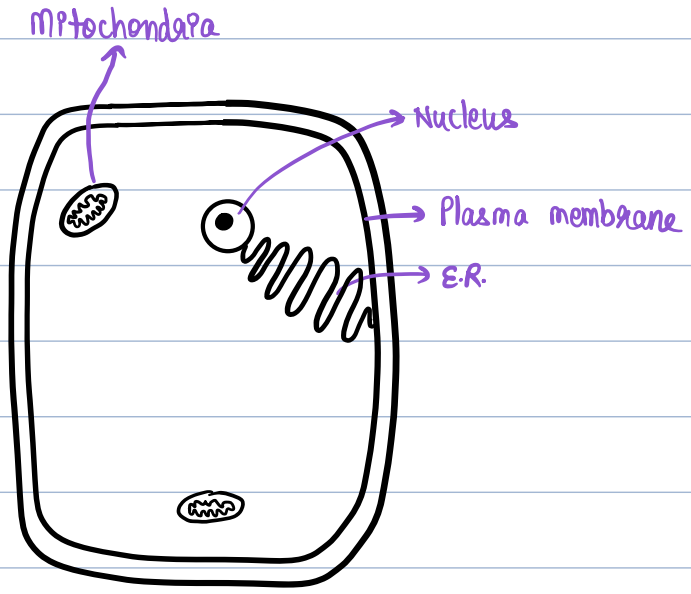
Cell Adaptations :



Causes of Cell Injury :

i) **Hypoxia** : decreased O_2 supply to a tissue
   ↳ most common cause of cell injury

→ most common cause of hypoxia ⇒ Ischaemia → decreased blood supply
→ cells most sensitive to hypoxia ⇒ neurons
→ cells least sensitive to hypoxia ⇒ fibroblast / skeletal muscle

ii) **Physical Agents** : → Radiation, mechanical stress, etc.
iii) **Chemical Agents** : → Carcinogens, CCl_4, etc.
iv) **Infectious Agents** : → bacteria, viruses, parasites, etc.
v) **Genetic Abnormalities**
vi) **Immunologic abnormalities**
vii) **Nutritional Imbalance**

- Anorexia nervosa (a psychological disorder of inadequate food consumption)
- food shortage
- poor diet

Cell Injury

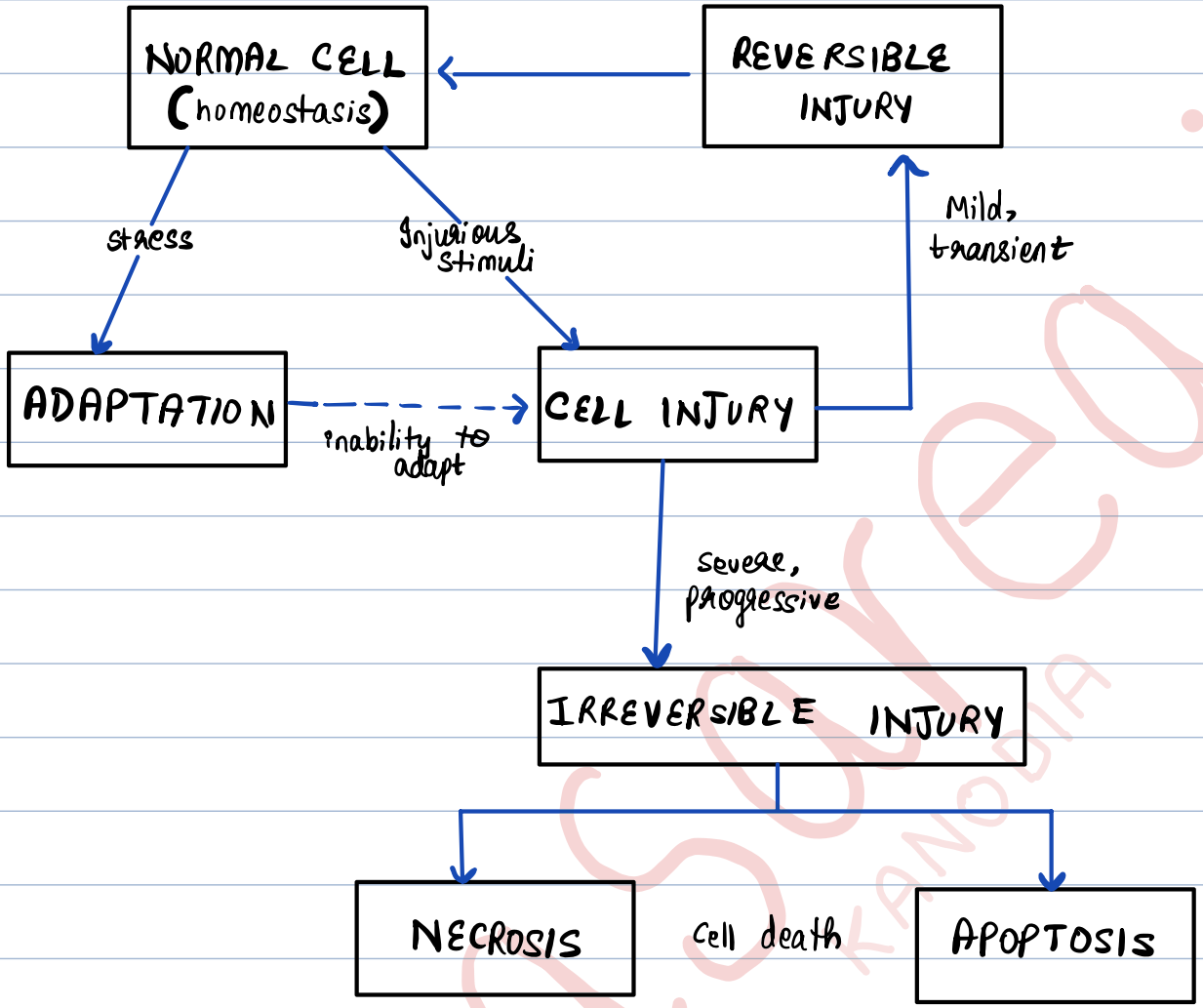
- **Adaptation**
  - Hypertrophy
  - Hyperplasia
  - Metaplasia
  - Atrophy
  - Dysplasia
- **Reversible cell injury**
- **Irreversible cell injury** → **Cell death**
  - Necrosis
  - Apoptosis
  - Pyroptosis
  - Necroptosis
  - Ferroptosis
- **Pathologic calcification**
- **Intracellular accumulation**

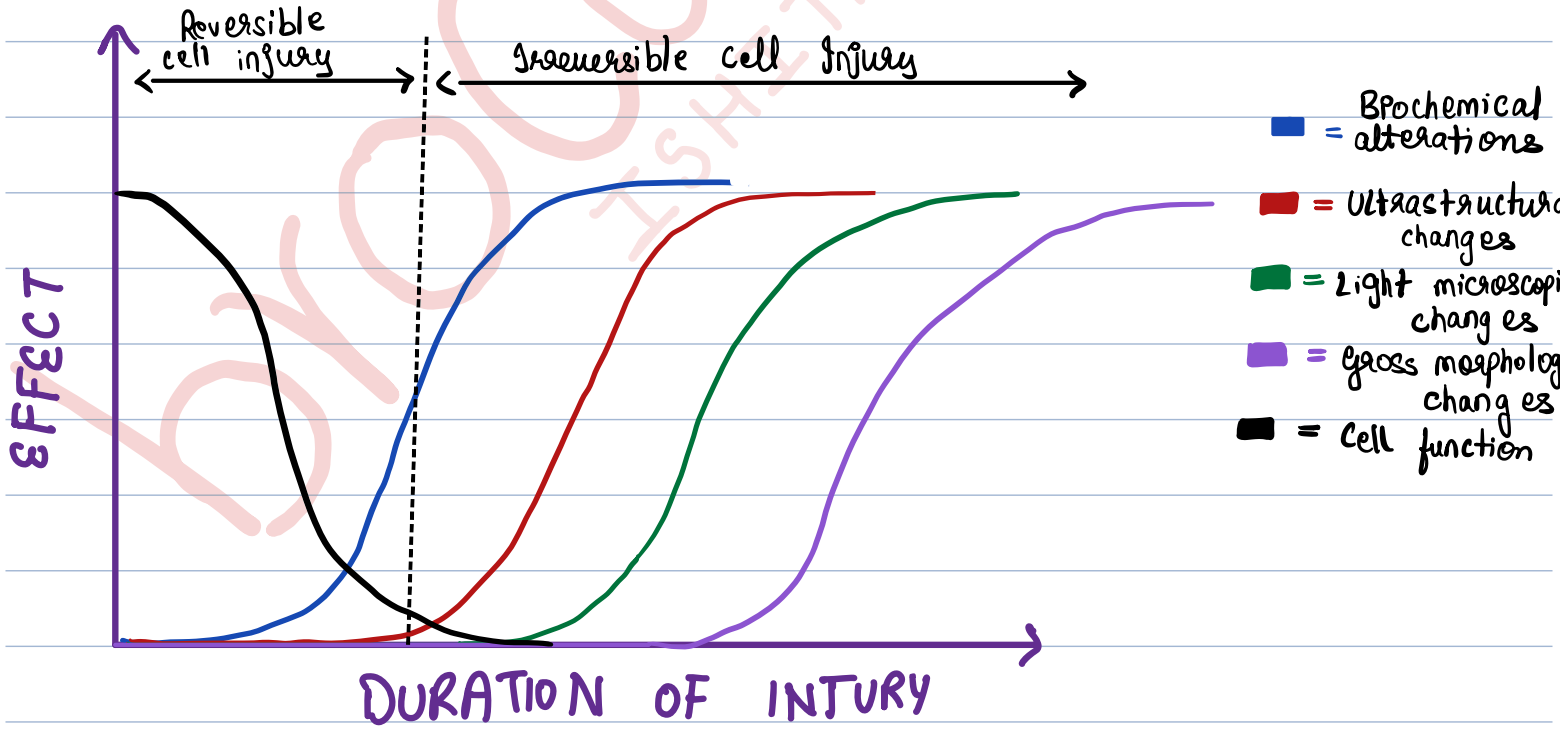
4 aspects of a disease process:

- **Etiology (causation)**
- **Pathogenesis**: biochemical & molecular mechanism
- **Morphologic changes**
- **Clinical manifestations**: functional alterations in cells & organs, & the resulting clinical consequences

Rudolf Virchow: Father of modern pathology
→ gave the **Cellular Basis of Disease Concept**
↓
virtually all diseases start with molecular or structural alterations in cells

Stages of Cellular Response to Noxious Stimuli:





(definition, mechanism, examples)

ISHITA KANODIA

Hypertrophy:

→ increased cell size but no increase in number of cells

↓ ∴

increased size of organ

Mechanism: increased synthesis of cellular proteins

GATA 4
NFAT
MEF 2
} 3 proteins usually involved in hypertrophy

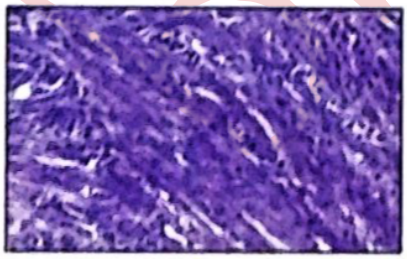
→ cells that cannot divide adapt by hypertrophy

→ hypertrophy occurs in Permanent (non-dividing) cells – cardiac cells
– skeletal cells
& dividing cells.

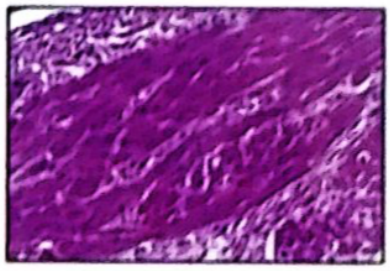
Examples:

Physiologic Hypertrophy

i. Uterus during pregnancy
ii. Breast during lactation
iii. Skeletal muscle in body builders



Normal smooth muscles cells of uterus



Hypertrophied smooth muscle cells

Pathologic Hypertrophy

i. Left ventricular hypertrophy (in hypertension).
ii. In case of bladder outlet obstruction, due to stone, area proximal to stone undergoes hypertrophy.

Hyperplasia: response to certain viral infections (like papilloma-viruses) which cause skin warts & several mucosal lesions composed of masses of hyperplastic epithelium.

→ Increase in the number of cells

⇓

Increase in organ size

Mechanism: growth factor induced proliferation of mature cells.

→ occurs in dividing cells

Examples:

Physiologic Hyperplasia

HORMONAL

i. Breast during puberty

ii. Breast during pregnancy

COMPENSATORY

i. Liver after partial hepatectomy

Pathologic Hyperplasia

→ Usually induced by imbalance of hormones

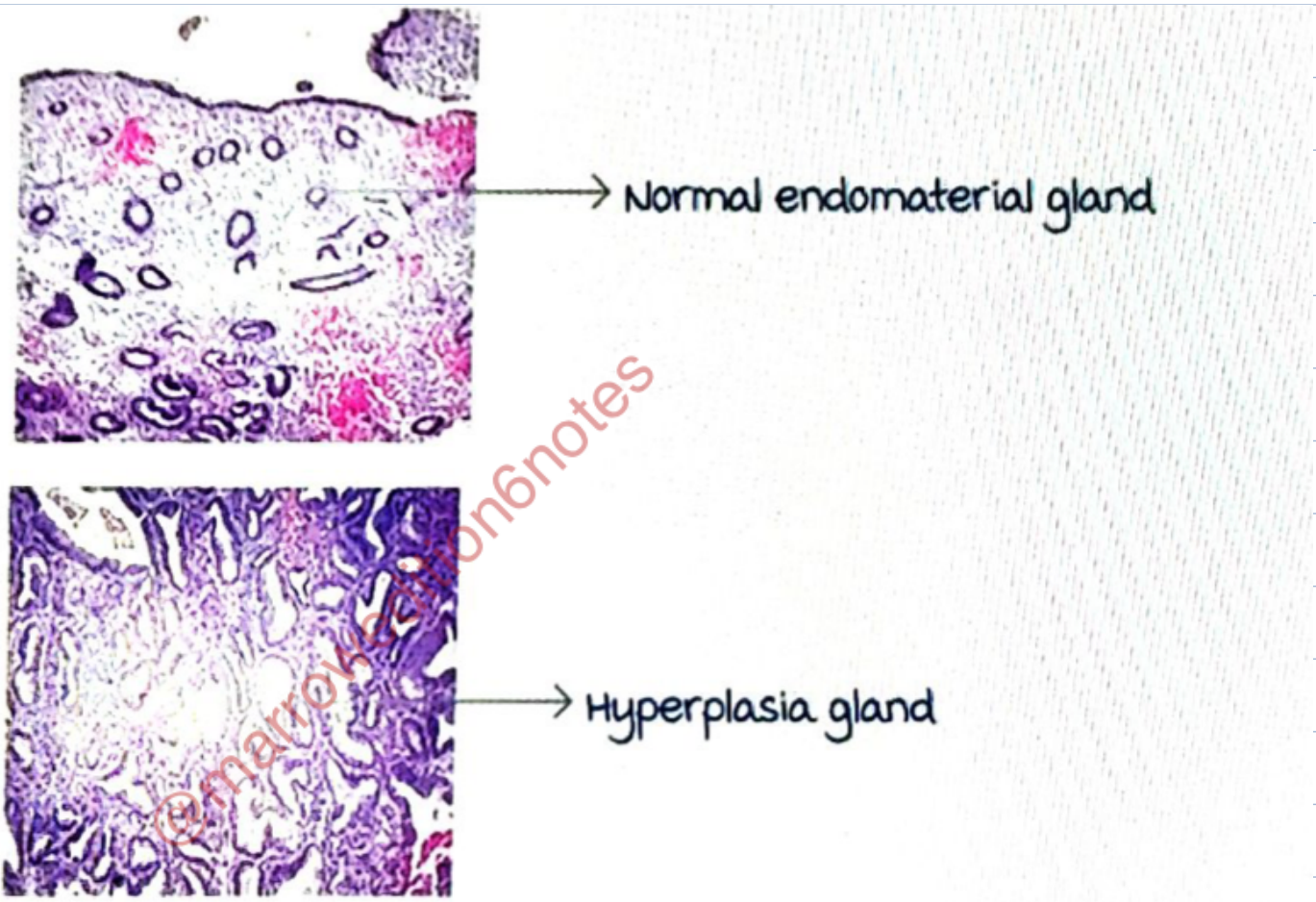
Androgen Excess ⇓ Benign Prostatic Hyperplasia

Oestrogen excess ⇓ Endometrial hyperplasia

- Hyperplastic proliferations are a fertile soil in which cancers can develop.

Example of both hypertrophy & hyperplasia

- Uterus during pregnancy [hypertrophy > hyperplasia]
- Breast during puberty / pregnancy



Atrophy:

→ decreased cell size
→ decreased cell number
} ⇒ decreased organ size

Mechanism: i. decreased protein synthesis
ii. increased protein degradation
iii. autophagy

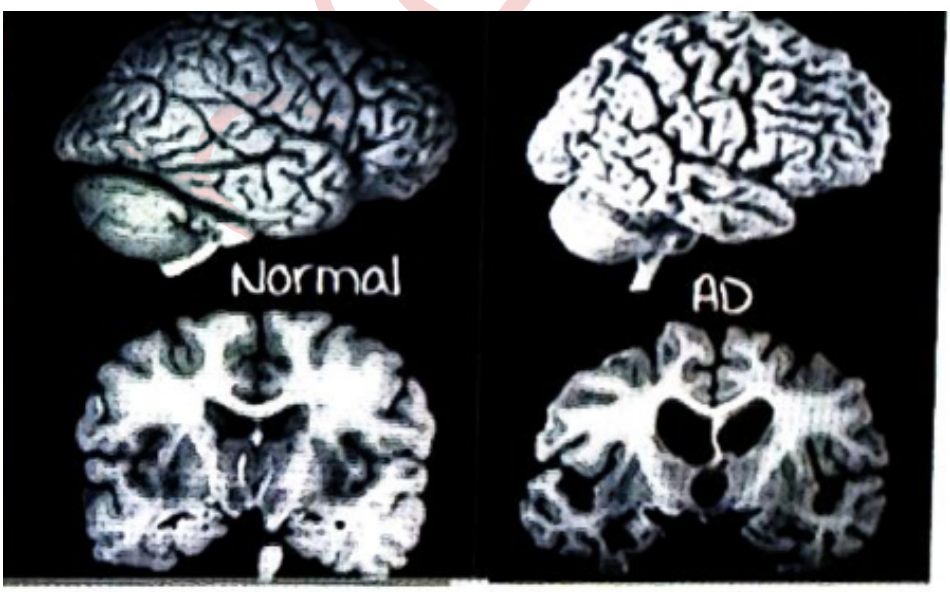
Examples:

Physiologic Atrophy

i. disappearance of notochord, thyroglossal duct at puberty
ii. involution of uterus after parturition
iii. Thymus

Pathologic Atrophy

i. Senile atrophy (ageing)
ii. Ischaemic atrophy
iii. Denervation atrophy
iv. Pressure atrophy
v. Disuse atrophy
vi. Nutritional atrophy



Normal brain Atrophied brain

→ In many situations, atrophy is also accompanied by increased autophagy which is marked by appearance of increased numbers of autophagic vacuoles

→ Some of the cell debris within autophagic vacuoles may resist digestion & persist in cytoplasm as membrane-bound residual bodies (eg: lipofuscin granules)

→ When these residual bodies are present in sufficient amount, they impart a brown discolouration to the tissue ⇒ Brown atrophy.

Metaplasia:

→ **Reversible** change in which one differentiated cell type is converted into another

epithelium ⟶ another epithelium

mesenchyme ⟶ another mesenchyme

Mechanism:

→ usually occurs due to re-programming of stem cells.

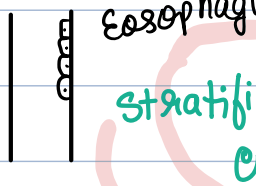
Examples:

i. Respiratory Tract:

- Pseudostratified ciliated glandular columnar epithelium —(SMOKERS / Squamous metaplasia)→ Stratified squamous epithelium

ii. Barret's Oesophagus:

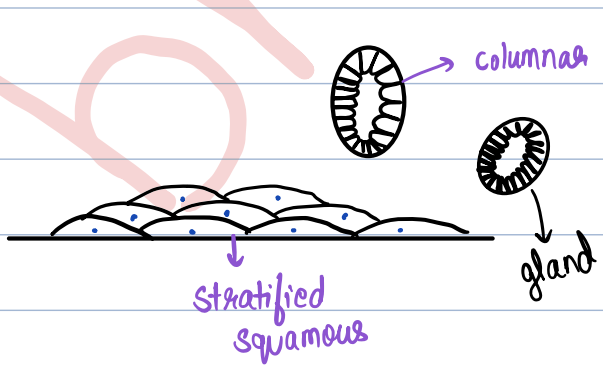
Columnar metaplasia



Stratified squamous epithelium —(GERD / any other irritating factor)→ Columnar epithelium

⇓

Barret's oesophagus / Columnar lined oesophagus.



⇒ if seen in biopsy

⇓

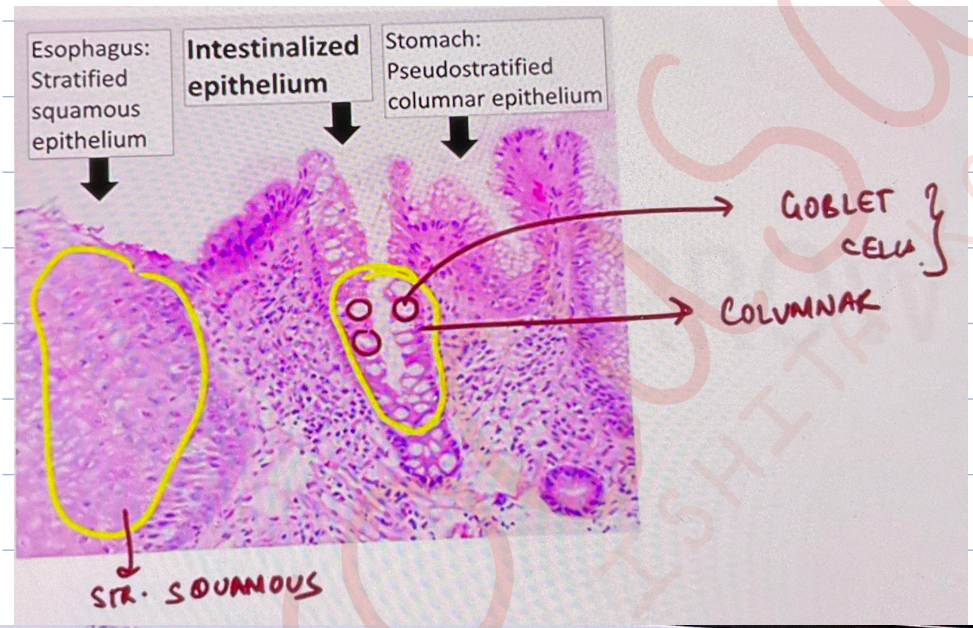
Metaplasia

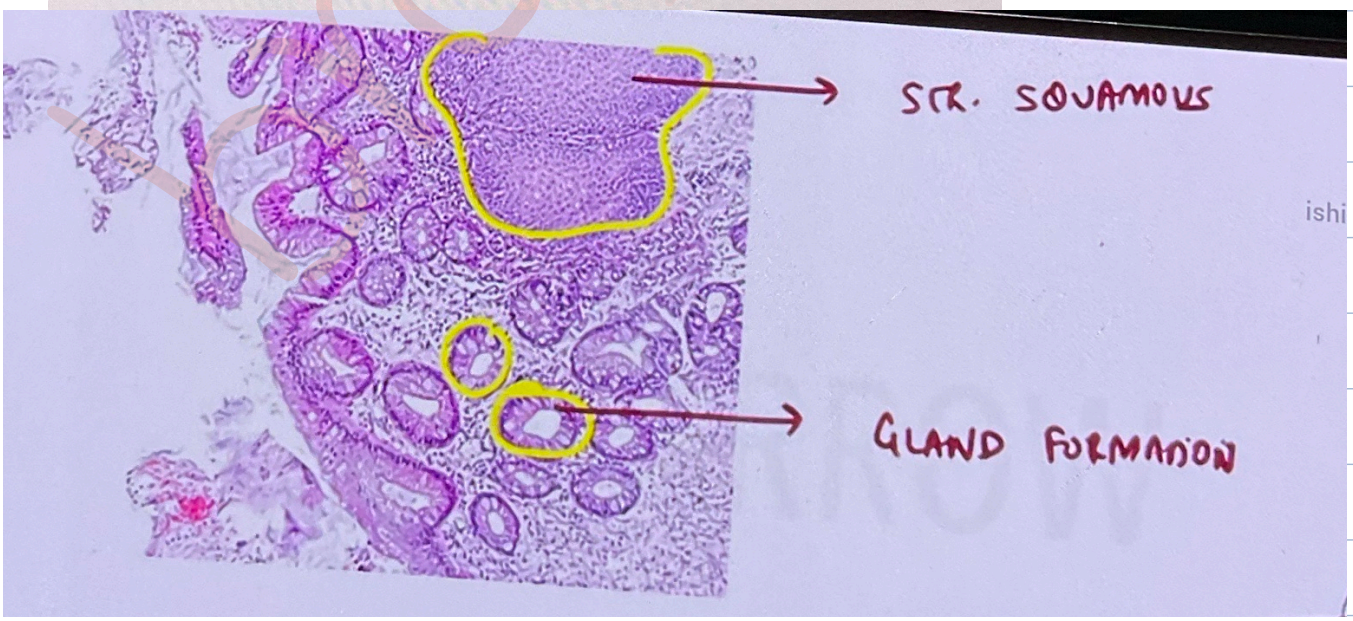
Microscopic Hallmarks of Barrett's Oesophagus:

i. Intestinal metaplasia (∵ intestine has columnar epithelium in their glands)
ii. Presence of goblet cells.

- Goblet cells (contain a lot of mucin) are absent in normal oesophagus
- Special Stain of Barrett's Oesophagus = Alcian blue
  (∵ mucin is Alcian blue +ve)

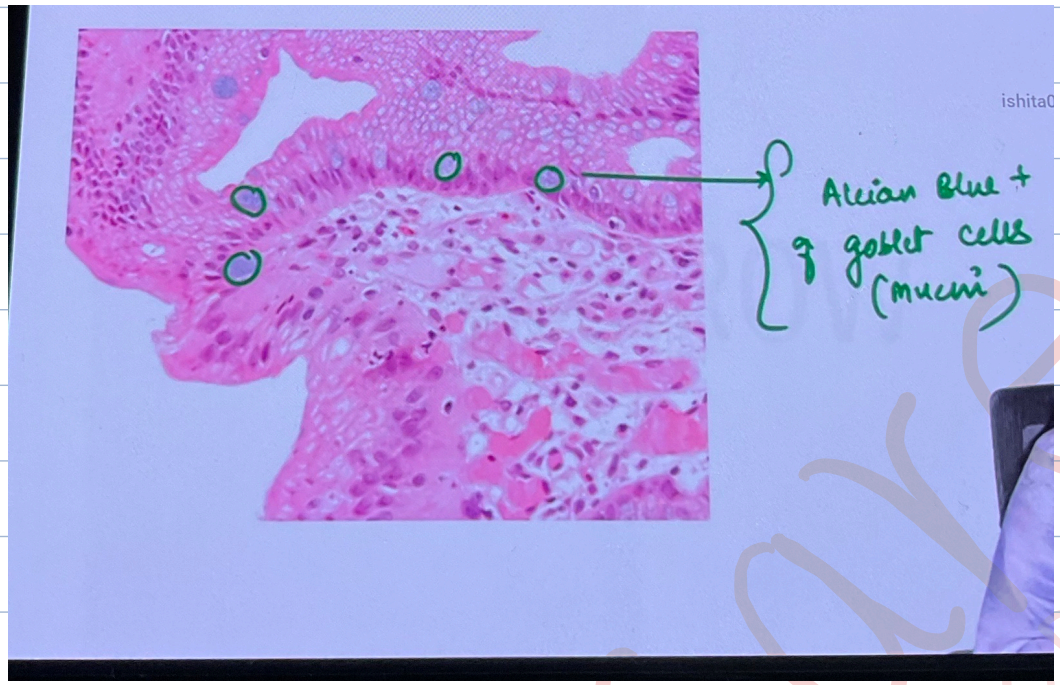
Barrett's Oesophagus: risk factor for ADENOCARCINOMA





→ vit. A deficiency can lead to metaplasia

→ Connective tissue metaplasia : myositis ossificans



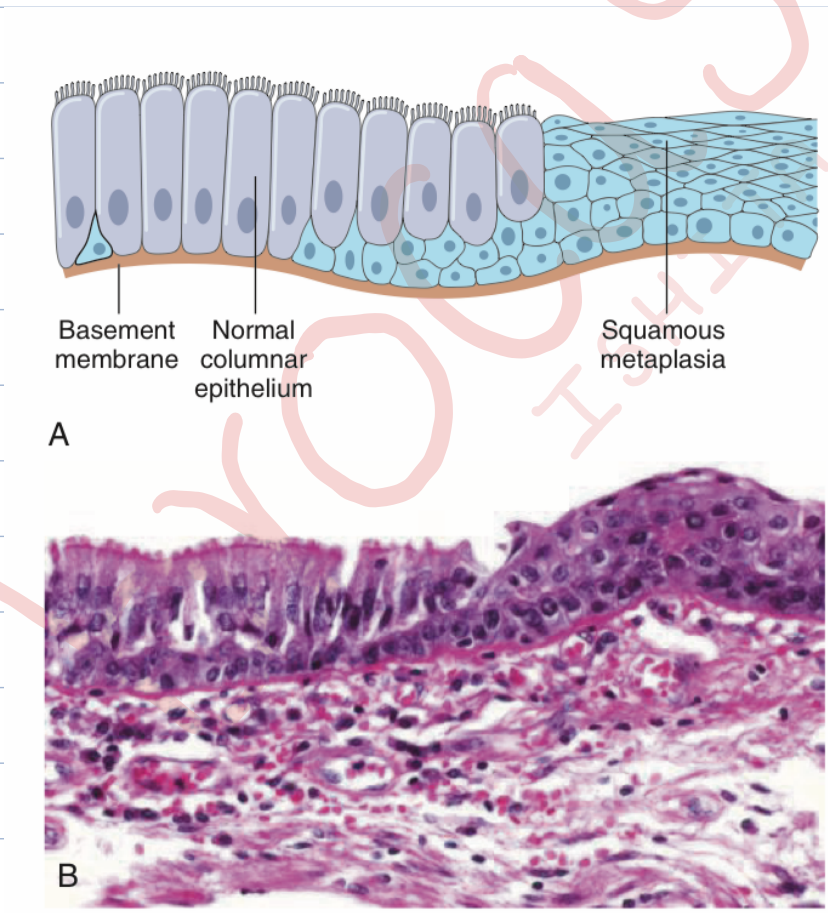


Figure 2.28 Metaplasia of columnar to squamous epithelium. (A) Schematic diagram. (B) Metaplasia of columnar epithelium *(left)* to squamous epithelium *(right)* in a bronchus (as often occurs with smoking).

Dysplasia: cells showing cytological features of malignancy

↳ disordered growth.

- Cellular polymorphism
- Large hyperchromatic nuclei
- High nuclear-to-cytoplasmic ratio
- Loss of polarity.

CELL INJURY

→ Reversible cell injury
→ Irreversible cell injury
→ E.R. stress
→ Free radical oxidation

Reversible Cell Injury: [cell can go back to normal state once the injurious stimulus is removed]

Mechanism: decreased oxygen [injurious/noxious stimulus]

↓

mitochondria is affected

↓

decreased ATP synthesis

↓

$Na^+ - K^+$ ATPase pump's reduced activity

↓

- Influx of Na^+ & H_2O
- Efflux of K^+

↓

cellular swelling [HYDROPIC CHANGE / CLOUDY CHANGE / VACUOLAR DEGENERATION]

- ER swelling
- Mitochondrial swelling
- Membrane bleb formation
- Myelin figure formation

↑ anaerobic glycolysis

↓

Lactic acidosis

↓

decreased pH

↓

Clumping of chromatin in nucleus

Detachment of ribosomes from RER

↓

decreased protein synthesis

Increased lipid synthesis

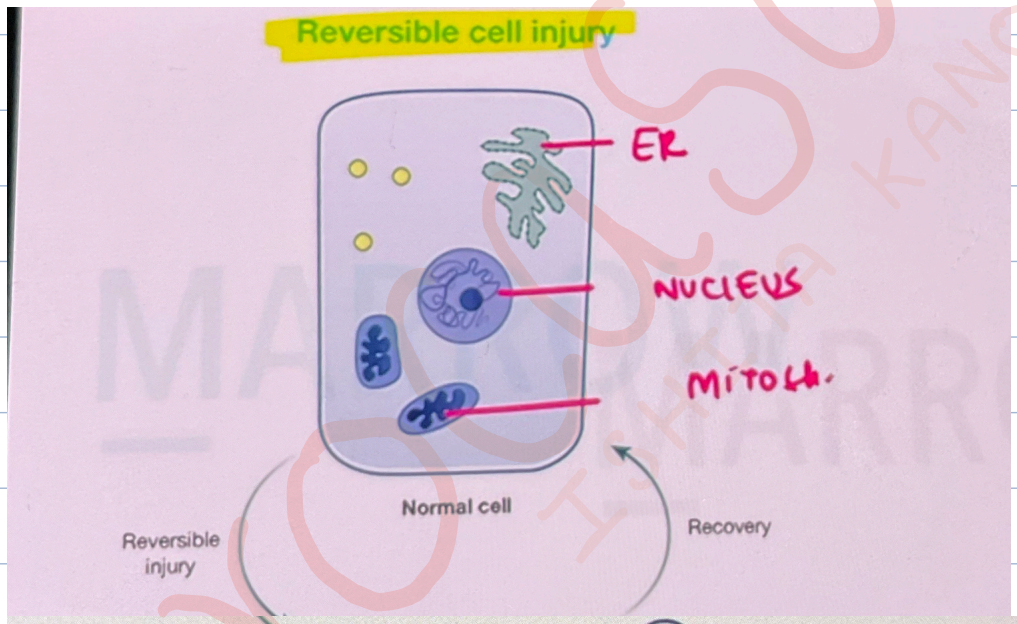
↓

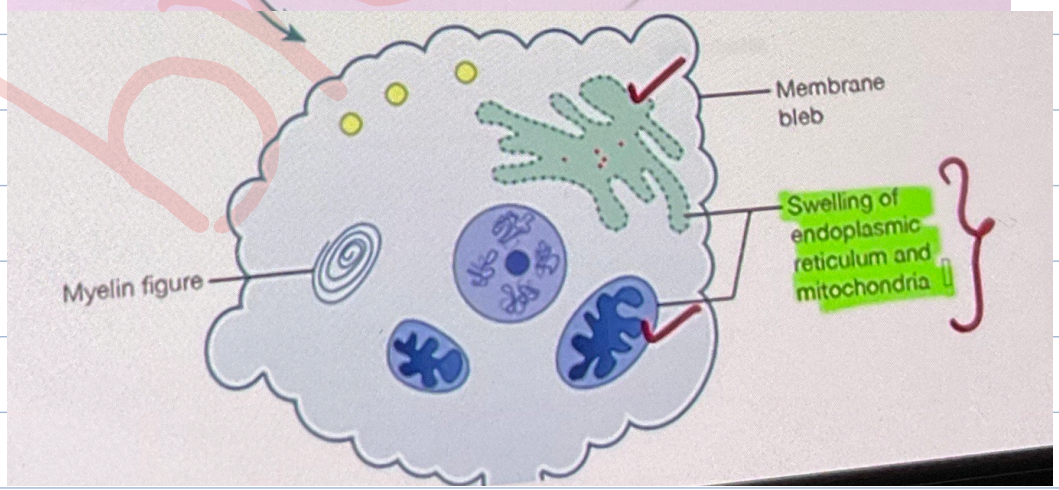
fatty change

MYLEIN FIGURES:

→ formed due to damage to phospho lipid bilayer
→ composed of Ca^{2+} & phospholipid
→ seen in both reversible & irreversible cell injury
→ these figures look like 'Myelin' (have a laminated configuration)

- Most common organelle affected in reversible cell injury: Mitochondria
- Most important morphological feature of reversible cell injury: Cellular Swelling / hydropic change





Irreversible Cell Injury:

→ even if injurious stimulus is removed, cell cannot return back to normal state

→ 2 things characterize irreversible cell injury: [2 M's]

- Mitochondrial dysfunction
- Membrane dysfunction

Membrane Dysfunction:

→ normal: selectively permeable, bilayer

Injurious stimulus

↓

membrane will loose its selective permeability [freely permeable]

↓

Ca^{2+} will leak out of organelles

↓

Increased cytosolic Ca^{2+}

↓ production of enzymes

- Phospholipases → breakdown of phospholipids → membrane dysfunction, reproduction of myelin figures
- Endonucleases → DNA damage
- DNAase → DNA damage
- Protease → Protein breakdown
- ATPase → decreased ATP

- In disorders like MI or liver diseases (AST/CKMB, etc.), enzymes can be measured in blood since there is membrane dysfunction & they can leak out of the cell.

Mitochondrial Dysfunction: → decreased ATP synthesis
→ mitochondrial membrane becomes freely permeable *
→ formation of large, floculent, amorphous densities
↳ CHARACTERISTIC FEATURE OF IRREVERSIBILITY.
↳ only seen on electron microscopy

Nuclear Changes:

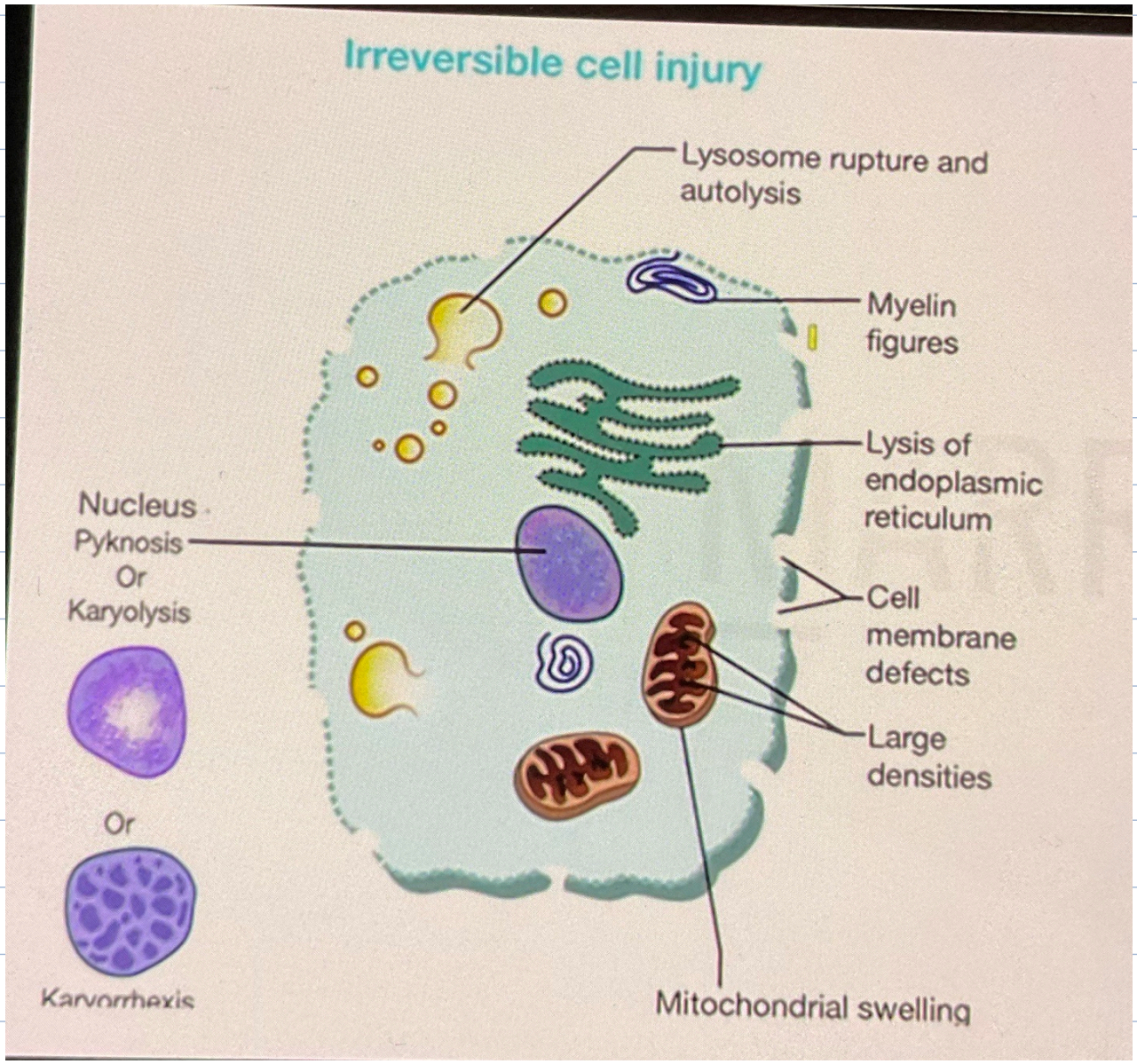
Pyknosis
→ shrinkage / clumping of nuclear chromatin
→ hyperchromasia

Karyorrhexis (nucleus + fragmentation)
→ fragmentation of nuclear chromatin

Karyolysis
→ dissolution of nucleus

- Most important light microscopic feature of irreversibility: Nuclear changes

* due to formation of a high-conductance channel in the mitochondrial membrane ⇒ Mitochondrial permeability transition pore.



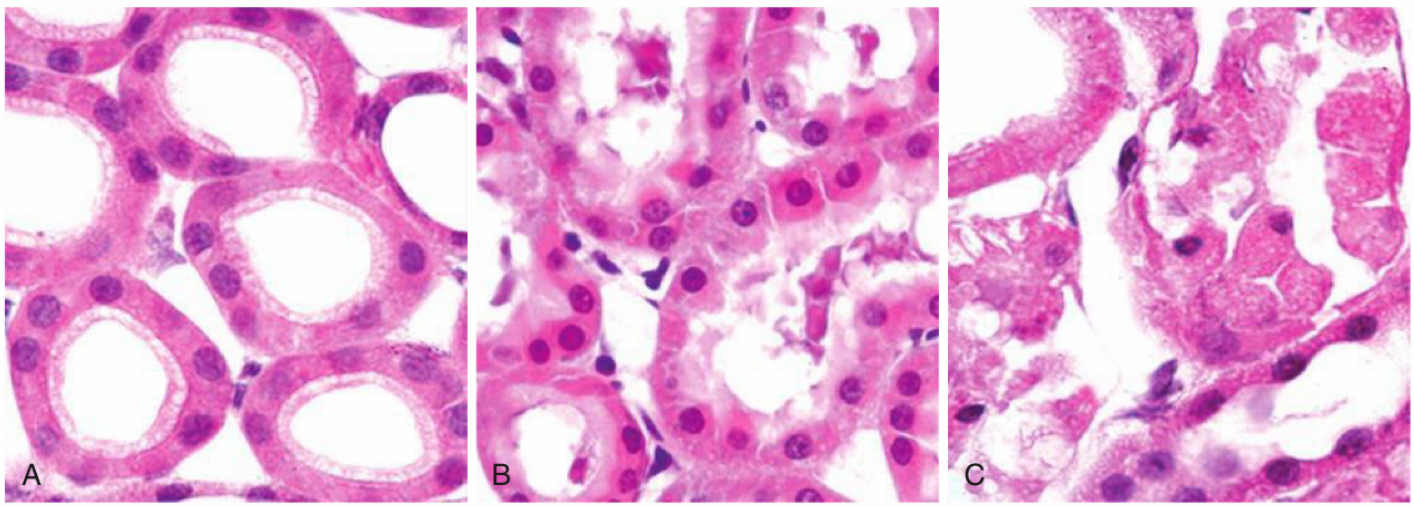


Figure 2.5 Morphologic changes in reversible cell injury and necrosis. (A) Normal kidney tubules with viable epithelial cells. (B) Early (reversible) ischemic injury showing surface blebs, increased eosinophilia of cytoplasm, and swelling of occasional cells. (C) Necrosis (irreversible injury) of epithelial cells, with loss of nuclei, fragmentation of cells, and leakage of contents. The ultrastructural features of these stages of cell injury are shown in Fig. 2.6. (Courtesy Drs. Neal Pinckard and M.A. Venkatachalam, University of Texas Health Sciences Center, San Antonio, Tex.)

Structure	Reversible Injury	Irreversible Injury
Plasma membrane changes	Blebbing, blunting, loss of microvilli	Discontinuities in plasma & organelle membrane
mitochondrial changes	Swelling & appearance of small amorphous densities.	Marked dilatation with app. of large amorphous densities (precipitates of Ca), aggregates of fluffy material (denatured protein)
ER	Dilatation with detachment of polysomes	Swelling & fragmentation
myelin figure	May be present	Usually present
Nuclear changes	Disaggregation of granular & fibrillar elements	Pyknosis, Karyorrhexis, Karyolysis

Free Radical Injury:

Free radicals: molecules with one or more unpaired electron in their outermost orbit

→ $O_2^{-\cdot}$ (super-oxide), H_2O_2, $OH^{-\cdot}$, $OONO^{-\cdot}$ (peroxy-nitrite)

→ MOST POTENT FREE RADICAL : $OH^{-\cdot}$ (hydroxyl)

→ Excess free radicals ⇒ produce oxidative stress

↓

DISEASES:

a) Aging
b) Cancers
c) Neurodegenerative disorders
d) Reperfusion injury

Production of Free Radicals (FR):

O_2

↓

$O_2^{-\cdot}$ —(superoxide dismutase (SOD))→ H_2O_2 —(Fenton's reaction: Fe^{2+} ⇄ Fe^{3+}; Cu; glutathione peroxidase)→ $OH^{-\cdot}$ (MOST POTENT FR)

Enzymes which lead to FR Production:

i) NADPH oxidase
ii) Xanthine oxidase
iii) Super oxide dismutase [SOD]

Mechanism by which FR cause injury:

- OXIDATIVE MODIFICATION OF PROTEINS
- DAMAGE TO DNA
- LIPID PEROXIDATION OF MEMBRANES

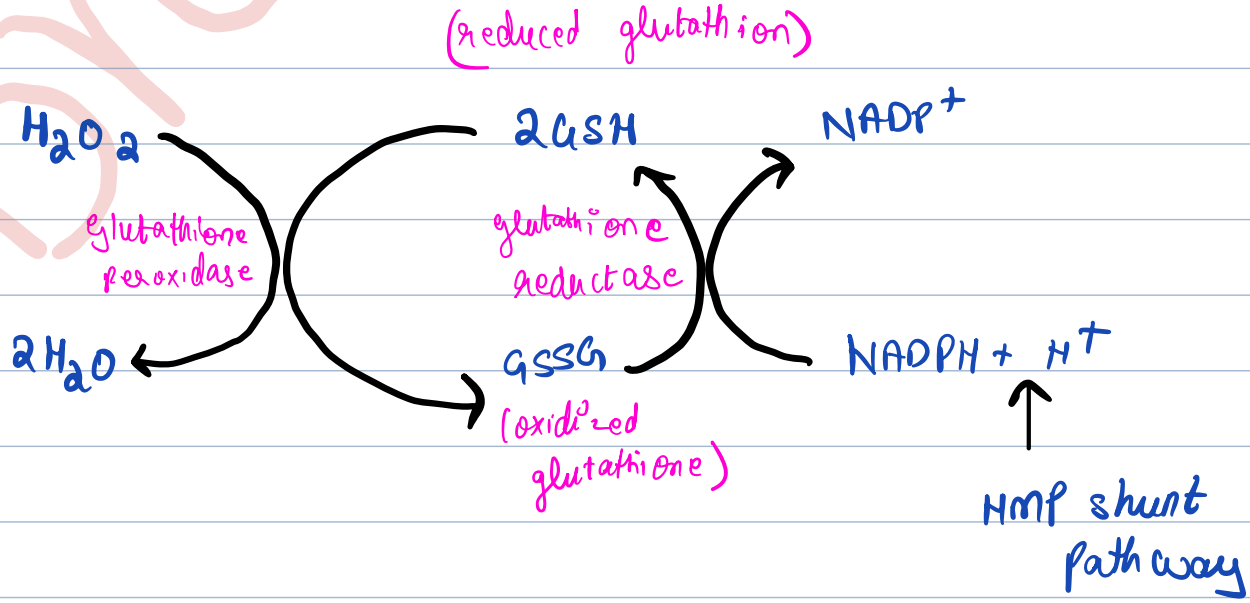
Antioxidants:

Non-Enzymatic

- vit. E, A, C
  [vit. A is not an antioxidant in aqueous humor of eye]
- Se, Mn, Zn

Enzymatic

- SOD
- Glutathione peroxidase
- Catalase



Super Oxide Dismutase [SOD]: inactivates $O_2^{-\bullet}$

Cu-Zn SOD (SOD_1)

→ present in cytoplasm

→ brain is protected from FR injury by SOD_1

Mn - SOD (SOD_2)

→ present in mitochondria

- Mutation of SOD_1: Amyotrophic lateral sclerosis of brain

Catalase: inactivates H_2O_2

→ present in peroxisomes

Glutathione Peroxidase:

→ It inactivates both H_2O_2, $OH^{-\bullet}$

→ present in both cytoplasm & mitochondria

- Intracytoplasmic Ratio of oxidised glutathione to reduced glutathione is an important indicator of oxidative state of the cell

Ischaemia–Reperfusion Injury: → Contributes to tissue damage during myocardial & cerebral infarction following therapies that restore blood flow.

→ Restoration of blood flow to ischaemic tissues can promote recovery of cells if they are reversibly injured, but can also paradoxically exacerbate cell injury & cause cell death

→ Mechanisms:

- OXIDATIVE STRESS ⇒ New damage may be initiated during reoxygenation by increased generation of ROS & RNS (reactive nitrogen species)
- INTRACELLULAR CALCIUM OVERLOAD
- ACTIVATION OF COMPLEMENT SYSTEM ⇒ some IgM antibodies have a propensity to deposit on ischaemic tissue
  ⇒ on reperfusion, circulating complement proteins bind to the deposited antibodies

E.R. Stress:

→ ER = site of protein synthesis

→ folding of these proteins is caused by Chaperons present in ER leading to formation of mature folded proteins

PROTEIN MISFOLDING

↓

Proteins from ER are transported to cytoplasm

↓

These Proteins are degraded by Ubiquitin - Proteosome Pathway

→ Excess Protein misfolding ⇒ MISFOLDED PROTEIN DISEASES (MPD)

MPD	Misfolded Protein
i) Familial Hypercholesterolemia	LDL receptor
ii) Tay Sachs disease	Hexosaminidase β - subunit
iii) α1 anti-trypsin deficiency	α1 antitrypsin
iv) Creutz-Felt Jacob Disease (CJD)	Prion protein
v) Alzheimer's disease	Aβ amyloid
vi) Cystic fibrosis	CFTR (cystic fibrosis transmembrane conductance receptor)

Cell Death:

→ Necrosis
→ Apoptosis
→ Necroptosis
→ Pyroptosis
→ Ferroptosis
→ Autophagy

Necrosis: always pathological

Mechanism: i. Denaturation of proteins
ii. Enzymatic digestion of cells
} ⇒ Damage to plasma membrane
↓
plasma membrane becomes freely permeable
↓
cellular contents leak out of the cell
↓
elicits an inflammatory reaction

[ACCIDENTAL CELL DEATH]

[inflammatory cells come to the site of accident in order to clear the debris]

- In necrosis, some specific substances released from injured cells are called DAMPs (damage associated molecular patterns)
  eg: ATP, uric acid, etc.
- These DAMPs trigger phagocytosis of the debris & trigger the production of cytokines that induce inflammation.

Types of Necrosis:

i) Coagulative
ii) Liquefactive
iii) Caseous
iv) Fat
v) Fibrinoid
vi) Gangrenous

Coagulative Necrosis: MOST COMMON TYPE OF NECROSIS

→ ischaemia

↳ occurs in all solid organs except brain.
[Kidney, spleen, heart, liver]

→ Most common organ affected by coagulative necrosis: HEART
→ Infarct: localised area of coagulative necrosis
→ can be considered to be a type of dry gangrene
↳ wedge-shaped

Examples:
- burns
- gangrene
- Zenker's degeneration: seen in typhoid
  ↳ usually affects rectus abdominis / skeletal muscle.

H & E: → cell outlines are preserved* (due to non-destruction of collagen & proteins)
→ densely eosinophilic appearance (due to loss of cytoplasmic RNA)
→ glassy appearance (due to loss of glycogen)
→ moth-eaten appearance (due to digestion of organelles by lysosomal enzymes)

* since, injury denatures not only structural proteins but also enzymes & so, blocks the proteolysis of dead cells.

Liquefactive Necrosis: [Colliquative Necrosis]

→ mostly due to enzymatic digestion of cells

Examples – Brain
– Abscess
– Fungal infections

H & E: → cell outlines are not preserved

→ necrotic material is frequently creamy yellow because of the presence of leukocytes & is called ⇒ **PUS**.

→ can be considered to be a type of wet gangrene

Caseous Necrosis: [caseous = cheese-like appearance]

Example:- Tuberculosis (TB)
– Fungal infections like histoplasmosis & coccidiomycosis

→ combination of coagulative + liquefactive necrosis

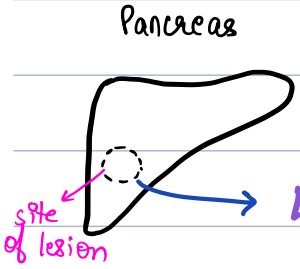
→ densely eosinophilic due to loss of cytoplasmic RNA.

{ Mycobacterium tuberculosis' cell wall is composed of micolic acid.
micolic acid, on degeneration, leads to caseation. }

Fat Necrosis: focal areas of fat destruction, typically resulting from release of activated pancreatic lipases into substance of pancreas & peritoneal cavity.

TRAUMATIC
- Breast

ENZYMATIC
- Omentum
- Pancreas
- Mesentery



Lipase → release of free fatty acids

↓

combine with Ca^{2+}

↓

saponification of fat

↓

CHALKY WHITE DEPOSITS

Fibrinoid Necrosis:

→ Seen in: Type II & Type III hypersensitivity reactions

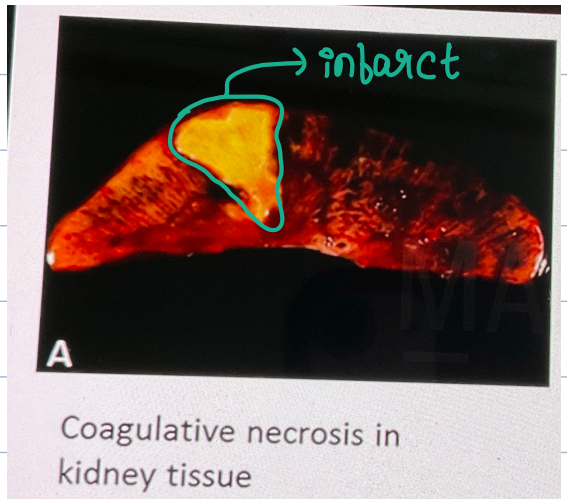
→ occurs when there is immune complex deposition which has a fibrin like appearance (thin pink threads)

Examples:
- Aschoff nodules in rheumatic heart disease
- Malignant hypertension
- Polyarteritis Nodosa

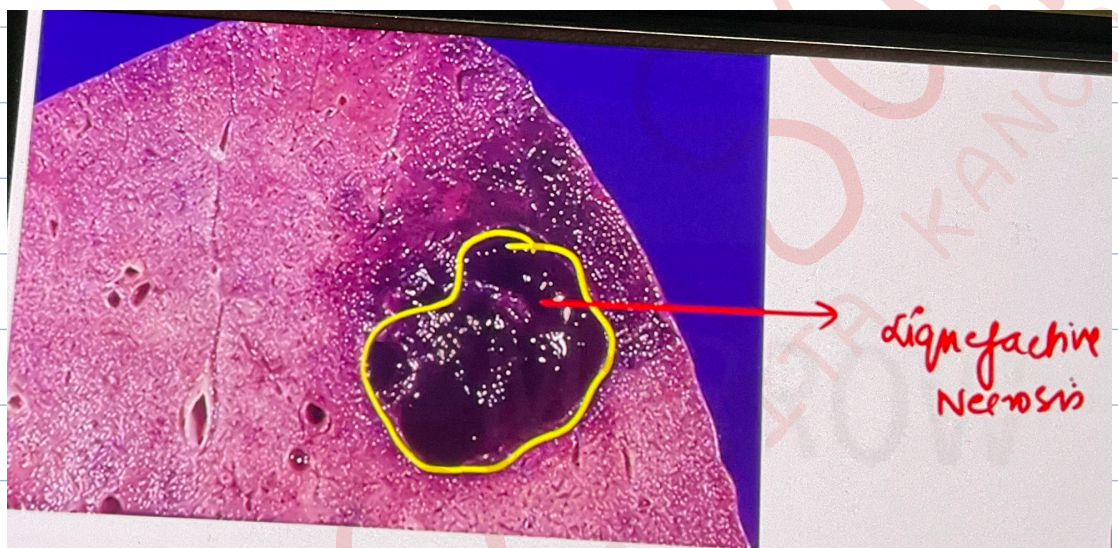
Gangrenous Necrosis : (Ischaemic coagulative necrosis + superimposed bacterial (putrefaction) infection)

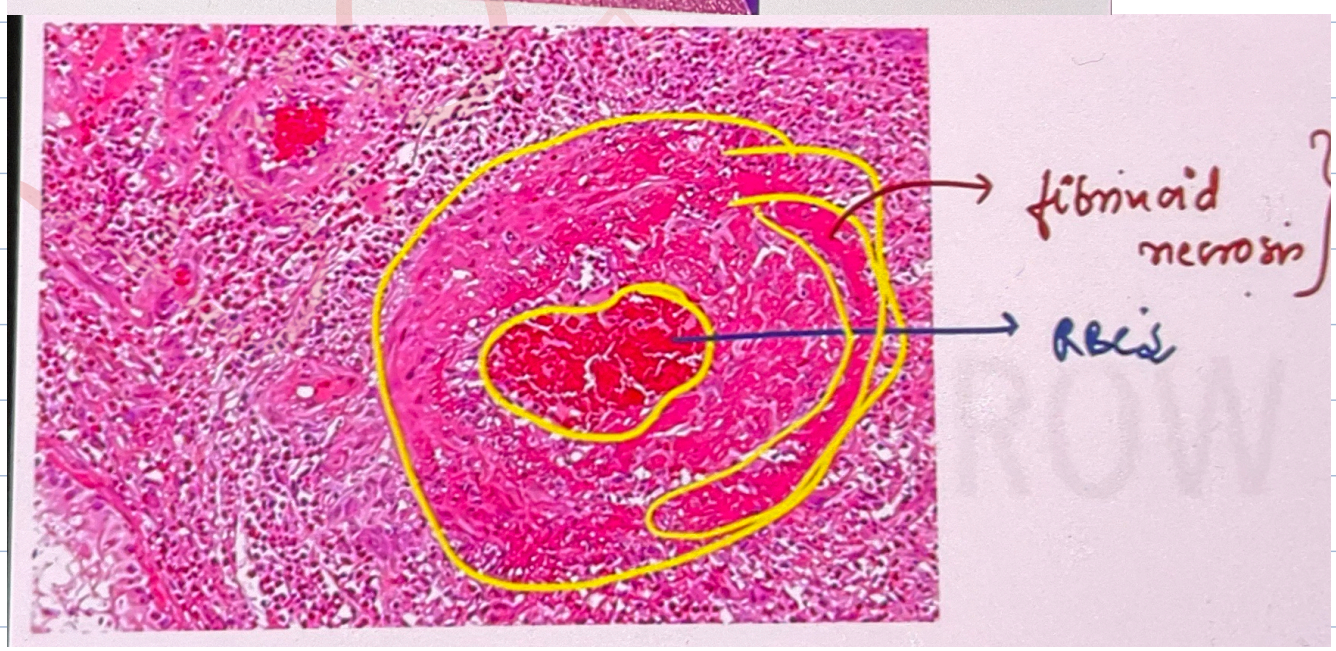
→ seen in Limbs

→ dry gangrene or wet gangrene

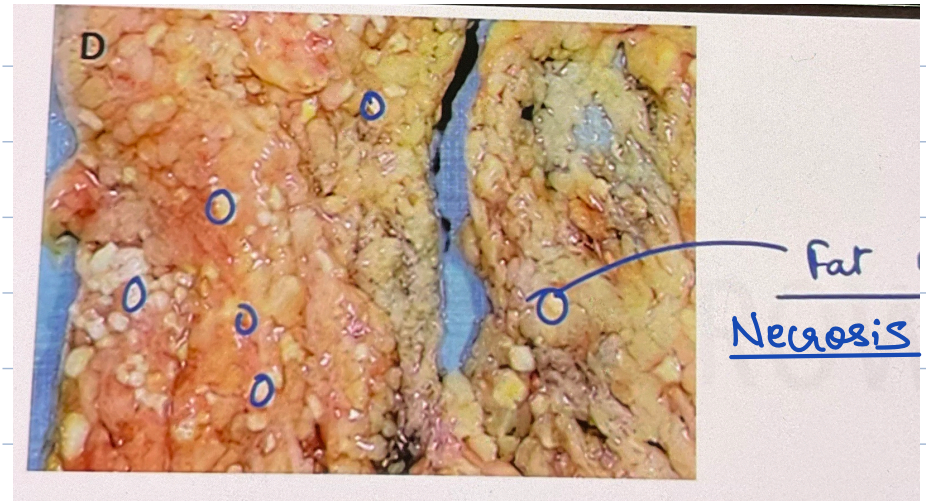


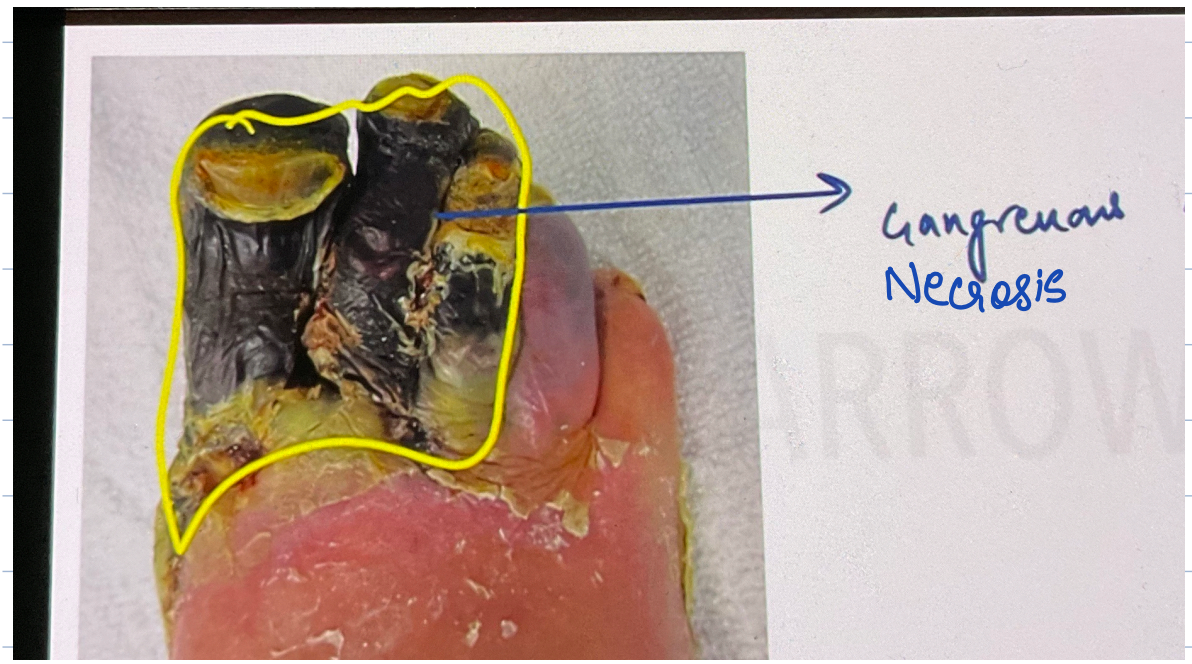
Coagulative necrosis in kidney tissue

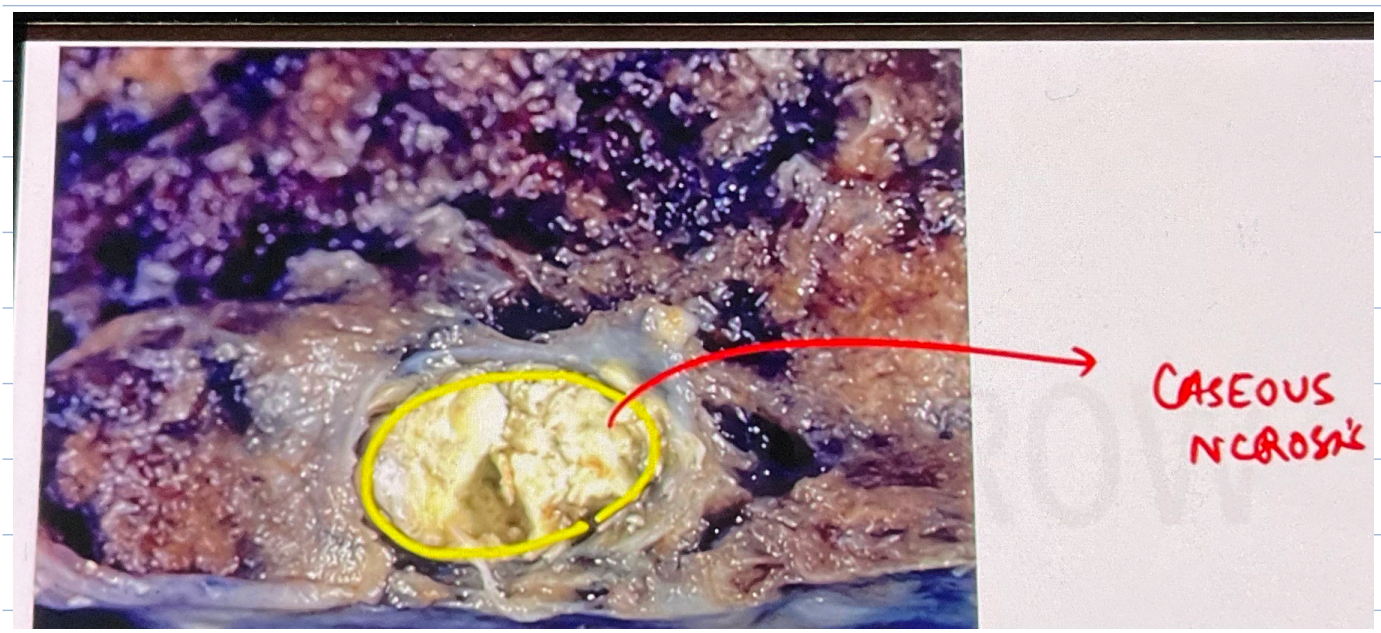




Characteristics	Dry Gangrene	Wet Gangrene
Common site	Limbs	Bowels (seen in structures with dual blood supply)
Examples	• Gangrene due to atheroscleotic narrowing of blood vessel of lower limb	• Volvulus, intussusception
Cause of ischaemia	Arterial obstruction	Commonly venous obstruction
Rate of obstruction		
Appearance of involved part	Shriveled dry (mummification) & black	Swollen, soft, moist
Line of Demarcation	Clear cut	Not clear cut
Spread	Slow	Rapid
Prognosis	Fair	Poor due to severe septicaemia







"falling off"

Apoptosis: genetically programmed cell death (tightly regulated)

→ most studies on apoptosis have been done on a nematode: Caenorhabditis elegans

→ single cell death [necrosis ⇒ multiple cells die together]
→ mechanism to eliminate unwanted cells
→ both physiological & pathological

Physiological Apoptosis

i. during embryogenesis / organogenesis
ii. involution of hormone dependant tissue upon hormone withdrawal
iii. endometrial shedding during menstrual cycle
iv. death of harmful self-reactive lymphocytes
v. death of cells which have fulfilled their purpose (eg: neutrophils in acute inflammatory response)

Pathological Apoptosis

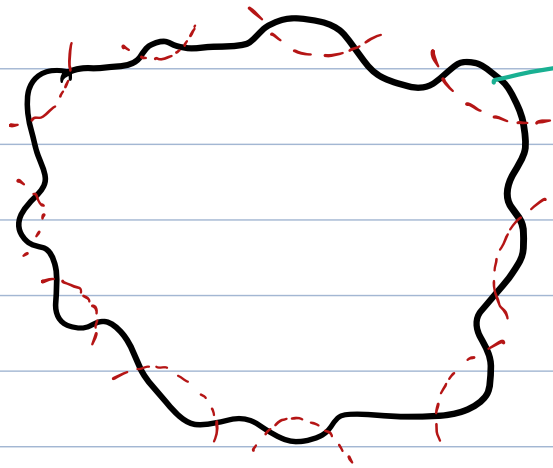
i. DNA damage
ii. Misfolded protein diseases
iii. Infections like Hep. B (councilman body)

⇓

eosinophilic apoptotic globule of hepatocyte

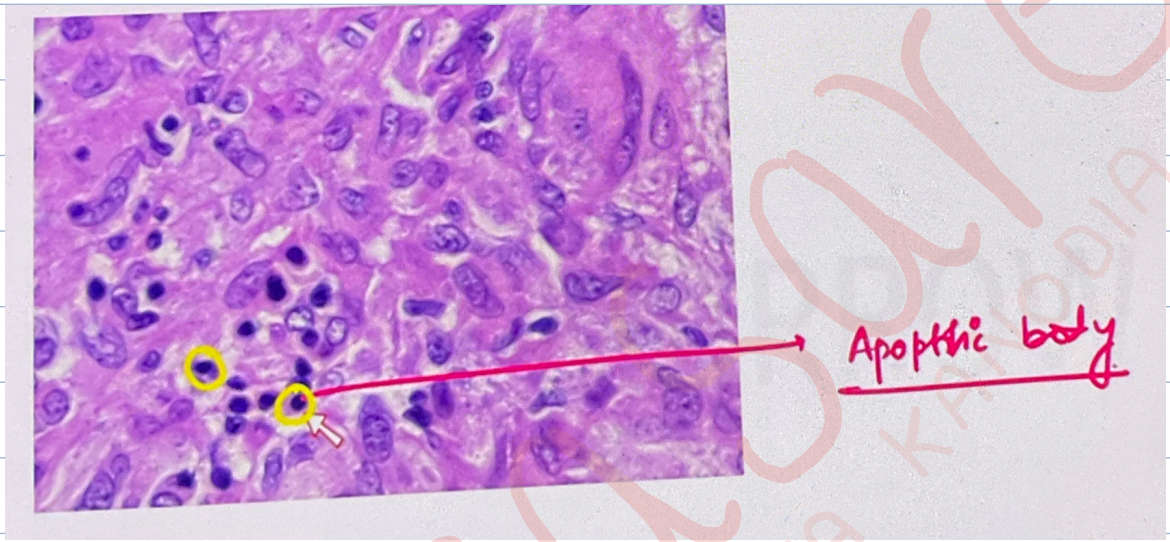
Morphological Features of Apoptosis:

→ cell size reduces (earliest morphological feature)
→ plasma membrane is intact
→ inflammation is absent (∴ difficult to detect apoptosis by light microscopy)
→ chromatin condensation (most characteristic morphological feature)
→ formation of cell membrane blebs



Blebs
↓
fall off
↓
APOPTOTIC BODIES → finally eaten up by phagocytes.

- membrane-bound
- Contain organelles
- eosinophilic



Mechanism of Apoptosis:

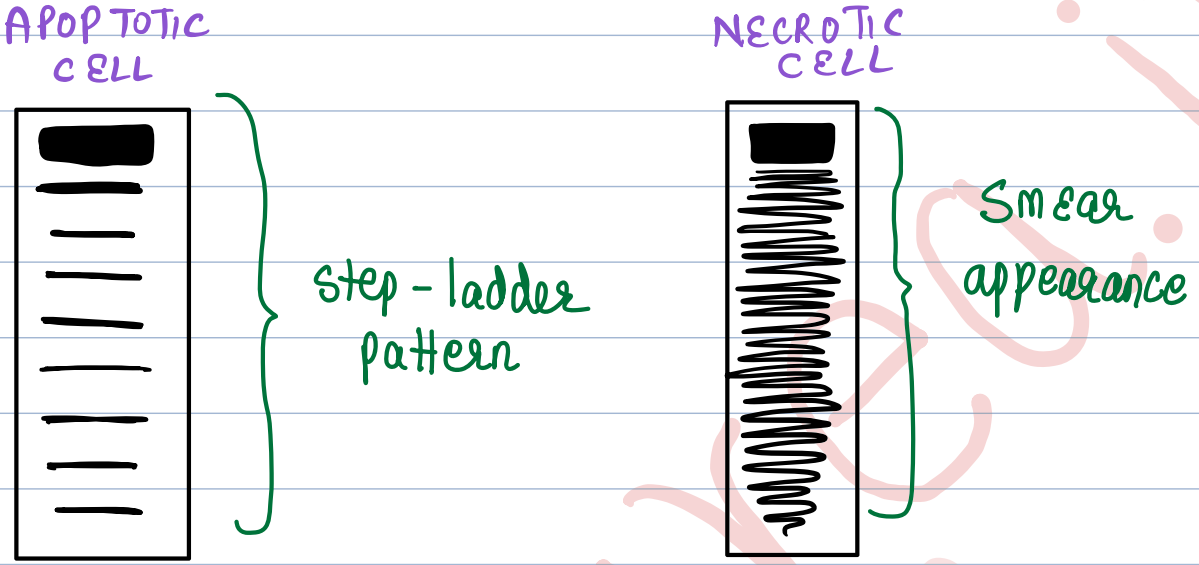
3 phases – Initiation
– Execution
– Removal of apoptotic body

2 enzymes important in apoptosis – Caspases
– Endonucleases

Caspase: contains cysteine
↳ cleaves near aspartic acid residue
→ 2 types – Initiator → Casp 8, 9, 10
– Executor → Casp 3, 6, 7

Endonuclease: DNA breakdown into fragments

→ DNA electrophoresis / PAGE of apoptotic cell



[PAGE = Poly-acrylamide gel electrophoresis]

Regulators of Apoptosis:

Pro-apoptotic Factors
- BAX
- BAK

Anti-Apoptotic factors
- BCL-2 family:
  - Bcl-2
  - Bcl-xL
  - Mcl-1

Regulated Initiators of apoptosis / stress sensors
- BIM
- BID
- BAD
- PUMA
- NOXA

- DNA damage activates p53, which arrests cells in G1 phase of cell cycle & activates DNA repair mechanism.
- If these mechanisms fail to correct the DNA damage, p53 triggers apoptosis by mitochondrial pathway.

INITIATION PHASE

INTRINSIC PATHWAY (MITOCHONDRIA ")

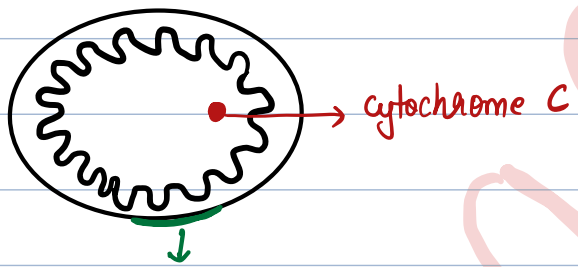
→ 90% cases

Most common organelle affected in aptosis : mitochondria

EXTRINSIC PATHWAY (DEATH RECEPTOR MEDIATED PATHWAY)

→ 10% cases

MITOCHONDRIAL / INTRINSIC PATHWAY :



↓

permeability of mitochondrial membrane is maintained by BCL-2 family proteins

(∴ prevent cyt. C from coming out of mitochondria & causing apoptosis)

Signals for Apoptosis –
- DNA damage
- Viral infection
- radiation injury
- lack of growth signals

Signal for Apoptosis

↓

Activation of stress sensors
(BIM, BAD, BID, PUMA, NOXA)

↓

Activation of BAX & BAK
(pro-apoptotic)

↓

BAX & BAK will form a channel b/w inner & outer mitochondrial membrane
↳ BAX-BAK channel

↓

Release of cytochrome C through this channel into cytoplasm

↓

Cyt. C + Apaf 1
(Apoptosis activating factor)

↓

APOPTOSOME

↓

Activation of Caspase 9 (Initiation Caspase)

↓

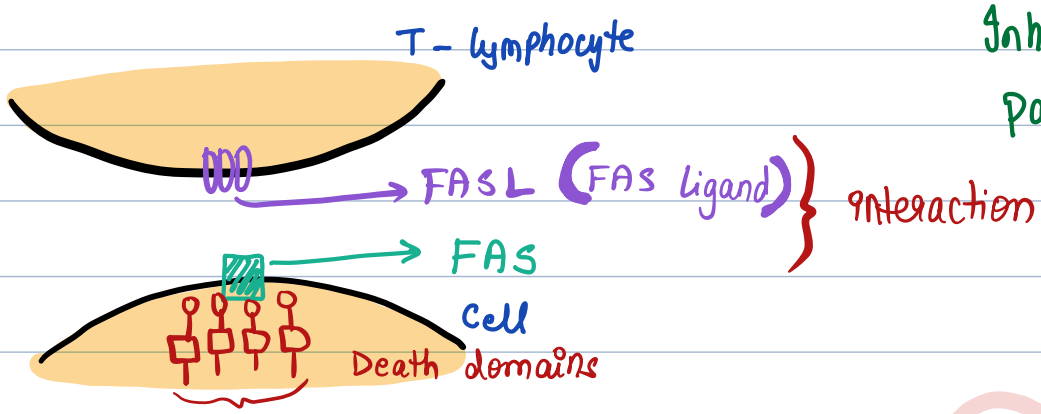
Activation of caspase 3,6,7 (Execution caspases)

↓

APOPTOSIS

- Inhibitors of intrinsic pathway : IAP
- (SMAC, DIABLO) → Inhibit IAP
  ↳ ∴ pro-apoptotic.

EXTRINSIC PATHWAY:



Inhibitor of extrinsic pathway: FLIP

4 Death domains combine to form

FADD (FAS associated death domains)

↓

Procaspase 8 ⟶ Active caspase 8

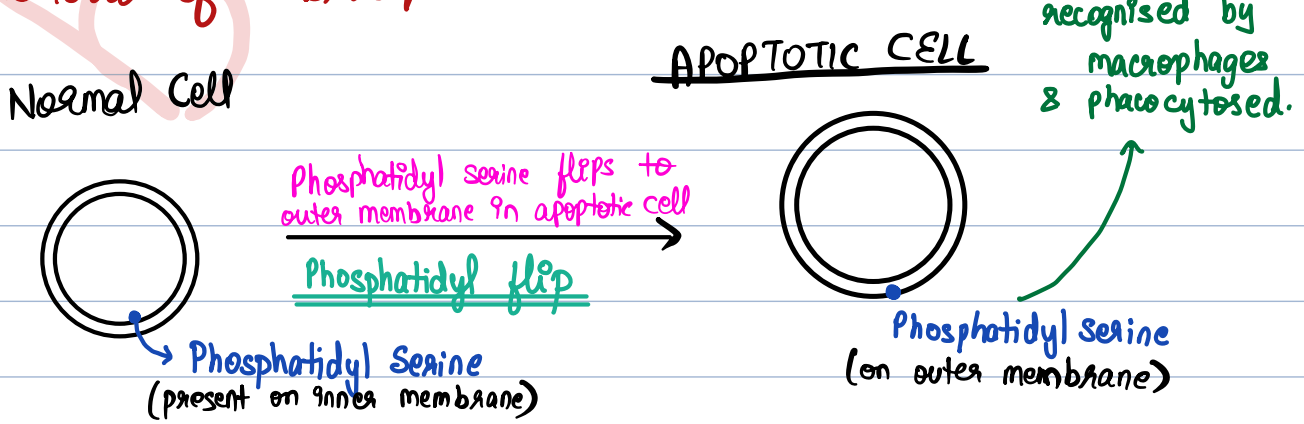
↓

Activation of Caspase 3, 6, 7

↓

APOPTOSIS

Removal of Debris / Dead Cell:



ANNEXIN V = Marker for Apoptotic Cells

↓

recognizes the phosphatidyl serine on outer membrane of apoptotic cell & binds to give colour.

Apoptosis V. Necrosis :

Feature	Necrosis	Apoptosis
Def.	• Enzymatic or ischaemic process • Passive • Group of cells • always pathological	• Genetically programmed cell death • Active • Single cell • physio & pathological
Cell size	Increases	Decreases
Cell membrane	Affected	Intact
Inflammation	Present	Absent
Marker	No	Annexin V, CD 95
PAGE	Smear	Step ladder
Nucleus	Pyknosis, Karyorrhexis, Karyolysis	Fragmentation into nucleosome - size fragments

Efferocytosis : phagocytosis of apoptotic cell

Necroptosis : combination of necrosis & apoptosis

→ cell will starts as apoptosis (Mechanism of apoptosis)

→ cell will end as necrosis (Morphological features of necrosis)

→ Mechanism is Caspase independent

→ a.k.a programmed necrosis.

MECHANISM:

TNF + TNF R1

↓

Activation of RIP K1 & RIP K3

↓

Phosphorylation of MLKL

↓

MLKL enters plasma membrane

↓

- Damage of plasma membrane
- Inflammation
- Free radical injury

} apoptosis

} necrosis

[RIP K = receptor - interacting - protein Kinase]

MLKL = Mixed lineage Kinase Domain - like protein.

Examples:

PHYSIOLOGICAL

- development of mammalian growth plate

PATHOLOGICAL

- Acute pancreatitis
- Acute steatohepatitis
- Neurodegenerative disorders

Pyroptosis:

[IL = interleukin]

→ cell death associated with release of fever-inducing cytokine (IL-1)

Microbial toxin

↓

enters the cell

↓

toxin is recognized by NOD like receptor

↓

Formation of Inflammasome

↓

Activation of Caspase 1

↓

Activation of IL-1

↓

Fever, Inflammation

Ferroptosis:

→ Cell death caused by excess Iron (Fe)

→ discovered in 2012

(Iron in Fe^{2+} form causes Fenton's reaction leading to production of free radicals)

Excess intracellular Iron

↓

Lipid peroxidation of membrane

↓

Free radical injury

↓

Plasma membrane damage

↓

Cell death

Normally, in the body, Iron is present in Fe^{3+} form or bound to proteins like ferritin, transferrin, etc.

∴ Fenton's reaction cannot occur.

Autophagy: "self-eating"

→ cell eats its own contents

→ survival mechanism of cell in nutrient deprivation

Formation of Initiation membrane called PHAGOPHORE (derived from ER)

↓

Formation of autophagosome (due to release of vesicle)

↓

Autophagosome + Lysosome

↓

Digestion of cellular contents

For formation of autophagosome:

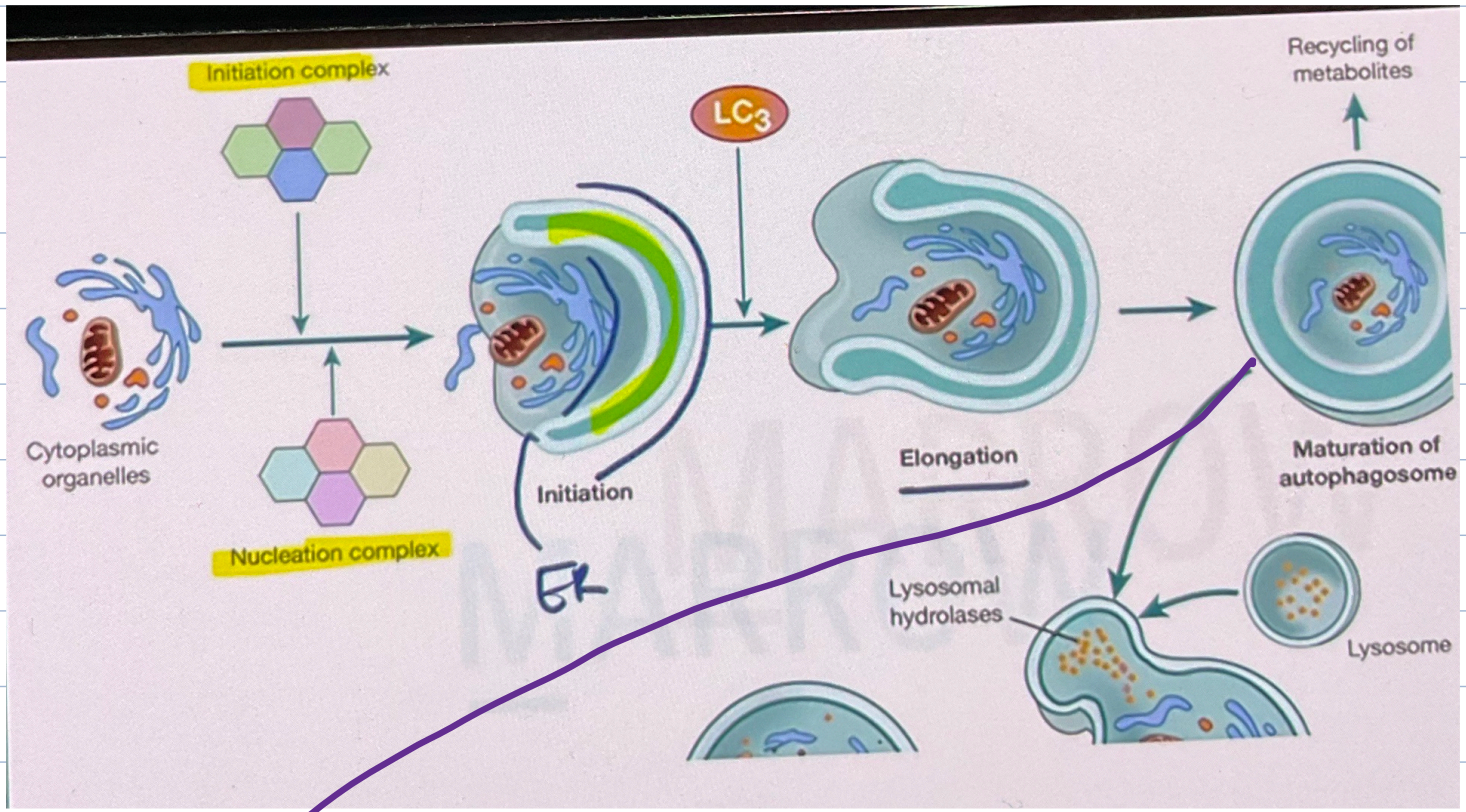
- genes required ⇒ ATGs (Autophagy related genes)

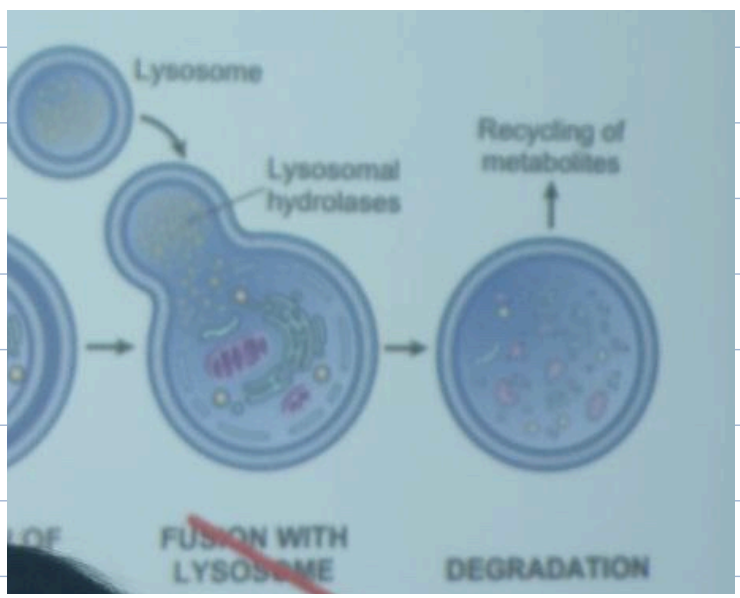
↓

LC3 → marker for autophagy

CROHN'S DISEASE: ATG 16L1 gene

- **Autophagy plays a role in:**
  - cancer
  - neurodegenerative disorders
  - infectious diseases (mycobacteria, shigella, HSV-1)
  - inflammatory bowel diseases





Intracellular Accumulations:

- Proteins
- Lipids
- Glycogen
- Water
- Hyaline
- Calcium
- Pigments

Mechanisms leading to intracellular accumulations:

→ inadequate removal of a normal substance

→ accumulation of an endogenous substance (as a result of genetic/acquired defects)

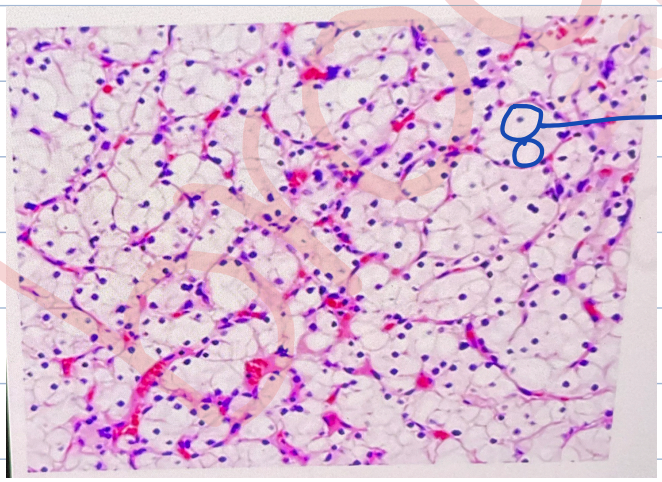
→ failure to degrade a metabolite due to inherited enzyme deficiencies

→ deposition & accumulation of an abnormal exogenous substance

GLYCOGEN:

→ can be deposited in
- glycogen storage disorders
- severe diabetic nephropathy (Armani Ebstein lesions in PCT)

H&E: glycogen appears as clear vacuoles (because it dissolves in the aqueous fixative)



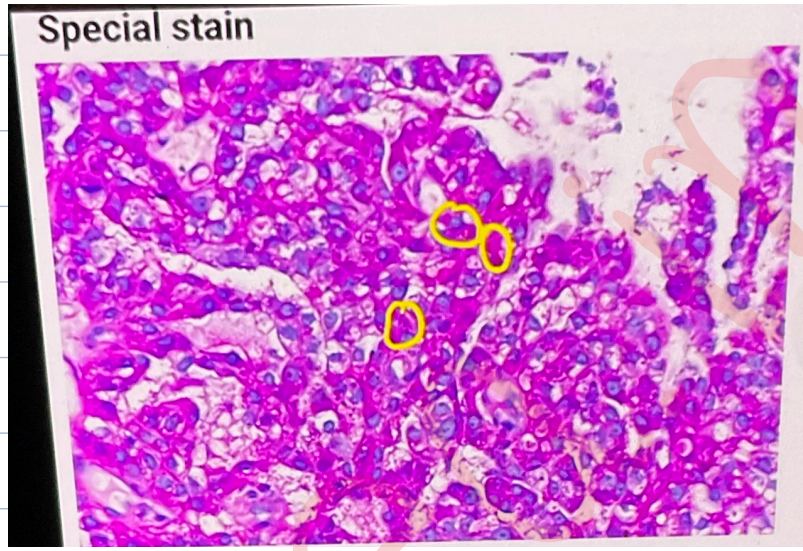
CLEAR-CELL RENAL CELL CARCINOMA

Special Stain for Glycogen:

- PAS (Per-Iodic Acid Schiff)

↓

Pink / magenta coloured

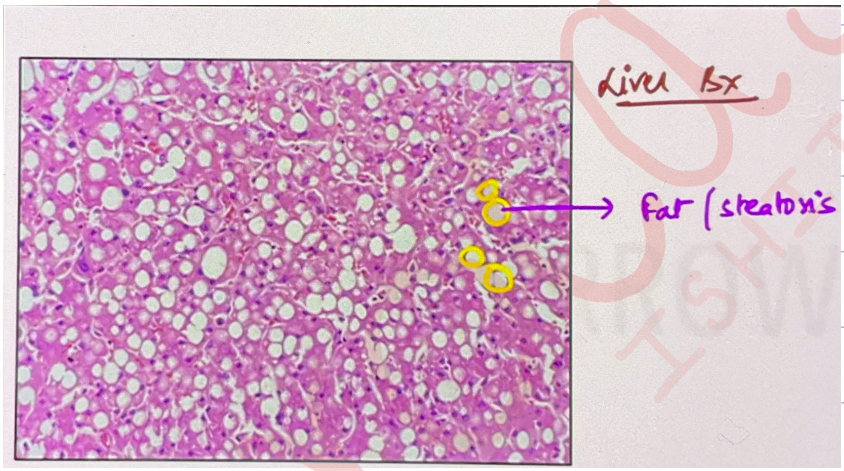


PAS +ve substances:

- Glycogen
- Lymphoblasts
- Basement membrane
- Fungi

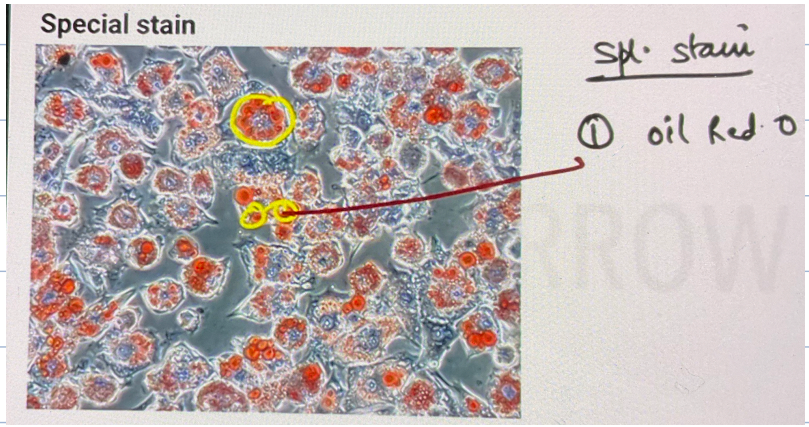
LIPID / FAT:

- TRIGLYCERIDES – Fatty Liver / Steatosis
- CHOLESTEROL – Atherosclerosis
  - Xanthomas
  - Cholesterolosis



[Fatty liver appears yellowish due to pigment lipochrome].

Special Stain for Lipid: – OIL RED O – SUDAN BLACK



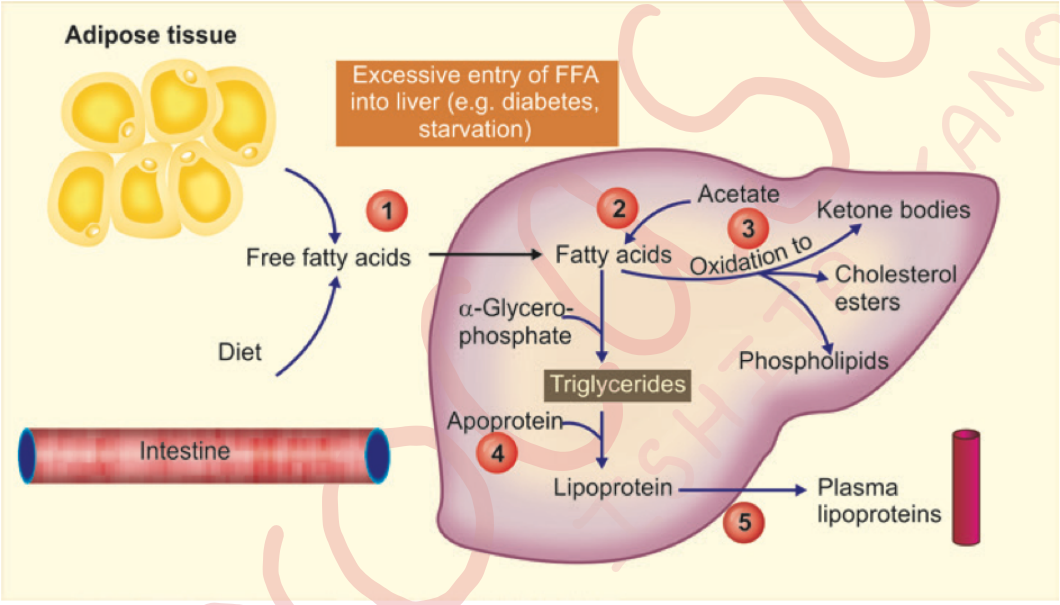
Xanthoma: intracellular accumulation of cholesterol within macrophages

Cholesterolosis: focal accumulations of cholesterol-laden macrophages in lamina propria of gall bladder

Niemann-Pick Disease [Type C]: lysosomal storage disorder caused by mutation of an enzyme involved in cholesterol trafficking, resulting in cholesterol accumulation in multiple organs.

Morphology of Fatty Liver: Liver enlarges, becomes yellow, soft & greasy

Microscopy:- fat is seen as small vacuoles in the cytoplasm around the nucleus (displacement of nucleus to periphery)



Starvation: Increases fatty acid mobilization from peripheral stores.

Steatosis of liver may be due to:
1. Excessive entry free fatty acids
2. Defective metabolism of lipids
3. Defective export of lipoproteins.

Alcohol is the most common cause of steatosis of liver.

Hypoxia inhibits fatty acid oxidation.

PROTEINS:
- Russel Body
- Dutcher Body

} seen in multiple myeloma

- Reabsorption droplets in Proteinuria in renal tubules

Russel Body: intracytoplasmic inclusion

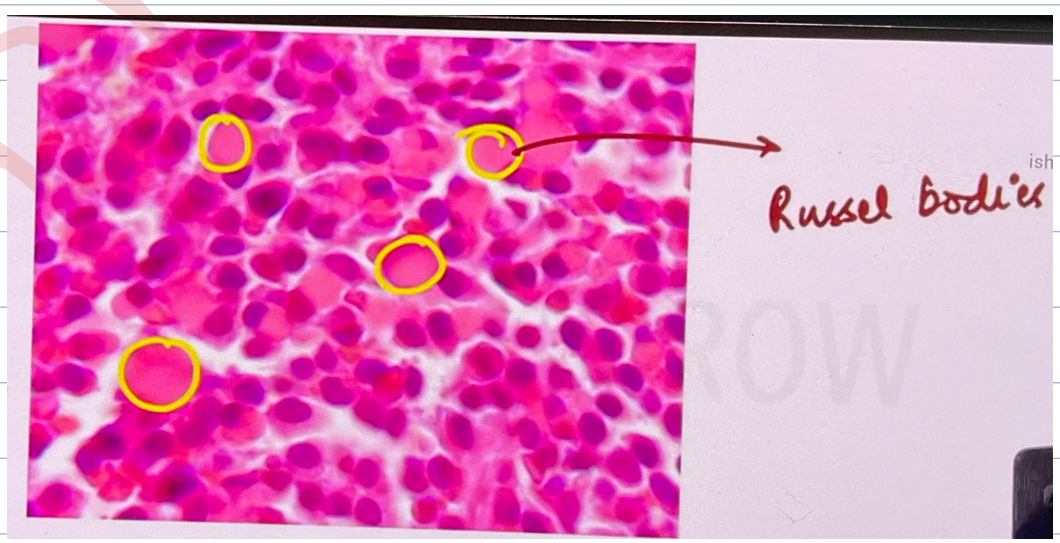
Dutches Body: intranuclear inclusion.

H & E: eosinophilic, granular appearance

HYALINE: → INTRACELLULAR
→ EXTRACELLULAR

H & E: eosinophilic (pink), smooth appearance (homogeneous)
↳ ground glass appearance

MALLORY HYALINE BODY: commonly seen in Alcoholic Liver Disease
↳ other conditions in which it is seen: New INDIAN WATCH.



- New → Non-alcoholic Steatohepatitis (NASH)
- INDIAN → Indian childhood cirrhosis
- W → Wilson's disease
- A → Alcoholic liver disease
- T → Tumours (like hepatocellular carcinoma)
- C → Cirrhosis (like primary biliary cirrhosis)
- H → focal nodular Hyperplasia.

Mallory Hyaline Bodies:

[CK = cytokeratin]

→ composed of intermediate filaments like CK 8 & CK 18

CALCIFICATION [CALCIUM]: < DYSTROPHIC / METASTATIC

→ deposition of Ca with small amounts of other minerals

Dystrophic Calcification:

→ occurs in dead tissues
→ no abnormality of calcium metabolism
→ serum Ca^{2+} is normal.

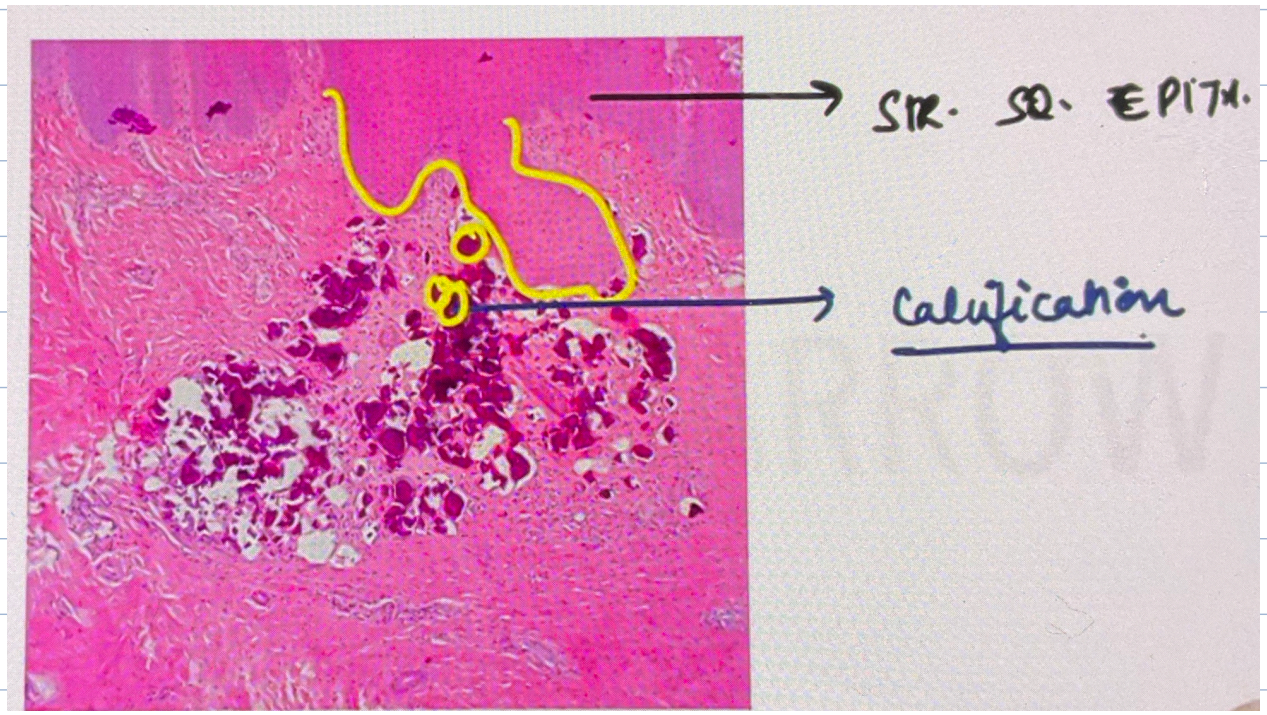
Examples: { Rheumatic vegetations
A Atheromatous plaque
T Tubercular lymph node

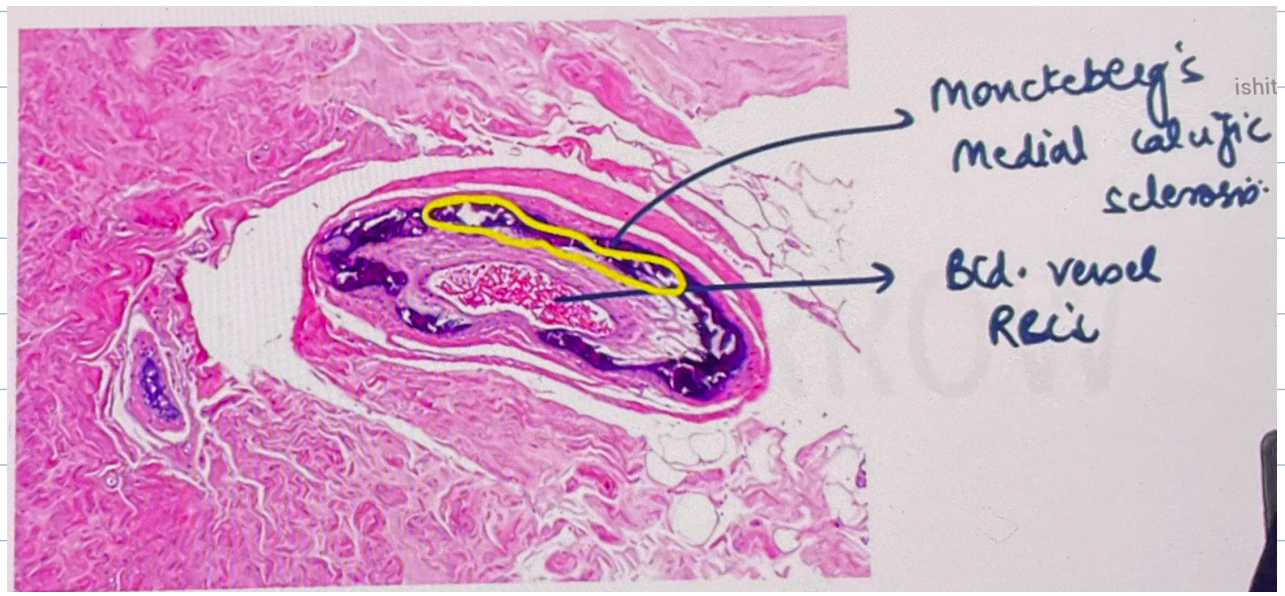
- Necrosis
- Dead parasite
- Monckeberg's medial Calcific Sclerosis (calcification in tunica media of blood vessel)
- Psamomma bodies

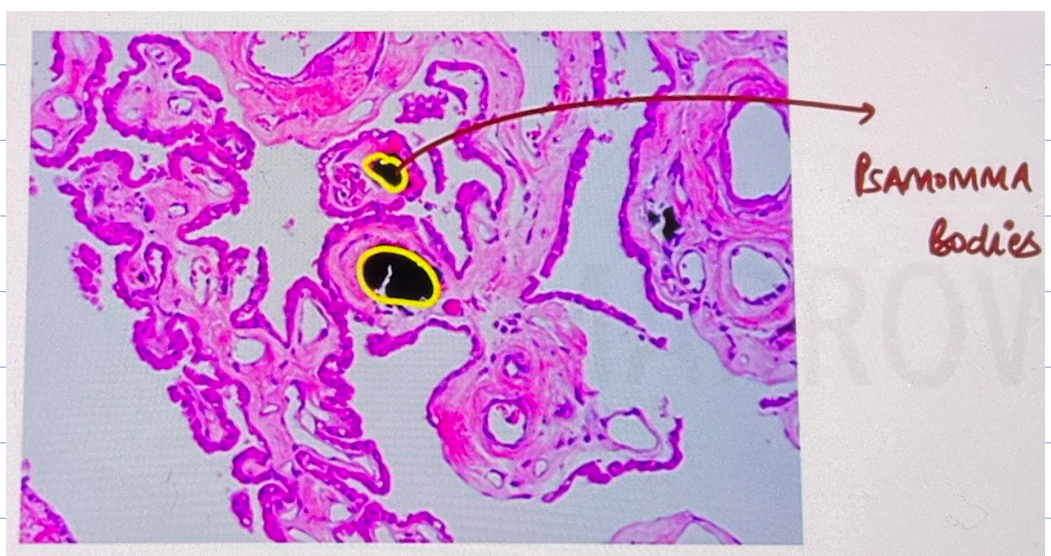
Psamomma bodies: Concentric lamellations of Ca^{2+} 

→ seen in – papillary cancer of thyroid
– papillary renal cell cancer
– meningioma
– Prolactinoma
– Serous cystadenocarcinoma of ovary.

H & E: of Calcium
↳ densely basophilic
→ gritty







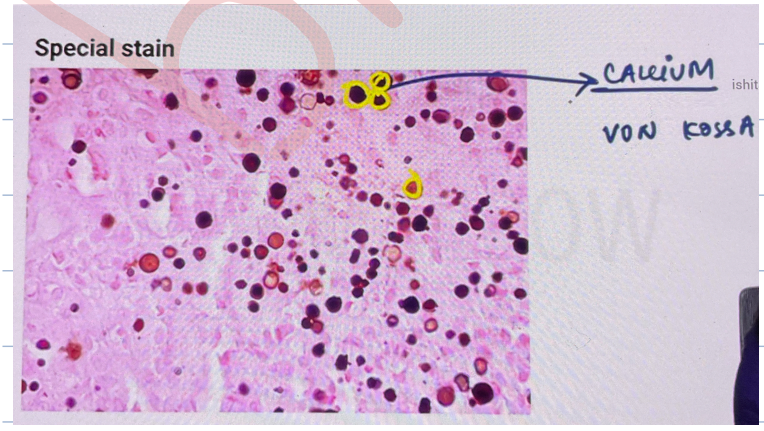
Metastatic Calcification:

→ occurs in living tissues
→ abnormality of Ca metabolism
→ serum Ca^{2+} is high [hypercalcemia]

Examples:
- vit. D related disorders
- Bone diseases (multiple myeloma)
- Hyperparathyroidism
- Renal cell carcinoma / CA Breast
- Sarcoidosis
- Milk alkali Syndrome

- Calcification begins in: MITOCHONDRIA
  [except kidney where it begins in basement membrane of renal tubules]
- Most common site of calcification:
  - Lung alveoli
  - Gastric mucosa

Special Stain for Ca^{2+}:
- Von Kossa (black)
- Alizarine Red S (red)
  ↳ it can pick up even small quantities of Ca^{2+}

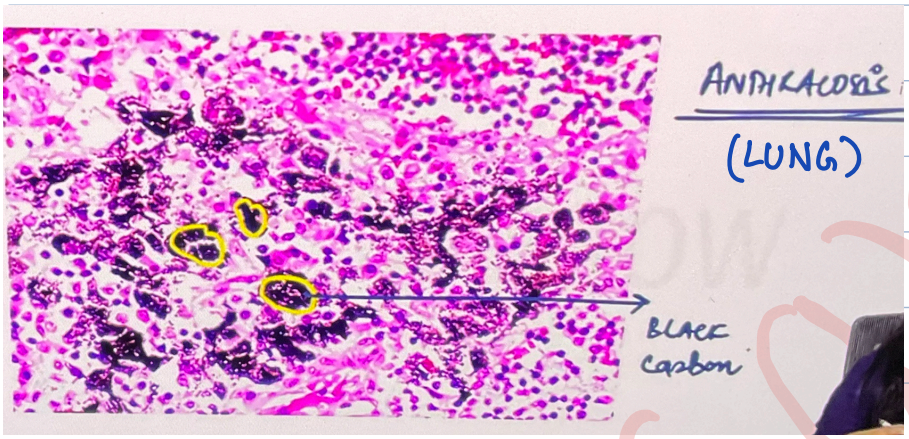


- Test for Bone mineralisation:
  Tetracycline labelling index

PIGMENTS: coloured substances deposited in various tissues & organs

EXOGENOUS
- Tattoo
- Anthracosis

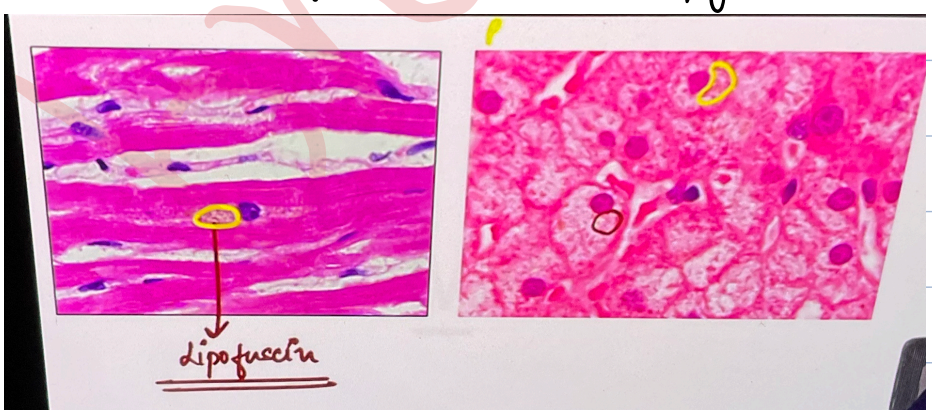
ENDOGENOUS
- Lipofuscin
- Hemosiderin
- melanin



→ brown in colour

Lipofuscin: derived from lipid peroxidation of membranes
↳ telltale sign of free radical injury
→ a.k.a aging pigment / wear & tear pigment
→ responsible for BROWN ATROPHY OF LIVER & HEART

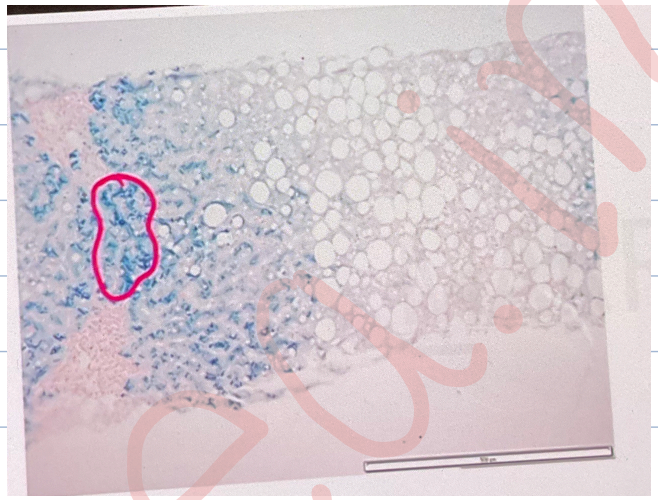
H & E: perinuclear brown pigment



Special Stain for Lipofuscin:
Oil red O.

Hemosiderin:

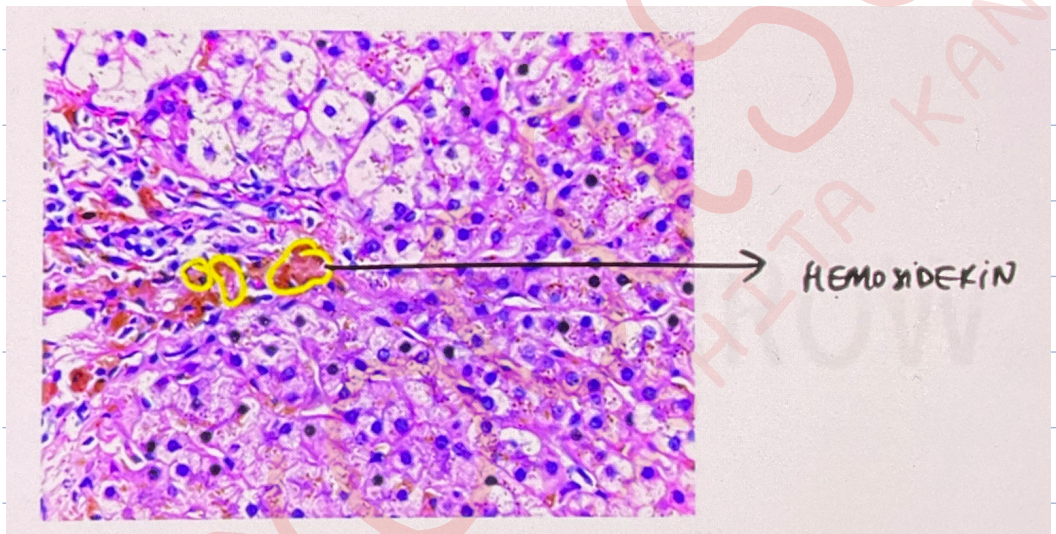
→ deposited in conditions of Fe overload
- repeated blood transfusion
- haemorrhage / bruise

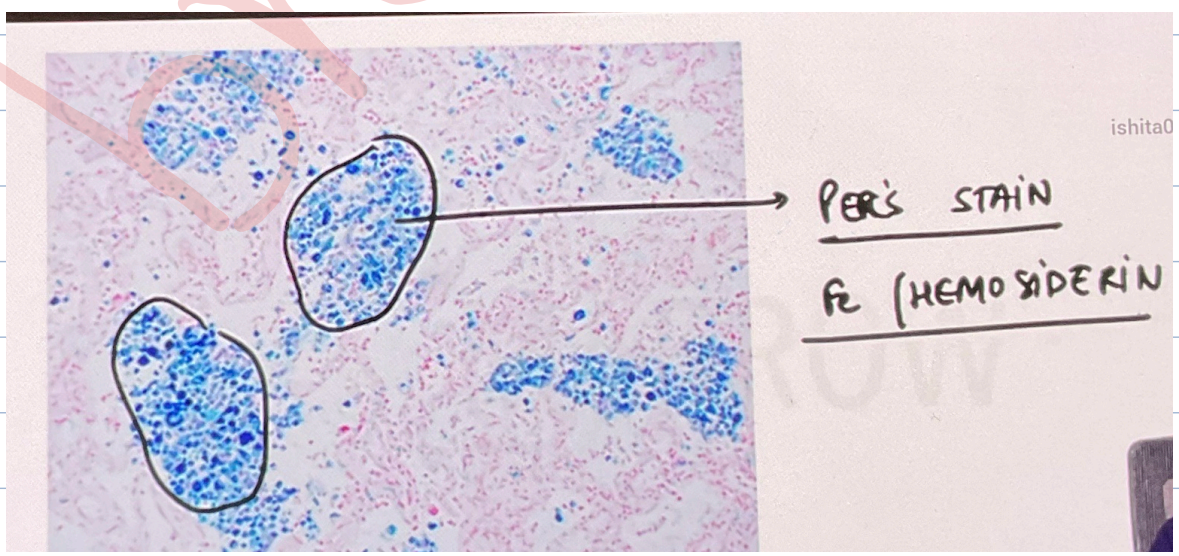


H & E: golden-yellow / brown
↳ refractile

Special Stain: PRUSSIAN BLUE [PERL's REACTION]

Principle: potassium ferrocyanide
↓
ferric ferrocyanide





Melanin: present in skin, hair, eyes, substantia niagra of brain

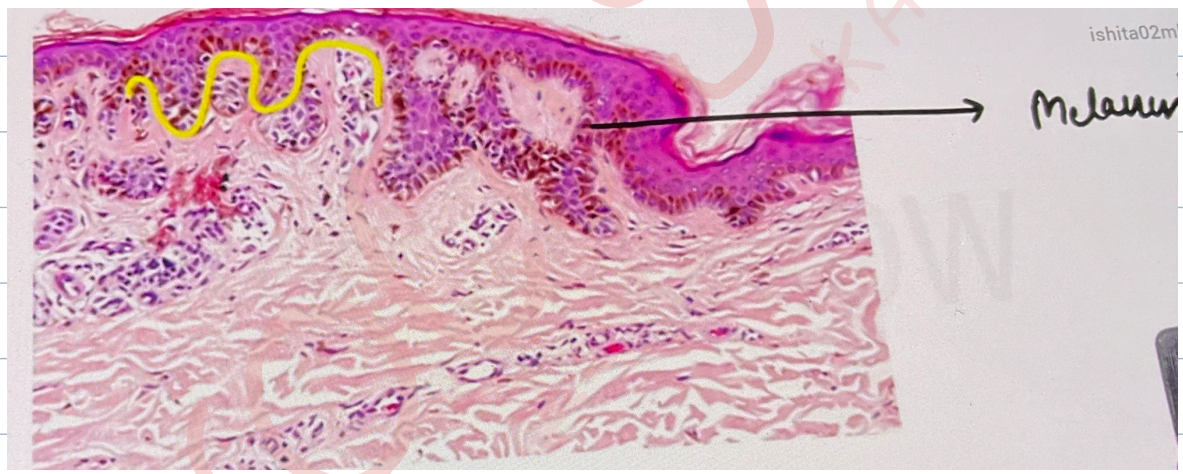
(Pale substantia Niagra ⇒ PARKINSON's DISEASE)

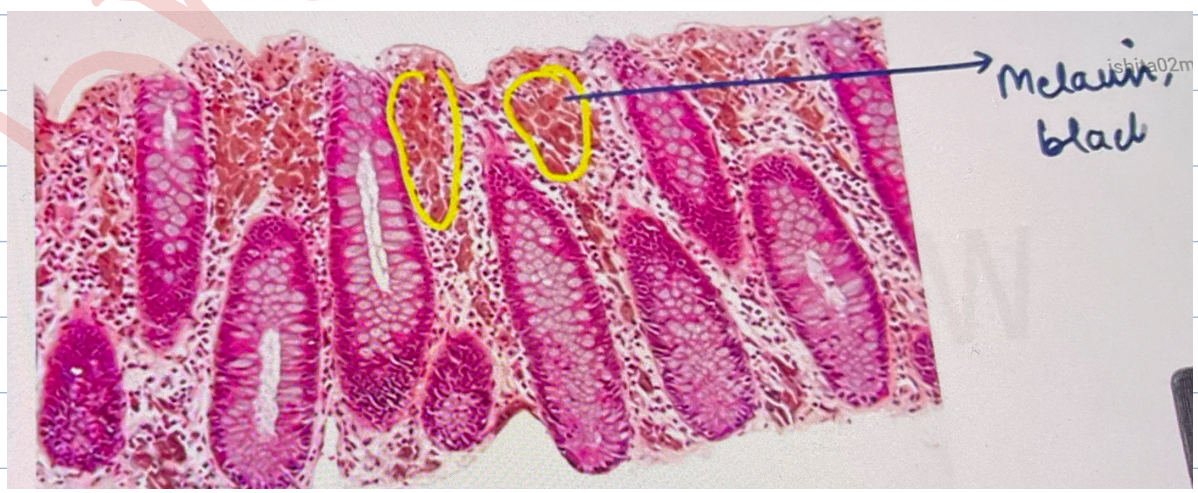
→ black coloured

→ derived from tyrosine

Special Stain:
- MASSON FONTANNA (MF)
- DOPA REACTION (most specific)
- Schmorl's Test

Markers for malignant melanoma:
- HMB-45
- S-100
- Melan A





In haemachromatosis, bronze like pigmentation is not due to hemosiderin, but due to melanin.

Exogenous Pigments: most common exogenous pigment is **Carbon** (coal dust)

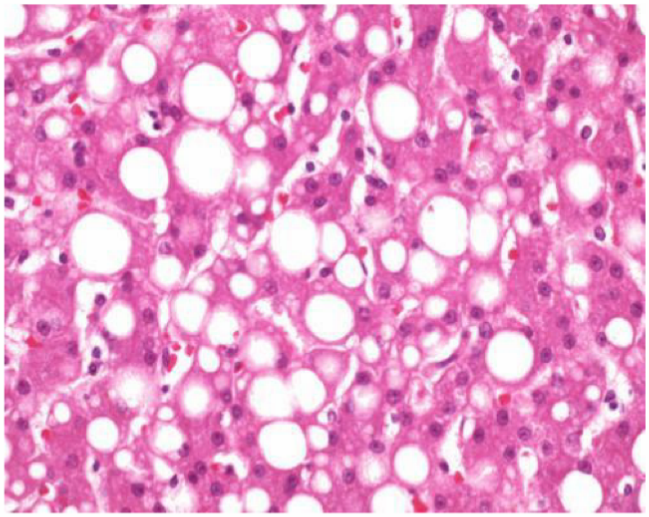


Figure 2.30 Fatty liver. High-power detail of fatty change of the liver. In most cells, the well-preserved nucleus is squeezed into the displaced rim of cytoplasm about the fat vacuole. (Courtesy Dr. James Crawford, Department of Pathology, Hofstra Northwell School of Medicine, NY)

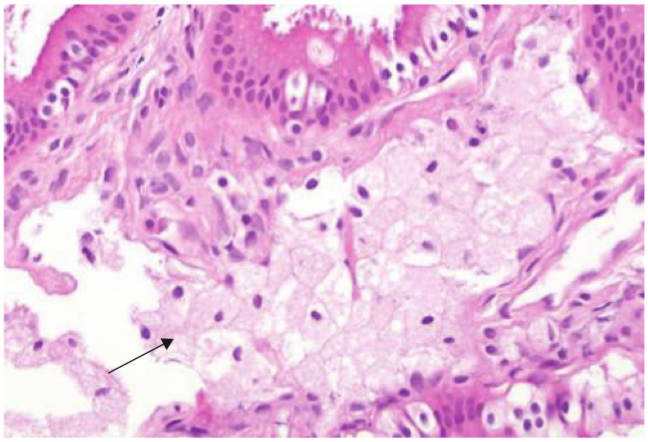


Figure 2.31 Cholesterolosis. Cholesterol-laden macrophages (foam cells, *arrow*) in a focus of gallbladder cholesterolosis. (Courtesy Dr. Matthew Yeh, Department of Pathology, University of Washington, Seattle, Wash.)

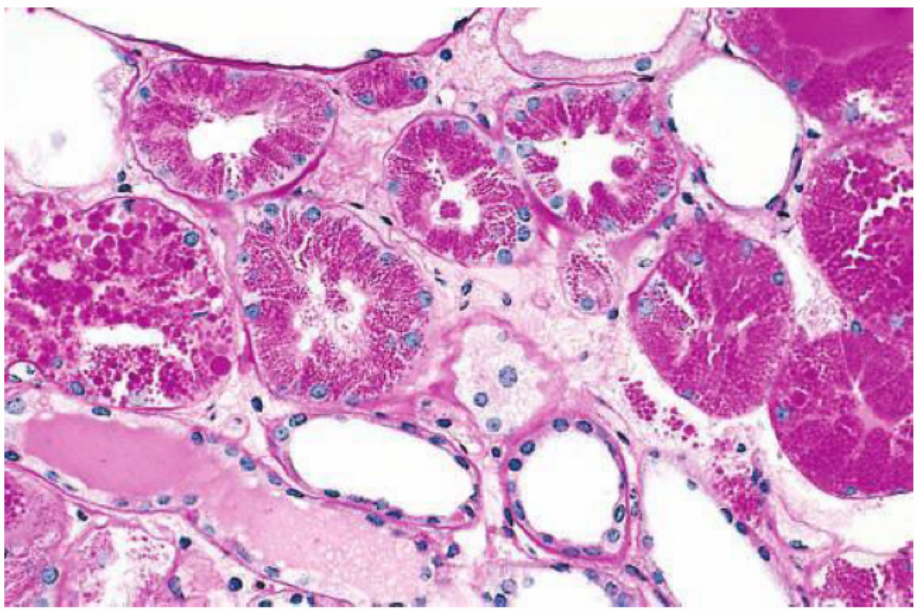


Figure 2.32 Protein reabsorption droplets in the renal tubular epithelium. (Courtesy Dr. Helmut Rennke, Department of Pathology, Brigham and Women's Hospital, Boston, Mass.)

Xanthoma:

Intracellular accumulation of cholesterol within macrophages is found in acquired and hereditary hyperlipidemic states. The tumor mass produced by the macrophages filled with cholesterol is termed xanthomas. Microscopically, it consists of clusters of foamy cells in the subepithelial connective tissue of the skin and in tendons.

Q. Write short note on lipofuscin and brown atrophy of heart.

- Lipofuscin is an **insoluble golden-brown endogenous pigment**. It also called as **lipochrome or wear and tear pigment**.
- **Composition:** It is composed of mixture of lipids, phospholipids and proteins. **It is accumulated by accretion of peroxidized unsaturated lipids and oxidized cross-linked proteins**. The term lipofuscin is derived from the Latin (*fuscus*, brown), and refers to brown lipid.
- **Significance:** It indicates a product of free radical injury and lipid peroxidation. Lipofuscin does not injure cell or its functions. It is observed in cells undergoing slow, regressive changes and is particularly prominent in the **liver and heart (often called brown atrophy of heart)** of aging patients or patients with severe malnutrition and cancer cachexia.
- **Appearance:** Microscopically, it appears as a yellow-brown, finely granular cytoplasmic pigment, often present in the perinuclear region.

Commonly used histochemistry (special stains) in histopathology are listed in Table 1.9.

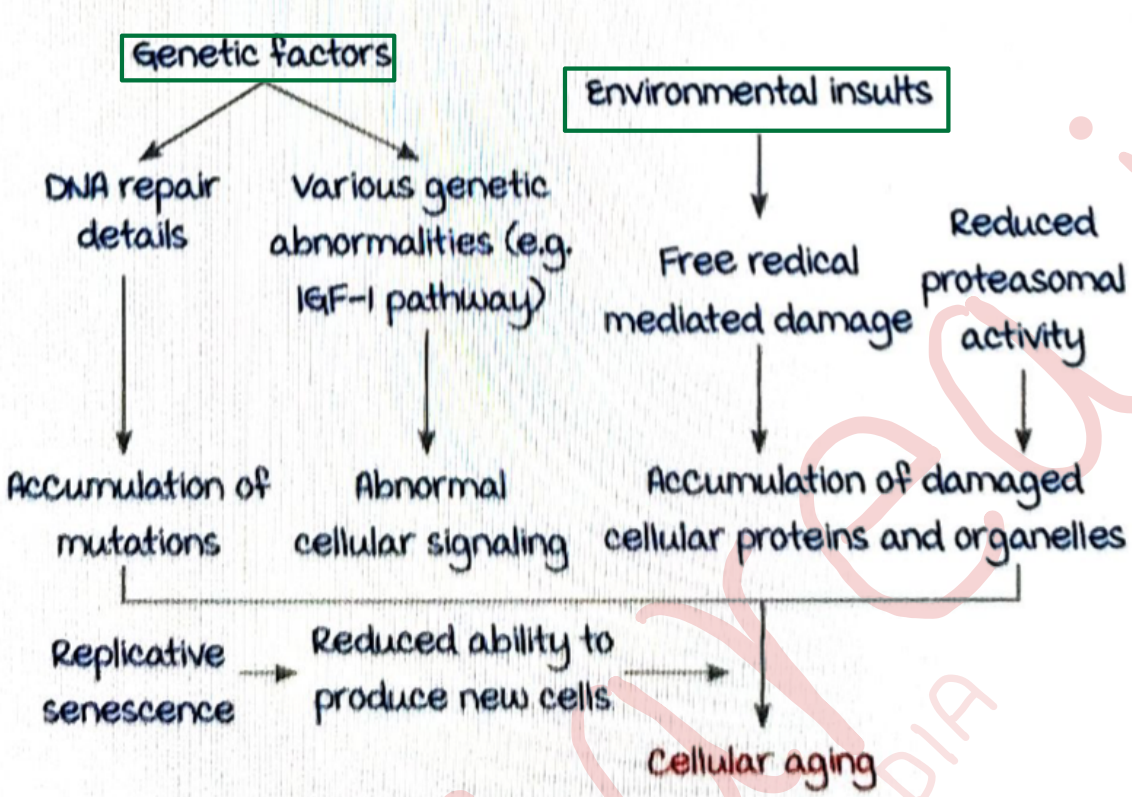
Lipochrome/lipofuscin: Wear and tear pigment seen in old age, severe malnutrition, and cancer cachexia. Perinuclear in location. Derived through lipid peroxidation.

Lipofuscin: Important indicator of free radical injury.

Pigmentation of liver may be caused by:

1. Lipofuscin
2. Malaria pigment
3. Wilson disease
4. Bile pigment
5. Pseudomelanin

Cellular Aging:



DNA damage:

WERNER SYNDROME: premature aging

→ due to defect in DNA Helicase enzyme

↓

for DNA unwinding & replication

- Bloom syndrome
- Ataxia – telangiectasia

} defect in base excision repair mechanism

Cellular Senescence:

HAYFLICK LIMIT: average cells undergo 60-70 divisions in their lifetime
→ by activation of tumor suppressor gene present in CDK-2NA gene.

Telomere Atrition:

Telomeres: short repeated sequences of nucleotides at the end of chromosomes [TTAGGG]

→ It prevents chromosomes from breaking, fusion, etc.

→ telomere shortens with each cell division ⇒ CELLULAR AGING
∴ cell dies when telomere gets exhausted

Telomerase: enzyme which synthesizes telomeres.

↳ inhibits cellular aging

→ a.k.a Immortality gene

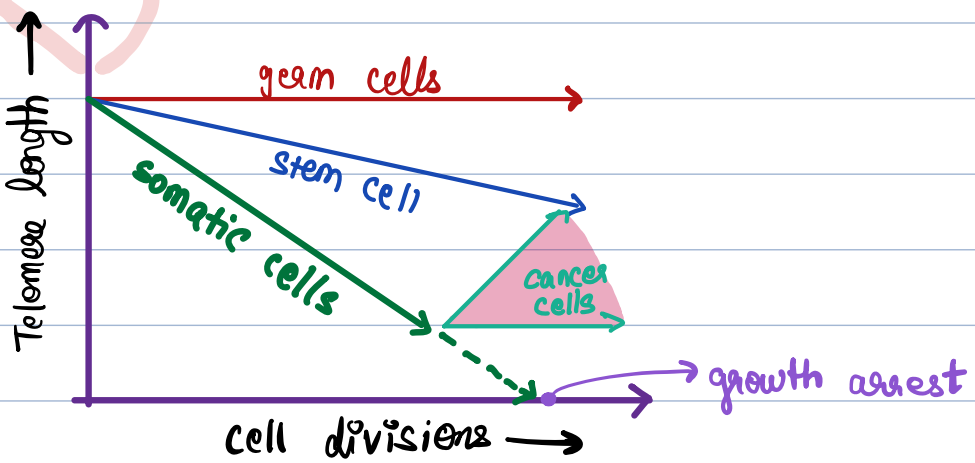
Cells with high telomerase activity:

- germ cells
- stem cells

} rapidly dividing cells

→ Somatic cells ⇒ nil telomerase activity

→ Cancer cells usually have a high telomerase activity



Dysregulated Nutrient Sensing:

- calorie restriction ⇒ ↑ life span

SIRTUINS: inhibit cellular aging by
- reducing free radical injury
- increasing insulin sensitivity of cells
- increasing DNA repair

↓

NAD dependent protein deacetylases

How to increase Sirtuin Levels:

- CALORIE RESTRICTION
- WINE CONSUMPTION

Role of Sirtuins:
- Aging
- Diabetes Mellitus
- Cancer

Calorie Restriction

Attenuation of IGF-1 signaling → ↓ cell growth & metabolism → reduced cellular damage (mimicked by Rapamycin)

Increase in Sirtuins (sirtuin 6) → Dual functions → 1. metabolic adaptations 2. genomic integrity

Defective Protein Homeostasis:

→ Mechanisms – maintenance of correct folding of proteins (by chaperones)
– degradation of misfolded proteins by autophagy-lysosome system & ubiquitin protease system.

Cell/Condition	Stain
m/c Stain in Histopathology	Hematoxylin and Eosin.
m/c in Hematology	Romanowsky like Leishman Geimsa.
Reticulocyte	Supravital (Brilliant cresyl blue), New methyl blue.
Lymphoblast	PAS.
myeloblast	NSE, SBB, Oil Red-O.
monoblast	NSE.
Hairy cell	TRAP.
Lipid	Oil red O, sudan Black.
Iron	Prussian Blue.
Calcium	Von Kossa, alizarin red S.
Glycogen	PAS.
Copper	Rhodamine, rubeanic acid.
mast cell	Toluidine blue.
mucin	Mucicarmine, Alcian blue.
Reticulin Fibres	Silver.
Elastin fibres	Van geison, VVG.
Collagen	masson trichrome.
melanin	masson Fontana.
H pylori	Warthin starry silver.
Cryptococcus	Indian ink.
Fungi	Silver methenamine, PAS, GMS.
Amyloid.	Congo red.

[SOURCE : MARROW E6]