Introduction to pathology

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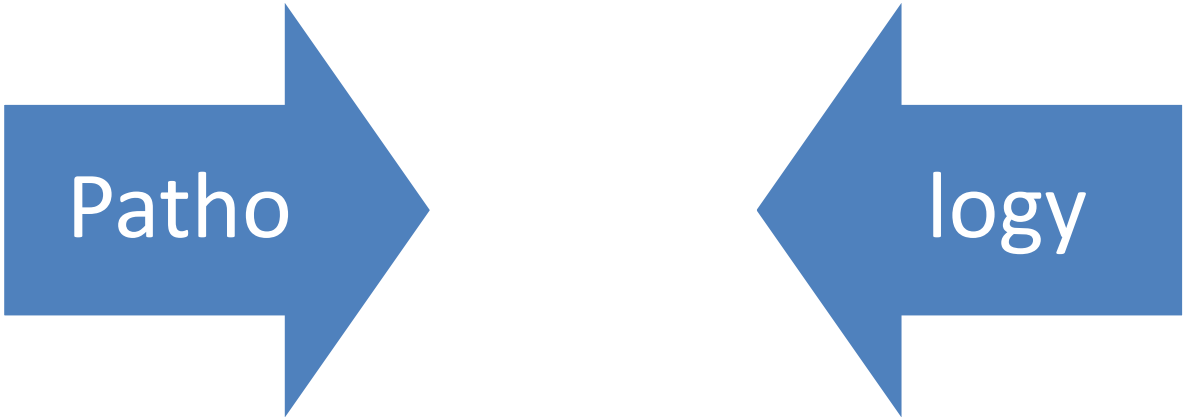
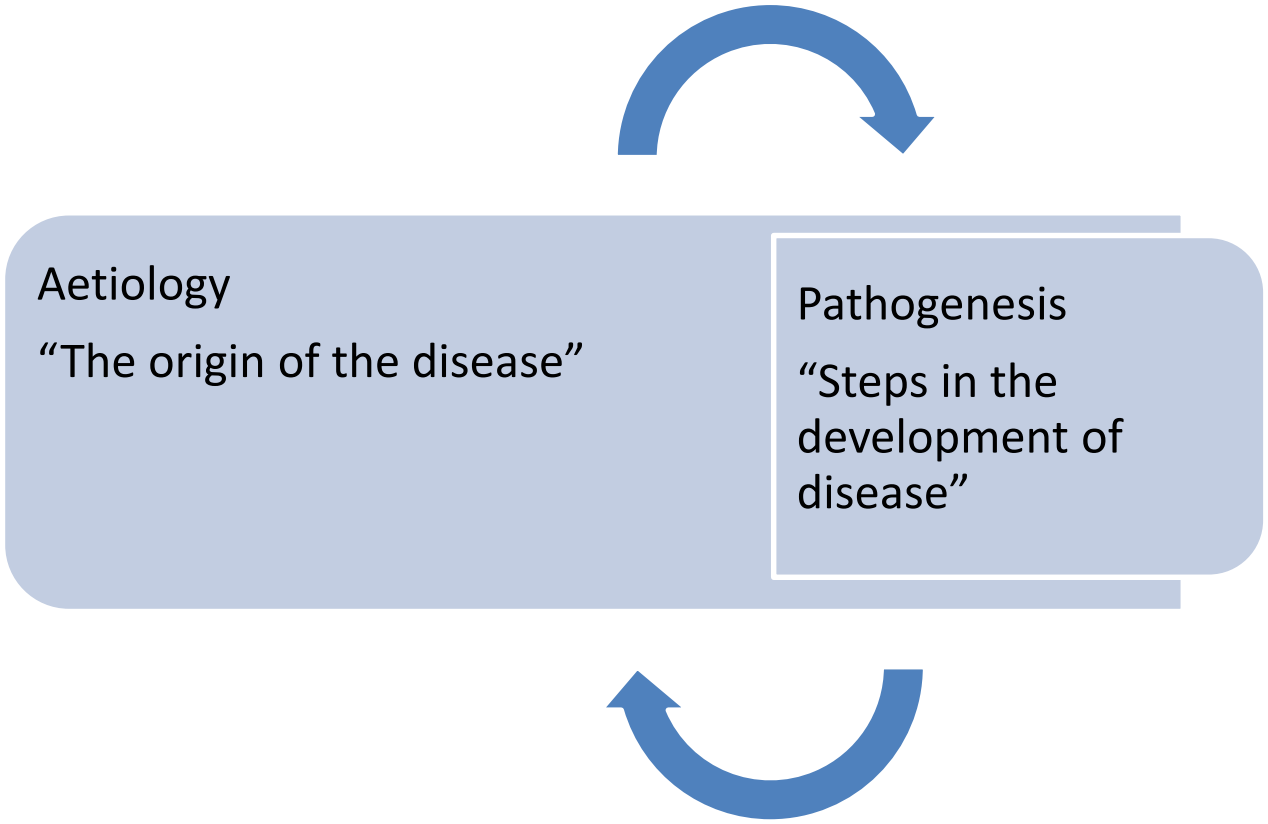
?????

What is pathology?

Who is a pathologist?



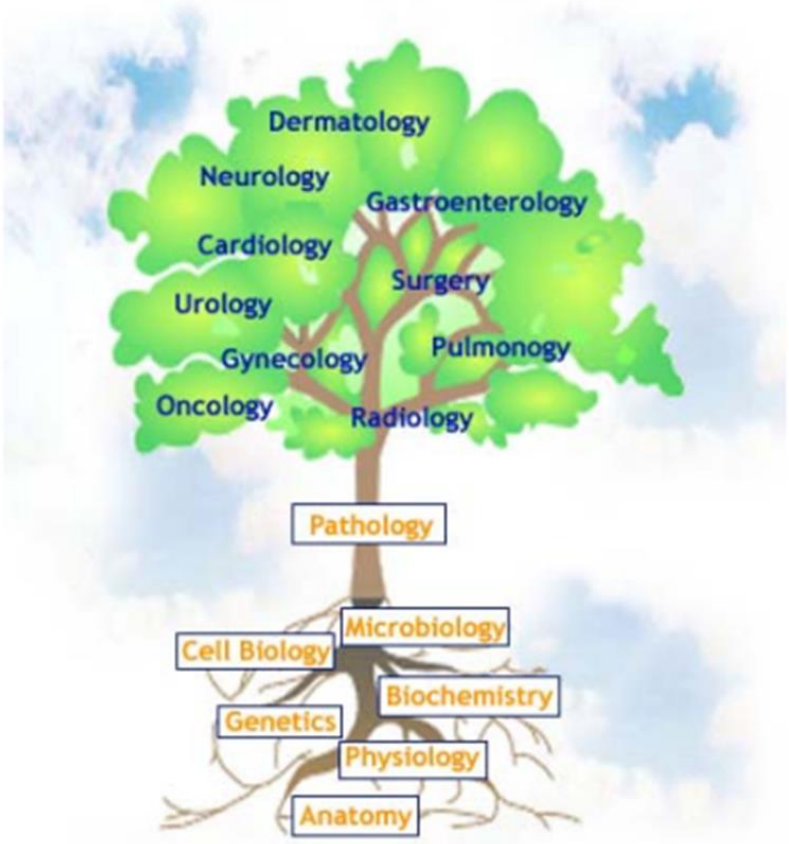
Pathology (pathos “**disease/suffering**” + logos “ **reason/study of**”) is the study of the links between diseases and the reasons.



The scientific foundation for the practice of medicine

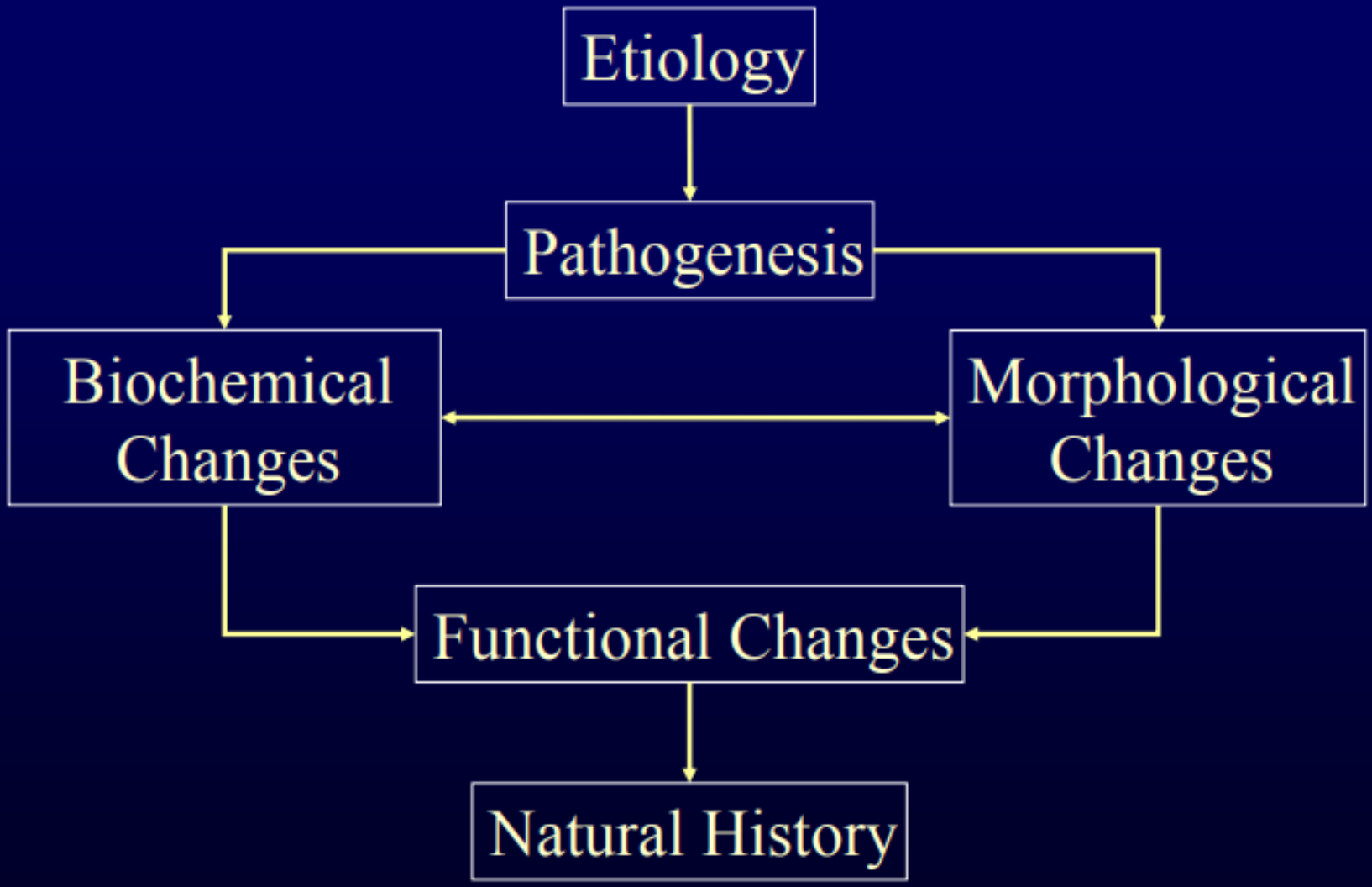
The Tree of Medicine

(After G. Diamandopoulos)



Pathology (pathos “disease” + logos “word, reason”) is the study of the links betwee
diseases and the basic science

The Disease Paradigm



Aspects of a disease process

- Cause (**etiology**)
- Biochemical and molecular mechanisms of its development (**pathogenesis**)
- Structural alterations induced in the cells and organs of the body (**morphologic changes**)
- Functional consequences of these changes (**clinical manifestations**).

(These four steps are the core of pathology)

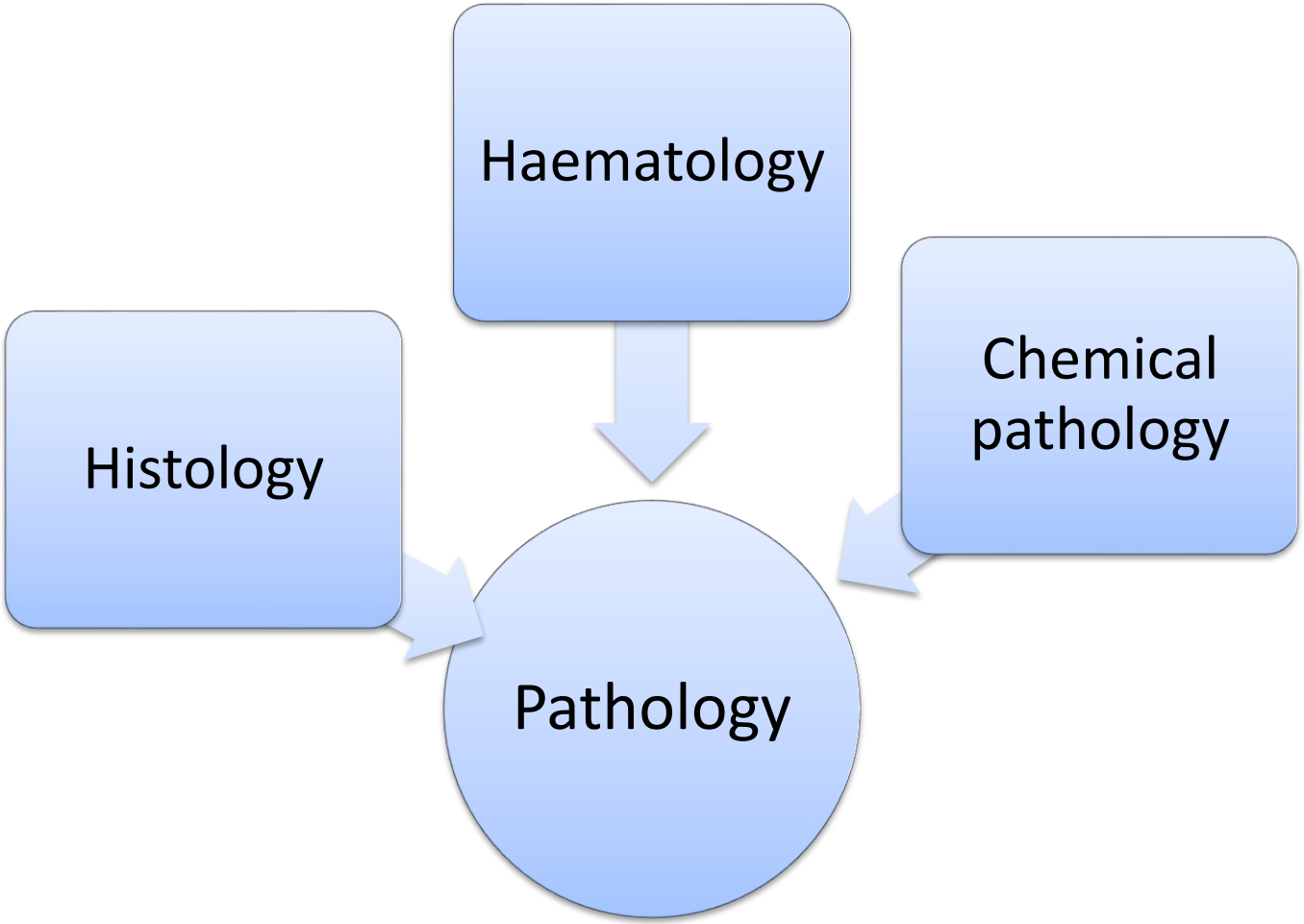
Pathology

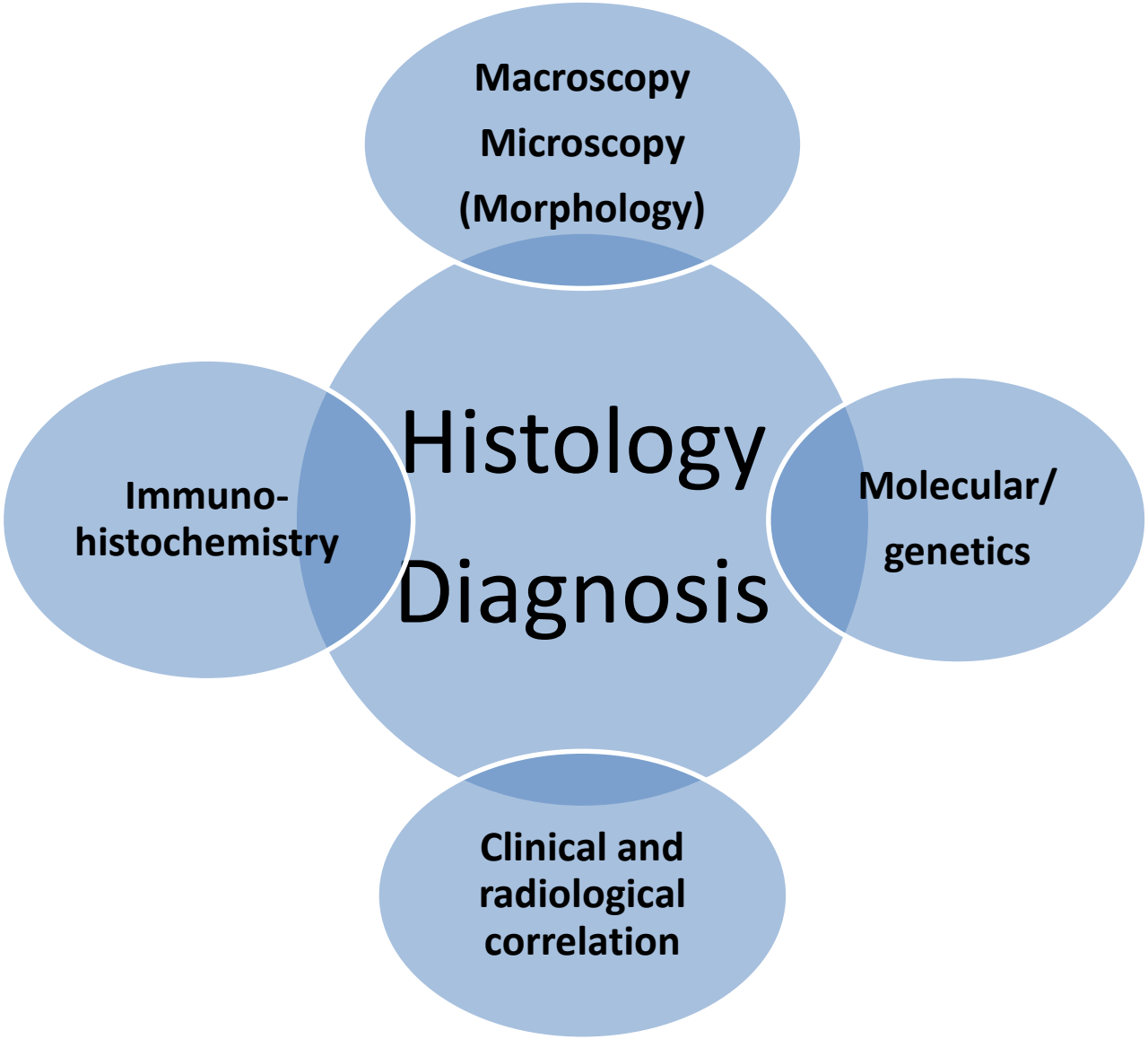
General pathology

Systemic pathology

- Respiratory
- Cardiovascular
- Renal
- GIT
- Male and female genital
- Breast
- Neuro
- Lymphoid
- endocrine

Pathologist is a person identifying diseases based on the examination of cells , tissues, fluids removed from the body.


Haematology
Histology
Chemical pathology
Pathology


Macroscopy
Microscopy
(Morphology)
Immuno-
histochemistry
Histology
Diagnosis
Molecular/
genetics
Clinical and
radiological
correlation

Cell injury- I

2018/01/02

Learning outcomes

- Define cell injury, reversible cell injury and irreversible cell injury.
- List the causative agents / injurious stimuli.
- Briefly outline the mechanisms of cell injury.
- Describe the different morphological patterns / appearances of cell injury and list the clinical situations in which they occur

  Reversible cell injury

  Irreversible cell injury - Necrosis and its patterns,
  - Apoptosis,

Homeostasis

- The normal cell is confined to a fairly **narrow range of function and structure** by its state of metabolism, differentiation, & specialization;

- **by constraints** of neighboring cells; and by the availability of metabolic substrates.
- But able to handle physiologic demands, **maintaining a steady state** called homeostasis.

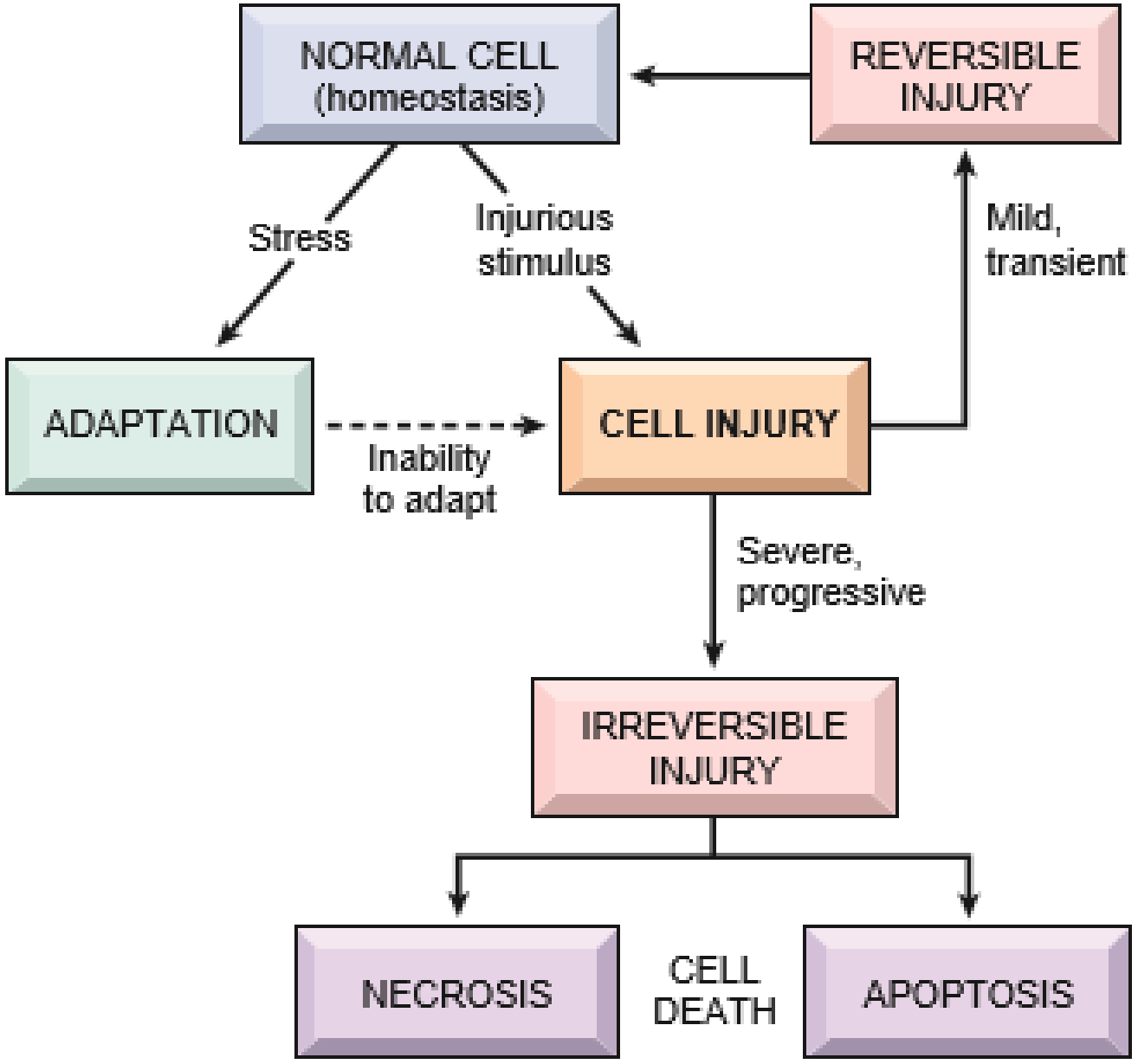
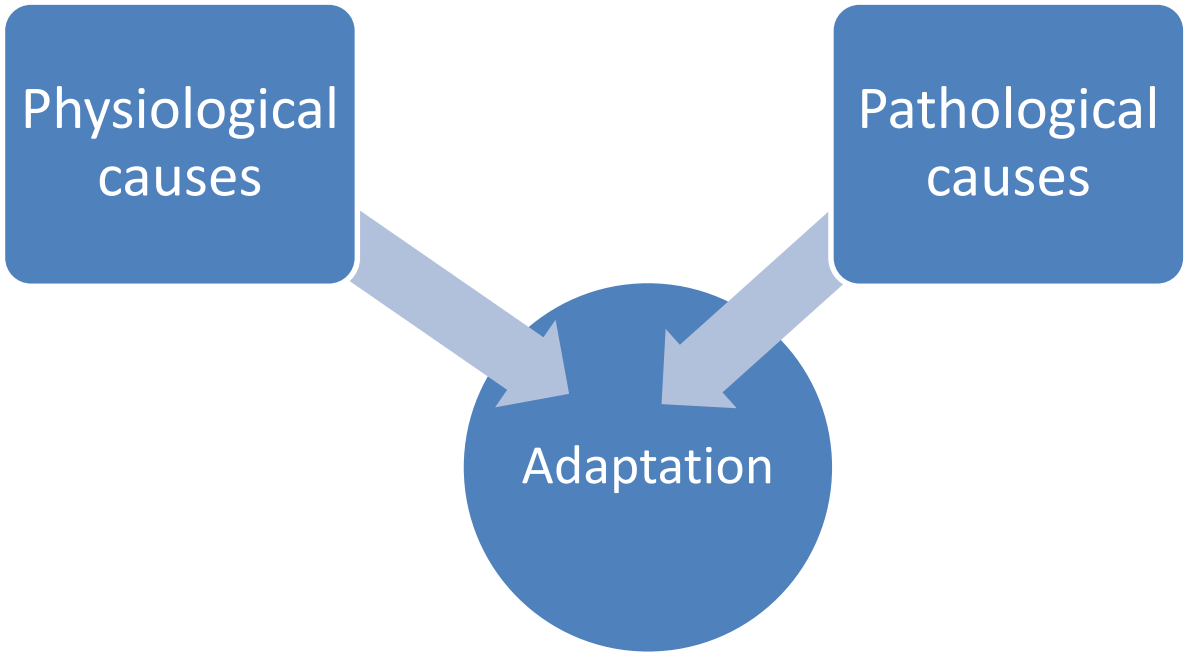


Figure 2-1 Stages of the cellular response to stress and injurious stimuli.

Cellular adaptations to stress

- **Adaptation-** Reversible changes in the number, size, phenotype, metabolic activity or functions of cells in response to changes in the environment.



Adaptive responses

1) Increase in the size of cells and its functional activity **(hypertrophy)**
2) Increase in their number **(hyperplasia)**
3) Decrease in the size and metabolic activity of cells **(atrophy)**
4) Change in the phenotype of cells **(metaplasia)**

When the **stress is eliminated** the cell **can recover** to its original state **without** any **harmful consequences.**

Cell injury

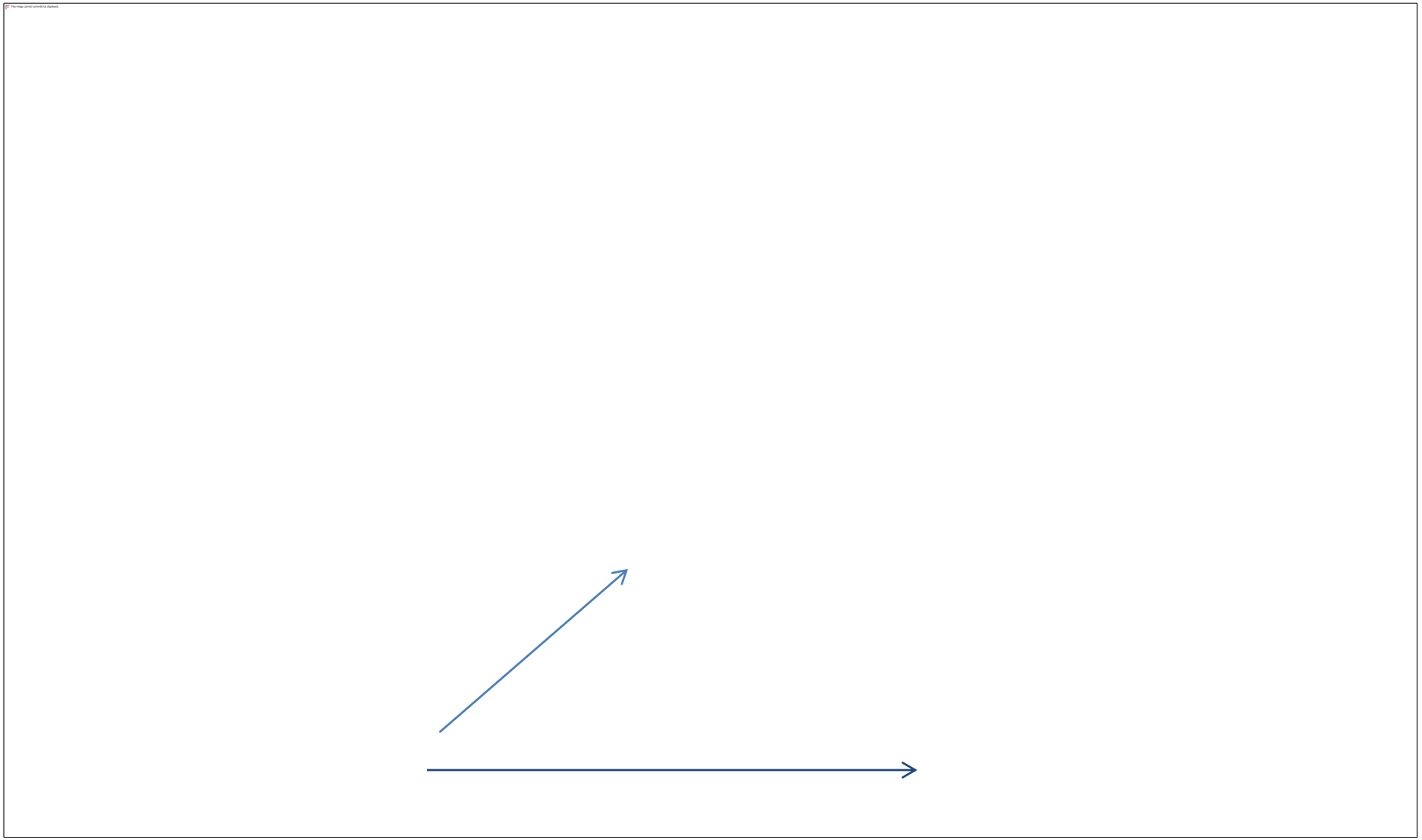
If the cells are stressed to a limit exceeding the **adaptive responses** or

When cells are exposed to inherently damaging agents or suffer from intrinsic abnormalities

(DNA, proteins)

a **sequence of events** follows that is termed cell injury.

- Cell injury is **reversible** up to a certain point, but if the stimulus persists or is severe enough from the beginning, the cell suffers **irreversible injury** and ultimately undergoes **cell death**.



Eg: Cell injury-cardiac muscle

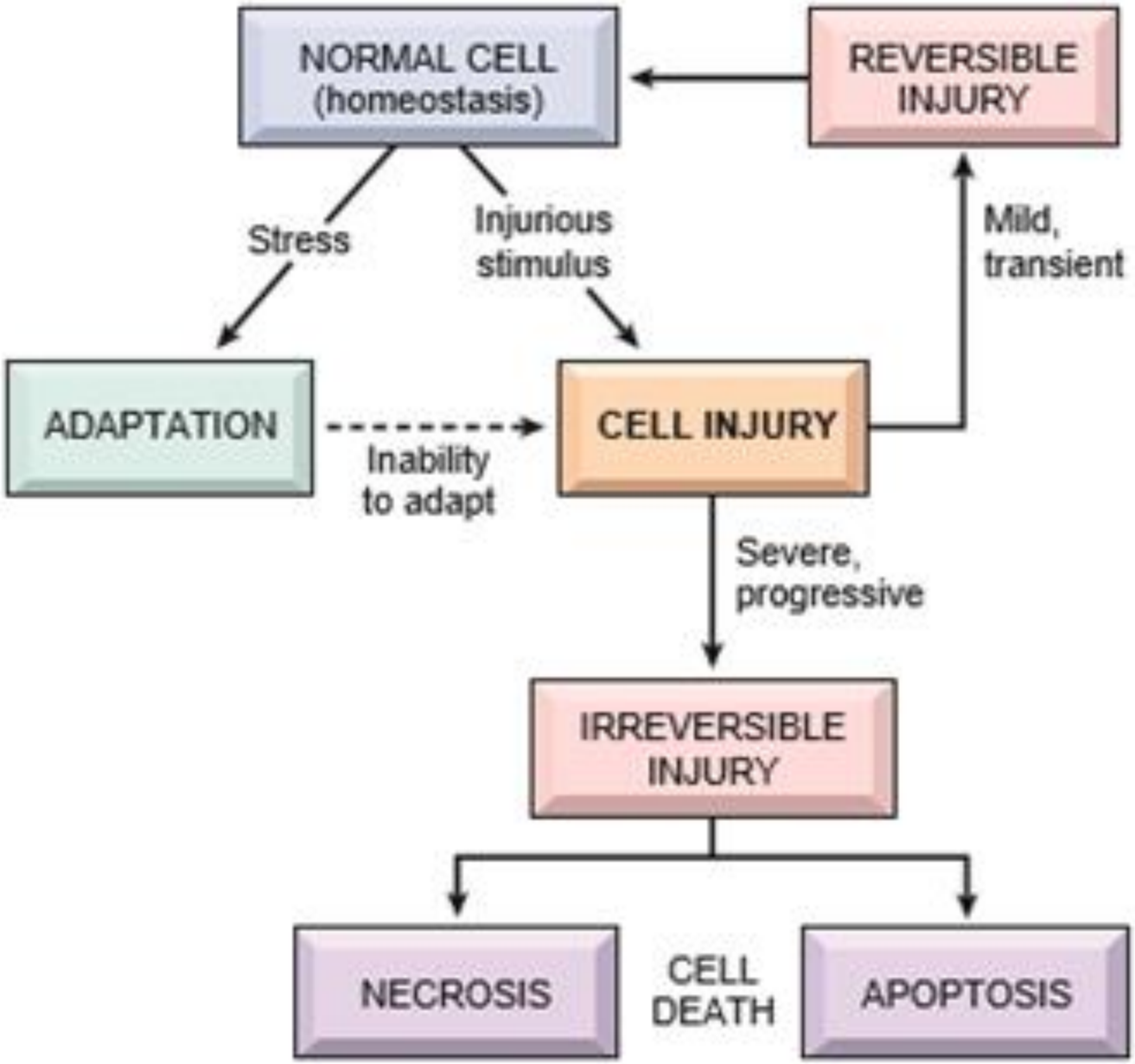


Figure 2-1 Stages of the cellular response to stress and injurious stimuli.

Causes of cell injury

- Oxygen deprivation
- Physical agents
- Chemical agents and drugs
- Infectious agents
- Immunological reactions
- genetic derangements
- Nutritional imbalances
- Aging

- **Reduced oxyen**

  oxygen deficiency in blood

  ischemia (blood flow deficiency)

  loss of O2 carrying capacity (CO, anaemia)

  poisoning

- **Chemical agents**:

  drugs (Eg-Paracetamol - P450 catalyzed oxidation to toxic metabolite)

  alcohol, narcotics

- **Physical agents**: trauma, heat, radiation, electric shock

- **Infections-** Viruses, bacteria, fungi, protozoa

- **Immunological reactions**: including anaphylaxis and loss of immune tolerance that results in autoimmune disease
- **Genetic defects:** sickle cell disease, inborn errors of metabolism

- **Nutritional defects**: malnutrition, vitamin deficiencies,
  obesity leading to type II DM,
  defective fat metabolism leading to atherosclerosis

- **Aging:** degeneration as a result of repeated trauma, and intrinsic cellular senescence

Cell injury……

These aetiological factors cause cell injury by **different mechanisms**.

Cell response to injury is **not an all-or-nothing** phenomenon.

Response to a given stimulus depends on

type, duration and severity of injury and

type, state, genetic make up and adaptability of cell.

Multiple biochemical alterations may be triggered by any on injurious insult.

All stresses and noxious influences exert their **effects first at the molecular or biochemical** level.

- The first effect of all injuries is on the biochemical and molecular level.
- Functional derangement happens next.
- Ultrastructural changes seen by electron microscopy follow.
- Then light microscopic changes occur.
- The last visible change is at the gross; macroscopic level.

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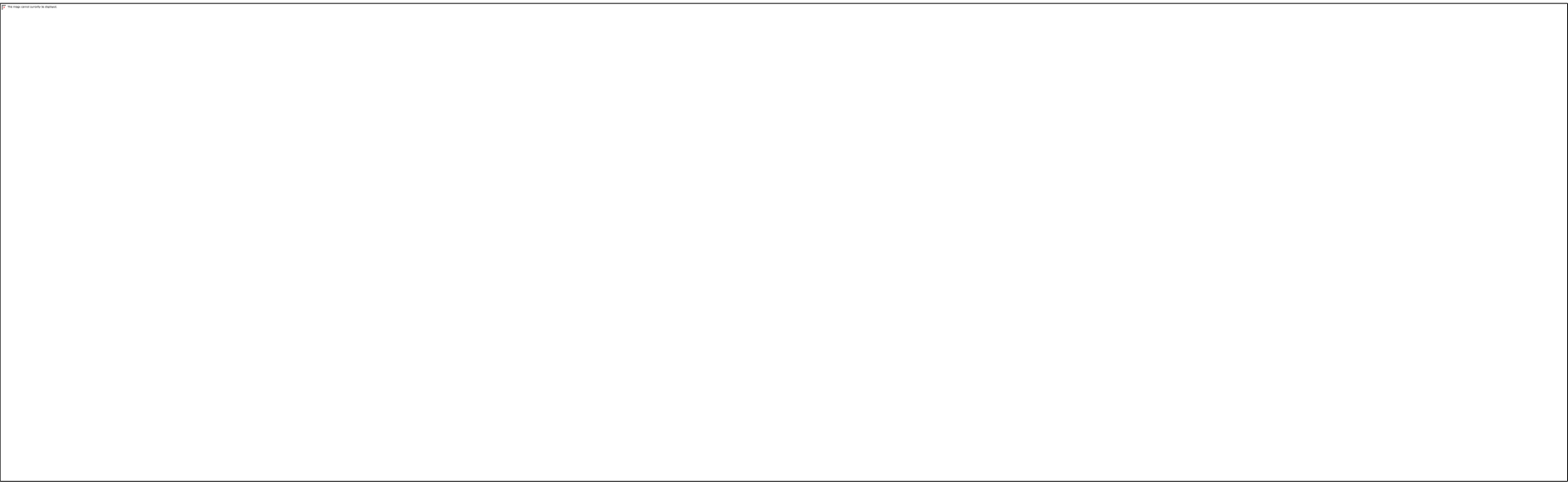
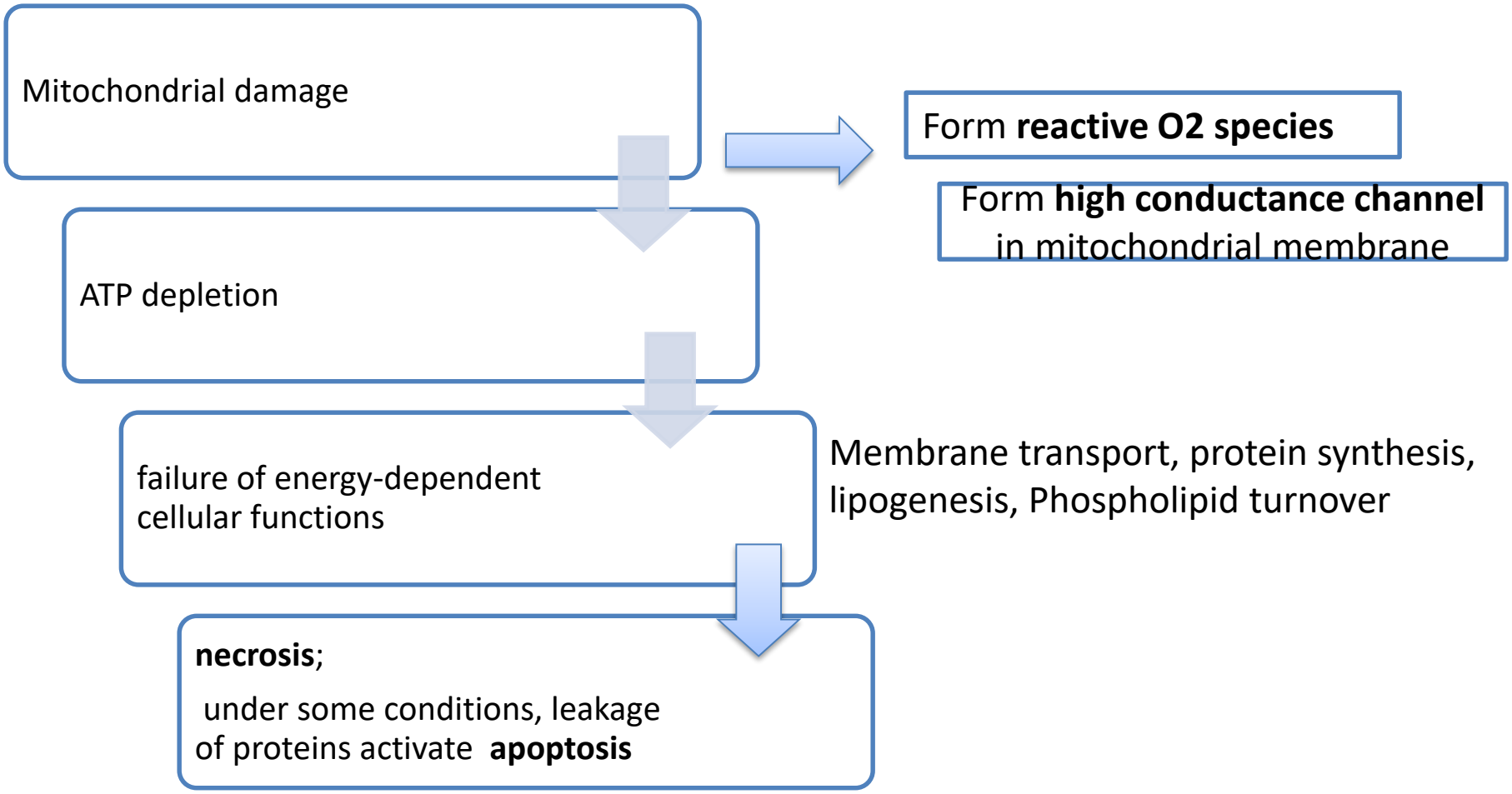
Mechanism of cell injury



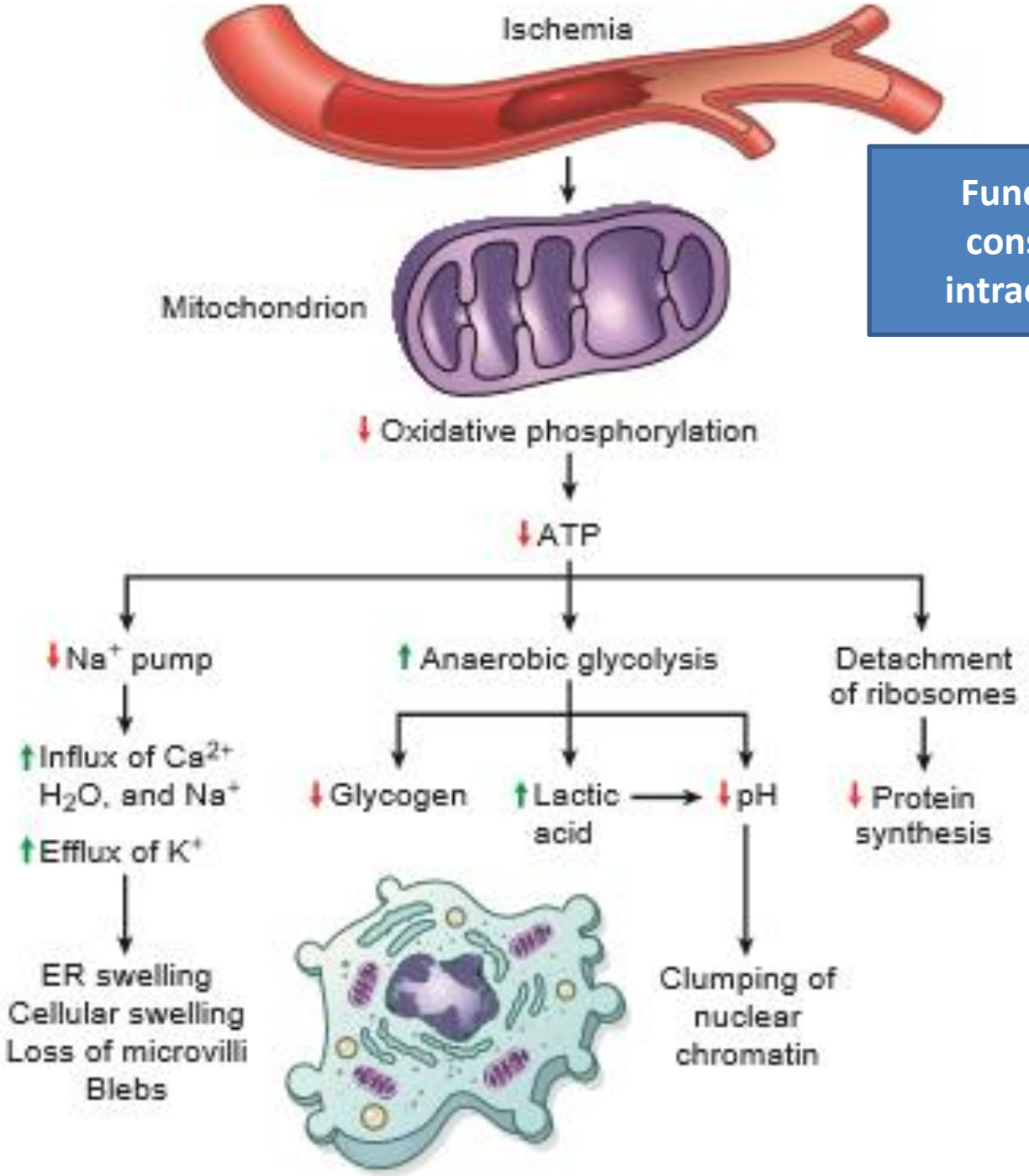
Biochemical mechanisms responsible for reversible cell injury

- Mitochondrial damage
- Influx of intracellular calcium
- Increased permeability of cell membranes
- Accumulation of damaged DNA and misfolded proteins

1) Mitochondrial damage



Functional and morphologic consequences of decreased intracellular ATP during cell injury



- Damage to mitochondria cause high conductance channel formation in the mitochondrial membrane. (mitochondrial permeability transition pore).

- Leads to loss of mitochondrial membrane potential and pH changes.

- This further compromise oxidative phosphorylation.

Free radicles

- Free radicals are chemical species with a single unpaired electron in an outer orbit.

- Free radicals are chemically unstable and therefore readily react with other molecules, resulting in chemical damage.

Intracellular Sources of Free Radicals

- Normal redox reactions generate free radicals
- Ionizing radiation (UV, X-rays) can hydrolyze water into hydroxyl (OH •) and hydrogen (H •) free radicals
- Metabolism of exogenous chemicals (Eg CCl_4) can generate free radicals
- Nitric oxide (NO) can act as a free radical

Free radical generation is a “physiological” antimicrobial reaction.

Neutralization of Free Radicals

- **Spontaneous** decay
- **Superoxide dismutase** (SOD): $2O_2^{\bullet} + 2H \rightarrow O_2 + H_2O_2$
- **Glutathione** (GSH): $2OH^{\bullet} + 2GSH \rightarrow 2H_2O + GSSG$
- **Catalase**: $2H_2O_2 \rightarrow O_2 + H_2O$
- Endogenous and exogenous **antioxidants** (Vitamins E, A, C and β-carotene)

Free radical induced injury

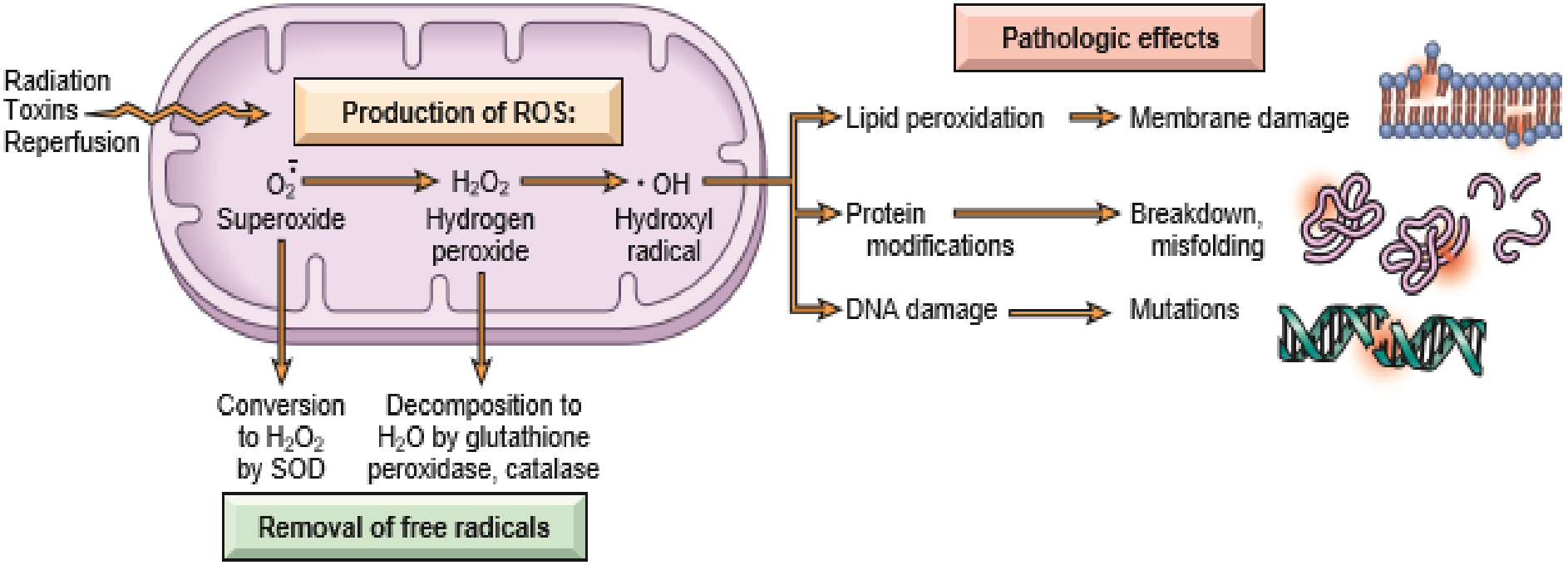
If not adequately neutralized, free radicals can damage cells by three basic mechanisms:

1. **Lipid peroxidation of membranes**: double bonds in polyunsaturated membrane lipids are vulnerable to attack by oxygen free radicals. Lipid-radical interaction yield peroxides.

2. **DNA fragmentation**: Free radicals react with thymine in nuclear and mitochondrial DNA to produce single strand breaks.

3. **Protein cross-linking:** Free radicals promote sulfhydryl-mediated protein cross-linking, resulting in increased degradation or loss of activity.

Role of ROS in cell injury

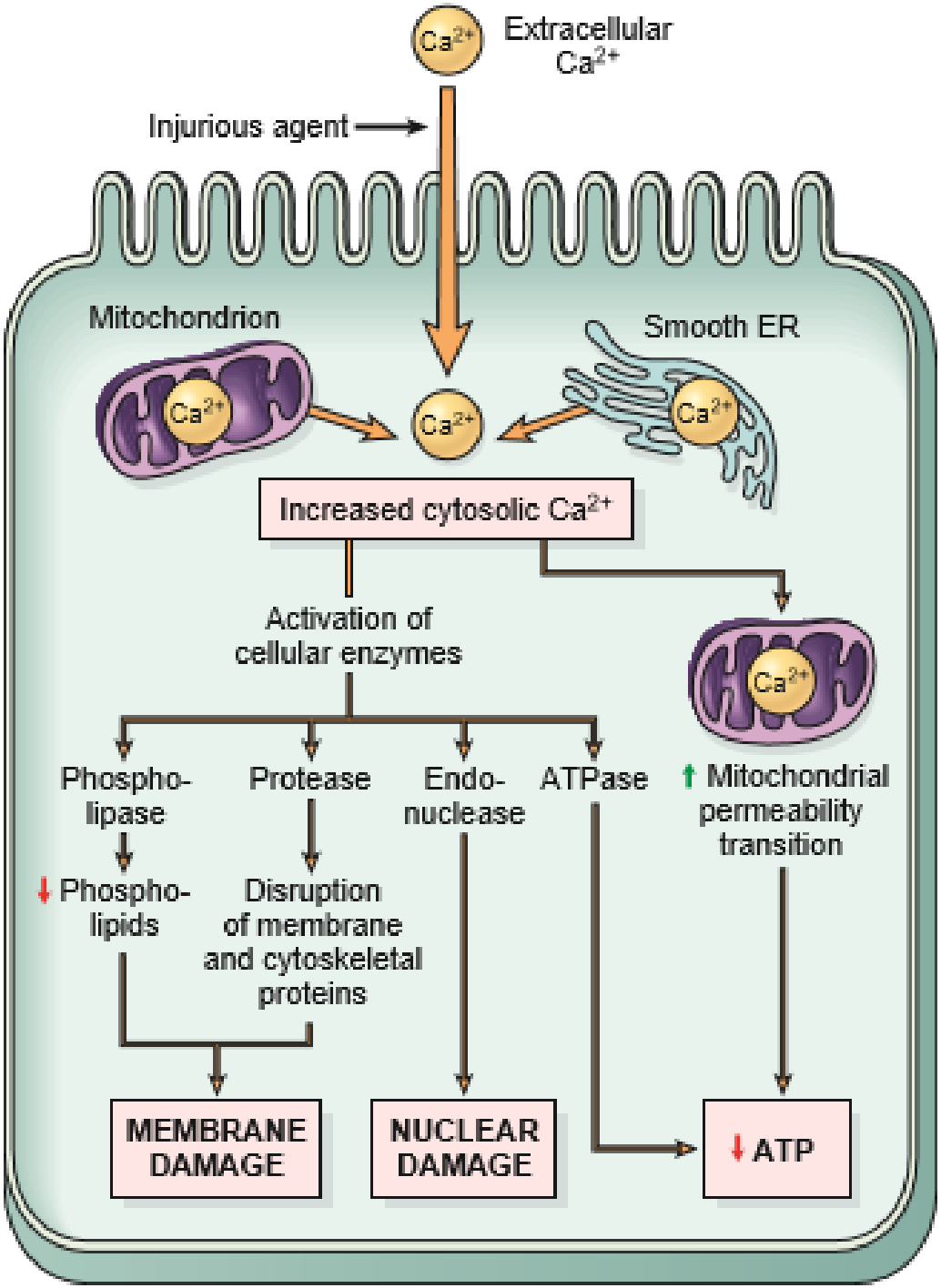


2) Influx of calcium

- Cytosolic free calcium is maintained(Very low concentration) by ATP dependent calcium transporters.
- Ischemia and some toxins cause increased cytosolic ca+.
- Increased cytosolic ca+ activate enzymes that damage cellular components.

  (phospholipase, endonuclease,ATPase, Protease)
- May also trigger apoptosis.

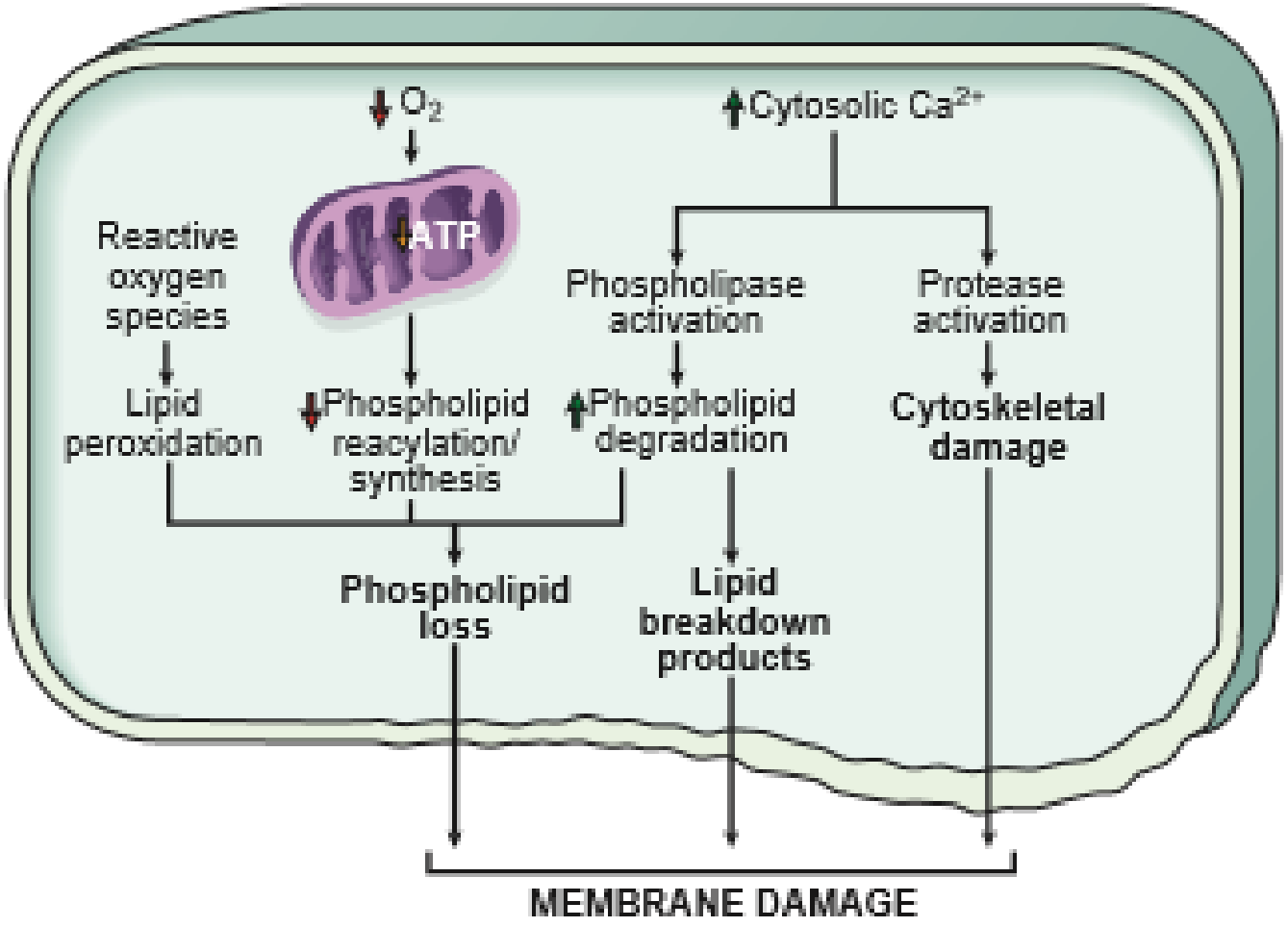
Role of cytosolic calcium in cell injury



3) Increased permeability of cellular membranes:

- Increased membrane permeability lead to membrane damage.
- May affect
  - plasma membrane (loss of osmotic balance, loss of cellular contents)
  - lysosomal membranes (leakage of enzymes into cytoplasm)
  - mitochondrial membranes.(discussed)
- Typically cause in necrosis

Mechanism of membrane damage



- Decreased PL synthesis
- Increased PL breakdown
- ROS
- Increased cytosolic ca+ activate proteases which damage cytoskeleton
- Lipid breakdown products causing changes in membrane permeability.

4) Damage to DNA and Proteins

- Cells have **mechanisms that repair** damage to DNA.
- But if DNA **damage is too severe** to be corrected (e.g., after exposure to DNA damaging drugs, radiation, or oxidative stress), the cell initiates a suicide program that results in death by **apoptosis**.

Clinico-pathologic correlations-

Eg-

- Ischemic and hypoxic injury
- Ischemia - reperfusion injury
- Chemical injury

(refer Robbins basic pathology)

Pathogenesis of hypoxic injury

- Hypoxia is a condition in which the body or a region of the body is deprived of adequate O_2 supply.

- The most common cause of cell injury.

- Hypoxia leads to cell injury mainly by reducing aerobic respiration.

- Cells need O2 to generate energy and perform metabolic functions.
- Deficiency in 02 result in failure to carry out these activities.

- Cell related and injury related factors decide the degree of injury

Ischemia-reperfusion injury

If cells are reversibly injured due to ischemia, restoration of blood flow can recover the cells.

But some instances paradoxically this accelerates injury leading to irreversible injury.

- Eg: In myocardial and cerebral infarctions after thrombolytic therapy.

Mechanisms of cell damage in

Ischaemic reperfusion injury.

- Increased generation of reactive oxygen species.
- Subsequent inflammatory reaction.
- Calcium overload

Pathogenesis of chemical injury

- Direct cytotoxic effect

- By converting to reactive toxic metabolites.

Eg: cyanide - poisons mitochondrial cytochrome oxidase

CCl_4 - conversions to free radical $CCl_3\cdot$ causing lipid peroxidation

Biochemical mechanisms responsible for reversible cell injury

- Mitochondrial damage
- Influx of intracellular calcium
- Increased permeability of cell membrane
- Accumulation of damaged DNA and misfolded proteins

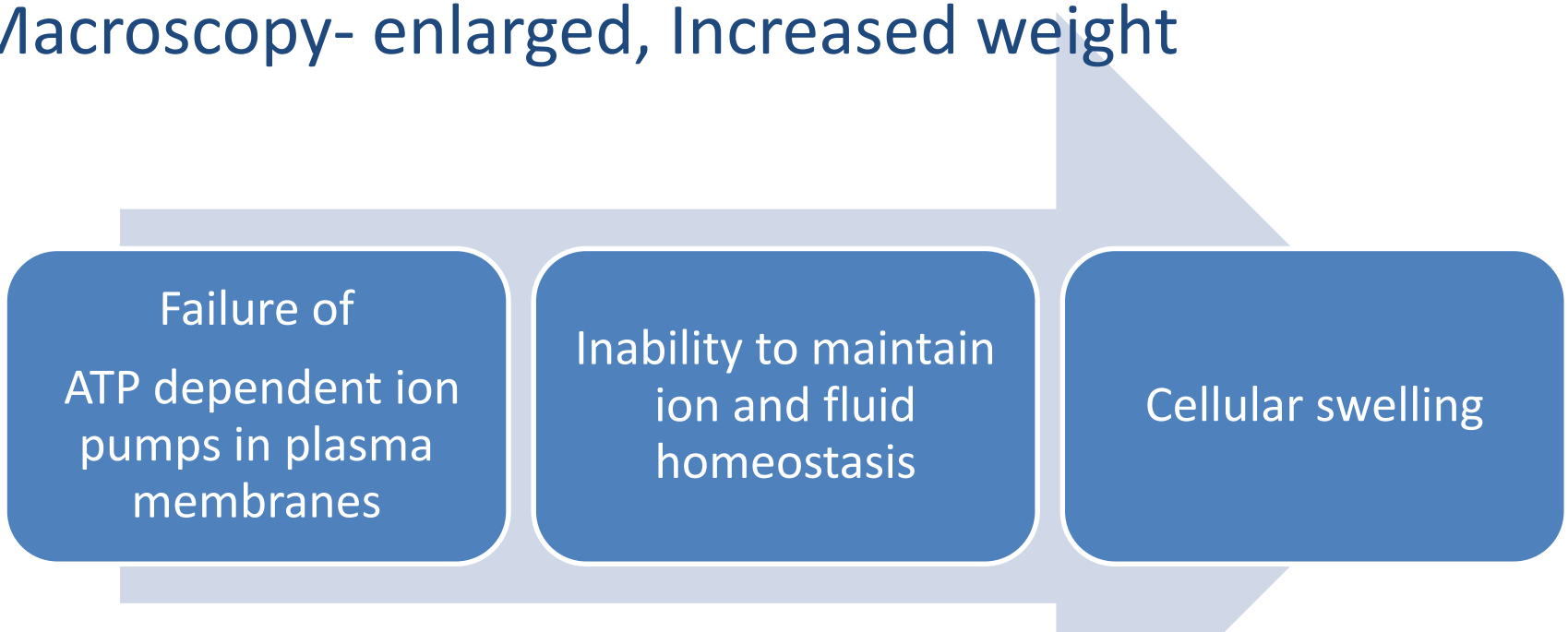
Morphology

Two patterns of reversible cell injury can be recognized under the light microscope:

- Cellular swelling
- Cellular fatty change

- **Cellular swelling** appears whenever cells are incapable of maintaining ionic and fluid homeostasis.
- This is the first manifestation of cell injury.
- It is the result of loss of function of plasma membrane energy-dependent ion pumps.
- It is reversible.

- Macroscopy- enlarged, Increased weight

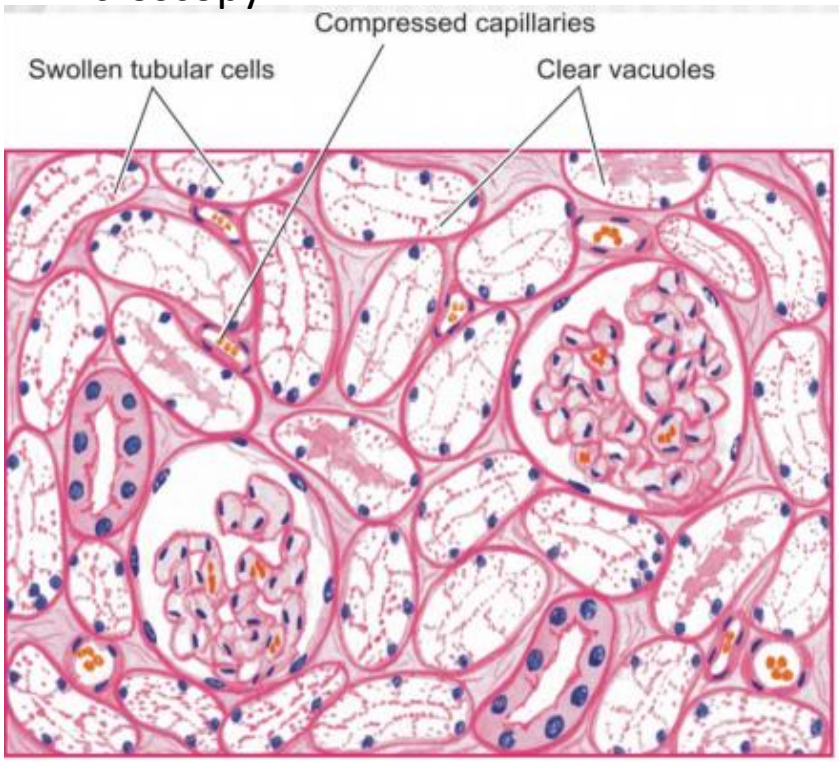


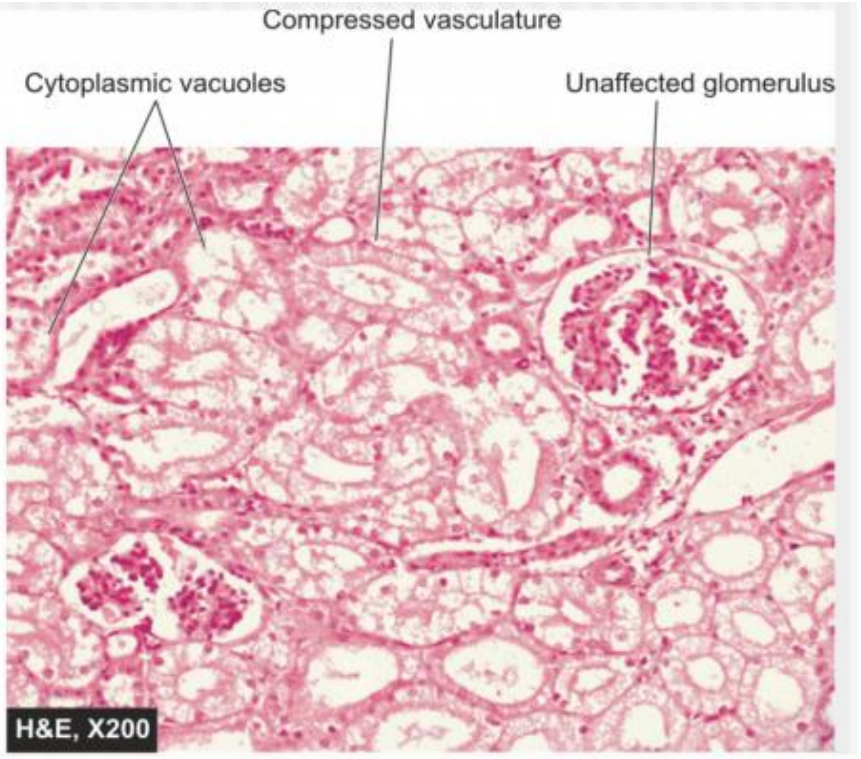
Microscopy

- Small clear vacuoles may be seen within the cytoplasm (these represent distended and pinched-off segments of the ER).
- Organelles within the cell are also swollen.
- Cells may also show increased eosinophilic staining, which becomes much more pronounced with progression to necrosis (described later).
- This pattern is called hydropic change or vacuolar degeneration as well.

Hydropic change

Microscopy



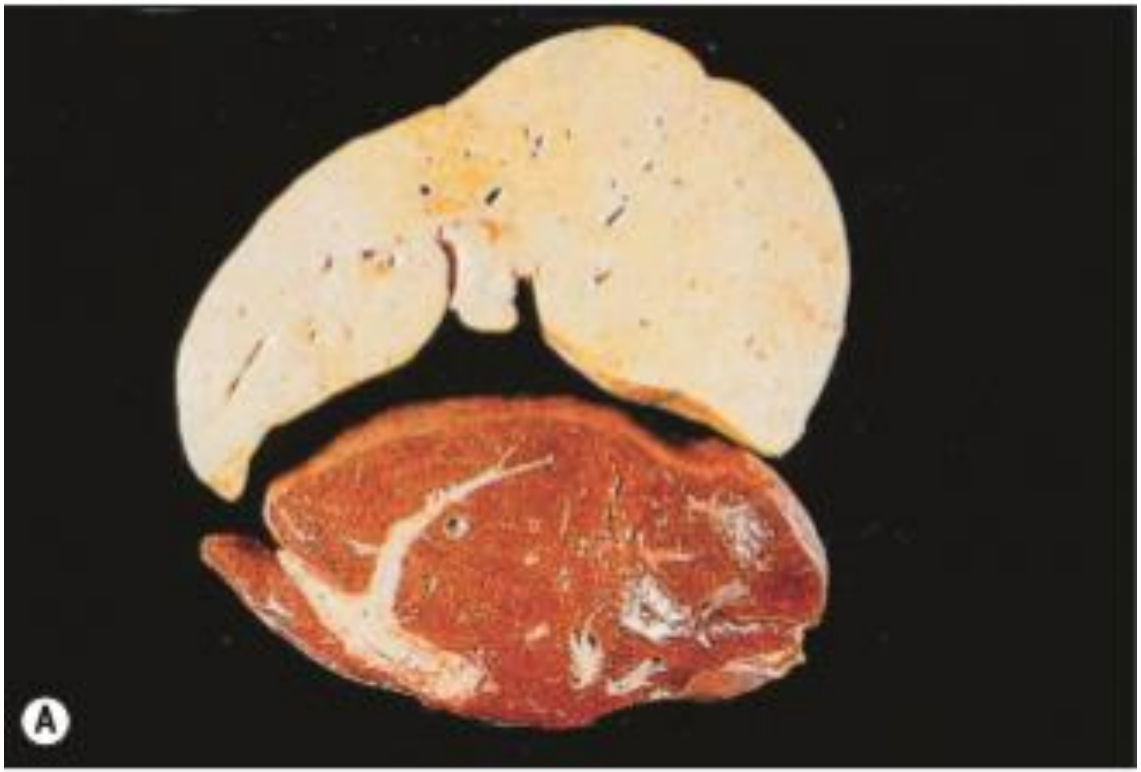


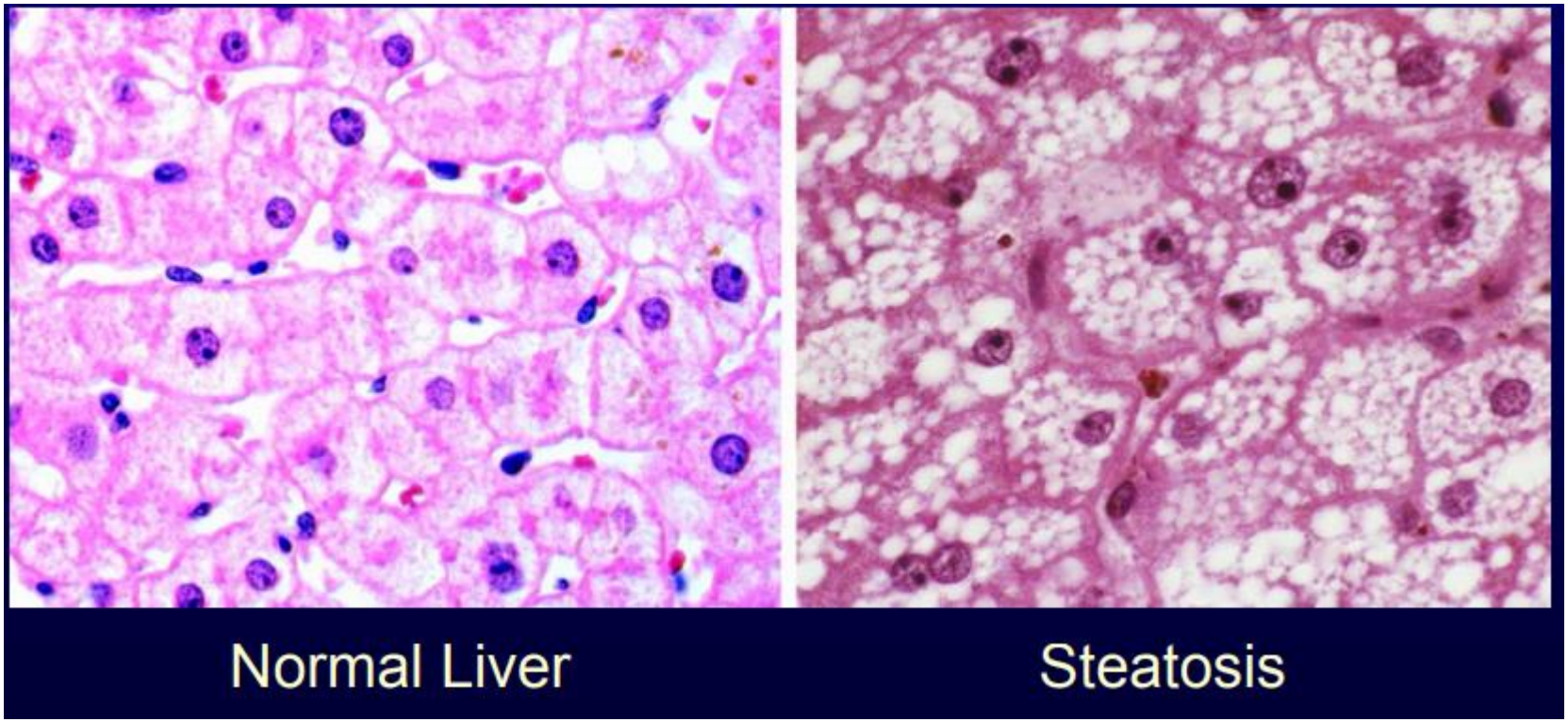
- **Fatty change** occurs in hypoxic injury and various forms of toxic or metabolic injury.
- It is manifested by the appearance of small or large lipid vacuoles in the cytoplasm.
- Abnormal intracellular accumulation of triglycerides.

Eg: hepatocytes, myocardial cell.

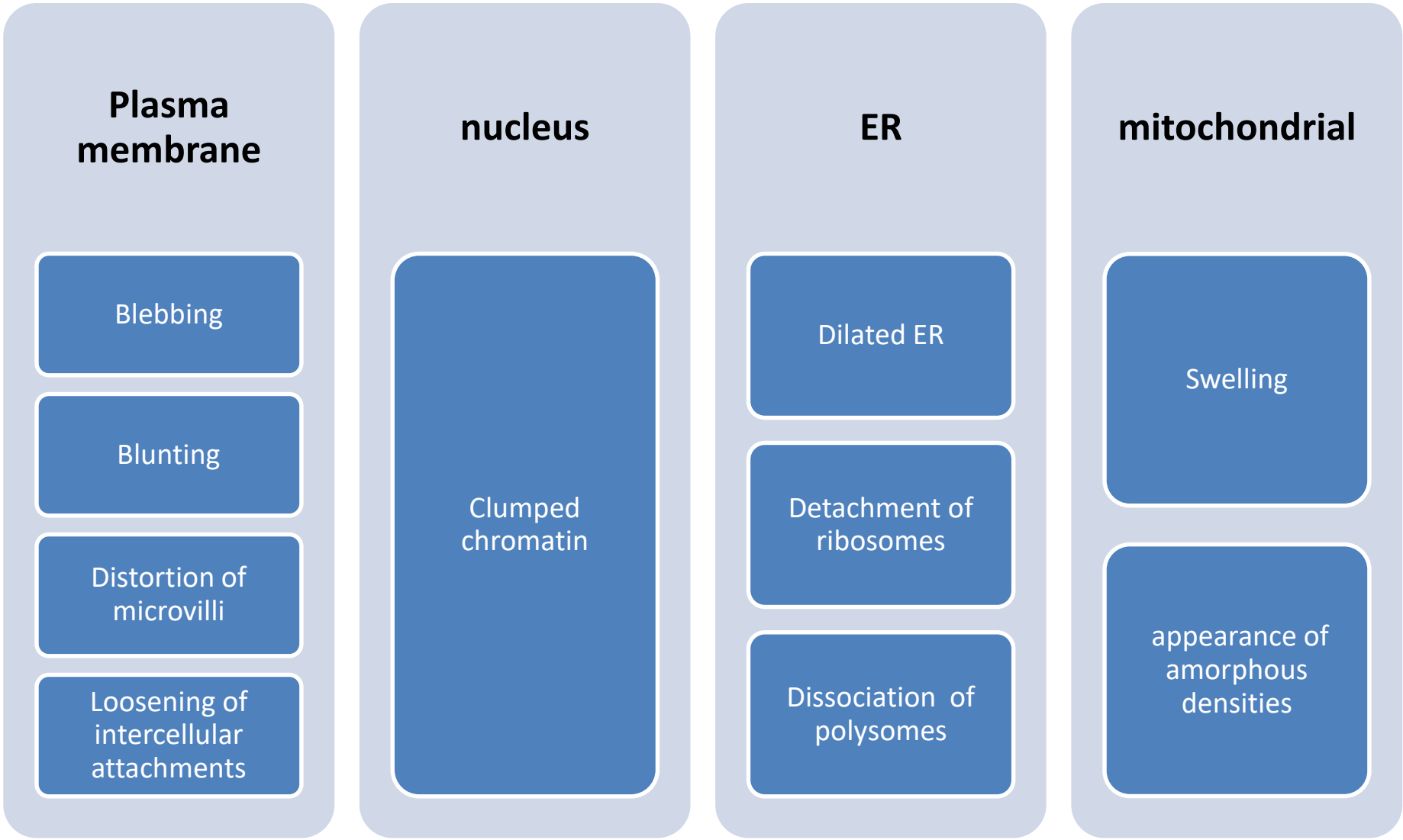
- Macroscopy

Fatty liver – enlarged, greasy, yellow cut surface




Normal Liver
Steatosis

Reversible injury- intracellular changes(Electron microscopic)



Electron microscopic changes of reversible cell injury

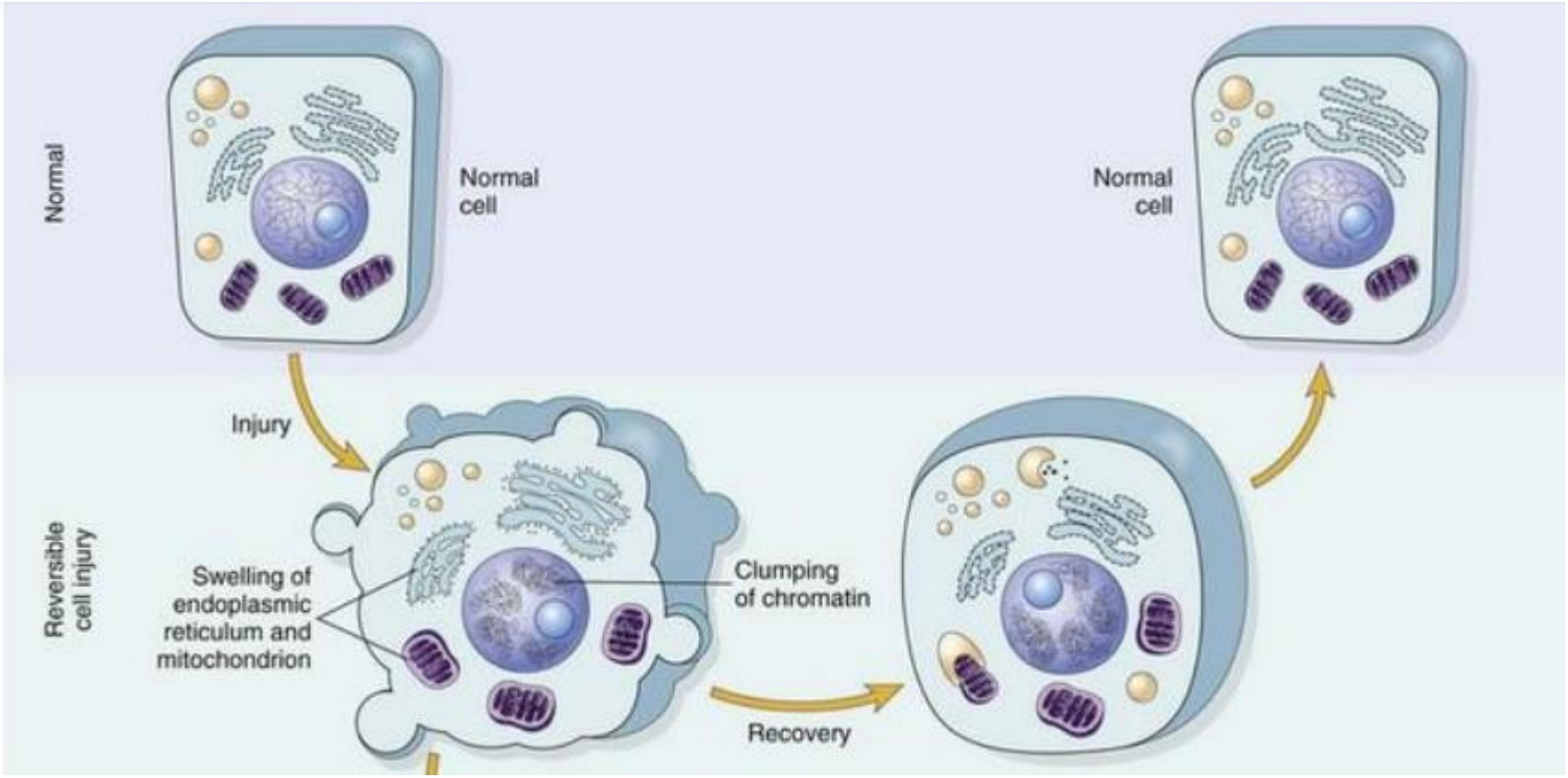
1. **Plasma membrane** alterations, such as blebbing, blunting, and loss of microvilli

2. **Mitochondrial** changes, including swelling and the appearance of small amorphous densities

3. Dilation of the **ER,** with detachment of polysomes; intracytoplasmic myelin figures may be present

4. **Nuclear** changes with clumping of chromatin.

5. Formation of phospholipid aggregates called myelin figures which are derived from damaged cellular membranes



These changes are reversible

Cell injury –Part 11

Irreversible cell injury

Learning outcomes

- Define cell injury, reversible cell injury and irreversible cell injury.
- List the causative agents / injurious stimuli.
- Briefly outline the mechanisms of cell injury.
- Describe the different morphological patterns / appearances of cell injury and list the clinical situations in which they occur

  Reversible cell injury

  Irreversible cell injury - Necrosis and its patterns,
  - Apoptosis,

T/F

- Macroscopic changes can be seen before microscopic changes following cell injury.
- Mitochondrial damage cannot cause apoptosis.
- Free radicles can cause DNA fragmentation.
- Hypoxia leads to cell injury mainly by reducing aerobic respiration.

T/F

- Fatty change is a morphological manifestation of irreversible cell injury.
- Hydropic change occur when cells are incapable of maintaining ionic an fluid homeosatsis.
- Blebbing and blunting of the plasma membrane can only be seen in irreversible cell injury.
- Clumped chromatin can be seen in reversibly injured cells.

Answers

- Macroscopic changes can be seen before microscopic changes following cell injury. **F**
- Mitochondrial damage cannot cause apoptosis.**F**
- Free radicles can cause DNA fragmentation
- Hypoxia leads to cell injury mainly by reducing aerobic respiration. **T**

T/F

- Fatty change is a morphological manifestation of irreversible cell injury. **F**
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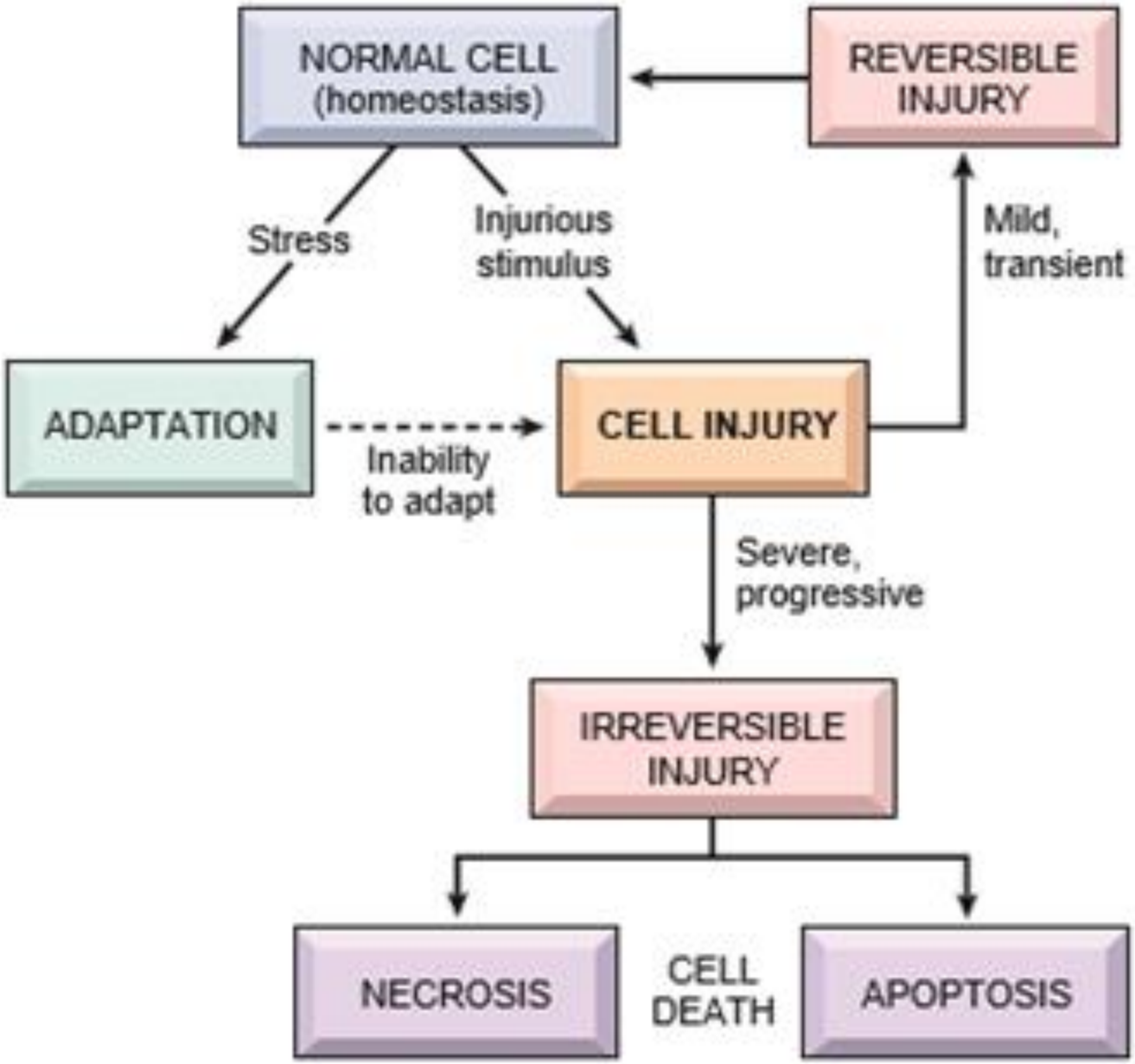
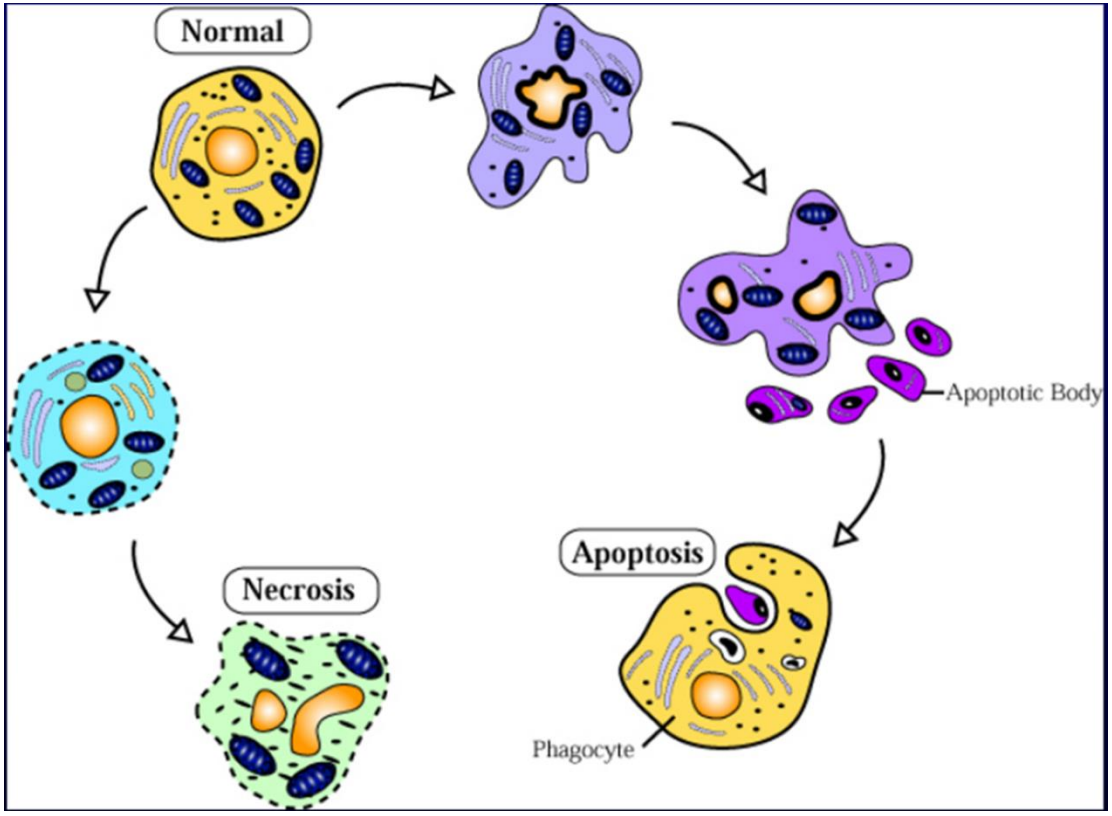


Figure 2-1 Stages of the cellular response to stress and injurious stimuli.

Irreversible cell injury patterns

Necrosis

Apoptosis



Necrosis

- A spectrum of morphological changes that follow cell death in living tissues.

- Necrotic cells are unable to maintain membrane integrity and their **contents leak out**.
- Leaked out cellular contents lead to **inflammation** in the surrounding tissue trying to remove the dead cells.

- Lysosomal enzymes of the dying cells and the lysosomal enzymes of leucocytes recruited as part of the inflammatory reaction to dead cells are responsible for digestion of cells.

Necrosis…..

The morphologic appearance of necrosis is the result of

denaturation of intracellular proteins and enzymatic digestion of the lethally injured cell.

Necrosis-microscopy

- **Increased eosinophilia of cytoplasm**

  (loss of cytoplasmic RNA which binds hematoxylin and in part to denatured cytoplasmic proteins which bind the eosin).

- **A glassy homogeneous appearance**

  (as a result of the loss of glycogen particles).

- **Cytoplasm becomes vacuolated and appears moth eaten**

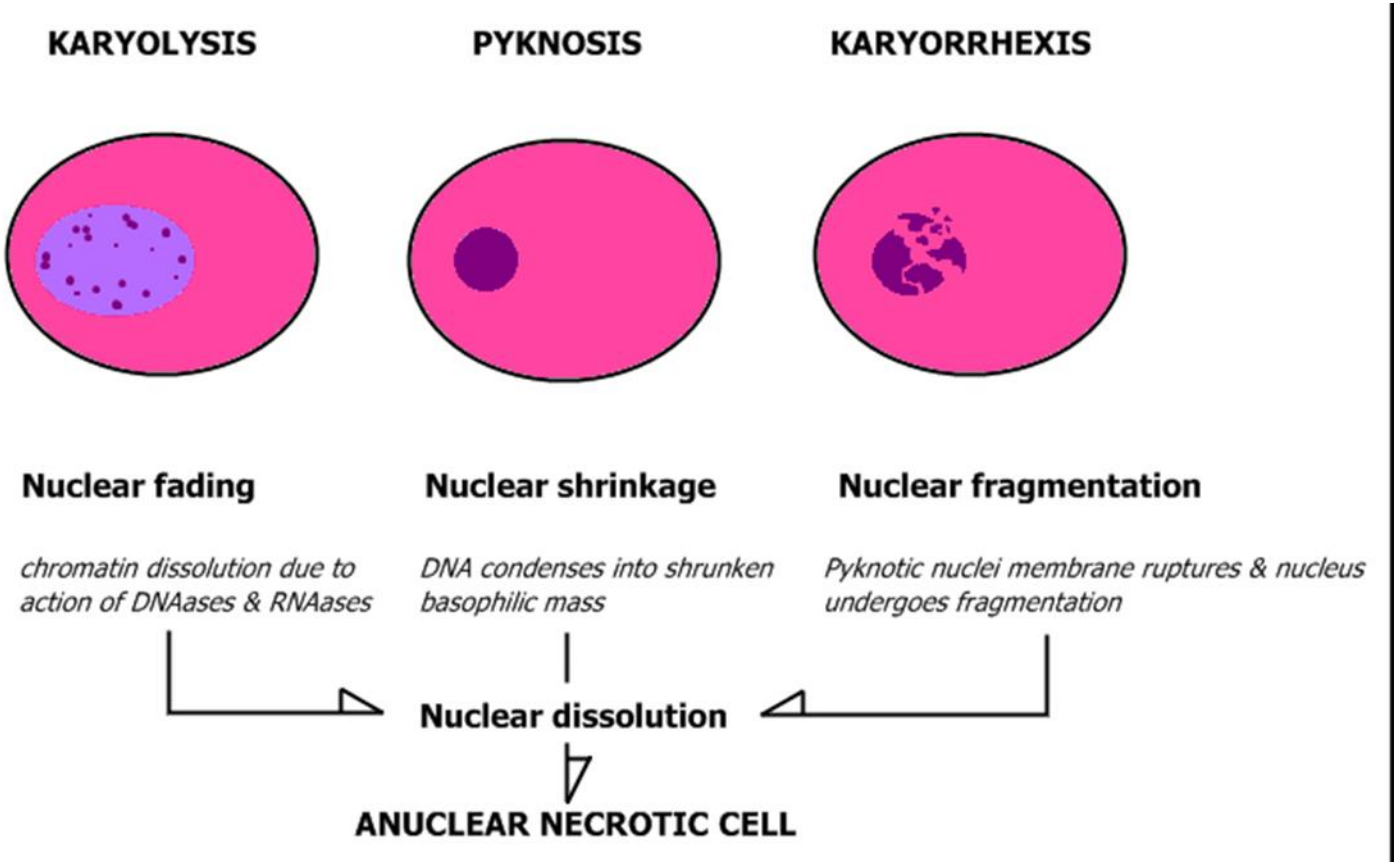
  (enzymes have digested the cytoplasmic organelles)

- **Breakdown of plasma membrane and organelle membranes**

- **Abundant myelin figures**.

(Dead cells may be replaced by large, whorled phospholipid masses called myelin figures that are derived from damaged cell membranes)

- **Nuclear changes**



Electron microscopic changes of reversible cell injury

1. **Plasma membrane** and organelle membranes become discontinus

2. **Mitochondrial** changes, marked swelling and the appearance of large amorphous densities

3. Intracytoplsmic myelin figures
4. Disrupted lysosomes
5. **Nuclear** changes – dissolved nuclei

Fate of necrotic cell

- May persist for sometime or may be digested by enzymes and disappear.
- May be replaced by myelin figures which are phagocytosed or further degraded to fatty acids.
- Fatty acids can bind calcium and become calcified.

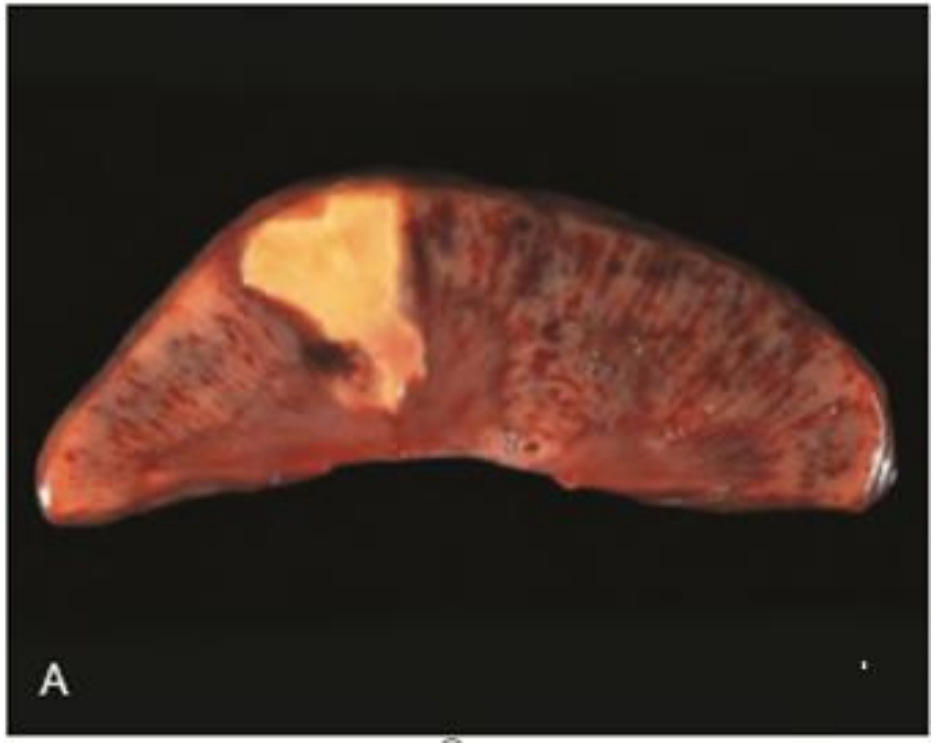
Patterns of tissue necrosis:

Necrosis of tissues has several morphologically distinct patterns.

These are important to recognize because they may provide clues about the underlying cause.

- Coagulative
- Liquefactive
- Caseous
- Fat necrosis
- Fibrinoid

Coagulative necrosis



wedge-shaped kidney infarct (yellow).

- The most common cause is hypoxia.
- All parenchymal tissues undergo coagulative necrosis.

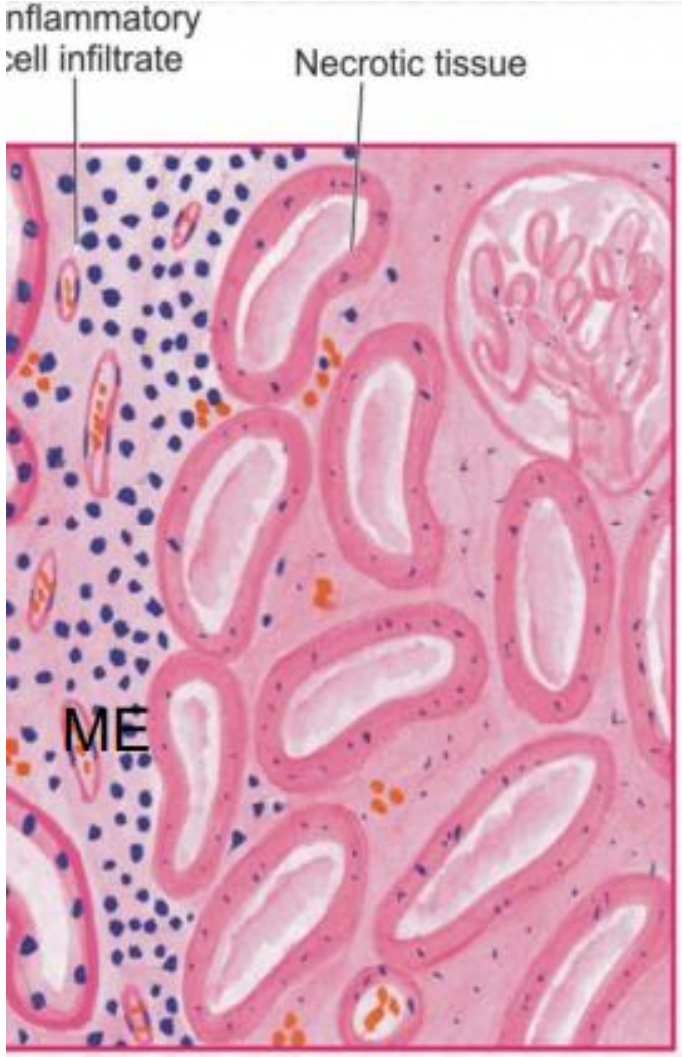
(except brain and abscesses)

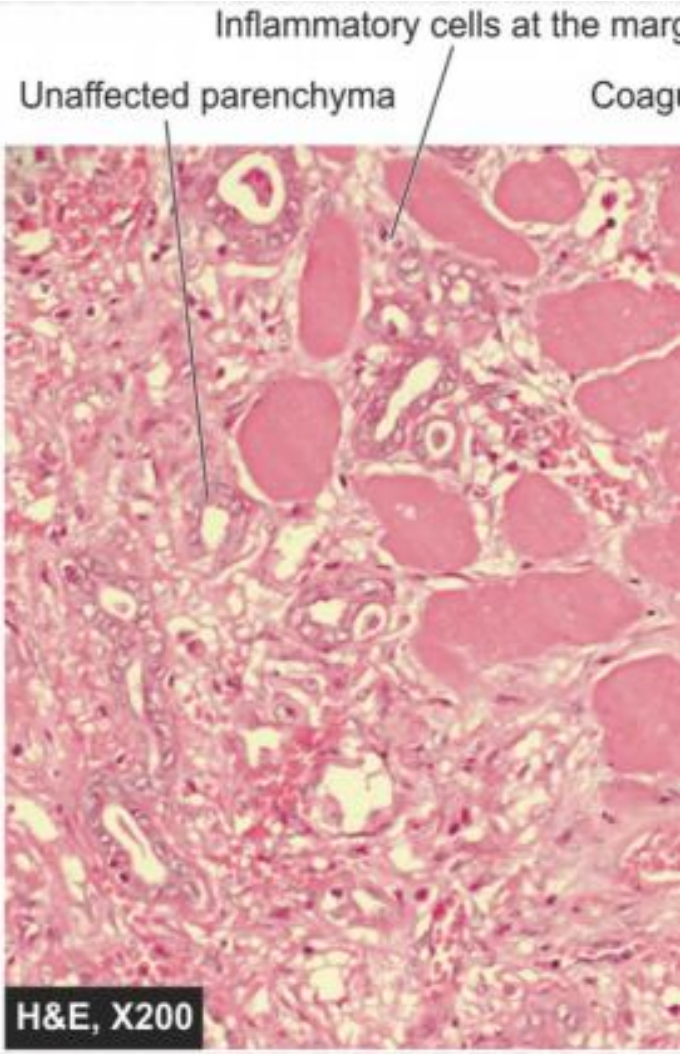
- Injury results denaturing of proteins.

(structural proteins and enzymes).

- Anucleated cells persist for days (proteolysis blocked).

- Leucocytes migrate to the site and digest the cells by enzymes. Debris removed by phagocytosis.

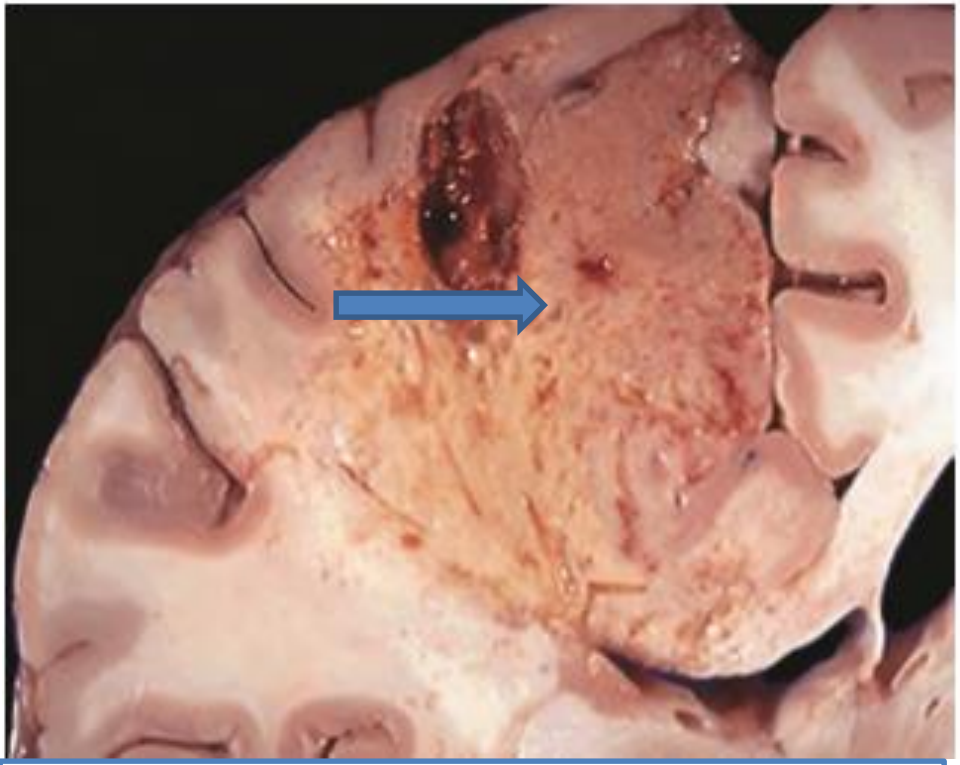




Coagulative necrosis

- Necrotic cells in the infarct show preserved cellular outlines (ghost outline)
- Eosinophilic cytoplasm
- loss of nuclei
- Adjacent inflammatory infiltrate

Liquefactive necrosis



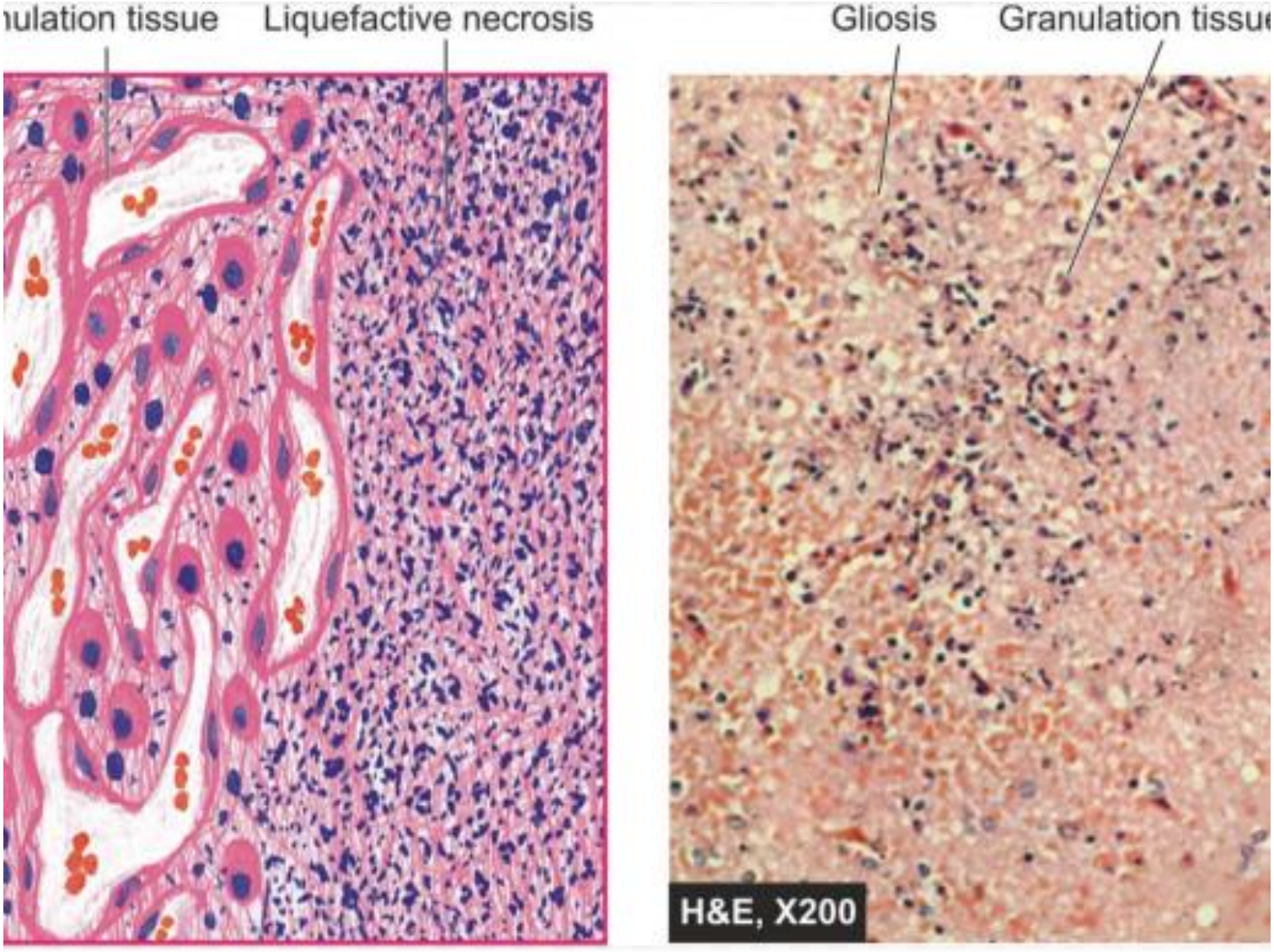
An infarct in the brain, showing dissolution of the tissue.

- Tissues in which the initial digestion of cells and tissues predominates, with loss of structure.
- Transform the tissue into a liquid mass.

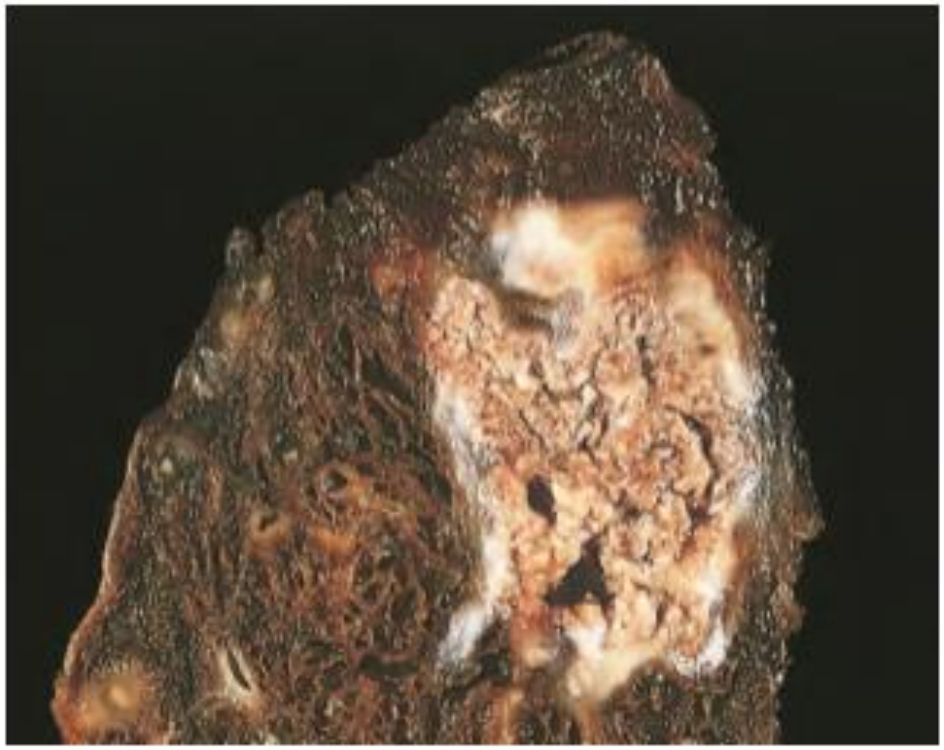
➢ Necrosis due to bacterial infection - substances released by bacteria and by PMNs attracted to the area result in rapid dissolution of the tissue - i.e. pus

➢ Brain - infarction of brain tissue is followed by rapid dissolution, resulting in a liquid-filled space - unknown reason?

Colliquative (Liquefactive) Necrosis

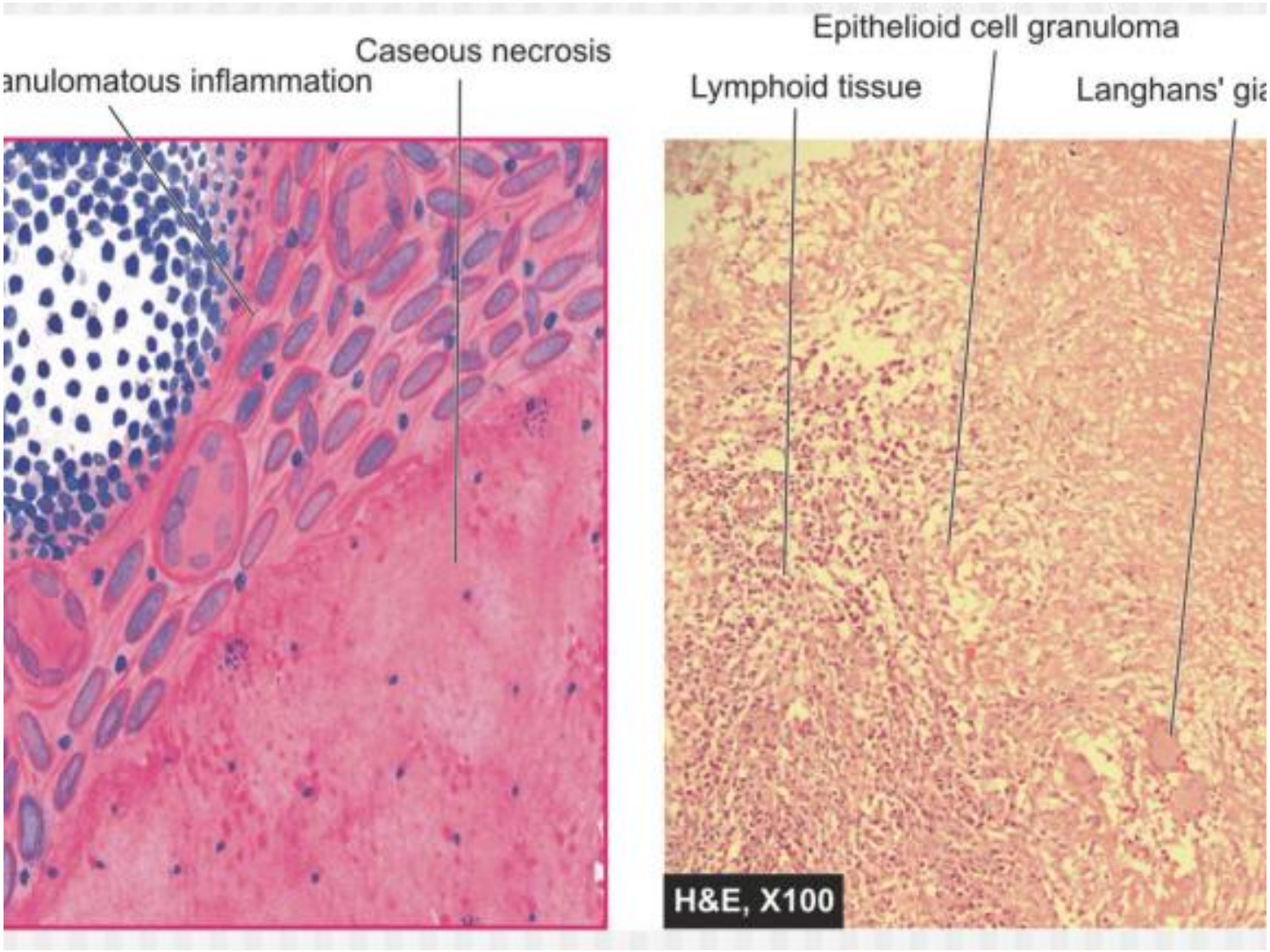


Caseous necrosis.



Tuberculosis of the lung, with a large area of caseous necrosis containing yellow-white and cheesy debris.

- Appear ‘cheese like’
- process starts as coagulative necrosis and the necrotic tissue is broken down. This results loss of the structure.
- Seen in Tuberculosis and some fungal infections.



Microscopy-

tissue architecture completely lost.

necrotic material appear as a pink granular substance.

The area of necrosis enclosed by inflammatory cells

Fat necrosis

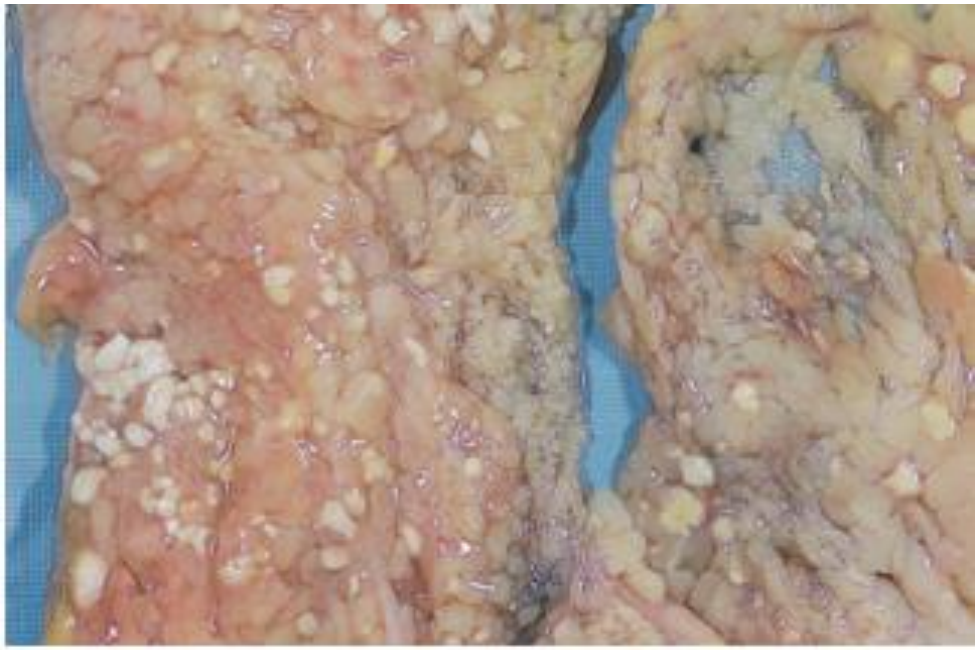
Related to fatty tissues

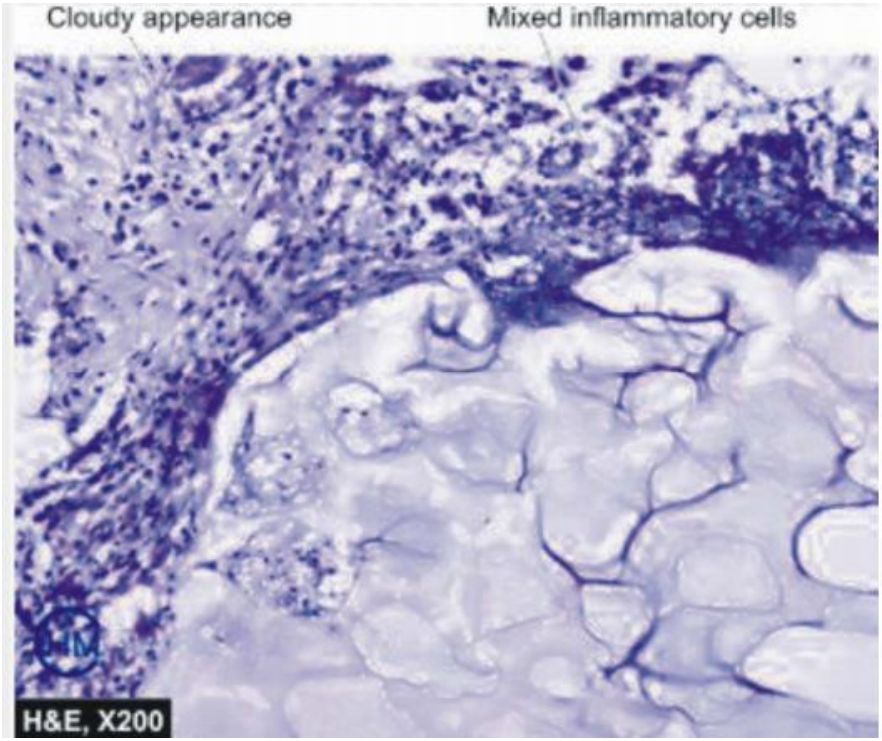
- Enzymatic- Mesentric fat necrosis due to acute pancreatitis
- Non enzymatic- traumatic fat necrosis of breast

Fat necrosis

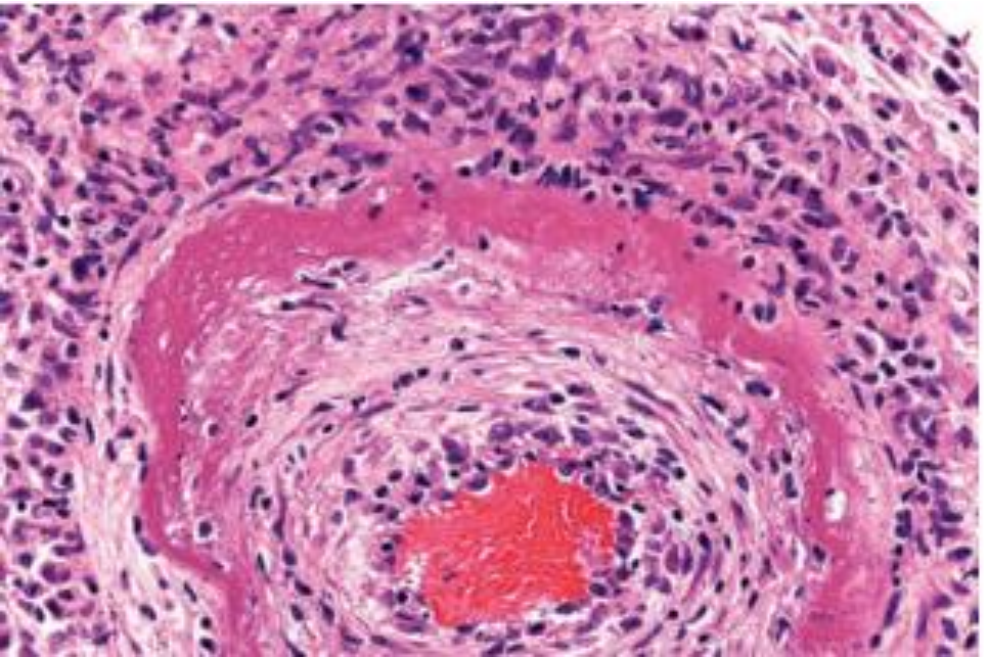
In acute pancreatitis lipases released from the pancreas act on fatty tissues causing digestion of fat into the free fatty acids and glycerol.

Free FA combine with calcium to foam calcium soaps which appear as **firm white chalky masses.**





Fibrinoid necrosis



A type of connective tissue necrosis.

seen in vessel walls during hypertension, autoimmune diseases.

Micro-

Loss of normal structure and replacement by bright pink necrotic material similar to fibrin.

Gangrene

Not a distinctive pattern of cell death.

Gangrene - special type of coagulation necrosis

- Gradual ischemia of distal extremities, esp. foot and leg
- “dry” - black-brown, mummified appearance
- “wet” - bacterial superinfection of the necrotic material (Liquefactive Necrosis)

Effects of necrosis

Abnormal function

- Myocardium: Heart failure
- Brain : paralyze

Bacterial infection

- gangrene

Release contents within necrotic material

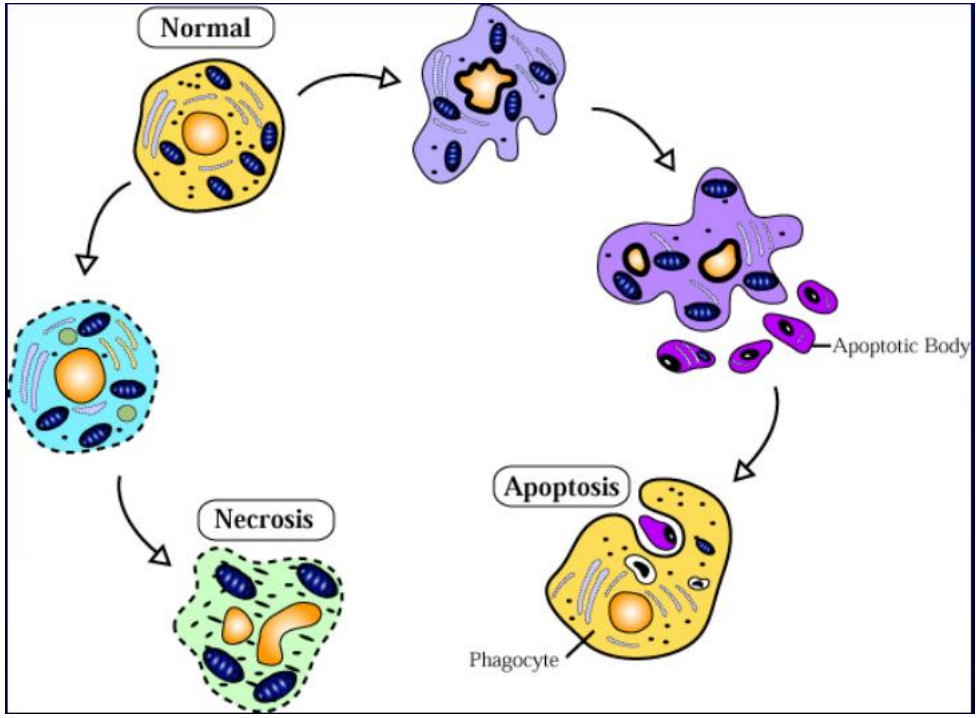
- Myocardial infarction -Creatinine kinase
- AST, ALT from hepatocytes

Systemic effects: fever, leucocytosis

Local effects: Eg Ulcers

Apoptosis

- Programmed cell death.
- A distinctive morphological pattern of cell death.



- Occur in single cells
- An energy dependent process

Apoptosis

- Definition

Apoptosis is a pathway of cell death.
It is induced by a **tightly regulated suicide** program.
in this cells are destined to die due to activated intrinsic **enzymes that degrade the cells'** own nuclear DNA and nuclear and cytoplasmic proteins.

Causes of apoptosis

Physiologic conditions

- During embryogenesis
- Involution of hormone dependent tissues upon hormone deprivation
- Cell loss in proliferation of cell populations
- Elimination of cells which have served their purpose
- Elimination of self reactive lymphoctes

Pathologic conditions

- DNA damage
- Accumulation of misfolded proteins
- Cell injury in infections. eg-viral
- In organ atrophy after duct obstruction

Refer- examples for each of these conditions.

If apoptosis fail??

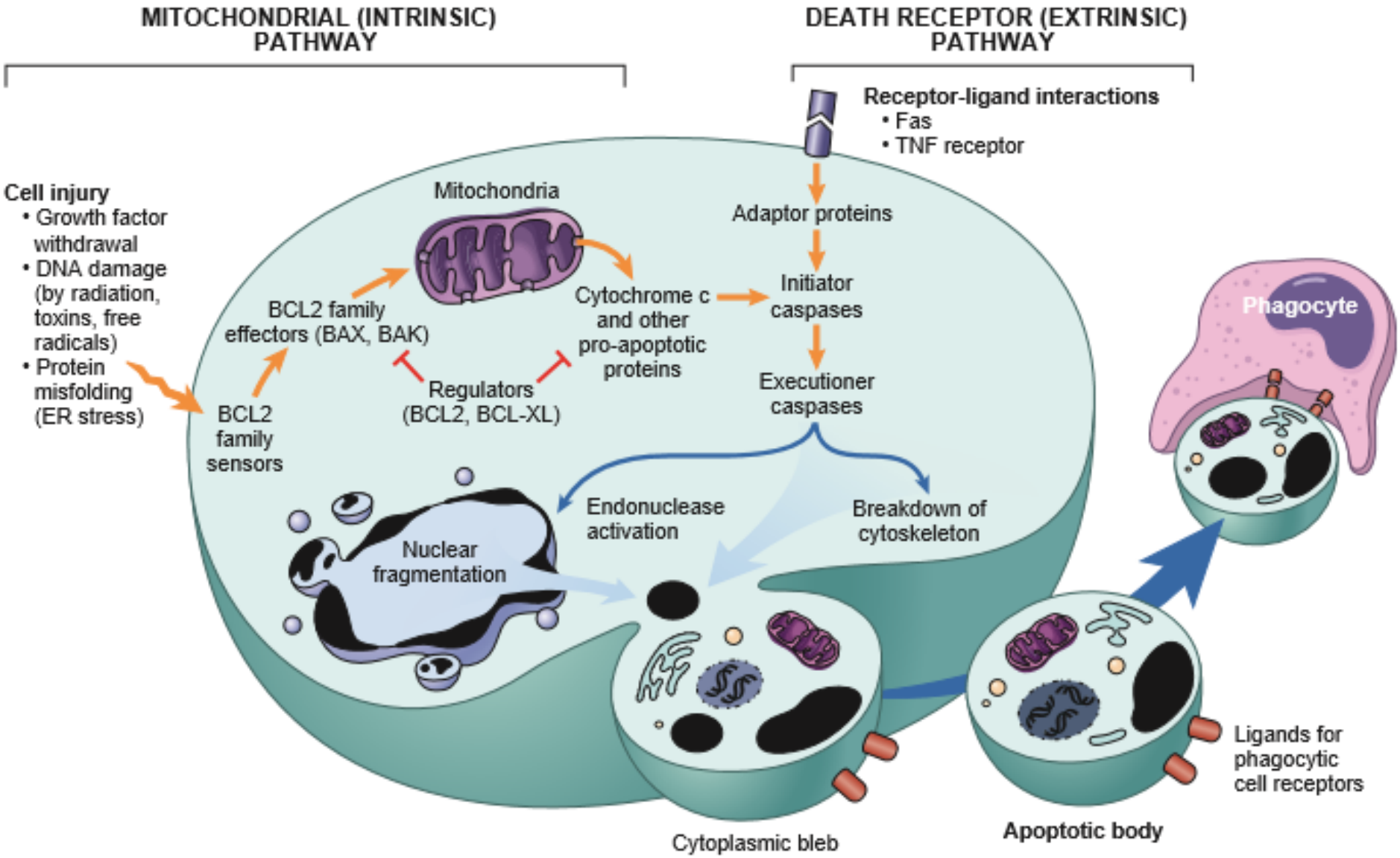
- Aberrant development
- Tumour proliferation
- Autoimmune diseases

Mechanisms of apoptosis.

- There are two pathways of apoptosis.
- They differ in their induction and regulation.
- But both activate caspases.

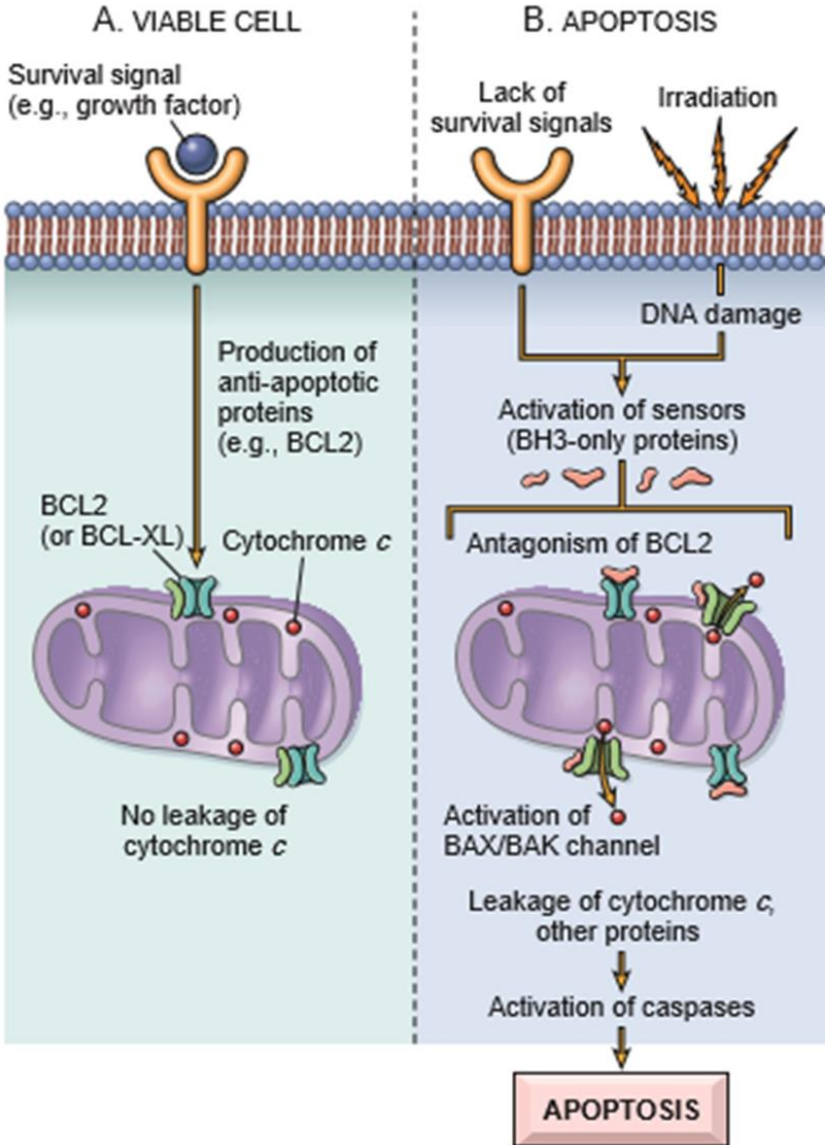
- **Mitochondrial pathway**
- **Death receptor pathway**

Mechanisms of apoptosis



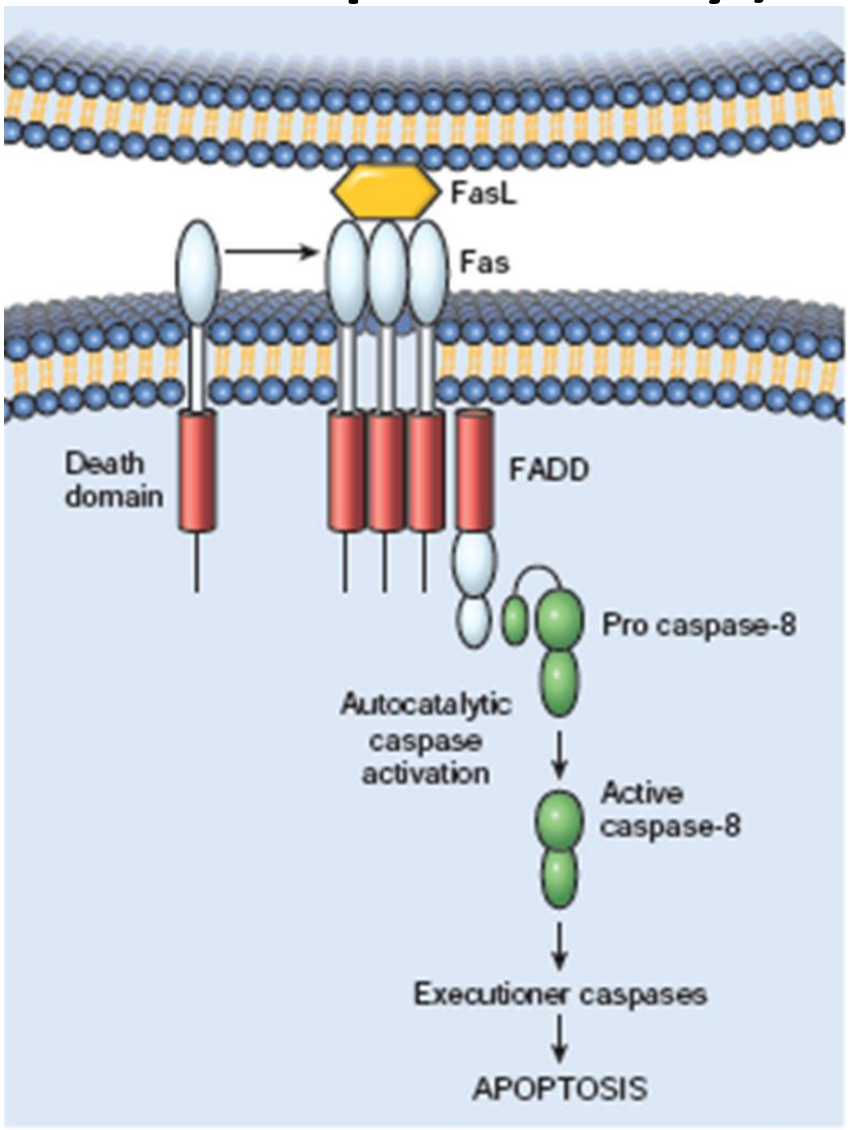
Mitochondrial (intrinsic)pathway of apoptosis

- Cell viability is maintained by the induction of **anti-apoptotic proteins such as BCL2** by survival signals.
- These proteins maintain the integrity of mitochondrial membranes and prevent leakage of mitochondrial proteins.
- Loss of survival signals, DNA damage, and other insults activate sensors that antagonize the anti-apoptotic proteins and activate the **pro-apoptotic proteins BAX and BAK**, which form channels in the mitochondrial membrane.
- The subsequent leakage of cytochrome c and other proteins leads to caspase activation and apoptosis.



Death receptor (Extrinsic pathway)

- Death receptor are surface molecules on many cells that trigger apoptosis.(type 1 TNF, Fas/CD95)
- Fas Ligand (Fas L)is a membrane protein expressed on activated T lymphocytes.
- When these lymphocytes bind to Fas receptor expressing targets(Fas- Fas L), recruit and activate caspases.

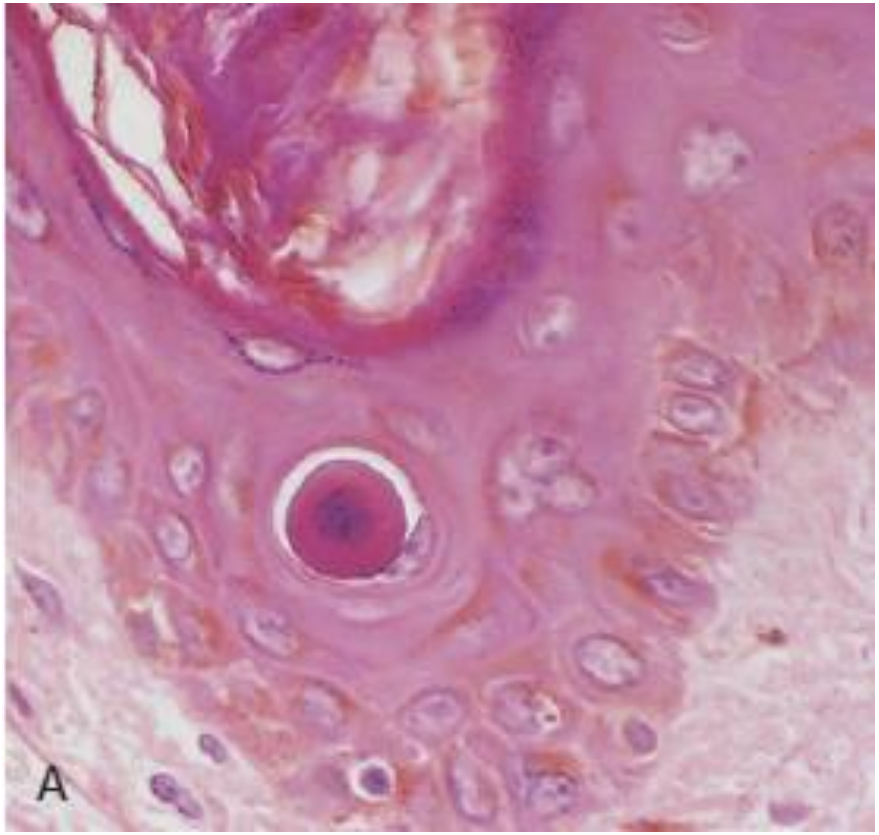


- Caspases activate nucleases that degrade DNA an nucleoproteins.
- Caspases also degrade nuclear matrix and cytoskeleton.
- Cause cell fragmentation
- Apoptotic cells removed fast by phagocytosis.

Morphology

Microscopy

- Shrunken cells
- Form cytoplasmic buds and apoptotic bodies(membrane bound vesicles of cytosol and organelles).
- Chromatin condensed and aggregated.
- No inflammatory response (because fragmented cells are quickly eliminated by phagocytosis).



Apoptosis of an epidermal cell in an immune reaction. The cell is reduced in size and contains brightly eosinophilic cytoplasm and a condensed nucleus.

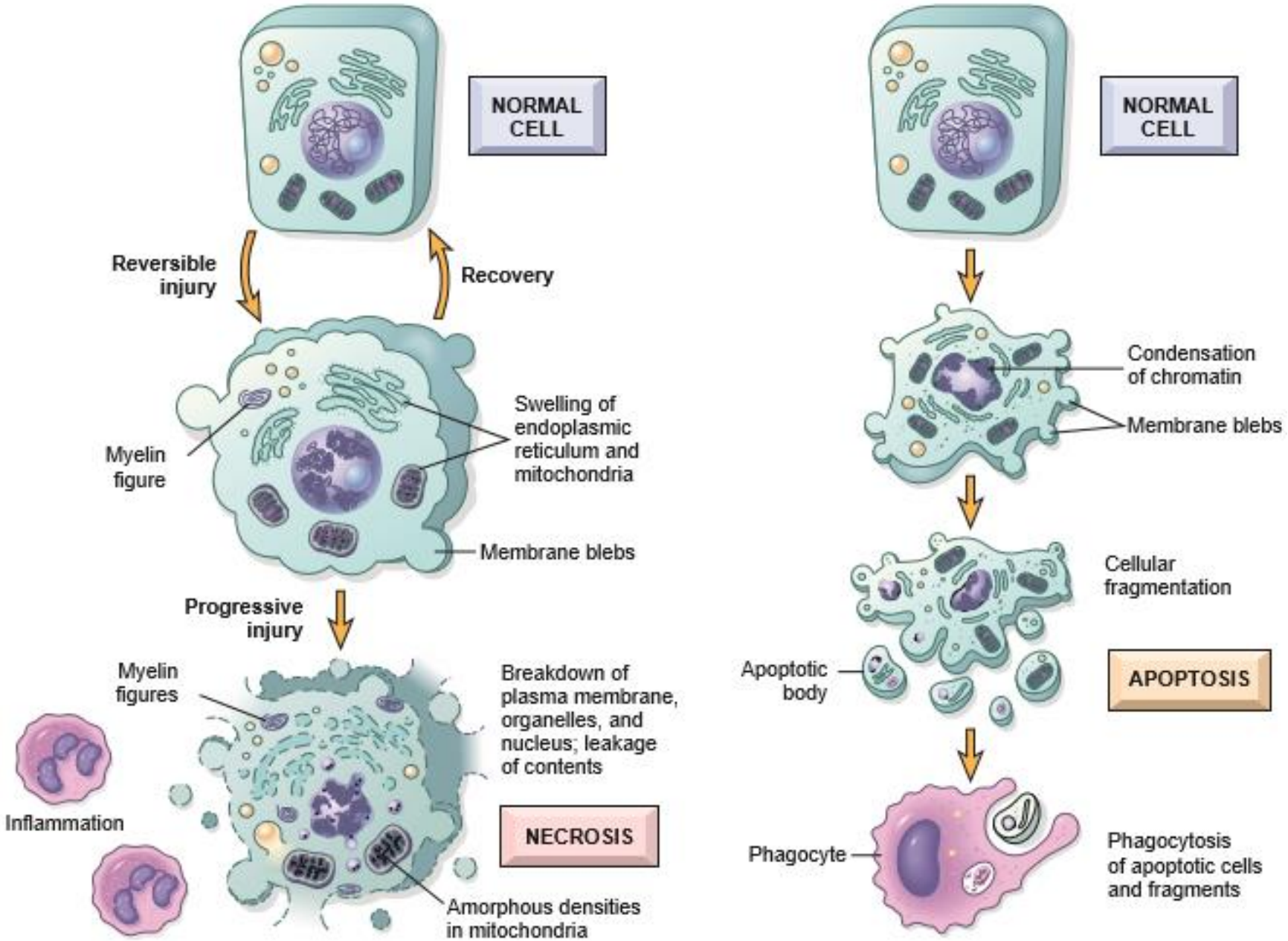


Figure 2-8 Schematic illustration of the morphologic changes in cell injury culminating in necrosis or apoptosis.

	necrosis	Apoptosis
Stimuli	Always pathological	Physiological and pathological
mechanisms	Not Energy dependent process	Energy dependent
	ATP depletion Membrane injury Free radical damage	Gene activation Endonucleases, proteases
	Large groups of cells	Single cells
Histology	Cell swelling	Cells shrink Chromatin condense Apoptotic bodies form
DNA breakdown	Random/diffuse	Intra nucleasomal
Cell membrane	Integrity is lost	Integrity is maintained
Tissue reaction	Inflammation	No inflammation Phagocytosis of apoptotic bodies

summary

Further reading

- What is necroptosis?
- What is autophagy?

Questions

- Compare and contrast necrosis and apoptosis
- List different types of necrosis. Give example for each.
- Compare and contrast coagulative type necrosis vs liquefactive necrosis.
- What are the two apoptotic pathways??