CELLULAR RESPONSES TO STRESS AND TOXIC INSULTS

Adaptation, Cell injury & Cell Death

@2024

PRECLINICALS MBS 220

LEARNING OBJECTIVES

- To discuss cellular adaptation to stress
- To discuss cell injury, causes and mechanisms of cell injury
- To discuss the morphologic alteration of reversible cell injury
- To discuss the morphologic alteration of irreversible cell injury, necrosis and the types of necrosis
- To discuss the cell death and the two pathways of cell death
- Discuss various forms of intracellular accumulations that contribute to cell death

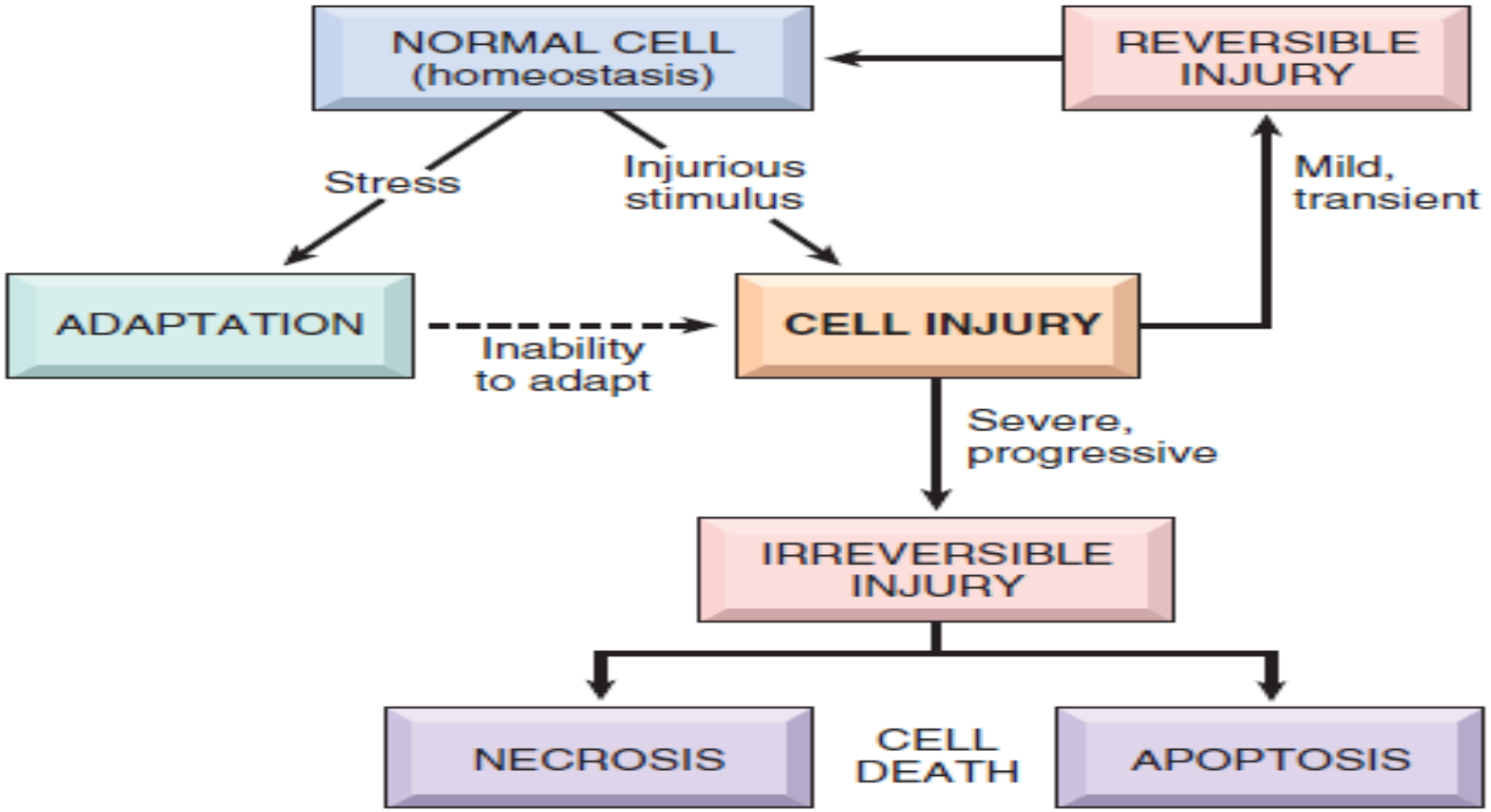
INTRODUCTION

- **Normal homeostasis** – Cells are able to maintain normal structure and function (e.g. ion balance, pH, and energy metabolism) in response to normal physiologic demands.
- Cells are able to handle normal physiologic demands
- Exposure to excess physiologic stress or pathologic stimuli
- The possible outcomes are:
  - The cell may adapt to the situation
  - They cell may acquire a reversible injury
  - The cell may obtain an irreversible injury and may die. The cell may die via one of two ways: either by necrosis or by apoptosis.

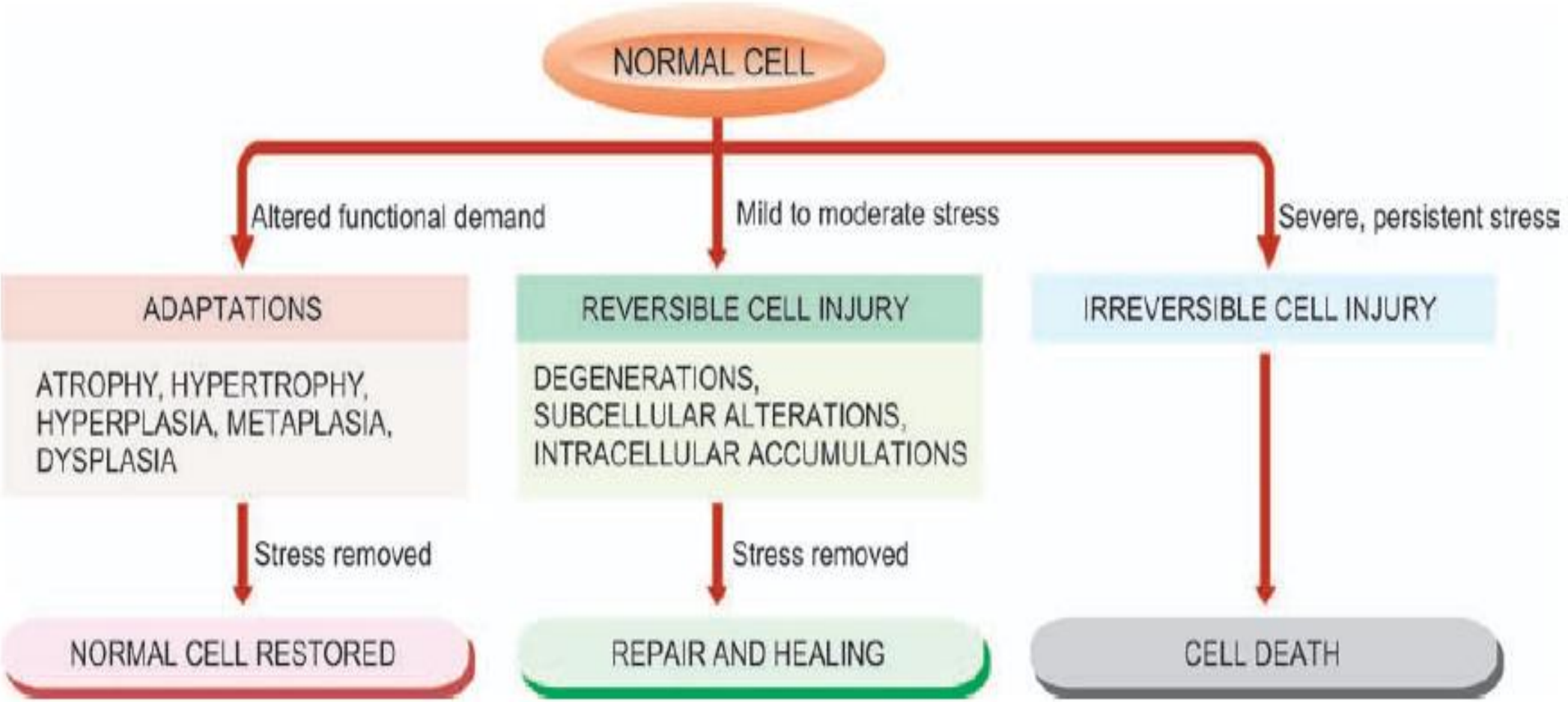
INTRODUCTION – CONT'D

- Excess physiologic stress may result in:
  - Cellular adaptation – in which new altered states are achieved preserving viability of cell
  - Reversible injury – cell injury reversible up to a point
- Residual effects of reversible cell injury may persist in the cell as evidence of cell injury at subcellular level (**subcellular changes**), or metabolites may accumulate within the cell (**intracellular accumulations**)
  - Irreversible injury – if stimulus persists or is severe from the outset
  - Cell death – ultimate result of cell injury i.e. **Necrosis & Apoptosis**
- All maybe stages of a progressive impairment following different types of insults

STAGES OF CELLULAR RESPONSES TO STRESS & INJURIOUS STIMULI



CELLULAR RESPONSES



CELLULAR ADAPTATIONS

- Are Normal reversible changes in the **size**, **number**, **phenotype**, **metabolic activity**, or **functions** of cells in response to changes in their environment
- Reversible **functional** & **structural responses** to more severe physiologic stress & pathologic stimuli
- A new and altered state is achieved.

  - preserves viability of cell and modulates function

1.HYPERTROPHY

- Refers to an increase in **size** of parenchymal cells (and organ)
- No new cells are formed, only larger cells
- Due to synthesis of more structural components of the cells
  - Due increased activation of genes responsible for production of these proteins
- Sometimes a subcellular organelle may undergo selective hypertrophy e.g. patients on barbiturates show hypertrophy of the SER in hepatocytes

Physiologic or **pathologic**

- Due to increased functional demand or hormonal stimulation
- Hypertrophy and hyperplasia may coexist
- Striated muscle cells incapable of cell division. When ↑workload →hypertrophy

TYPES OF HYPERTROPHY

PHYSIOLOGICAL HYPERTROPHY

1. Enlarged size of the uterus in pregnancy

- Estrogenic hormones → stimulate estrogenic receptors on the smooth muscles → ↑smooth muscles proteins → ↑ cell size

2. Hypertrophy of breasts during lactation

3. Skeletal muscle hypertrophy in body builders

TYPES OF HYPERTROPHY

PATHOLOGICAL HYPERTROPHY

1. Cardiac muscle hypertrophy --- Pressure load in diseases (e.g. hypertension, Coarctation of the aorta) or Volume load (e.g. Valvular diseases) ------Cardiomyopathy (familial, viral, toxic, metabolic)
2. Skeletal muscle hypertrophy --- as compensatory rxn following loss or atrophy of surrounding muscle due to myopathic or neuropathic disorder, Muscle fibres will be abnormal in pathologic hypertrophy
3. Smooth muscle hypertrophy ------ of the bladder wall due to ↑resistance to outflow e.g. in cases of enlarged prostate

2. HYPERPLASIA

- Refers to increase in number of **cells** in tissue or organ→↑mass of the organ
- Occurs in cells capable of cell division

Mechanism:

  - Results from growth factor induced proliferation of mature cells or
  - Increased output of new cells from stem cells

- **Physiologic** hyperplasia:

  - hormonal – female breast at puberty or pregnancy

  - compensatory – after hepatectomy

- **Pathologic** – excessive hormonal stimulation e.g. endometrium → abnormal menstrual bleeding
- May give rise to deviations of neoplasms

TYPES OF HYPERPLASIA

PHYSIOLOGICAL HYPERPLASIA

1. Enlarged size of uterus in pregnancy
2. Breast – puberty, pregnancy & lactation

Compensatory hyperplasia

- Regeneration of liver, kidney
- Marrow hyperplasia

TYPES OF HYPERPLASIA

PATHOLOGICAL HYPERPLASIA

1. Hormonal excess --- endometrial, prostate
2. Certain viral infections --- papillomavirus warts

3. METAPLASIA

- Reversible change in which one adult cell type is replaced by another cell type
- Cells sensitive to stress are replaced by cells capable of withstanding stress
- In respiratory tract in smokers
- In oesophageal reflux in Barret's oesophagus
- Malignant transformation in metaplastic epithelium may occur
- Arises from reprogramming of stem (reserve) cells
  - Due to signals generated by cytokines & growth factors & ECM
  - Leads to ↑ expression of genes that promote differentiation to a particular lineage

TYPES OF METAPLASIA

EPITHELIAL METAPLASIA

1. Squamous metaplasia
    1. BRONCHUS
    2. UTERINE CERVIX
    3. GALL BLADDER
2. Columnar metaplasia
    1. Barrett’s oesophagus
    2. Interstinal & gastric metaplasia

TYPES OF METAPLASIA

MESENCHYMAL METAPLASIA

1. Osseous metaplasia
    1. Arterial wall
    2. Myositis ossificans
    3. Stroma of the tumour
    4. Cartilage of larynx in elderly
2. Cartilagenous metaplasia
    1. Healing of fractures

4. ATROPHY

- **Reduced size** of an organ or tissue resulting from a decrease in cell size and number
  - Shrinkage in cell size due to loss of cell substance
- Mechanism
  - Decrease in protein synthesis and increase in protein degradation in cells
- Physiologic atrophy –e.g. thyroglossal duct
- Pathologic atrophy – local or generalized:
  - ↓workload (disuse atrophy) in immobilized limb
  - loss of innervation (denervation atrophy)
  - ↓blood supply →progressive cell loss 2° ischaemia
  - inadequate nutrition e.g. marasmus
  - loss of endocrine stimulation – after menopause
  - pressure – pressure atrophy

TYPES OF ATROPHY

PHYSIOLOGICAL ATROPHY

- During early development

1. Decrease in the size of the uterus during parturition
2. Aging – senile atrophy

TYPES OF ATROPHY

PATHOLOGICAL ATROPHY

- May be localised or generalised

1. ↓workload (disuse atrophy) in immobilized limb
2. Loss of innervation (denervation atrophy)
3. ↓blood supply →progressive cell loss 2° ischaemia atrophy
4. Inadequate nutrition e.g. marasmus
5. Loss of endocrine stimulation – after menopause
6. Pressure – pressure atrophy

DYSPLASIA: NOT A TRUE ADAPTIVE CHANGE

- Refer to abnormal changes in the size, shape, and organization of mature cells
- Not a true adaptive change, often called atypical hyperplasia
- Commonly encountered in the epithelial cells in the cervix & respiratory tract → strongly associated with common neoplastic growth, adjacent to cancer cell
- Classified as "low grade" or "high grade"
- Dysplastic changes are reversible

CELL INJURY

- Are sequences of events that follows when the cell's ability to adapt to physiologic stress or pathologic stimuli are exceeded
- Cell injury underlies all diseases
- These outcomes depend on both the injurious agent and on cellular factors
- **Reversible cell injury (Sub lethal)**
  - Removal of stress will result in complete structural and functional integrity to be restored
- **Irreversible cell injury (Lethal)**
  - Results in cell death

EFFECTS OF CELL INJURY

Stimuli that cause cell injury affect four intracellular systems:

1. Maintenance of integrity of cell membrane
2. Aerobic respiration - involves mitochondrial oxidative phosphorylation and production of ATP
3. Protein synthesis
4. Preservation of integrity of genetic apparatus of cell

CAUSES OF CELL INJURY

Depending on severity of stimulus, cells may adapt, undergo injury or die

1. Oxygen deprivation (Hypoxia, celled ischaemia)
2. Physical agents – trauma, temperature extremes, electric shock, radiation
3. Chemical agents and drugs
4. Infectious agents – bacteria, fungi, viruses e.t.c
5. Immunologic reactions – e.g. anaphylactic reaction
6. Genetic derangements – e.g. sickle cell anaemia
7. Nutritional imbalance – Vitamin or protein deficiency

1. HYPOXIA (OXYGEN DEPRIVATION)

- Most **important** & **common** causes of cell injury and cell death.
- Causes impairment of **oxidative respiration**
- Occurs with:
  - Deficient blood supply
- **Ischemia**: deficiency of blood supply from impeded arterial flow or reduced venous drainage results in hypoxia + ↓ delivery of nutrients and ↓ removal of metabolites.
- **Infarction**: localized area of **ischemic necrosis**.
  - Reduced oxygen - carrying capacity of the blood
- Due to **anaemia**: reduction in numbers or volume of erythrocytes or quantity of hemoglobin (Hb)

2. PHYSICAL AGENTS

- Direct mechanical trauma - lacerations or crush injuries.
- Temperature extremes - heat (thermal burn), cold (frostbite).
- Radiation - e.g. UV light, x-rays
- Electric Shock

3.CHEMICALS, DRUGS & TOXINS

- Inorganic poisons - e.g. lead (Pb), arsenic, selenium, mercury, etc.
- Organic poisons - e.g. nitrate/nitrite, oxalate, hydrocyanic acid, etc.
- Manufactured chemicals - e.g. drugs (overdose), pesticides, herbicides, rodenticides, etc.
- Physiologic compounds - e.g. salt, glucose, oxygen, etc.
- Plant toxins – wild mushroom,
- Animal toxins - e.g. snake or spider venom, tick toxin, etc.
- Bacterial toxins / mycotoxins - e.g. botulinum toxin, aflatoxin, ergot, etc.

4. INFECTOUS AGENTS

- Viruses
- Bacteria / rickettsiae / chlamydia
- Fungi
- Protozoa
- Metazoan parasites

5. IMMUNOLOGIC REACTIONS

- Hypersensitivity (allergic) reactions - e.g. anaphylactic reaction to a foreign protein or drug.
- Autoimmune diseases - reactions to self-antigens

6. GENETIC ABNORMALITIES

- Mutation, chromosomal abnormalities, disease-associated gene variants, polymorphism
- e.g. sickle cell anaemia, neoplasia, hypertension, coronary artery disease

7. NUTRITIONAL IMBALANCES

- Deficiencies - of protein/calories (starvation), vitamins, minerals (e.g. copper).
- Vitamin deficiency
  - Vitamin A - night blindness
  - Vitamin D - rickets and osteomalacia
  - Vitamin K - bleeding diathesis
  - Vitamin B12 - anaemia (e.g. pernicious anaemia), neuropathy and spinal cord degeneration
  - Folate - anaemia and neural tube defects
  - Niacin (Vitamin B3) - pellagra (diarrhoea, dermatitis and dementia)
- Over nutrition - e.g. excess lipids / calories - obesity, diabetes, atherosclerosis, etc.
  - Hypervitaminosis e.g. Hypervitaminosis D

GENERAL CELLULAR RESPONSES TO INJURY

- Adaptation
  - Reversible injury
  - Irreversible injury and cell death (necrosis/apoptosis)
- Cellular response to injury depends on several important factors

- The *type* of injury
- The *duration* of injury
- The *severity and intensity* of injury
- The *type of cell* injured
- The cell's *metabolic state*
- The cell's *ability* to *adapt*

REVERSIBLE CELL INJURY

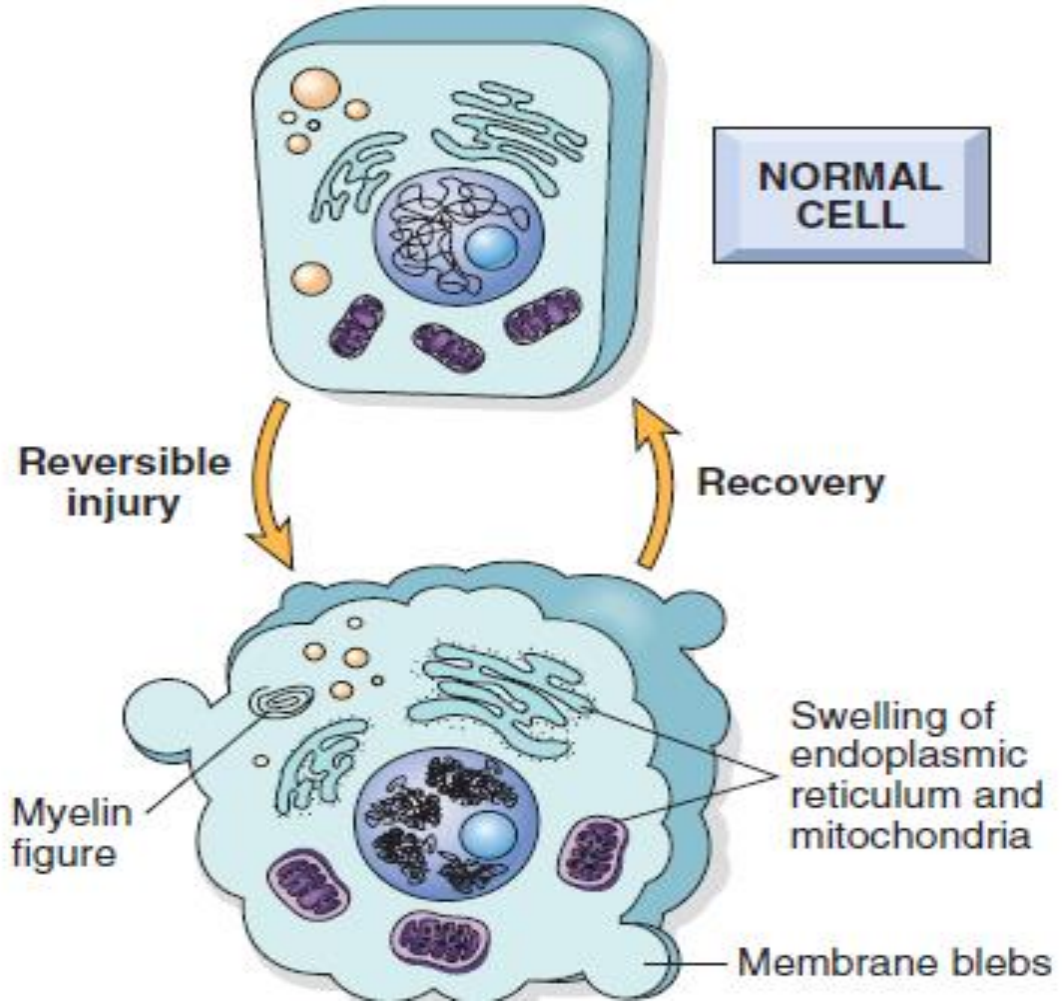
- First point of attack is cell's aerobic respiration
- Hypoxia causes ↓O_2 tension and ↓generation of ATP This results in:

- ↓activity of Na+, K+-ATPase causing Na+ to accumulate intracellularly →cell swelling
- Anaerobic (glycolysis) as energy source causes ↑lactic acid, inorganic phosphates leading to ↓intracellular pH → Nuclear clumping
- Detachment of ribosomes from RER →disruption of protein synthesis
- Formation of blebs and myelin figures

MORPHOLOGICAL ALTERATIONS IN REVERSIBLE CELL INJURY

- Cellular swelling – appears due to alterations in ionic and fluid homeostasis → failure of the ion pumps in the plasma membrane
- When affecting many cells it causes pallor, increased turgor & ↑ organ weight
- Fatty change - occurs in hypoxic injury & various forms of toxic/metabolic injury manifested by the appearance of lipid vacuoles in the cytoplasm
- This non lethal pattern of cell injury is called hydropic change or vacuolar degeneration

MORPHOLOGIC ALTERATION IN CELL INJURY



MORPHOLOGIC ALTERATION IN CELL INJURY (**DEGENERATION**)

- This is the morphological and functional changes in a cell as a result of injury(insult/Stress)
- It is also known as ‘ **cell sickness**’
- Such cells under go some morphological, biochemical and functional alteration
- Operations of such cells departs from normal operation
- In degeneration it can be said that the cells are sick
- The process is reversible if the insulting agent is removed

Cont’d

- The cells restores its structural and functional integrity
- E.g. In the heart the interruption of circulation for less than 30 minutes all the functional and structural alteration is restored

TYPES OF DEGENERATION

- There are various types of degeneration
- There are basically divided into:
- A. INTRACELLULAR degenerative changes
- B. EXTRACELLULAR degenerative changes

a. INTRACELLULAR DEGENERATIVE CHANGES

- Intracellular degenerative changes are further divided into

1. **Cloudy swelling**.

- This is a disturbance in protein metabolism in which the cells imbibe more water and swell
- Their cytoplasm become more granular than normal.
- This is the first sign of cell injury

Etiology

- caused by mild irritants
- Especially bacterial toxins, fever, diabetes, organic and inorganic poisons (Pb, As) , anaemia

CONT’D

Grossly

- cloudy swelling is easier to see in cellular organs e.g. in **heart**, **liver** and **kidney**.
- But it can occur in all organs and tissues of the body
- Organ is slightly enlarged, edges rounded more than usual .
- Organ appears anemic due to pressure.
- Cut surfaces bulges , capsule tight; cloudy and slightly opaque as if slightly scalded or cooked.
- But it can occur in all organs and tissues of the body

Ultrastructural changes includes:

- **Plasma membrane alterations** such as blebbing, blunting or distortion of the microvilli and loosening of the intercellular attachment
- **Mitochondrial changes** such as swelling and the appearance of phospholipid rich amorphous densities
- **Dilatation of the ER** with the detachment of the ribosomes
- **Nuclear alterations**, with disaggregation of the granular and fibrillar elements
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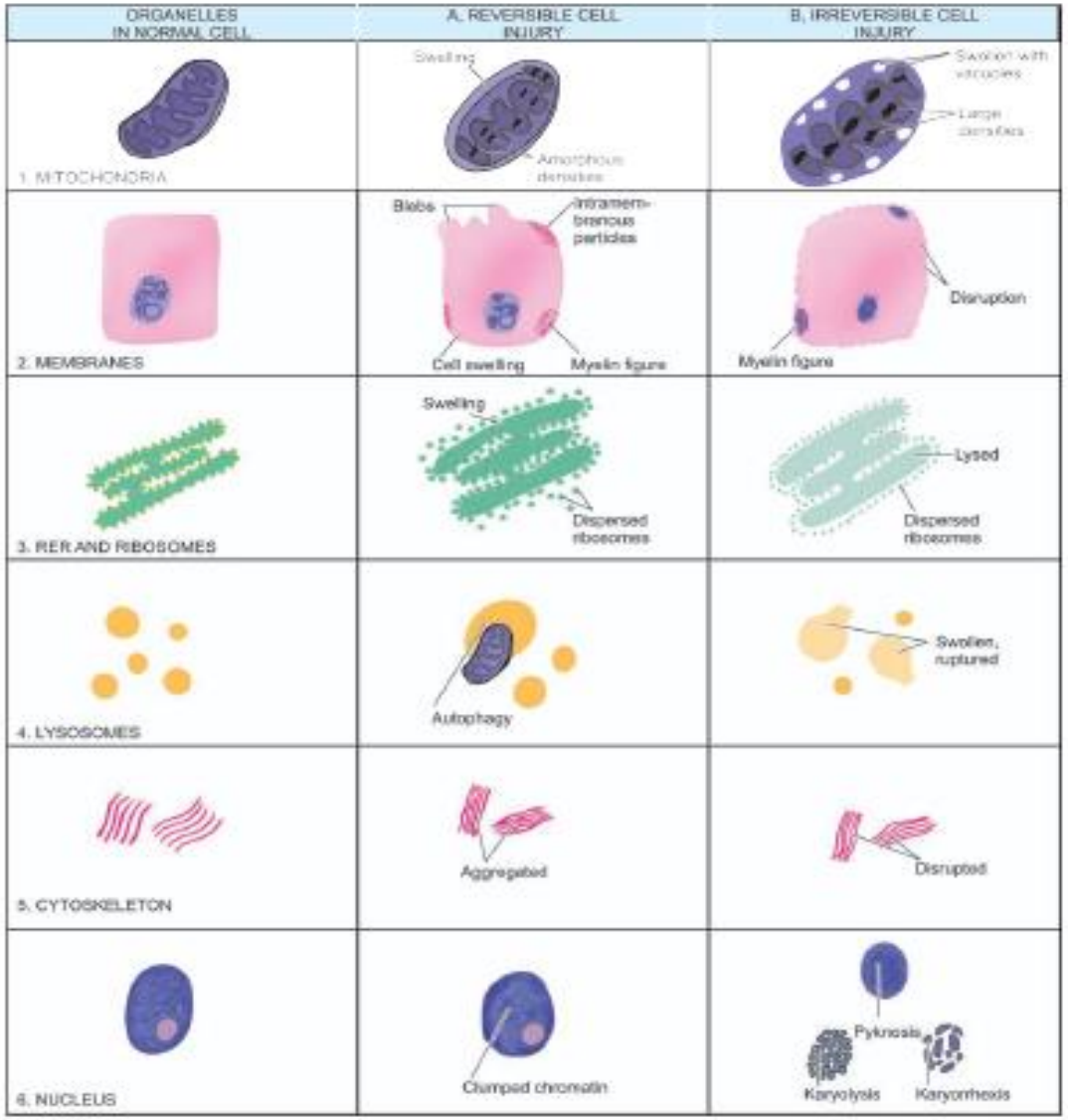
ULTRASTRUCTURAL CHANGES DURING CELL INJURY DUE TO HYPOXIA-ISCHAEMIA



CONT’D

Microscopy

- Best seen in renal convoluted tubules, skeletal and cardiac muscle.
- **Colloidal changes** in intracellular proteins cause increased colloidal pressure and the cells imbibe too much water and swells, edges become rounded , angular e.g. in cross section, loose their angularity becomes rounded or spherical.
- The cell becomes more **eosinophilic** but internal structure becomes hazy and cytoplasm more granular than normal
- The granules are solvents in acetic acid but not lipoid solvent.
- Cloudy swelling could be confused with postmortem autolysis where cells also swell but not so much.
- To avoid confusion remove tissues for histology quickly after death and fix in formalin

CONT’D

Significance

- Cloudy swelling represents mild tissue irritation
- It is of **little diagnostic** value because when cause is removed , the granules disappear and the cell becomes normal in structure and function

INTRACELLULAR DEGENERATIVE CHANGES

2. **Hydropic degeneration**

- Disturbance in protein metabolism in which the cells imbibe too much water and burst

Etiology:

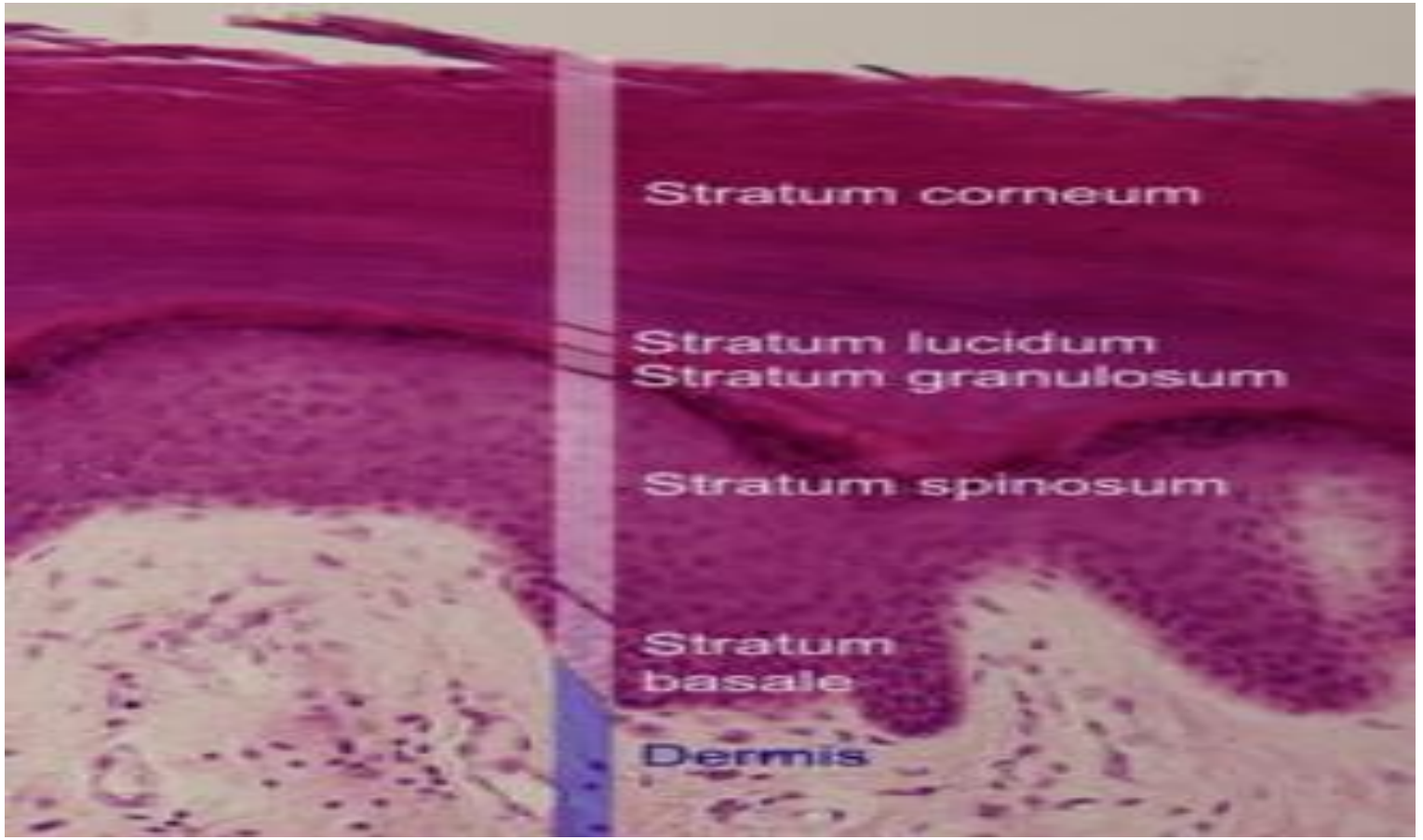
- Mechanical injury, especially rubbing type force, causes hydropic degeneration of stratified epithelial cell
- Thermal injury: heat or cold on skin---- hydropic degeneration
- Chemicals applied to mucosae by accident or for therapy
- Infectious agents especially viruses acts on stratified squamous epithelia, e.g. pox virus
- Neoplasms, cervical tumours-----hydropic degeneration

CONT’D

Grossly

- Skin has raised puffy appearance due to water in the prickle cell layer.
- Superficial epidermis is tight and stretched.
- When incised, fluid escapes and swelling collapses , but the stratum corneum does not retract to its former size and shape: its dead.

A **prickle cell** is an epidermal cell which lies above the basal cell layer, constituting a **stratum spinosum**, which forms innumerable intercellular bridges



CONT’D

- The underlying tissue is hyperemic and sensitive to touch
- In few days stratum corneum dries and sloughs
- New epidermis regenerates to cover the wound
- Lesion can be aggrevated by pyogenic organisms
- Hydropic degeneration is very difficult to recognize in cuboidal, columinar, transitional and pseudistratified epithelium.

CONT’D

Microscopically

- Begins with increased cell size and water droplets in the cytoplasm(small vacuoles).
- Droplets coalesce and get so big that it results in the bursting of the cell membrane.
- Water from the adjacent busted cells coalesce to form bigger reservoir usually in the prickle cell layer and then cause the epidermis above to bulge (blister, vesicle).
- The stratum corneum cannot undergo hydropic degeneration because the cells already dead and cornified.
- The stratum germintavum or basal cell layer is rarely affected because it is too close to the blood and lymphatic vessels

INTRACELLULAR DEGENERATIVE CHANGES

3. Mucinous or mucous degeneration

- Excess accumulation of mucin in degenerated cells.
- Mucin is a glassy, stringly, viscid, slimy glycoprotein usually produced by columnar and cuboidal cells especial in the mucous membranes.
- Mucin plus water or tissue fluid becomes what is known as mucous

Causes

- It occurs when mild irritants are applied to the mucous membranes such as:
  - Mechanical (trauma)
  - Chemical (disinfectants and soap)
  - Heat and cold in moderate amount
  - Neoplasms of columnar epithelia- adenocarcinoma

CONT’D

Grossly

- Clear transparent fluid which becomes opaque with accumulation of neutrophils in secondary bacterial invasion

Microscopically

- Mucin first appears as small droplets.
- Adjacent droplets in cells coalesce and form larger ones located in the apical pole and push nucleus to the basal pole of the cell where become crescentric and may die.
- The force of mucous ruptures cells

INTRACELLULAR DEGENERATIVE CHANGES

4. Fatty degeneration

- Is a retrogressive change in which **fat droplets** appear in the cytoplasm of cells

Etiology

- Causes are irritants which are more severe than those causing cloudy swelling.
- Change is so severe that **the colloidal fat** is unmasked and appear as droplets

Causes

- Inadequate amounts of oxygen frequently causes fatty degeneration e.g. in **anaemia**, hemorrhages of various origins including blood sucking parasites and hemoparasites

CONT’D

- In chemical poisons: Organic and Inorganic, bacterial toxins, poisonous plants e.g senecio, astragalus, crotalaria, chloroform, phosphorus, Pb, As
- Metabolic diseases: diabetes etc.

Gross

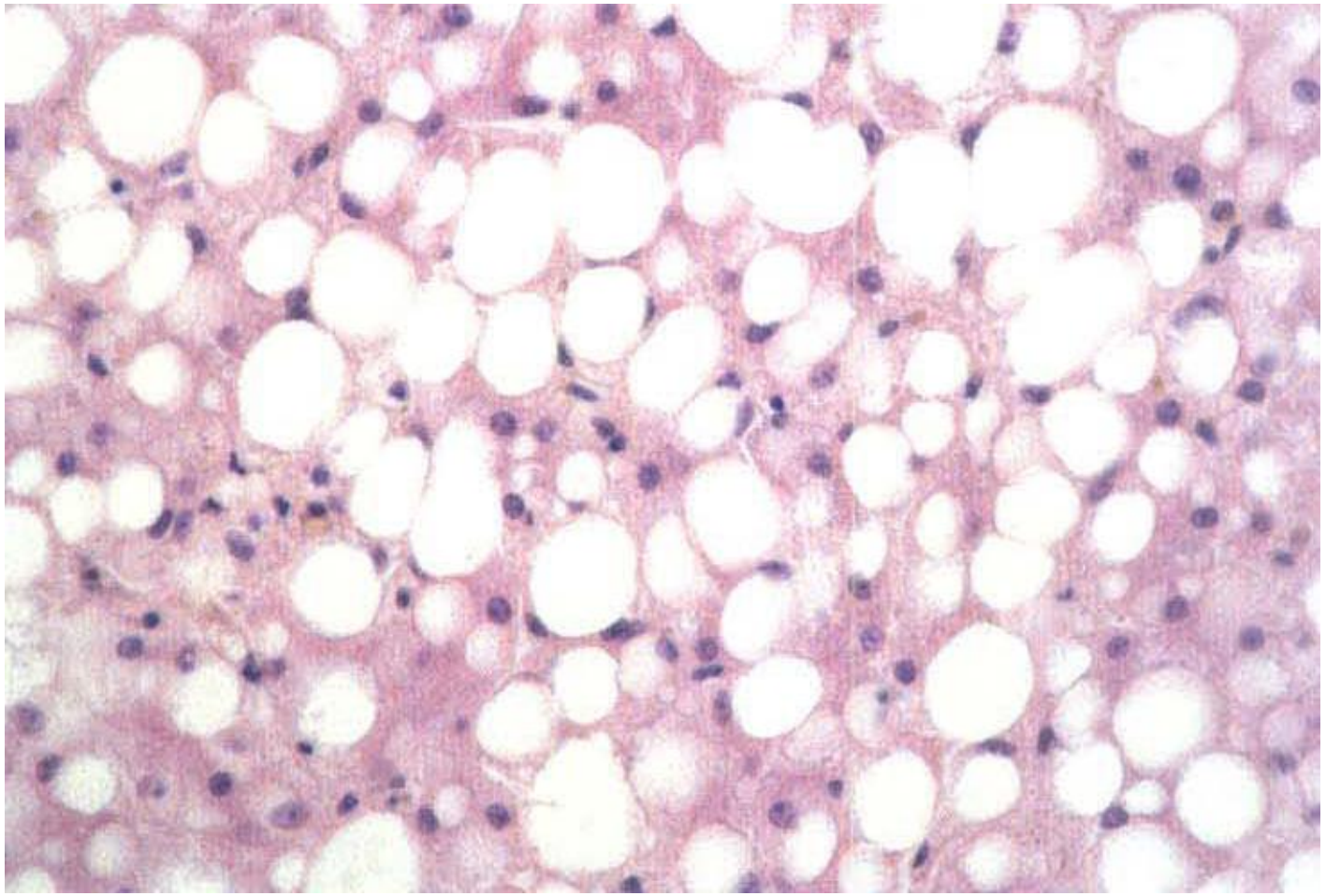
- Organ may be normal in size or enlarged.
- When enlarged it becomes rounded , friable, greasy and apiece floats in water

Microscopy

- Droplets of varying sizes appear as vacuoles nucleus may be central or pushed to the periphery

Fatty liver

note that the vacuoles displaces nucleus to the periphery



CONT’D

Significance

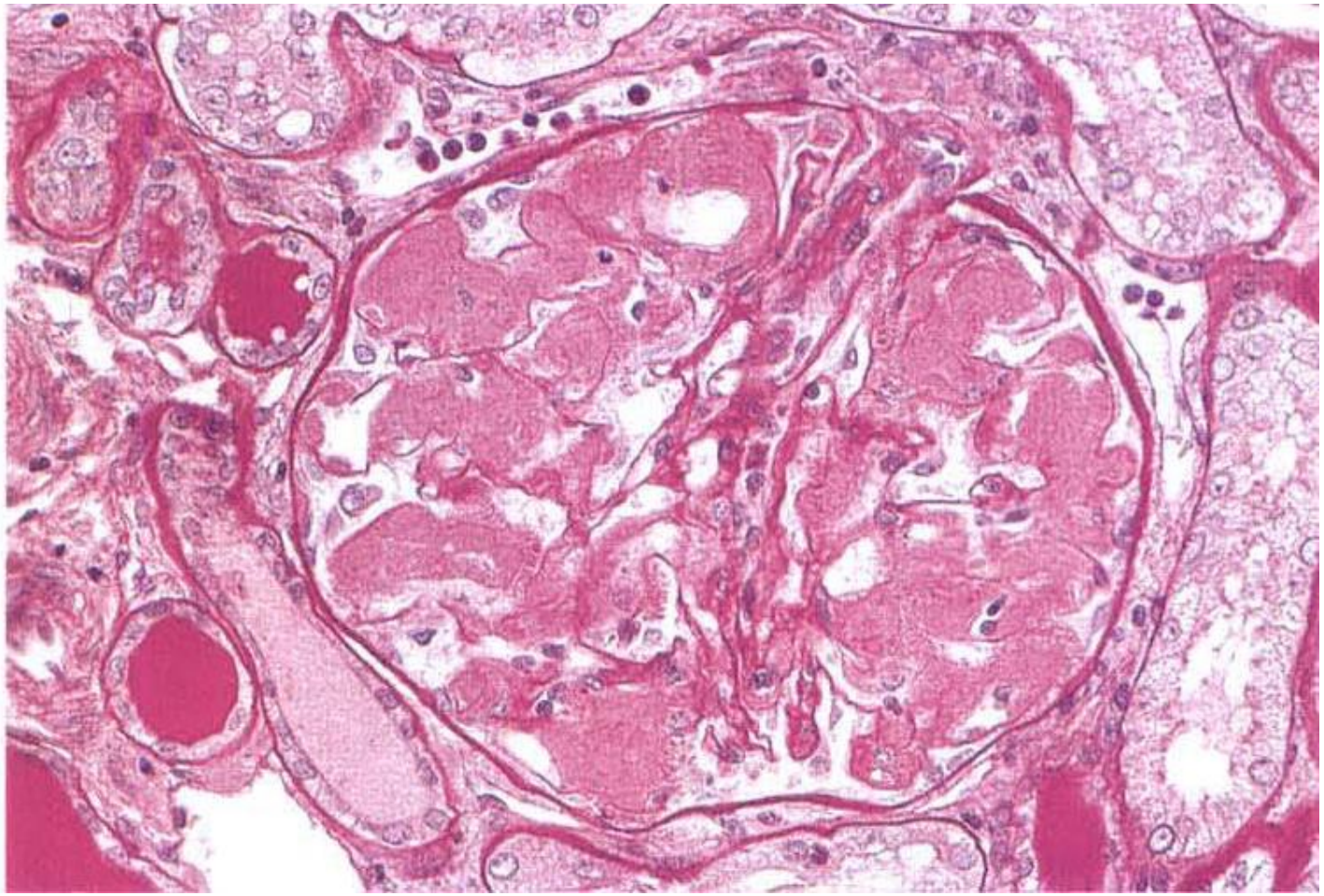
- Unless cause is removed early, **cell will die**.
- Too much fat inside the cell will disturb the affected organ which can’t function normally

b. EXTRACELLULAR DEGENERATION

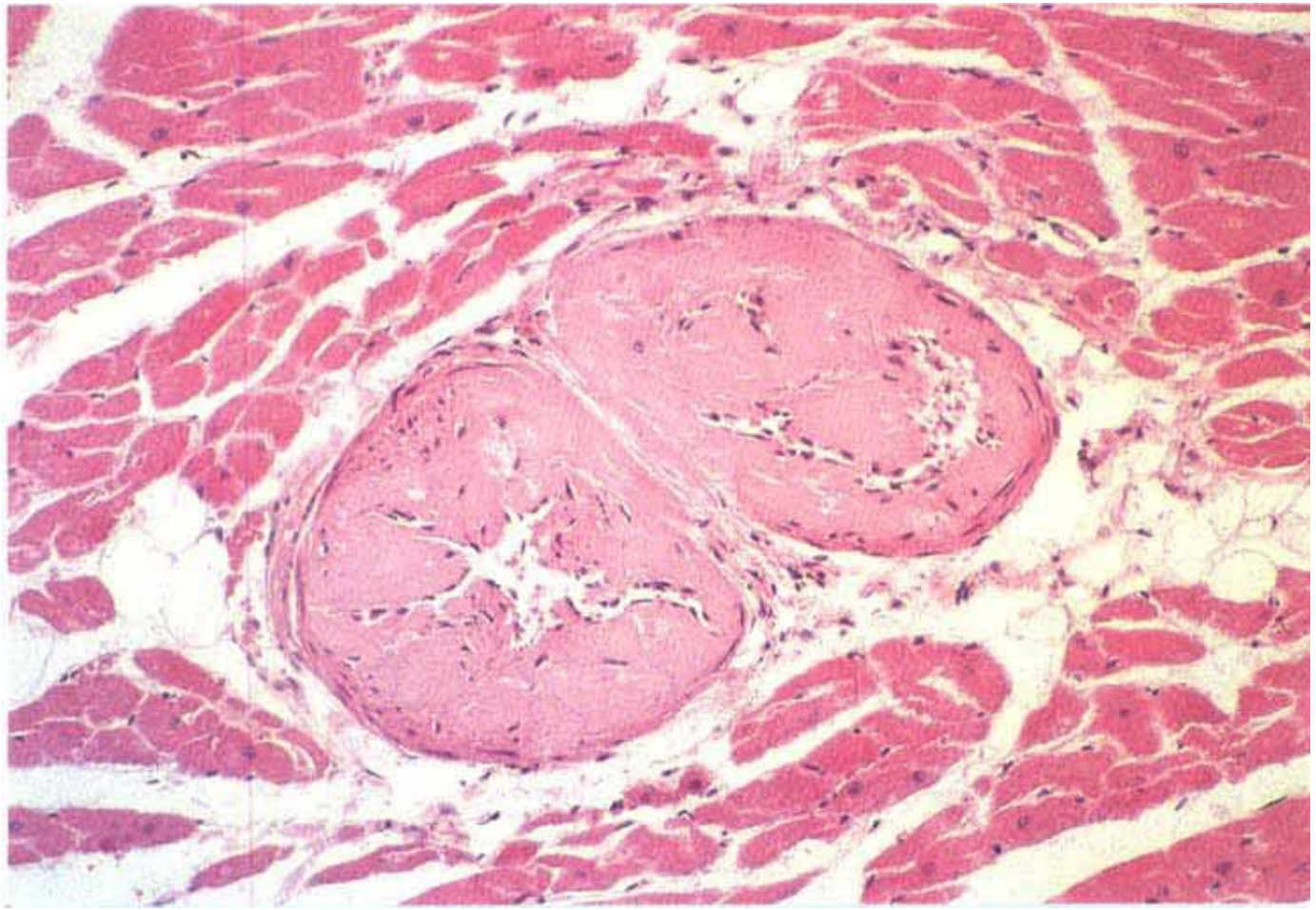
1. **Amyloid Infiltration**

- The deposition of a starch-like substance between the capillary endothelium and adjacent cells.
- The disease is called amyloidosis.
- The term amyloid means starch-like, because the substance reacts with iodine to give a brown, blue or black colour

Kidney amyloidosis



Coronary artery amyloidosis



CONT’D

Chemistry:

- Amyloid is a **protein polysaccharide**.
- It is quit resistant to digestive enzymes except artificial gastric juices which dissolve it slowly.
- Once deposited in tissue it for remains for life.
- Rarely does it ware off once cause is removed

Occurrence:

- It occurs in all animals.
- But today, because of tissue culture vaccines amyloids have become a rare condition

CONT’D

Pathogenesis:

- Deposition of amyloids thought to be the result of precipitation ag- ab complexes.
- Affected organs – mainly the spleen, liver , adrenals, kidneys and intestines .
- Amyloid is deposited between vascular endothelium and the adjacent cells of the affected organ especially around the capillaries but rarely around the arterioles and venules.

CONT'D

Results of amyloidosis:

- Amyloid is an unyielding and therefore press onto affected blood vessels and cause stenosis with resultant ischaemia in area supplied
- The amyloid cuff is impervious to gas and liquid.
- Therefore exchange of nutrient and waste products of cell metabolism are retarded and the result is **necrosis**, **degeneration** and **atrophy** of organs
- When massive, amyloid cause pressure atrophy of surrounding tissue

CONT’D

Grossly:

- The lesion may be single or multiple, in one or more organs especially the spleen, kidney, liver, adrenals and intestines.
- It may occur in areas of purulent inflammation e.g. lungs with tuberculosis.
- In the spleen amyloids form cuffs around the central arteries of the splenic corpuscle from which it extends to surrounding splenic pulp.

CONT’D

- In the liver it is usually extensive, leading to severe enlargement of the organ.
- Edges are rounded, **colour is cyanotic**, **yellow** and **very friable**, ruptures easily.
- Usually the mere attempt to remove it out of the cadaver sinuses it to rupture.
- Death is usually caused by hepatic rupture with resultant hemorrhages.
- They may be some old ruptures which have partially healed.
- It causes pressure atrophy of hepatocytes

CONT’D

The kidney

- In the glomeruli the most damage is due to obliteration of the glomeruli capillaries and tubular epithelium atrophy and necrosis.
- The kidney is swollen with bulging cut surface.

CONT’D

- In the pancreas, the **amyloids** occur in **islets of langerhans**.
- In the skin amyloidosis is a diffuse lesion in skin and subcutaneous connective tissue.

Microscopy:

- Amyloids **stain pink with eosin** , Van Gieson- amyloid yellow; Connective tissue and collagen-red
- Iodine reaction: place a piece of tissue in 3% acetic acid to take out the RBC (Iodine reacts with rbcs to mask the colour).

Significance:

- Amyloids is significant in the kidney where it causes uremia. In the pancreas it causes diabetes. In the liver it causes rupture

CONT’D

2. **Hyaline degeneration**

- A term used to describe heterogeneous tissue changes which are all translucent, homogeneous and stain deeply eosinophilic

Biochemistry:

- Hyaline = a **protein of varying** composition and it may living or dead.
- Its found in many organs, tissue and cells.
- It is the physical appearance of the tissue rather than its chemical composition which determines whether hyaline degeneration is present or not

CONT’D

Causes:

- There is no specific cause but it can be brought by a variety of conditions which produce **protein metabolic disturbances**.
- In some areas , hyaline is normal e.g. in the cornea, skin, ovaries, scars.
- Try to use the specific name of a particular type of hyaline degeneration rather than the general name of hyaline degeneration.

CONT’D

Three major hyaline

- *Connective tissue hyaline*
- *Keratohyaline*
- *Cellular hyaline*

Gross:

- Hyaline is difficult to see in small amounts, but it is a glassy or opaque streaks or pecks in tissues the hyalinized tissue is firm translucent and inelastic.
- The connective **tissue scar** remaining in the site of an injury is an example of hyaline

CONT’D

- Microscopy:
- Hyaline is a homogeneous mass which stains intensely with eosin.
- The affected cell and tissue have lost their architecture and fused into homogeneous mass.
- The process is that of coagulation and dehydration, with minimal metabolism remaining

CONT’D

Connective tissue hyaline

- Occurs in scar tissue, degenerating tumours, lymph nodes draining areas of chronic inflammation, renal glomeruli, media and intima of blood vessels

Gross:

- It appears smooth, dense, firm tough bluish-white, shiny layer of deposits

Microscopy:

- The mass contains a few cells and nuclei and devoid of capillaries.
- The mass stains pink with eosin.
- With van Gieson, the connective tissue stains red, but doesn’t stain red with methyl violet and congo red like amyloid

CONT’D

Significance:

- Hyaline indicates that the tissue has been injured or altered.
- The change is permanent for life and the consequences depends on organ or tissue affected

CONT’D

Cellular hyaline

- Hyaline which is different from that of connective tissue or keratohyline and results from fusion and dehydration of cells.
- Stain deeply with eosin

Occurrence.

- Form in glandular lumen.
- They also occur in lung in areas of infarcts and pneumonia.
- In that case the cellular exudates in the alveoli are compressed , dehydrated and rolled into spherical masses.

CONT’D

Grossly:

- Not easy to see but like grain of sand

Microscopy

- Laminated mass

Significance:

- corpora amylacea indicates that there has been excess desquamation of epithelial cells.
- No pathological significance.
- If secretory or excretory duct present, it is voided out

CONT’D

- Hyalinization of thrombocytes help in forming blood clot to arrest hemorrhages
- On the other side if it happens in some circumstances it causes thrombus

CONT’D

Keratohyaline

- Is produced by the normal skin = physiological keratohyalin

Causes

- Viruses of warts = papillomatosis
- Highly chlorinated naphthalenes = squamous cell carcinoma
- Friction of pressure of shoes = cones and bunions
- Cancer of the skin

CONT’D

Macroscopy

- Cornfield epithelia is firm, hard and almost colourless and glassy

Microscopy

- Homogeneous mass which stains pink

Significance

- A protective coating

CONT’D

3. Gout

- Deposition **of sodium** and **calcium urates** in connective tissue and serous membranes.

Causes

- It is associated with high protein diets

Macroscopy

- Man gets synovial gout

Microscopy

- Needle shaped crystals of sodium and calcium urates found in affected tissues unfortunately most is removed in paraffin sections leaving empty clefts.

Significance

- Prognosis is poor.
- The crystals may disappear if the cause is removed

EXTRACELLULAR DEPOSITION

4. Fibrinoid degeneration

- An **amorphous**, bright eosinophilic material found in the walls of blood vessels
- Fibrin is the major component along with serum proteins(immunoglobulins)

CONT’D

- There is a **strong association** between presence of **Fibrinoid** and **acute immunologic** reaction
- The lesion involves the antigen-antibody complexes
- The combination cause severe injury to the vessel wall and leakage of plasma proteins into lesions
- It is associated with severe injury in connective tissue as in mast cell tumours

CONT’D

- Other locations are spinal canal cerebral ventricles where they are **called brain sand**.
- Also in islet of Langerhans of patients with diabetes, in thrombi due to fusion of thrombocytes, in blood capillaries with sludged blood or in incompatible transfusion, when RBC form a roleux and fuse together

EXTRACELLULAR DEPOSITION

5. Fat infiltration

- is the accumulation of fat in white fibrous connective tissue, liver, kidney and adrenal parenchyma.
- The commonest site is the mesentery, omentum and subcutaneous connective tissue.
- They are fat deposits which get neutral fat composed of triglycerides of palmitic stearic and oleic amino acids

CONT’D

- When the body needs that fat, it has to get from the depot to the liver for transformation into colloid fat.
- Some of it may accumulate in the hepatocytes and form small droplets which coalesce and may push the nucleus to the side

EXTRACELLULAR DEPOSITION

6. Calcification

- Refers to the deposition of calcium salts in soft tissues
- The lesion occurs in a variety of circumstances and requires proper interpretation
- Special stains can specifically identify calcium in tissue
- It stains **blue** with hematoxylin eosin and can be confused with bacteria

CONT’D

- Pathologic calcification should be clearly separate from bone production
- Ectopic or metaplastic bone formation does occur
- But calcification in this context is used to refers deposition of phosphates and carbonate salts of calcium in soft tissues, but not bone formation

CONT’D

Forms of calcification

Two forms of calcification

i. **Dystrophic calcification**

- Implies **tissue damage**, degeneration or death of cells results into denaturation of proteins in tissues which allows the salts of calcium to precipitate
- Calcium enters rapidly in most of the degenerating cells

CONT’D

- But some tissues seem to have a higher affinity for taking up calcium
- Muscles have this particular affinity
- The calcium deposits appears **white** grossly and has a **grity texture**
- Dystrophic calcification may be prominent in some **chronic tissue destructive lesions** such as in **TB**
- It has some association with some specific diseases but also occurs in non degenerative or necrotic lesions

CONT’D

- It occurs in atheromas of advance atherosclerosis, in areas of intima injuries in aorta and large arteries
- It is old age related
- For example it develops in aging or damaged heart valves
- This results in severely compromised heart valve motion
- It can also result in aortic valve stenosis in the elderly

CONT’D

ii. Metastatic calcification

- Occurs as a deposition on the **basement membranes** and **elastic fibres** of several organs, particularly arteries
- It implies high levels of serum calcium, excess vitamin D or hyperparathyroidism
- The deposits may be smooth on all basement membranes or irregular in distribution

CONT’D

Causes of metastatic calcification

- Metastatic calcification occurs in tissues whenever there is **hypercalcemia**
- There four major causes of hypercalcemia
- i - Increased secretion of **parathyroid hormone**, due to either primary parathyroid tumours or other malignant tumours
- ii - Destruction of bone due to effects of **accelerated turnover**(e.g. Pagets disease), immobilization, or tumours(increased bone catabolism associated with multiple myelomas, leukemia's or diffuse skeletal metastasis

CONT’D

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CONT’D

- iii - Vitamin D- related disorders, including vitamin D intoxication
- vi - Renal failure, in which there is phosphate retention which leads to **hyperparathyroidism**

- Calcium may also be deposited intracellularly
- In this case it tends to be deposited on organelles like the mitochondria
- The term **calcinosis** is used to describe extensive metastatic calcification

CONT’D

Microscopically

- Appearance of calcium will vary from a smooth, shiny blue colour along the basement membranes
- It may also appear as dark blue clumps depending on the location and type of calcification
- There is an association between metastatic calcification of the kidney and uremia
- Can use Von Kossa’s stain which makes calcium appear as black granules
- Stains blue with H&E

OTHER EXOGENOUS PIGMENTS

Anthracosis

- Deposition of carbon particles (especially from air pollution), into lungs / lymph nodes.
- Gives tissue a black discoloration to tissue; seen in macrophages in lungs and draining lymph nodes.
- Relatively harmless unless present in large quantities (e.g. chronic lung injury in coal miners).

EXOGENOUS PIGMENT – CON'TD

Silicosis

- Deposition of silica dust in the lungs is a special problem for miners; causes granulomatous pneumonia.

Asbestosis

- Deposition of asbestos into lung, associated with mesotheliomas, and chronic lung injury.

ENDOGENOUS PIGMENTS

Melanin

- Melanocytes and melanoblasts, located primarily in the basal layers of the skin convert L-tyrosine into melanin.
- Melanin is normally found in the skin, retina, choroid and iris as minute brown or black granules in the cytoplasm of epithelial cells.
- Melanin imparts colour to the body and serves as protection against ultra-violet rays of the sun.
- Complete lack of melanin is a congenital defect referred to as albinism.
- Grossly: melanosis will appear as a dark (pigmented) area of otherwise normal tissue

PATHOLOGICAL CONDITIONS ASSOCIATED WITH MELANIN

- **Melanosis** - the presence of melanin in the abnormal location e.g. leptomeninges, intestine, kidney, lung, base of aorta, etc.
- **Melanomas** - these are tumours of the melanocytes and melanoblasts. They may be benign or malignant but usually have a poor prognosis
- **Leukoderm** - is a focal area of the skin which lacks pigmentation. It could be due to copper deficiency (copper being an essential component of L-tyrosinase, scars and radiation burns. It is also known as **achromotricia**

HAEMOGLOBIN – DERIVED PIGMENTS

- **Hemosiderin**
  - Golden-yellow to brown pigment
  - Major storage form of iron
  - Hemosiderosis – hemosiderin deposited in cells and tissues in systemic overload of hemosiderin
- **Bilirubin**
  - Jaundice or icterus
    - Pre hepatic
    - Hepatic
    - Post hepatic

IRREVERSIBLE INJURY

If hypoxia persists irreversible injury ensues:

1. **Calcium influx**: Mitochondrial damage.
   - Severe swelling of mitochondria
   - Extensive damage to cell membrane and swelling of lysosomes
   - Large amorphous densities in mitochondrial matrix
   - Massive influx of calcium into cell
2. **Activated phospholipases**: Membrane damage.
3. **Intracellular proteases**: Cytoskeletal damage
   - Normal cytoskeleton of the cell (microfilaments, microtubules and intermediate filaments) which anchors the cell membrane is damaged due to degradation by activated intracellular proteases

IRREVERSIBLE INJURY - CONTD

4. **Activated endonucleases**: Nuclear damage.
   - Damage caused by proteases and endonuclease
5. **Lysosomal hydrolytic enzymes**: Lysosomal damage, cell death and phagocytosis.
   - Injury to lysosomes causes enzyme leakage and activation of acid hydrolases →digestion of cell components
   - Enzyme leakage used in clinical diagnosis e.g. CK MB, troponin, in myocardial infarction

NECROSIS

- “The morphological changes that follow cell death in living tissue or organ, resulting from the progressive degradative action of enzymes on the irreversibly injured cell”.
- Results from:
  - enzymatic digestion of the injured cell
  - denaturation of intracellular proteins
- Autolysis – enzymes derived from cell itself
- Heterolysis – enzymes derived from leukocytes outside dying cell

MORPHOLOGY OF NECROTIC CELLS

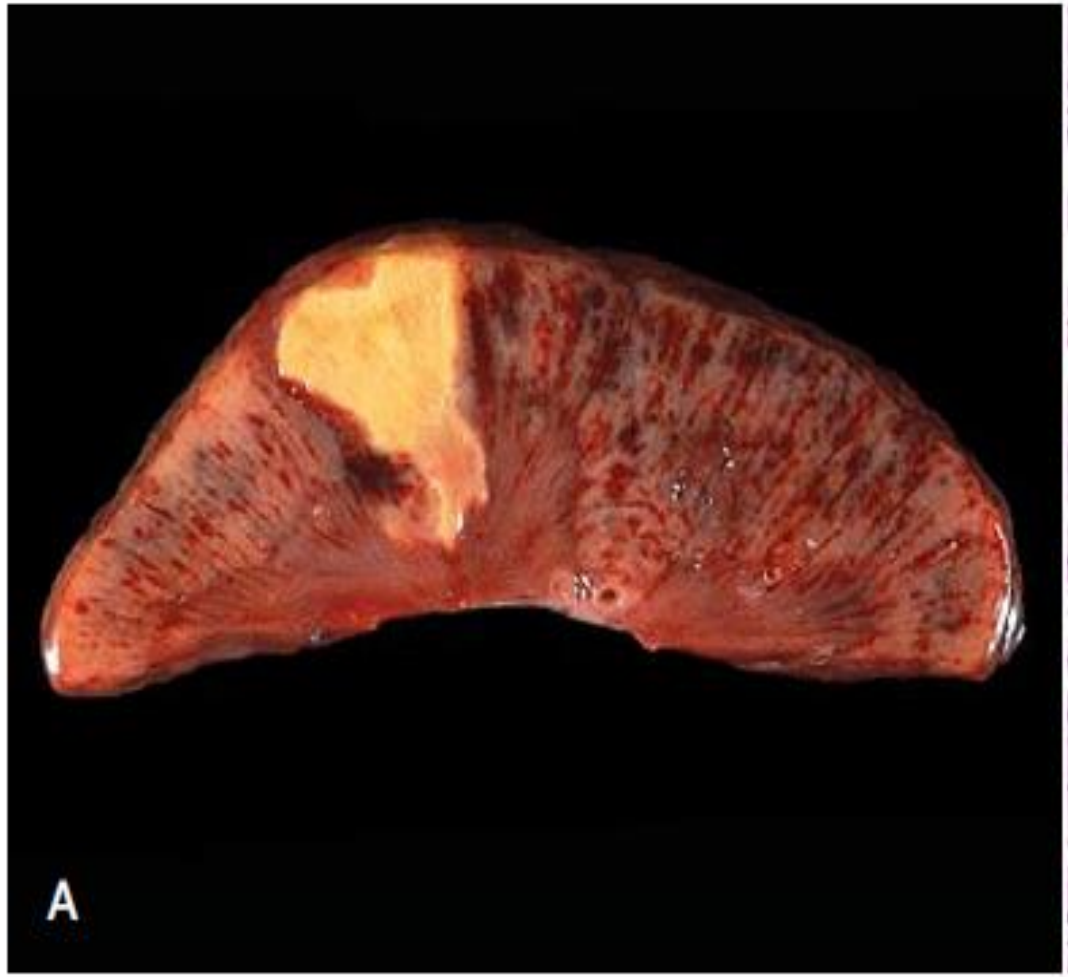
- Increased eosinophilia
- Glassy homogenous appearance
- Cytoplasm is vacuolated and moth eaten
- Calcification of dead cells may occur
- Nuclear changes:
  - **Pyknosis** – shrinkage of nucleus and ↑basophilia
    (condensation of the nucleus, homogenous and black)
  - **karyolysis** – fading of basophilia of chromatin (dissolution of nucleus).
  - **karryorexis** – fragmentation of pyknotic nucleus (fragmentation of the nucleus)

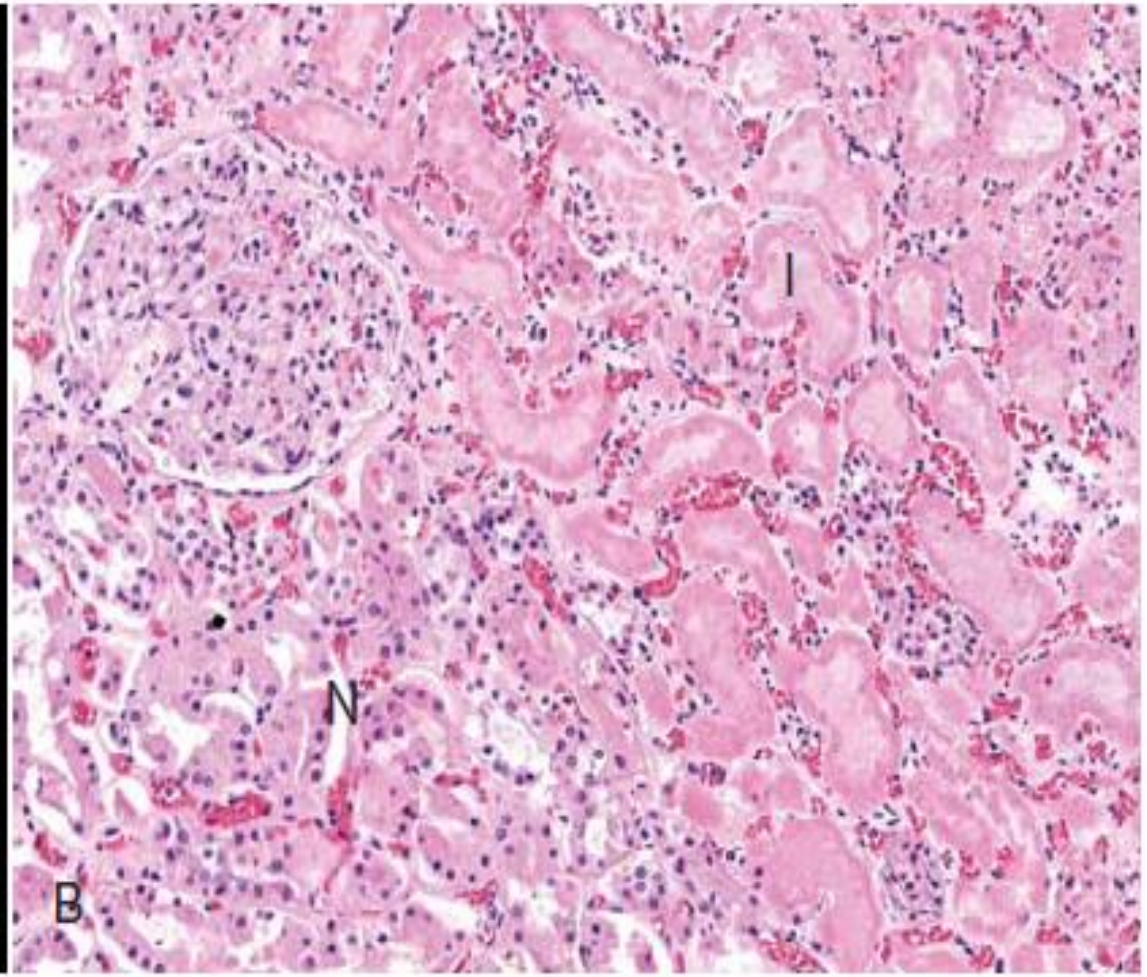
MORPHOLOGIC PATTERNS OF NECROSIS

Coagulation (coagulative) Necrosis

- Most common manifestation of cell death. characteristic of hypoxic / ischemic death of cells in all tissues (except brain).
- Basic outline of coagulated cell persists at least for a few days; protein denaturation predominates over enzymatic digestion
- **Gross appearance**
  - necrotic tissue resembles normal tissue, but color and texture may be visibly different.
  - Lighter in color (pale); due to coagulation of cytoplasmic proteins and decreased blood flow, e.g. **infarcts**
  - Usually firm texture.
  - May be swollen (due to swelling of individual cells) or shrunken (due to cell loss).
  - May see a local reaction to necrotic tissue (surrounding red zone of hyperemia or white layer of inflammatory cells).
- It is exemplified well in infarcts of solid organs, e. g. heart, spleen, and kidney

COAGULATION (COAGULATIVE) NECROSIS



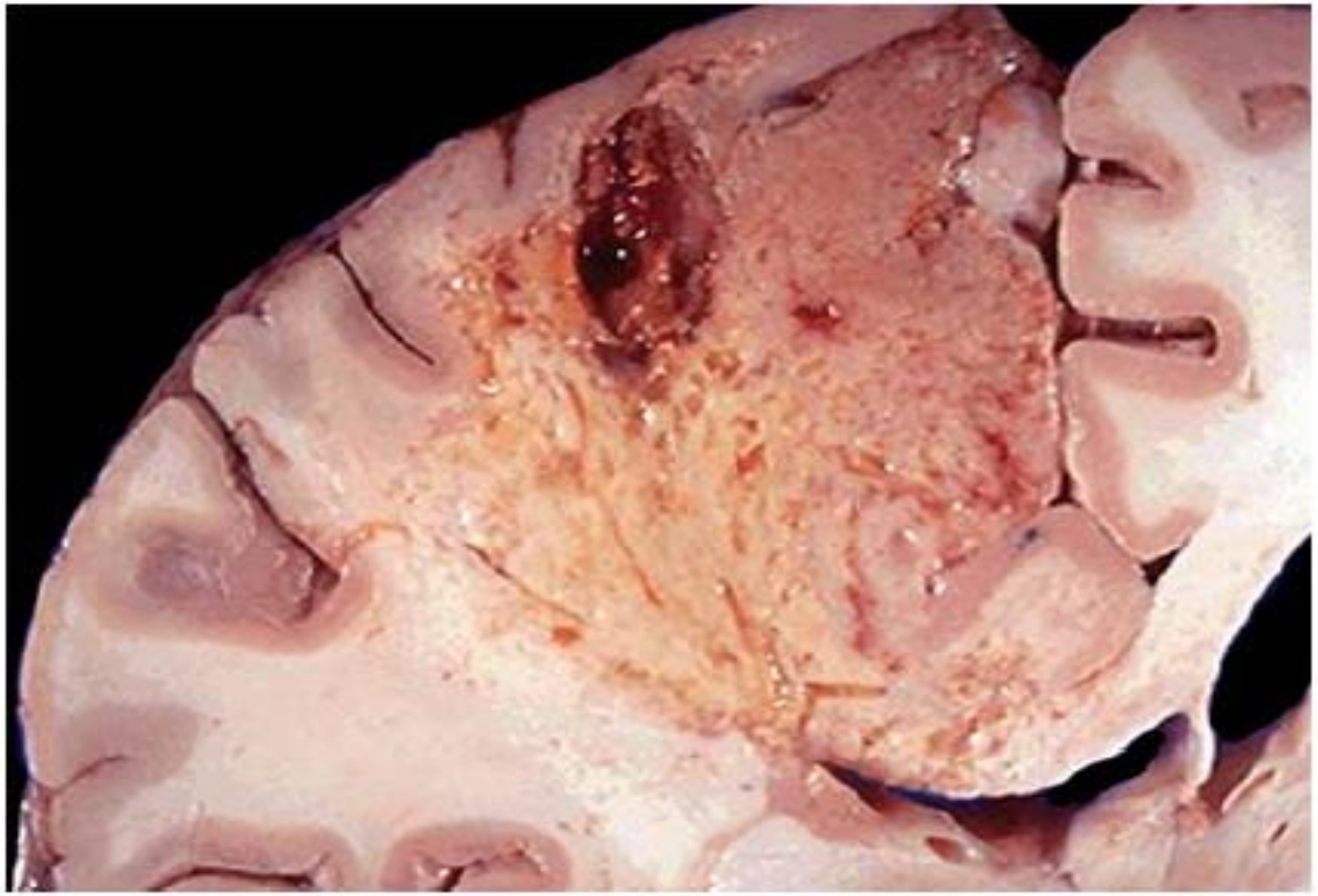


MORPHOLOGIC PATTERNS OF NECROSIS – CONT'D

Liquefactive Necrosis

- **Enzymatic digestion** of necrotic cells predominates over protein denaturation
- Seen in ischaemic injury and bacterial infections, due to attraction of neutrophils which contain potent hydrolases which are capable of digesting dead cells
- However, coagulative necrosis of the brain as a result of cerebral artery occlusion is usually followed by liquefactive necrosis without the involvement of an acute inflammatory response (neutrophils)
- **Gross appearance**
  - Affected tissue is liquefied; becomes a soft to viscous fluid
  - If process was initiated by acute inflammation, then the liquid (creamy yellow) is often dead WBC's **(PUS).**
  - This forms a semi-solid or fluid mass undergoing self-digestion e.g. an abscess.

LIQUEFACTIVE NECROSIS

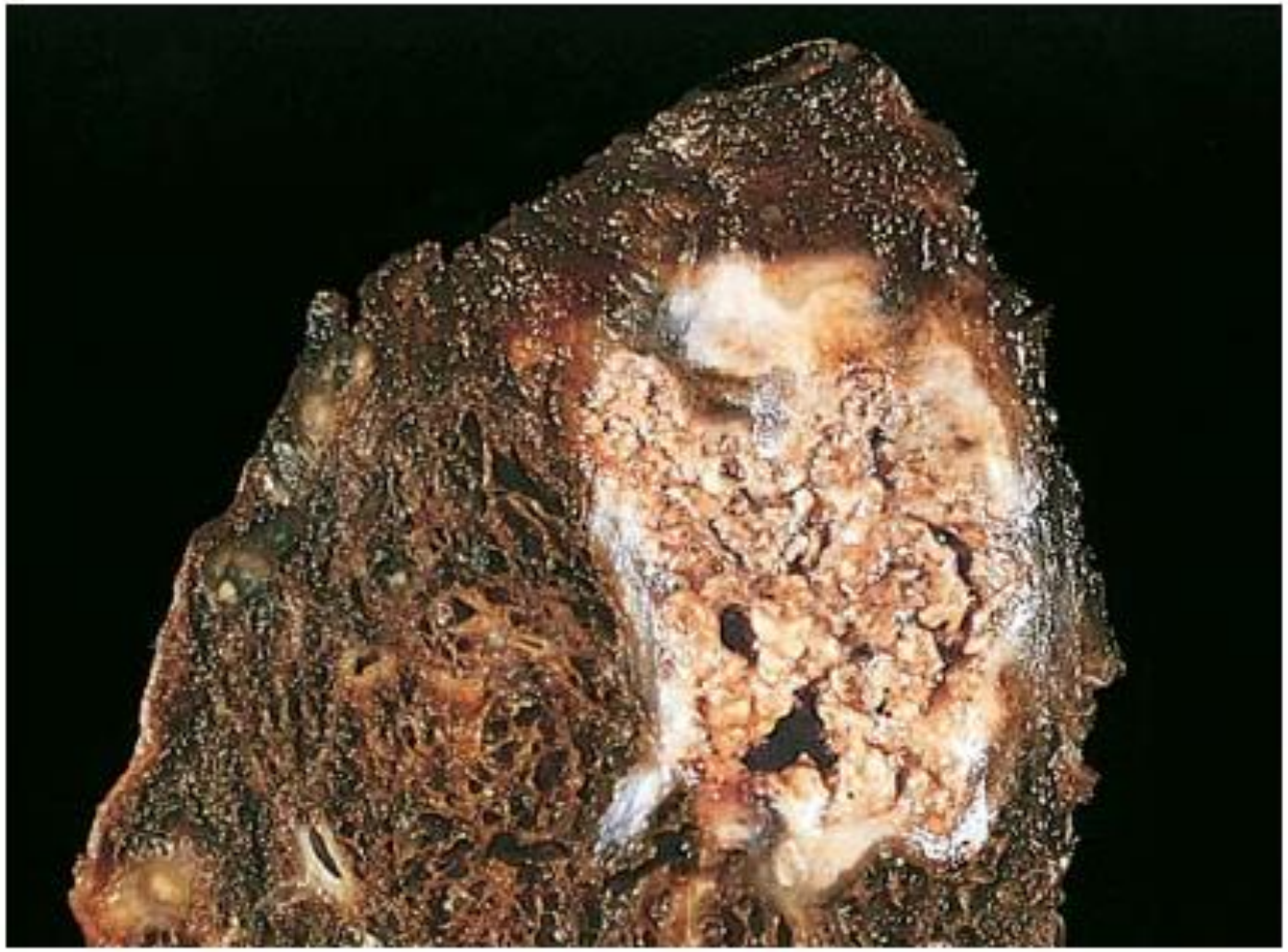


MORPHOLOGIC PATTERNS OF NECROSIS – CON'TD

Caseous necrosis

- Typical lesion seen with specific bacterial diseases, e.g. tuberculosis.
- It combines features of both coagulative and liquefactive necrosis.
- Manifested by loss of recognizable tissue architecture, where the necrotic cells fail to retain their cellular outline.
- The dead cells persist indefinitely as amorphous, coarsely granular debris; appearance characteristic of **granuloma**
- **Gross appearance**
  - Grey-white and dry with friable (crumbly) to pasty (caseous = cheese like) texture.
  - It resembles clumpy cheese, hence the name caseous necrosis.

CASEOUS NECROSIS



MORPHOLOGIC PATTERNS OF NECROSIS – CON'TD

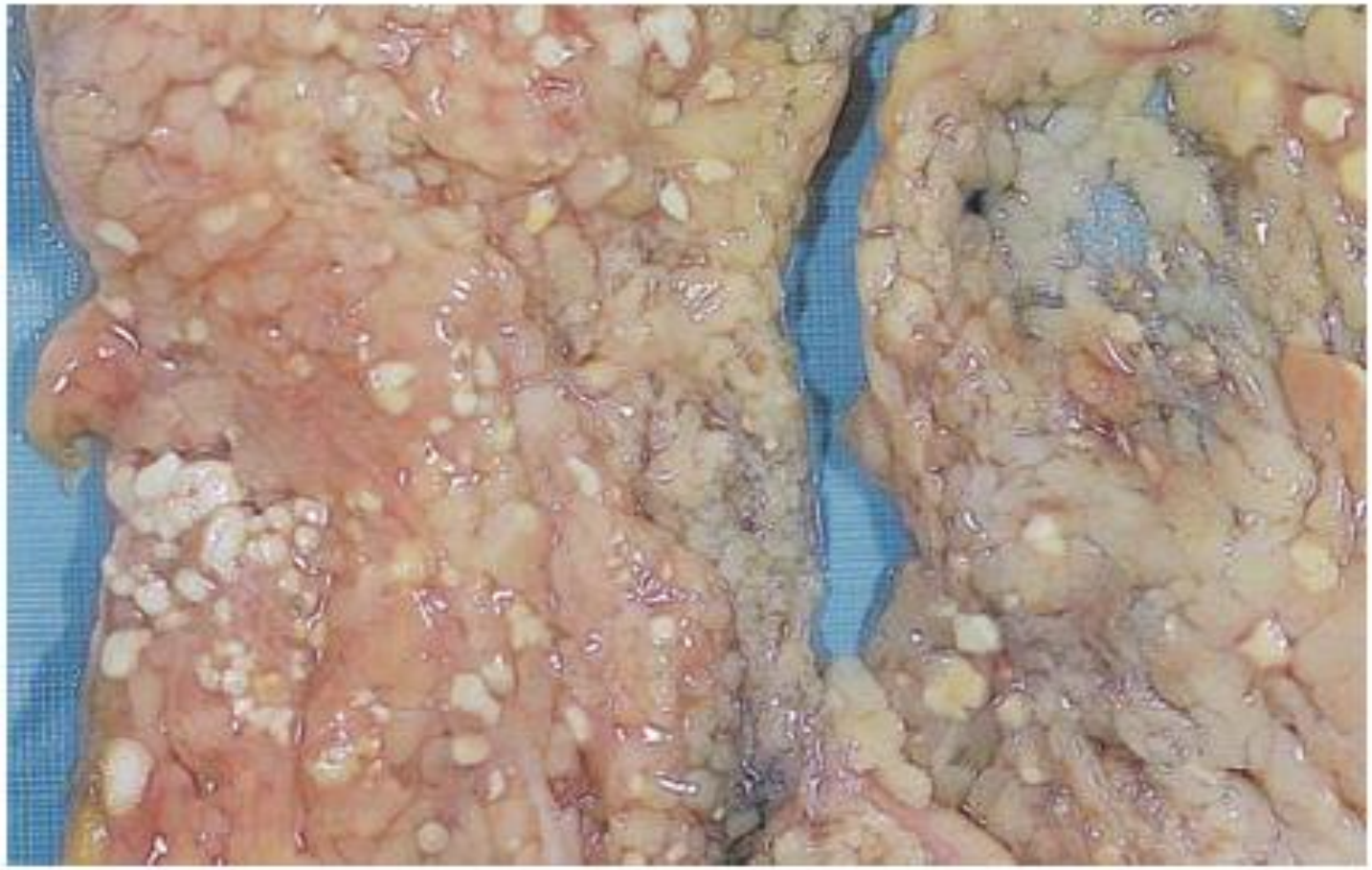
Fat Necrosis

- Type of necrosis distinguished by its location **within body fat stores**, especially abdominal or subcutaneous fat, follow *acute pancreatic necrosis* & *traumatic fat necrosis* commonly in breasts
- **Etiology**
  - Inflammation (especially **pancreatitis**), **trauma**, unknown causes (**idiopathic**).
- **Example**: in pancreatitis digestive enzymes are activated extra-cellularly → adipose and pancreatic tissues are digested → fatty acids combine with calcium → precipitated as insoluble calcium soaps (so called "**saponification of fat**").
- **Gross appearance**

➢ Affected adipose tissue loses its shiny, translucent appearance

➢ It appears firm to hard, opaque, white / chalky, ± gritty areas (often adjacent to normal fat).

FAT NECROSIS



MORPHOLOGIC PATTERNS OF NECROSIS – CON'TD

Gangrenous necrosis

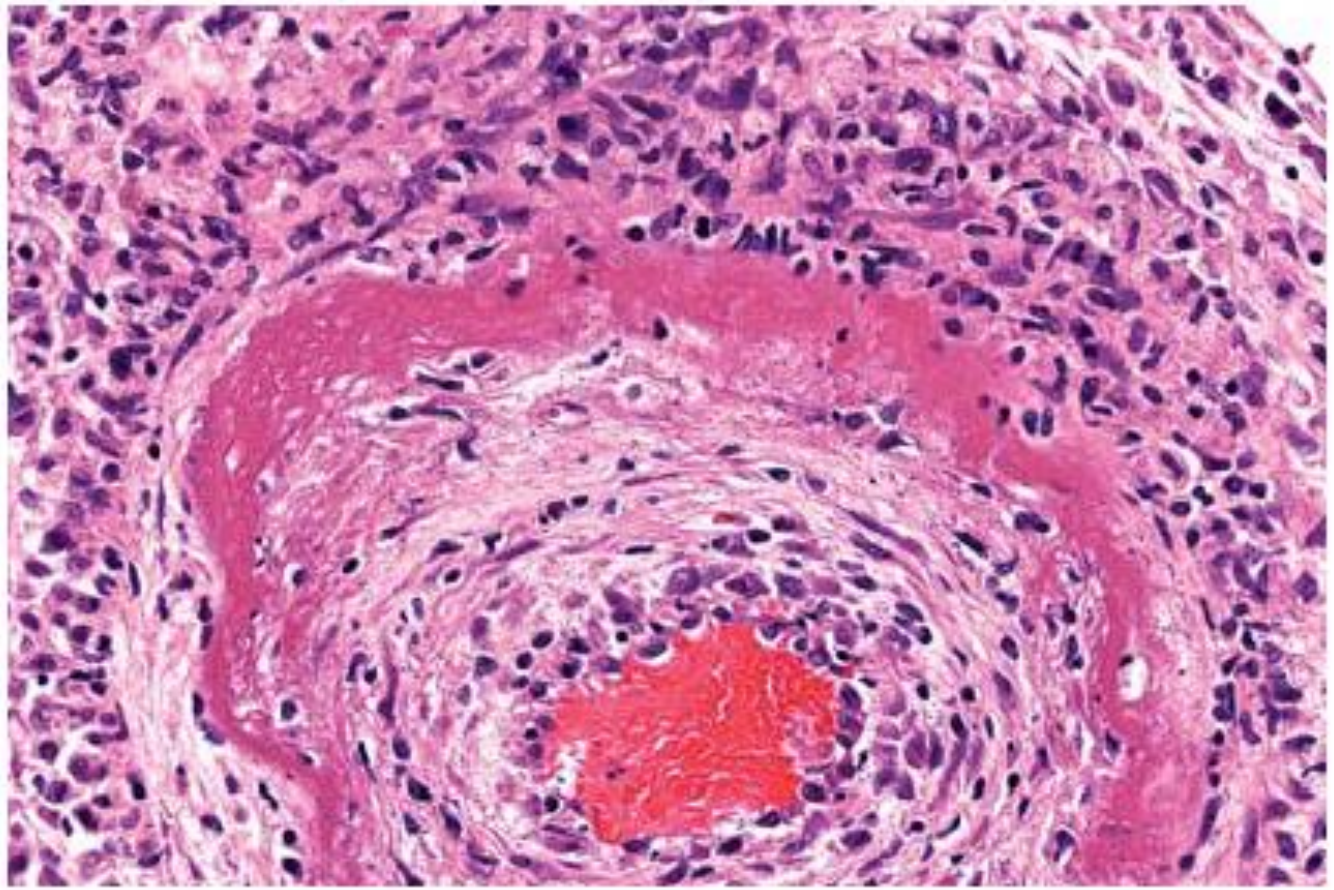
- Is not a distinctive pattern of cell death; the term is still commonly used in surgical clinical practice.
- Usually applied to a limb, generally the lower leg, that has lost its blood supply and has undergone coagulative necrosis, but with a distinct black colour due to deposition of **iron sulphide** from breakdown of **haemoglobin**.
- Infection of the gangrenous area causes liquefactive necrosis "**wet gangrene**" and produces a very foul odour.
- Is caused by ischaemia and saprophytic bacterial infections (Necrosis + putrefaction by saprophytes).

MORPHOLOGIC PATTERNS OF NECROSIS – CON'TD

Fibrinoid necrosis

- Seen in immune reactions involving blood vessels
- Due to deposition of immune complexes in the walls of blood vessels
- Complexes together with fibrin result in bright pink appearance
  - E.g. immunologically mediated vasculitis

FIBRINOID NECROSIS



FATE OF NECROTIC TISSUE

- Liquefaction: pus or abscess formation
- Encapsulation: without liquefaction
- Desquamation: of skin or oral mucosa
- Replacement: with scar tissue
- Mineralization: as in dystrophic calcification
- Gangrene formation: due to invasion by saprophytic anaerobic bacteria (e.g. *Clostridium* spp)
  - Gas
  - Dry
  - Moist (wet)

APOPTOSIS

- Pathway of cell death induced by a tightly regulated intracellular suicide program
- Cells destined to die activate enzymes that degrade cell's own nuclear DNA and protein
- Cell's plasma membrane remains intact
- Its structure is altered in a way as to become target of phagocytosis
- Dead cell is rapidly cleared before contents leak out
- Does not elicit inflammatory reaction in host

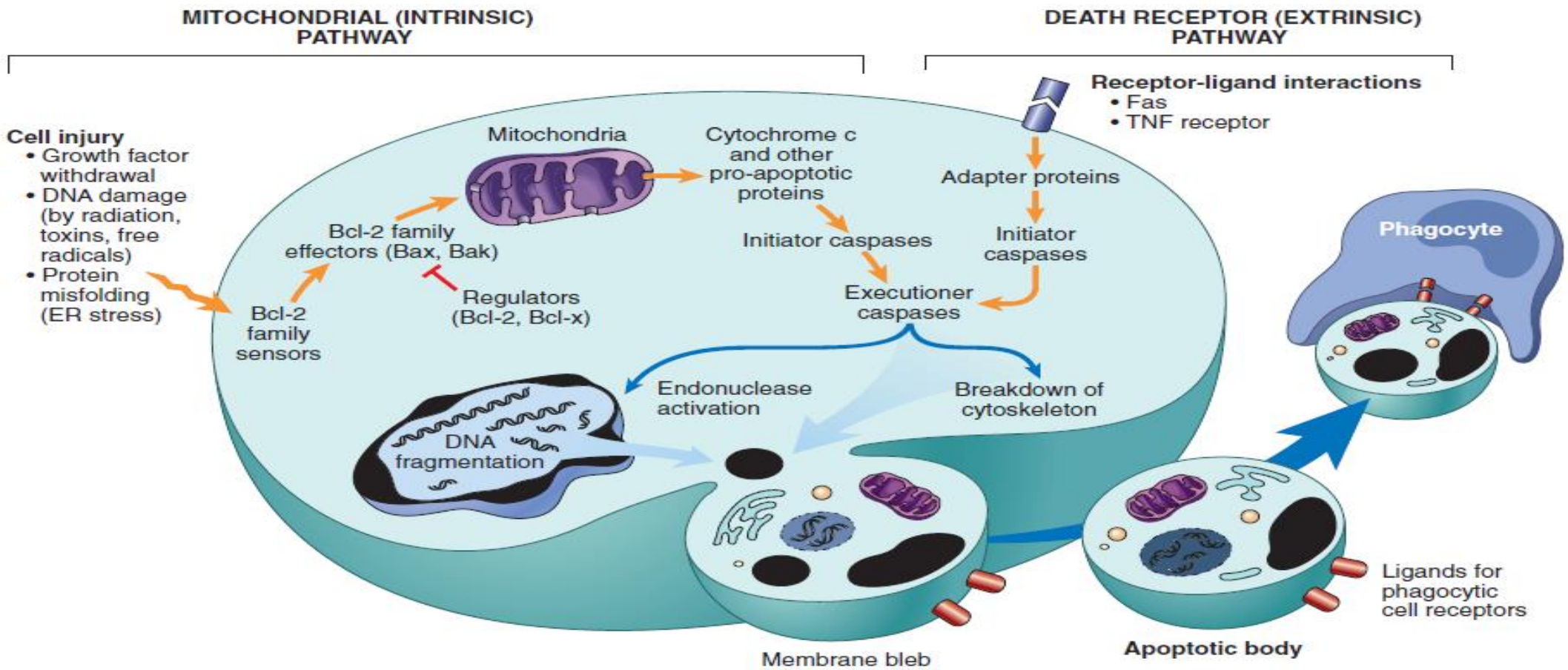
CAUSES OF APOPTOSIS

- Occurs in physiologic & Pathologic conditions
- Elimination of unwanted or potentially harmful cells
- Elimination of cells that have outlived their usefulness, RBCs, inflammatory cells
  - Destruction of cells during embryogenesis
  - Involution of hormone dependent tissues upon hormone withdrawal
  - Elimination of self-reacting lymphocytes in BM

CAUSES OF APOPTOSIS

- Pathologic event when diseased cells become damaged beyond repair and are eliminated in the following;
- DNA damage
- Accumulation of misfolded proteins : ER stress
- Cell injury in viral infections

SUMMARY OF INITIATION OF APOPTOSIS



MECHANISMS AND BIOCHEMICAL PATHWAY OF CELL INJURY

- Mechanisms are complex, one or more may contribute to cell injury
  - Nature, severity & duration of the injurious agent
  - Type, state & adaptability of the cell
  - Biochemical mechanisms activated by different injurious stimuli acting on several essential cellular components
  - Any injurious stimulus may simultaneously trigger multiple interconnected mechanism that damage cells

1. DEPLETION OF ATP

- Depletion of ATP or reduced synthesis
- Associated with toxic and chemical injury
- ATP is produced by oxidative phosphorylation of ADP
- Or the glycolytic pathway using glycogen without requirement for oxygen
- Damage to mitochondria reduces synthesis of ATP
- Many energy dependent functions of cells are affected

DEPLETION OF ATP – CONT'D

- ATP depletion → to wide spread effects on many critical cellular systems such as
- Reduced activity of the plasma membrane energy-dependant sodium pump (ouabian-sensitive Na^+ k^+ -ATPase)
- Cellular energy metabolism is altered
- Failure of the Ca^+ pump → influx of calcium
- ↓ ATP → Structural disruption of the protein synthetic apparatus
- Unfolded protein response trigger by misfolded proteins → cell injury or death due to oxygen or glucose deprivation

2. MITOCHONDRIAL DAMAGE

- Mitochondria synthesizes ATP which provides energy to the cell, important in cell injury & death
- Mitochondria can be damaged by
  - Increases of cytosolic calcium
  - Reactive oxygen species (Oxidative stress)
  - Oxygen deprivation
- High-conductance channel → mitochondrial permeability transition pore → membrane potential → failure to generate ATP
- Release of cytochrome C

3. INFLUX OF CALCIUM & LOSS OF CALCIUM HOMEOSTASIS

- Ischaemia and toxins can cause increase in cytosolic calcium:
  - Increases mitochondrial permeability pores
  - Activates enzymes which break down components
    - Phospholipases
    - Proteases
    - Endonucleases
    - ATPases
  - Induction of apoptosis by direct activation of caspases

4. DEFECTS IN MEMBRANE PERMEABILITY

- Membrane damage affects integrity of all cellular membranes
  - Plasma membrane – influx of fluids and ions
  - Mitochondrial membrane – mitochondrial damage
  - Lysosomal membranes – leakage of enzymes

5. DAMAGE TO DNA AND PROTEINS

- DNA damage can be caused by radiation, drugs, oxidative stress
  - Repair mechanisms are activated and if repair fails, apoptosis is activated
  - Misfolded proteins also causes apoptosis

6. FREE RADICAL-INDUCED CELL INJURY

- Free radicals - chemical species with single unpaired electron in outer orbit
- Are unstable and can initiate autocatalytic reactions by converting molecules they react with into free radicals
- Free radicals can be initiated within cells by:

- absorption of radiant energy. $H_2O \rightarrow OH^{\cdot} + H$
- enzymatic metabolism of chemical/drugs $CCl_4 \rightarrow CCl_3$
- redox reactions occurring during normal metabolism
- Nitric Oxide (NO) $\rightarrow ONOO^{\cdot}$, NO_2, NO_3

EFFECTS OF FREE RADICALS ON CELLS

- Lipid peroxidation of membranes in presence of O_2 →damage to plasma membranes
- Oxidative modification of proteins →degradation of enzymes
- Reactions with thymine in DNA causing single stranded breaks (mutations)
  - Fatty acids → oxidation → generation of lipid peroxidases → disruption of plasma membrane organelles
  - Proteins → oxidation→ loss of enzymatic activity, abnormal folding
  - DNA→ oxidation→ mutation, breaks

MECHANISMS OF REMOVING FREE RADICALS

- Antioxidants – Vitamin A, C, E

Enzymes that act as free radical-scavenging system

- catalase decomposes $H_2O_2 \rightarrow O_2 + H_2O$
- superoxide dismutases convert superoxide$\rightarrow H_2O_2$
- glutathione peroxidase breaks down free radicals

AUTOPHAGY

- Process by which the cell digests its own contents
- Survival mechanism in times of nutrition deprivation
- The digested contents are recycled in order for the cell to remain viable
- Autophagy appears to trigger cell death that is distinct from necrosis and apoptosis
- It is thought to be responsible for cell loss in a number of degenerative conditions of the CNS

CELLULAR AGING

- With age comes physiologic and structural alterations in tissues and organs
- Result of progressive decline in cellular function and viability
  - Genetic abnormalities
  - Accumulation of cellular and molecular damage
- Aging process affected by genetic factors, diet, social conditions, age-related diseases
- Progressive accumulation of sublethal cell injury leading to cell death

CHANGES THAT CONTRIBUTE TO AGING

- **Decreased cellular replications**
  - After a fixed number of cell divisions cells become arrested in terminally non-dividing state – senescence
  - Attributed to telomere shortening
  - Werner syndrome – premature aging due to defect in DNA replication
- **Accumulation of metabolic and genetic damage**
  - Reactive oxygen species accumulate due to exposure to radiation, or reduction in antioxidants
  - Oxidative damage may contribute to senescence