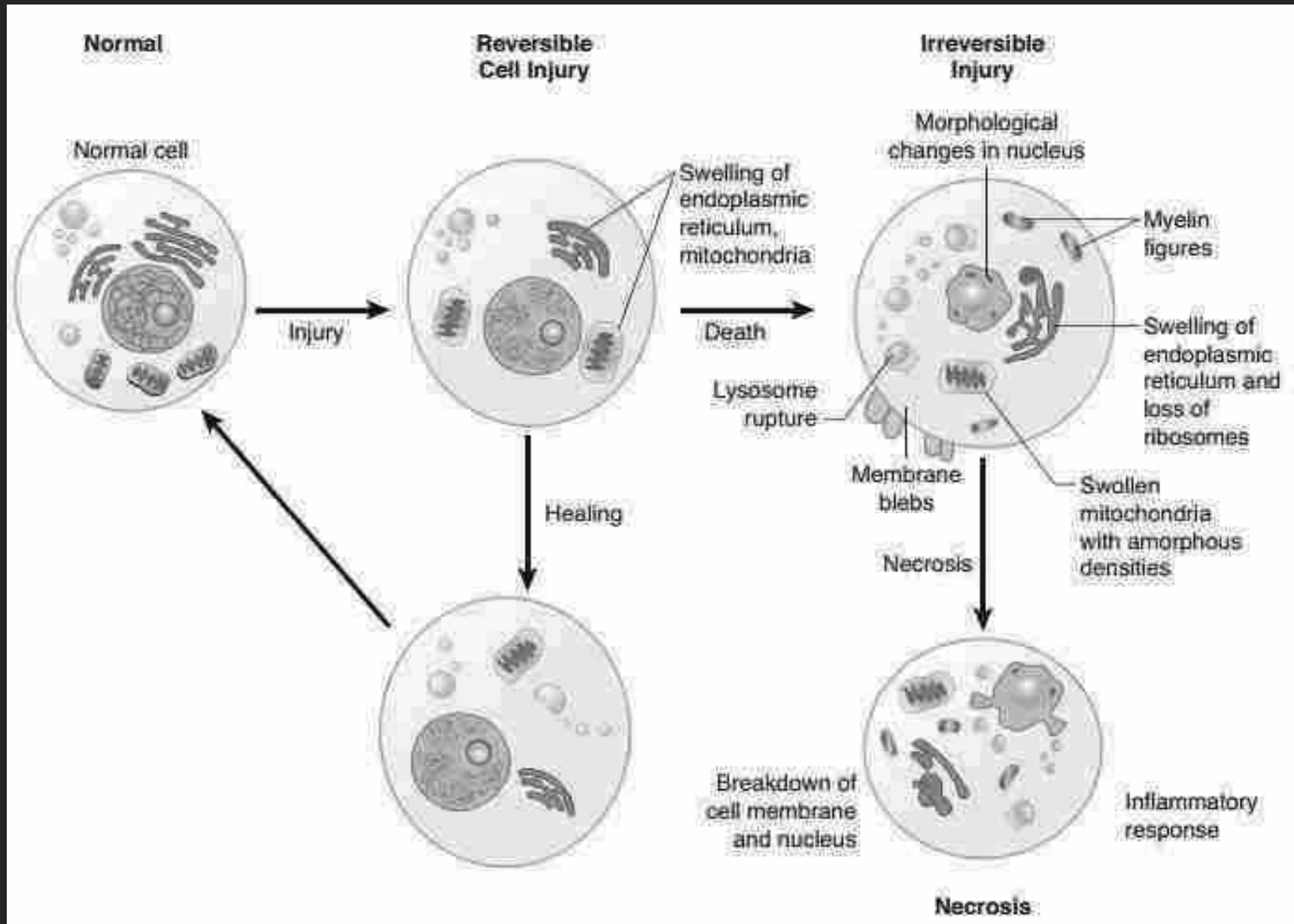


Figure 2-4. Cell injury

Stages in cellular response to stress and injurious stimuli

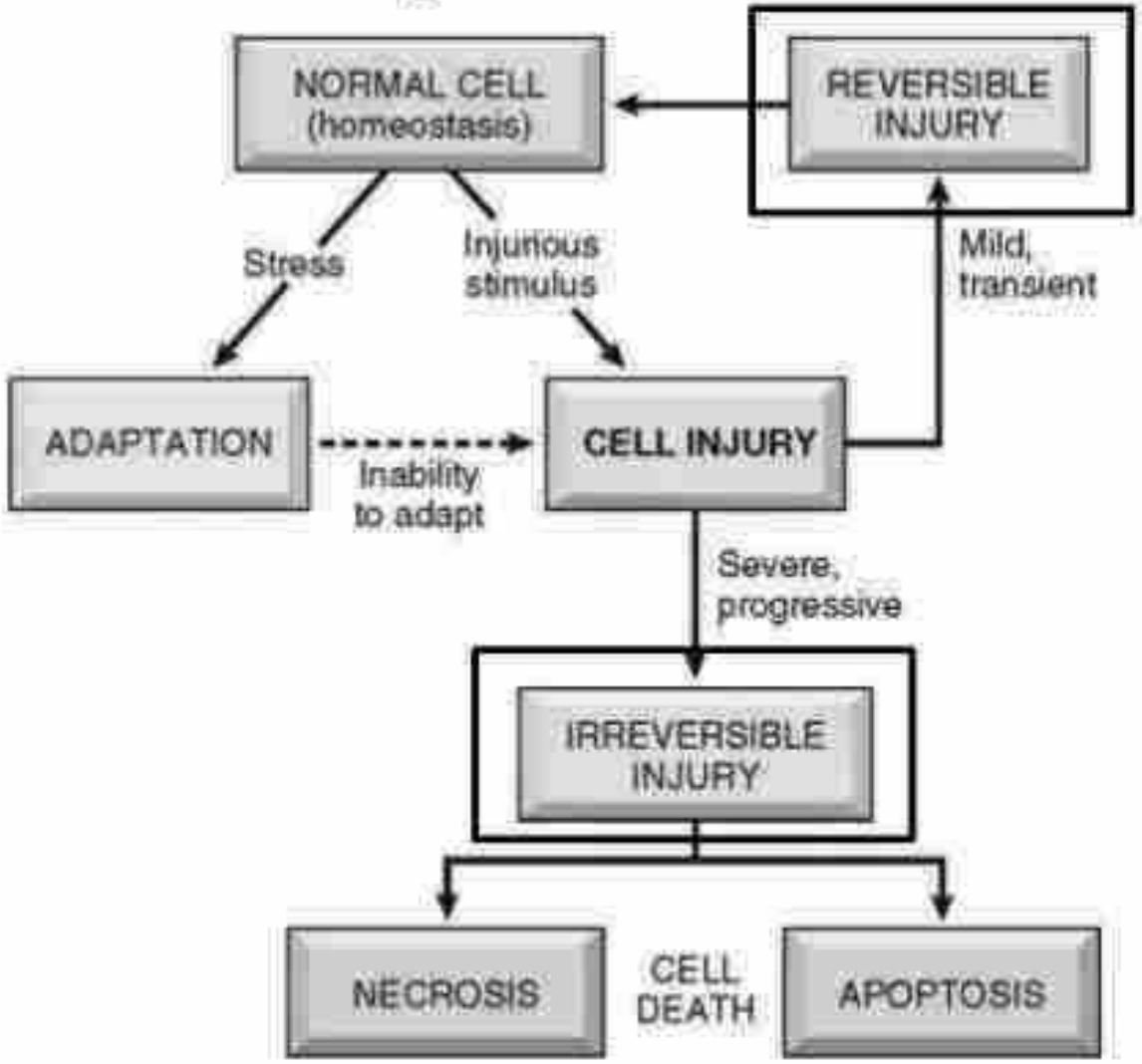


Fig 1.1 (5)

Causes of cell injury

PA 2.1

Introduction

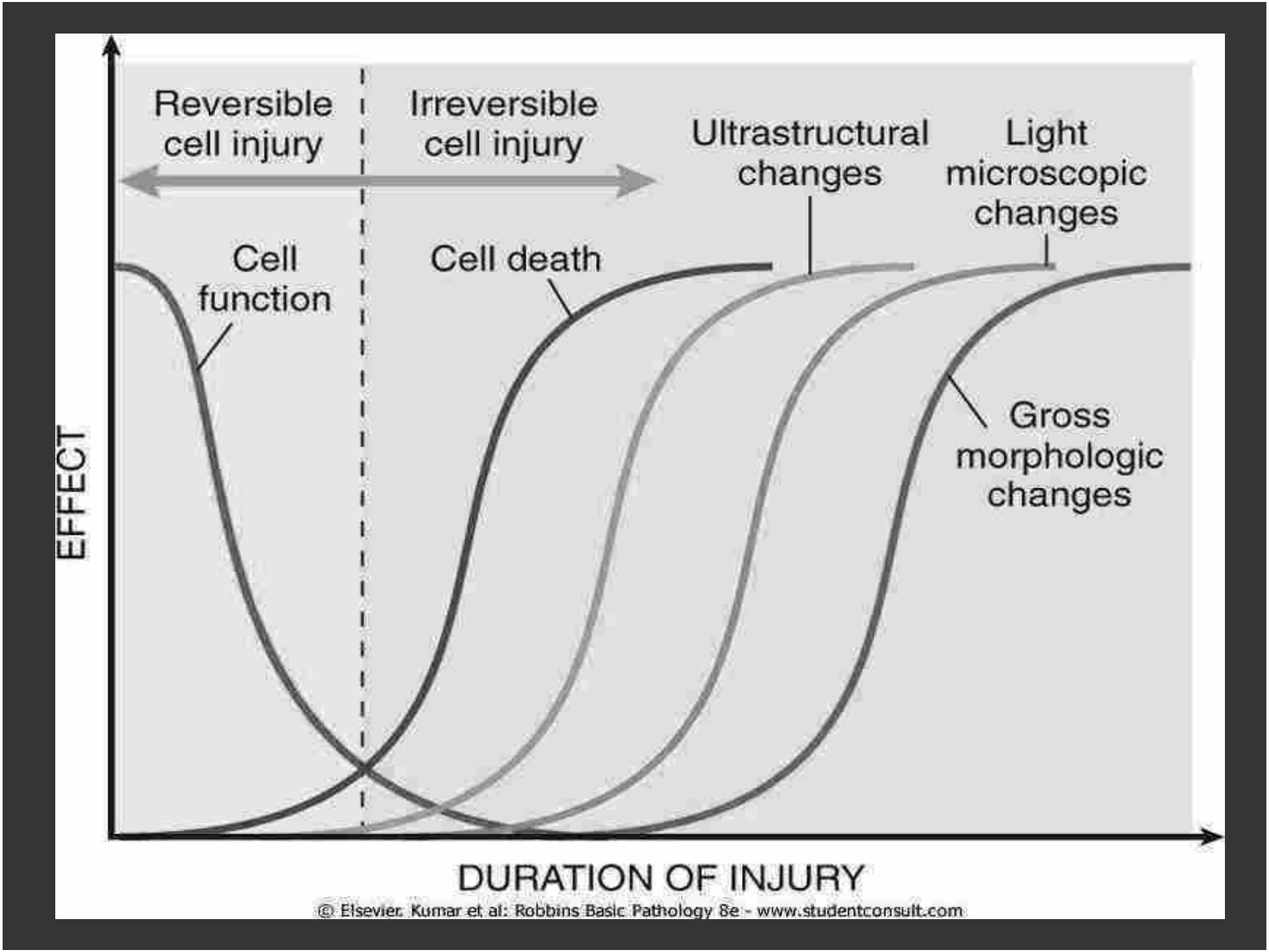
- **Cell injury is defined as a variety of stresses a cell encounters as a result of changes in its internal and external environment.**
- **Virchow's Cellular theory of disease (1859): diseases occur due to abnormalities at the level of cells**
- **The cellular response to stress may vary and depends upon the following:**
  - **The type of cell and tissue involved.**
  - **Extent and type of cell injury**

Cell Injury

- Functional and morphological effects of variety of stresses due to aetiological agents
- Resulting in changes of internal & external environment.

Cellular responses to stress vary & depends upon

1. Host factors: type of cell & tissue involved
2. Factors pertaining to injurious agent : extent & type of cell injury.


Reversible cell injury
Irreversible cell injury
Ultrastructural changes
Light microscopic changes
Cell function
Cell death
Gross morphologic changes
EFFECT
DURATION OF INJURY
© Elsevier. Kumar et al: Robbins Basic Pathology 8e - www.studentconsult.com

ETIOLOGY OF CELL INJURY

Genetic causes

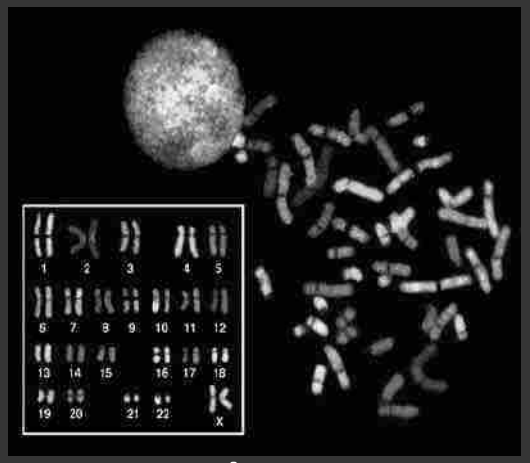
- Developmental defects:
- Errors in morphogenesis
- Cytogenetic (Karyotypic) defects: chromosomal abnormalities
- Single-gene defects:
- Mendelian disorders
- Multifactorial inheritance disorders

Acquired causes

- Hypoxia and ischaemia
- Physical agents
- Chemical agents /drugs
- Microbial agents
- Immunologic agents
- Nutritional derangements
- Ageing
- Psychogenic diseases
- Iatrogenic factors

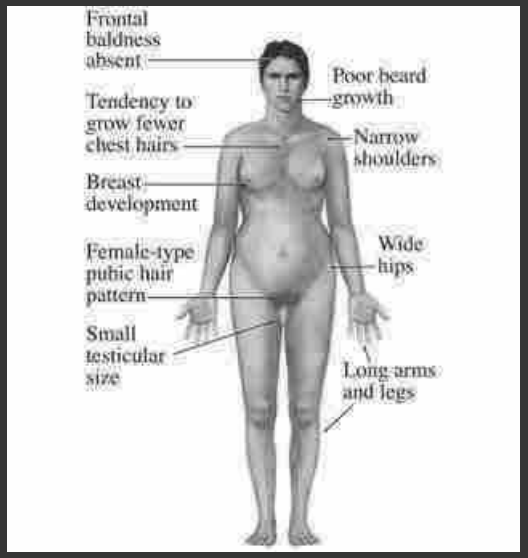
Genetic Causes

Hurler Syndrome



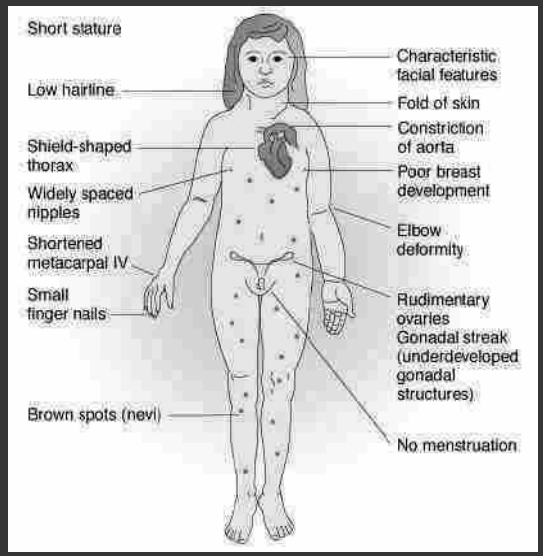
Spectral Karyotype

Hunter Syndrome



Klinefelter Syndrome
(XXY, XXXY)

Down's Syndrome
(Trisomy 21)



Turner Syndrome
(X0)

Oxygen deprivation- Hypoxia, Ischaemia

- **Inadequte oxygenation-** Cardio respiratory failure
- **Reduced oxygen carrying capacity -**Anemia or CO poisoning

- Loss of oxidative phosphorylation and ATP generation by mitochondria.
- Decreased ATP (with increase in AMP): stimulating fructokinase and phosphorylation, resulting in aerobic glycolysis.
- Depleted glycogen.
- Reduced intracellular pH: Lactic acid and inorganic phosphate.
- Clumping of nuclear chromatin.

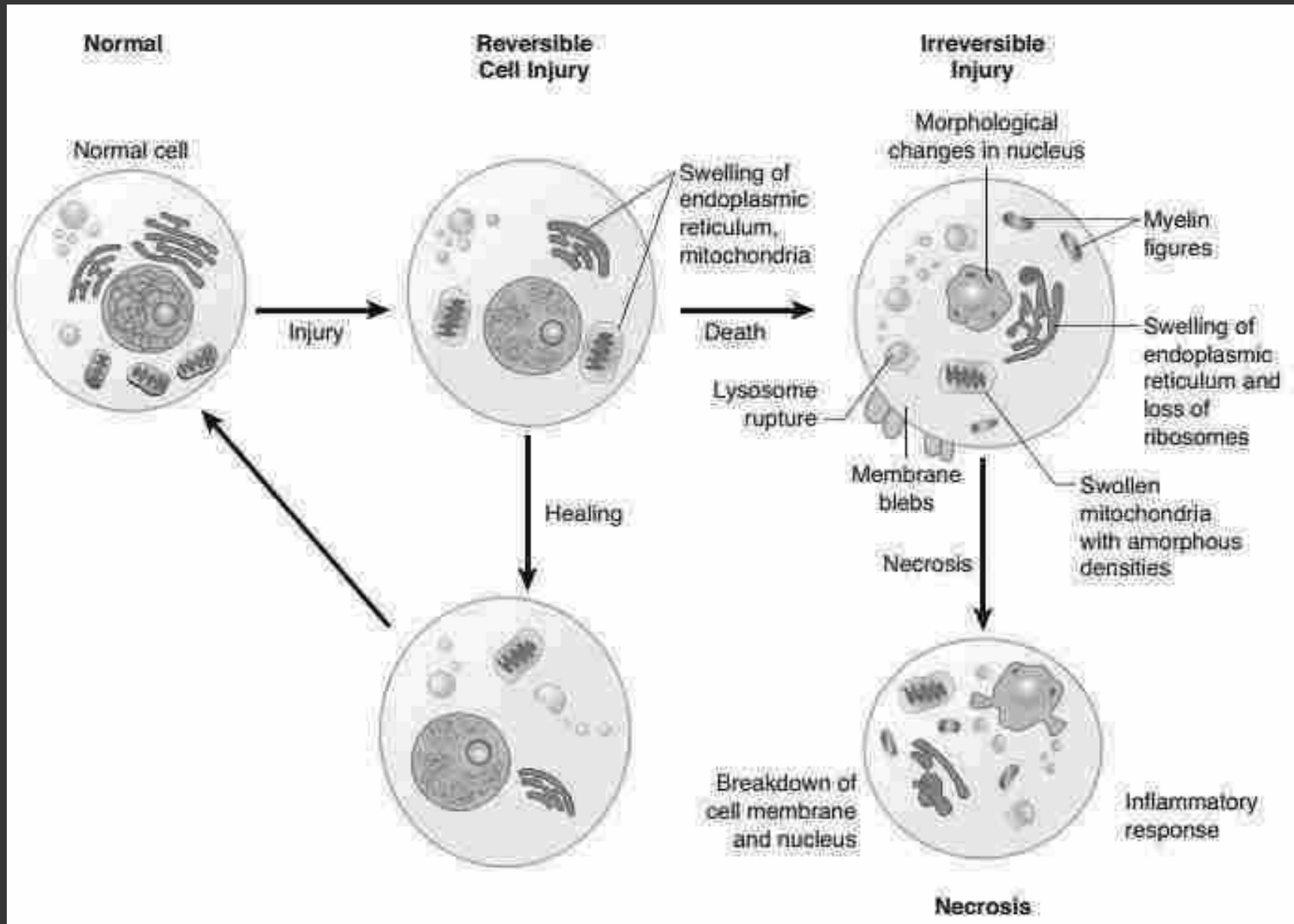


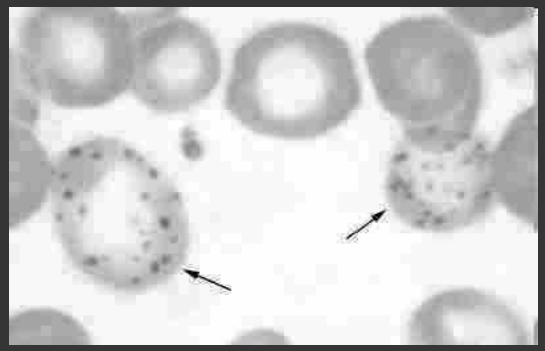
Figure 2-4. Cell injury

PHYSICAL AGENTS

- Trauma
- Heat
- Cold
- Radiation
- Electric shock

CHEMICAL AGENTS AND DRUGS

- Endogenous products: urea
- Exogenous agents
  - Therapeutic drugs: hormones
- Non therapeutic agents: lead , alcohol, OPP



FREE RADICAL INJURY

Absorption of energy (UV light and x-rays)

Oxidative metabolic reactions

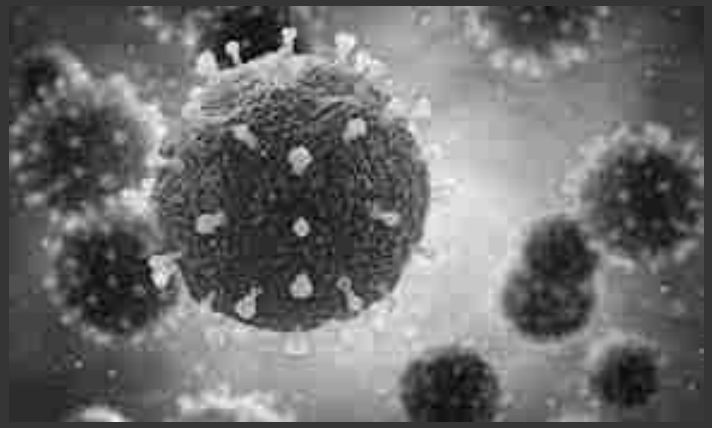
Enzymatic conversion of exogenous chemicals or drugs (CCl_4>CCl_3)

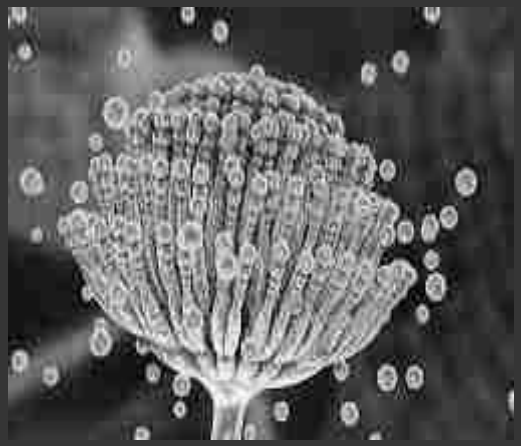
Oxygen-derived radicals

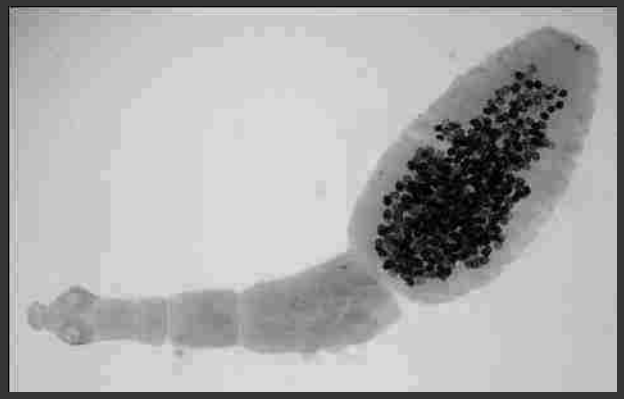
Superoxide

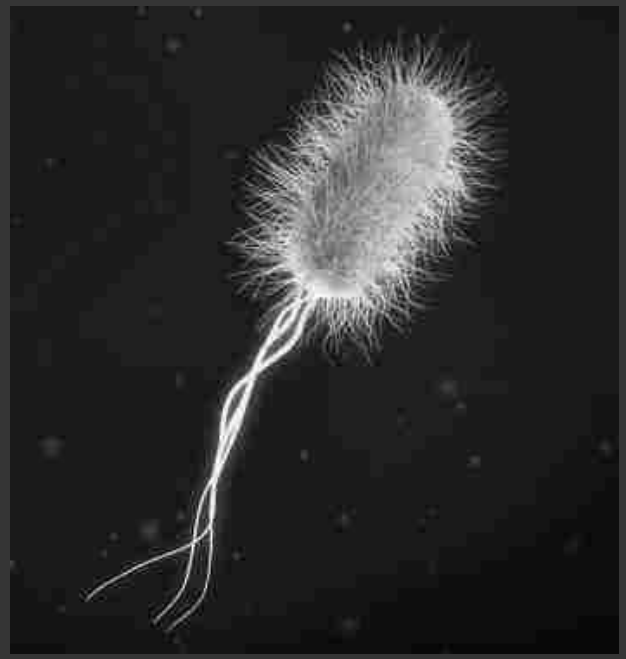
INFECTIOUS AGENTS

Viruses
Rickettsiae
Bacteria
Fungi
Parasites









Immunological

- Hypersensitivity reactions (I-IV)
- Autoimmune diseases

Nutritional

- Protein calorie malnutrition
- Vitamin deficiencies
- Hypermetabolic syndrome & obesity

Ageing

- Faulty DNA repair mechanisms
- Increased susceptibility to somatic mutations

Stages in cellular response to stress and injurious stimuli

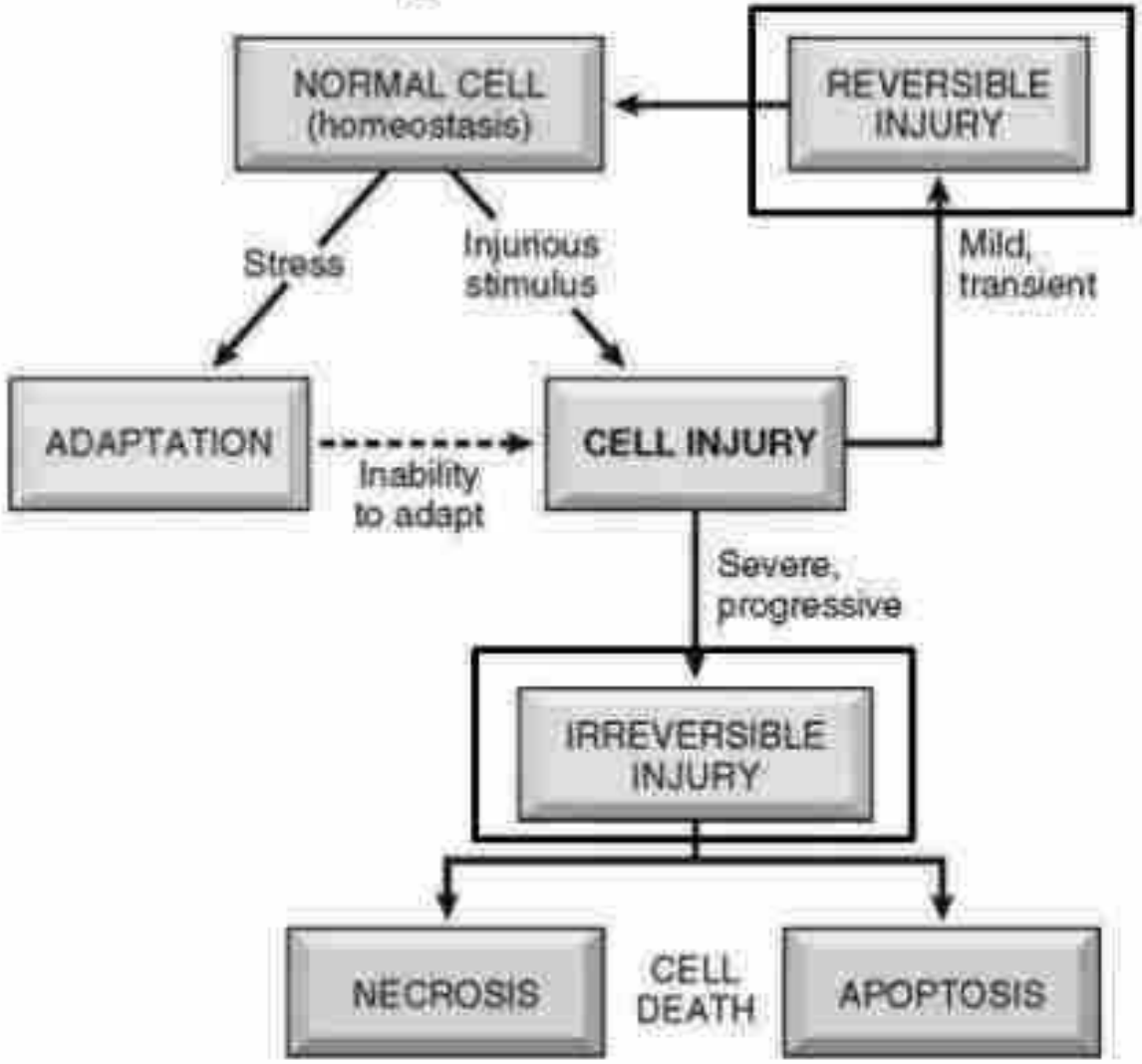


Fig 1.1 (5)

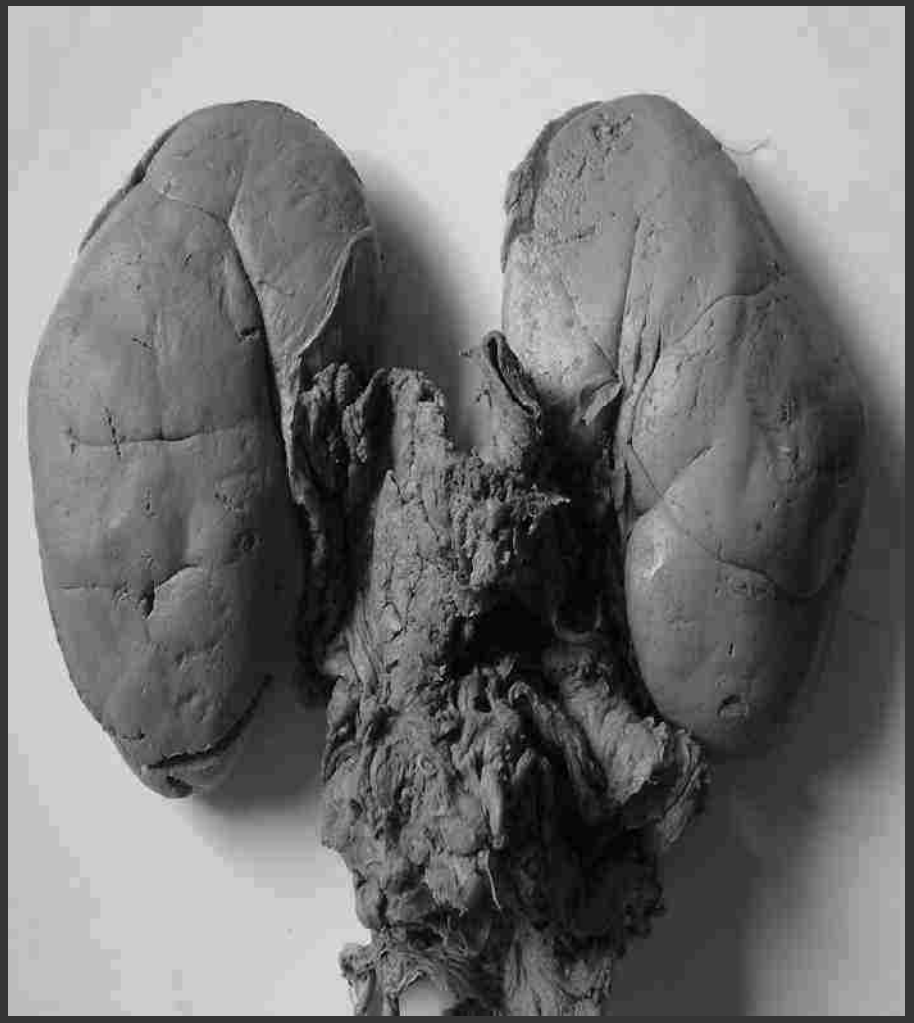
Reversible Injury

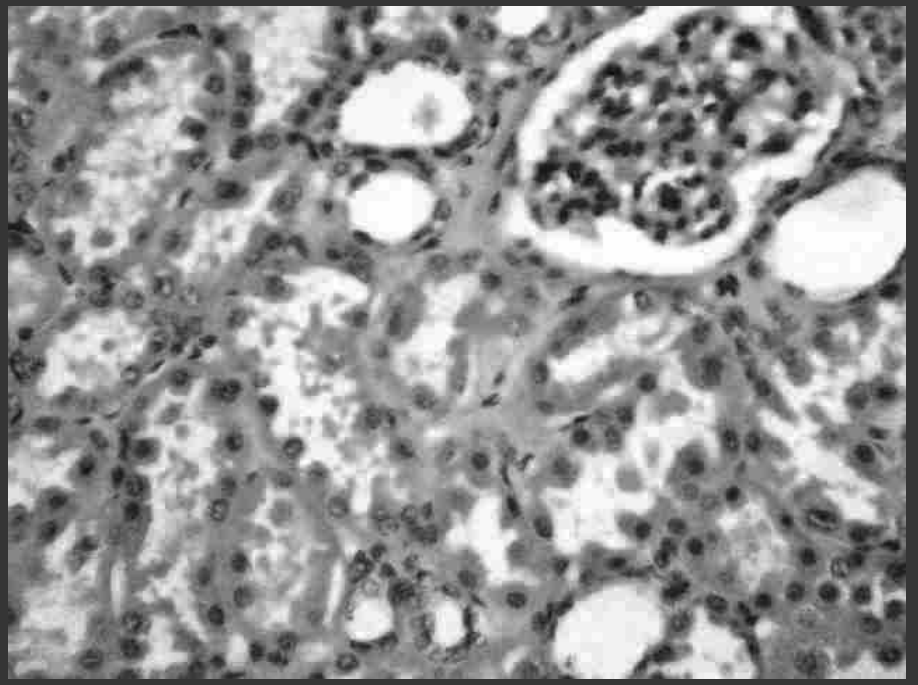
- Hydropic change
- Fatty change
- Hyaline change
- Mucoid Degeneration
- Amyloidosis
- Pathologic Pigments

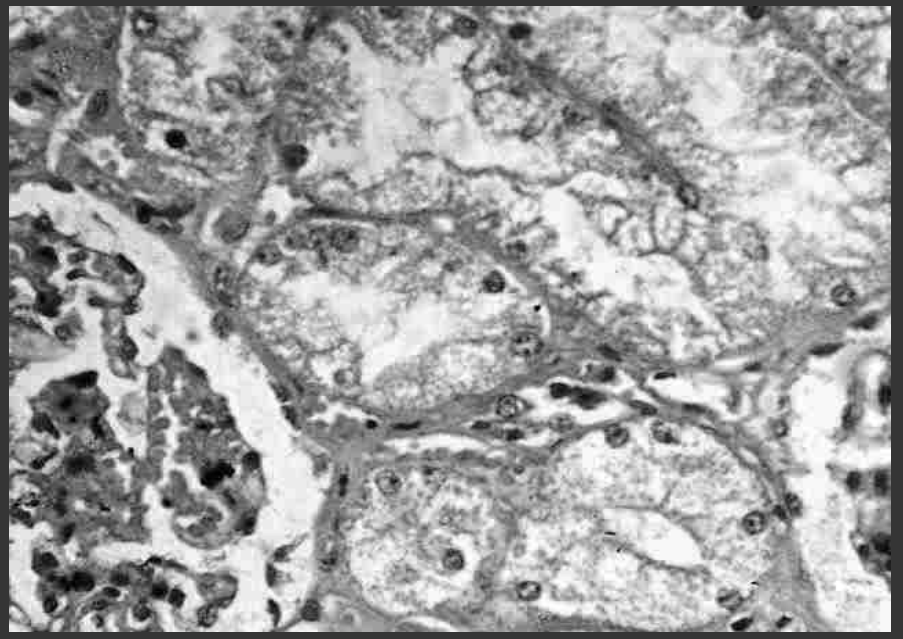
Hydropic Change

- Also known as cloudy swelling.
- Accumulation of watery fluid in cells.
- Commonest and earliest form of cell injury
- Caused by bacterial toxins, chemicals, poisons, burns, high fever, intravenous administration of hypertonic glucose or saline.
- Impaired regulation of sodium and potassium at the level of cell membrane.

Cloudy change

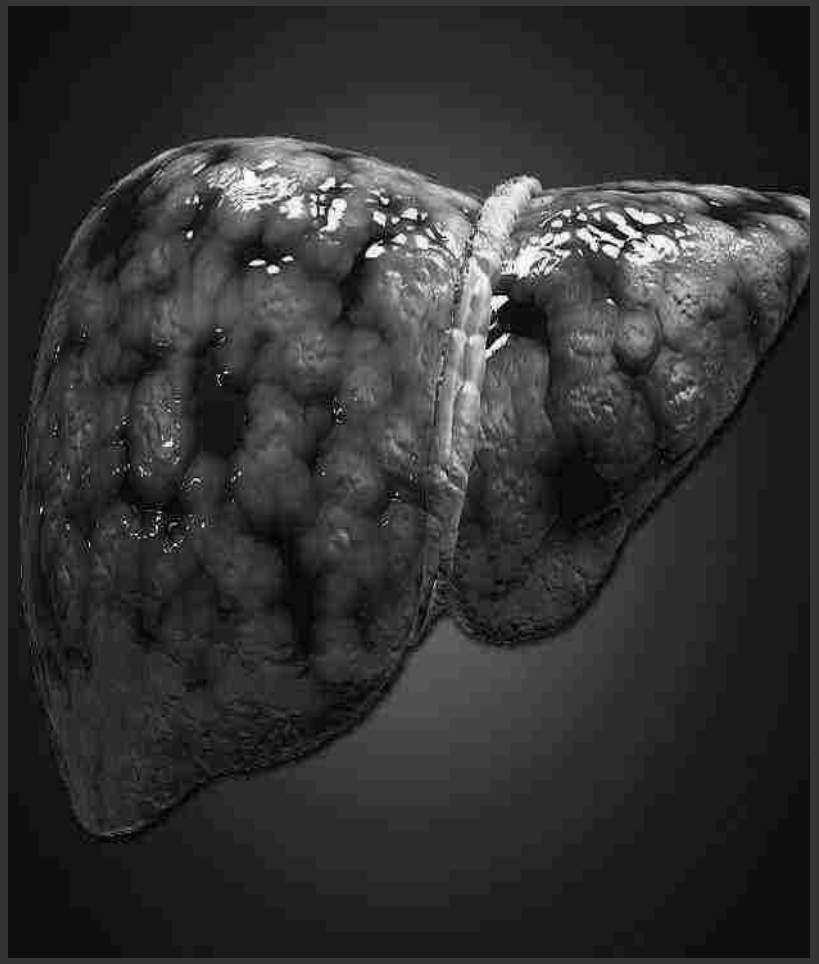






Fatty change (Steatosis)

- Conditions with excess fat
  - Obesity
  - Diabetes mellitus
  - Congenital hyperlipidaemia
- Liver cell damage:
  - Alcoholic liver disease (most common)
  - Starvation ,Protein calorie malnutrition
  - Chronic illnesses (e.g. tuberculosis)
  - Acute fatty liver in late pregnancy
  - Hypoxia (e.g. anaemia, cardiac failure)
  - Hepatotoxins
  - Drug-induced liver cell injury
    (methotrexate, steroids, CCl_4)
  - Reye's syndrome



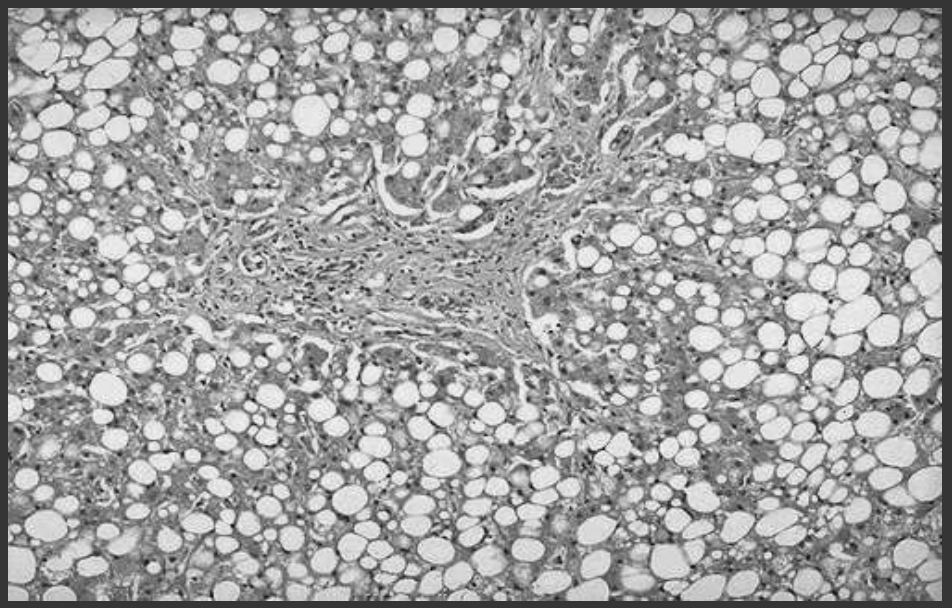
Fatty change

Gross features:

The organ enlarges and becomes yellow, soft, and greasy.

HPE:

Fatty change appears as clear vacuoles within parenchymal cells.

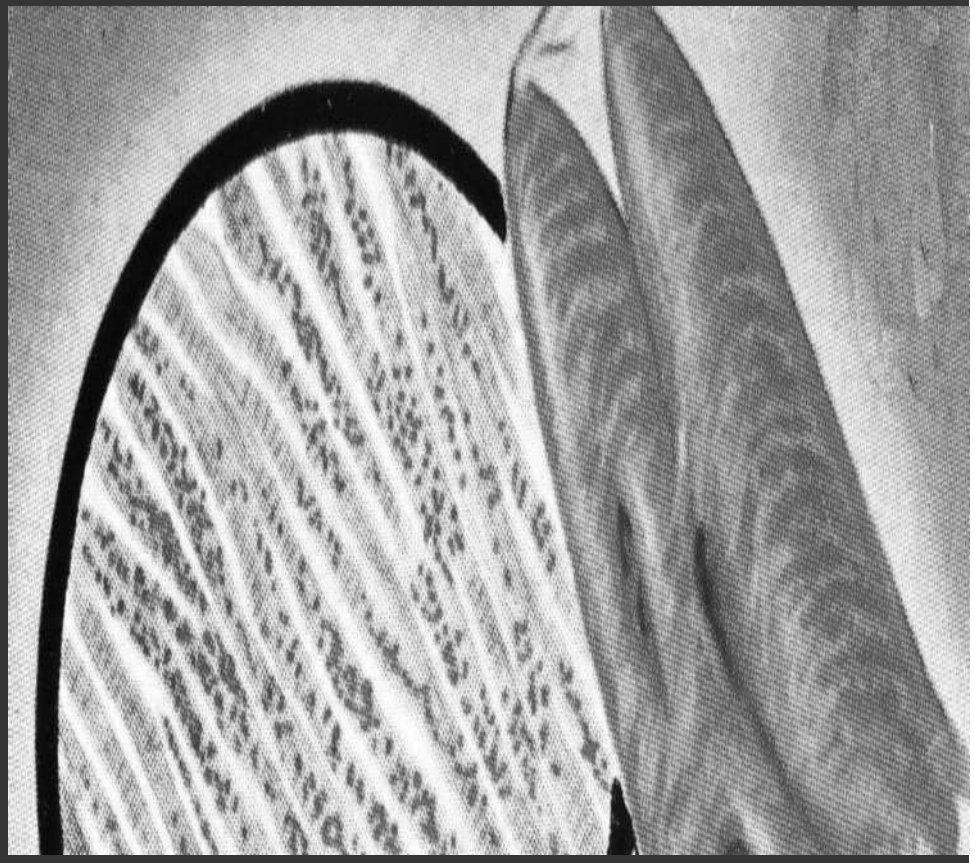


Fatty degeneration: Heart

Prolonged moderate hypoxia causes intracellular deposits of fat

Gross- bands of yellowed myocardium alterations with bands of darker, red-brown, uninvolved myocardium (Tigered effect).

Profound hypoxia or diphtheritic myocarditis,myocardial cells are uniformly affected.



Fatty degeneration: Kidney

In most cases fatty change is confined to the epithelium of the convoluted tubules,
but in severe poisoning it may affect all structures including the glomerulus.

- Causes:

Nephrotoxic poisons

Infections

Congestive cardiac failure

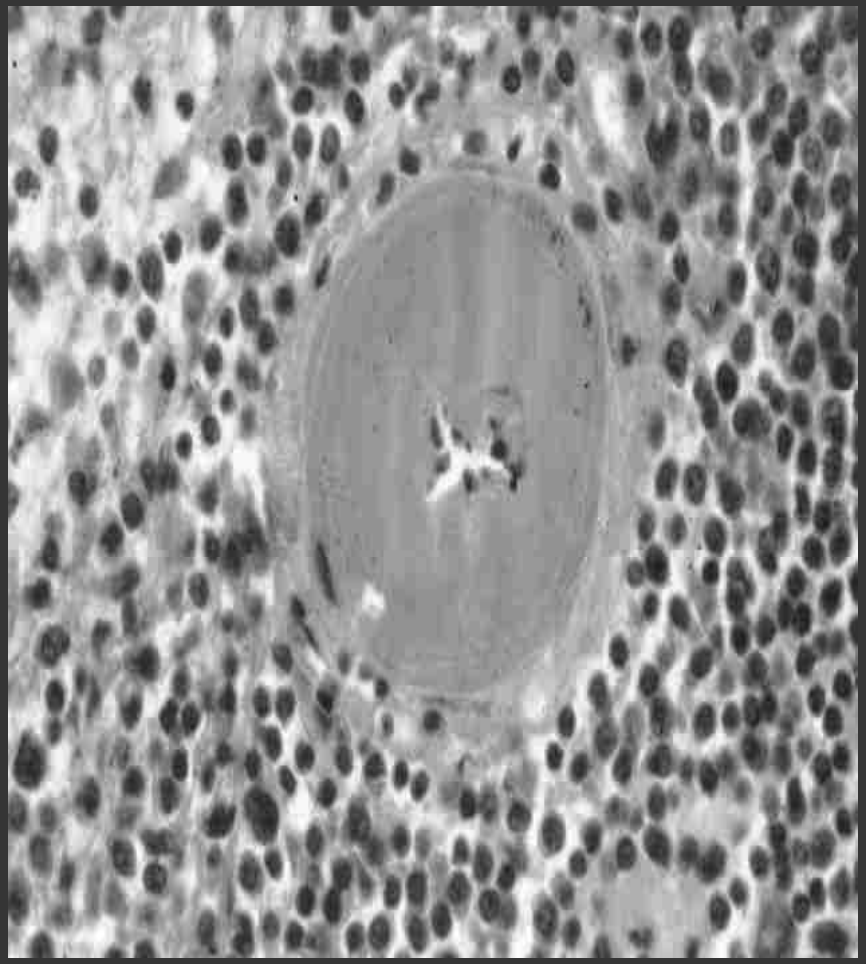
Severe anaemia ,Ischaemia

Diabetes mellitus

Malnutrition and wasting disease.

Hyaline degeneration

- Not a distinct chemical entity.
- Various histological or cytological alterations characterized by homogeneous, glasslike appearance in hematoxylin and eosin-stained sections.



INTRACELLULAR HYALINE

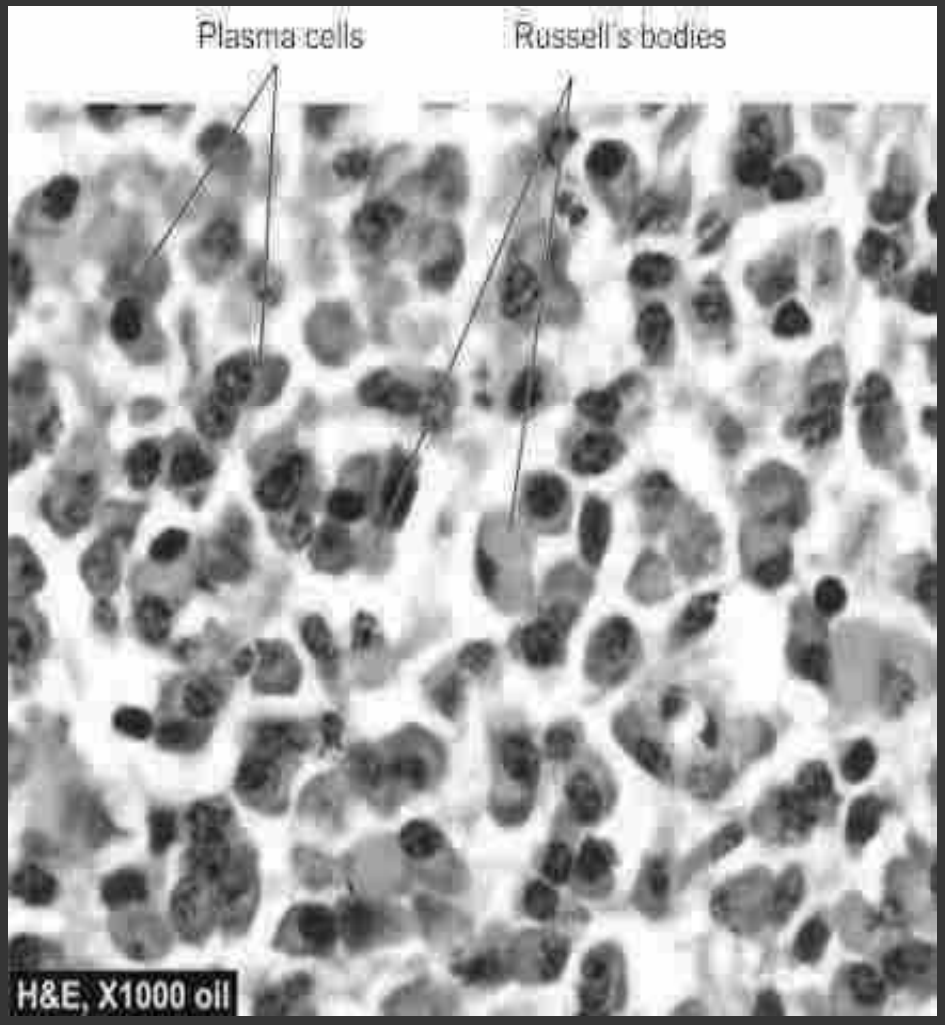
- Hyaline droplets in the proximal tubular epithelial cells
- Rectus abdominis muscle

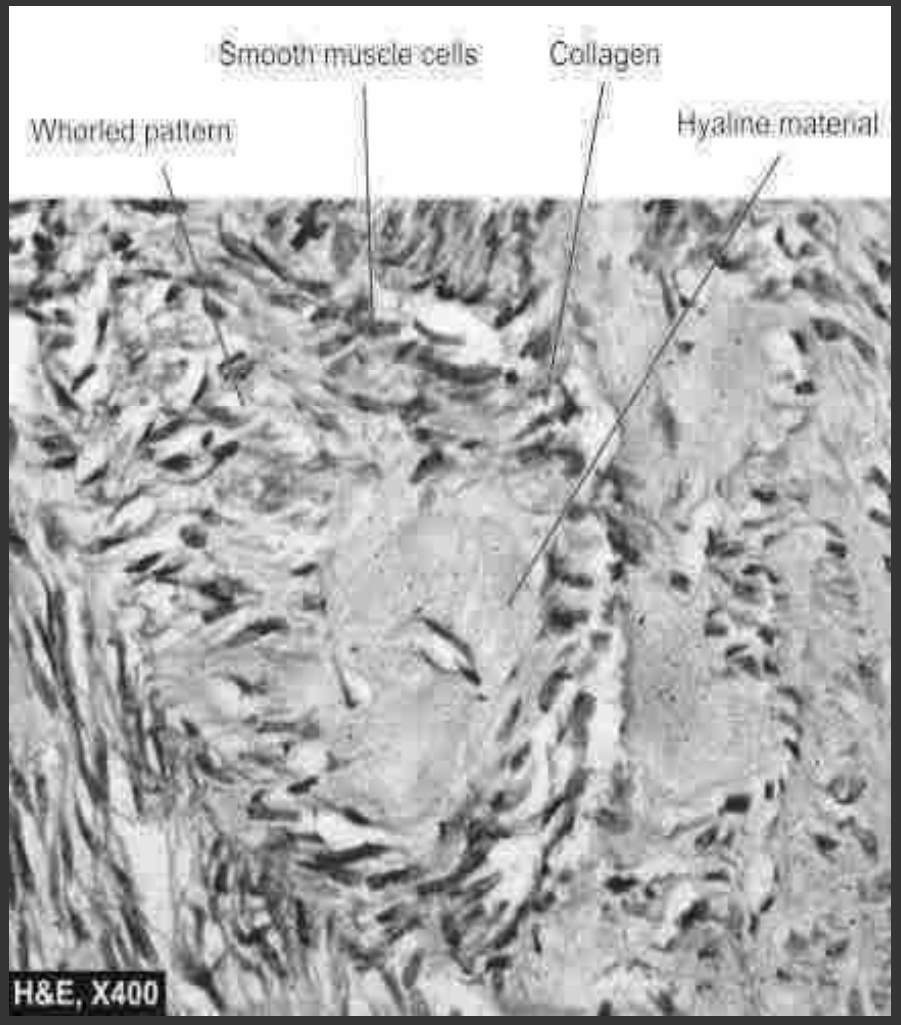
  Zenker's degeneration- typhoid fever.
- Mallory's hyaline - aggregates of intermediate filaments in the hepatocytes in alcoholic liver
- Hyaline -Nuclear or cytoplasmic - Viral infections.
- Russell's bodies excessive immunoglobulins in plasma cells

EXTRACELLULAR HYALINE

- Hyaline degeneration in leiomyomas
- Hyalinised old scar
- Hyaline arteriolosclerosis
- Hyalinised glomeruli
- Corpora amylacea
- old infarcts

Hyaline degeneration





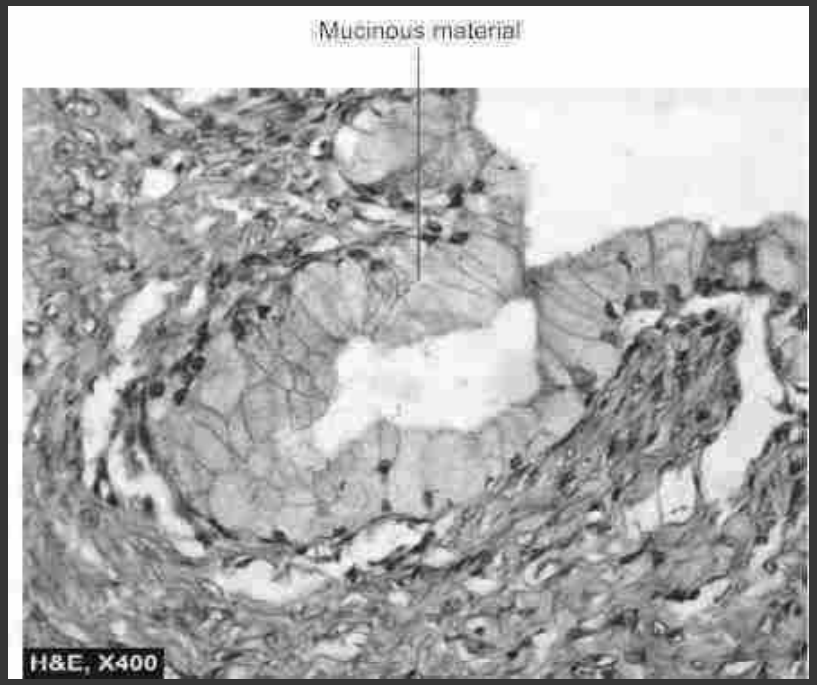
Mucoid degeneration

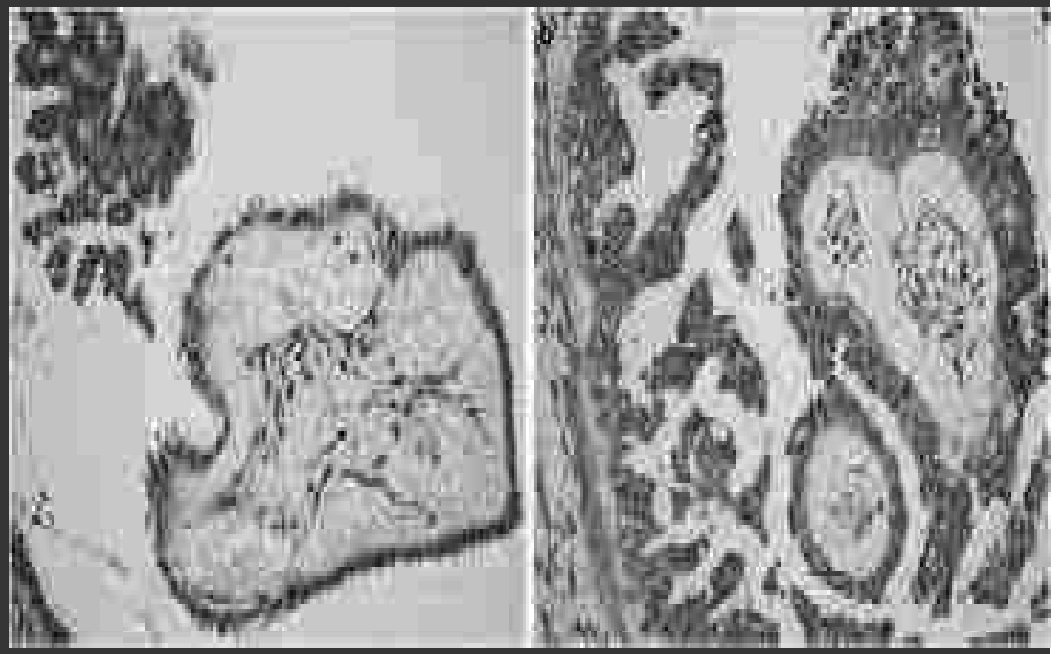
Epithelial mucin

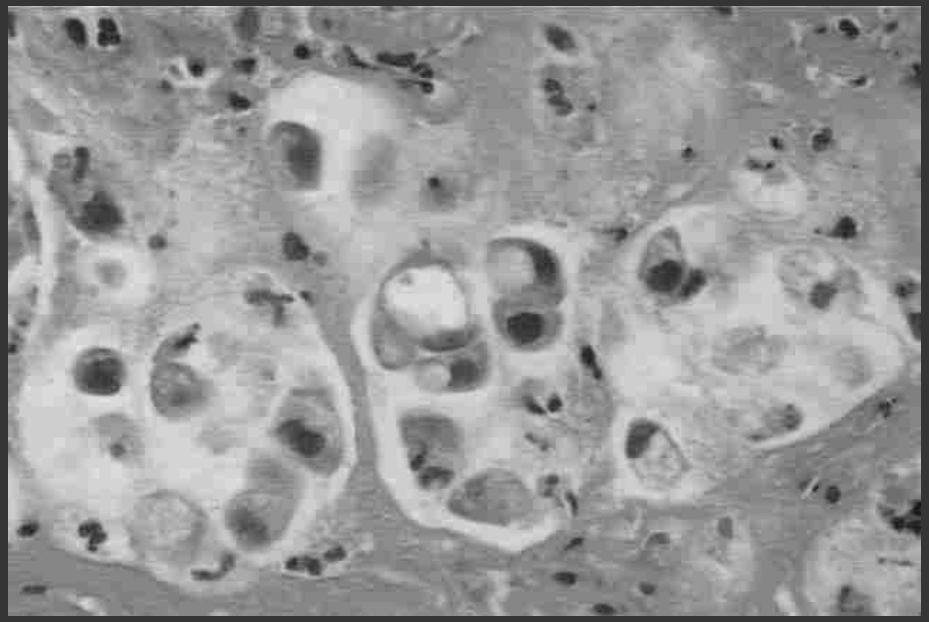
- **Catarrhal inflammation of mucous membrane (e.g. of respiratory tract, alimentary tract, uterus).**
- **Obstruction of duct leading to mucocele in the oral cavity and gallbladder.**
- **Cystic fibrosis of the pancreas.**
- **Mucin-secreting tumours of ovary, stomach, intestine**

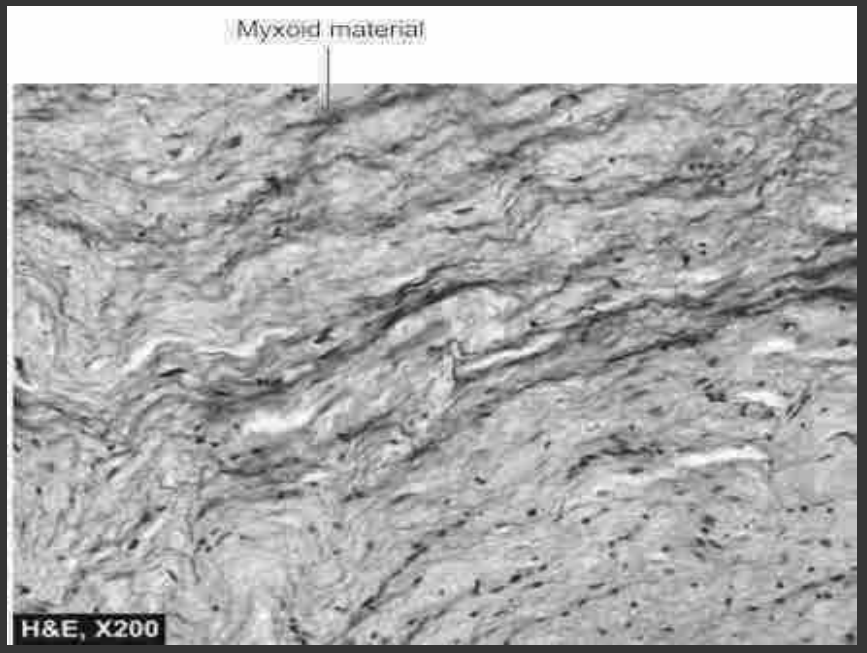
Connective tissue mucin

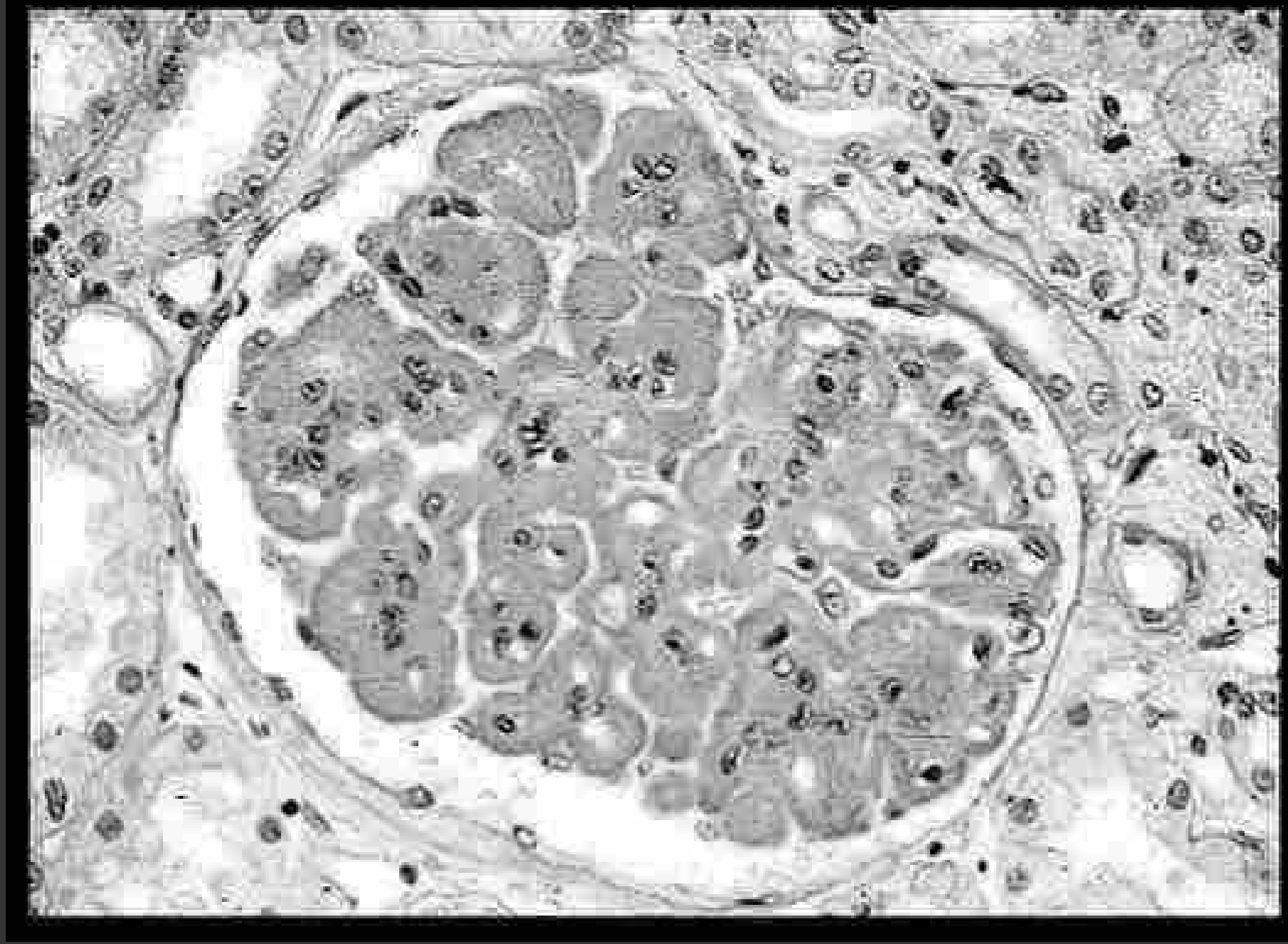
- **Mucoid or myxoid degeneration** in tumours e.g. myxomas, neurofibromas, fibroadenoma
- **Dissecting aneurysm** of the aorta due to **Erdheim's medial degeneration** and **Marfan's syndrome**.
- **Myxomatous** change in the dermis in **myxoedema**.
- **Myxoid** change in the **synovium in ganglion**


Mucinous material
H&E, X400






Myxoid material
H&E, X200



Causes of cell injury

PA 2.1

Introduction

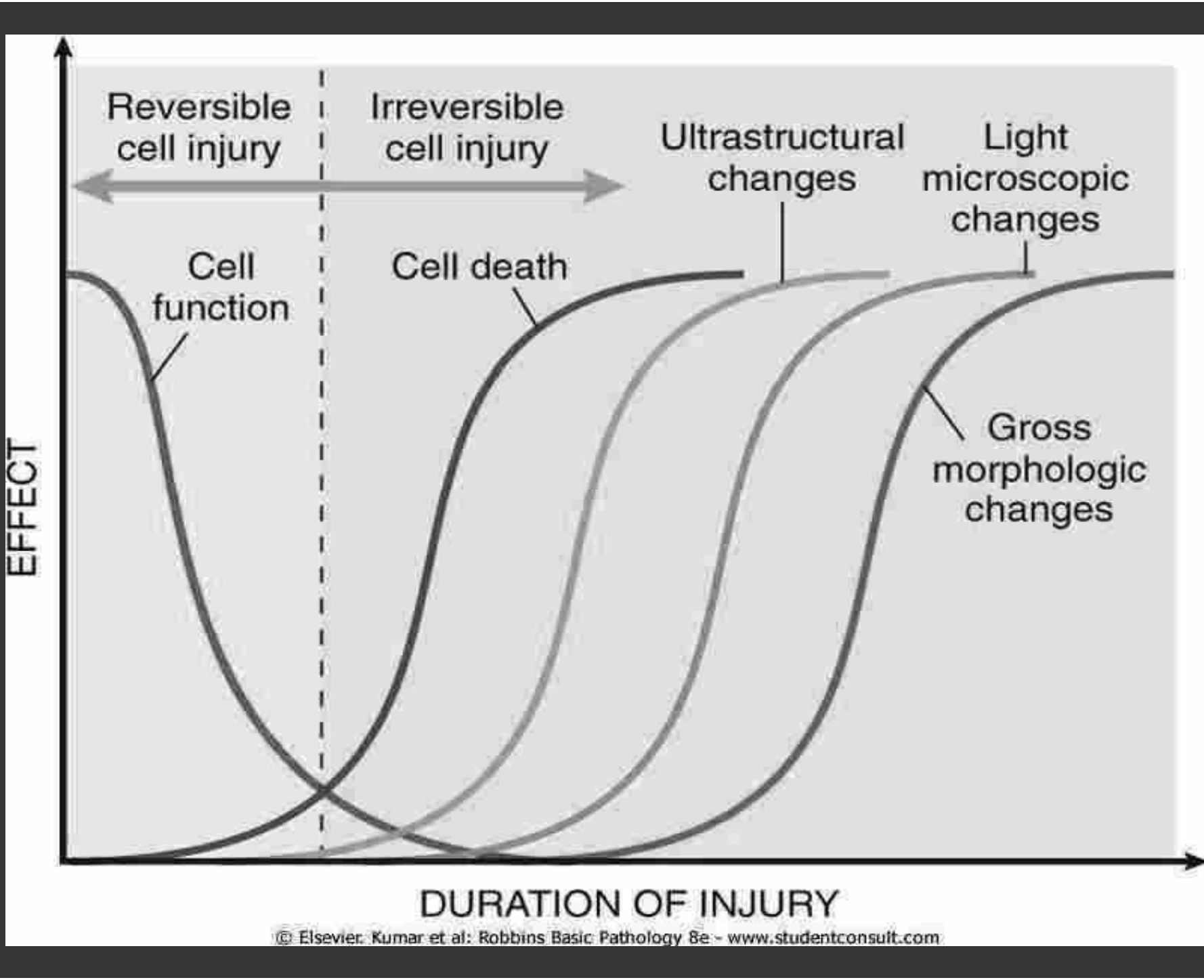
- **Cell injury is defined as a variety of stresses a cell encounters as a result of changes in its internal and external environment.**
- **Virchow's Cellular theory of disease (1859): diseases occur due to abnormalities at the level of cells**
- **The cellular response to stress may vary and depends upon the following:**
  - **The type of cell and tissue involved.**
  - **Extent and type of cell injury**

Cell Injury

- Functional and morphological effects of variety of stresses due to aetiological agents
- Resulting in changes of internal & external environment.

Cellular responses to stress vary & depends upon

1. Host factors: type of cell & tissue involved
2. Factors pertaining to injurious agent : extent & type of cell injury.


Reversible cell injury
Irreversible cell injury
Ultrastructural changes
Light microscopic changes
Cell function
Cell death
Gross morphologic changes
EFFECT
DURATION OF INJURY
© Elsevier. Kumar et al: Robbins Basic Pathology 8e - www.studentconsult.com

ETIOLOGY OF CELL INJURY

Genetic causes

- Developmental defects:
- Errors in morphogenesis
- Cytogenetic (Karyotypic) defects: chromosomal abnormalities
- Single-gene defects:
- Mendelian disorders
- Multifactorial inheritance disorders

Acquired causes

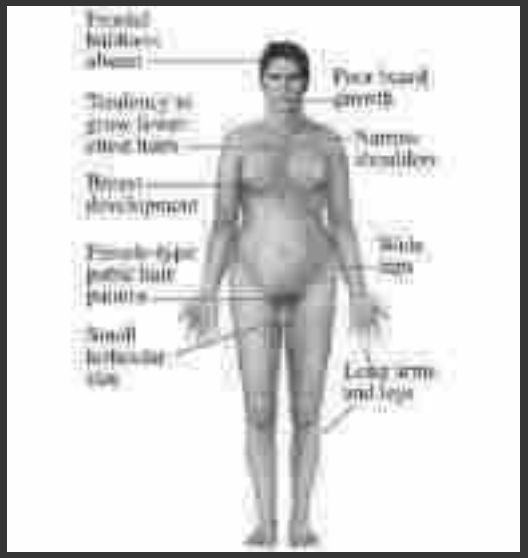
- Hypoxia and ischaemia
- Physical agents
- Chemical agents /drugs
- Microbial agents
- Immunologic agents
- Nutritional derangements
- Ageing
- Psychogenic diseases
- Iatrogenic factors

Genetic Causes

Hurler Syndrome

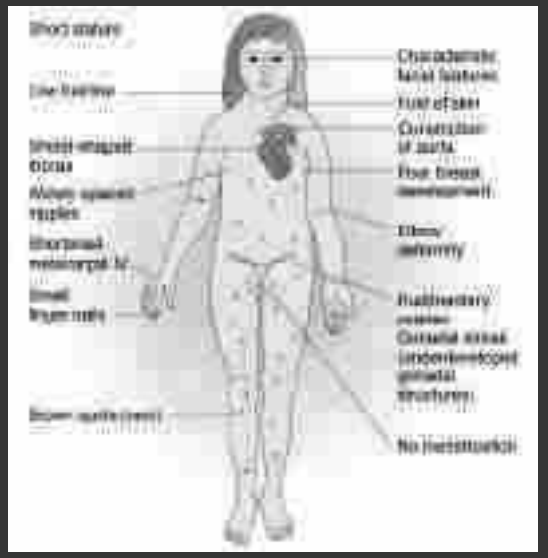
Spectral Karyotype

Hunter Syndrome



Klinefelter Syndrome
(XXY, XXXY)

Down's Syndrome
(Trisomy 21)



Turner Syndrome
(X0)

Oxygen deprivation- Hypoxia, Ischaemia

- **Inadequte oxygenation-** Cardio respiratory failure
- **Reduced oxygen carrying capacity -**Anemia or CO poisoning

- Loss of oxidative phosphorylation and ATP generation by mitochondria.
- Decreased ATP (with increase in AMP): stimulating fructokinase and phosphorylation, resulting in aerobic glycolysis.
- Depleted glycogen.
- Reduced intracellular pH: Lactic acid and inorganic phosphate.
- Clumping of nuclear chromatin.

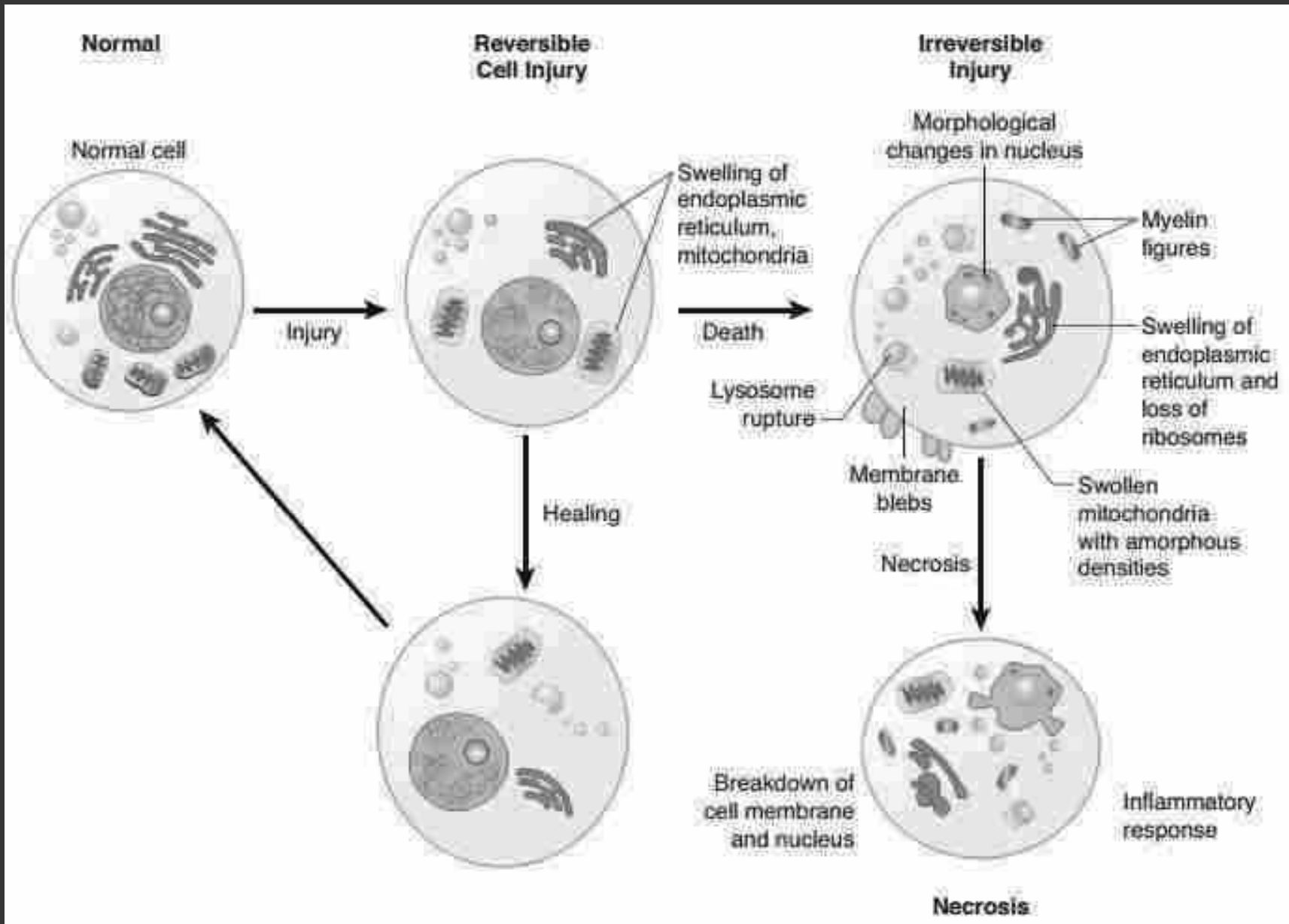


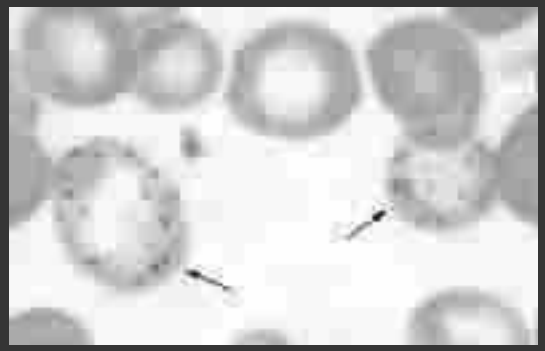
Figure 2-4. Cell Injury

PHYSICAL AGENTS

- Trauma
- Heat
- Cold
- Radiation
- Electric shock

CHEMICAL AGENTS AND DRUGS

- Endogenous products: urea
- Exogenous agents
  - Therapeutic drugs: hormones
- Non therapeutic agents: lead , alcohol, OPP



FREE RADICAL INJURY

Absorption of energy (UV light and x-rays)

Oxidative metabolic reactions

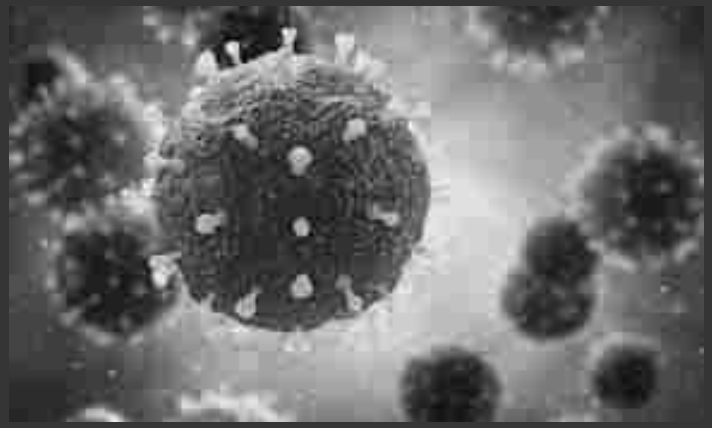
Enzymatic conversion of exogenous chemicals or drugs (CCl_4>CCl_3)

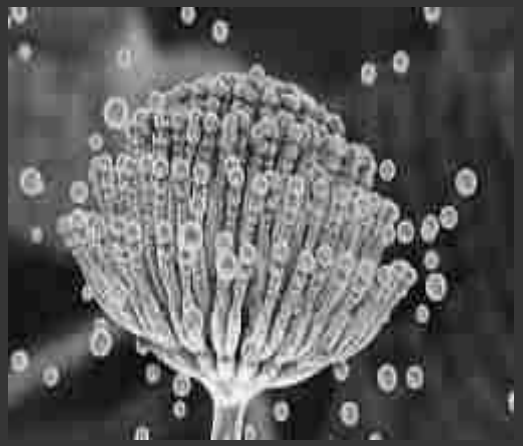
Oxygen-derived radicals

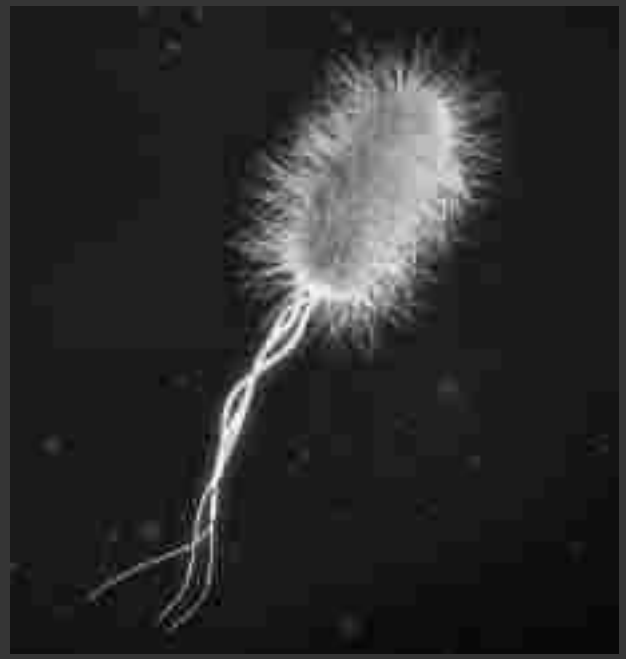
Superoxide

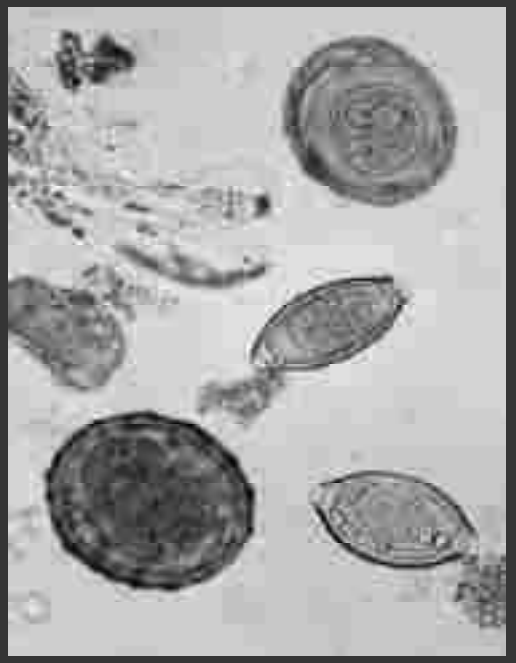
INFECTIOUS AGENTS

Viruses
Rickettsiae
Bacteria
Fungi
Parasites









Immunological

- Hypersensitivity reactions (I-IV)
- Autoimmune diseases

Nutritional

- Protein calorie malnutrition
- Vitamin deficiencies
- Hypermetabolic syndrome & obesity

Ageing

- Faulty DNA repair mechanisms
- Increased susceptibility to somatic mutations

Stages in cellular response to stress and injurious stimuli

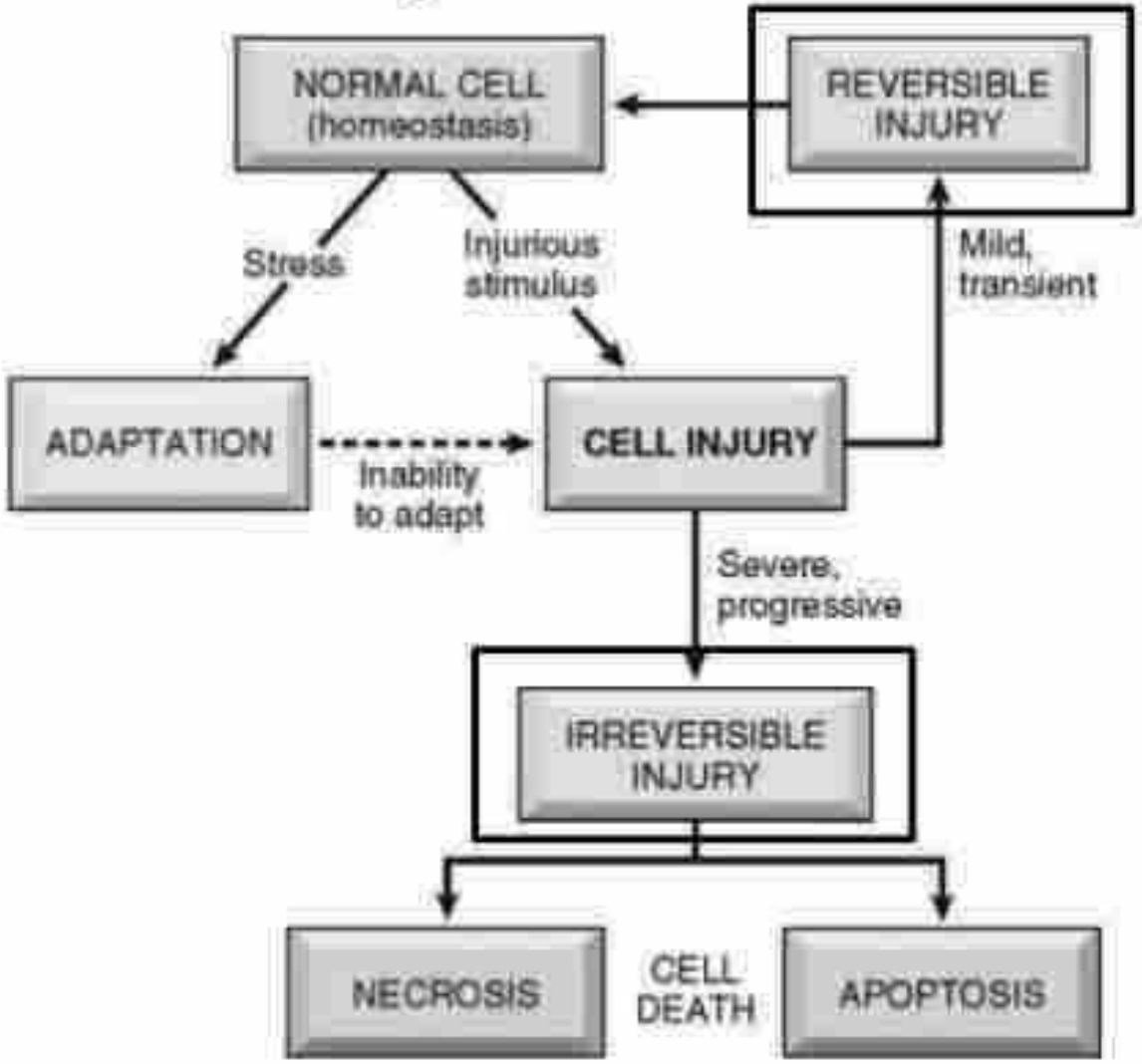


Fig 1.1 (5)

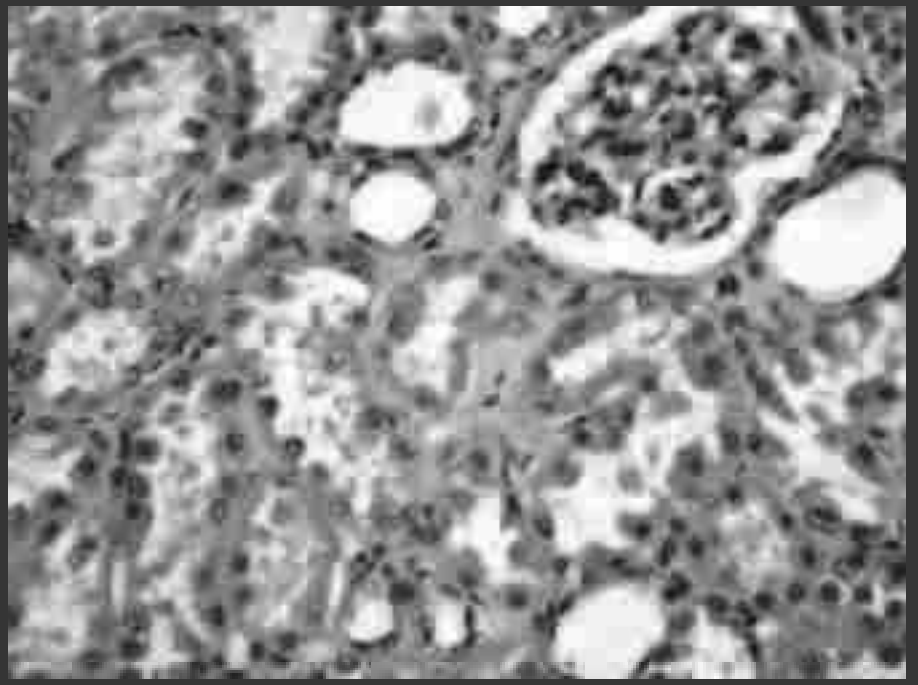
Reversible Injury

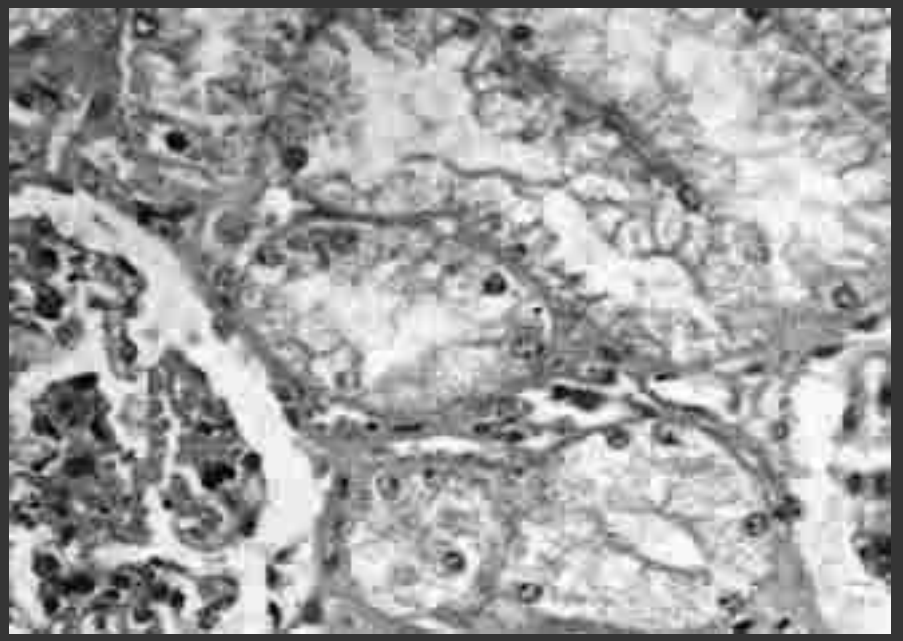
- Hydropic change
- Fatty change
- Hyaline change
- Mucoid Degeneration
- Amyloidosis
- Pathologic Pigments

Hydropic Change

- Also known as cloudy swelling.
- Accumulation of watery fluid in cells.
- Commonest and earliest form of cell injury
- Caused by bacterial toxins, chemicals, poisons, burns, high fever, intravenous administration of hypertonic glucose or saline.
- Impaired regulation of sodium and potassium at the level of cell membrane.

Cloudy change





Fatty change (Steatosis)

- Conditions with excess fat
  - Obesity
  - Diabetes mellitus
  - Congenital hyperlipidaemia
- Liver cell damage:
  - Alcoholic liver disease (most common)
  - Starvation ,Protein calorie malnutrition
  - Chronic illnesses (e.g. tuberculosis)
  - Acute fatty liver in late pregnancy
  - Hypoxia (e.g. anaemia, cardiac failure)
  - Hepatotoxins
  - Drug-induced liver cell injury
    (methotrexate, steroids, CCl_4)
  - Reye's syndrome

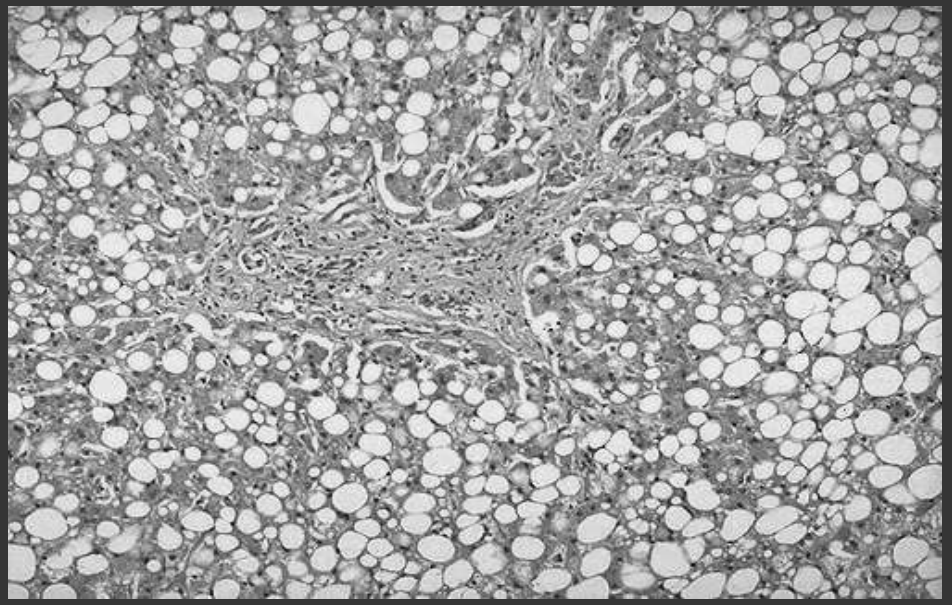
Fatty change

Gross features:

The organ enlarges and becomes yellow, soft, and greasy.

HPE:

Fatty change appears as clear vacuoles within parenchymal cells.

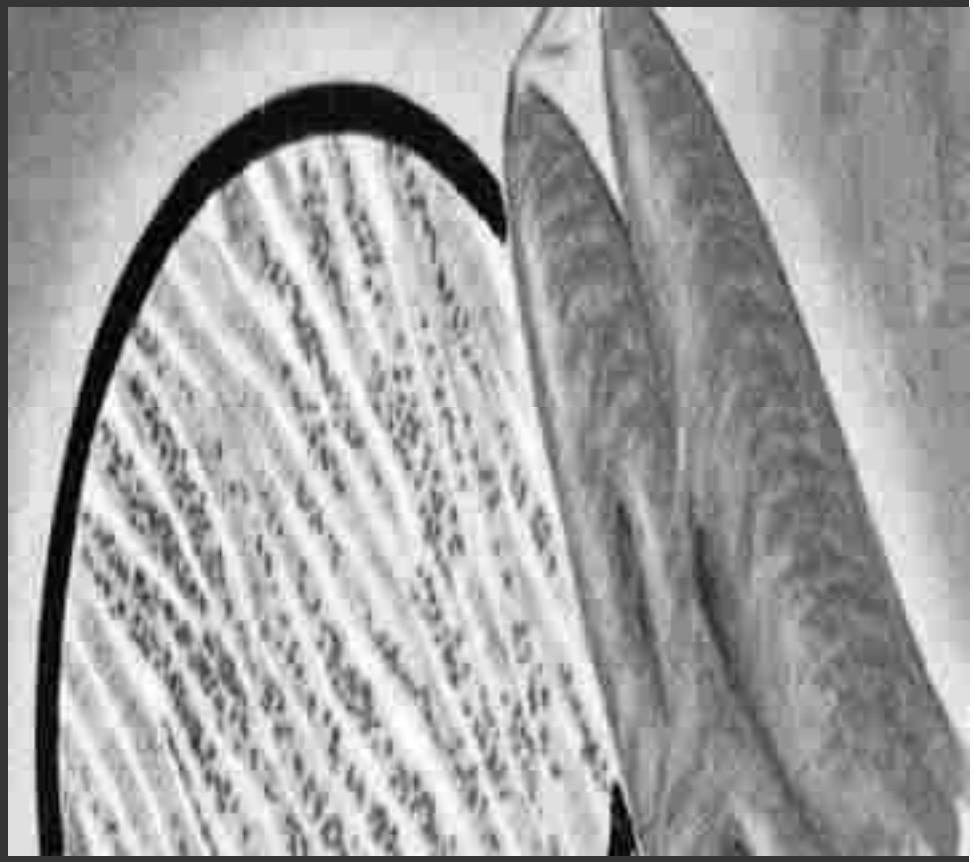


Fatty degeneration: Heart

Prolonged moderate hypoxia causes intracellular deposits of fat

Gross- bands of yellowed myocardium alterations with bands of darker, red-brown, uninvolved myocardium (Tigered effect).

Profound hypoxia or diphtheritic myocarditis,myocardial cells are uniformly affected.



Fatty degeneration: Kidney

In most cases fatty change is confined to the epithelium of the convoluted tubules,
but in severe poisoning it may affect all structures including the glomerulus.

- Causes:

Nephrotoxic poisons

Infections

Congestive cardiac failure

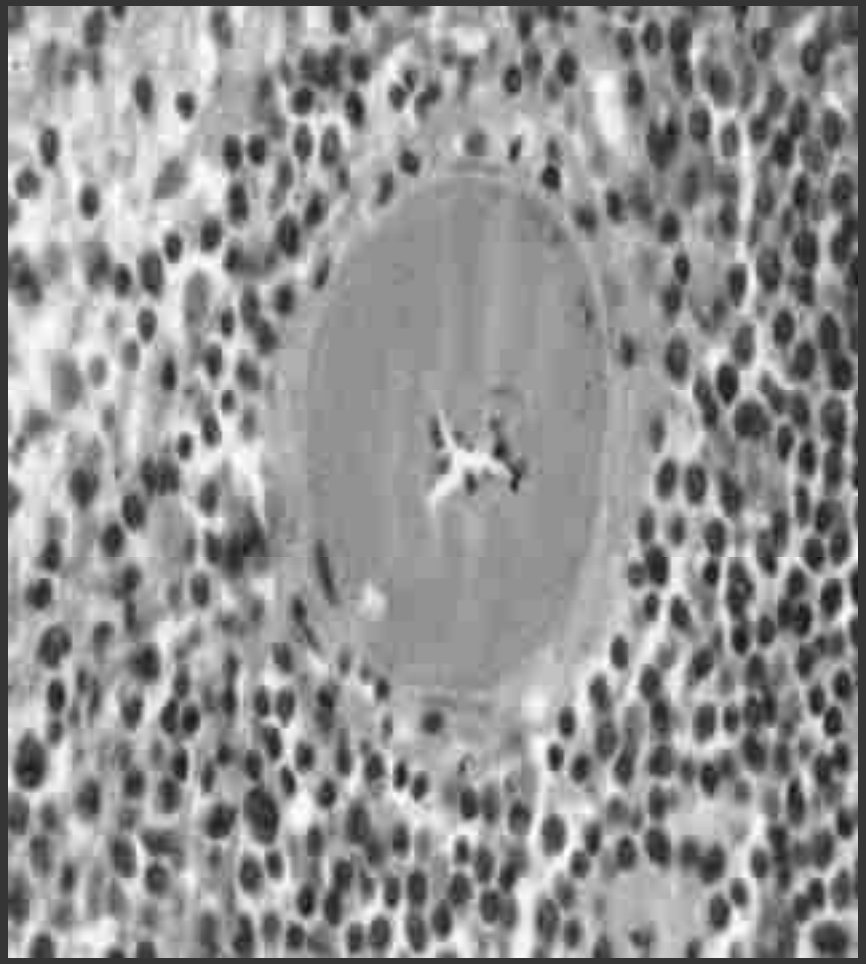
Severe anaemia ,Ischaemia

Diabetes mellitus

Malnutrition and wasting disease.

Hyaline degeneration

- Not a distinct chemical entity.
- Various histological or cytological alterations characterized by homogeneous, glasslike appearance in hematoxylin and eosin-stained sections.



INTRACELLULAR HYALINE

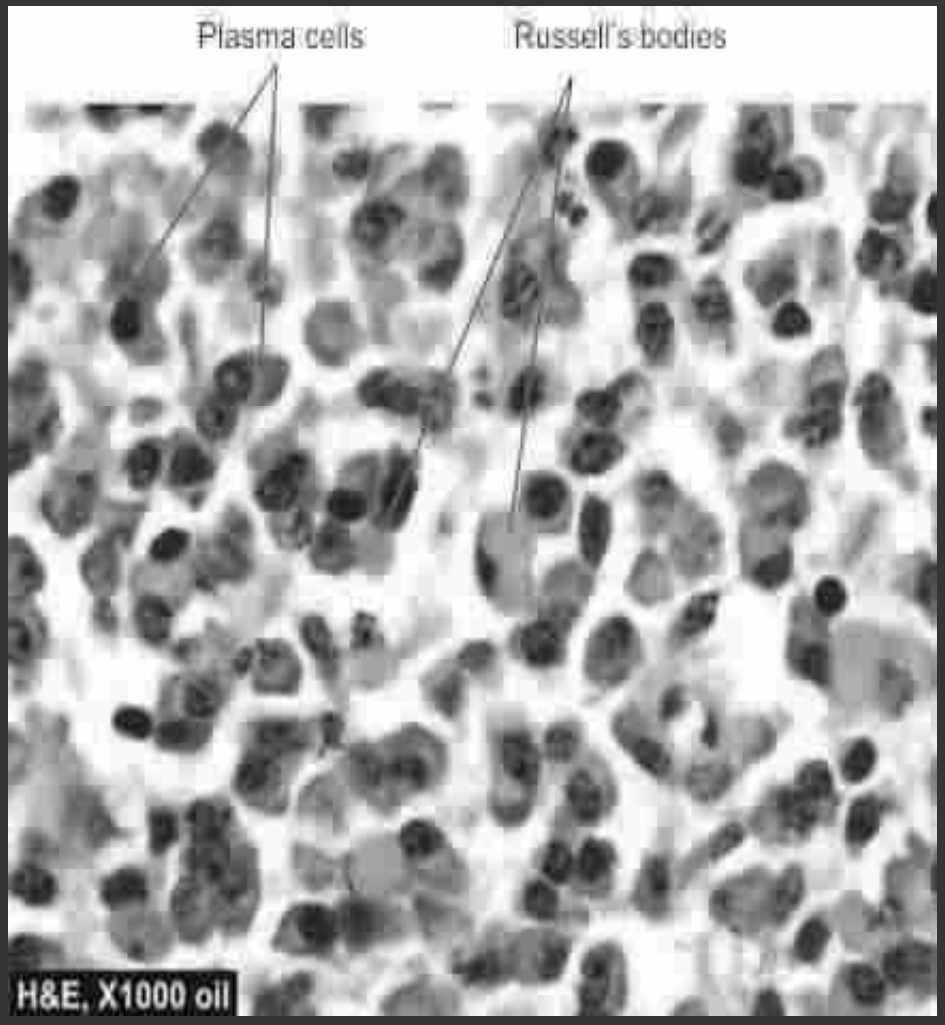
- Hyaline droplets in the proximal tubular epithelial cells
- Rectus abdominis muscle

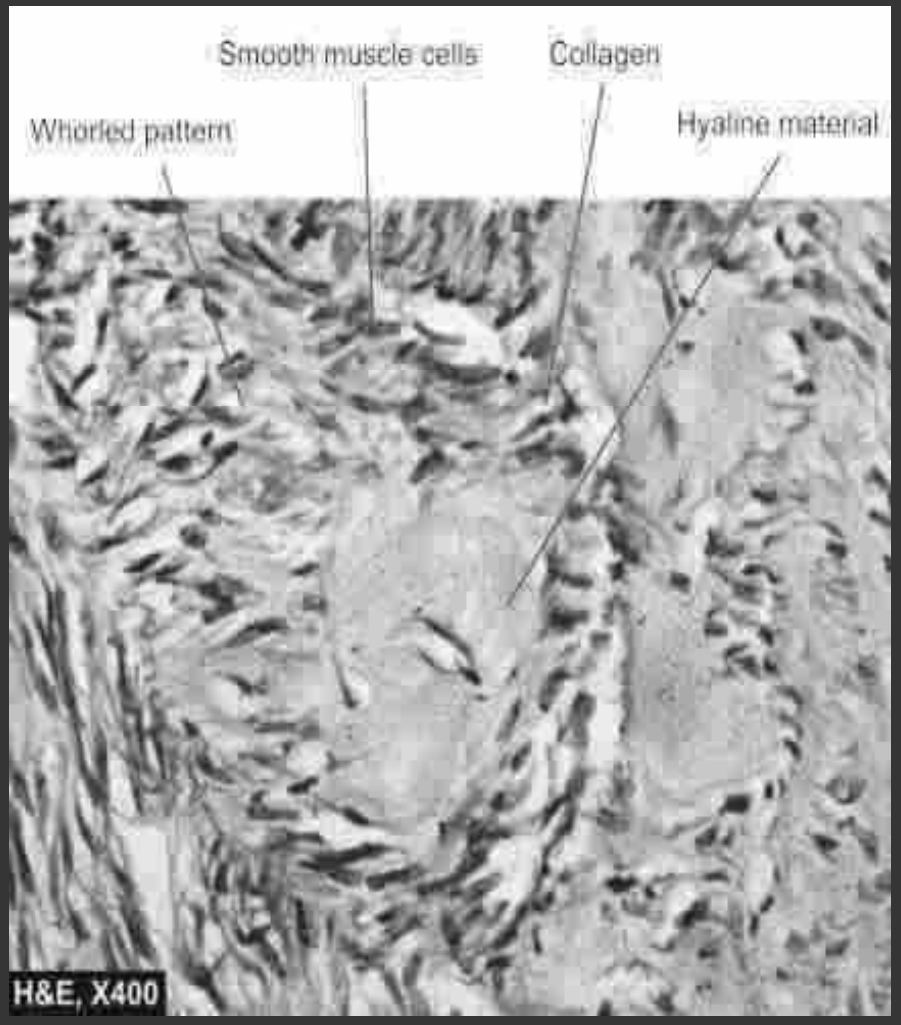
  Zenker's degeneration- typhoid fever.
- Mallory's hyaline - aggregates of intermediate filaments in the hepatocytes in alcoholic liver
- Hyaline -Nuclear or cytoplasmic - Viral infections.
- Russell's bodies excessive immunoglobulins in plasma cells

EXTRACELLULAR HYALINE

- Hyaline degeneration in leiomyomas
- Hyalinised old scar
- Hyaline arteriolosclerosis
- Hyalinised glomeruli
- Corpora amylacea
- old infarcts

Hyaline degeneration





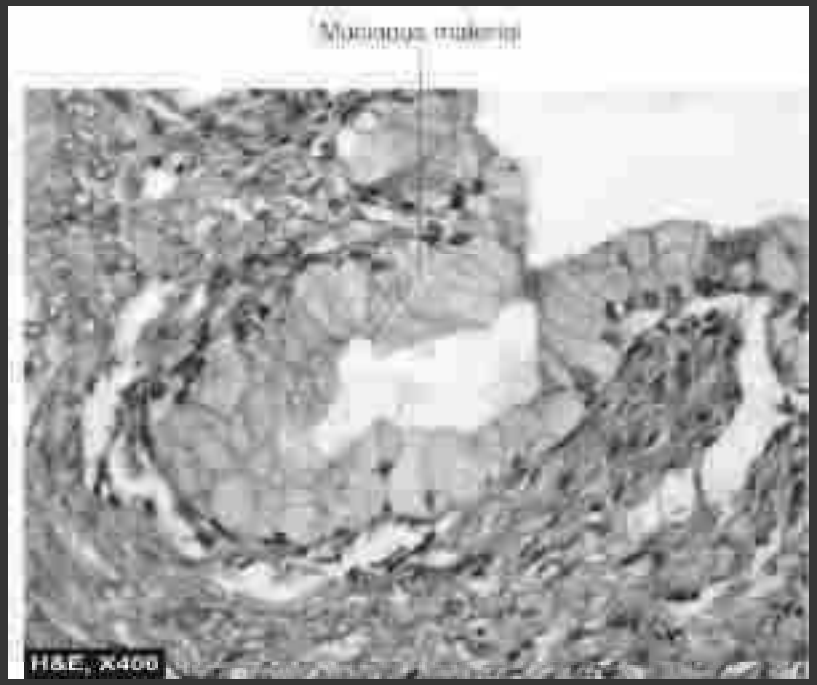
Mucoid degeneration

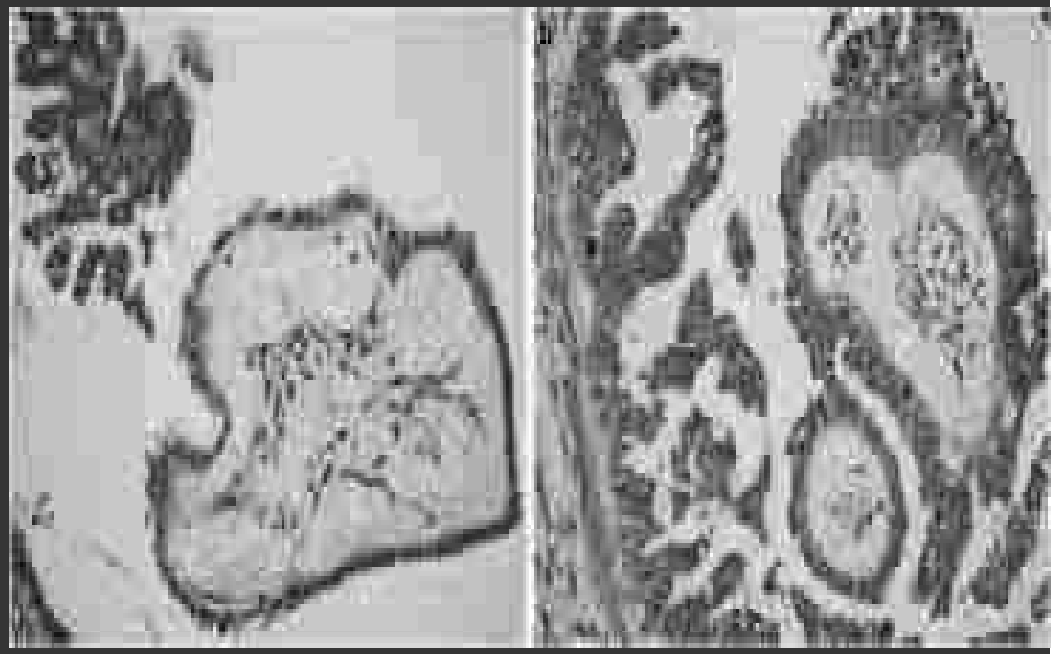
Epithelial mucin

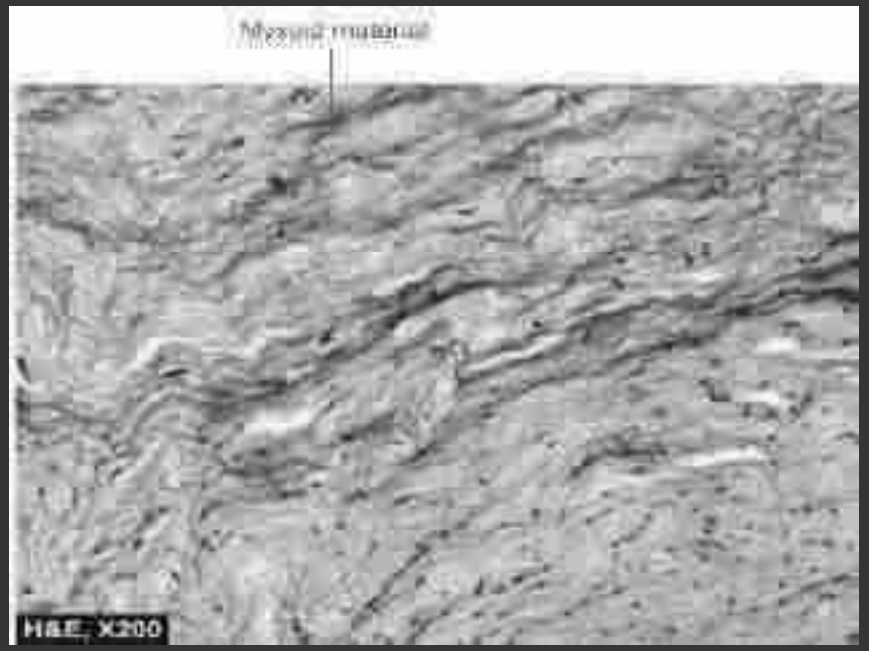
- **Catarrhal inflammation of mucous membrane (e.g. of respiratory tract, alimentary tract, uterus).**
- **Obstruction of duct leading to mucocele in the oral cavity and gallbladder.**
- **Cystic fibrosis of the pancreas.**
- **Mucin-secreting tumours of ovary, stomach, intestine**

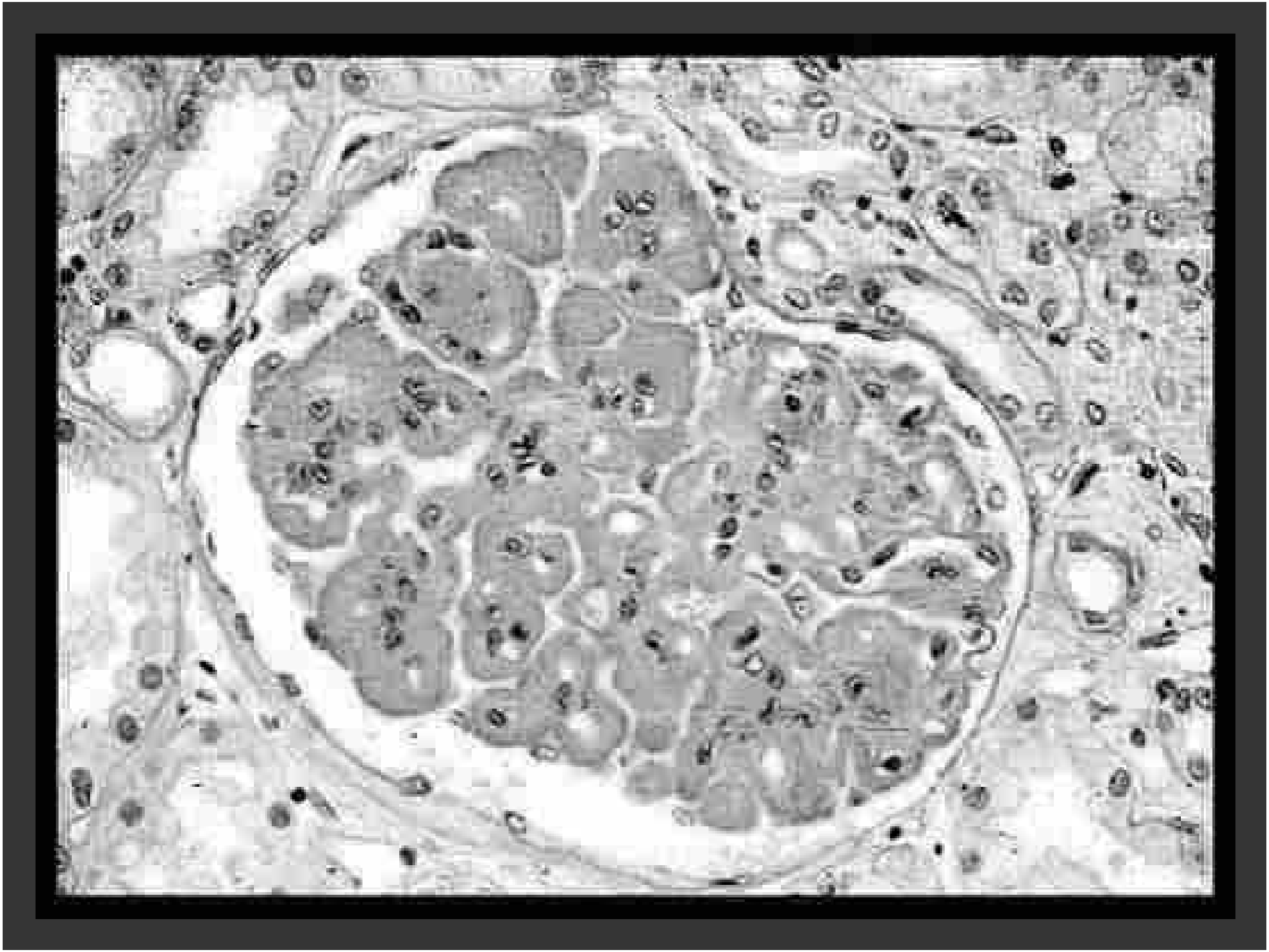
Connective tissue mucin

- **Mucoid or myxoid degeneration** in tumours e.g. myxomas, neurofibromas, fibroadenoma
- **Dissecting aneurysm** of the aorta due to **Erdheim's medial degeneration** and **Marfan's syndrome**.
- **Myxomatous** change in the dermis in **myxoedema**.
- **Myxoid** change in the **synovium in ganglion**


Mucinous material
H&E, x400




Mucinous material
H&E, x200



Gen Path Pract IV

Pigment disorders

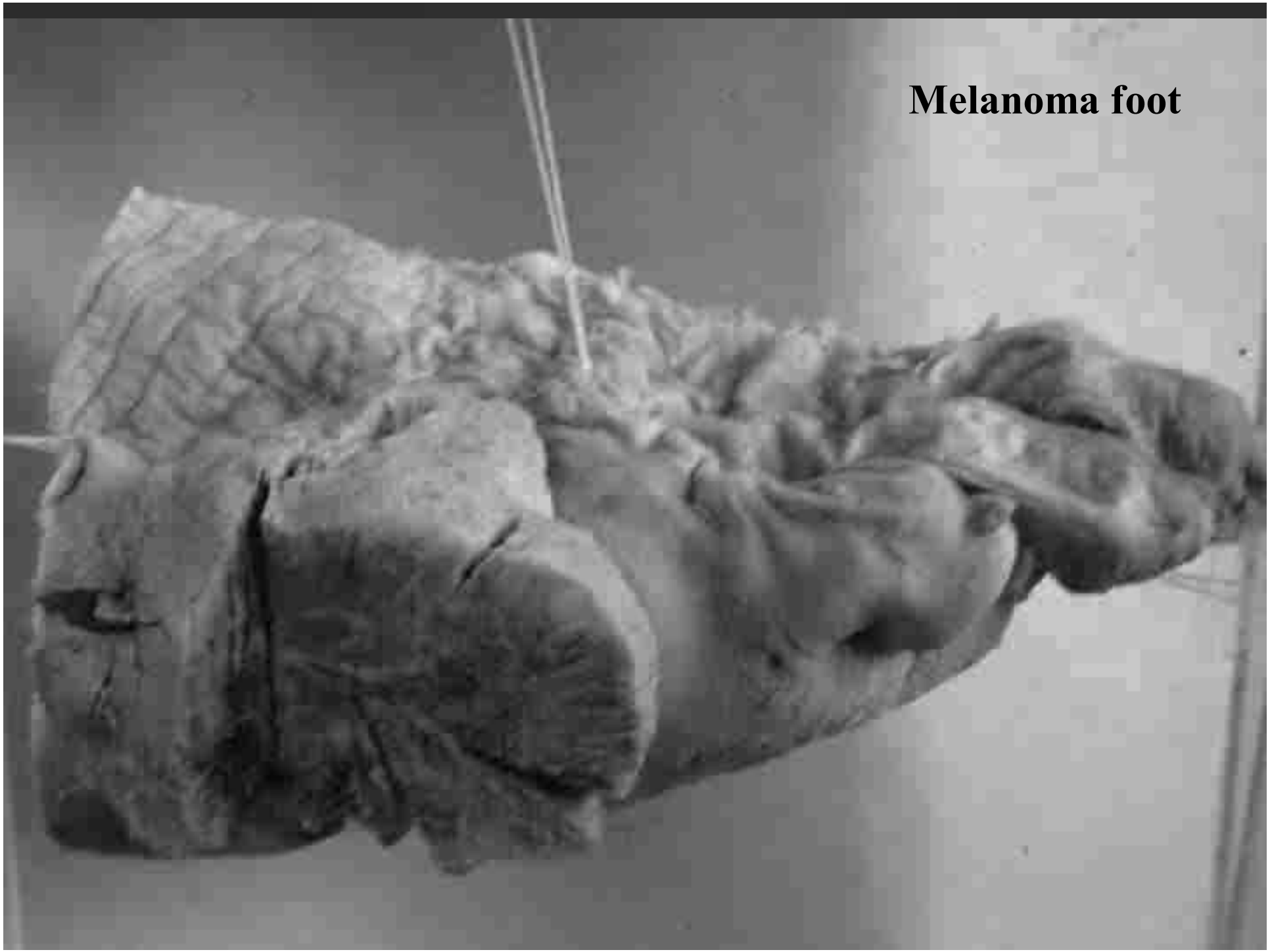
ENDOGENOUS PIGMENTS

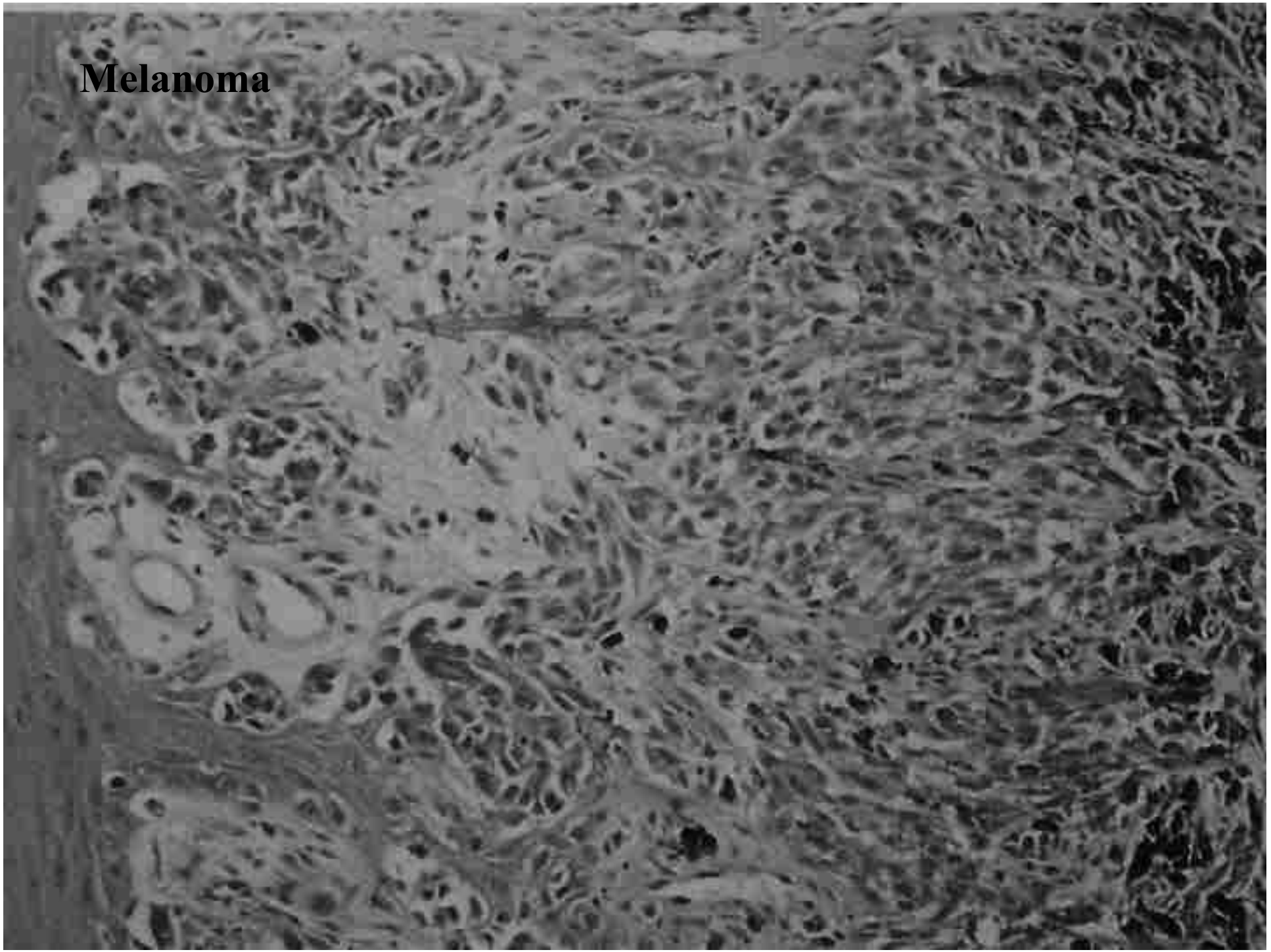
Melanins	Hb & derivatives	Lipopigments
1. Melanin	1. Bilirubin	1. Lipofuscin
2. Pigment in melanosis coli	2. Hemoglobin	2. Ceroid
3. Pigment of ochronosis	3. Hematin	3. Hemofuscin
	4. Malarial pigment	
	5. Porphyrin	

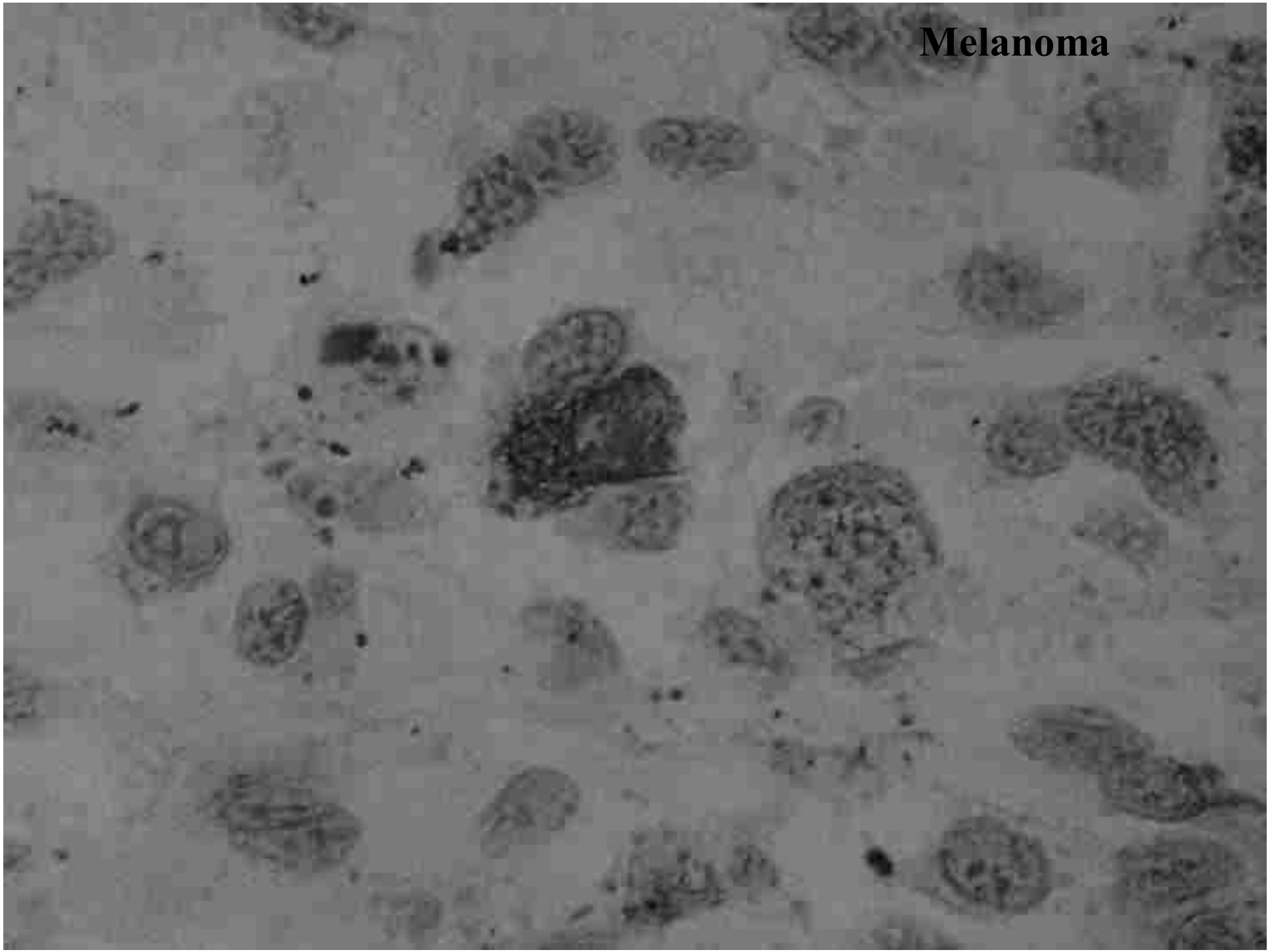
ENDOGENOUS PIGMENTS

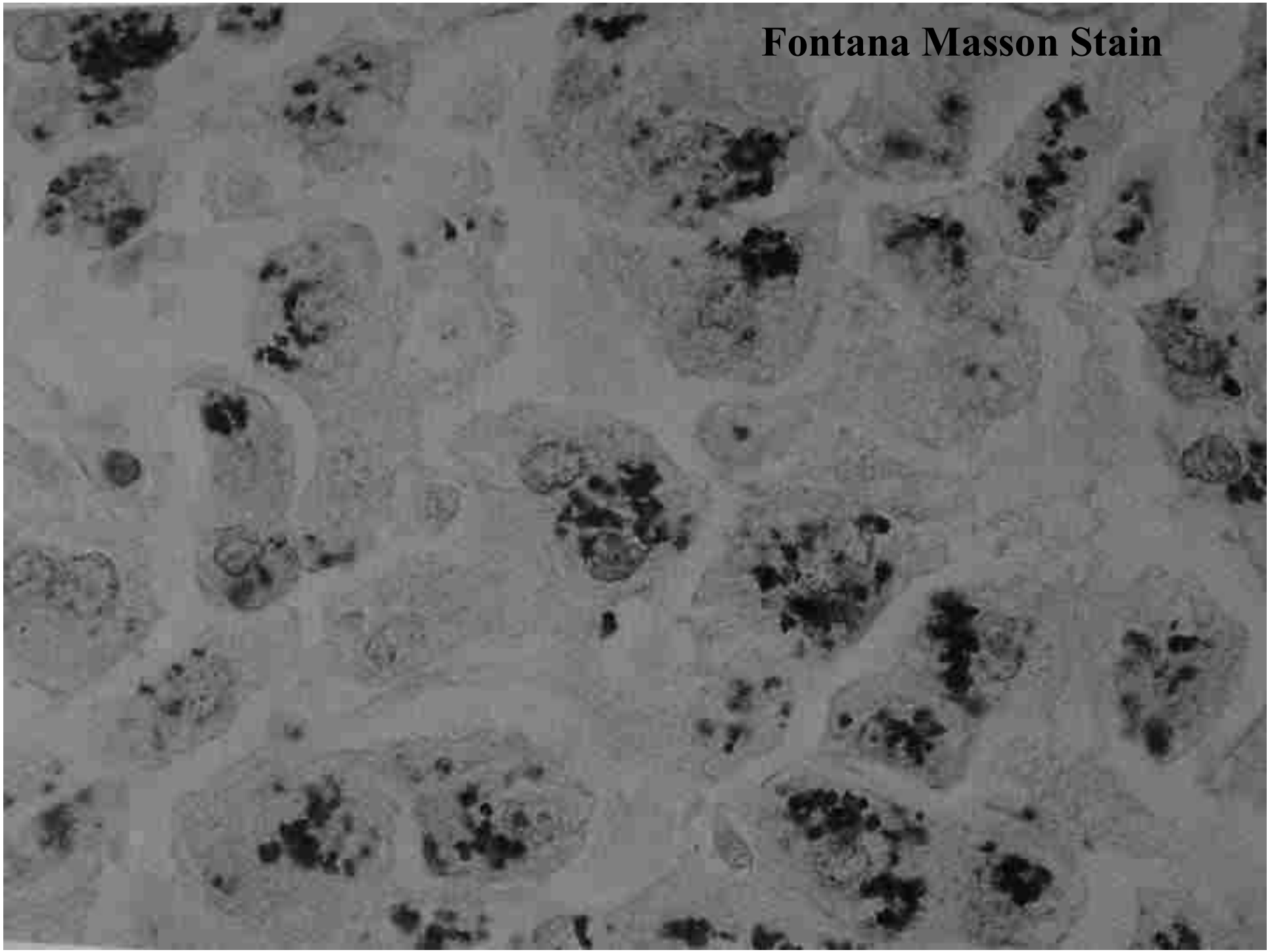
- **Melanin Pigments**
  - **Brown-black**
  - **Bleached by H_2O_2, Chlorine water, $KMnO_4$**
  - **Fontana Masson argentaffin technique: black granules.**

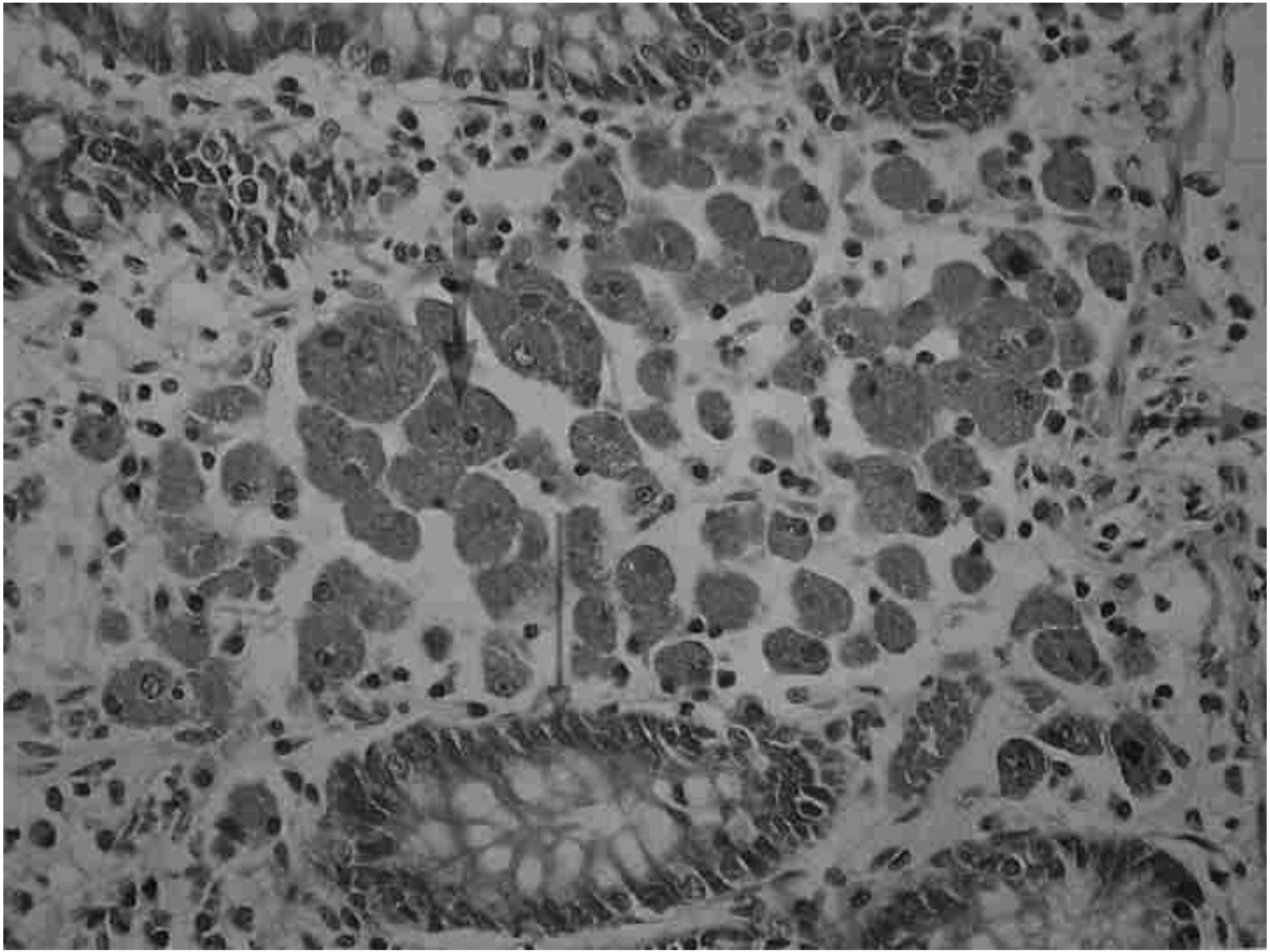
- **Melanosis Coli: Pseudomelanosis**
  - **Brownish-black pigment in submucosa of intestine.**


Melanoma foot


Melanoma

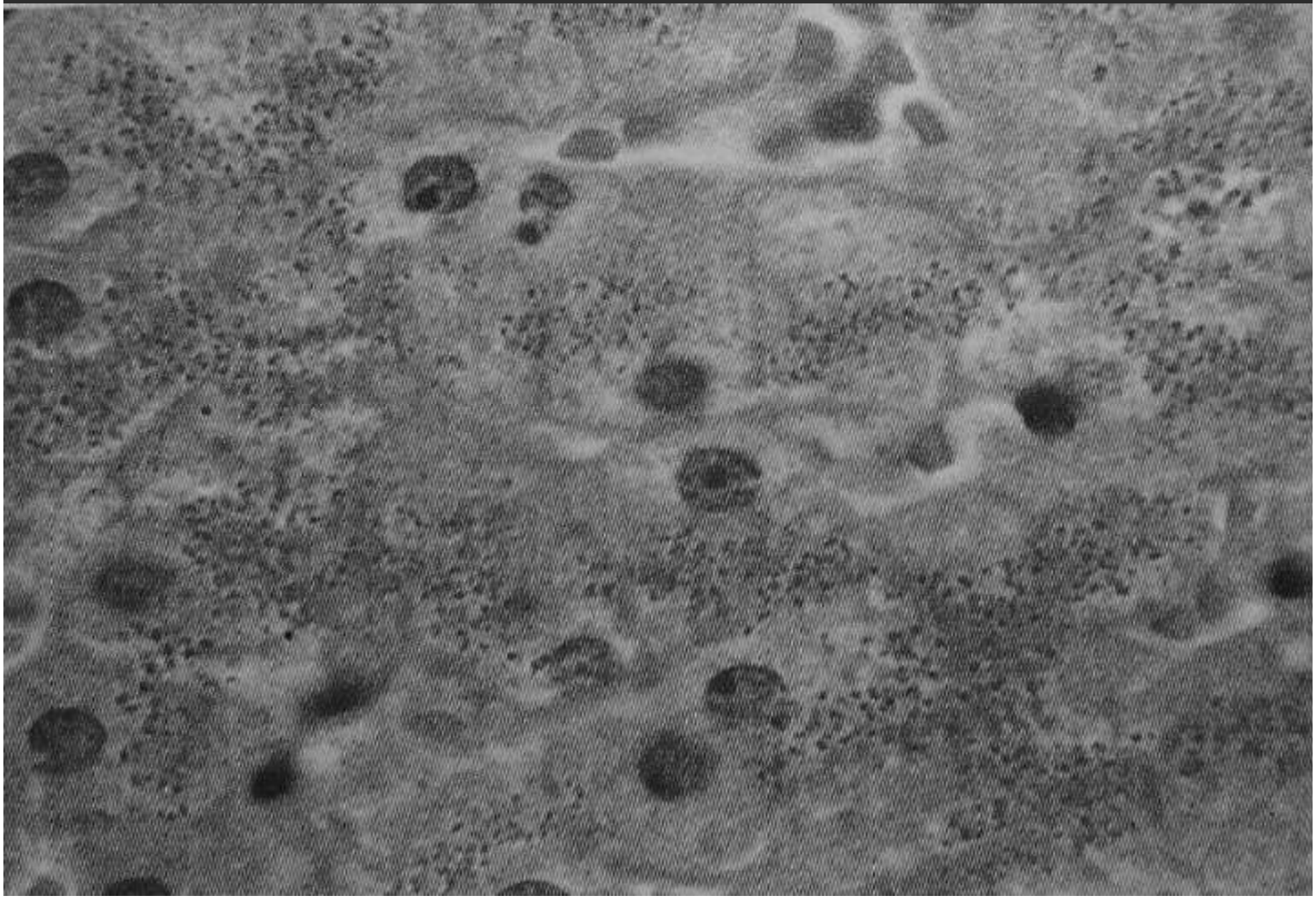

Melanoma


Fontana Masson Stain

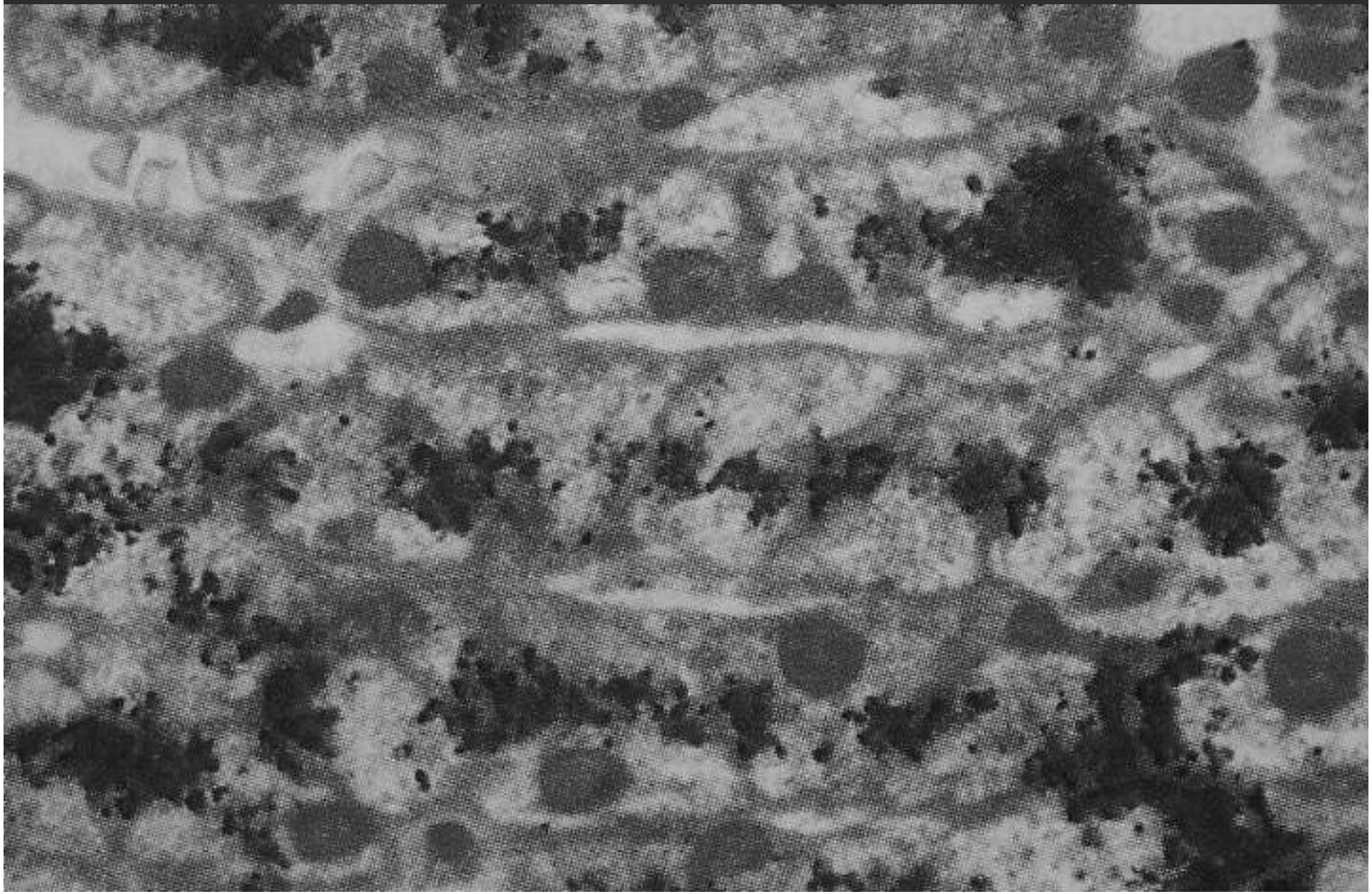


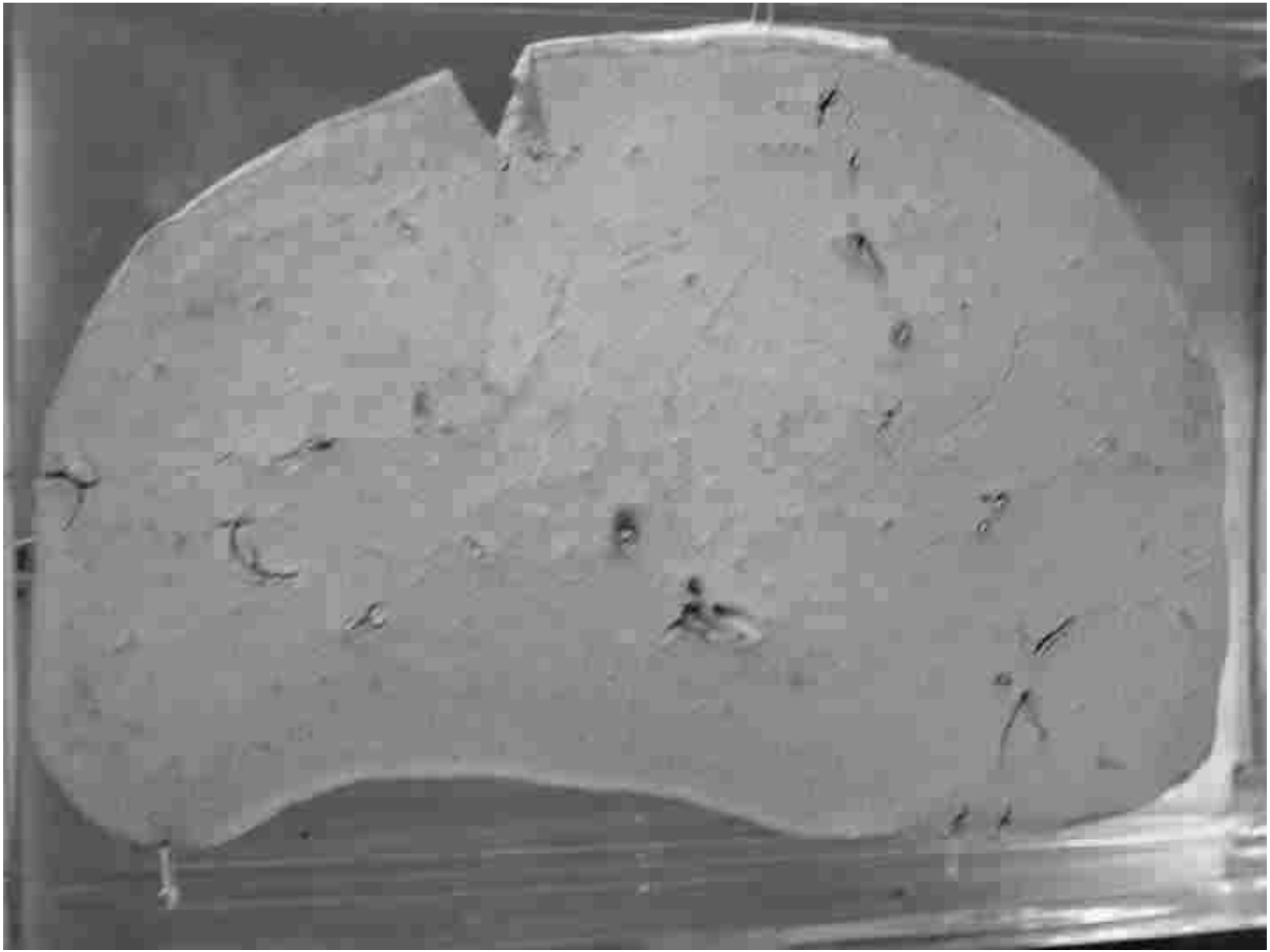
Haemoglobin and its derivatives

Haemosiderin in liver

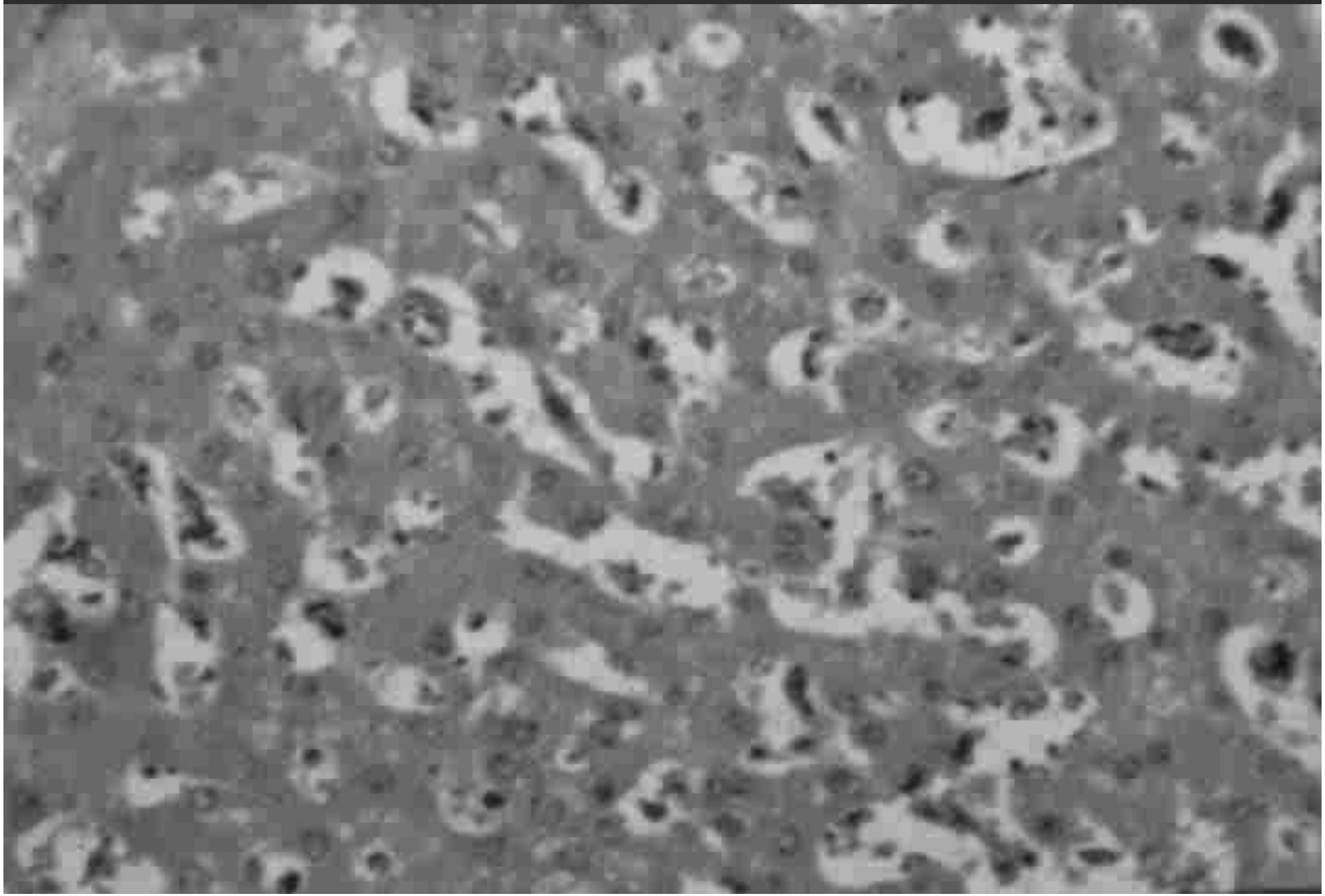


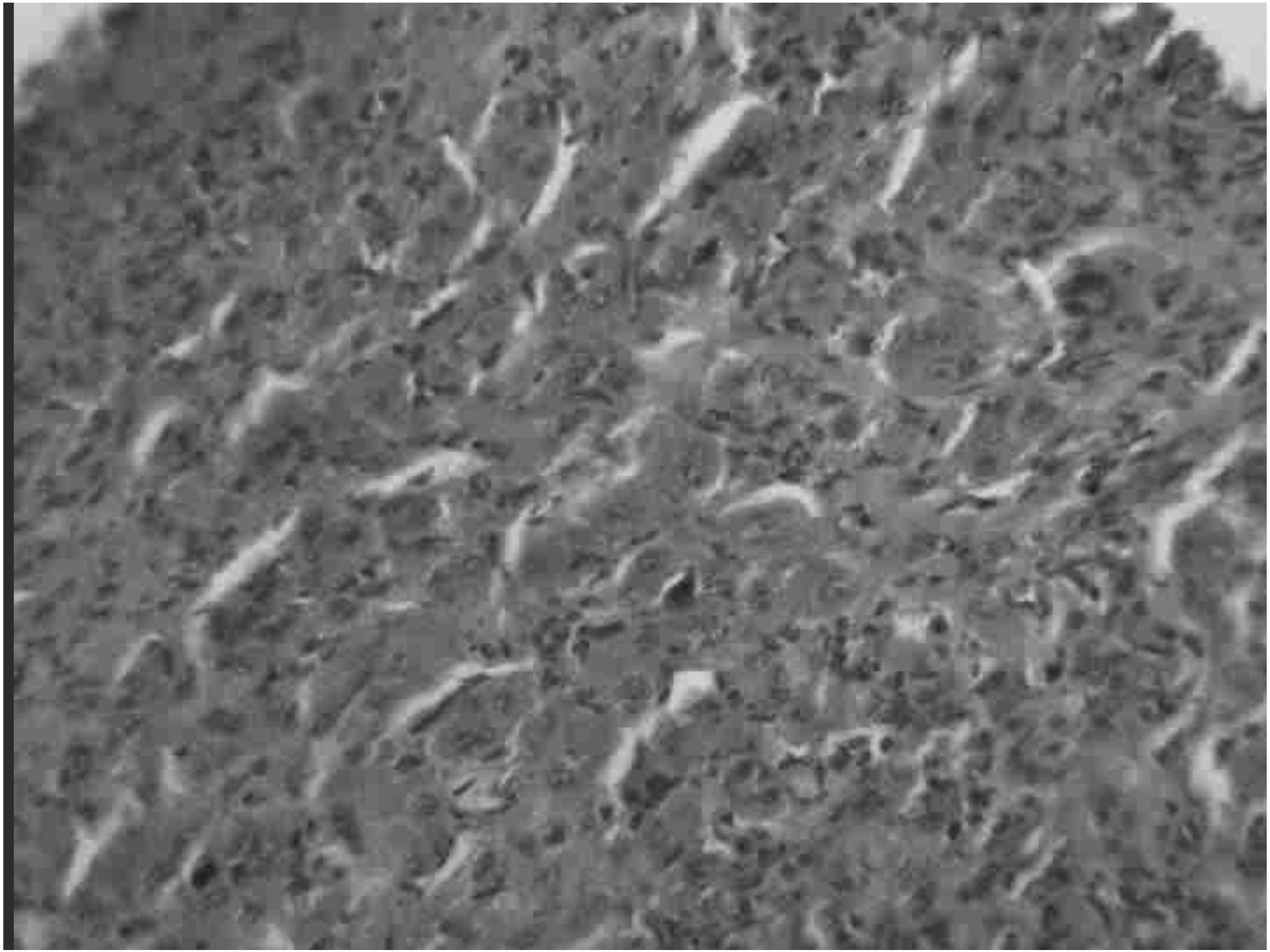
Prussian blue



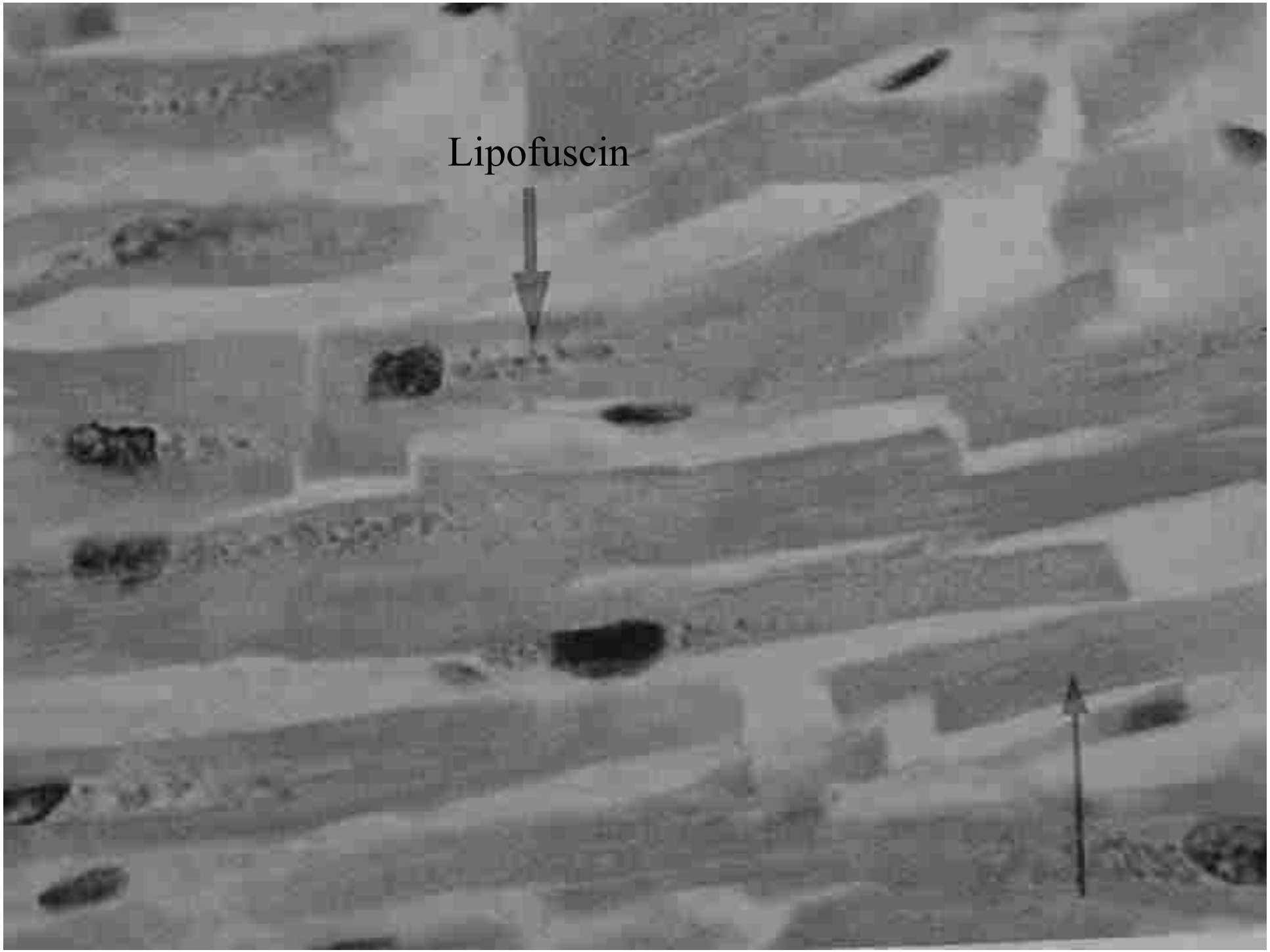


Haemozoin



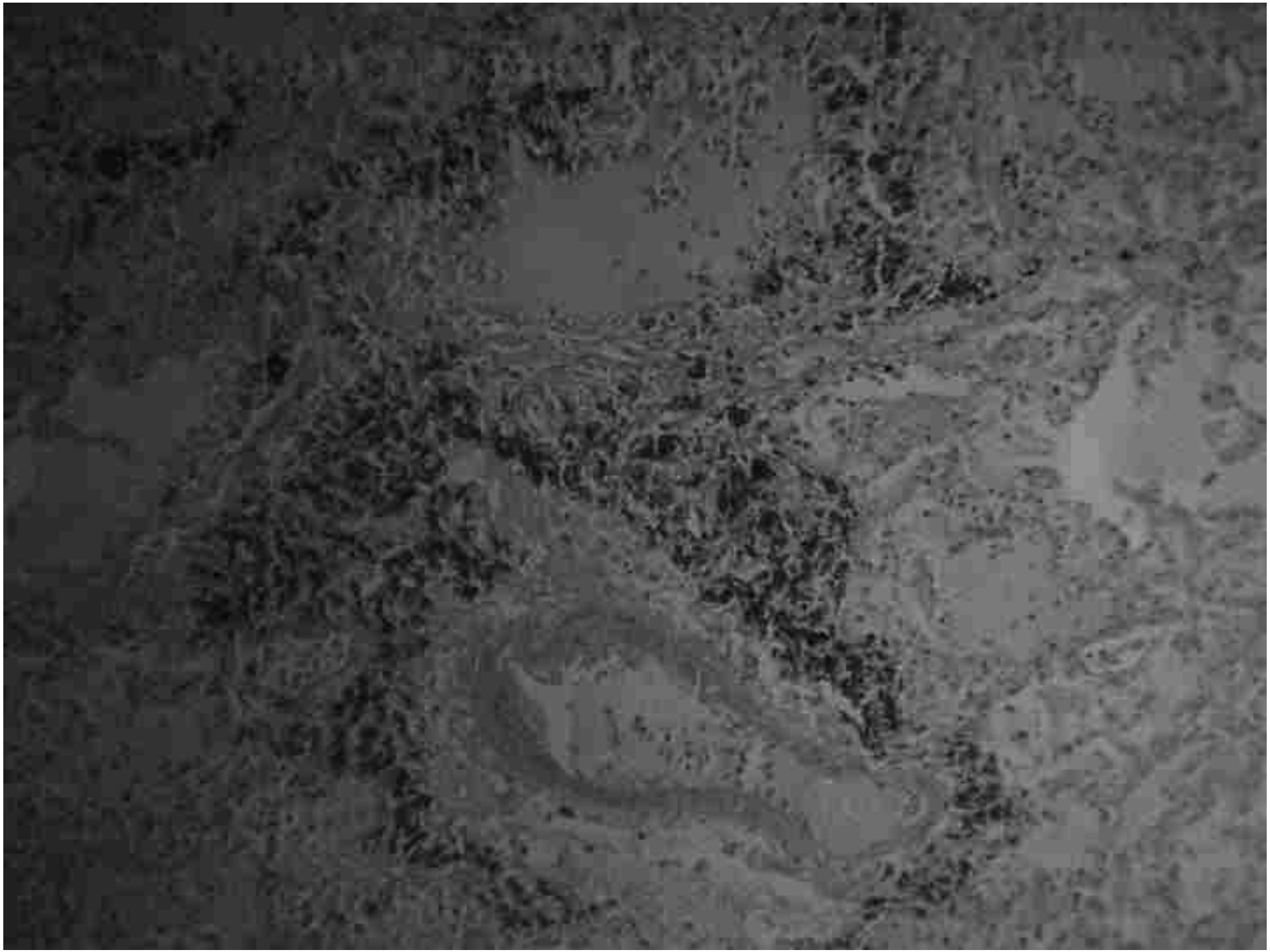


Lipofuscin


Lipofuscin

EXOGENOUS PIGMENTS

- **Introduced by**
  - **Inhalation – Anthracosis**
  - **Ingestion – Lead & mercury**
  - **Inoculation – Tattoing-Intradermal with India ink.**



CALCIFICATION

- **Types:** **1. Physiological**

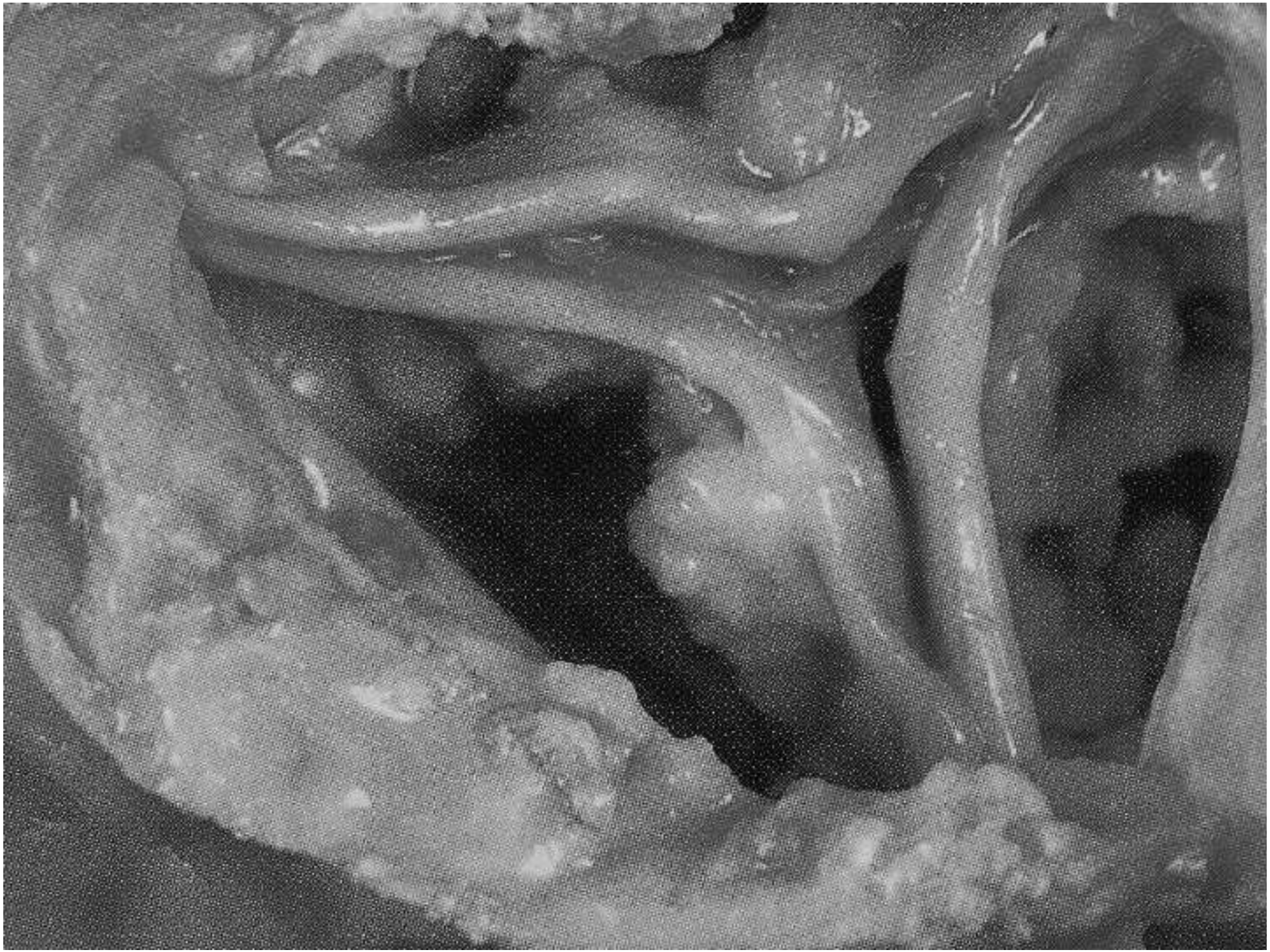
2. Pathological:

a) Metastatic

b) Dystrophic

Gross: Chalky-white, gritty deposits.

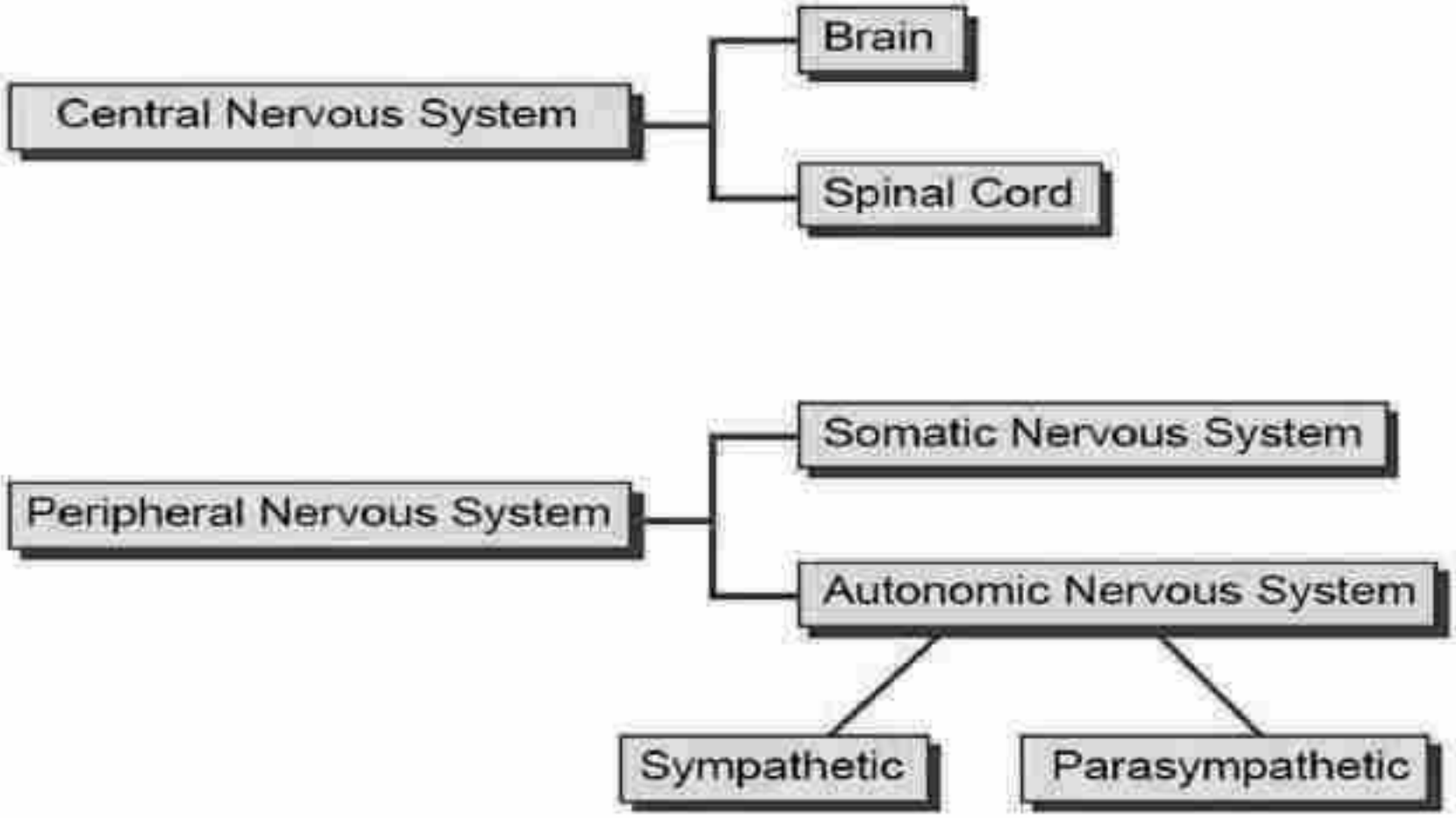
Staining in H & E: Deep violet & granular material either finely divided or as coarse clumps.



INTRODUCTION TO AUTONOMIC NERVOUS SYSTEM

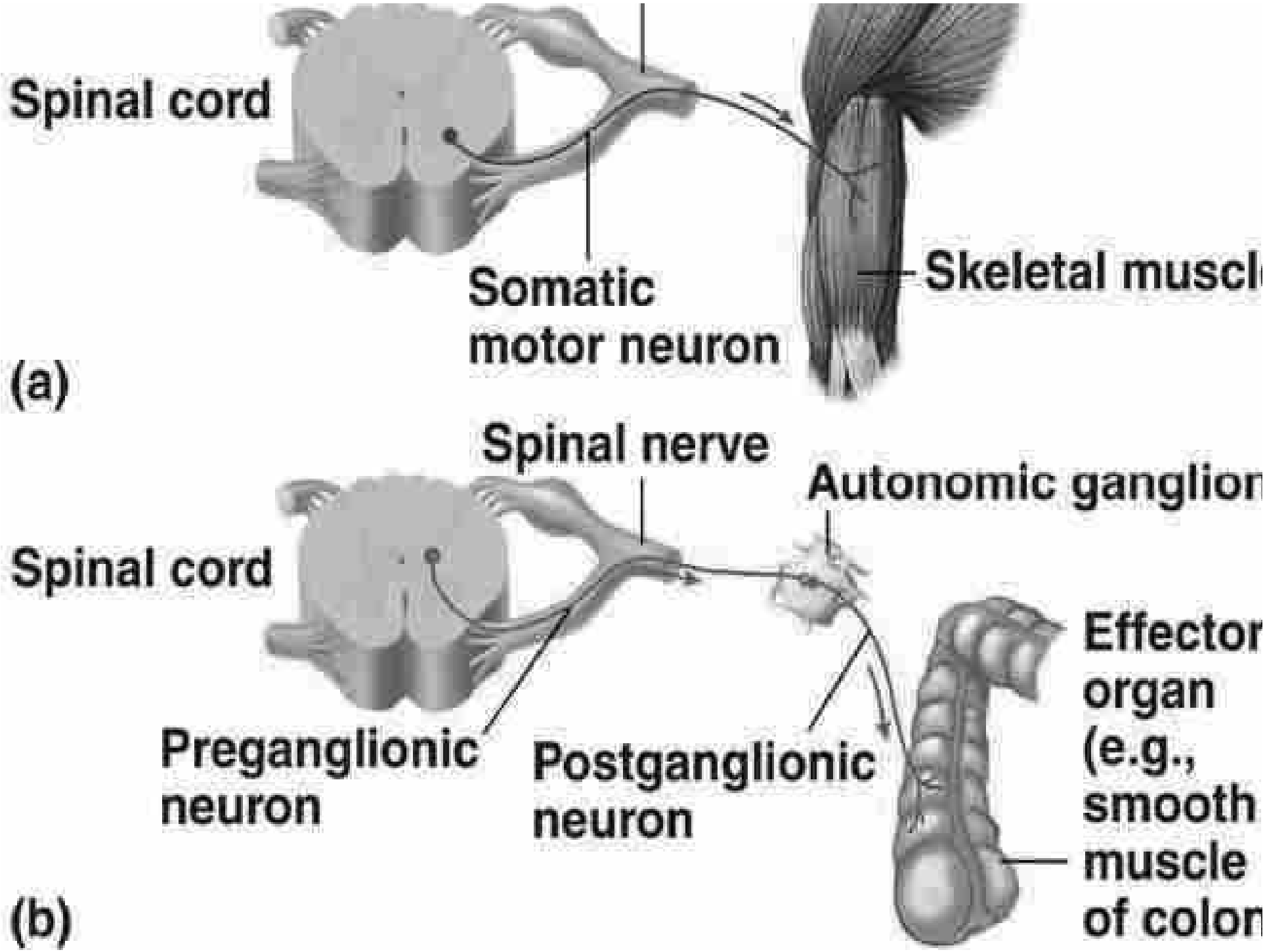
Human Nervous System

The divisions of nervous system



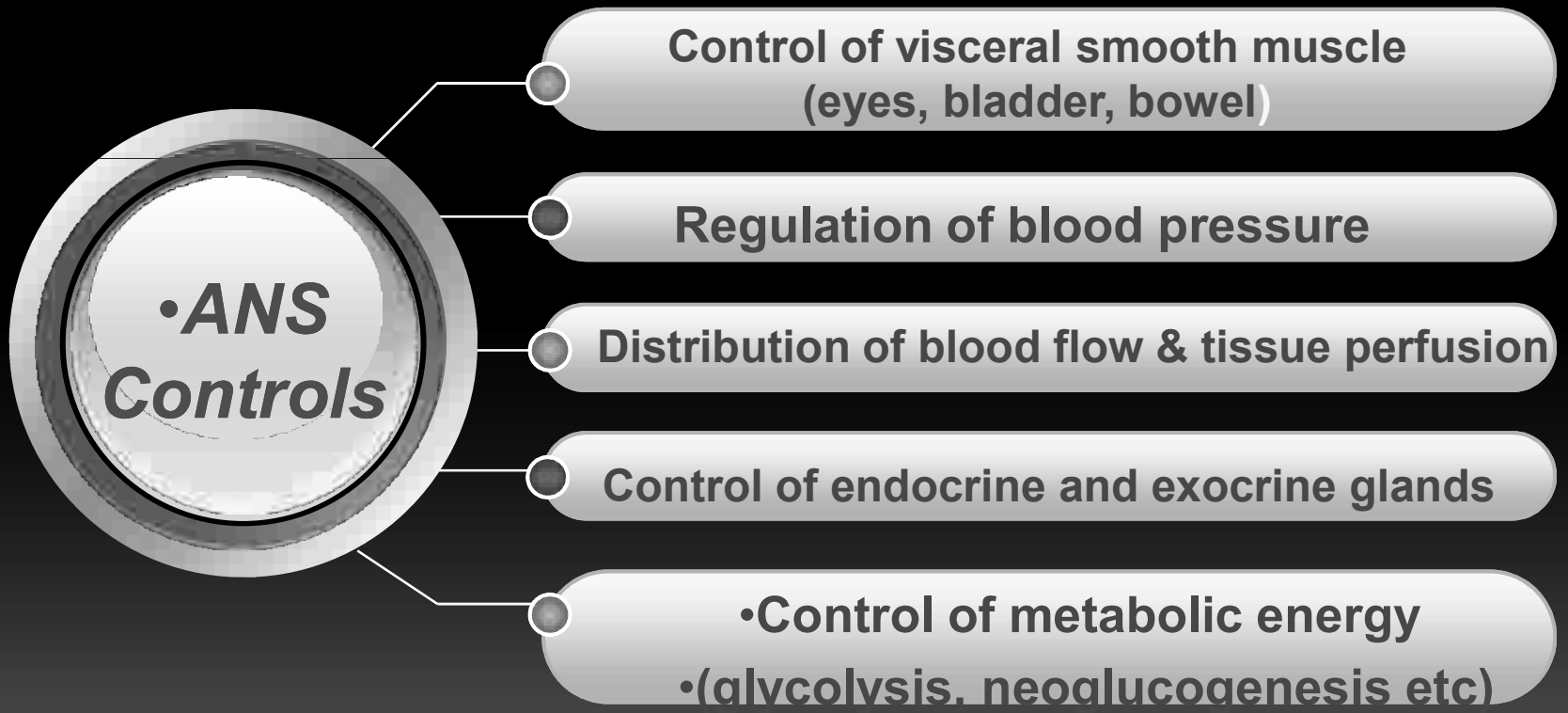
Two or "Three" Subdivisions of the Nervous System

Innervates	skeletal muscle	smooth muscle cardiac muscle secretory glands	intestine controls intestinal motility secretion absorption
Neurotransmitter	ACh	norepinephrine ACh neuropeptides	norepinephrine ACh serotonin neuropeptides


Spinal cord
Somatic
motor neuron
Skeletal muscl
(a)
Spinal nerve
Autonomic ganglion
Spinal cord
Effector
organ
(e.g.,
smooth
muscle
of colon
Preganglionic
neuron
Postganglionic
neuron
(b)

What Is It ?

•**The component of the Nervous System that functions below the conscious level, controlling several key physiological processes**



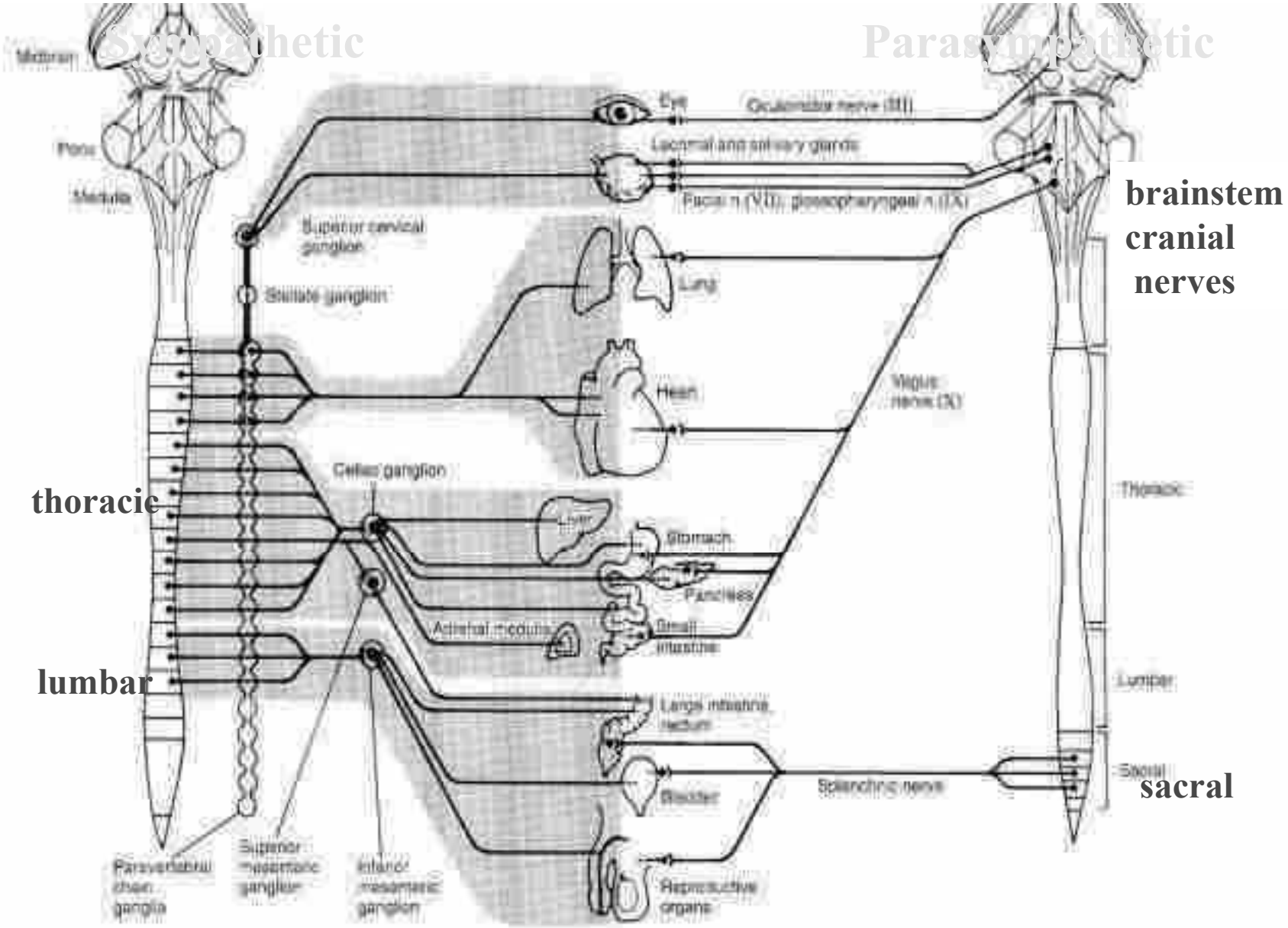


FIGURE 49–2

Sympathetic and parasympathetic divisions of the autonomic nervous system. Preganglionic neurons of the sympathetic division extend from the first thoracic spinal segment to lower lumbar segments. Parasympathetic preganglionic neurons are located within the brain stem and in segments S2 to S4 of the spinal cord. This figure also illustrates the coordinate innervation of a subset of targets by these two divisions of the autonomic nervous system.

Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.

Table 16.2 Comparison of the Sympathetic and Parasympathetic Divisions

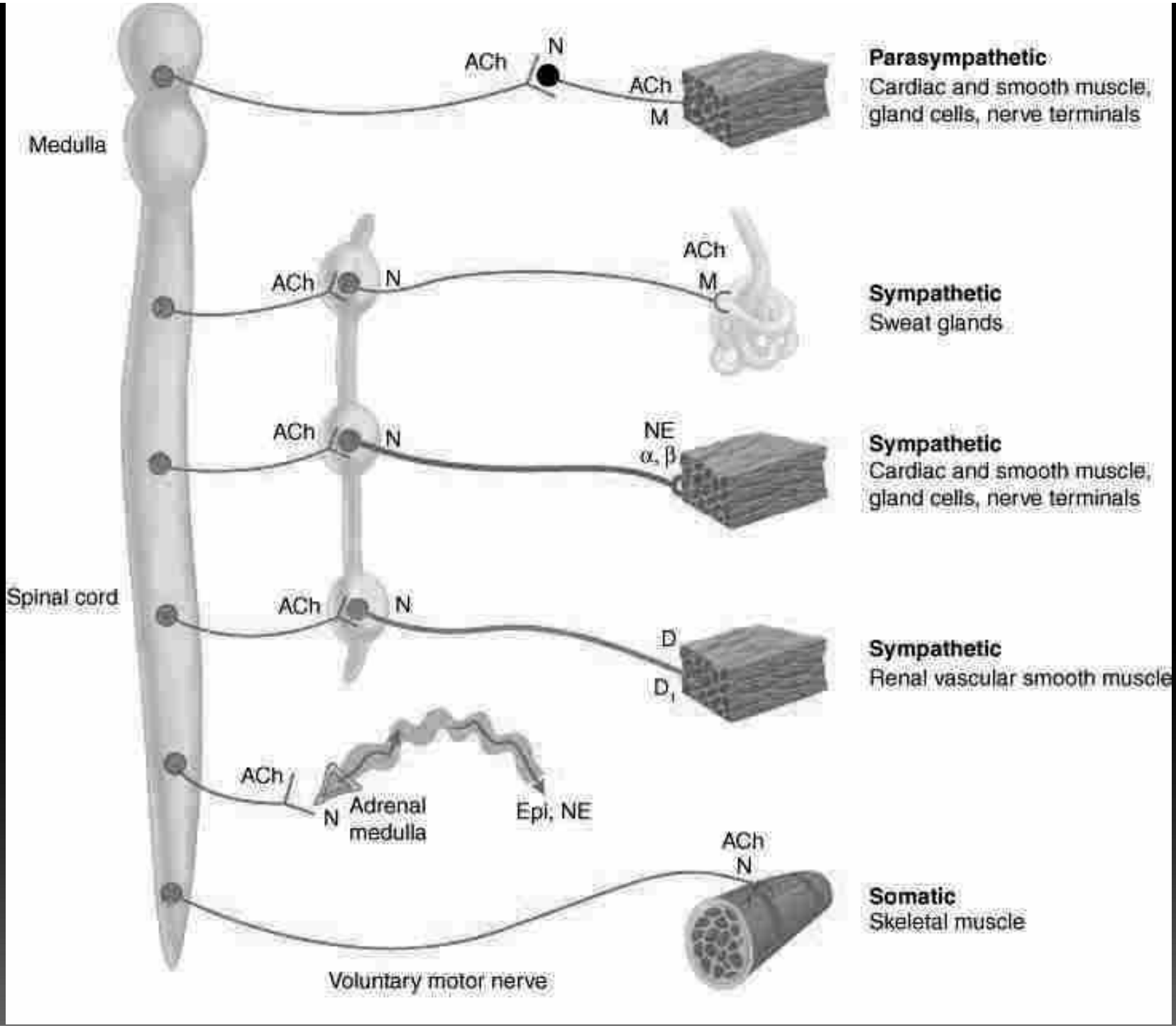
Features	Sympathetic Division	Parasympathetic Division
Location of preganglionic cell body	Lateral horns of spinal cord gray matter (T1–L2)	Brainstem and lateral parts of spinal gray matter (S2–S4)
Outflow from the CNS	Spinal nerves Sympathetic nerves Splanchnic nerves	Cranial nerves Pelvic nerves
Ganglia	Sympathetic chain ganglia along spinal cord for spinal and sympathetic nerves; collateral ganglia for splanchnic nerves	Terminal ganglia near or on effector organ
Number of postganglionic neurons for each preganglionic neuron	Many (much divergence)	Few (less divergence)
Relative length of neurons	Short preganglionic Long postganglionic	Long preganglionic Short postganglionic

Neurotransmitters

Neurotransmitters Present In Peripheral Nervous System

- Acetylcholine
- Norepinephrine
- Epinephrine

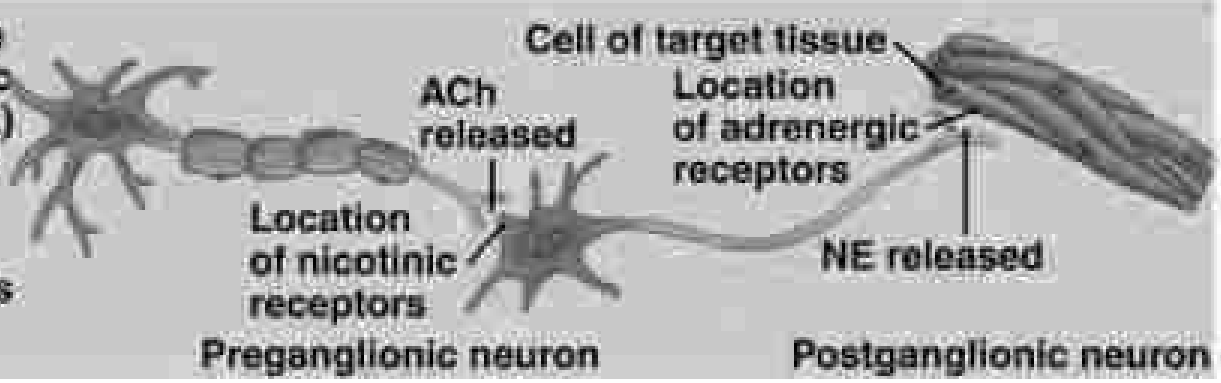
- Definition
  - Neurotransmitters are endogenous chemicals which transmit signals from a neuron to a target cell across a synapse


Medulla
ACh
N
ACh
M
Parasympathetic
Cardiac and smooth muscle, gland cells, nerve terminals
ACh
N
ACh
M
Sympathetic
Sweat glands
ACh
N
NE
α, β
Sympathetic
Cardiac and smooth muscle, gland cells, nerve terminals
Spinal cord
ACh
N
D
D1
Sympathetic
Renal vascular smooth muscle
ACh
N
Adrenal medulla
Epi, NE
ACh
N
Somatic
Skeletal muscle
Voluntary motor nerve

Copyright © The McGraw-Hill Companies, Inc. Permission required for reproduction or display.

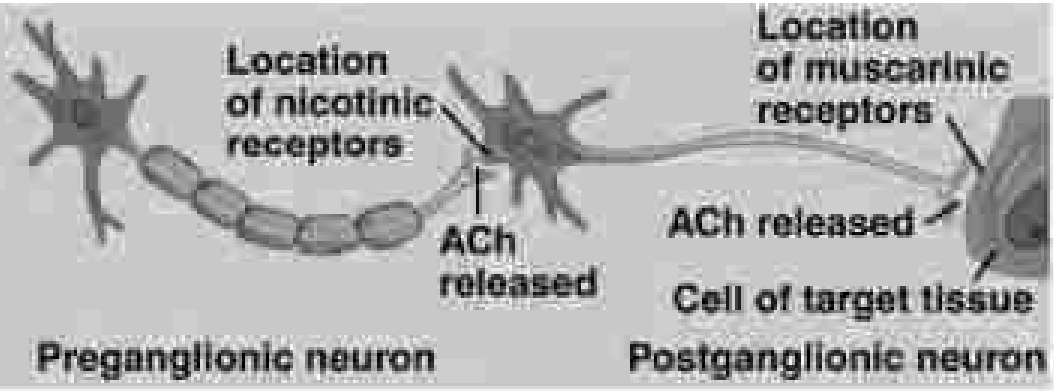
Sympathetic division

Most target tissues innervated by the sympathetic division have adrenergic receptors. When norepinephrine (NE) binds to adrenergic receptors, some target tissues are stimulated, and others are inhibited. For example, smooth muscle cells in blood vessels are stimulated to constrict, and stomach glands are inhibited.



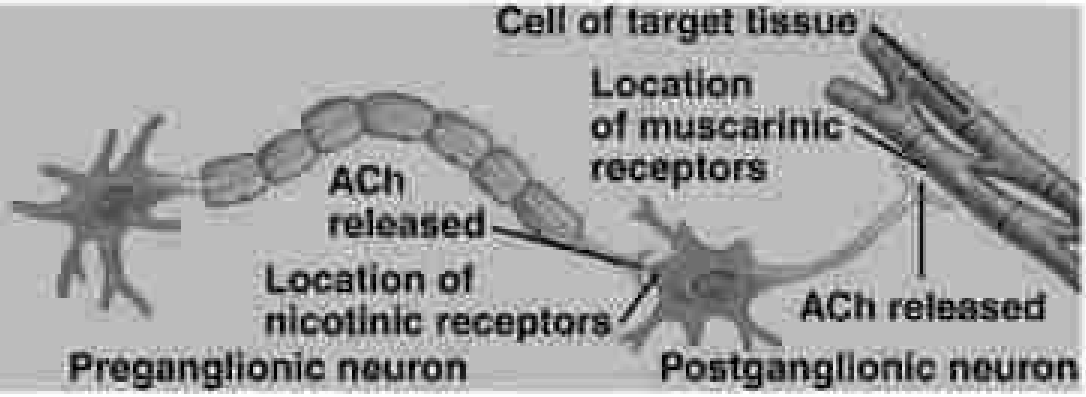
Sympathetic division

Some sympathetic target tissues, such as sweat glands, have muscarinic receptors, which respond to acetylcholine (ACh). Stimulation of sweat glands results in increased sweat production.

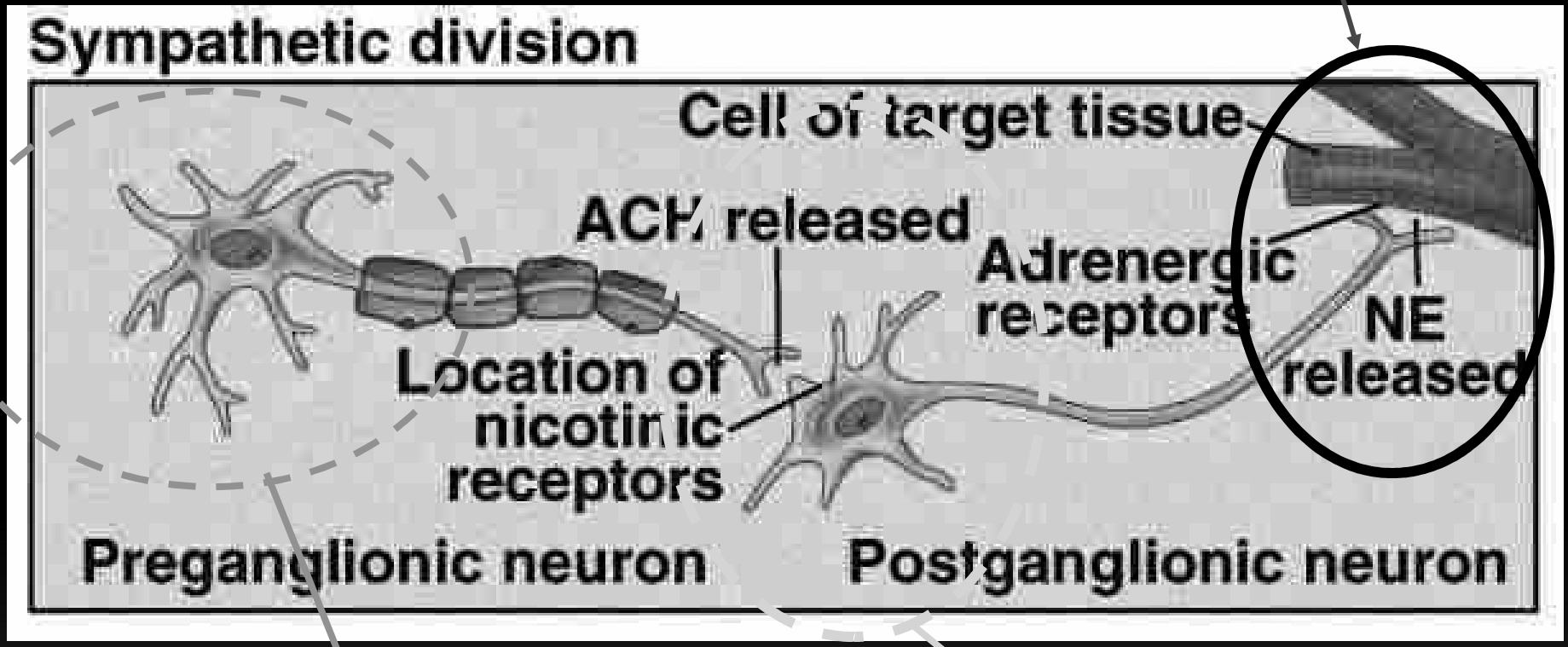


Parasympathetic division

All parasympathetic target tissues have muscarinic receptors. The general response to ACh is excitatory, but some target tissues, such as the heart, are inhibited.

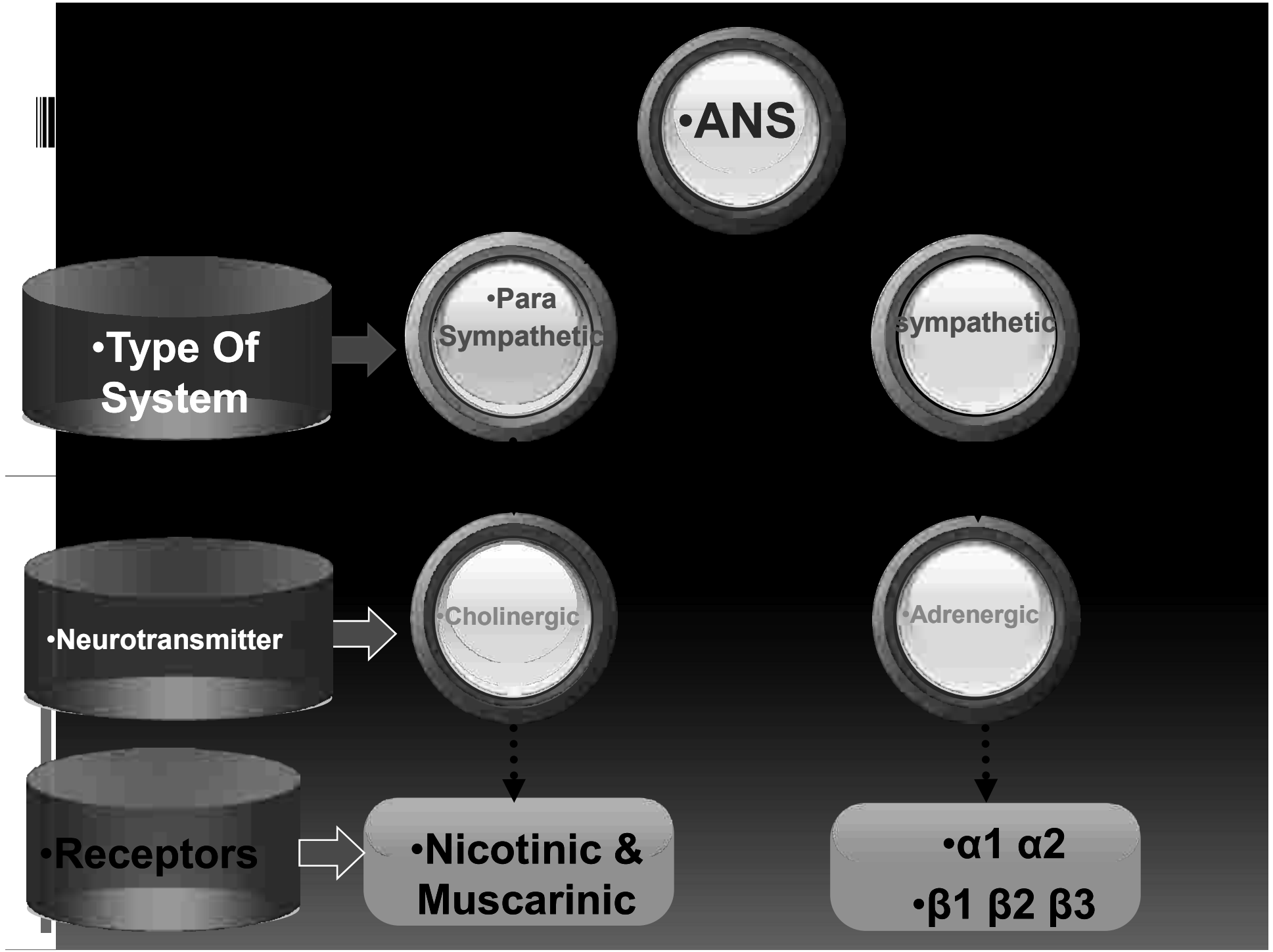


Focus on this synapse

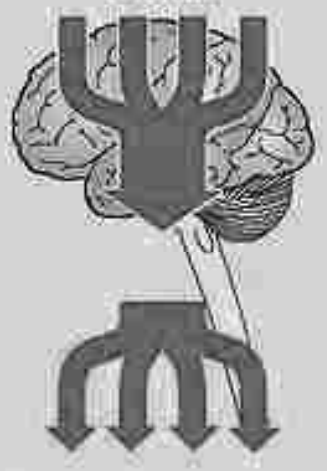


autonomic ganglion

Cell body in spinal cord

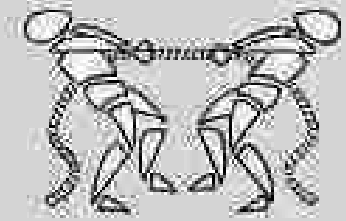

•ANS
•Type Of System
•Para Sympathetic
Sympathetic
•Neurotransmitter
•Cholinergic
•Adrenergic
•Receptors
•Nicotinic & Muscarinic
•α1 α2
•β1 β2 β3

"Fight or flight" stimuli

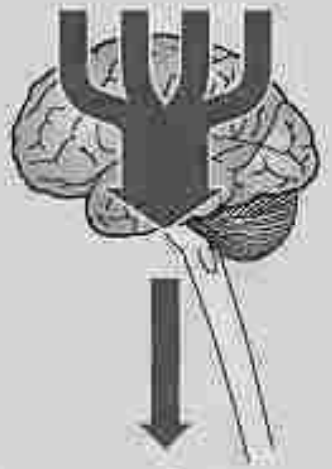


Sympathetic output
(diffuse because postganglionic neurons may innervate more than one organ)

Sympathetic and para-sympathetic actions often oppose each other



"Rest and digest" stimuli



Parasympathetic output
(discrete because postganglionic neurons are not branched, but are directed so a specific organ)

Cholinergic Nervous System: (parasympathetic).

•Acetylcholine neurotransmitter releasing nerve fibres •The parasympathetic nervous system innervates both smooth and cardiac muscle as well as exocrine glands such as salivary and sweat glands.

Biologic responses to parasympathetic stimulation:

•Constriction of pupil , ciliary body (accommodation of lens)
•Contraction of smooth muscle in the GI and urinary tract
•Constriction of the bronchioles (“bronchoconstriction”)
•Slowing of heart rate (“bradycardia”)
•Increased secretion by the glands

Cholinergic agonist: Agents that bind to acetylcholine receptors and stimulates the parasympathetic system

Cholinergic antagonist: Agents that bind to acetylcholine receptors but exhibit no intrinsic activity (“parasympatholytic” – competitive)

Parasympathetic

The regulatory functions of PNS affect these sites

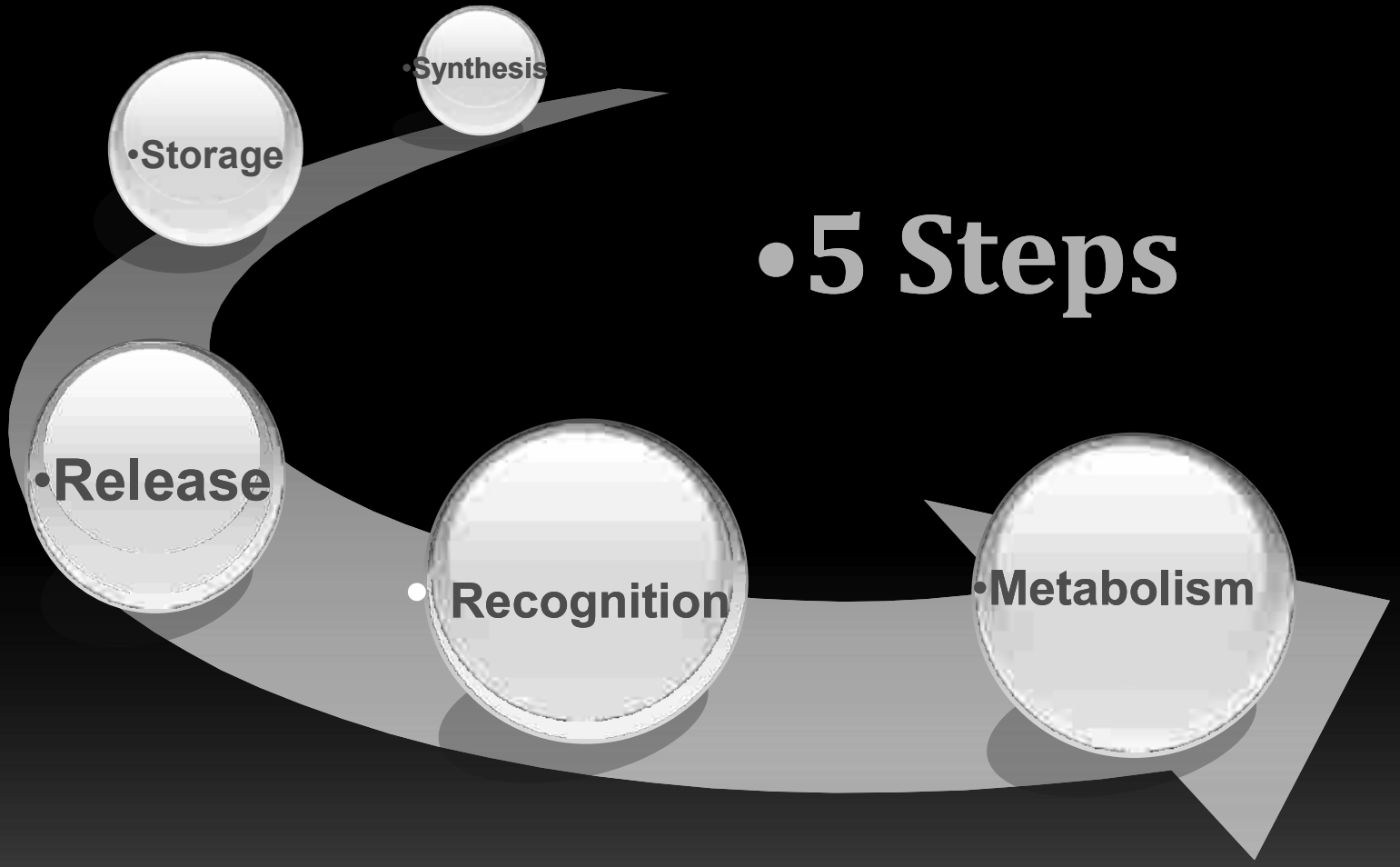
- Heart rate
- Gastric secretions
- Bladder and bowel
- Vision
- Bronchial smooth muscle

Sympathetic

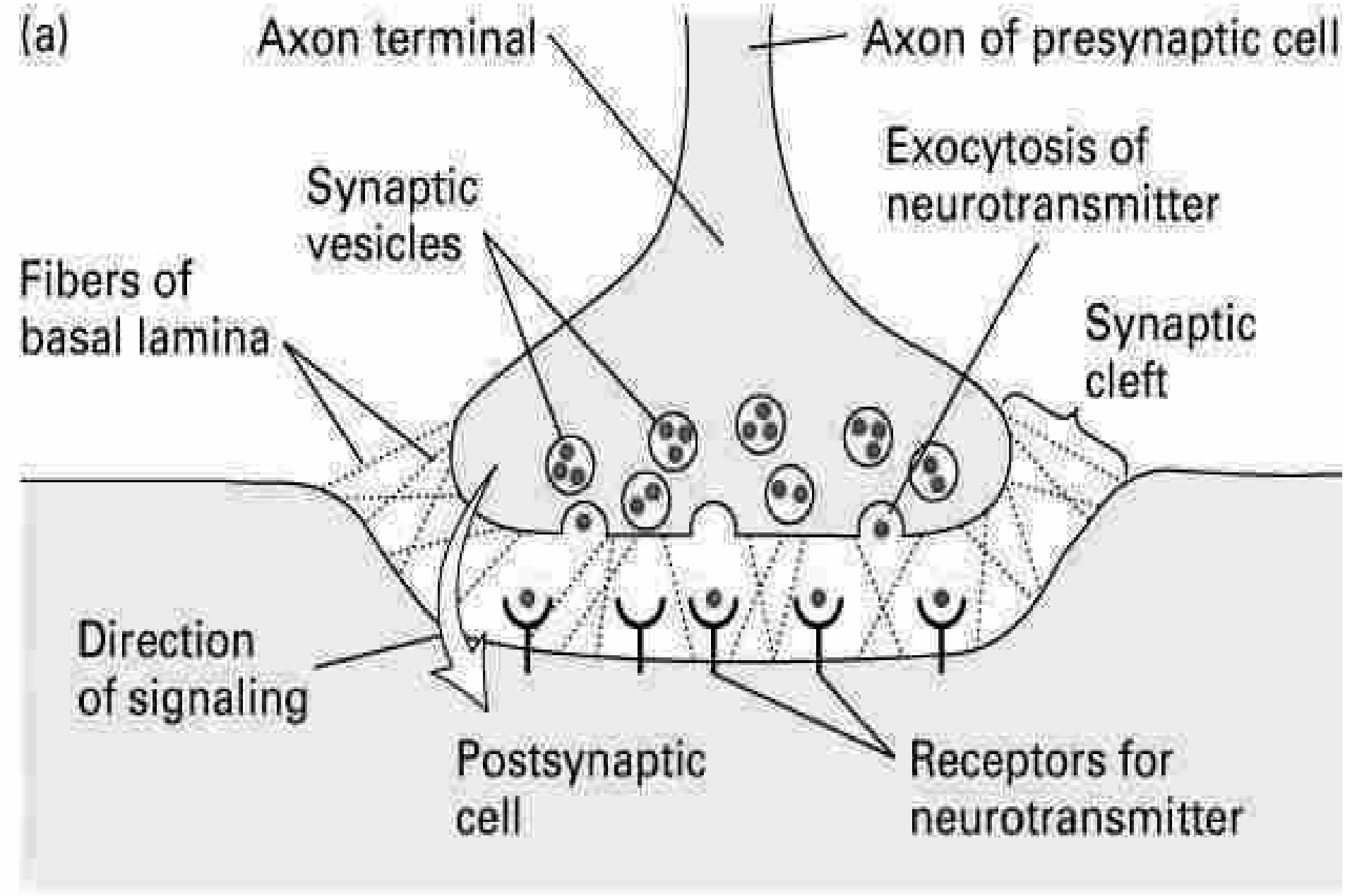
Main functions of the SNS

- Regulation of cardiovascular system
- Regulation of body temperature
- Implementation of “fight or flight” reaction

Neurohumoral transmission



Chemical neurotransmission



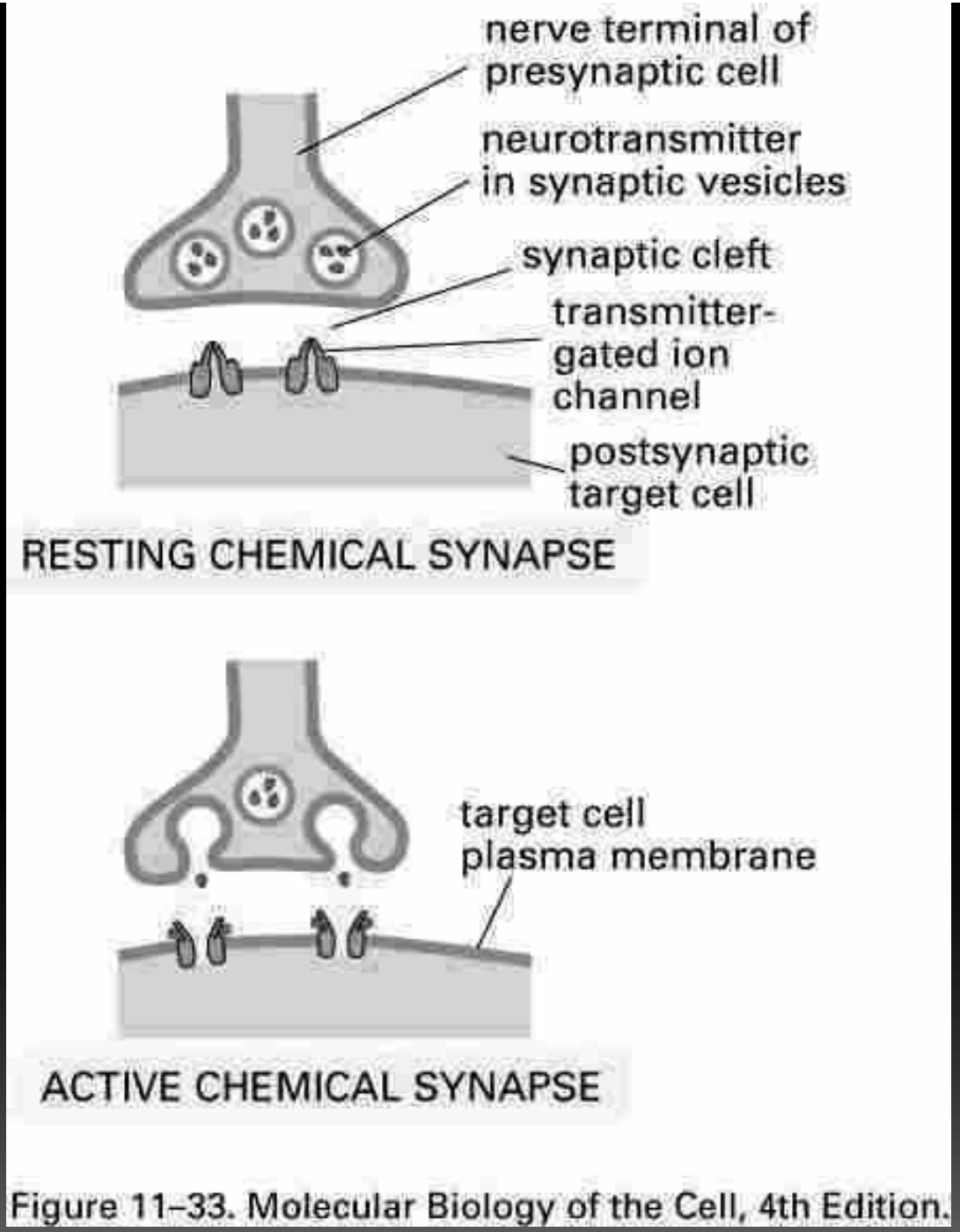
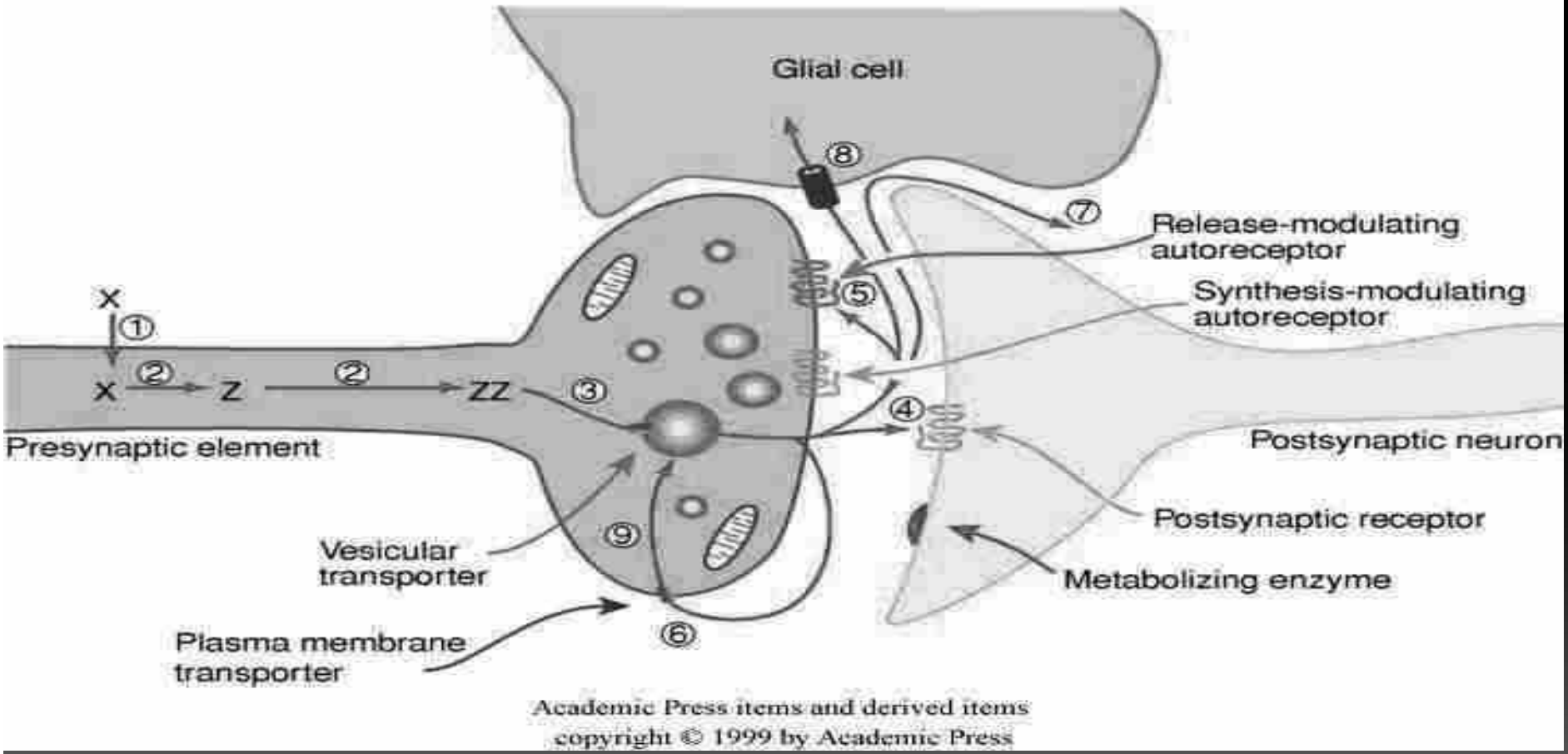


Figure 11–33. Molecular Biology of the Cell, 4th Edition.

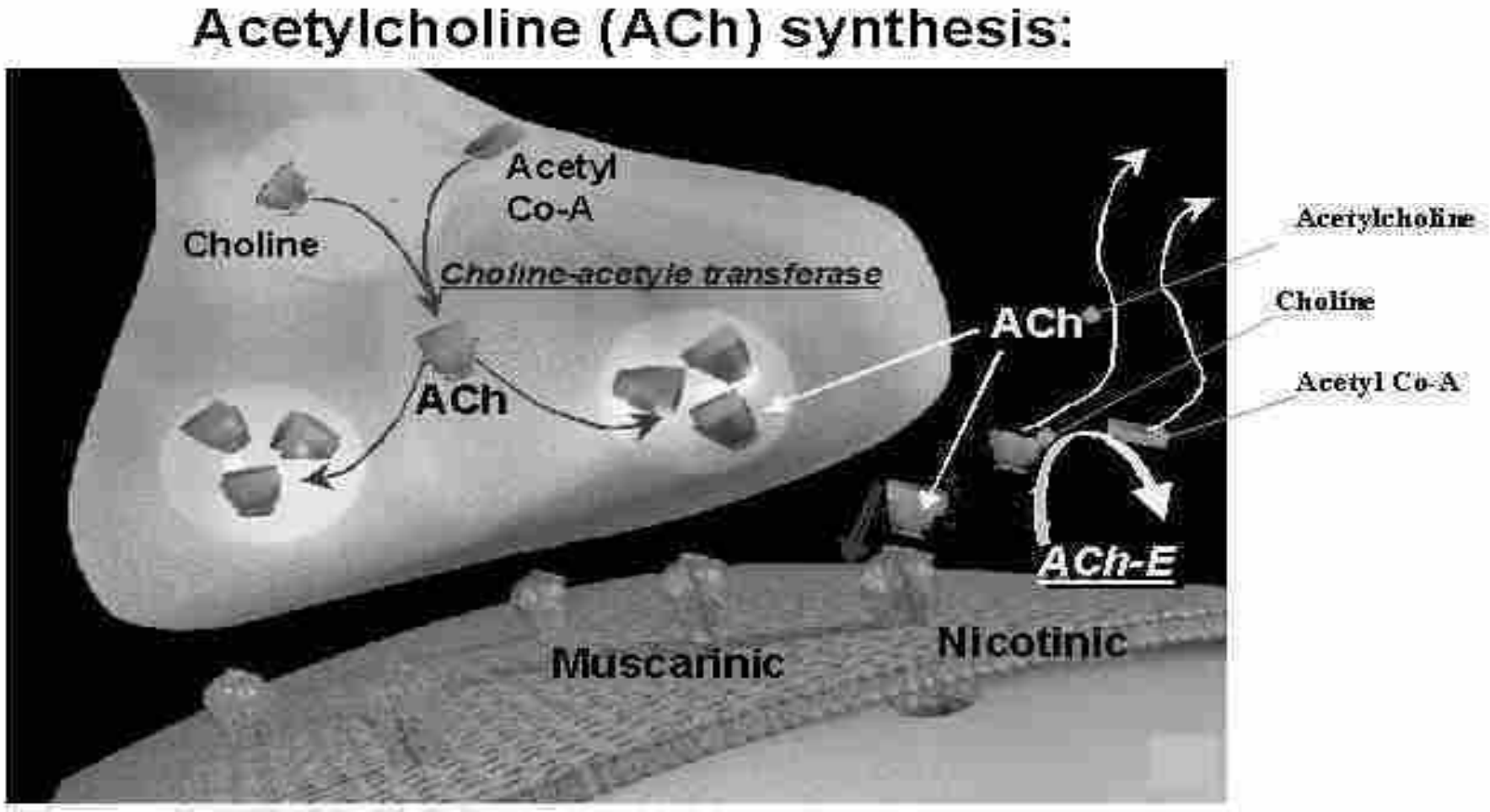
the presynaptic terminal and uptake into glial cells



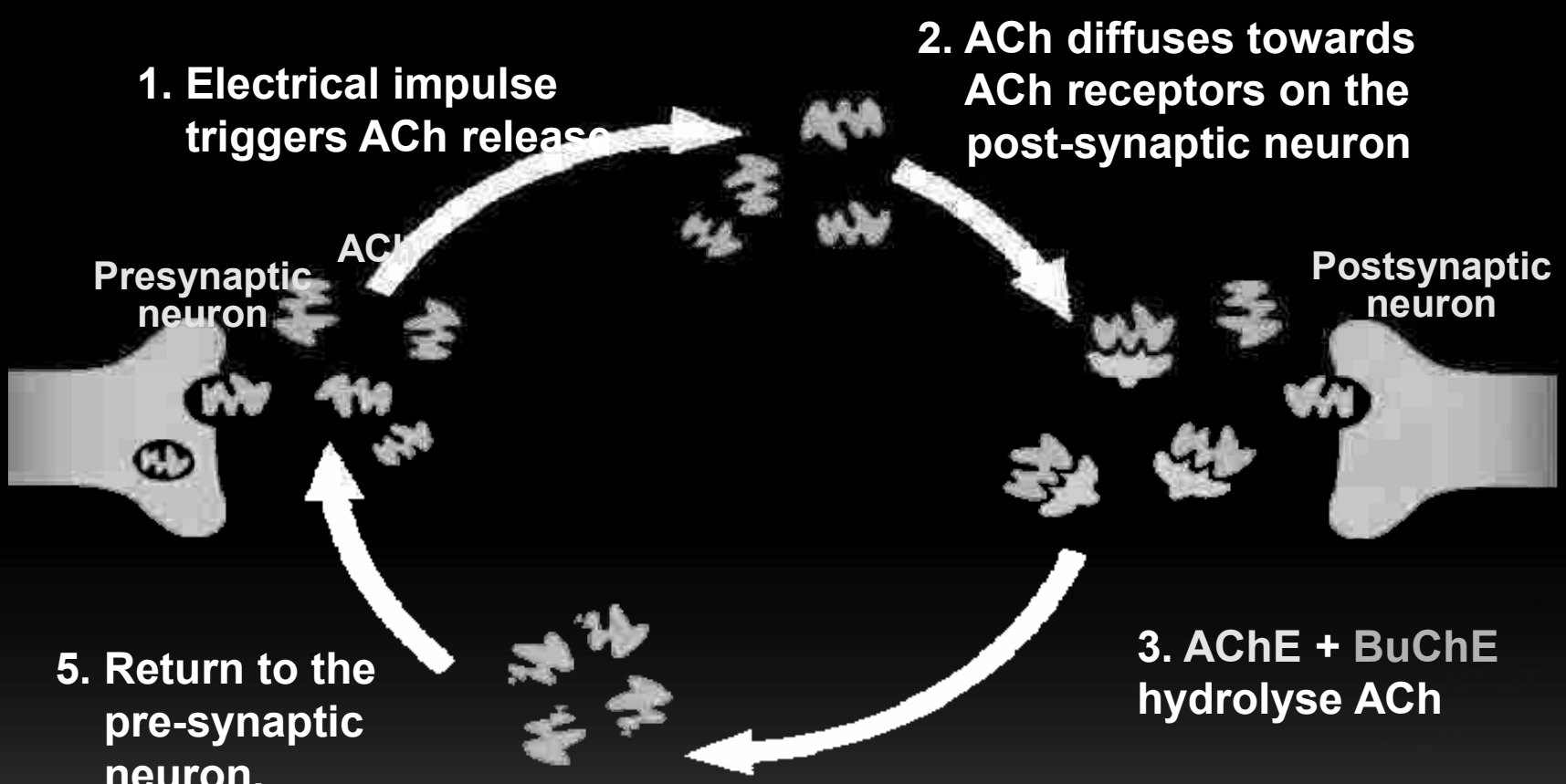
Academic Press items and derived items
copyright © 1999 by Academic Press

CHOLINERGICS

Synthesis, storage and release



CHOLINERGIC TRANSMISSION

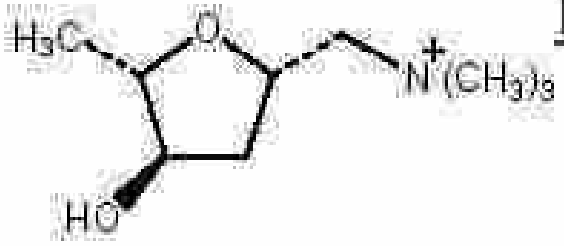


AChE=acetylcholinesterase
BuChE=butyrylcholinesterase

Cholinergic Receptors: Two distinct receptor types → Muscarinic and Nicotinic

Differs in composition, location, pharmacologic function
Agonists / antagonists for each structural type.

Muscarinic Receptors: Receptor sites where muscarine binds, eliciting the same qualitative effects as acetylcholine.



Muscarine
(toxic compound found in mushrooms)

Location:

(a) Postganglionic in parasympathetic nervous system (End organ)
(b) CNS

Characteristics: G-Protein coupled receptors

Sub-Types:

M_1-M_5 (five): Differing in tissue location and chemical specificities.

Chinoceptors

- Muscarinic- M1-M5

M1-ganglionic cells, central neurones..,gastric glands
-Plays role in gastric secretions, and relaxation of LES.

M2-Heart
-mediate vagal bradycardia.

M3- Visceral smooth muscle (contractions)
-glands (secretions)
-vascular endothelium (vasodilatation)

- Nicotinic-Nm and Nn

Muscarinic Acetylcholine Receptors					
	M_1	M_2	M_3	M_4	M_5
Distribution	Cortex, hippocampus	Heart	Exocrine glands, GI tract	Neostriatum	Substantia nigra
Antagonists	Pirenzepine	AF-DX 116	pF-HHSiD		
Agonists	Xanomeline, CDD-0097				
G protein	$G_{\alpha q/11}$	$G_{\alpha i/o}$	$G_{\alpha q/11}$	$G_{\alpha i/o}$	$G_{\alpha q/11}$
Intracellular response	Phospholipase Cβ	Adenylyl cyclase inhibition	Phospholipase Cβ	Adenylyl cyclase inhibition	Phospholipase Cβ

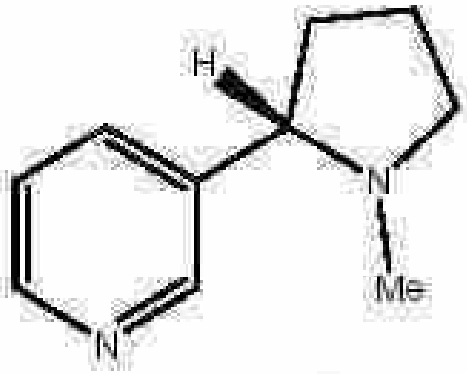
Nicotinic Receptors: Receptor sites acted upon by nicotine.

Location: (Distribution)

(a) Preganglionic in the autonomic nervous system (sympathetic and parasympathetic)

(b) Neuromuscular Junctions (Somatic)

(c) CNS



Nicotine

Characteristics:

Ligand gated ion channels (modulates Na^+ / K^+ transport)

Sub-Types:

N_1 (somatic) and N_2 (neural)

NICOTINIC RECEPTORS

- Nicotinic receptors are ligand gated ion channels linked to sodium ions.
- Nm: Present on skeletal muscle end plates.Mediate skeletal muscle contraction.
- Nn: Present on ganglionic cells, adrenal medullary cells and in spinal cord.mediate transmission in ganglion.

Cholinergic Agonists

- Choline esters: Acetylcholine
  Methacholine
  Bethanechol
  Carbachol

- Alkaloids Muscarine
  Pilocarpine

Pharmacological actions

A)MUSCARINIC

- Heart- bradycardia,slowed conduction, reduced force of contraction.

- Blood vessel- vasodilatation, fall in BP.

- Smooth muscle-
  1. contracted, tone and peristalsis in Git is increased-abd.cramps an bowel evacuation.
  2. urinary bladder detrusors contracts, trigone and sphincter relax – voiding of bladder.
  3. Bronchus –contraction

- Glands-
  secretions increased
  (salivation, lacrimation, tracheobronchial, gastric)

- Eyes-
  circular muscle of iris contracts -miosis

B) Nicotinic

Autonomic ganglia

Skeletal muscles

CHOLINE ESTERS

Ach-

- Hydrolyzed both by acetyl & butryl cholinesterase.
- Non selective i.e. acts on both muscrinic aswellas nicotinic receptors.
- Not used clinically because it is hydrolysed very fast (micro sec) by acetylcholiesterase.

Methacholine

- Hydrolyzed only by acetylcholinesterases.
- Relatively higher affinity for muscrinic receptors (CVS).
- Occasionally used for termination of paroxymal supraventricular tachycardia

Carbachol and Bethnachol-

- Resistant to hydrolysis by AchE.
- Affinity for muscarinic receptors (git, bladder).
- **Uses -** Postop / postpartum urinary retention,

  Neurogenic bladder atony,

  Congenital megacolon,

  GER.
- **A/E-** colic ,involuntary urination/

  defecation, flushing, sweating, fall in BP, bronchospasm
- **Dose :** 10-40mg oral

CHOLINOMIMETIC ALKALOIDS

PILOCARPINE

- Obtained from the leaves of pilocrapus microphyllus.
- Prominent Muscarinic action.
- Increases all the secretions .
- Have complex effect on CVS, small doses decreases BP but larger doses have opposite action. (Ganglionic stimulation)

Pilocarpine action on eye

- Penetrates cornea
- Promptly causes miosis
- Ciliary muscle contracts and IOP reduces.
- **Uses:**
  1. 0.5-4% eye drops for open angle glaucoma.
  2. To counteract mydriatics after refraction testing.
  3. To prevent or break adhesions of iris with lens or cornea by alternating with mydriatics.
- **A/E:** stinging sensations, painful spasms of accomodation.

- Muscarine : source Amantia muscaria

  Not used therapeutically

- Arecoiline: Found in Beetel nuts Areca catechu

  Muscrinic as well as nicotinic action

  Not used therapeutically

Cholinergic Agents: Side Effects

Side effects are a result of overstimulation of the PSNS.

- Cardiovascular:
  - Bradycardia, hypotension, conduction abnormalities (AV block and cardiac arrest)
- CNS:
  - Headache, dizziness, convulsions
- Gastrointestinal:
  - Abdominal cramps, increased secretions, nausea, vomiting

Cholinergic Agents: Side Effects

Side effects are a result of overstimulation of the PSNS.

- Respiratory:
  - Increased bronchial secretions, bronchospasms
- Other:
  - Lacrimation, sweating, salivation, loss of binocular accommodation, miosis

Beta Blockers

β Adrenergic blocking agents

- Inhibit adrenergic responses mediated through β receptors.
- All are competitive antagonists.
- Propranolol first drug introduced in 1963

Classification

- Nonselective (β1& β2):
  a. Without intrinsic sympathomimetic activity: Propranolol, Sotalol, Timolol
  b. With intrinsic sympathomimetic activity: Pindolol
  c. With additional α blocking activity: Labetalol, Carvedilol
- Cardioselective (β_1):
  - Metoprolol, Atenolol, Acebutolol, Bisoprolol, Esmolol, Betaxolol, Celiprolol
- Selective (β_2): Butoxamine

Pharmacological Actions
Propronolol

1. CVS

a) Heart –

- Propranolol ↓es HR, FOC & CO. Effect is more prominent under sympathetic over activity. Ventricular dilatation occur in pt with reduced reserve – CHF may be precipitated.
- Cardiac work & oxygen consumption are reduced (HR & aortic pressure ↓) Total coronary flow reduced- but restricted to subepicardial region & subendocardial area remains unaffected (Site of ischemia in angina pts)
- Overall effect in angina Pts- improvement of O_2 supply, exercise tolerance is increased.
- At higher doses direct depressant & membrane stabilizing action is exerted.
- It blocks cardiac stimulant action of adrenergic drugs but not of digoxin Ca+ etc.

b) **Blood vessels**:

- Cause fall in BP of Isoprenaline. Also cause re-reversal of vasomotor reversal.
- On prolonged administration – gradually fall in BP in hypertensives but not in normotensives**.**
- **Mechanism** : Total peripheral resistance initially ↑ed & CO ↓ed . On continued treatment, resistance vessels adapt to chronically reduced CO so that t.p.r. ↓es - both systolic & diastolic BP fall.
- ↓ed NA release – blockade of β receptor mediated facilitation of release process.
- ↓ed renin release from kidney(β1).
- Central action reduces sympathetic outflow

2) Respiratory tract:

Increases bronchial resistance by blocking β2 receptors. In asthmatics severe attack may be precipitated.

3) CNS:

Decreases central sympathetic outflow May produce lethargy, forgetfulness, nightmares.

Suppresses anxiety in short term stressful situations but this is due to peripheral rather than central action.

4) Local anesthetic:

It is local anesthetic but not used clinically because of its irritant property.

5) Metabolic:

Blocks adrenergically induced lipolysis and increases plasma free fatty acid levels - Triglyceride level and LDL/HDL ratio ↑.

Inhibits glycogenolysis, occurs due to adr during hypoglycaemia – recovery from insulin action is delayed.

Decrease insulin release.

6) Skeletal muscle:

Inhibits adrenergically provoked tremor – due to direct action on muscle fibers.

7) Eye:

Reduces secretion of aqueous humor & i.o.t. is lowered.

8) Uterus:

Normal uterine activity is not significantly affected.
Isoprenaline induced relaxation of uterus is blocked.

- **PK:** Well absorbed orally – low oral bioavailability
  High first pass metabolism.
  Lipophilic in nature – penetrates brain

Interactions

1) Depression of sinus node & AV conduction with digitalis and verapamil – aggravated. Cardiac arrest can occur.
2) Delays recovery from hypoglycaemia due to insulin & oral antidiabetic.
3) α agonist present in cold remedies can cause marked rise in BP.
4) Cimetidine inhibits Ppnl metabolism.
5) Propranolol retards lidocaine metabolism by reducing hepatic blood flow.
6) Increases bioavailability of CPZ by decreasing its first pass metabolism.
7) Indomethacin & other NSAIDs oppose the antihypertensive action of Ppnl.

Adverse Drug Reactions

1) Accentuate myocardial insufficiency & precipitate CHF.
2) Bradycardia
3) Worsens COPD precipitates acute attack of bronchial asthma.
4) Exacerbates variant angina.
5) Carbohydrate tolerance may be impaired.
6) Lipid profile is altered on long term use.
7) Acute withdrawal can cause rebound hypertension, worsening of angina even sudden death.
8) Contraindicated in partial and complete heart block.
9) Tiredness and reduced exercise capacity.
10) Worsening of peripheral vascular disease
11) G. I. upset, lack of drive & nightmares

Cardio selective β Blockers

Advantages :

1. Lower propensity to cause bronchoconstriction (Still avoided in asthmatics if possible).
2. Less interfere with carbohydrate metabolism & glycogenolysis – Safer in diabetics
3. Lower incidence of cold hands & feet, less chances of precipitating Raynaud's phenomenon.
4. No/less deleterious effect on lipid profile.
5. Less liable to impair exercise capacity
6. Ineffective in suppressing essential tremors.

Partial agonistic /Intrinsic sympathomimetic activity

1) Bradycardia and depression in contractility at rest are not prominent- preferred in those prone for bradycardia.
2) Withdrawal is less likely to precipitate rebound HTN/angina
3) Plasma lipid profile is less/not worsened.
4) Not effective in migraine prophylaxis.
5) Not suitable for secondary prophylaxis of MI.

- **Sotalol** - Lower lipid solubility. Additional k+ channel blocking and class III antiarrhythmic.
- **Timolol** - Preferred for topical use in glaucoma
- **Pindolol** - Potent β blocker with intrinsic sympathomimetic activity. Used as antihypertensive – advantageous in patients who develop marked bradycardia.
- **Metoprolol** - Prototype of cardio selective blockers. Less likely to worsen asthma, but not entirely safe. Preferred in diabetics.

  Side effects are milder. Given orally. In MI given by i.v. route (bradycardia is ruled out)

- **S(-) Metoprolol** – active enantiomer. Half the dose is needed.

- **Atenolol** – low lipid solubility. Incompletely absorbed orally. Longer duration of action. Once a day administration sufficient. Commonly used for HTN & angina

- **S(-)Atenelol** – active enantiomer, effective in half dose, better tolerated.

- **Acebutolol**: Cardio selective with partial agonistic & membrane stabilizing activity. Effect on resting HR is less. Longer half life, once a day dose is sufficient.

- **Bisoprolol**: Cardio selective. Suitable for once a day administration in angina, HTN, CHF.

- **Esmolol**: Ultra short acting β1blocker. Plasma t1/2<10min.Rapid onset & short lasting fall in BP. Used to terminate supraventricular tachycardia, episodic atrial fibrillation or flutter during anaesthesia. Given by IV infusion.

- **Celiprolol**: Selective β1 blocker with additional weak β2 agonist activity. Safe in asthmatics, ↓es vascular resistance. Non adrenoceptor mediated vasodilatation adds to action.

- **Nebivolol**: Highly selective β1 blocker also acts as NO donor. Produces vasodilatation, improves endothelial function which may delay atherosclerosis. Hypotensive response is rapid. Used in hypertension & CHF.

Uses

1) **Hypertension** – Mild anti hypertensive. Good patient acceptability, cardio protective potential. Gradual fall in BP with no postural hypotension. Used alone or with diuretics.
2) **Angina pectoris** – They decreases cardiac workload, ↓ O_2 demand & ↑ exercise tolerance. So given in angina of effort. In Prinzmetal's angina, there is coronary spasm due to increased sympathetic over activity without any fixed stenosis, so beta blockers contraindicated.
3) **Cardiac arrhythmias** –Suppress extrasystoles & tachycardias due to high levels of CAs. Effective due to increased refractory period of AV node & direct membrane stabilizing action

.

4) **Myocardial Infarction (MI):**

a) Secondary prophylaxis – Decrease mortality by 20%.

i) Prevent reinfarction

ii) Prevent sudden ventricular fibrillation.

b) Myocardial salvage during evolution of MI – Administered within 4-6 hrs.

i) May limit infarct size by reducing O_2 consumption.

ii) Prevent arrhythmias – ventricular fibrillation

5) **Congestive cardiac failure** – previously C/I . Now it is established that these may be beneficial in pts. With mild to moderate CCF.

   Given gradually with slow advancing doses, maintained for long term. Benefit is due to antagonism of damaging effects of cardiac β receptor over activity which promotes unfavorable remodeling of myocardium (Carvedilol or Metoprolol)

6) **Dissecting aortic aneurysm.**

7) **Pheochromocytoma** – With α blockers.

8) **Thyrotoxicosis** – Propranolol preferred.

9) **Migraine** – For chronic prophylaxis.

10) **Anxiety** – Useful in conditions which provoke nervousness and panic e.g. Exam, unaccustomed public appearance.

11) **Essential tremors**

12) **Glaucoma** – Ocular β blockers are preferred

13) **Hypertrophic obstructive cardiomyopathy**

α+β Adrenergic Blocker

- **Labetelol** – First α+β blocker.
- 5 Times more potent in blocking β than α receptors (at low dose) but high dose equally block both the receptors. Fall both systolic & diastolic receptors.
- At relatively high doses reduce both C.O. and t.p.r. HR is unchanged.
- Limb blood flow is increased. Inhibit NA uptake.
- Use: Pheochromocytoma, clonidine withdrawal.
- S/E – Postural hypotension, failure of ejaculation, rashes & liver damage.

Carvedilol:

- β1+β2+α1 blocker.
- Produces vasodilatation, by α1blockade & CCB.
- Has antioxidant property.
- Use – Hypertension, CHF (as cardio protective).

Cell

Dr Hemant Makone

M.D.

PY1.1- LEARNING OBJECTIVES

At the end of this lecture student shall be able to-

- List the components of cell and describe their functions
- Describe the structure of cell membrane and function of its various components.

Principles of the Cell Theory

■ The cell is the smallest **structural and functional unit** capable of carrying out life processes.

■ The functional activities of each cell depend on the specific structural properties of the cell.

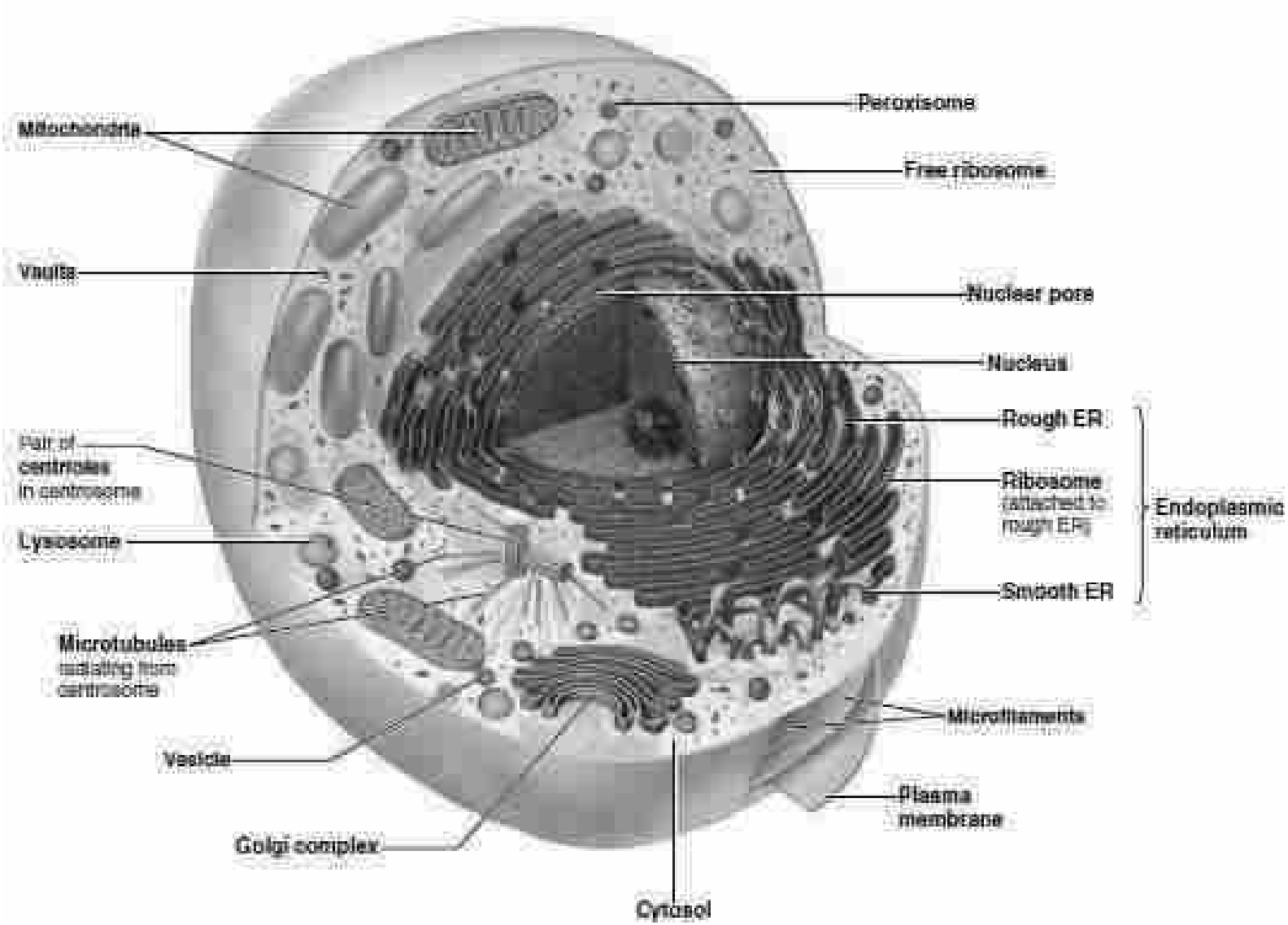
■ Cells are the living **building blocks** of all multicellular organisms.

■ An organism's structure and function ultimately depend on the collective structural characteristics and functional capabilities of its cells.

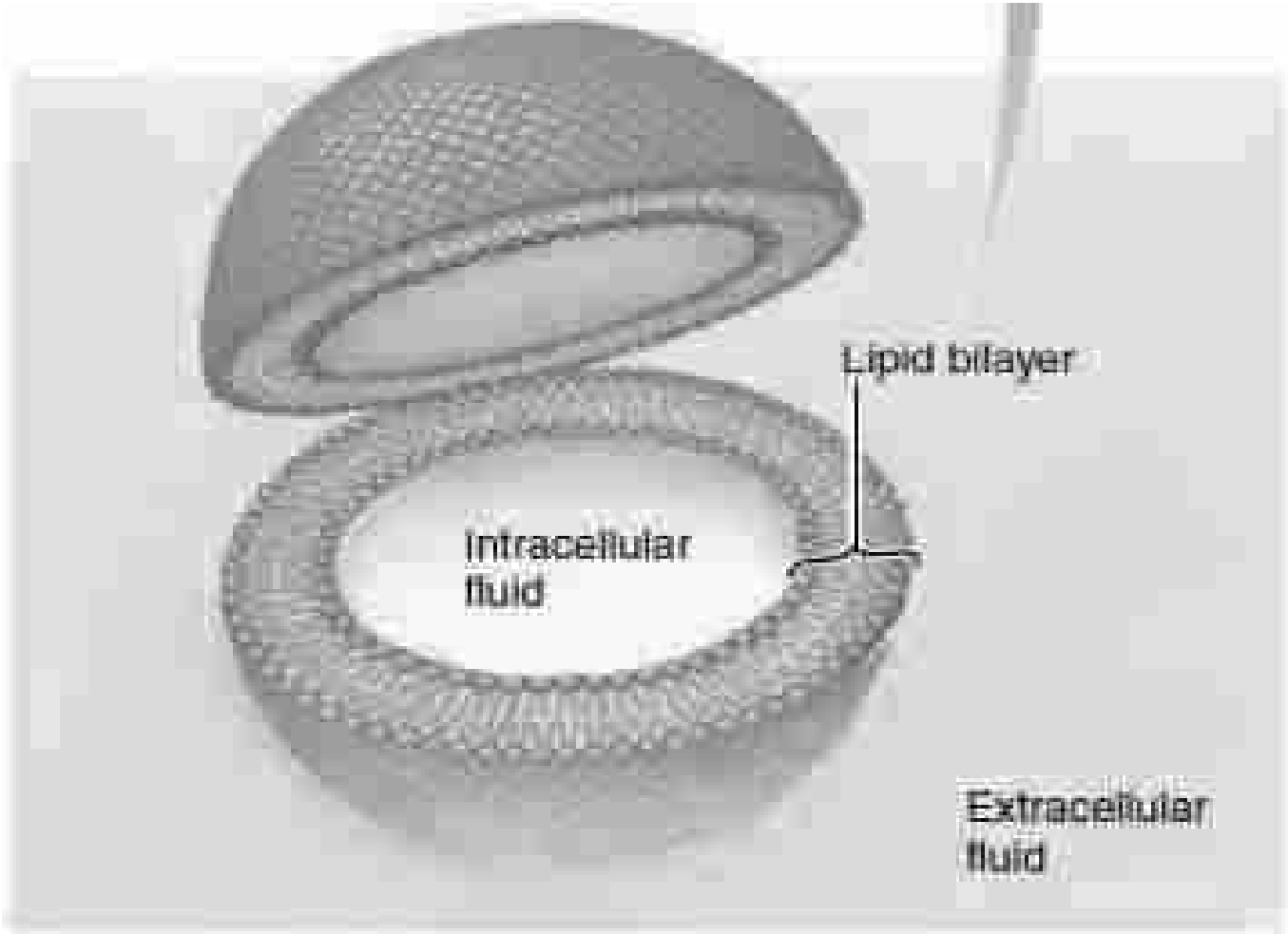
■ All new cells and **new life arise only from preexisting** cells.

■ Because of this continuity of life, the cells of **all organisms** are fundamentally **similar in structure and function**.

Diagram of cell structures visible under an electron microscope

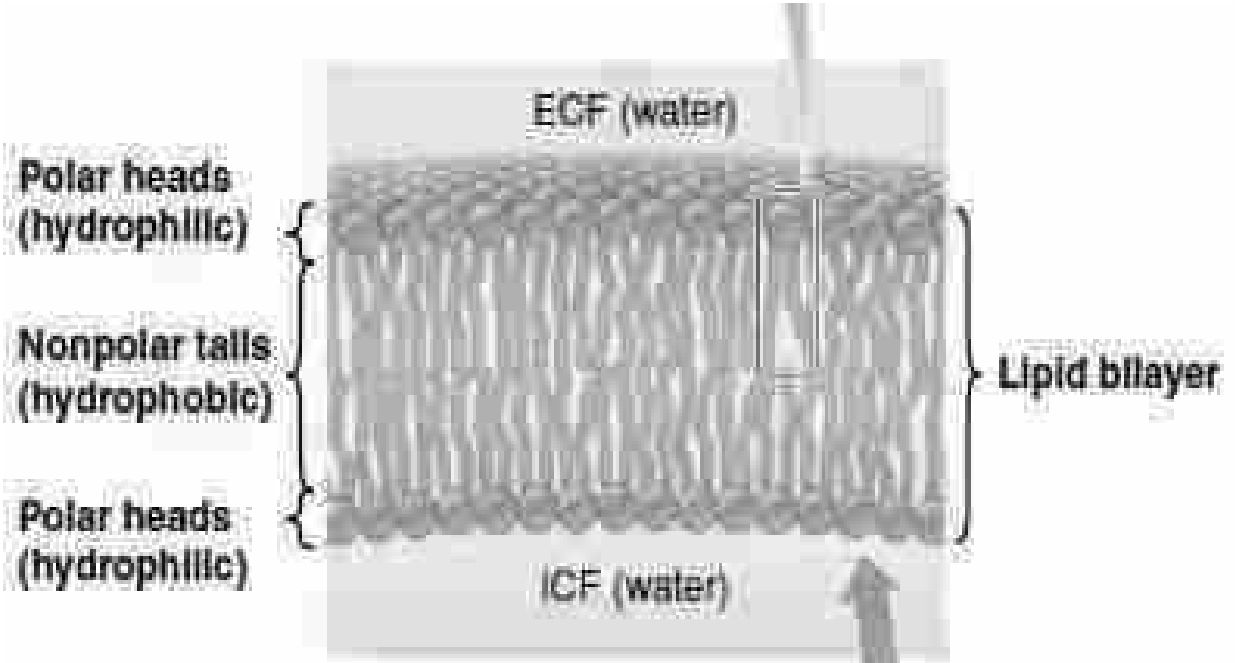


Cell membrane

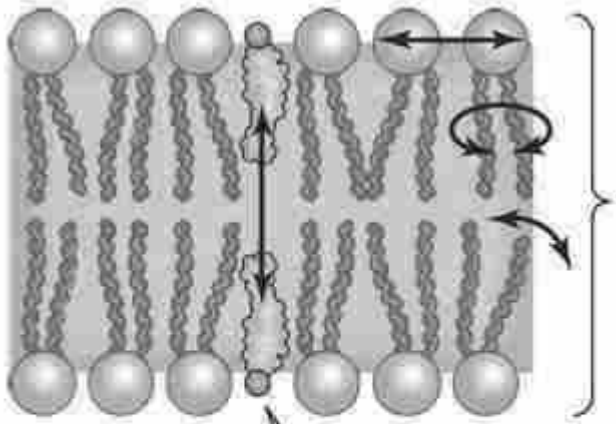


(c) Separation of ECF and ICF by the lipid bilayer

- 7-10 nm
- 55% protein, 4% lipid, 3% carb
- 25% PL, 13% cholesterol

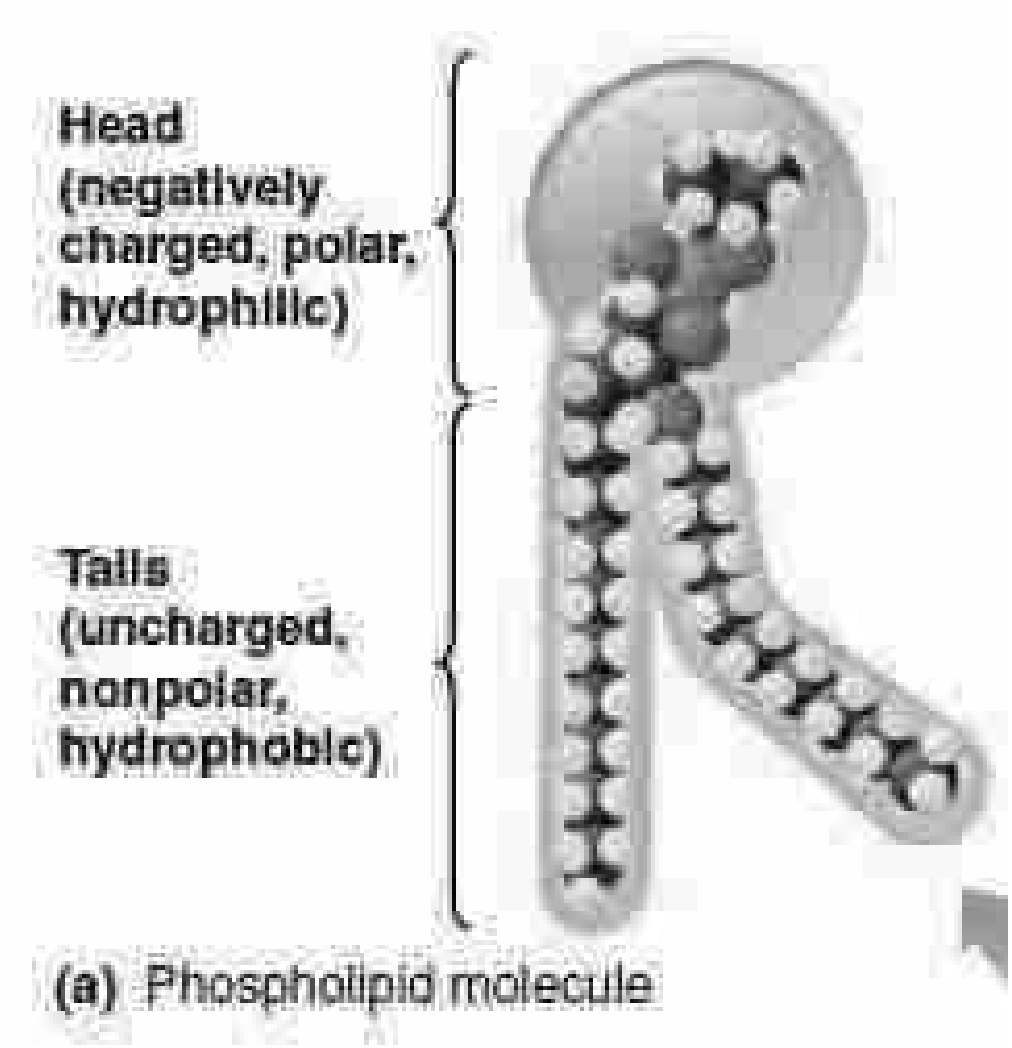


(b) Organization of phospholipids into a bilayer in water

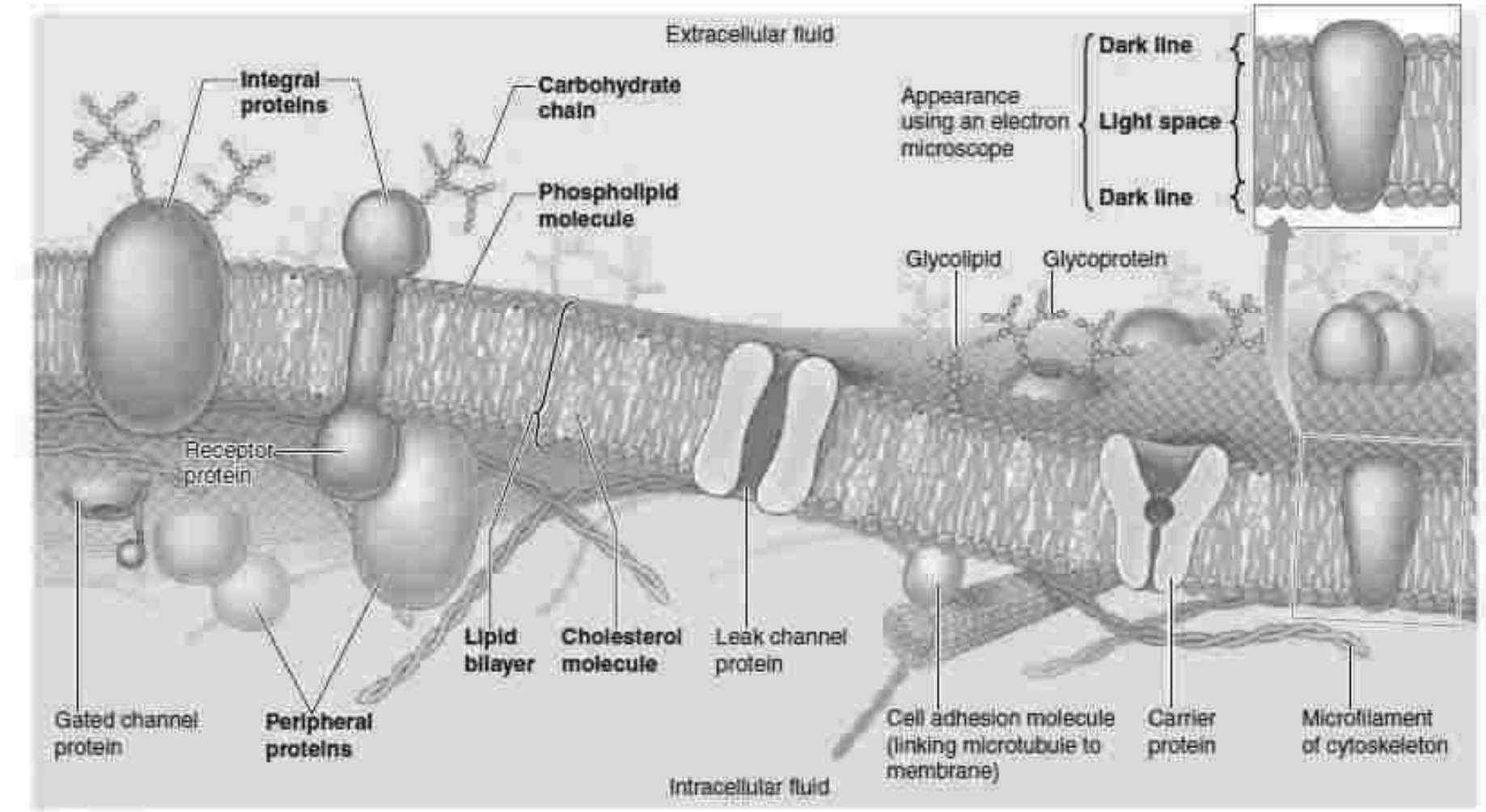


Phospholipids can move laterally, rotate, or flex. Rarely do they flip to the other leaflet.

Cholesterol aids in stiffening the membrane and can flip easily.


Head
(negatively
charged, polar,
hydrophilic)
Tails
(uncharged,
nonpolar,
hydrophobic)
(a) Phospholipid molecule

Fluid mosaic model



● **FIGURE 3-3 Fluid mosaic model of plasma membrane structure.** The plasma mem-

Function of protein

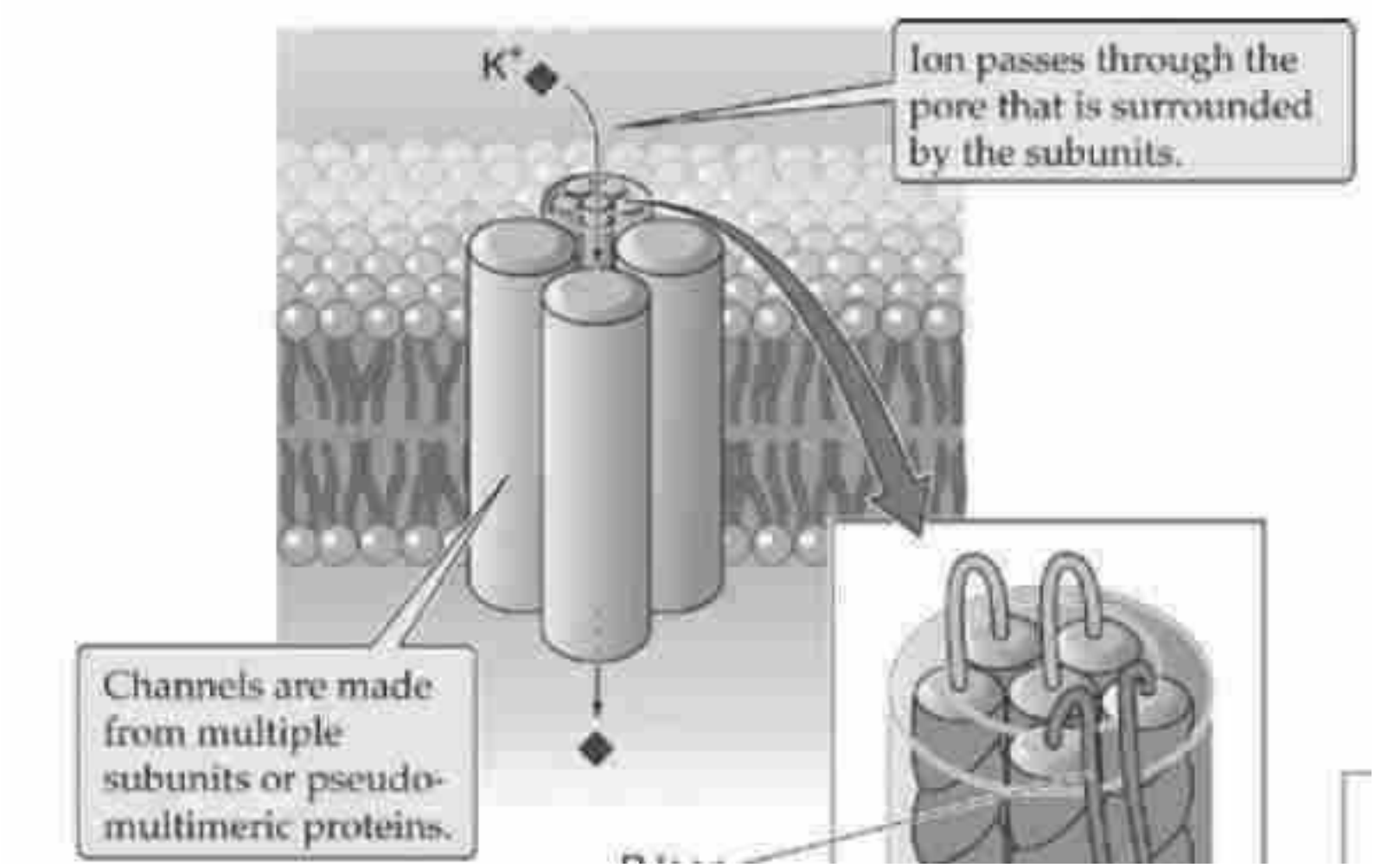
Integral –

- Channel protein
- Carrier protein
- Receptor
- As enzymes
- Linker protein
- Structural integrety

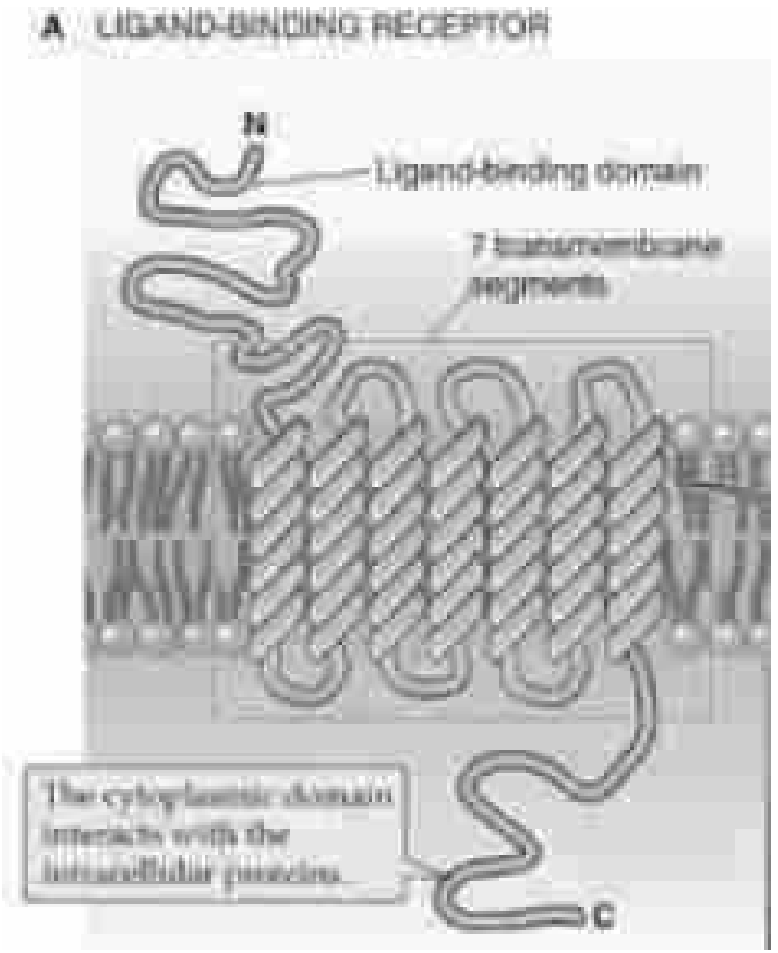
Peripheral

- **Intrinsic** – as enzymes , control transport
- **Extrinsic**- as CAM

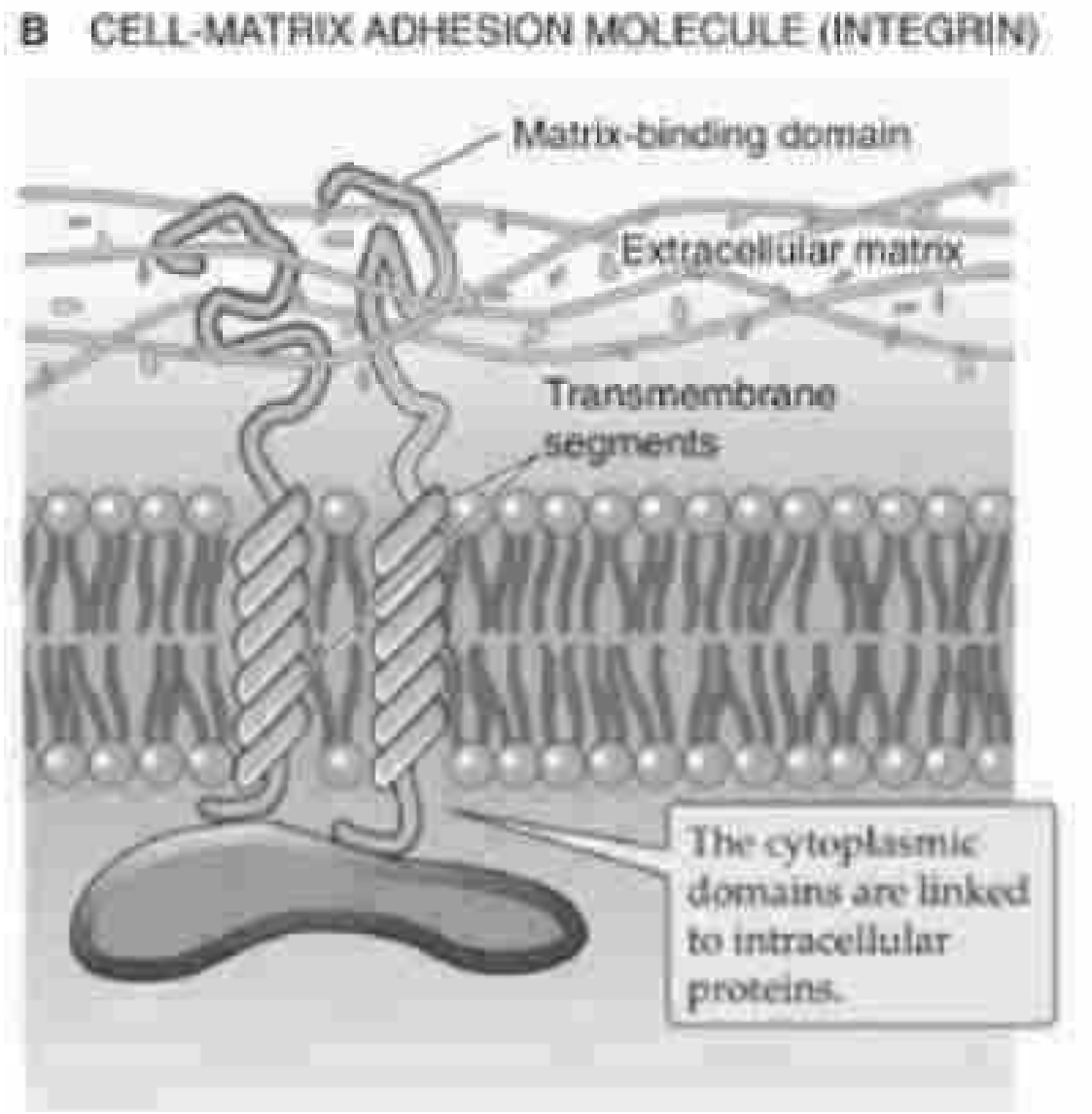
Integral membrane proteins can carry out the transmembrane movement of water-soluble substances



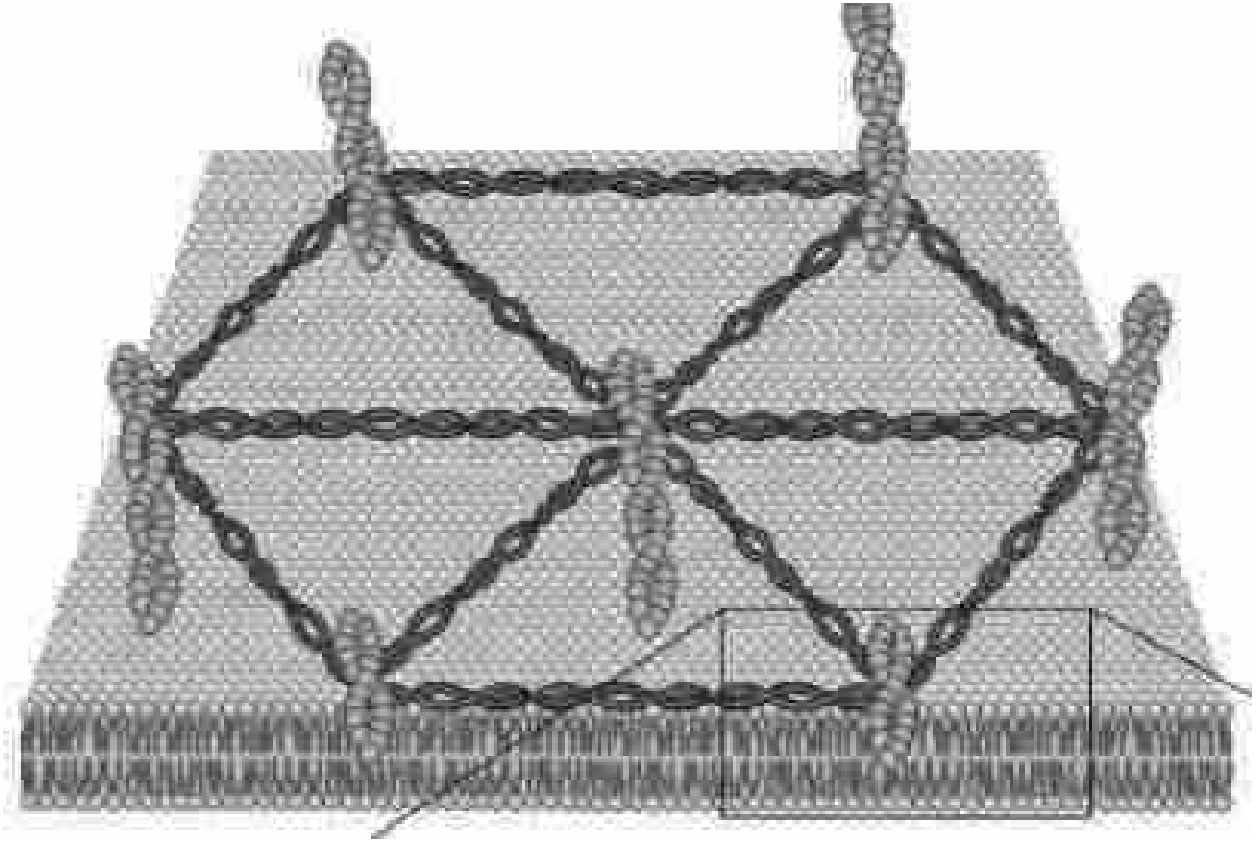
- **Integral membrane proteins can serve as receptors**



Integral membrane proteins can serve as adhesion molecules



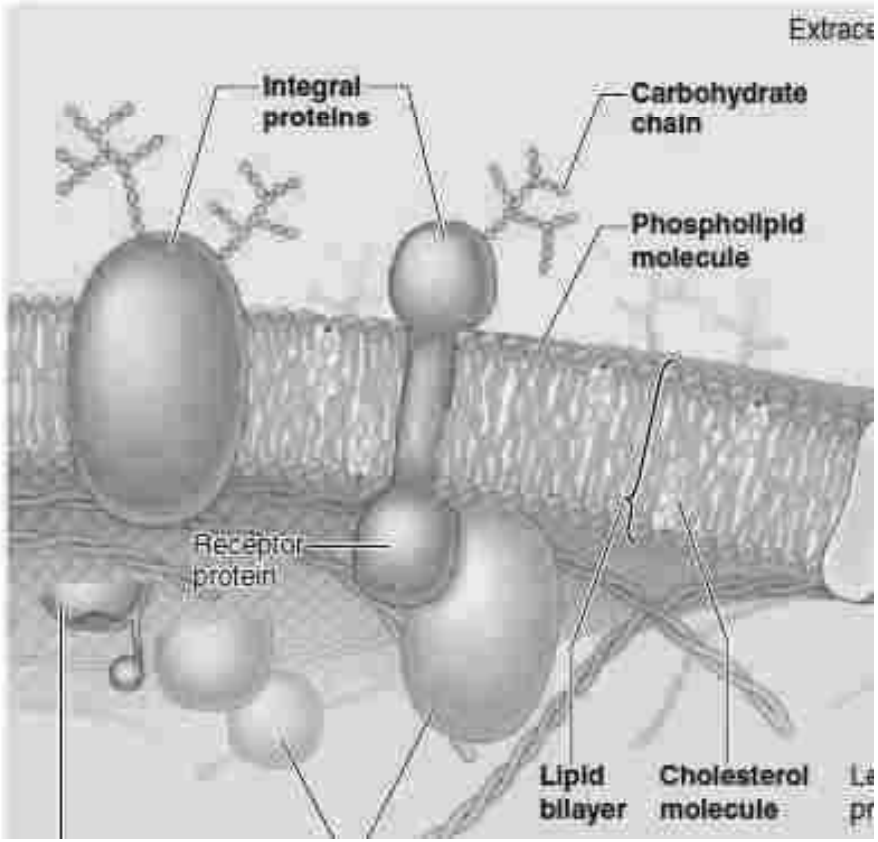
- **Peripheral membrane proteins participate in intracellular signaling and can form a submembranous cytoskeleton**



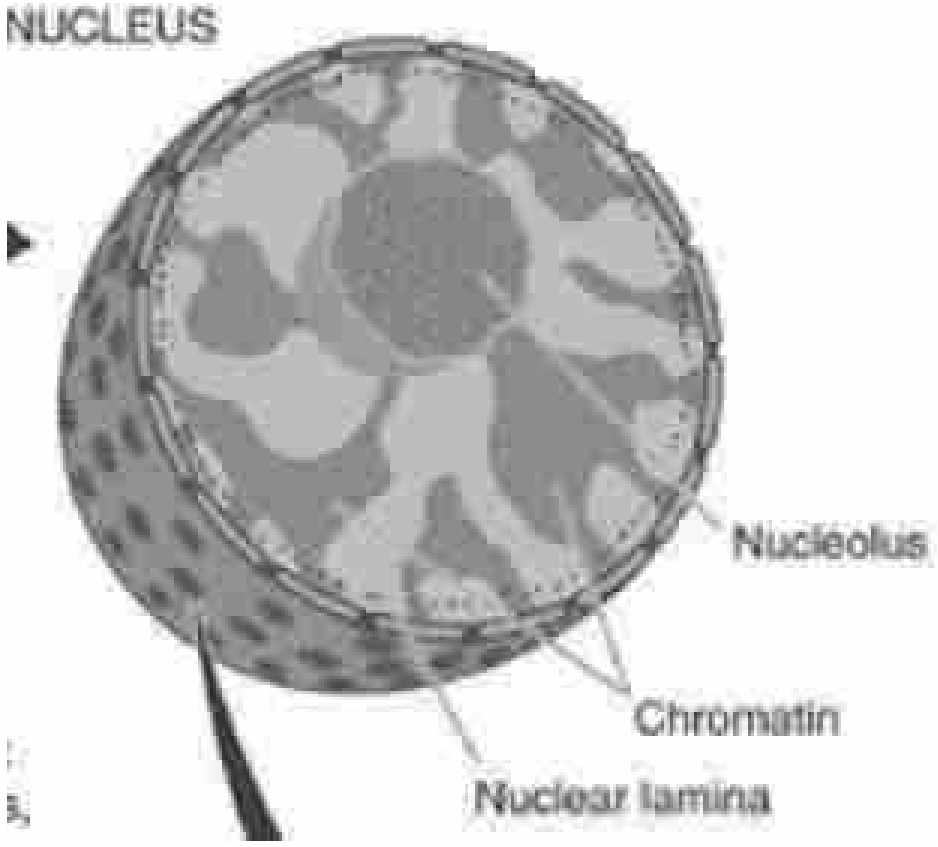
- **Integral membrane proteins can also be enzymes**
- **Integral membrane proteins can participate in intracellular signaling**

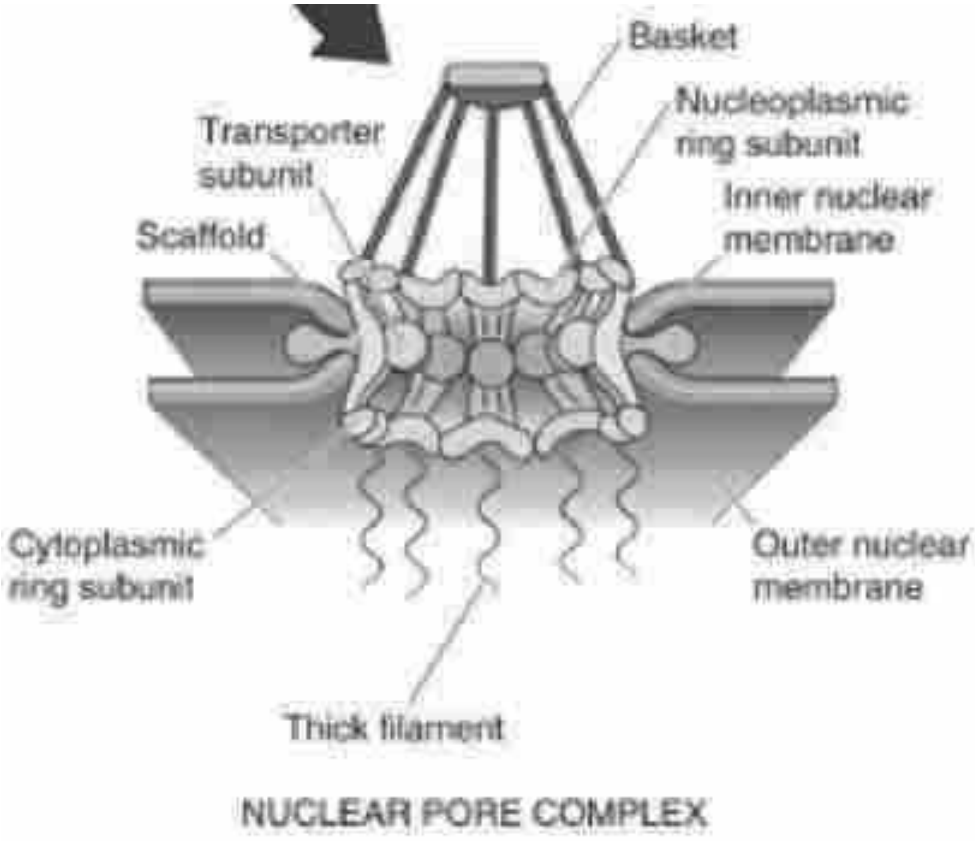
Carbohydrate

- Glycoprotein / glycolipids
- Form glycocalyx- give surface –ve charge
- Functions-
- Receptor for hormone
- Antigen
- CAM



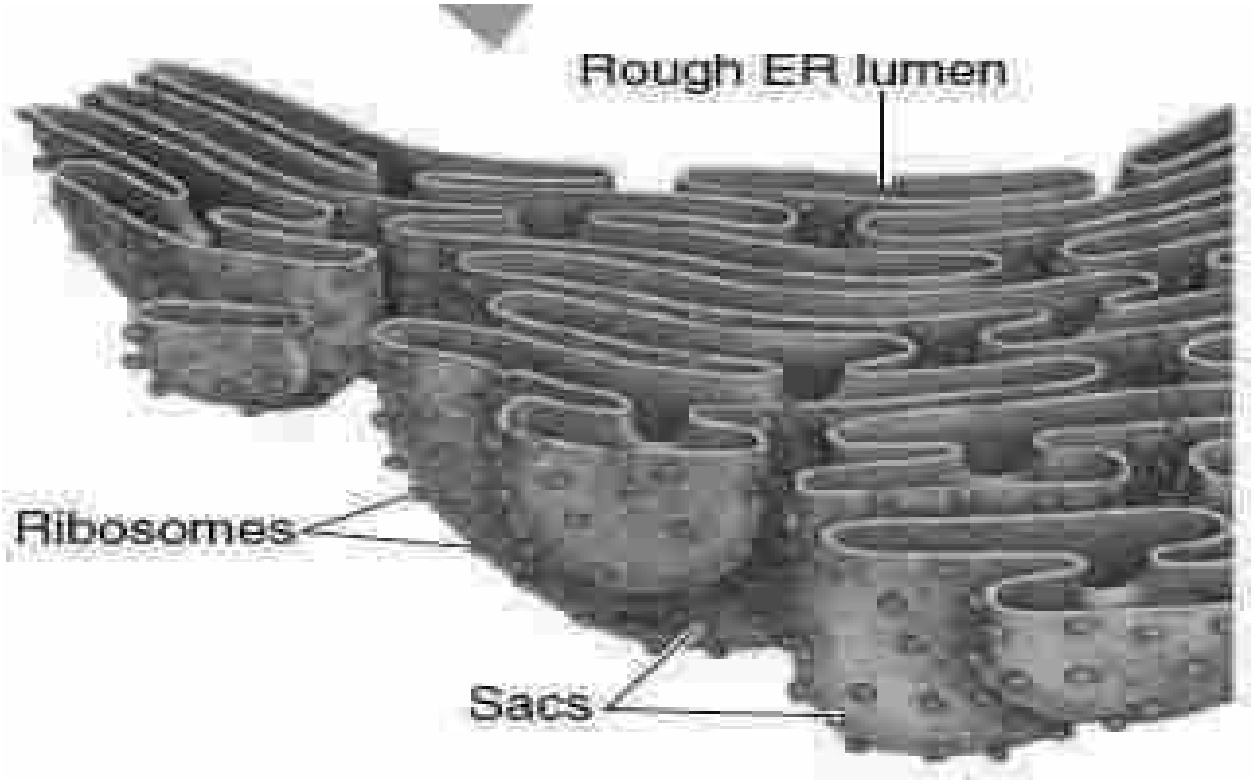
Nucleus


NUCLEUS
Nucleolus
Chromatin
Nuclear lamina


Basket
Nucleoplasmic ring subunit
Transporter subunit
Inner nuclear membrane
Scaffold
Cytoplasmic ring subunit
Outer nuclear membrane
Thick filament
NUCLEAR PORE COMPLEX

Rough ER

The rough ER synthesizes proteins to be secreted to the exterior or to be incorporated into cellular membrane.



Functions-

- Protein synthesis
- Abundant in- cell active in protein synthesis

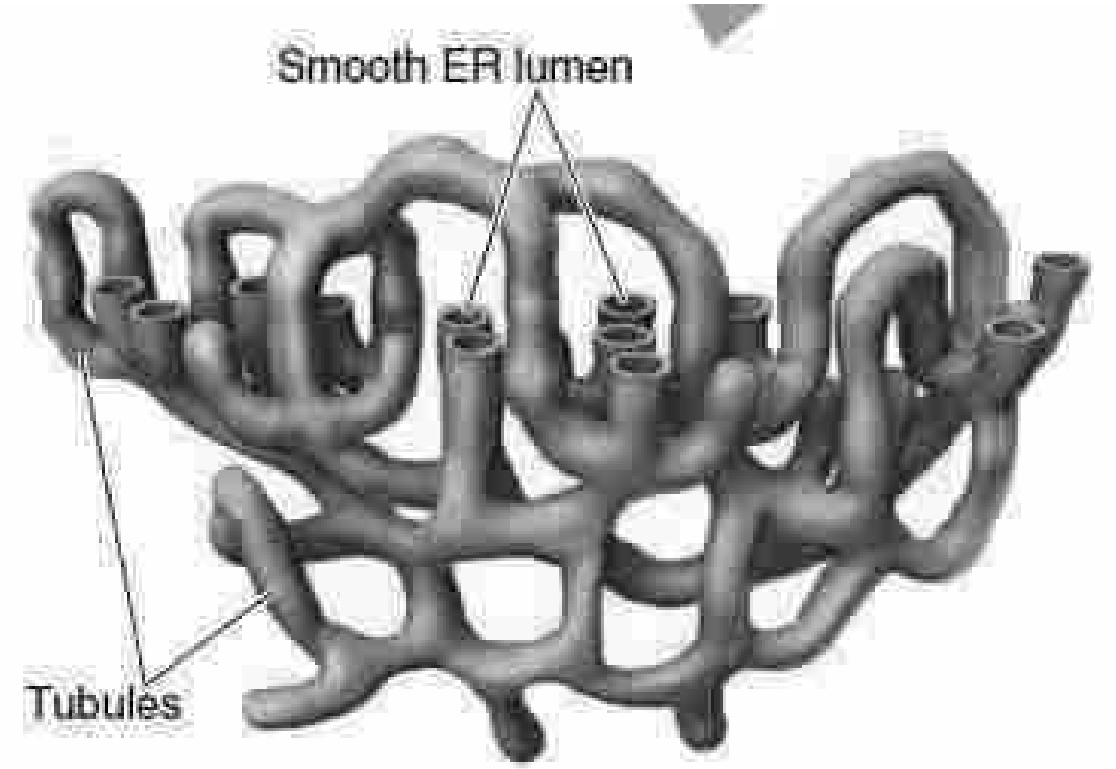
  Ex- Russel bodies of plasma cell

  Nissl granules of nerve cell

  acinar cell of pancreas
- Absent in RBC and spermatozoa

Smooth ER

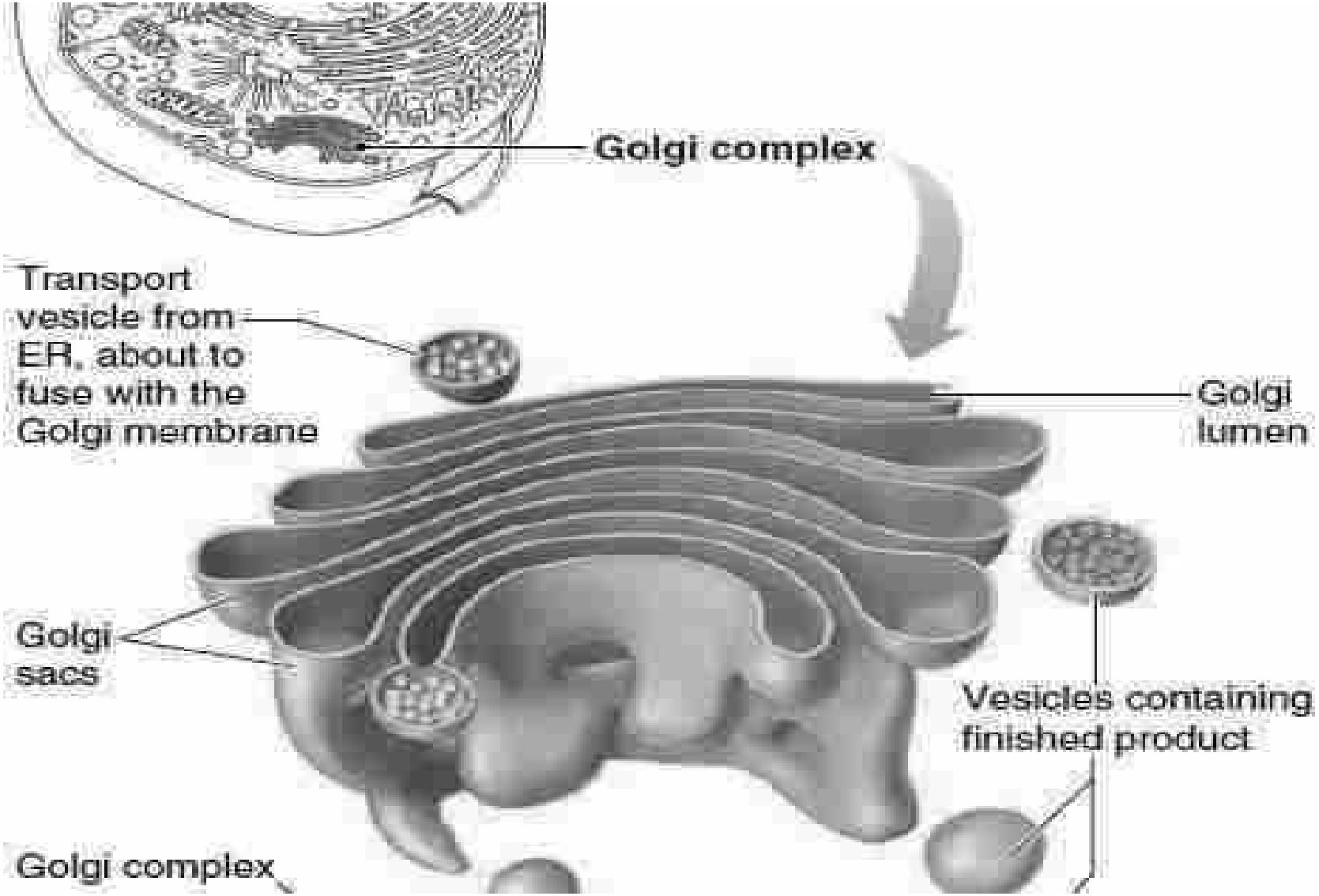
The smooth ER packages the secretory product into transport vesicles, which bud off and move to the Golgi complex



Functions –

- Lipid synthesis- phospholipid and cholesterol
- Steroid synthesis
- Abundant in – leydig cell and cells of adrenal cortex
- Sarcoplasmic reticulam – modification of SER in skeletal and cardiac muscle

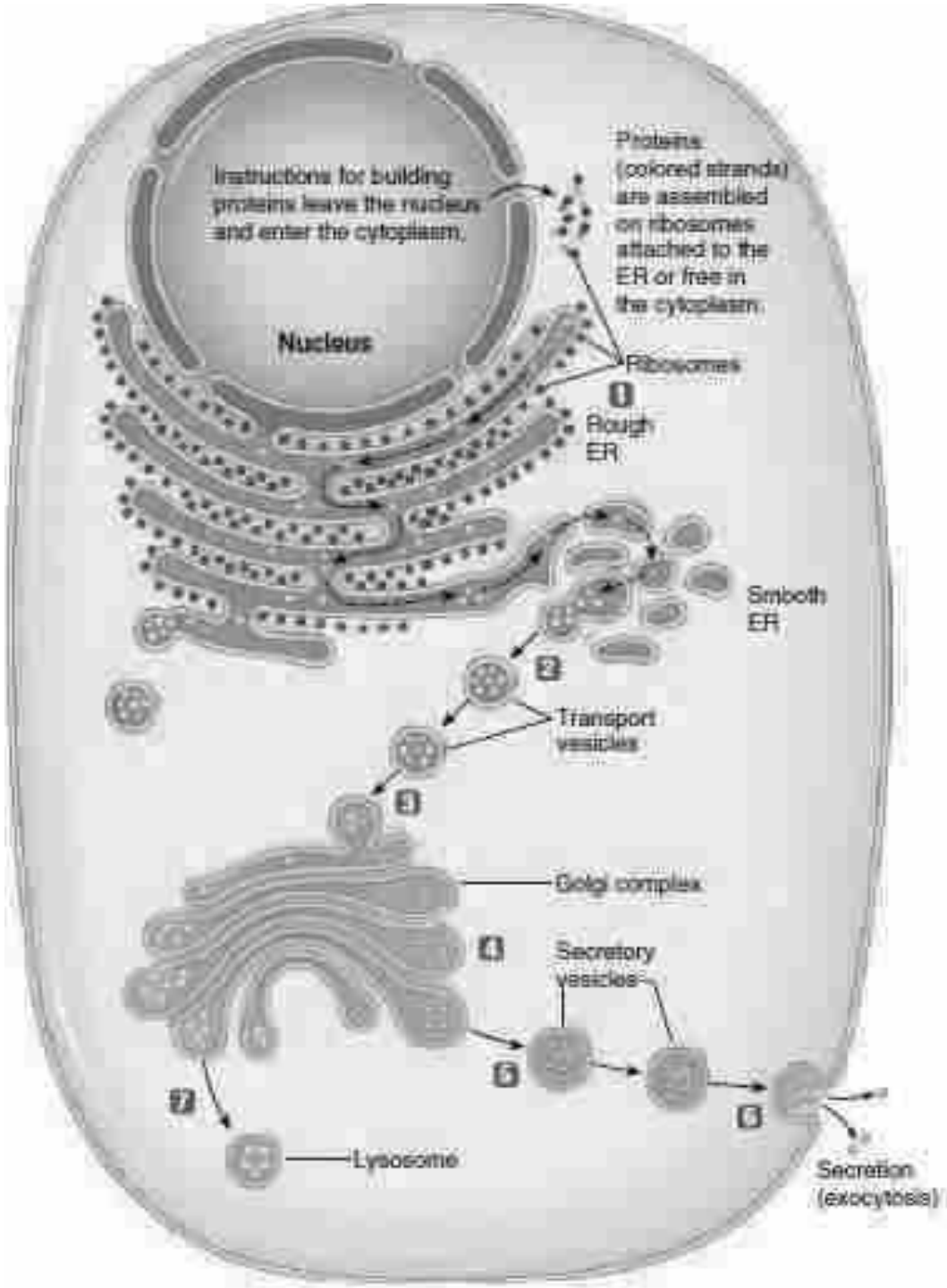
GOLGI COMPLEX



Function-

- Synthesis of – certain carbohydrates to form glycoprotein (e.g. hyaluronic acid, chondrointin sulphate)
- Processing of ER secretion- vesicles of ER secretion fuse with golgi, carbohydrates are added
- Packaging of - ER secretions in compacted and concentrated form
- Vesicle formation- 1.lysosomes 2.secretary vesicle
- Replenish cell membrane- golgi vesicle fuse with cell membrane

Secretory pathway

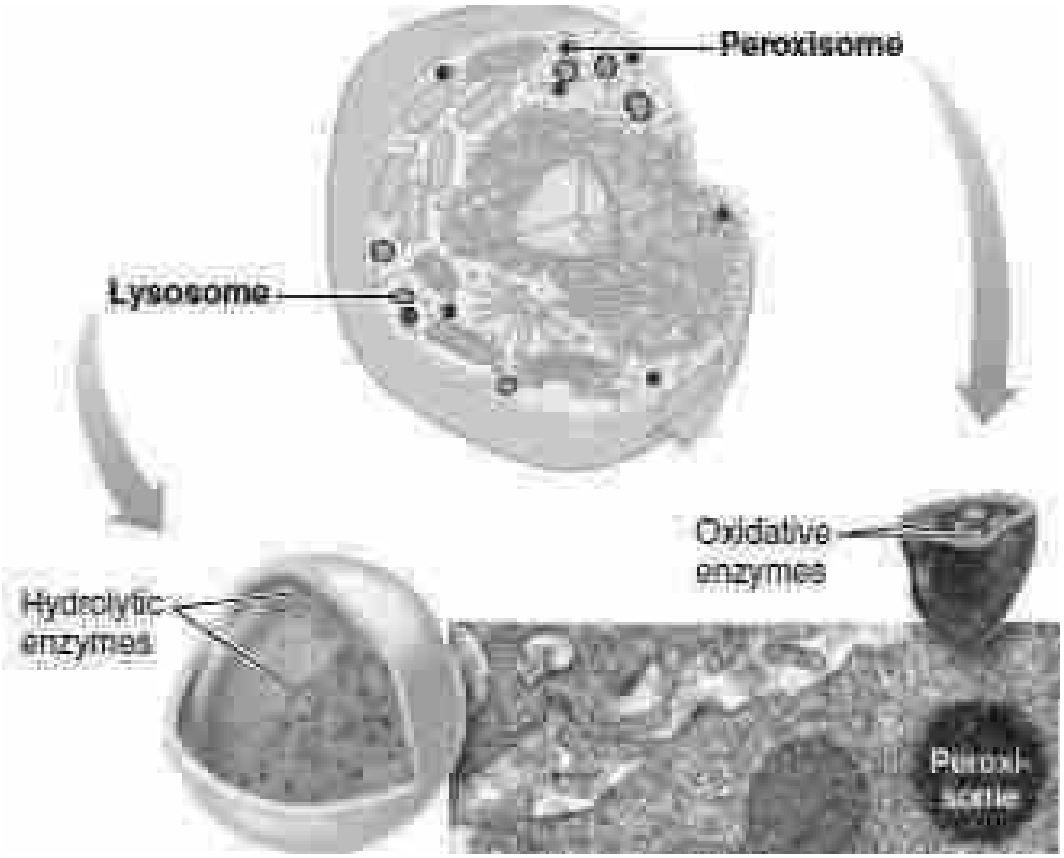


Secretory pathway

- The transport vesicles fuse with the Golgi complex, open up, and empty their contents into the closest Golgi sac.
- The newly synthesized proteins from the ER travel by vesicular transport through the layers of the Golgi complex,which modifies the raw proteins into final form and sorts and directs the finished products to their final destination by varying their wrappers.
- Secretory vesicles containing the finished protein products bud off the Golgi complex and remain in the cytosol, storing the products until signaled to empty.

L ysosomes and Endocytosis

- **Lysosomes digest extracellular material brought into the cell by phagocytosis.**
- formed by budding from the Golgi complex.
- 40 different powerful **hydrolytic enzymes**

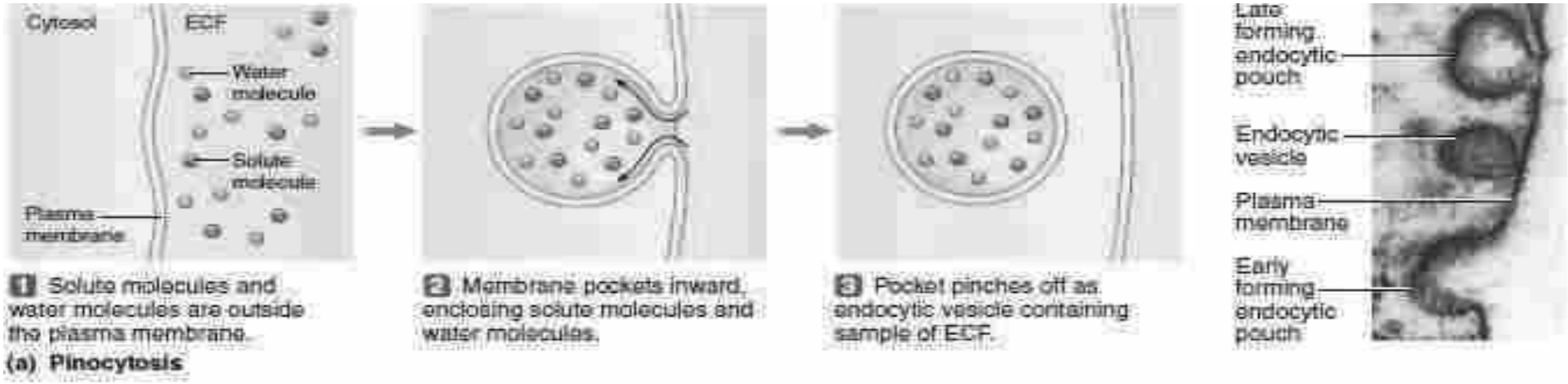


Types of lysosomes

- Primary/storage vacuoles- contain hydrolytic enzyme
- Secondary/autophagic vacuoles- fused primary with damaged cell component
- Residual bodies- undigestible material , excreted by exocytosis.

Functions

- Intracellular digestive system- food particle, bacteria, damaged cell structure
- Regression of tissue- uterus, muscle, mammary gland
- Autolysis –
- Bactericidal agents- lysizyme, lysoferrin, acid

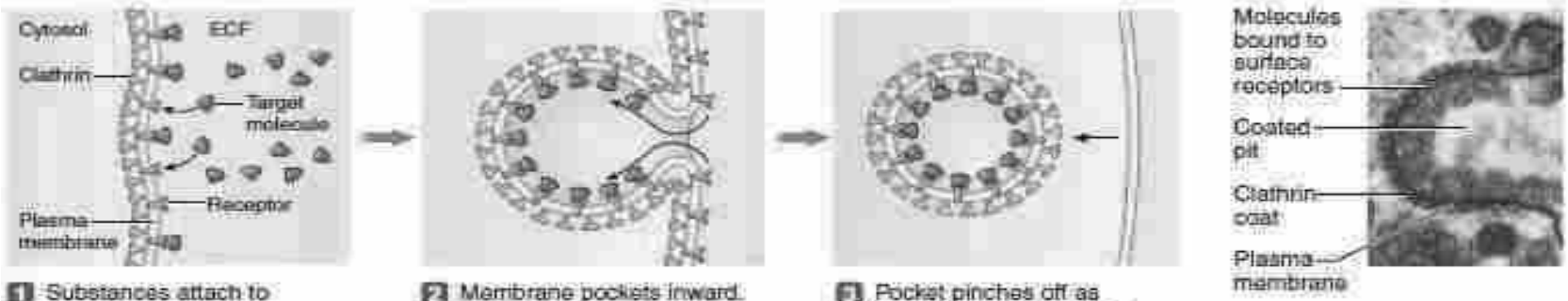


1 Solute molecules and water molecules are outside the plasma membrane.

2 Membrane pockets inward, enclosing solute molecules and water molecules.

3 Pocket pinches off as endocytic vesicle containing sample of ECF.

(a) Pinocytosis

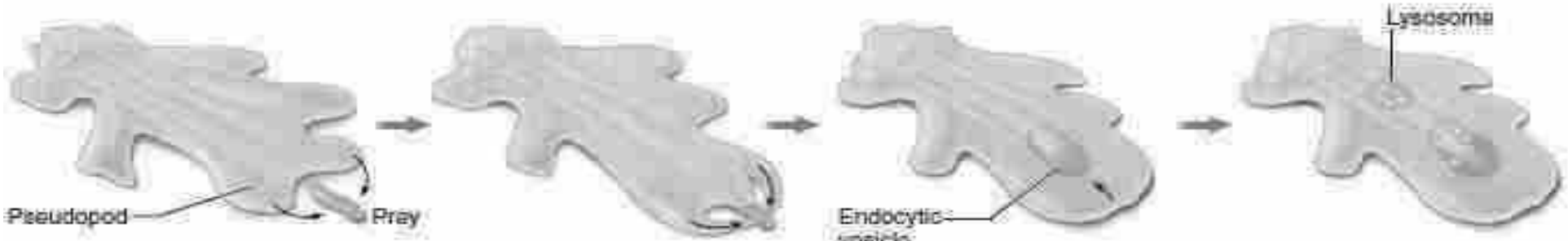


1 Substances attach to membrane receptors.

2 Membrane pockets inward.

3 Pocket pinches off as endocytic vesicle containing target molecule.

(b) Receptor-mediated endocytosis

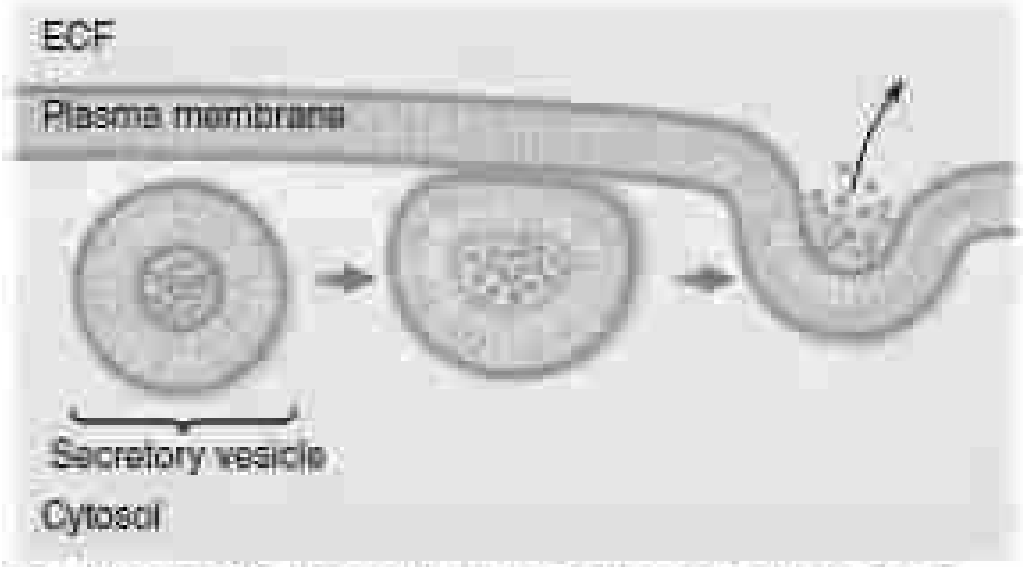


1 Pseudopods begin to surround prey.

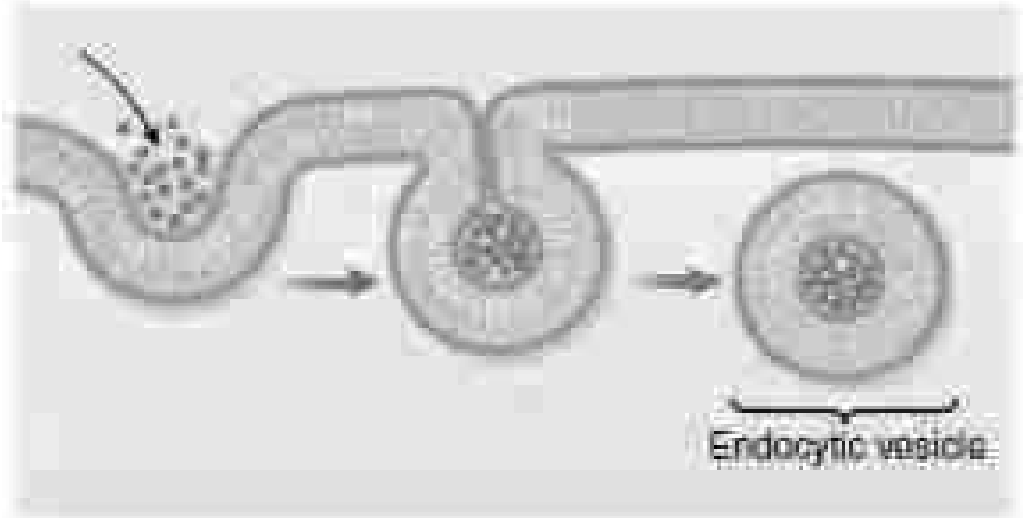
2 Pseudopods close around prey.

3 Prey is enclosed in endocytic vesicle (phagosome) that sinks into cytoplasm.

4 Lysosome fuses with vesicle, releasing enzymes that attack material inside vesicle.

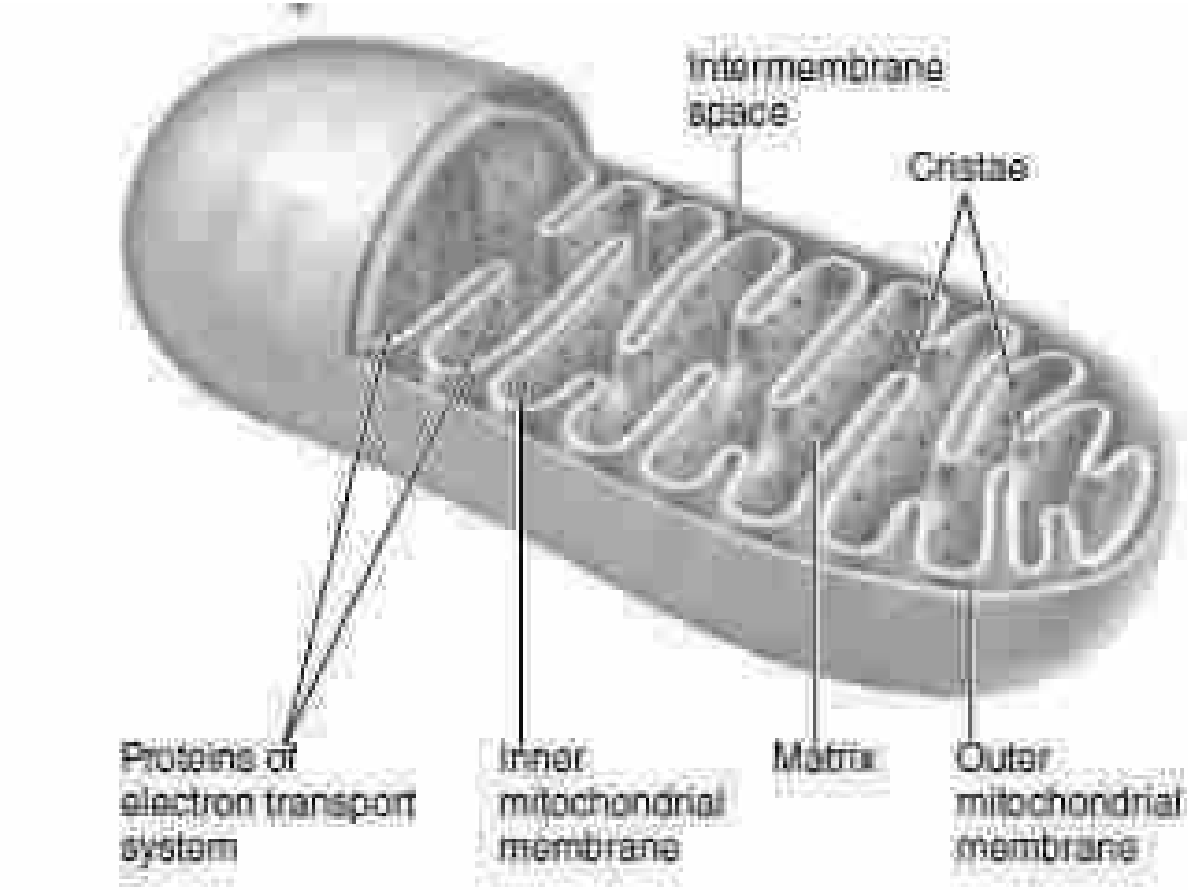


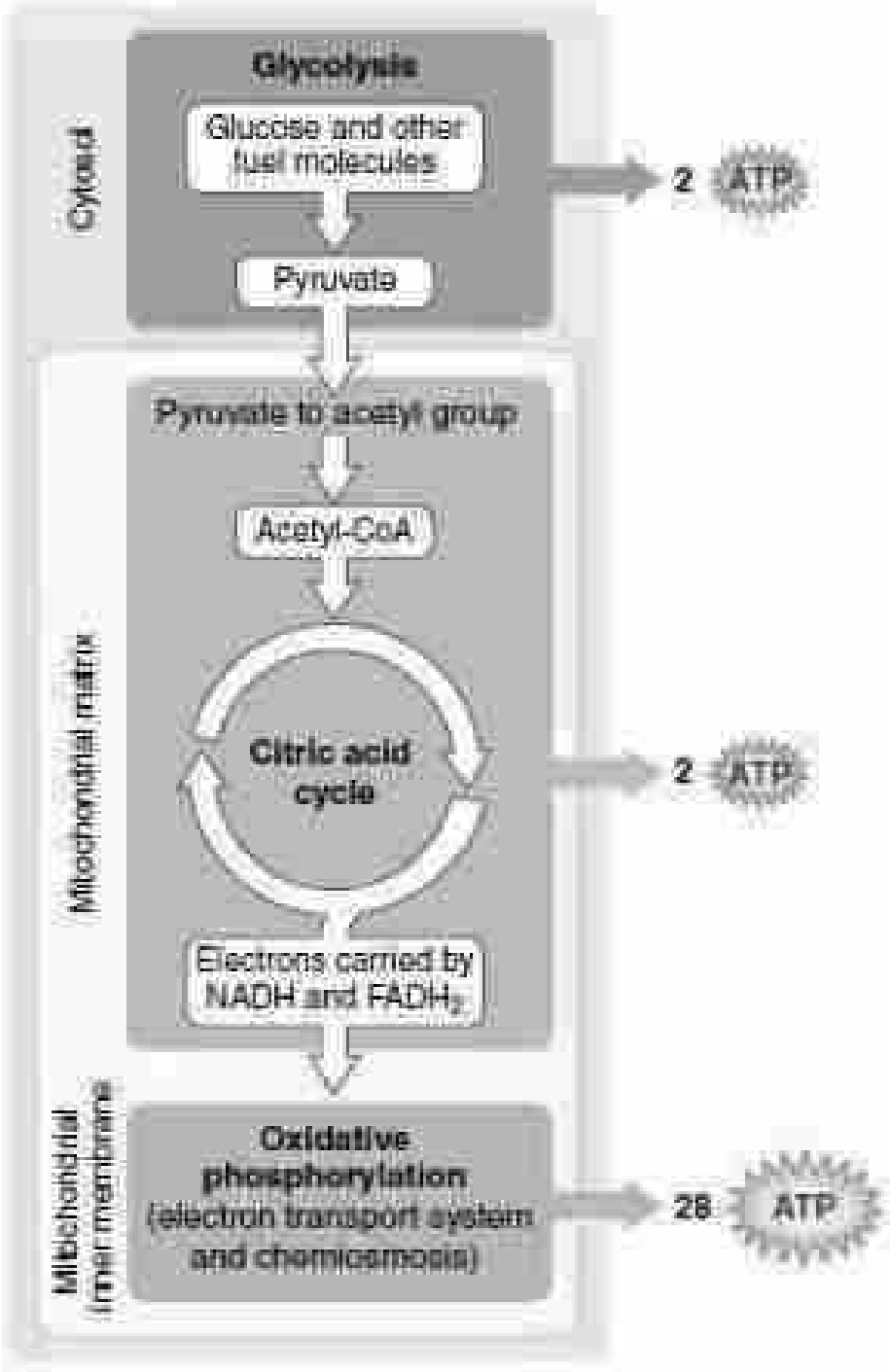
(a) Exocytosis: A secretory vesicle fuses with the plasma membrane, releasing the vesicle contents to the cell exterior. The vesicle membrane becomes part of the plasma membrane.

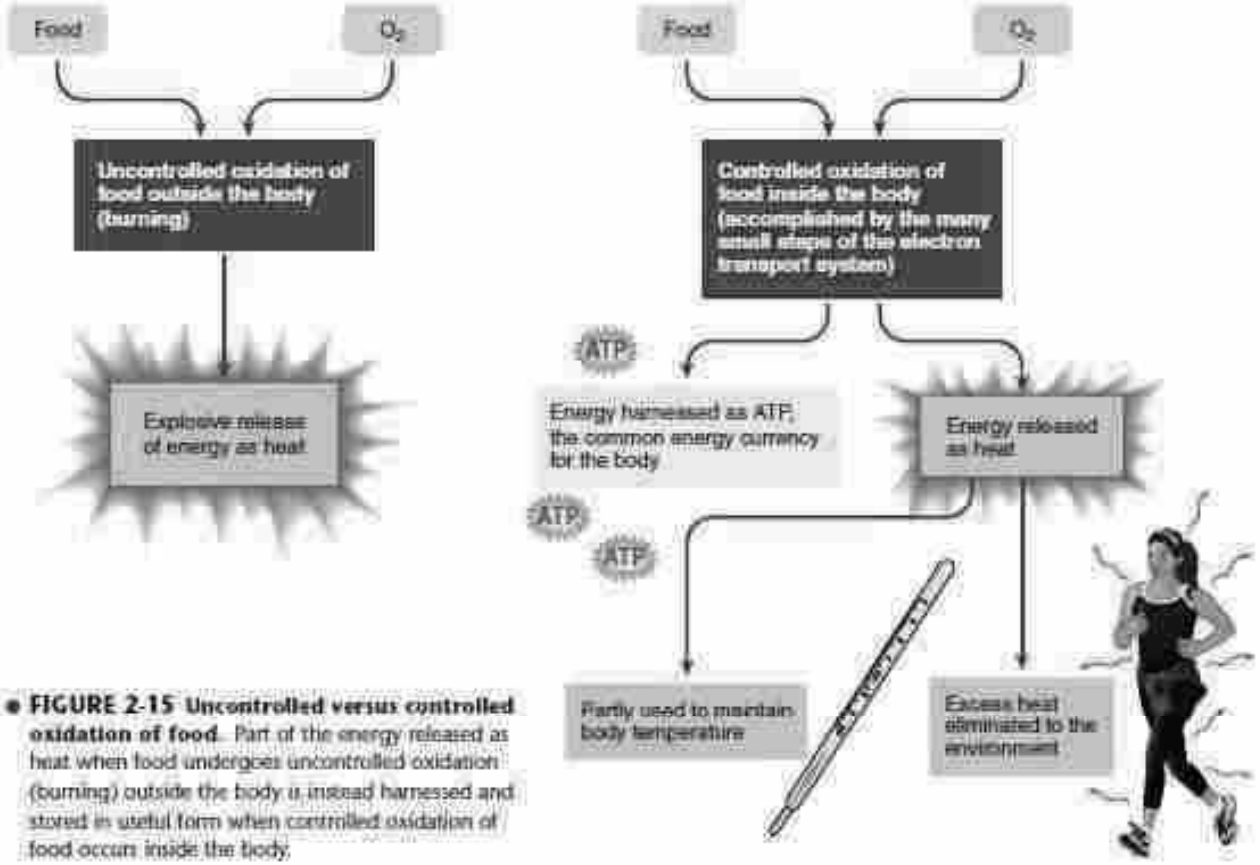


(b) Endocytosis: Materials from the cell exterior are enclosed in a segment of the plasma membrane that pockets inward and pinches off as an endocytic vesicle.

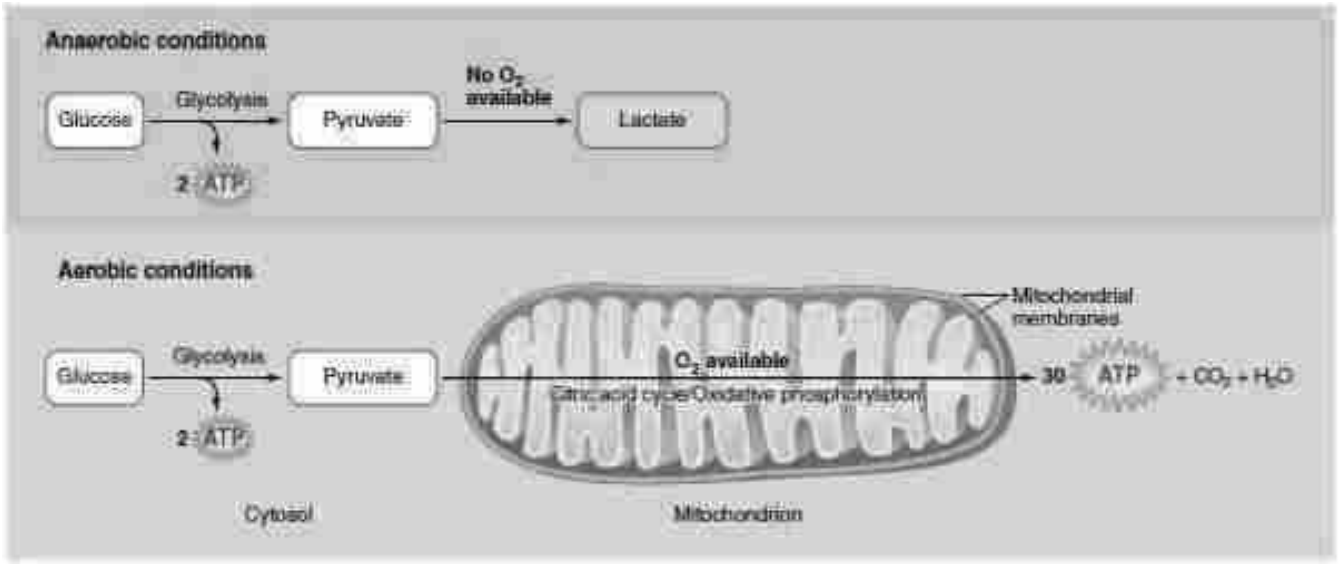
Mitochondria

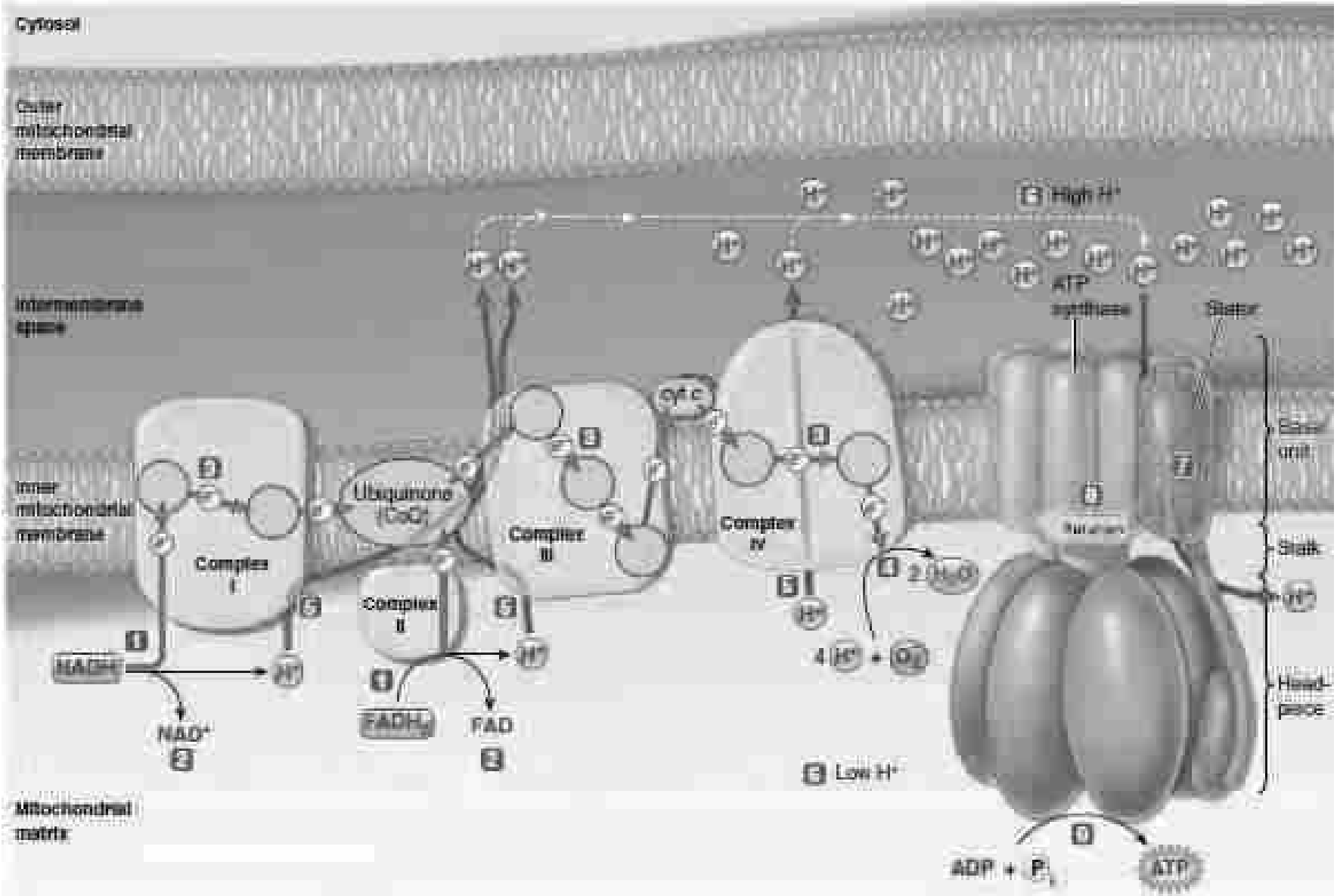



Cytosol
Glycolysis
Glucose and other fuel molecules
Pyruvate
2 ATP
Mitochondrial matrix
Pyruvate to acetyl group
Acetyl-CoA
Citric acid cycle
2 ATP
Electrons carried by NADH and FADH2
Mitochondrial inner membrane
Oxidative phosphorylation (electron transport system and chemiosmosis)
28 ATP

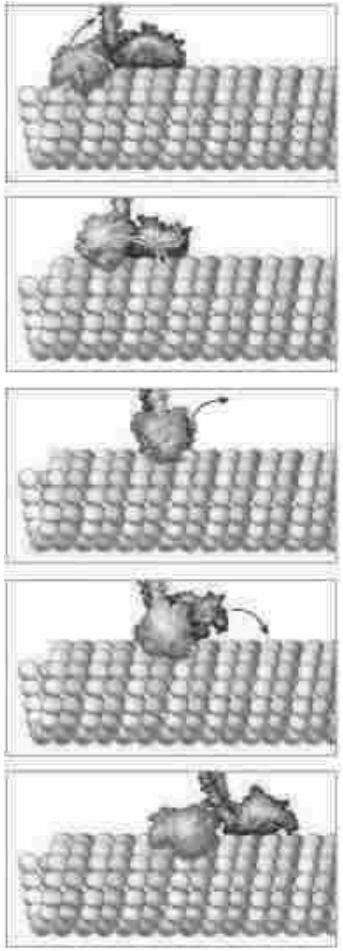


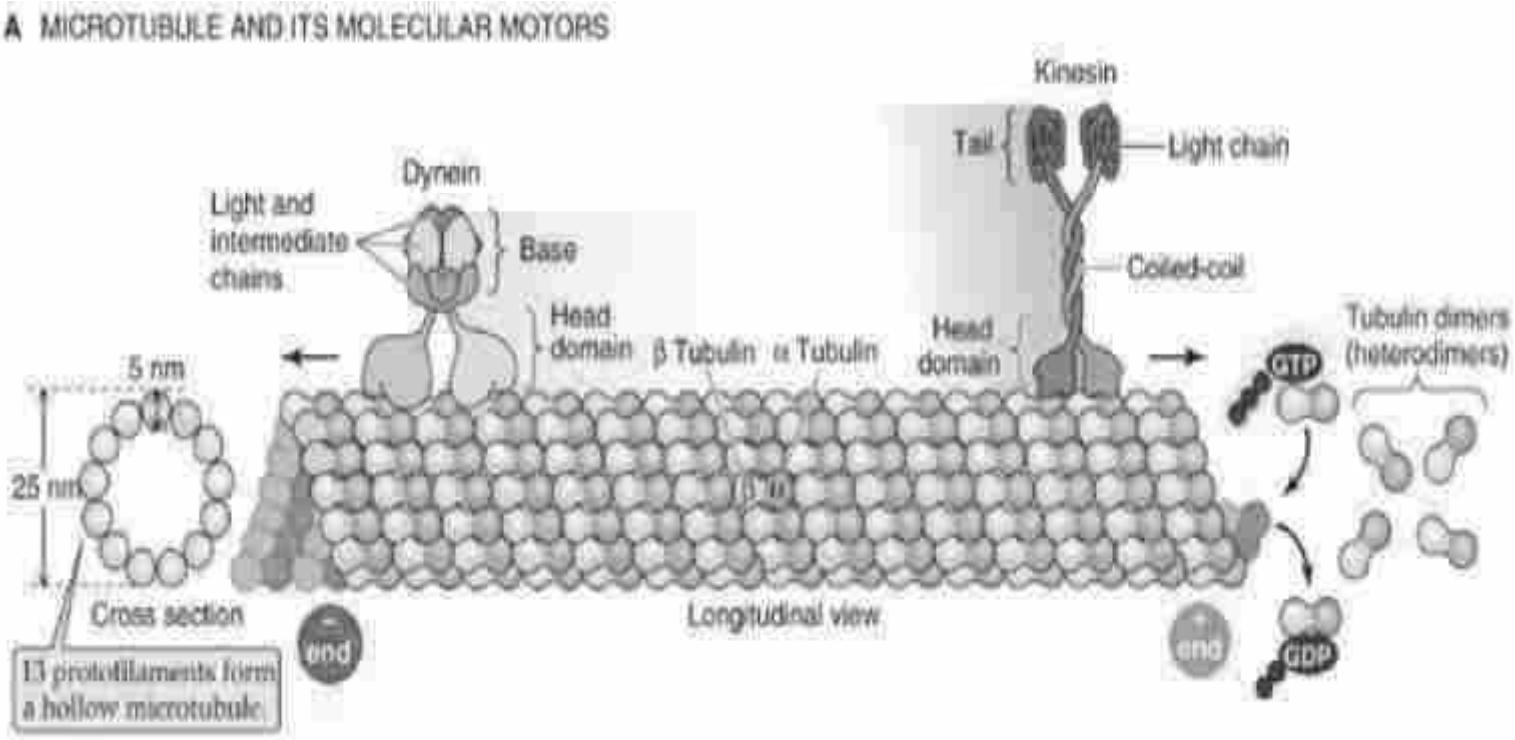
● **FIGURE 2-15 Uncontrolled versus controlled oxidation of food.** Part of the energy released as heat when food undergoes uncontrolled oxidation (burning) outside the body is instead harnessed and stored in useful form when controlled oxidation of food occurs inside the body.




Cytosol
Outer mitochondrial membrane
Intermembrane space
Inner mitochondrial membrane
Mitochondrial matrix
High H+
Low H+
Complex I
Complex II
Complex III
Complex IV
Ubiquinone (CoQ)
Cyt c
NADH
NAD+
FADH2
FAD
2 H2O
4 H+ + O2
ATP synthase
Stator
Rotor
Stalk
Head-piece
ADP + Pi
ATP
Electron transport system
Electrons flow through a series of electron carriers from high-energy to low-energy levels; the energy released builds an H+ gradient across the inner mitochondrial membrane.
Chemiosmosis
ATP synthase catalyzes ATP synthesis using energy from the H+ gradient across the membrane.
Oxidative phosphorylation

Molecular motors





Microtubule based-

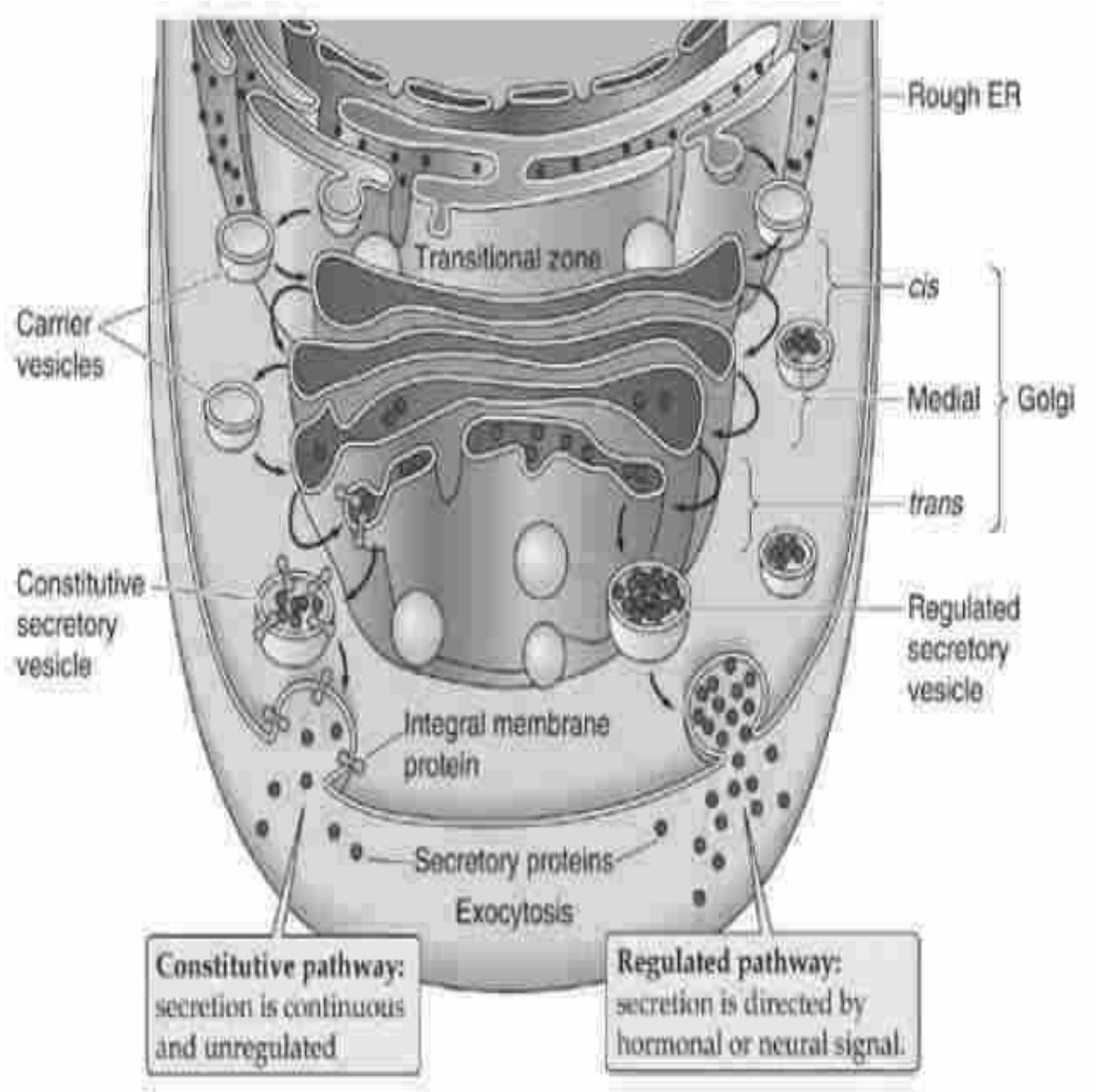
1.kinesin- towards +ve end

2.dyneins- towards -ve end

Actin based-

1. Myosin l –single headed
2. Myosin ll – double headed
3. Myosin V- transport of vesicle

THANK YOU.


Rough ER
Transitional zone
cis
Medial
trans
Golgi
Carrier vesicles
Constitutive secretory vesicle
Regulated secretory vesicle
Integral membrane protein
Secretory proteins
Exocytosis
Constitutive pathway: secretion is continuous and unregulated
Regulated pathway: secretion is directed by hormonal or neural signal.

Principles of Homeostasis

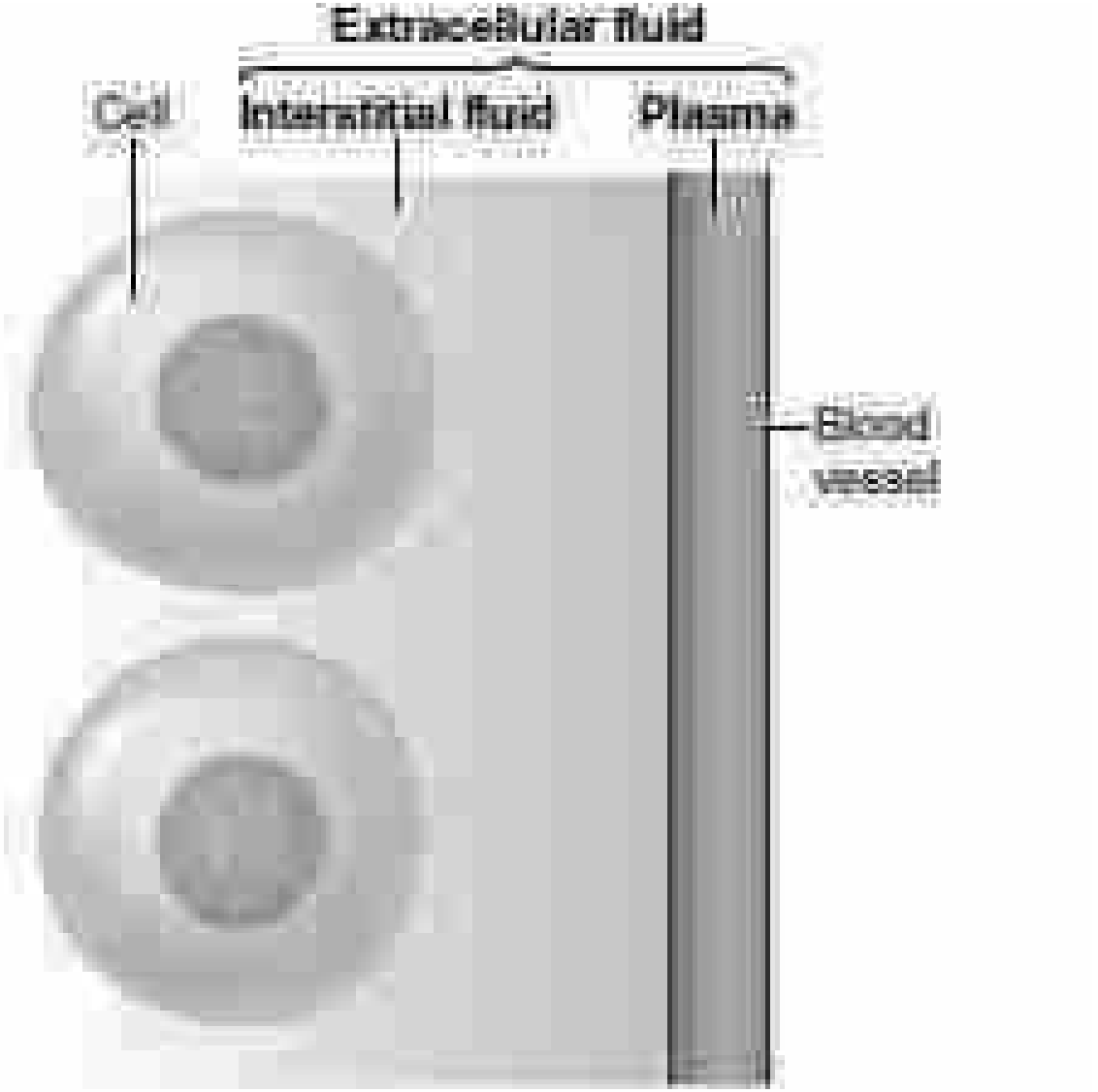
PY1.2-
The student will be able to

- Define homeostasis.
- Give examples of homeostatic regulations.
- Explain feedback mechanisms
- Explain dysfunctions due to failure of homeostatic regulations.

GENERAL CONCEPT

- Homeostasis is ***defined*** as the maintenance of constancy of the internal environment of the body

- In the nineteenth century, **Claude Bernard,** a French physiologist, was first to introduce the concept of ***milieu intérieur***, which means the internal environment of the body



Components of the extracellular fluid (internal environment)

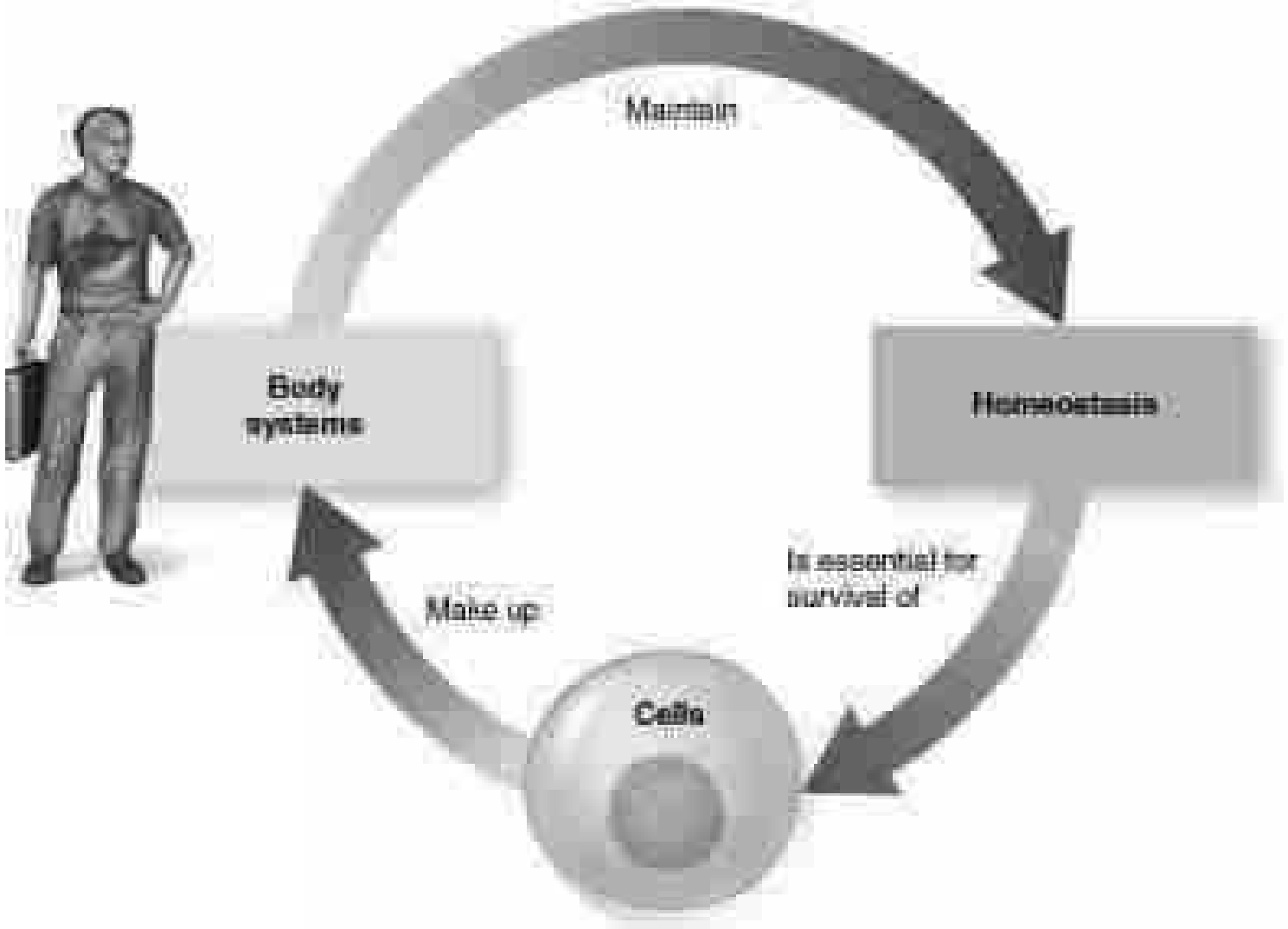
Observations made by Claude Bernard:

- He noted that the **volume and composition**

of the fluid are maintained constant, independent

of the changes in the environment in which the animal lives.

- He observed the **fluid in the body as the *internal environment***, to differentiate it from the external environment of the body.
- He suggested that the ability to regulate internal environment is the major reason for humans and animals to live a normal life despite changes and challenges imposed on them by the external environment.
- He **distinguished between intracellular and extracellular fluid** and proposed extracellular fluid as the internal environment of the body.


Maintain
Body systems
Homeostasis
Is essential for survival of
Make up
Cells

- In 1929, an American physiologist **W.B Cannon** coined the term ***'homeostasis'*** *(homoios* means 'like' and *stasis* means 'standing still') to describe the phenomenon of the constancy of the internal environment

Scientist contributed

Walter Bradford Cannon (1871-1945) was an American physiologist, Professor and Chairman of the Department of Physiology at Harvard Medical School. He coined the term fight or flight response, and he expanded on Claude Bernard's concept of homeostasis.

WB Cannon

HOMEOSTATIC MECHANISMS

- Homeostasis is the stability of the internal environment of the body
- It is not only the equilibrium of the internal environment, but also the **balance of the composition and components** of the environment and the physiological variables that influence the environment.
- Abnormal **deviation** from these homeostatic processes **leads to bodily dysfunctions**.

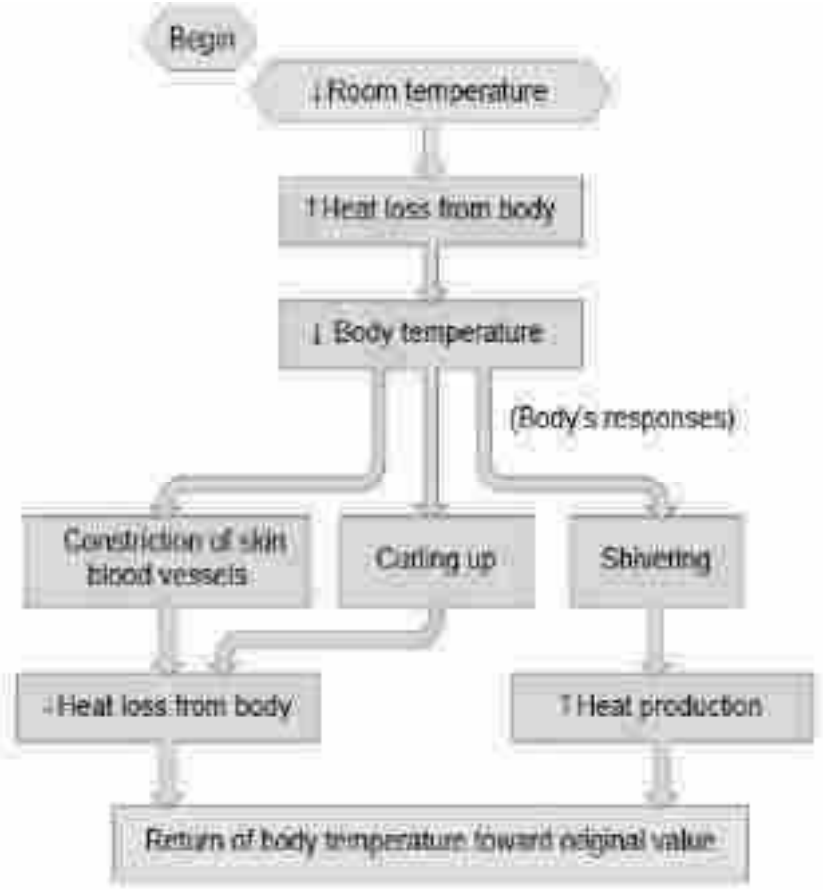
Table 3.1: Normal range and approximate short-term nonlethal range of some important parameters of the body.

Parameters (Unit) limit	Normal range	Approximate short-term nonlethal limit
Plasma pH	7.35–7.45	6.9–8.0
Plasma K^+ (mmol/L)	3.5–5.0	1.5–9.0
Body temperature (°F)	97–98.8	65–110
CO_2 (Venous blood) (mm Hg)	35–45	5.0–80
Plasma Na^+ (mmol/L)	135–146	115–175
Plasma HCO_3^- (mmol/L)	24–32	8.0–45
Blood glucose (mg/dL)	60–99	25–1500

Important Examples of Homeostatic Regulations

- Important **examples** of homeostatic regulations → control of **pH, osmolarity, and water and electrolyte** composition of the body fluids, regulation of solutes in the blood like **blood sugar**, balance of **body weight**, etc

- ***<u>Body temperature regulation</u>:***
- **96°F to 98.4°F.**
- When temperature is **above normal**, the body activates controlling mechanisms to **increase heat loss** through **cutaneous vasodilation and sweating.**
- When **hypothermia** develops, the body responds by decreasing heat loss through **vasoconstriction** and by increasing heat production through **shivering**

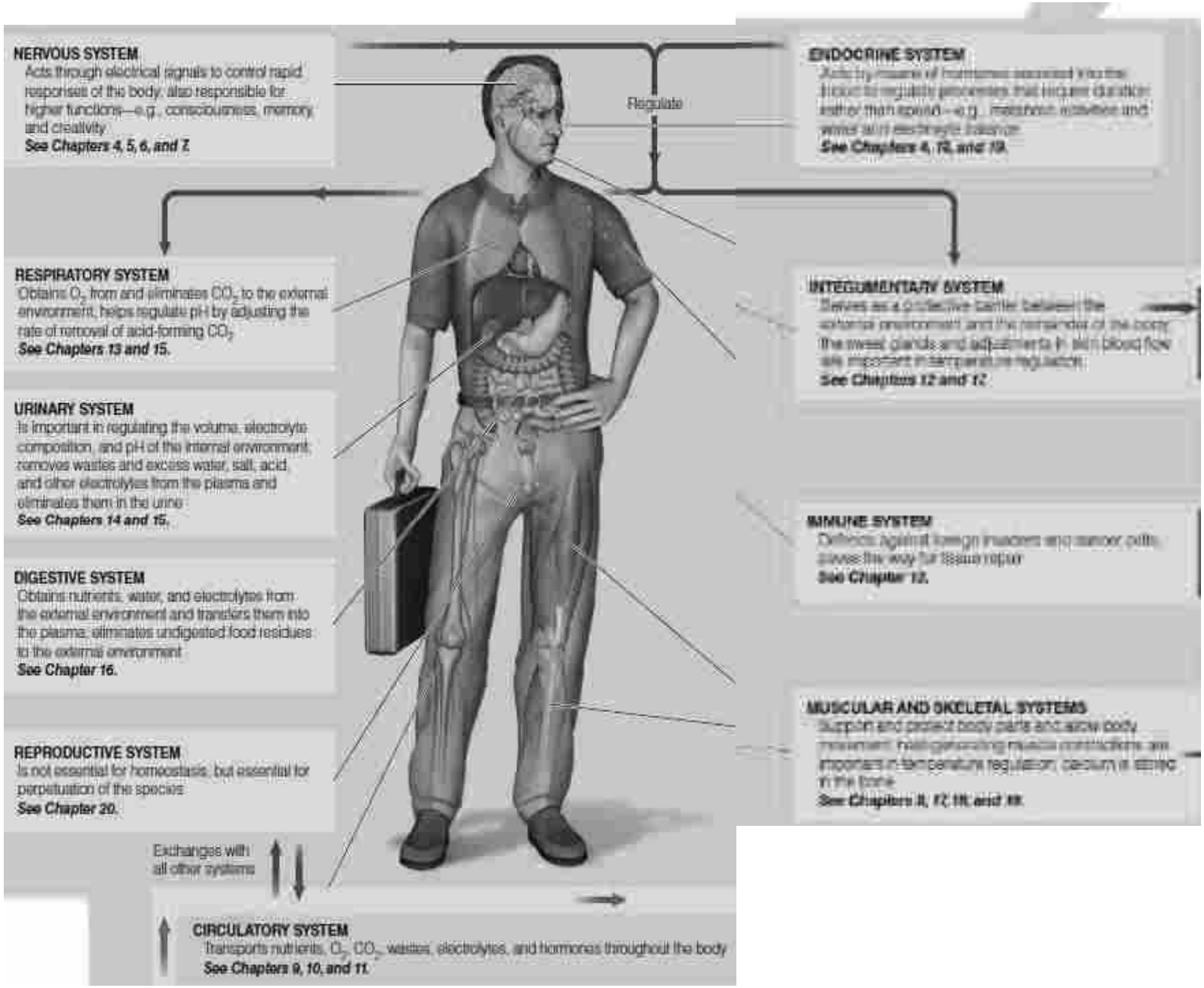


- ***Blood pressure regulation:***
- systolic between 100 and 119 mmHg
- diastolic between 60 and 79 mmHg

- **Kidney Plays an Important Role**

The kidneys are important organ for the homeostasis of many physiological variables such as

- **blood volume**
- **blood pressure,**
- **pH balance,**
- **electrolyte** composition of body fluids
- **osmolarity** of fluid compartments.


NERVOUS SYSTEM
Acts through electrical signals to control rapid responses of the body; also responsible for higher functions—e.g., consciousness, memory, and creativity
See Chapters 4, 5, 6, and 7.
Regulate
ENDOCRINE SYSTEM
See Chapters 4, 12, and 19.
RESPIRATORY SYSTEM
Obtains O_2 from and eliminates CO_2 to the external environment; helps regulate pH by adjusting the rate of removal of acid-forming CO_2
See Chapters 13 and 15.
INTEGUMENTARY SYSTEM
See Chapters 12 and 17.
URINARY SYSTEM
Is important in regulating the volume, electrolyte composition, and pH of the internal environment; removes wastes and excess water, salt, acid, and other electrolytes from the plasma and eliminates them in the urine
See Chapters 14 and 15.
IMMUNE SYSTEM
See Chapter 12.
DIGESTIVE SYSTEM
Obtains nutrients, water, and electrolytes from the external environment and transfers them into the plasma; eliminates undigested food residues to the external environment
See Chapter 16.
MUSCULAR AND SKELETAL SYSTEMS
See Chapters 8, 12, 18, and 19.
REPRODUCTIVE SYSTEM
Is not essential for homeostasis, but essential for perpetuation of the species
See Chapter 20.
Exchanges with all other systems
CIRCULATORY SYSTEM
Transports nutrients, O_2, CO_2, wastes, electrolytes, and hormones throughout the body
See Chapters 9, 10, and 11.

INTRACELLULAR HOMEOSTASIS

- It is important to maintain a balance intracellular fluid volume and composition.

- As intracellular compartment is in close contact with extracellular compartment, any **change in ECF is immediately reflected in ICF**

- Finally, the **primary objective of homeostasis is to maintain cellular functions** that in turn promotes tissue or organ functions.

Feedback Systems of Homeostatic Regulations

- Homeostatic regulation is mainly achieved through the feedback mechanisms that operate **to safeguard a set point** already set for the physiological variable.

- There are **two types** of feedback regulations:

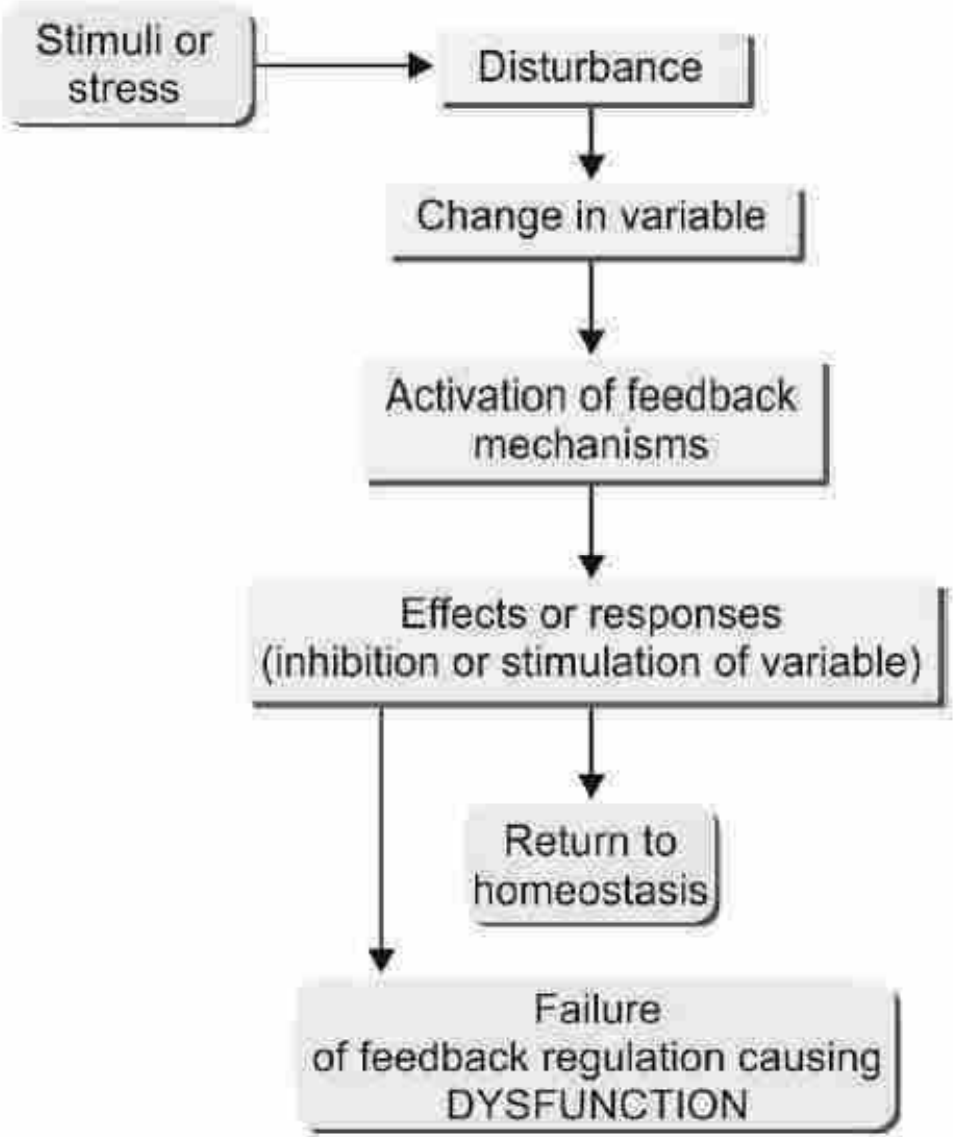
  The **negative feedback** and the **positive feedback**

Negative Feedback System

- The negative feedback mechanism is the **usual mechanism** of homeostatic regulation.
- When the **variable is raised above the set point**, the negative feedback mechanism **triggers processes** that inhibit the formation of the variable

- When the **variable is lowered below** the set point, negative feedback system **withdraws the inhibition** to allow production of the variable.

Flowchart 3.1: Feedback mechanism of homeostatic regulation. Note that failure of feedback control leads to the dysfunctions or diseases.

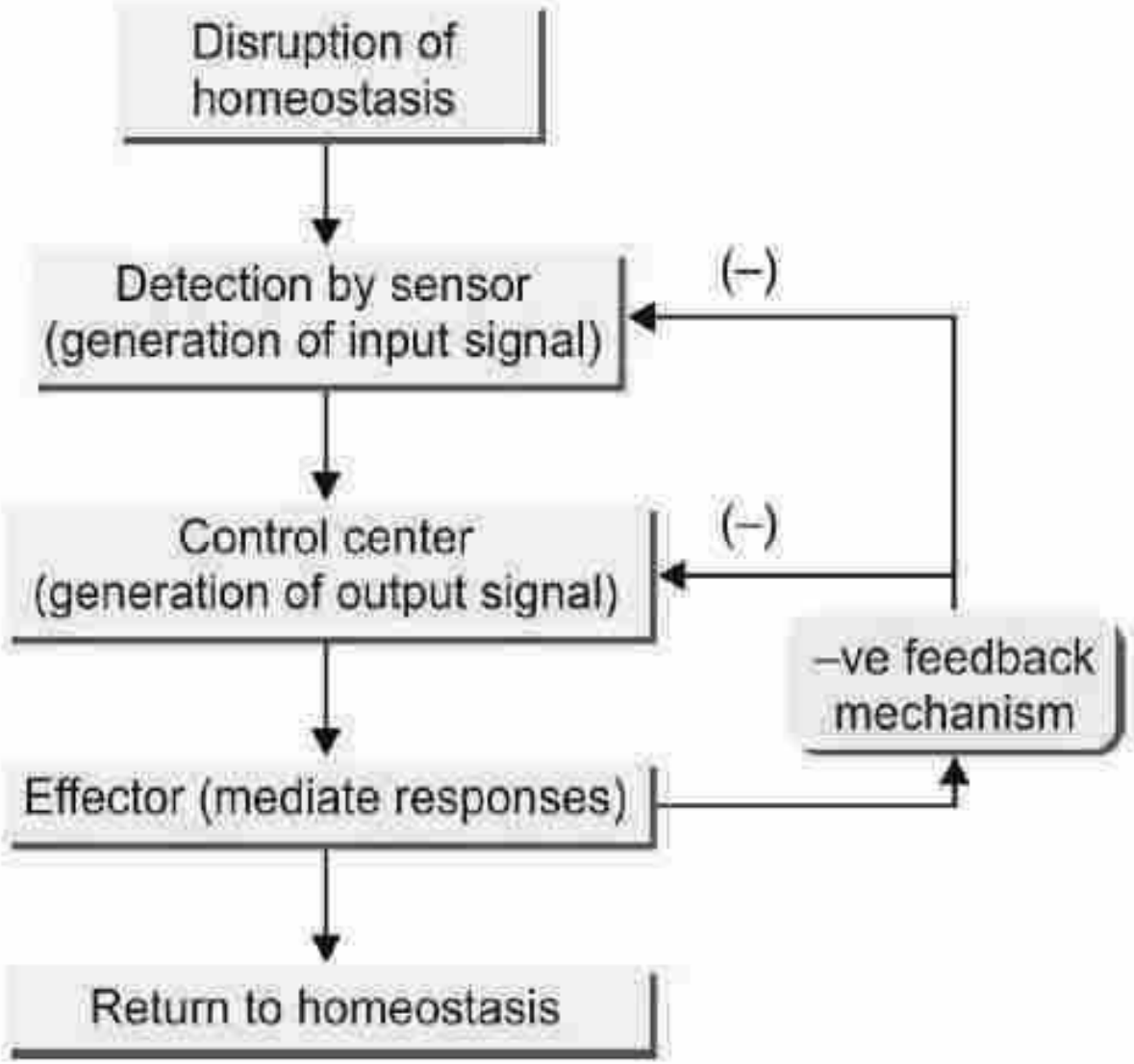


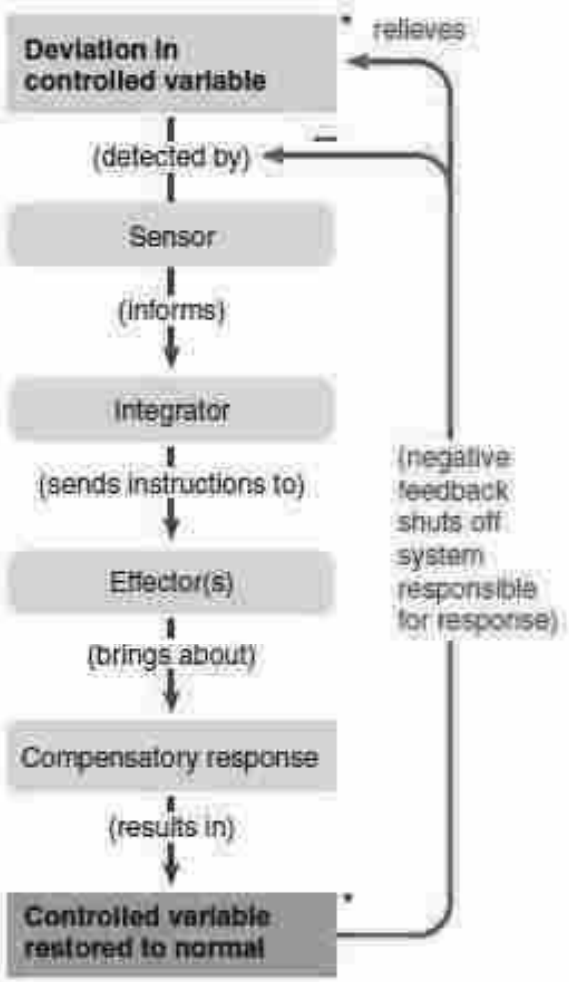
Components of Negative Feedback System

- Negative feedback control system requires a **sensor** that detects the change in variable, a **control center** that receives input from the sensor and initiates command signal, and an **effector** that brings in responses according to the command signal directed from the control center

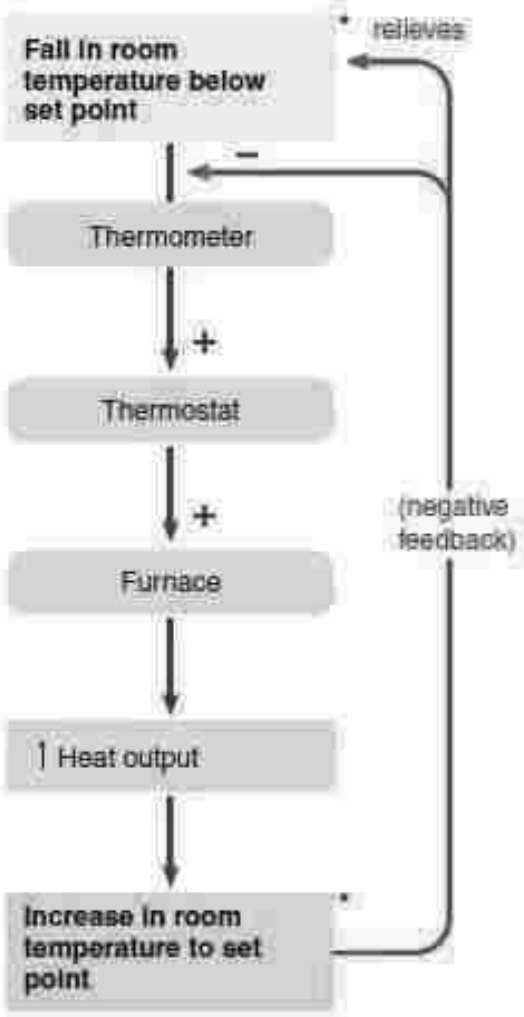
Sensor→ control center→ effector

Flowchart 3.2: Components of negative feedback regulation system.

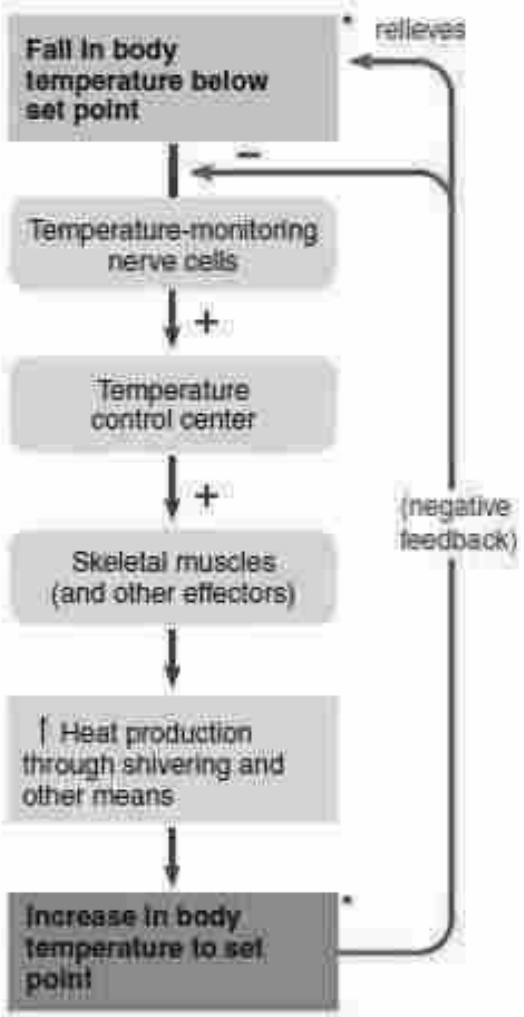




(a) Components of a negative-feedback control system

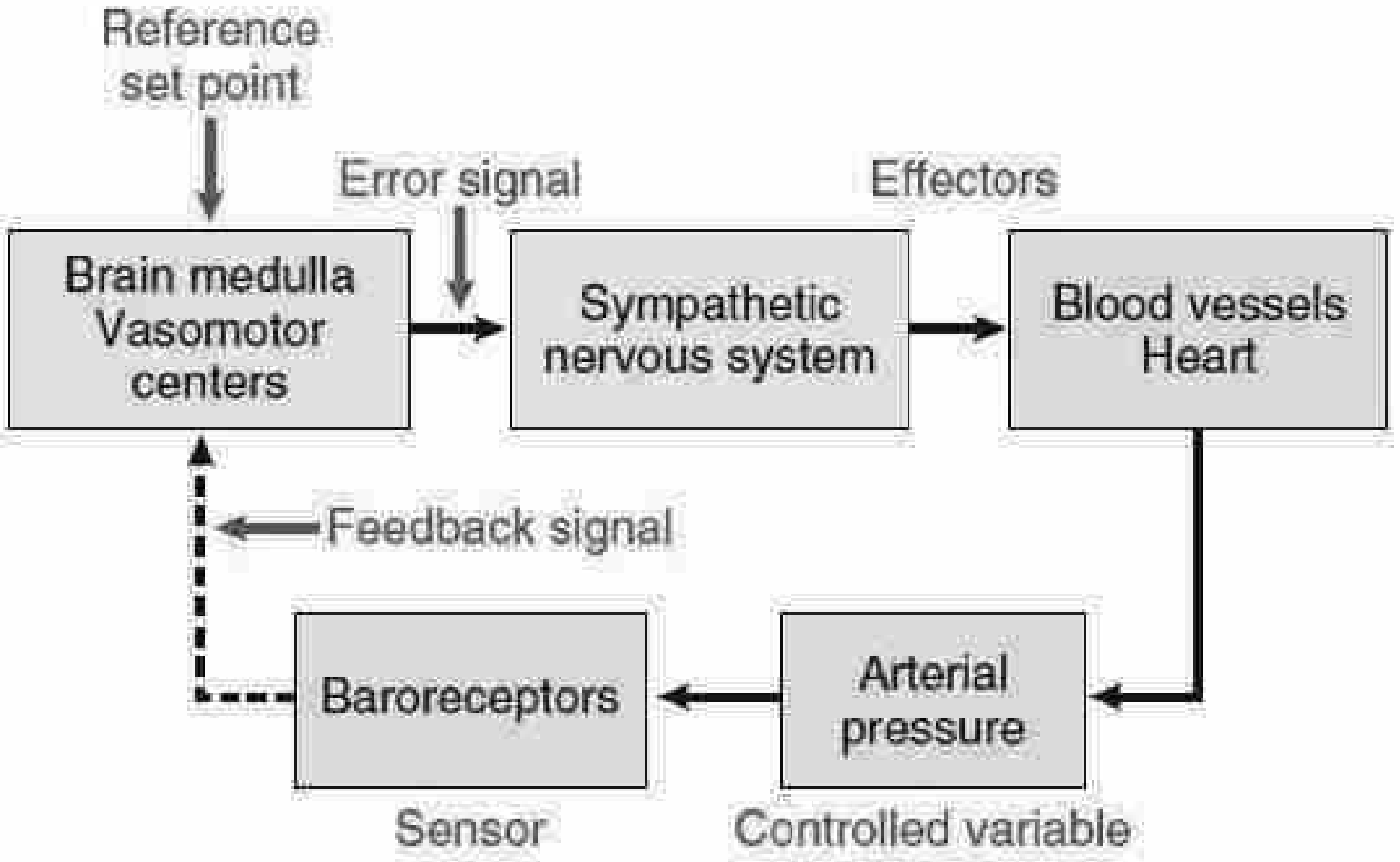


(b) Negative-feedback control of room temperature

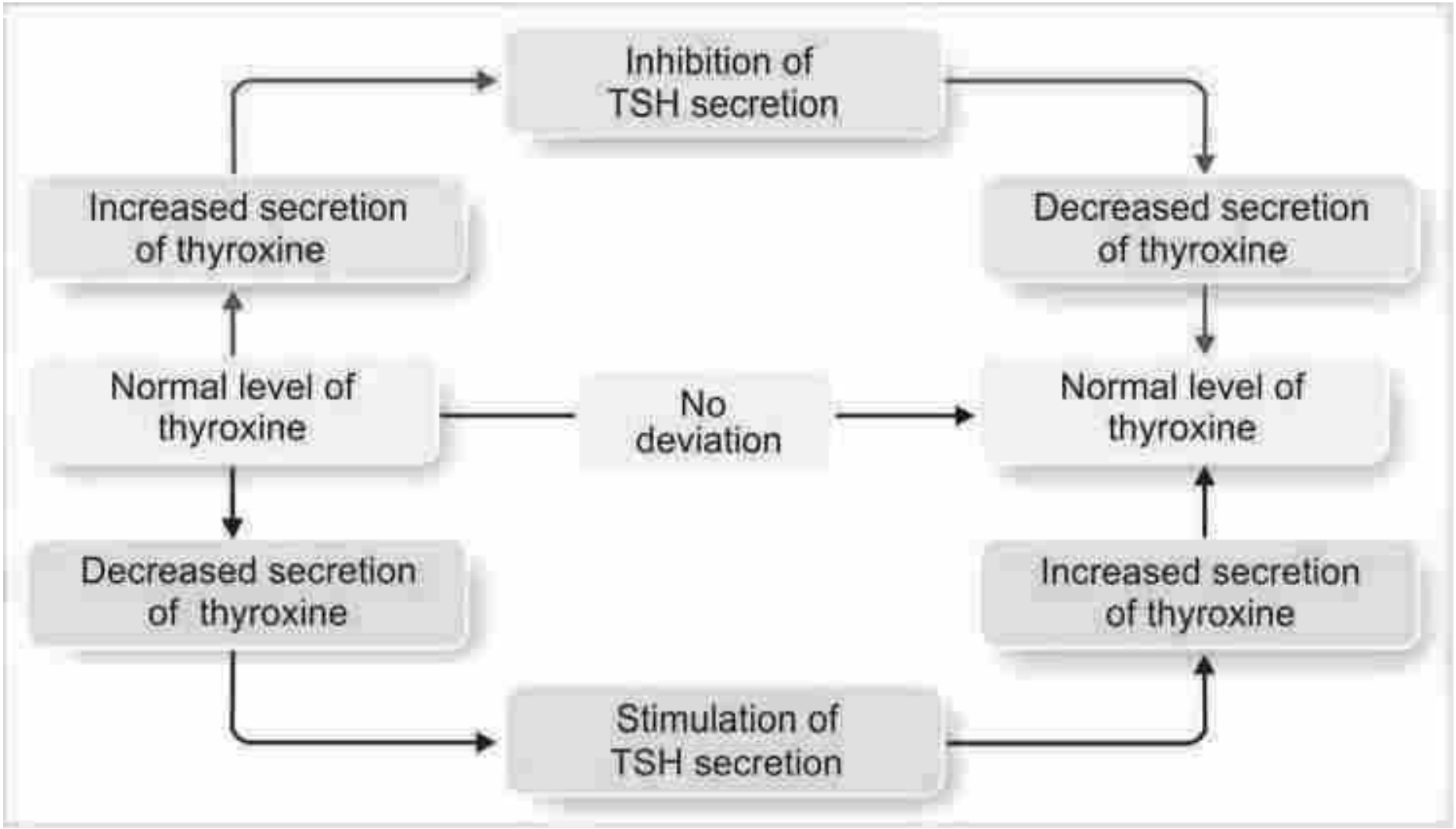


(c) Negative-feedback control of body temperature

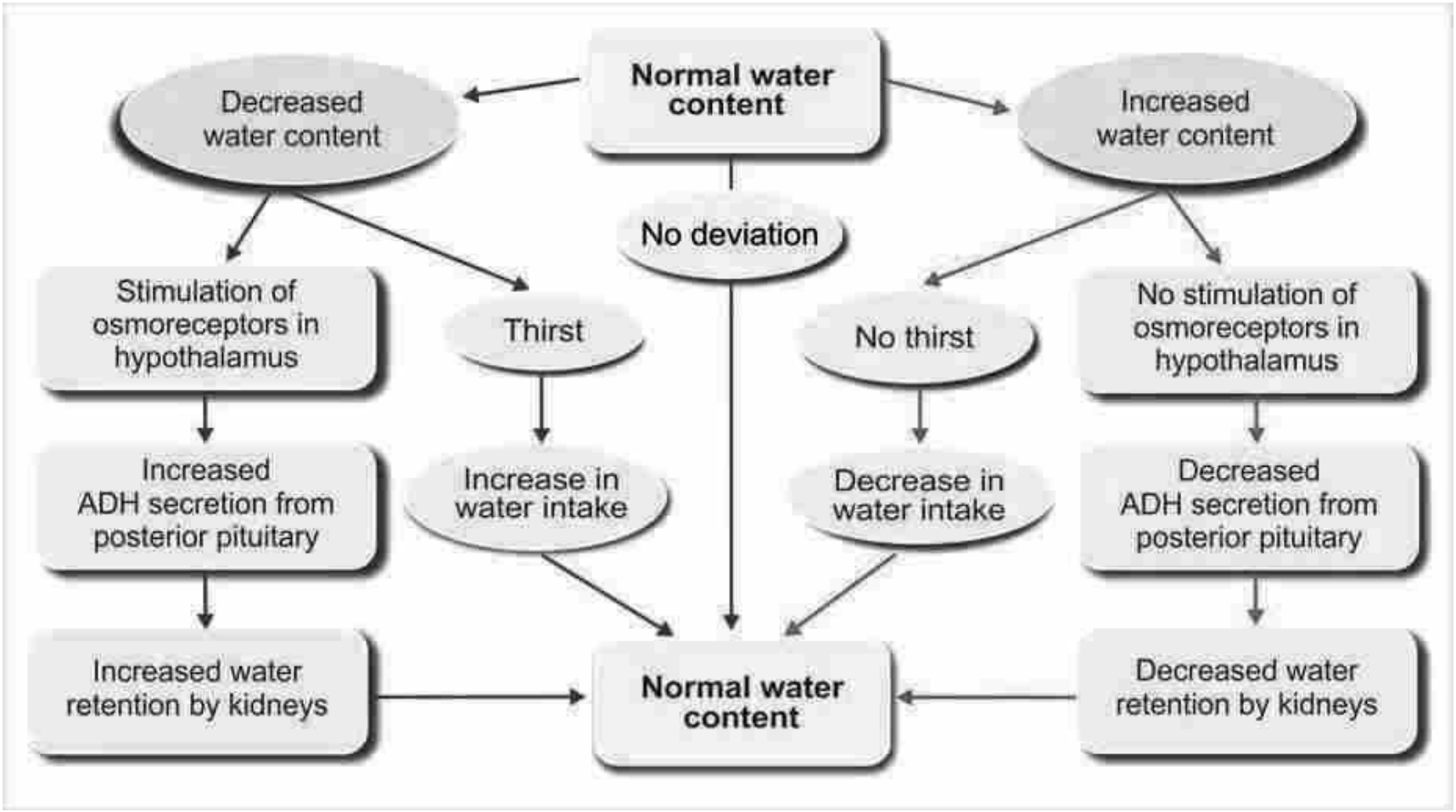
Negative feedback control of arterial pressure by the arterial baroreceptors.



Negative feedback mechanism – secretion of thyroxine



Negative feedback mechanism – maintenance of water balance.



Gain of a Control System

- The degree of effectiveness with which a control system maintains constant conditions

$$\text{Gain} = \frac{\text{Correction}}{\text{Error}}$$

- let us assume that a large volume of blood is transfused into a person whose **baroreceptor** pressure control system is **not functioning**, and the arterial pressure rises from the normal level of **100 mm Hg up to 175 mm Hg**. Then, let us assume that the same volume of blood is injected into the same person when the baroreceptor **system is functioning**, and this time the pressure **increases only 25 mm Hg**. Thus the feedback control system has caused a **"correction" of –50** mm Hg—that is, from 175 mm Hg to 125 mm Hg. There remains **an increase in pressure of +25 mm Hg, called the "error,"** which means that the control system is not 100 percent effective in preventing change.

- gain of the person’s baroreceptor system for control of arterial pressure is −50 divided by +25, or −2.
- gain of the system controlling internal body temperature when a person is exposed to moderately cold weather is about −33.
- much more effective than the baroreceptor

Positive Feedback System

- In a positive feedback system, increase in the variable triggers processes that further increase the variable.
- This sets the vicious cycle of activation of the process. Therefore, this control mechanism does **not operate to provide homeostasis.**

- The positive feedback system is known as a "**Vicious Cycle System**" that terminates only when the stimulus applied to trigger is withdrawn or the process itself is self-terminated

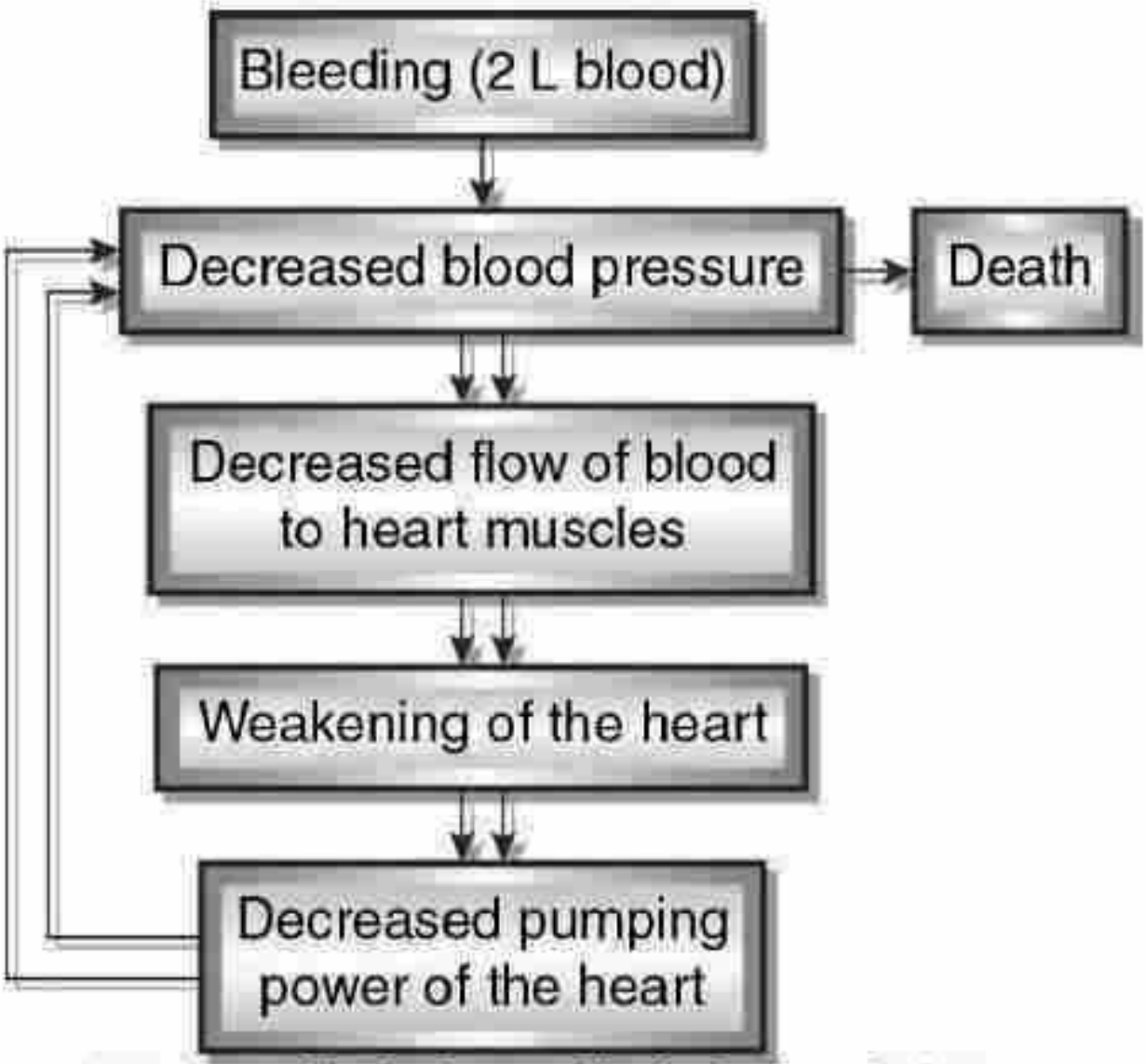
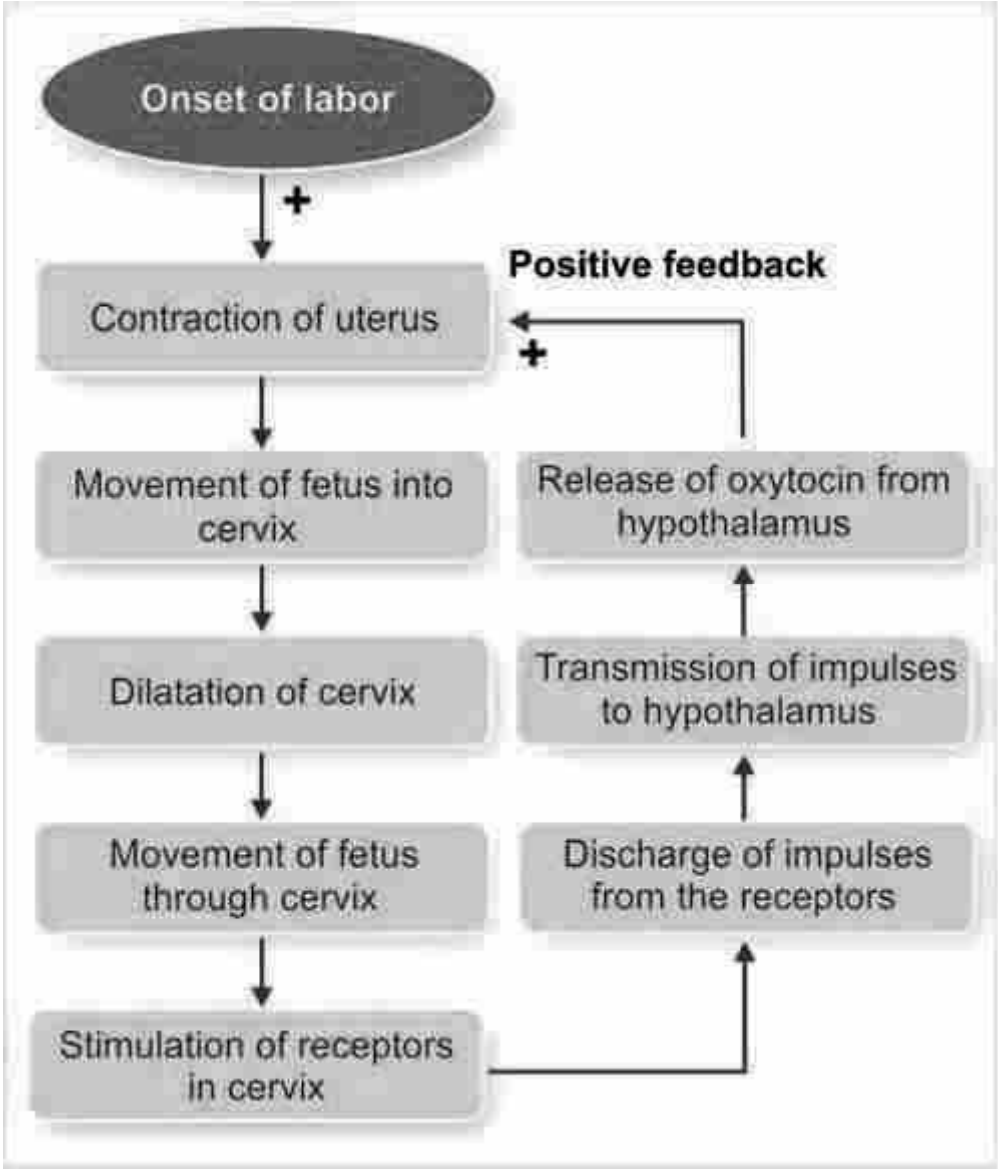


FIGURE 1.1-2 Showing how a positive feedback mechanism can cause death.

- Examples of positive feedback regulation are:

- The ***parturition reflex initiated by oxytocin***

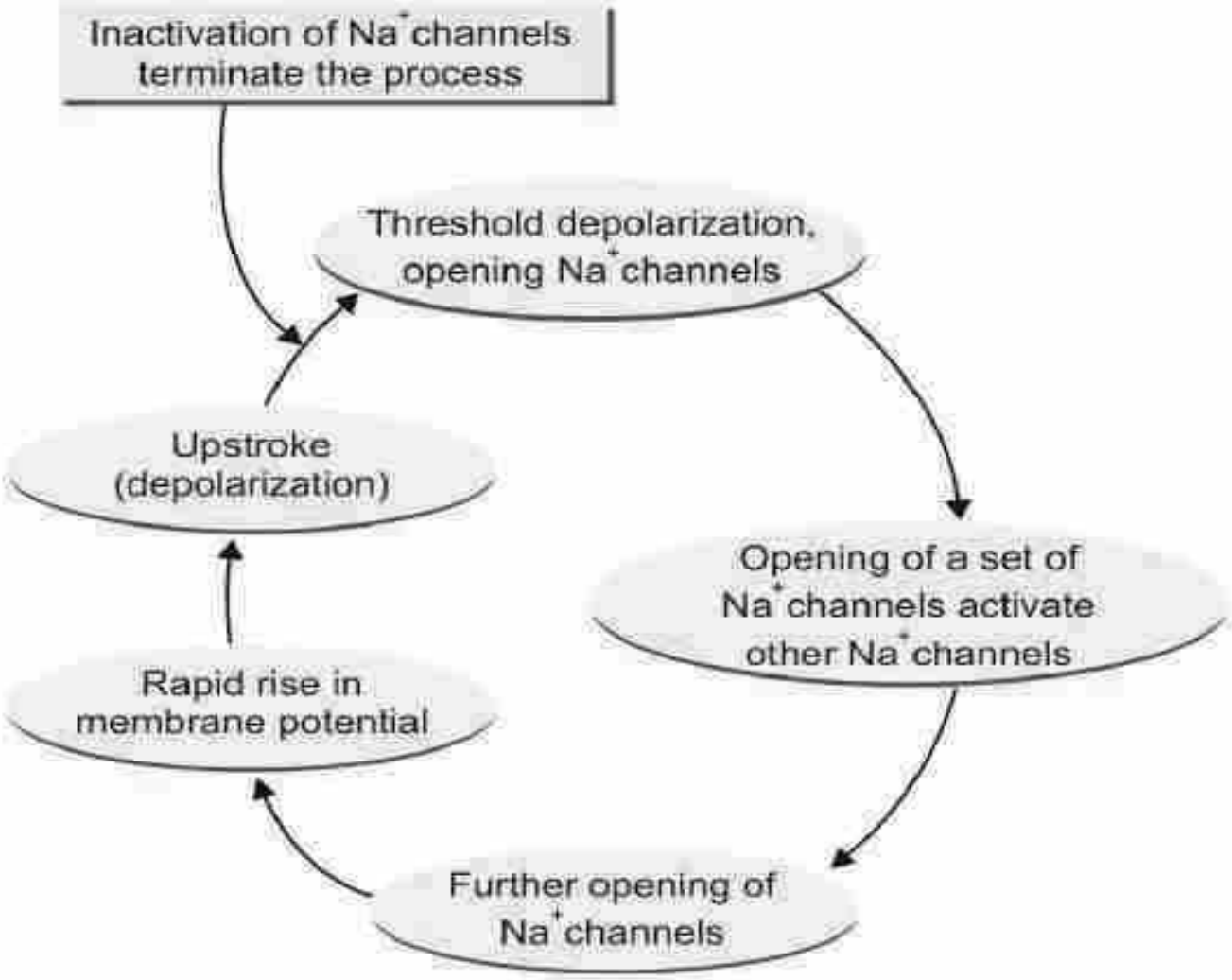
When the head of the matured fetus presses on the uterine cervix, the cervical distension sends signal to release oxytocin which increases the excitability of myometrium and causes uterine contraction. This vicious cycle continues till the baby is delivered.


Onset of labor
+
Positive feedback
Contraction of uterus
+
Movement of fetus into cervix
Release of oxytocin from hypothalamus
Dilatation of cervix
Transmission of impulses to hypothalamus
Movement of fetus through cervix
Discharge of impulses from the receptors
Stimulation of receptors in cervix

- **LH surge:**
- This is increased luteinizing hormone (LH) secretion that leads to ovulation. Normally, estrogen inhibits LH secretion. But, before ovulation, estrogen provides positive feedback to LH secretion, which results in LH surge.

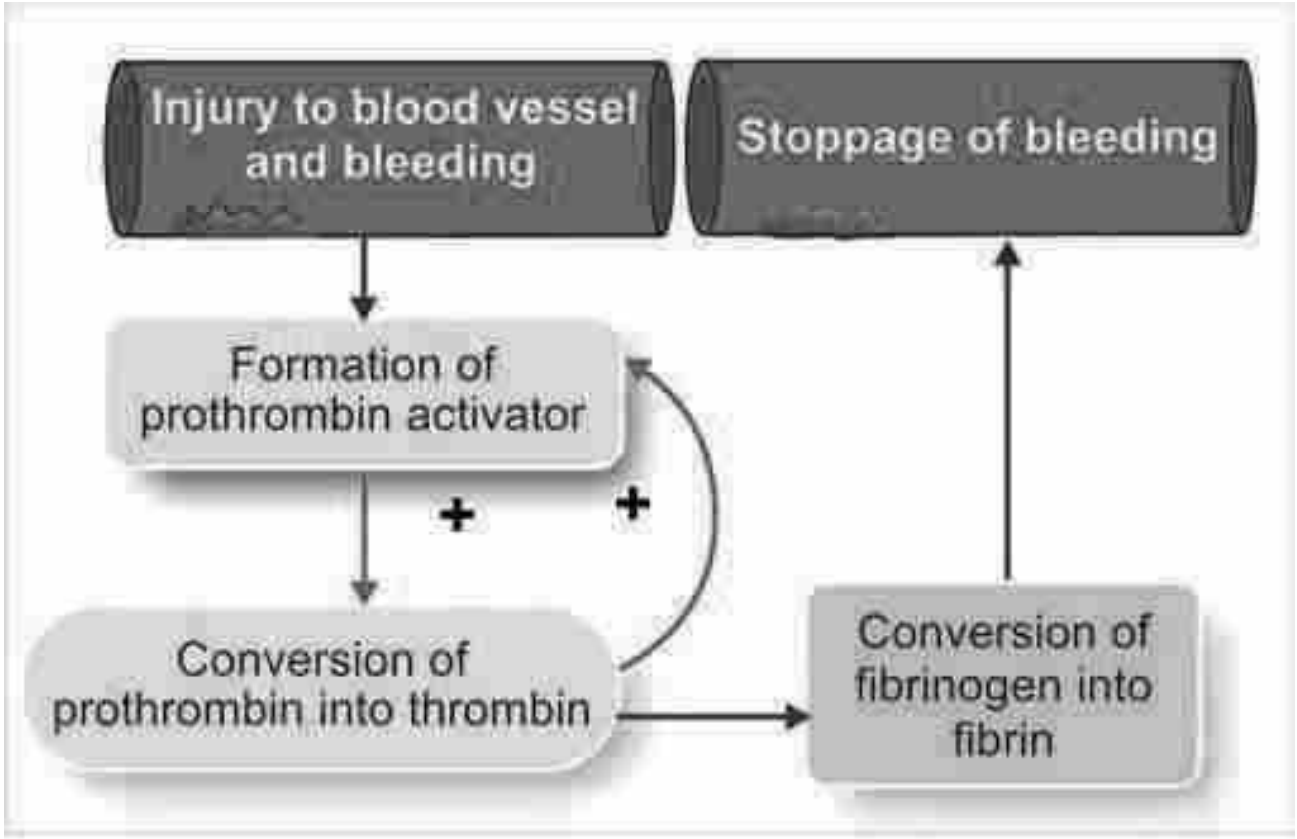
- **The Hodgkin's cycle:**
- Sodium influx that causes upstroke (depolarization) of nerve or muscle action potential (Flowchart 3.4). Opening of few sets of sodium channel provides positive feedback for opening of other sodium channels that results in massive sodium influx causing depolarization

Flowchart 3.4: Hodgkin's cycle as an example of positive feedback mechanism. In this, opening of a set of Na^+ channels activates opening of subsequent sets of Na^+ channels (+ve feedback) that leads to depolarization of the cell.



- **Activation of digestive enzymes:** The activation of digestive enzymes pepsinogen and trypsinogen by pepsin and trypsin respectively.

Enzyme cascade hypothesis of coagulation: Activation of one clotting factor, which acts as an enzyme to activate the next clotting factor in the coagulation cascade.



Thank you

Student's feedback

1. Which of the following activities is not carried out by every cell in the body?
a. obtaining O2 and nutrients
b. performing chemical reactions to acquire energy for the cell's use
c. eliminating wastes
d. largely controlling exchange of materials between the cell and its external environment
e. reproducing

2. Which of the following is the proper progression of the levels of organization in the body?

a. chemicals, cells, organs, tissues, body systems, whole body

b. chemicals, cells, tissues, organs, body systems, whole body

c. cells, chemicals, tissues, organs, whole body, body systems

d. cells, chemicals, organs, tissues, whole body, body systems

e. chemicals, cells, tissues, body systems, organs, whole body

3. Which of the following is *not a type of connective tissue?*
a. bone
b. blood
c. the spinal cord
d. tendons
e. the tissue that attaches epithelial tissue to underlying structures

4. The term *tissue can apply either to one of the four primary* tissue types or to a particular organ's aggregate of cellular and extracellular components. *(True or false?)*

5. Cells in a multicellular organism have specialized to such an extent that they have little in common with single-celled organisms. *(True or false?)*

6. Cell specializations are usually a modification or elaboration of one of the basic cell functions. *(True or false?)*

Use & Abuse of Antibiotics

Dr K R Gindodia

Box 1: Definition of terms as used in review

Definitions:

Rational use of drugs: requires that patients receive medications appropriate to their clinical needs, in doses that meet their own individual requirements for an adequate period of time and at the lowest cost to them and their community (Holloway, 2005; MSH, 1997).

Irrational drug use: is where there is over-prescribing, extravagant prescribing, incorrect prescribing, under-prescribing, and multiple prescribing as well as indiscriminate drug use. In this review it refers to prescribers (Holloway, 2005).

Drug misuse: Is the improper, unlawful or incorrect use and misapplication of drugs. In this review it refers to both irrational and inappropriate drug use (Hawkey, 2008)

Inappropriate use: Refers to use of wrong drug, indication, wrong patient and with wrong patient information as well as in inadequate doses, wrong duration and indiscriminate drug use. In this review it refers to drug use by consumers (Kardas *et al.*, 2005).

Antibiotic: They are chemical compounds produced by living cells of lower plant forms, such as bacteria, yeasts and molds and they inhibit or kill other micro-organisms (Brunton *et al.*, 2005).

Antibacterial: Are drugs that can suppress and kill bacteria and therefore the antibiotics are a subset of antibacterial drugs (Brunton *et al.*, 2005).

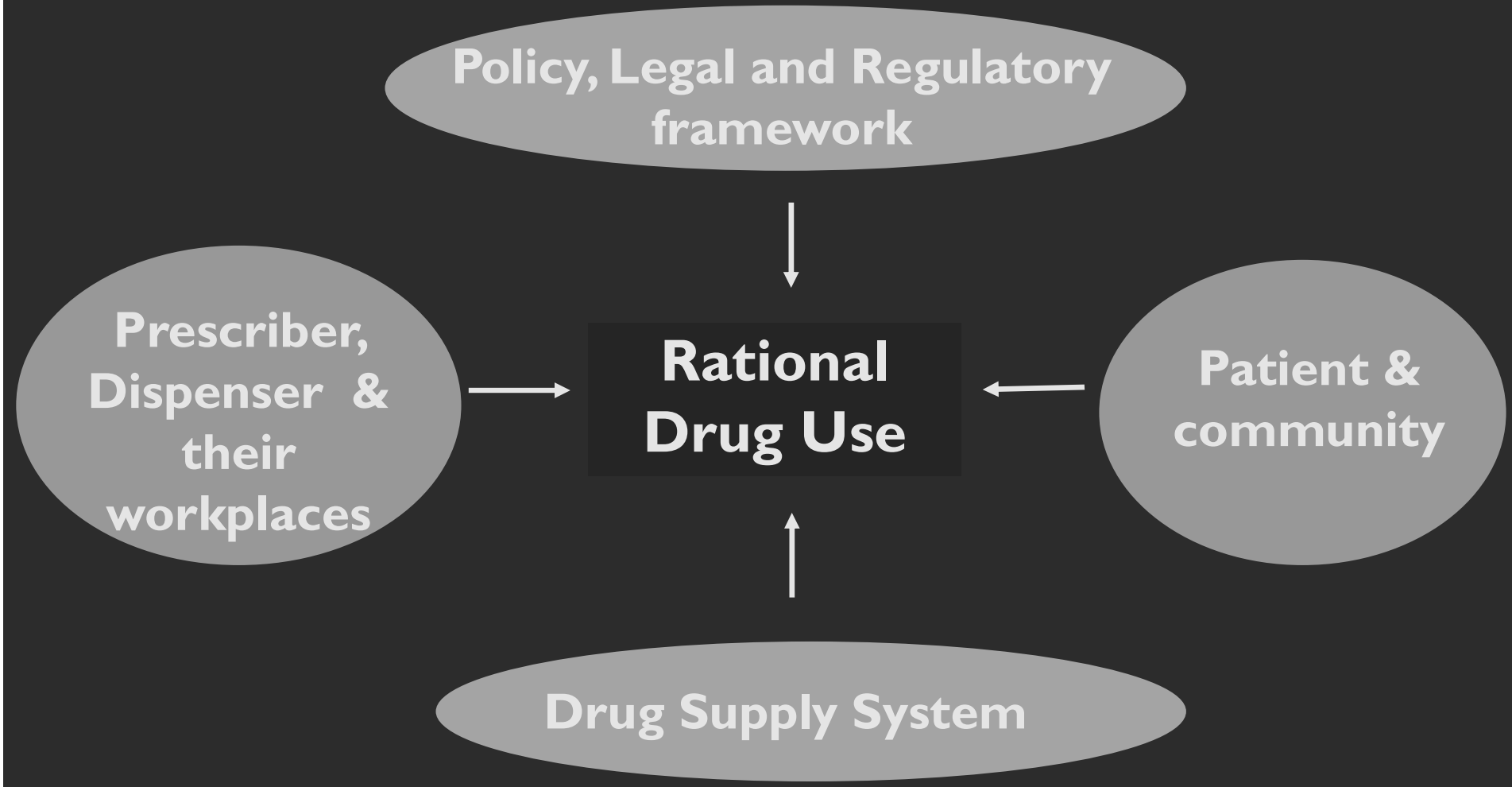
Intervention measures: Refers to the various mechanisms that can be used to prevent, reduce or avert the misuse of the antibiotics/antibacterial agents (Kardas *et al.*, 2005).

An antimicrobial agent: Is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi or protozoans (Abba, 2009).

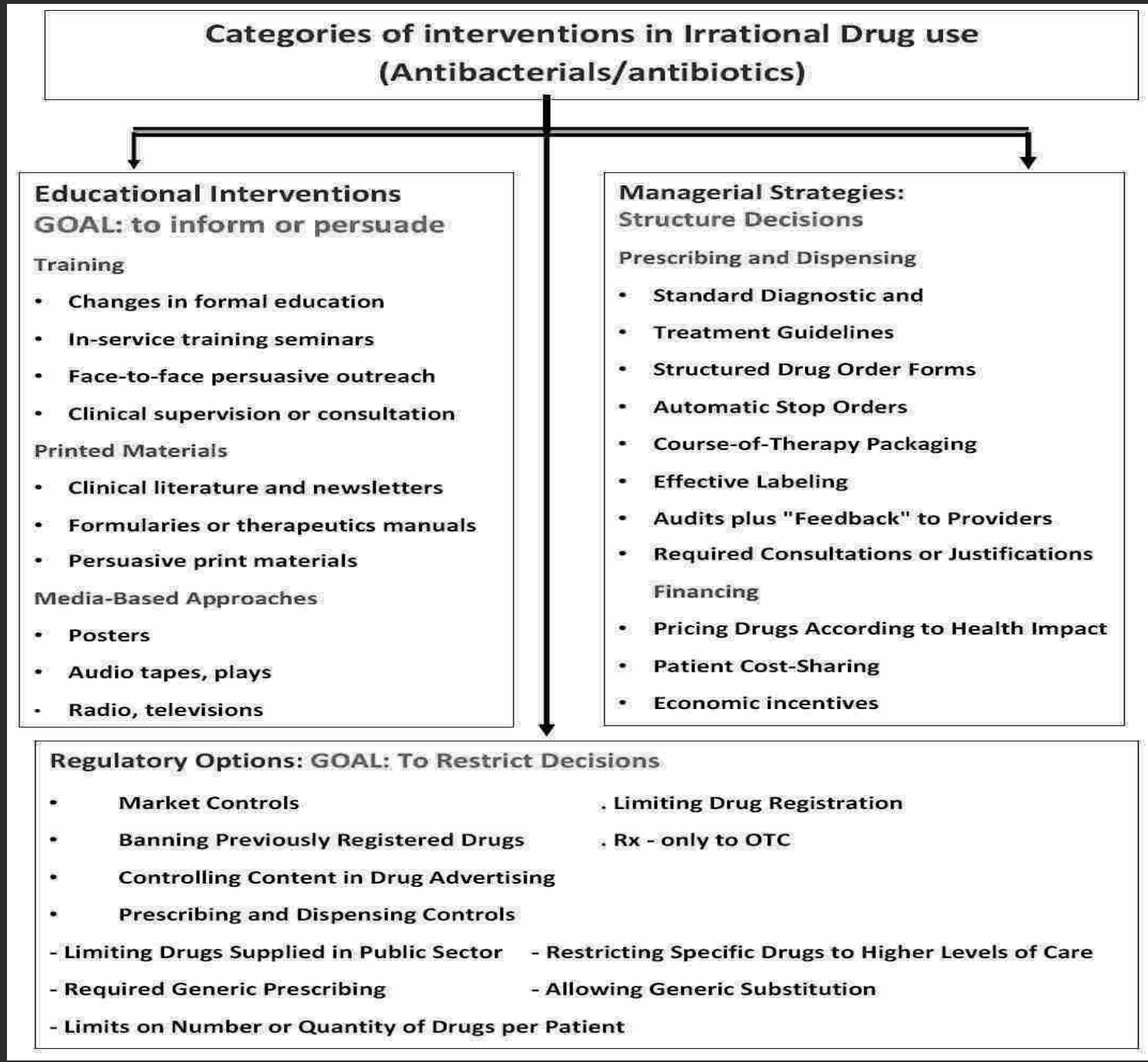
Introduction

- Misuse of antibiotics/antibacterial drugs is a global problem especially in developing countries with poor healthcare systems & corruption
- Occurs at all levels in both public & Private Healthcare facilities
- Reported up to 75 % of antibiotics are prescribed inappropriately in teaching hospitals in developing countries (Nambiar, 2003)
- Are used in conditons where not needed like flu etc.
- Resulted in failure of eradicating infectious bacteria, emergence of resistance, waste of resources, increased cost of treatment, ADR & death (Kardas *et al*., 2005)[3]

Many Factors Influence Use of Medicines



Interventions are directed at these components


Categories of interventions in Irrational Drug use
(Antibacterials/antibiotics)
Educational Interventions
GOAL: to inform or persuade
Training
• Changes in formal education
• In-service training seminars
• Face-to-face persuasive outreach
• Clinical supervision or consultation
Printed Materials
• Clinical literature and newsletters
• Formularies or therapeutics manuals
• Persuasive print materials
Media-Based Approaches
• Posters
• Audio tapes, plays
• Radio, televisions
Managerial Strategies:
Structure Decisions
Prescribing and Dispensing
• Standard Diagnostic and
• Treatment Guidelines
• Structured Drug Order Forms
• Automatic Stop Orders
• Course-of-Therapy Packaging
• Effective Labeling
• Audits plus "Feedback" to Providers
• Required Consultations or Justifications
Financing
• Pricing Drugs According to Health Impact
• Patient Cost-Sharing
• Economic incentives
Regulatory Options: GOAL: To Restrict Decisions
• Market Controls
. Limiting Drug Registration
• Banning Previously Registered Drugs
. Rx - only to OTC
• Controlling Content in Drug Advertising
• Prescribing and Dispensing Controls
- Limiting Drugs Supplied in Public Sector
- Restricting Specific Drugs to Higher Levels of Care
- Required Generic Prescribing
- Allowing Generic Substitution
- Limits on Number or Quantity of Drugs per Patient

Objectives of the review

- Review determined effect of various intervention studies on AB misuse in developing countries

Research question

1. What are the various interventions measures used in controlling irrational use of antibiotics/antibacterial agents in developing / poorer nations?

2. What is the impact of various intervention measures used in controlling the irrational use of antibiotics/antibacterial agents in developing / poorer nations?

Support Appropriate Antibiotic Use In India

Everyone Can Help Keep Antibiotics Working

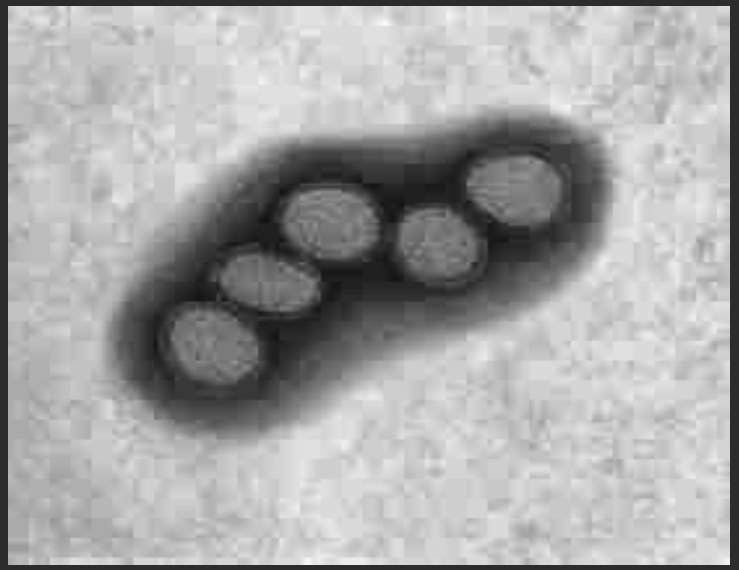
- By using antibiotics only when needed
- Following good hygiene practices

GET SMART VIRGINIA
Know When Antibiotics Work

What We'll Learn...

- Why children have more illness.
- How are viruses and bacteria different?
- How illness is spread.
- Myths and facts about antibiotics.
- Tips for for preventing the spread of illness.

GET SMART VIRGINIA
Know When Antibiotics Work



Influenza Virus
Flu

Streptococcus Pneumoniae Bacteria
- Ear infections
- Meningitis
- Pneumonia

A communicable disease is disease that can be passed from one person to another. These diseases are often caused by germs, such as bacteria or viruses.

GET SMART VIRGINIA
Know When Antibiotics Work

Some examples of contagious bacterial diseases are:

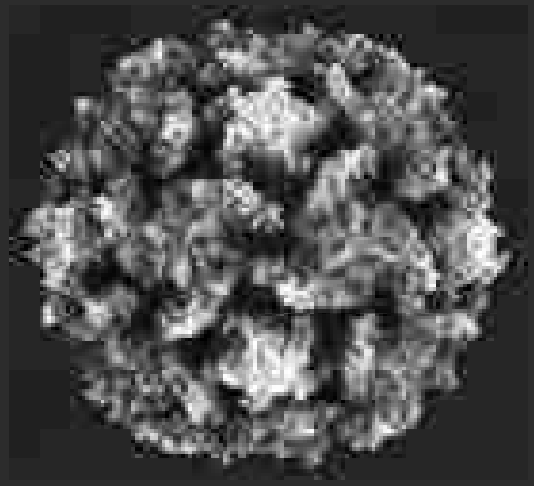
-strep throat
-impetigo
-pertussis (whooping cough)
-bacterial conjunctivitis (pink eye)

Antibiotics Work

GET SMART VIRGINIA
Know When Antibiotics Work

Some contagious viral infections include:

- **varicella (chicken pox)**
- **rubella (German measles)**
- **the common cold**
- **hepatitis**
- **mumps**
- **infectious mononucleosis**
- **rubeola (measles)**



Antibiotics Don’t Work

GET SMART VIRGINIA
Know When Antibiotics Work

Why do young children have more illness?

GET SMART VIRGINIA
Know When Antibiotics Work

Infection can result from sharing towels, dishes, or from handling contaminated objects. Indirect contact or skin to skin contact can also result in the spread of an illness.

GET SMART VIRGINIA
Know When Antibiotics Work

Sometimes an illness is passed to others by a carrier, or a person who has been infected by a germ but does not look or feel sick. This person may carry the germ in their nose, throat, or stomach. They can pass the germ to others by coughing, sneezing, or by not washing their hands properly.

GET SMART VIRGINIA
Know When Antibiotics Work

Light patches indicate germs carried on the hands

- **Your hands carry many germs even if you can't see them.**
- **Many people don't wash their hands because they look clean.**
- **As you can see this is not always the case.**

GET SMART VIRGINIA
Know When Antibiotics Work

How are Bacteria and Viruses Different?

GET SMART VIRGINIA
Know When Antibiotics Work

Viruses

- cause most common respiratory illnesses, such as colds and coughs
- cause a variety of infections from diarrhea to meningitis
- may take 2-3 weeks to resolve completely
- some viruses can be prevented by vaccines and immunizations
- antibiotics do not work for viruses

GET SMART VIRGINIA
Know When Antibiotics Work

Viruses Cause...

- Most bronchitis/chest colds
- Green/yellow nasal discharge
- Flu
- Most sore throats
- Ear aches and some sinusitis

....viruses clear up on their own - antibiotics won't help!

GET SMART VIRGINIA
Know When Antibiotics Work

Bacteria

- make you sick when they are where they don’t belong or grow more rapidly than normal
- live in your mouth, nose and throat
- are necessary for normal body functions like digestion
- are one of the oldest living things on Earth

GET SMART VIRGINIA
Know When Antibiotics Work

Bacteria Cause...

- Strep throat
- Some ear infections
- Some sinus infections
- Pneumonia

...these can be treated with antibiotics

GET SMART VIRGINIA
Know When Antibiotics Work

Virus or Bacteria: Who Knows?

- Let your healthcare provider decide!
- It is often hard to tell if illnesses are caused by a virus or bacteria
- Don't pressure healthcare providers or parents for antibiotics

GET SMART VIRGINIA
Know When Antibiotics Work

What are Antibiotics?

Powerful medicines with one very important job: to fight disease-causing bacteria.

When used properly, antibiotics can save lives.

GET SMART VIRGINIA
Know When Antibiotics Work

Antibiotic Resistant Bacteria Are:

Bacteria that mutate and are able to resist the antibiotics that are meant to kill them.

This is a normal process speeded up by the overuse and misuse of antibiotics.

GET SMART VIRGINIA
Know When Antibiotics Work

What is Antibiotic Misuse?

- Taking antibiotics when they are not needed:
  - for viral infections
- When needed, taking antibiotics incorrectly:
  - stopping the medicine when you feel better - not finishing the prescription
  - saving antibiotics for a future illness
  - sharing or using someone else's medicine

GET SMART VIRGINIA
Know When Antibiotics Work

Why is Antibiotic Misuse a Problem?

1. Antibiotics become less effective and may not work the next time you use them.
2. Improper use of antibiotics leads to more antibiotic resistant bacteria.
3. Antibiotic resistant bacteria can be spread throughout the community and from person to person.

GET SMART VIRGINIA
Know When Antibiotics Work

Antibiotic Resistant Bacteria

- Require stronger antibiotics
- Are more common in people who recently used antibiotics*
- Can be spread within a family or a community
- Can affect anyone
- Are becoming more common

*Pediatrics, Vol. 112 No. 4, p. 862-69, October 2003

GET SMART VIRGINIA
Know When Antibiotics Work

Myths & Facts about Antibiotics and Respiratory Illness

GET SMART VIRGINIA
Know When Antibiotics Work

Myths and Facts

- **Myth:** Taking antibiotics means I or my child can return to work or childcare sooner
- **Fact:** Antibiotics do not shorten the duration of viral illnesses

  Everyone should stay home until they are fever-free and well enough to participate in activities whether they have a viral or bacterial illness.

GET SMART VIRGINIA
Know When Antibiotics Work

Myths and Facts

- **Myth:** Cold and flu symptoms will feel better or get better faster on antibiotics
- **Fact:** Antibiotics cannot ease the symptoms of viral illnesses; these infections resolve on their own

  Children and adults need extra rest and care, extra fluids…not antibiotics, symptomatic relief is helpful

GET SMART VIRGINIA
Know When Antibiotics Work

Myths and Facts

- **Myth:** Illnesses with the same symptoms require antibiotics
- **Fact:** Illnesses with similar symptoms can be caused by different germs

  Let a healthcare provider decide if the illness is caused by a virus or bacteria - and if antibiotics are needed

GET SMART VIRGINIA
Know When Antibiotics Work

Myths and Facts

- **Myth:** If I take an antibiotic, I won't spread my illness to others
- **Fact:** Viral illnesses (colds, flu, etc.) usually spread from person to person before the onset of symptoms; before a person appears ill

  Antibiotics cannot stop the spread of viral illnesses

GET SMART VIRGINIA
Know When Antibiotics Work

How do we Stop Antibiotic Misuse?

- Don’t ask for antibiotics – let your doctor decide if you need them
- Always take antibiotics exactly as prescribed
- Finish the whole prescription - do not stop when you feel better
- Never save antibiotics for a future illness – or share with others

GET SMART VIRGINIA
Know When Antibiotics Work

How Can You Keep Yourself Well

Handwashing

Immunizations

GET SMART VIRGINIA
Know When Antibiotics Work

Wash Your Hands...

- Upon arrival to and before leaving work
- Before and after handling food
- Before and after toileting

- After:
  - working outside
  - handling pets
  - handling any body fluids
  - wiping a child's nose or your own
  - using play dough, sand or water tables

GET SMART VIRGINIA
Know When Antibiotics Work

Hand Washing is Important Because…

- **80% of disease is spread by your hands.**
- **Hand washing is the most effective way to prevent the spread of respiratory illness.**
- **Some germs can live on dry surfaces for several hours and moist surfaces like, sinks for 3 days.**

GET SMART VIRGINIA
Know When Antibiotics Work

Handwashing Guidelines

- Wet hands with warm, running water
- Add soap (preferably liquid), rub hands together to make a lather, away from water, for at least 15 seconds
- Rinse hands well
- Dry thoroughly with a clean, disposable towel
- Use a hand sanitizer (alcohol-based) when soap and running water is not available (when outside or on trips, wash hands upon return from trips)

GET SMART VIRGINIA
Know When Antibiotics Work

Despite Good Handwashing,

Children and adults will still
get sick...

GET SMART VIRGINIA
Know When Antibiotics Work

Immunizations

- Immunizations are another important part of infection control for vaccine preventable disease
- Make sure you and your family is up-to-date on immunizations
- Get an annual flu shot

GET SMART VIRGINIA
Know When Antibiotics Work

Take Home Messages

- Viruses cause most common respiratory illnesses
- Viral illness needs time to heal - antibiotics cannot help

GET SMART VIRGINIA
Know When Antibiotics Work

Take Home Messages

- Taking antibiotics for viral illnesses will not:
  - cure the infection
  - keep others from getting the illness
  - make you feel better

But it will make it more likely you will have resistant bacteria in your body.

GET SMART VIRGINIA
Know When Antibiotics Work

Bottom Line

- Antibiotics are powerful medicines, but they're not always the answer!
- Misusing antibiotics now means they may not work when needed later to fight a bacterial infection
- We all need to play a role to help keep antibiotics working!

GET SMART VIRGINIA
Know When Antibiotics Work

Conclusion

- Misuse of antibacterial/antibacterial drugs is on increase especially in developing countries
- Variety of interventions are used for irrational use of AB drugs & had some impact
- Most of interventions were done in Asia
- Multifaceted interventions are effective in reducing misuse & inappropriate use of AB drugs & reduce emergence of resistance to commonest bacteria in developing countries
- Some studies showed a tendency of reverting once intervention programme stops

Get Smart India

Know When Antibiotics Work

GET SMART VIRGINIA
Know When Antibiotics Work

END

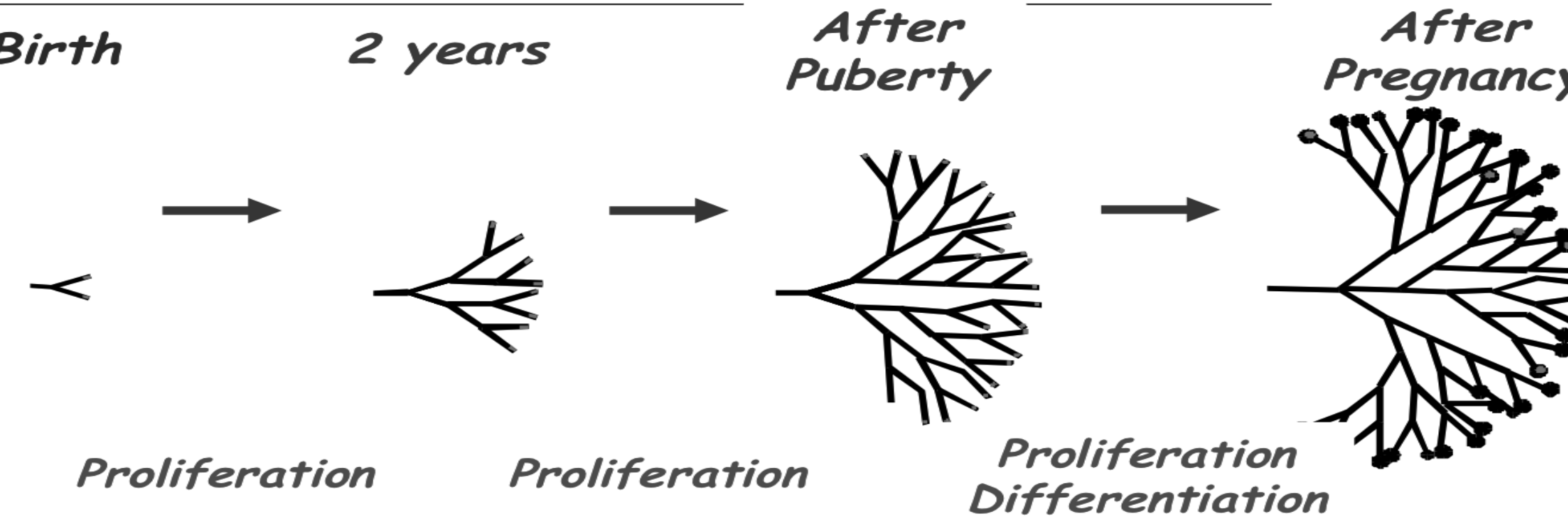
Illustration: Don Smith

Benign Diseases Of Breast

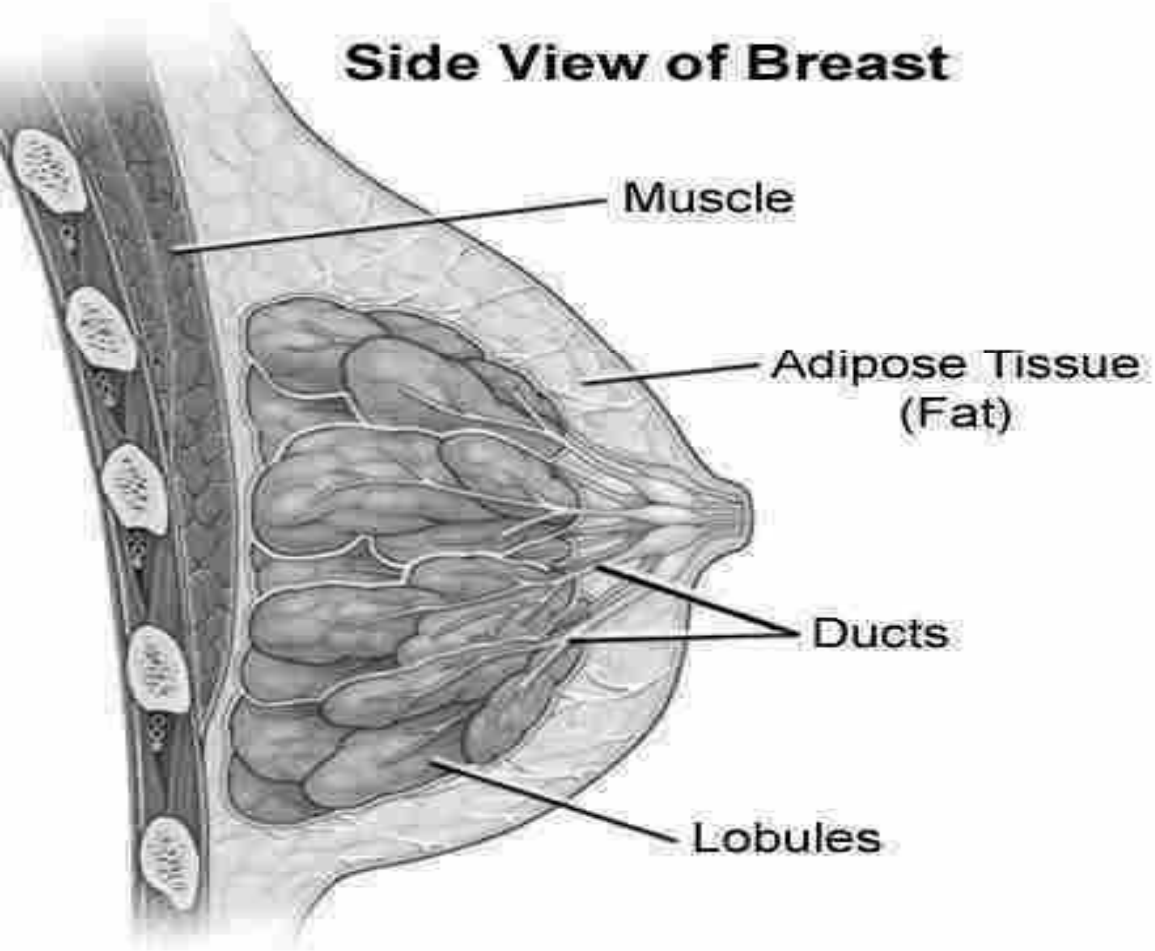
Dr K R GINDODIA

Development of the Breast Ductal Tree

Occurs mainly after birth



Female Breast Anatomy

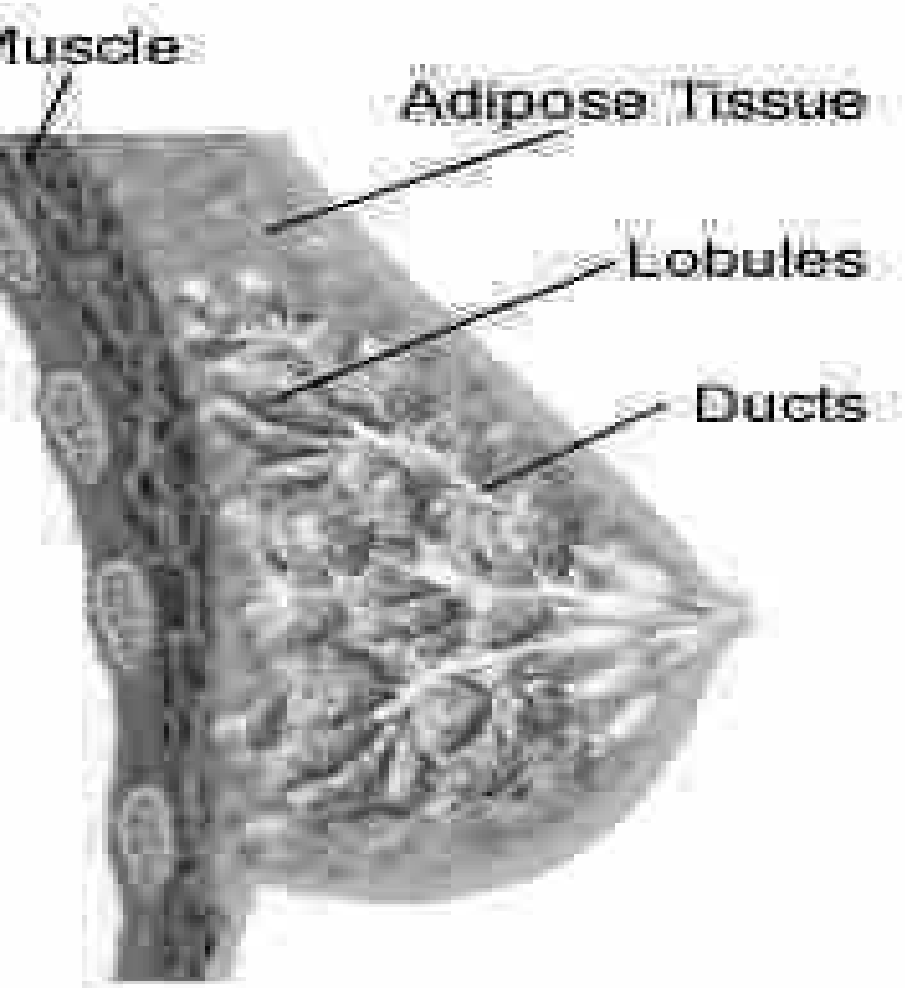


The bulk of the breast tissue is adipose tissue interspersed with connective tissue

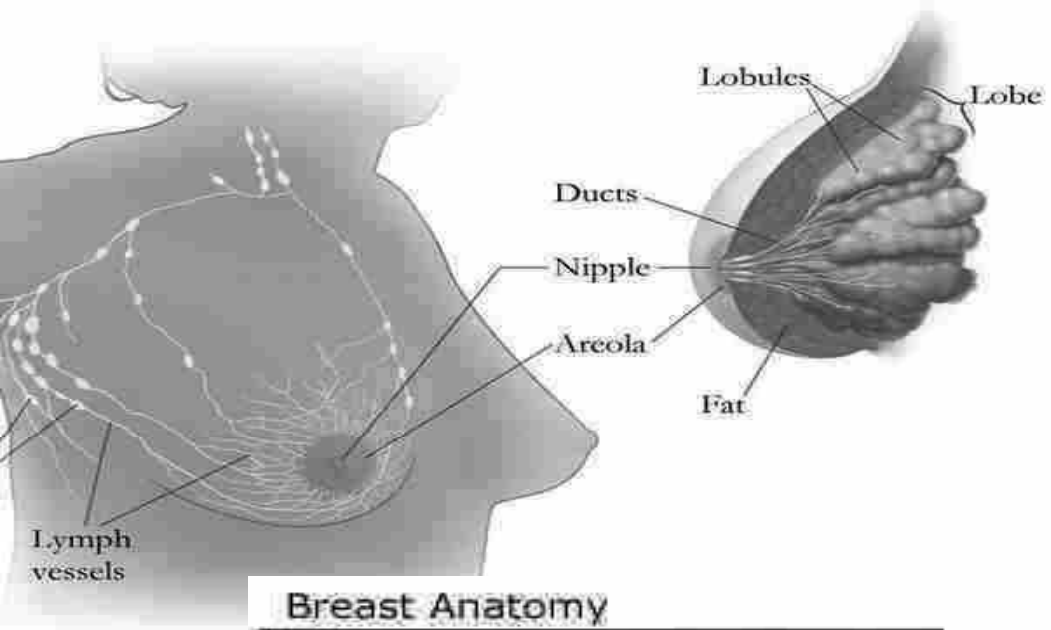
Breast ducts comprise only abou 10% of the breast mass

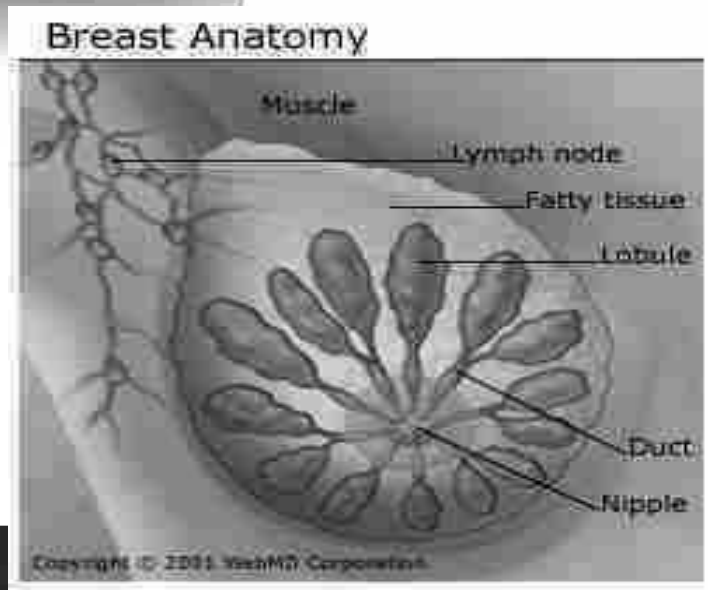
- lobes
- ducts
- lymph nodes

Side View of Breast



- Breast has no muscle tissue
- There are muscles underneath the breast separating them from the ribs



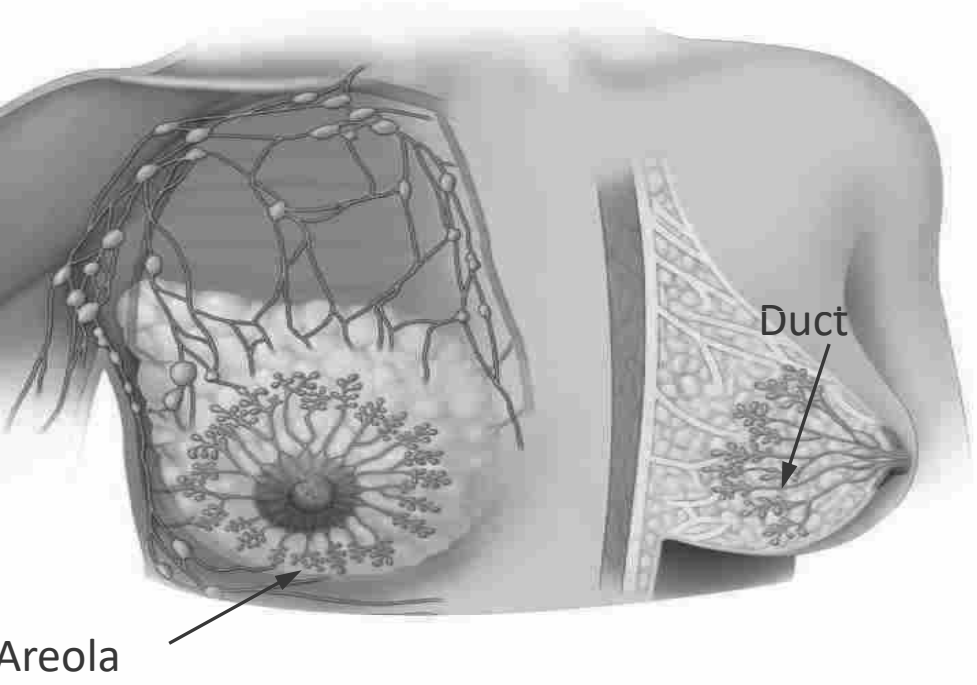


- Each breast has 8 to 10 sections (lobes) arranged like the petals of daisy

- Inside each lobe are many smaller structures called lobules

- At the end of each lobule are tiny sacs (bulbs) that can produce milk

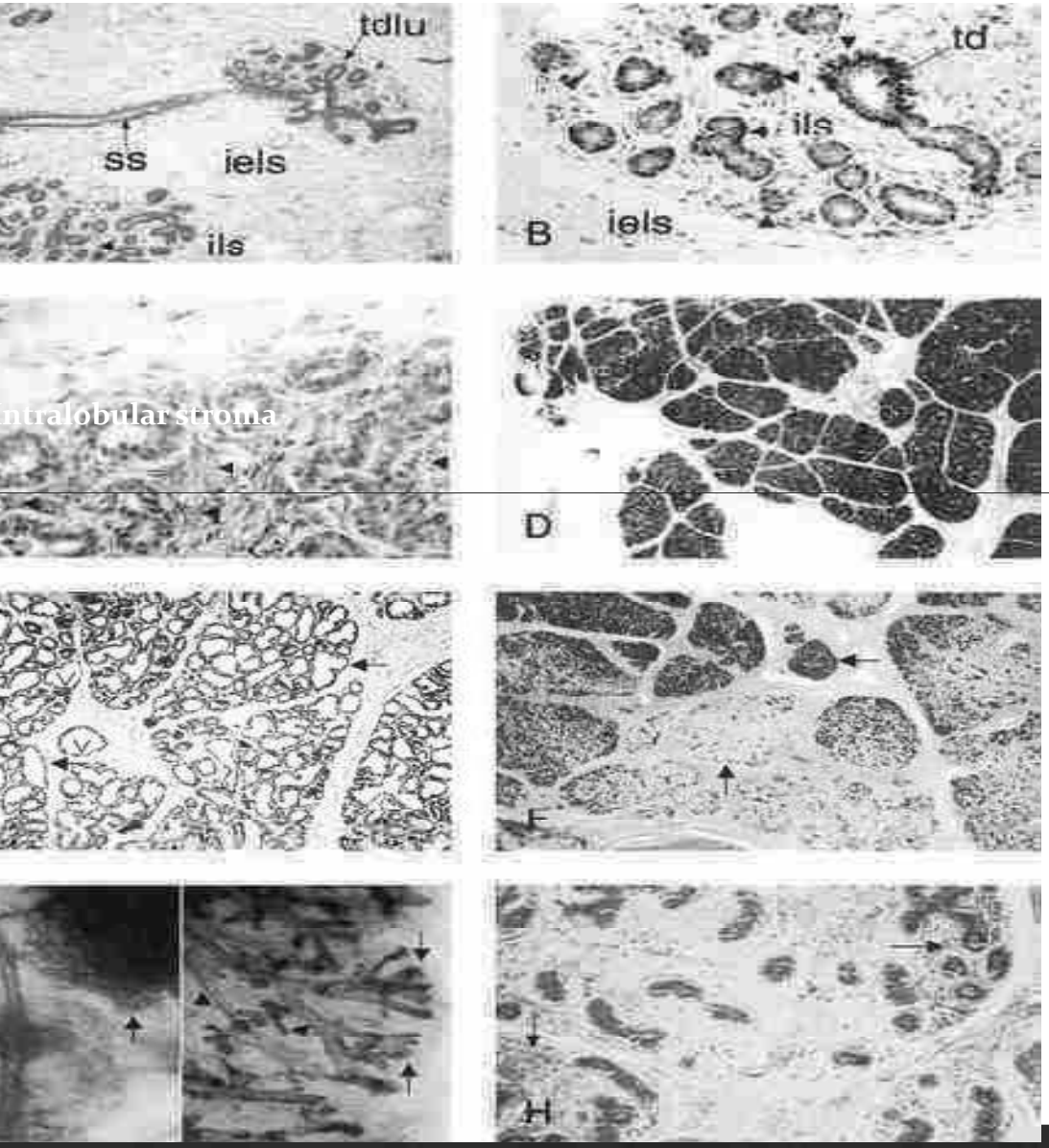
Ducts



- Lobes, Lobules and bulbs, are linked network of thin tubes (ducts)
- Ducts carry milk from bulbs toward area of skin in the center of the breas (areola)

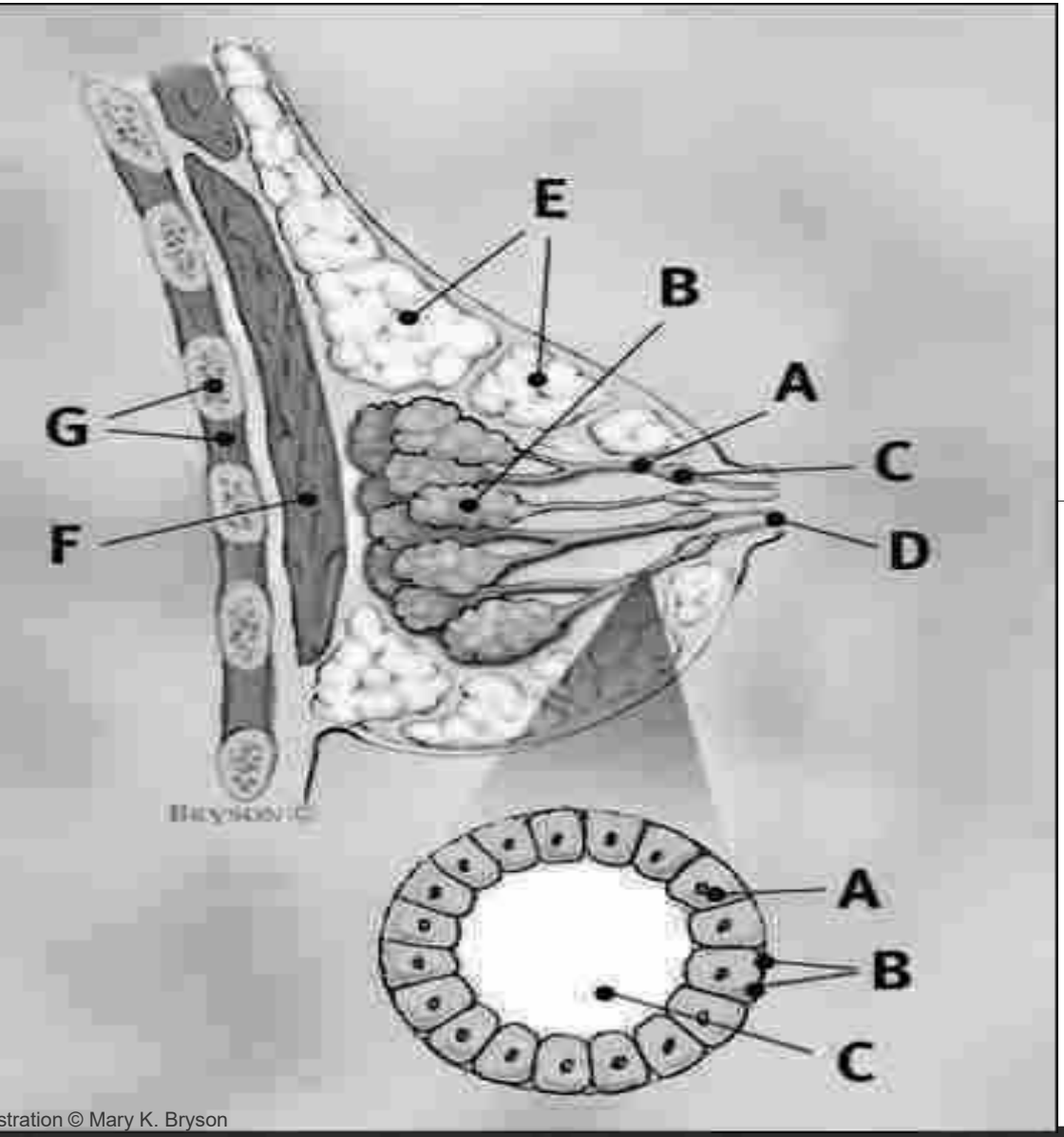
- Ducts join together into larger ducts ending at the nipple, where milk is delivered

Breast development-Adult



- 4A: Premenopausal adult breast section (H and showing a terminal duct (td) entering a TDLU. i intralobular stroma; iels: interepithelial lobular stroma
- 4B: High power of A.
- 4C: Intralobar stroma reactive antibody
- 4D: Increase in number of lobules with loss of fa still separated by intralobular connective tissue
- 4E: Lactating mammary gland composed of dila acini containing milk
- 4F: Following weaning, involution occurs. The t layered epithelium of the resting breast is reform in cycles of pregnancy and lactation
- 4G, H: Virginal hypertrophy

Normal Breast



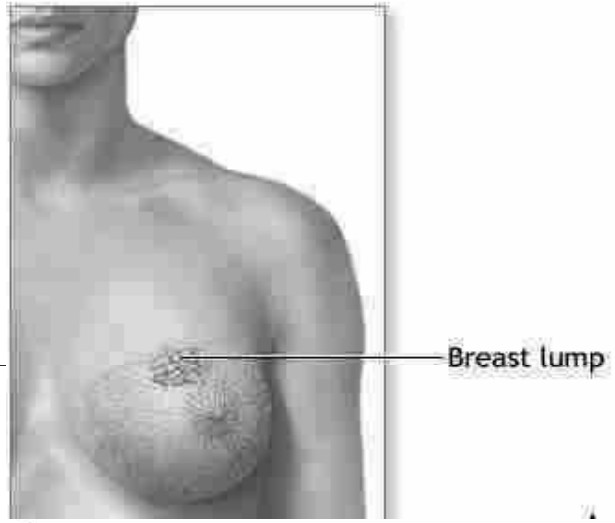
stration © Mary K. Bryson

Breast profile	
A	ducts
B	lobules
C	dilated section of duct to hold milk
D	nipple
E	fat
F	pectoralis major muscle
G	chest wall/rib cage

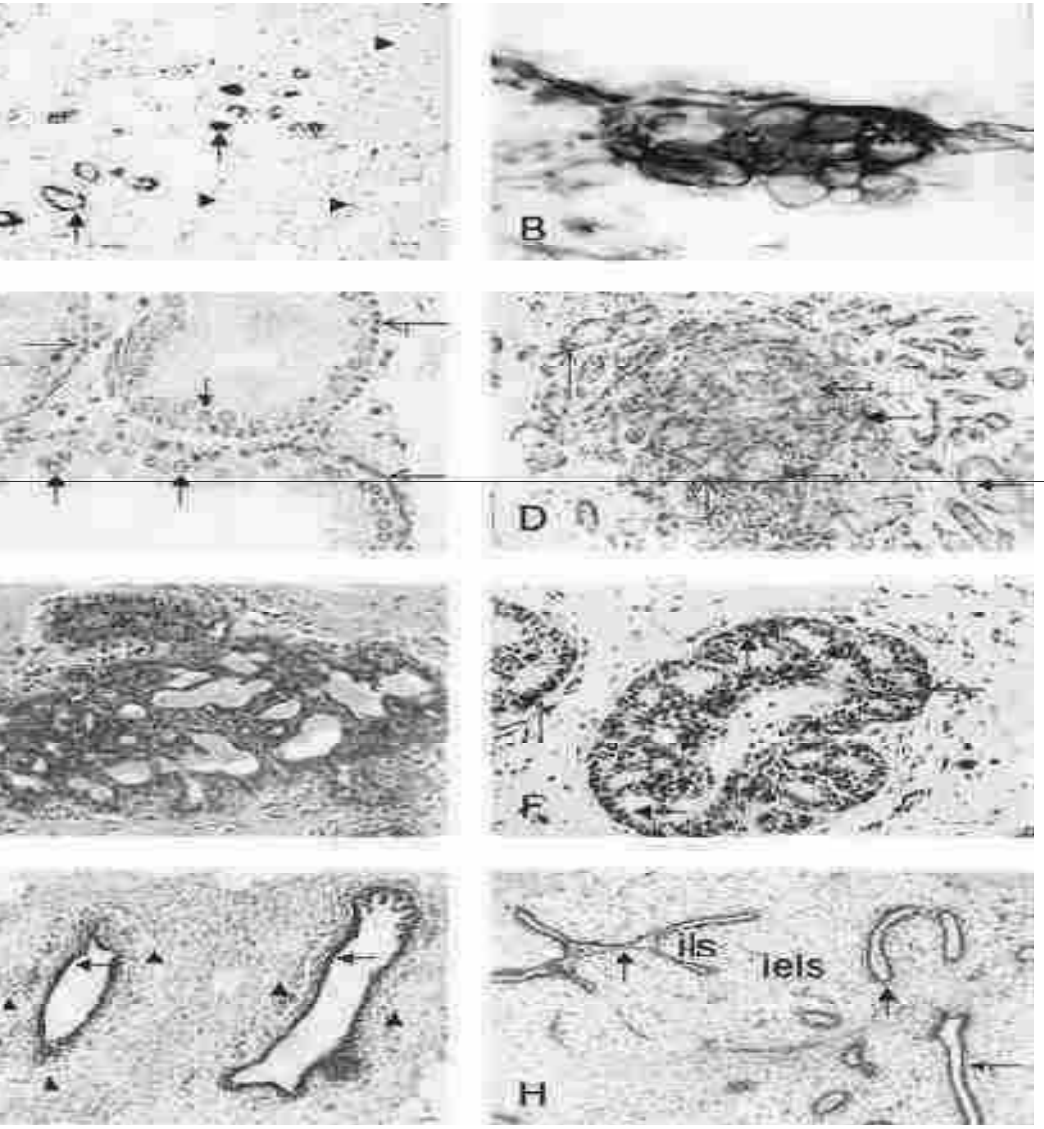
Enlargement	
A	normal duct cells
B	basement membrane (duct wall)
C	lumen (center of duct)

Features Of Benign Breast Disease

- Most common cause of breast problems.
- 30% of women suffer from a benign breast disorder
- The most common symptoms are
  - Pain,
  - Lumpiness or
  - A lump.
- Exclude cancer and to treat any existing symptoms



reast development- Involution, and benign breast conditions



Postmenopausal breast- both lobules and ducts a
reduced in number. Intralobular stroma is replace
with collagen

•5A: Few acini and ducts remain, embedded in thi
strands of collagen, widely dispersed in the fat.
Connective tissue regresses, replaced by fat

Benign breast conditions-

•5B:Cysts containing secretions

•5C: Apocrine metaplasia-lining epithelium takes
features of apocrine glands of the axilla. Granular
cytoplasm, large nuclei, nucleoli.

•5D:Sclerosing adenosis-lobular proliferation with
are infiltrative at the margins

•5F: Epithelial hyperplasia-expansion of lobules

Congenital disorders	Non-breast disorders	ANDI	Pregnancy-related	Misc
Inverted nipple Supernumerary breasts/nipples	• Tietze's disease (costochondritis) • Sebaceous cysts and other skin conditions	• Cyclical nodularity and mastalgia • Cysts • Fibroadenoma	• Galactocele • Puerperal abscess	• Duct ectasia/periductal mastitis • Injury • Inflammation

Congenital abnormalities

- ***Amazia***
  - Congenital absence of the breast .
  - Poland's syndrome.
- ***Polymazia***
  - Accessory breasts
    - The axilla
    - Groin,
    - Buttock and thigh.
  - Function during lactation

Contd…

- ***Mastitis of infants***
- At least as common in boys as in girls.
- On the third or fourth day of life, if the breast of an infant is pressed lightly, a drop of colourless fluid can be expressed.
- A few days later, there is often a slight milky secretion, which disappears during the third week. - ‘Witch’s milk’ .
- Stimulation of the fetal breast by prolactin in response to the drop in maternal oestrogens and is essentially physiological.

Diffuse Hypertrophy

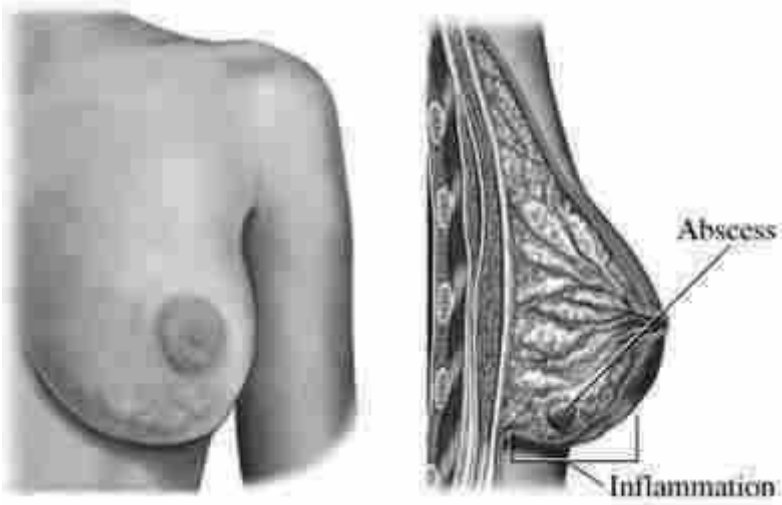
- Puberty (benign virginal hypertrophy) and, the first pregnancy.
- Attain enormous dimensions and may reach the knees when the patient is sitting.
- Rarely unilateral.

- An alteration in the normal sensitivity of the breast to oestrogenic hormones .
- Treatment -reduction mammoplasty.

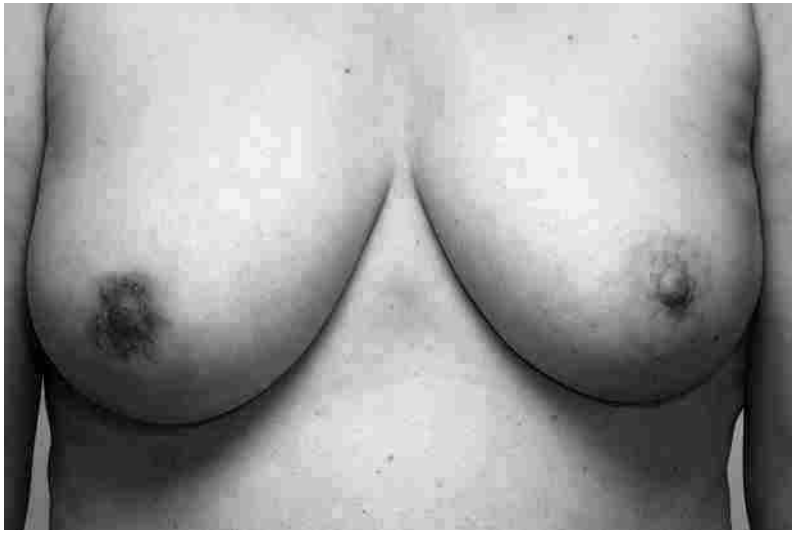
Injuries Of The Breast

- ***Traumatic fat necrosis***
- Acute or chronic
- In stout, middle-aged women.
- Following a blow, or even indirect violence (e.g. Contraction of the pectoralis major)
- A painless lump - mimic a carcinoma, (skin tethering and nipple retraction)
- Biopsy
- A history of trauma is not diagnostic.
- Seat belt injury.

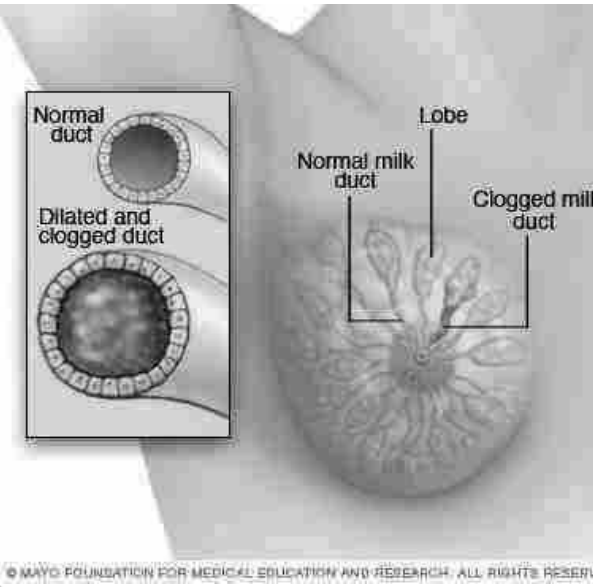
Inflammations Of Breast



- Bacterial mastitis - most common variety.
- Associated with lactation.
- The intermediary – infant harbouring staphylococci in the nasopharynx.
- Ascending infection from a sore and cracked nipple



Contd…



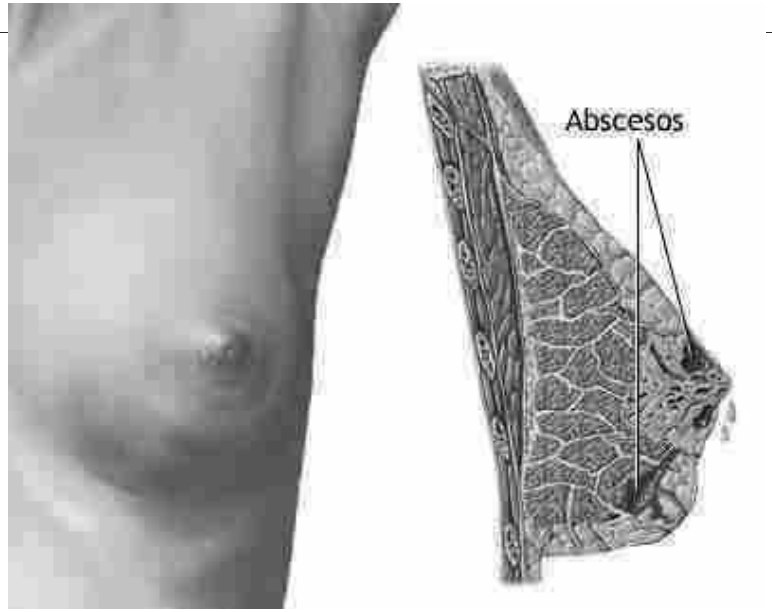
Lactiferous ducts will first become blocked by epithelial debris leading to stasis;

Once within the ampulla of the duct, staphylococci cause clotting of milk and,

Within this clot,organisms multiply.

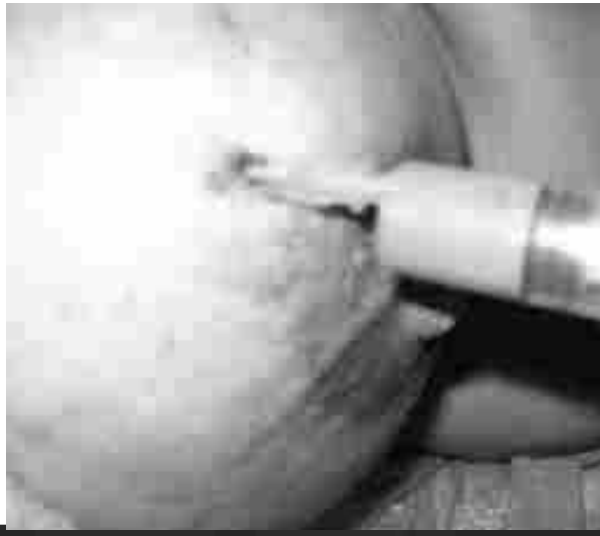
Contd…

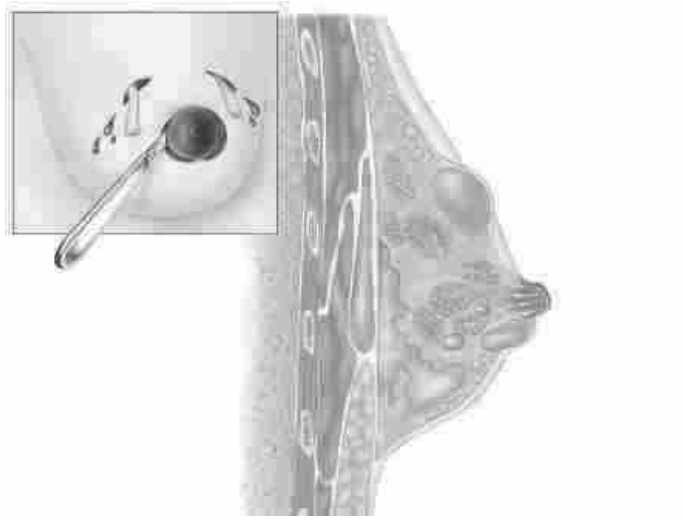
- Classical signs of acute inflammation.
- Initially generalised cellulitis but later an abscess will form.



Managment

- The cellulitic stage - appropriate antibiotic, (flucloxacillin or co-amoxiclav)
- Feeding from the affected side may continue.
- Support of the breast and symptomatic managament.
- ‘Antibioma’ - a large, sterile, brawny oedematous swelling .
- Repeated aspirations under antibiotic cover .
- **Fluctuation is a late sign**





Incision and drainage recommended

If the infection did not resolve within 48 hours or

if after being emptied of milk there was an area of tense induration or

other evidence of an underlying abscess.

Chronic Intramammary Abscess

- Inadequate drainage
- Injudicious antibiotic treatment,
- Encapsulated within a thick wall of fibrous tissue the condition cannot be distinguished from a carcinoma without the histological evidence from a biopsy.

Mondor's Disease

- Thrombophlebitis of the superficial veins of the breast and anterior chest wall & the arm.
- The absence of injury or infection, the cause is obscure.
- Subcutaneous cord, usually attached to the skin.
- The skin over the breast is stretched by raising the arm,
  - A narrow, shallow subcutaneous groove alongside the cord becomes apparent.

Contd…

- Differential diagnosis
  - Lymphatic permeation from an occult carcinoma of the breast.
- Rx - restricted arm movements
- Spontaneous regression a few months without recurrence, complications or deformity.
- Development of malignancy is co incidental.

Ductal Ectasia / Periductal Mastitis

- Dilatation of the breast ducts, which is often associated with periductal inflammation.
- Etiopathogenesis is obscure , the disease is much more common in smokers.
- Arteriopathy is a contributing factor in its aetiology.
- Periductal inflammation is the primary condition

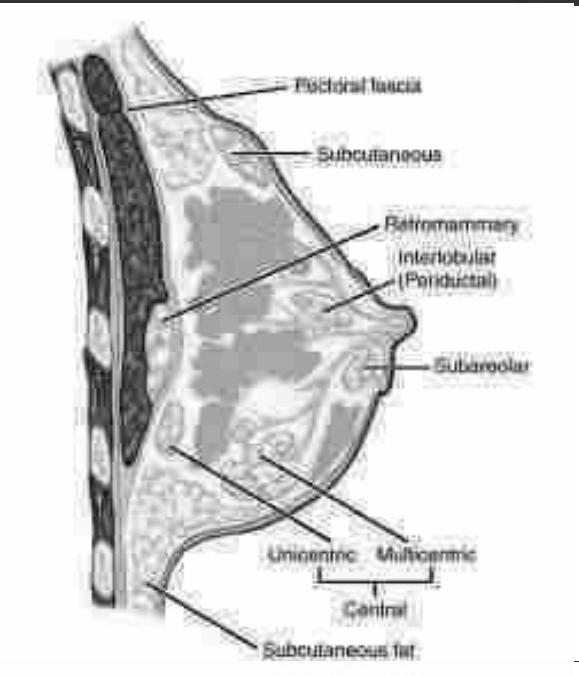
The first stage in the disorder is a dilatation in one or more of the larger lactiferous ducts,

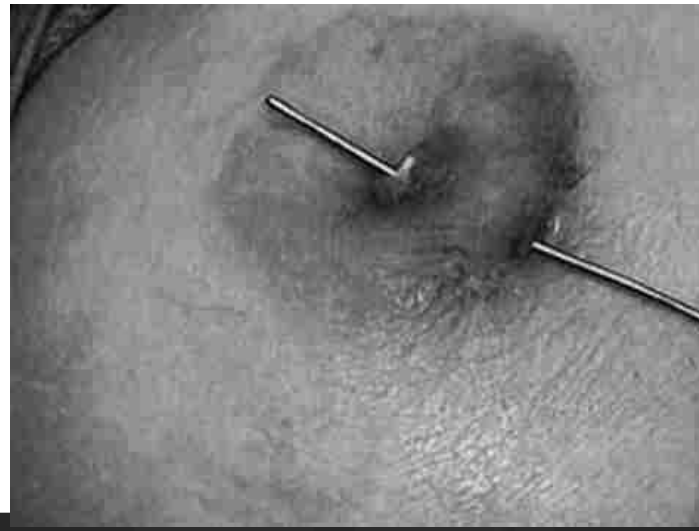
Which fill with a stagnant brown or green secretion and results in discharge.

Irritant reaction in surrounding tissue leading to periductal mastitis or even abscess and fistula formation

Fibrosis eventually develops-slit-like nipple retraction.

- Clinical features
  - Nipple discharge (of any colour),
  - A subareolar mass or abscess,
  - Mammary duct fistula and/or nipple retraction





Management

- In the case of a mass or nipple retraction,
  - A carcinoma must be excluded by obtaining a mammogram and negative histology.
  - If any suspicion remains, the mass should be excised.

- Antibiotic therapy may be tried,
  - Co-amoxiclav or flucloxacillin and metronidazole.
- Surgery - cure of this notoriously difficult condition;
- This consists of excision of all of the major ducts (**Hadfield's Operation**).
- Important to shave the back of the nipple to ensure that all terminal ducts are removed. .

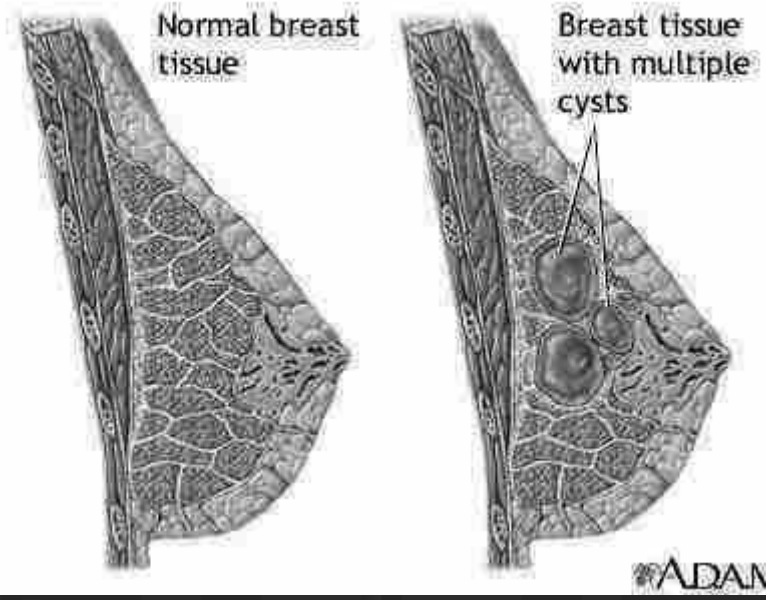
Microdochectomy

Fibroadenosis

Fibrocystic disease

Chronic mastitis and mastopathy

Aberrations Of Normal Development And Involution



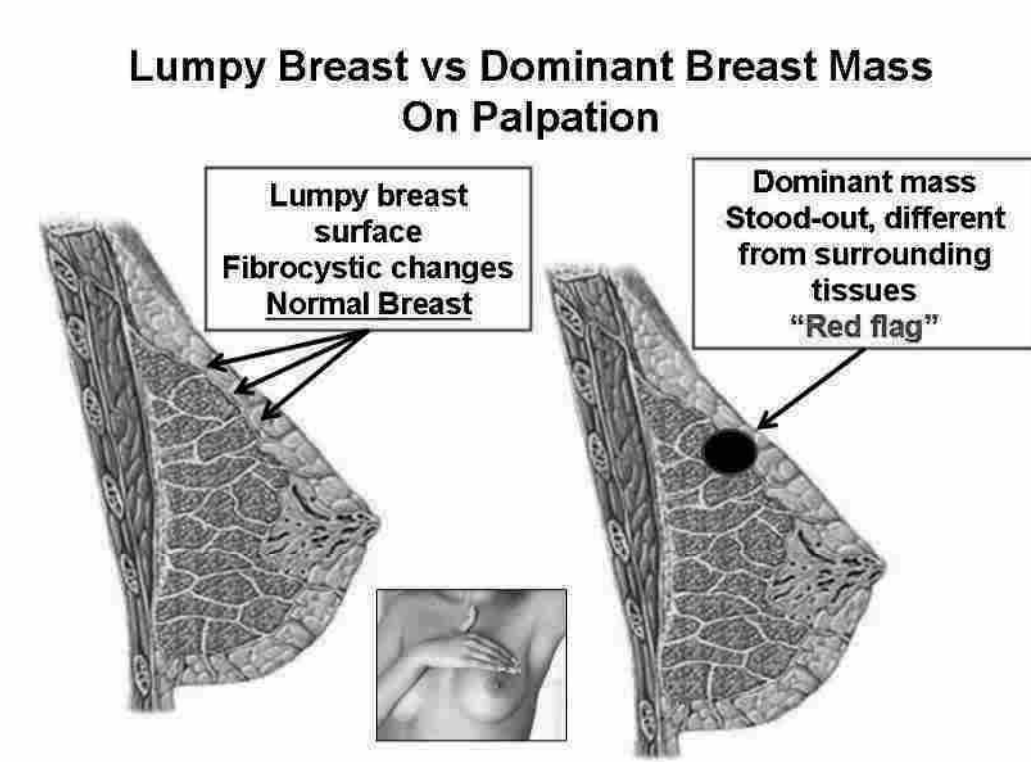
Ateiology

- Disturbances in the breast physiology extending from a perturbation of normality to well-defined disease processes.
- There is often little correlation between the histological appearance of the breast tissue and the symptoms

Cyst formation.	• Cysts are almost inevitable and very variable in size.
Fibrosis.	• Fat and elastic tissues disappear and are replaced with dense white fibrous trabeculae. • The interstitial tissue is infiltrated with chronic inflammatory cells.
Hyperplasia	• Epithelium in the lining of the ducts and acini may occur, with or without atypia.
Papillomatosis.	• The epithelial hyperplasia may be so extensive that it results in papillomatous overgrowth within the ducts.

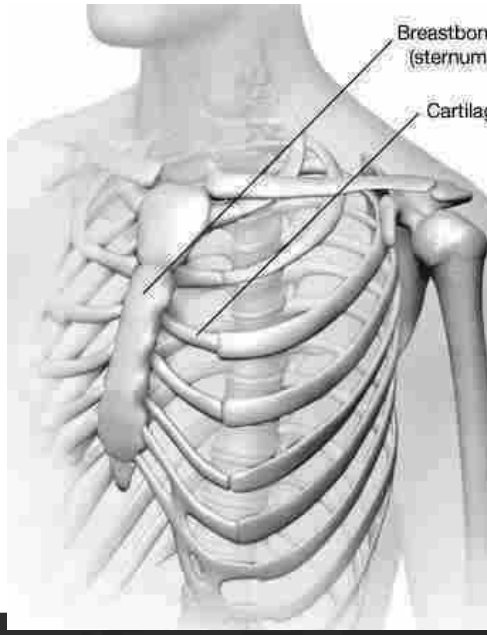
Clinical Features

- A benign discrete lump in the breast
  - a cyst or
  - fibroadenoma.



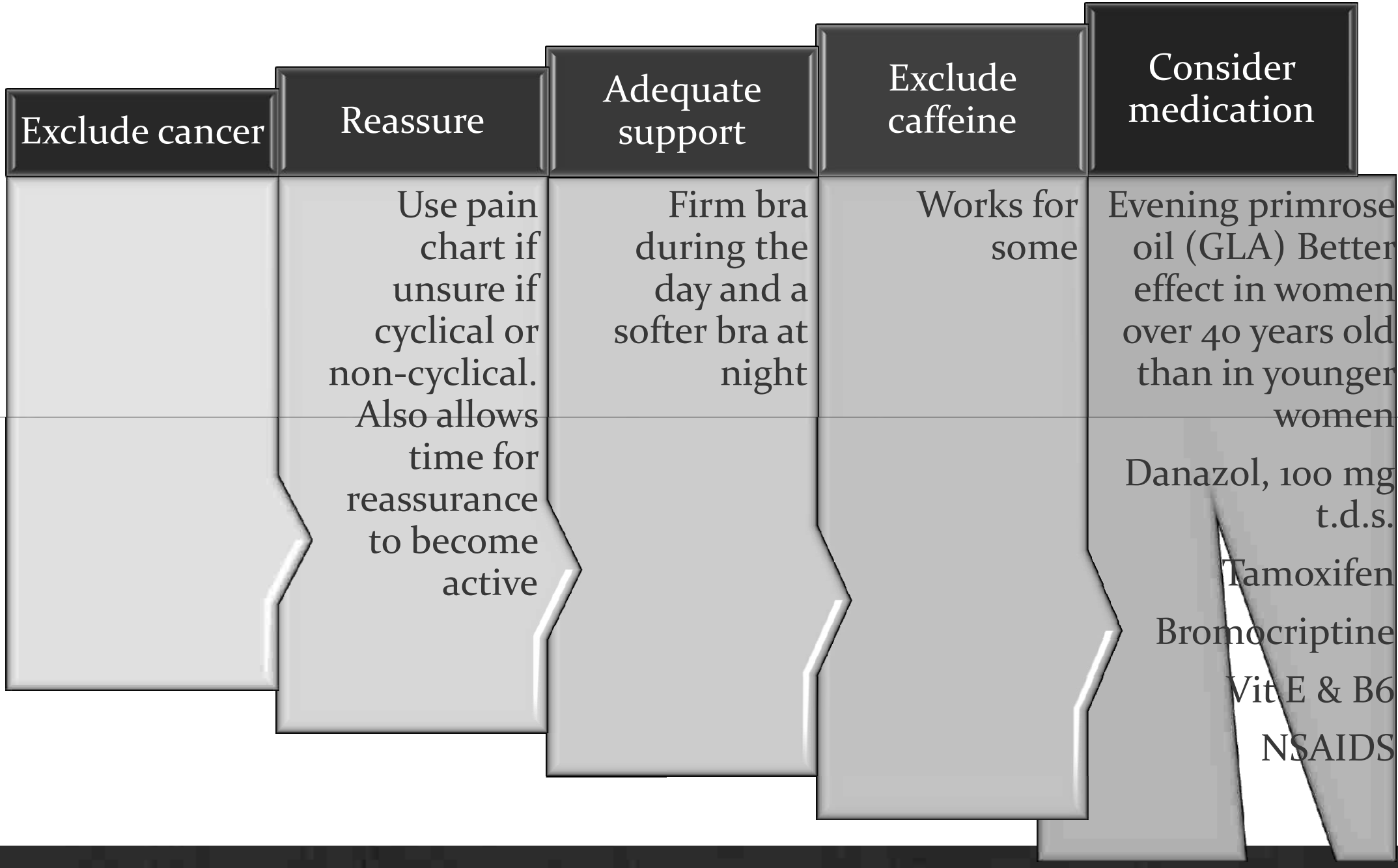
- Lumpiness
  - may be bilateral,
  - commonly in the upper outer quadrant
  - The changes may be cyclical, with an increase in both lumpiness and often tenderness before a menstrual period.

- Non-cyclical mastalgia
  - It may be associated with ANDI or with periductal mastitis.
  - It should be distinguished from referred pain, for example a musculoskeletal disorder.
  - About 5% of breast cancers exhibit pain at presentation.



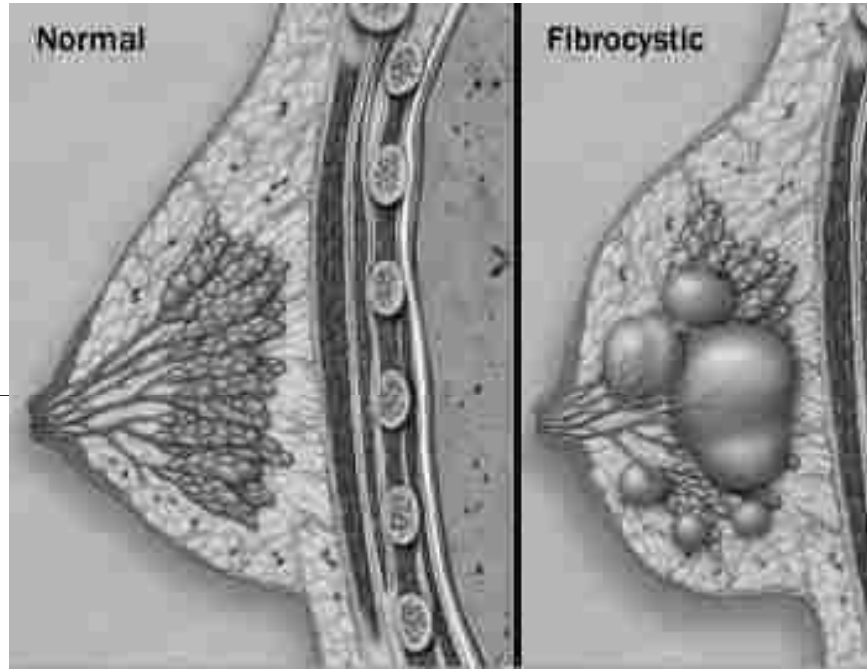
Management

- Treatment of lumpy breast
  - Reassurance.
  - Reviewing the patient at a different point in the menstrual cycle,
  - Pt will be unnecessarily anxious and to be submitted to multiple random biopsies because the clinician lacks the courage of his or her convictions.


Exclude cancer
Reassure
Use pain chart if unsure if cyclical or non-cyclical. Also allows time for reassurance to become active
Adequate support
Firm bra during the day and a softer bra at night
Exclude caffeine
Works for some
Consider medication
Evening primrose oil (GLA) Better effect in women over 40 years old than in younger women
Danazol, 100 mg t.d.s.
Tamoxifen
Bromocriptine
Vit E & B6
NSAIDS

Breast Cysts

- Last decade of reproductive life
- As a result of a non-integrated involution of stroma and epithelium.
- Multiple, may be bilateral and can mimic malignancy.
- Diagnosis can be confirmed by aspiration and/or ultrasound.



Treatment

A solitary cyst or small collection of cysts can be aspirated.

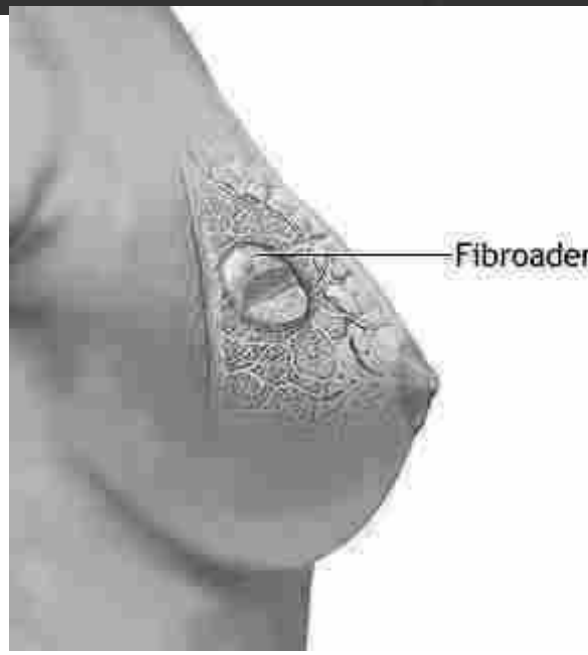
If they resolve completely, and if the fluid is not blood-stained, no further treatment is required.

If recur and require reaspiration.

If there is a residual lump or if the fluid is blood-stained, f the cyst reforms repeatedly

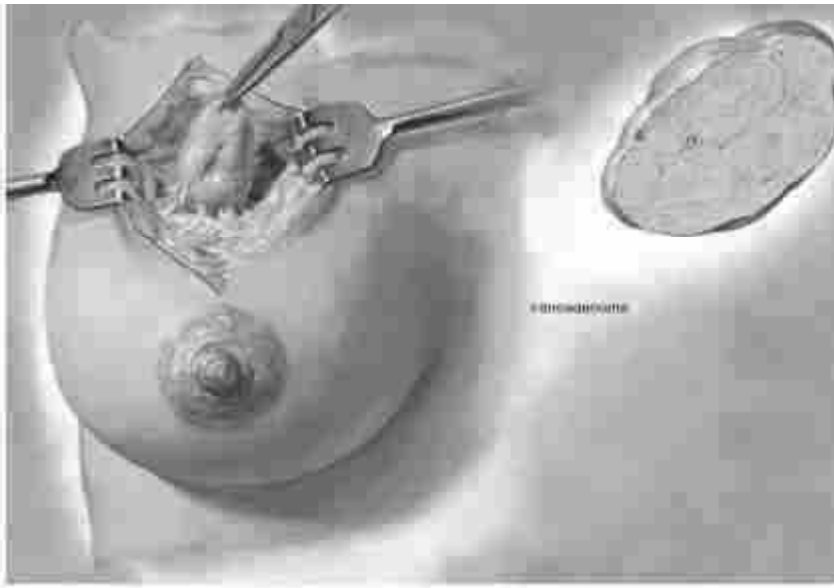
a core biops or local excision for histological diagnosis is advisable.

Fibroadenoma



- Fully developed breast
- Between the ages of 15 and 25 years
- They arise from hyperplasia of a single lobule and usually grow up to 2–3 cm in size.
- Wellmarked capsule
- Giant fibroadenomas - over 5 cm in diameter and are often rapidly growing

- A fibroadenoma does not require excision unless
  - Associated with suspicious cytology,
  - It becomes very large or
  - The patient expressly desires the lump to be removed.



Phylloides Tumor

- Serocystic disease of Brodie or cystosarcoma phylloides,
- Women over the age of 40 years
- Clinical features
  - Large, sometimes massive, tumour with an unevenly bosselated surface.
  - Ulceration of overlying skin occurs because of pressure necrosis.
  - Mobile on the chest wall.

- Histology
  - Low malignant potential resembling a fibroadenoma
  - Higher mitotic index,
  - They are rarely cystic and only very rarely develop features of a sarcomatous tumour.
  - These may metastasise via the bloodstream.
- **Treatment**
- Benign type is enucleation in young women or wide local excision.
- Massive tumours, recurrent tumours and those of the malignant type will require mastectomy

Cystic		Solid		
Lump disappears; clear fluid (many colours)	Residual thickening; blood-stained fluid	Benign	Atypical	Malignant
Discharge patient	Investigate – ?core biopsy	Offer excision or observe	Investigate – ?core biopsy	Treat for cancer

Aberrations Of Normal Development And Involution

ANDI CLASSIFICATION ENCOMPASSES ALL ASPECT OF THE BREAST CONDITION, INCLUDING PATHOGENESIS AND THE DEGREE OF ABNORMALITY.

Early Reproductive Years

Fibroadenomas in younger women aged 15 0 25
Nipple inversion is a disorder of development of the major ducts, which prevents normal protrusion of the nipple.
Mammary ducts fistulas arise when nipple inversion predisposes to major duct obstruction, leading to recurrent sub areolar abscess and mammary duct fistula.

Late Reproductive Years

Cyclical mastalgia and nodularity usually are associated with premenstrual enlargement of the breast and are regarded as normal.
In epithelial hyperplasia of pregnancy, papillary projections sometimes give rise to bilateral bloody nipple discharge.

Involution

Macrocytes are common, are often subclinical, and do not require specific treatment.
Sclerosing Adenosis is considered as a disorder of both the proliferative and the involutional phase of the breast cycle.
Duct ectasia (dilated ducts) at periductal mastitis are other important component.
Sixty percent of women 70 yearsof age exhibit some degree of epithelial hyperplasia.
Atypical proliferative disease includesductal and lobular hyperplasia, both of which display some features of carcinoma in situ.
Women with atypical ductal or lobular hyperplasia have a fourfold increase in breast cancer risk.

ANDI Classification Of Benign Breast Disorder			
	Normal	**Disorder**	**Disease**
Early Reproductive years (age 15-25 years)	Lobular development Stromal development Nipple eversion	**Fibroadenoma** Adolescent hypertrophy	Giant fibroadenoma Gigantomastia Subareolar abscess Mammary duct fistula

Later reproductive years (age 25-40 years)	Cyclical changes of menstruation. Epithelial hyperplasia of pregnancy.	**Cyclical mastalgia**[Q] Nodularity Bloody nipple discharge	**Incapacitating mastalgia**[Q]
Involution (age 35-55 years)	Lobular involution Duct involution Dilatation	Macrocysts Sclerosing lesions Duct ectasias	Periductal mastitis **Epithelial hyperplasia with atypia**[Q]
	Sclerosis Epithelial turnover	Nipple retraction **Epithelial hyperplasia**	

Thank You