# MJF COLLEGE OF VETERINARY AND ANIMAL SCIENCE, CHOMU, JAIPUR

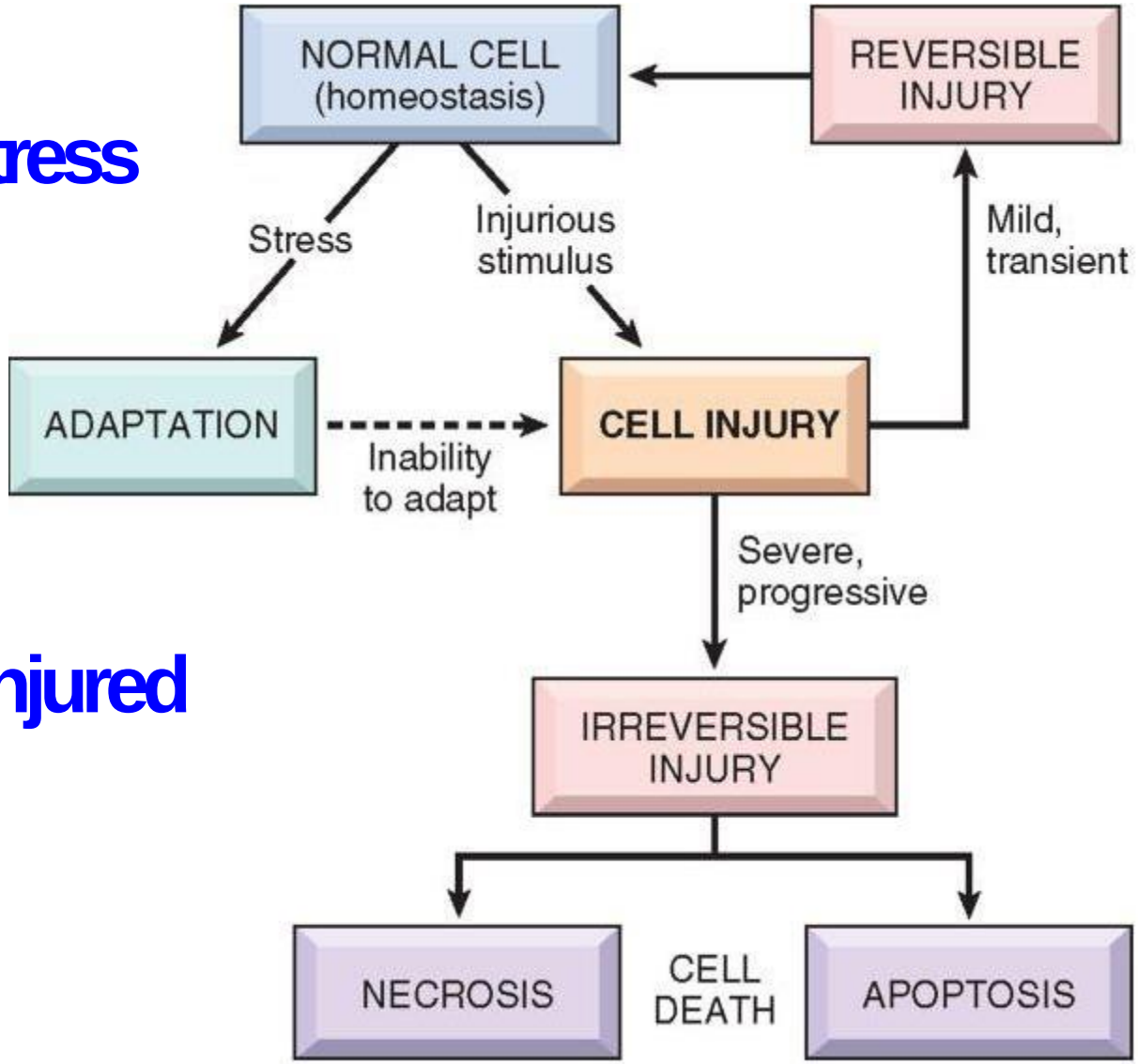
## DEPARTMENT OF VETERINARY PATHOLOGY

# CELLULAR RESPONSES TO STRESS

# Cellular responses to stress

## In response to stress

- Cells may:
- Adapt
- Be reversibly injured
- Die



# CAUSES OF CELL INJURY

- **Extrinsic** :- Trauma, viruses, and toxins, etc..
- **Intrinsic** :- Genetic mutations
- **Both Extrinsic or Intrinsic** :- Nutritional abnormalities, and immunologic dysfunctions

## ❶ Oxygen deficiency

- Common and important
- **Hypoxia:-** Partial reduction in the O2 concentration supplied to cells or tissue
- **Anoxia:-** Complete reduction in the O2 concentration supplied to cells or tissue

- Hypoxia reduces aerobic oxidative respiration
  - Cardio - Respiratory failure
  - loss of blood supply
  - Reduced transport of O2 in blood (i.e. Anemia or CO2 toxicity)
  - Blockage of cell enzymes (cyanide toxicosis)
  - Ischemia: loss of blood supply.

❷ **Physical agents**

- Trauma
- Extremes heat or cold
- Radiation
- Electrical energy

## ❸ Infectious agents

- Viruses
- Bacteria
- Mycotic agents
- Protozoa
- Metazoan parasites

## ❹ Nutritional deficiency and imbalances

- Protein-calorie deficiencies
- Protein-calorie excess
- Vitamin and mineral imbalances

## ❺ Genetic derangement

- Mutations, whatever their origin, may cause no disease, or deprive a cell of a critical protein (enzyme), or may be incompatible with cell survival.
  - E.g. defects of clotting factors (hemophilia)
  - Lysosomal storage disease (mannosidosis)

## ❻ Workload imbalance

- Overworked may adapt to the demand or eventually become exhausted and die
- Conversely, cells that are no longer stimulated to work may shrink in size and waste away

## ⑦Chemicals, drugs, toxins

- Influence cells by a multitude of mechanisms
  - Block or stimulate cell membrane receptors
  - Alter specific enzyme systems
  - Produce toxic free radicals
  - Alter cell permeability
  - Damage chromosomes
  - Modify metabolic pathways
  - Damage structural components of cells

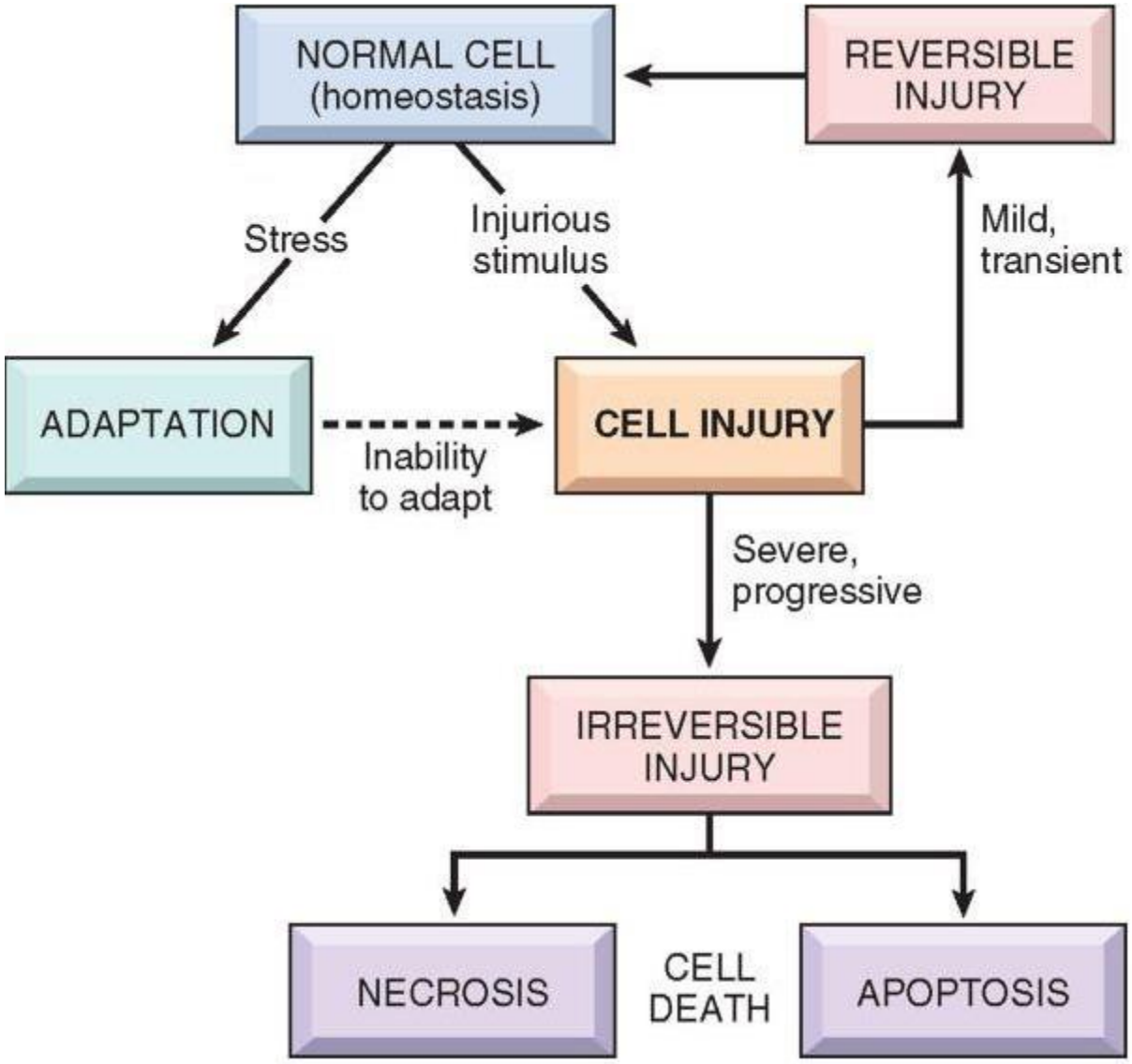
## ⑧Immune dysfunction

- Failure to respond due to congenital or acquired defects
- Autoimmunity
- Hypersensitivity

## ❻Aging

- Those lesions commonly found in aged animals
- lesions for which we have no other defensible mechanistic explanation
- E.g. nodular hyperplasia of parenchymal cells in the liver, pancreas, adrenal, spleen, and thyroid.
- Cancer (?)

# Mechanism of cell injury


NORMAL CELL
(homeostasis)
REVERSIBLE
INJURY
Stress
Injurious
stimulus
Mild,
transient
ADAPTATION
Inability
to adapt
CELL INJURY
Severe,
progressive
IRREVERSIBLE
INJURY
NECROSIS
CELL
DEATH
APOPTOSIS

# Definitions

**Homeostasis**

- Mechanism by which the body is kept in equilibrium **or**
- Cells maintain normal structure & function in response to physiologic demands

**Cellular Adaptation**

- As cells encounter some stresses they may undergo functional or structural adaptations to maintain viability / homeostasis
- Respond to some stimuli by *increasing or decreasing specific organelle content*
- Adaptive processes: atrophy, hypertrophy, hyperplasia and metaplasia

## Cell Injury

- If limits of the adaptive response are exceeded or if adaptation not possible, a sequence of events called cell injury occurs

## Reversible Cell Injury

- Reversible cell injury is injury from which the cell can adapt or recover and thus return to normal or nearly normal function.

## Irreversible Cell Injury / Cell Death

- If stimulus persists (or severe enough from the start) cell suffers irreversible cell injury and death
- 2 main morphologic patterns: necrosis & apoptosis

# General Considerations

**❶ Cellular response to injurious stimuli is dependant on:**

- Type of injury
- Duration of injury
- Severity of injury
  - Low doses or brief durations - reversible cell injury
  - High doses or longer intervals - irreversible injury / cell death

**❷Consequences of an injurious stimulus are dependent on:**

  - Type of cell injured
  - Current status of the cell (glycogen, hormonal, metabolic, O2 needs)
  - Adaptability
  - Genetic makeup of the injured cell

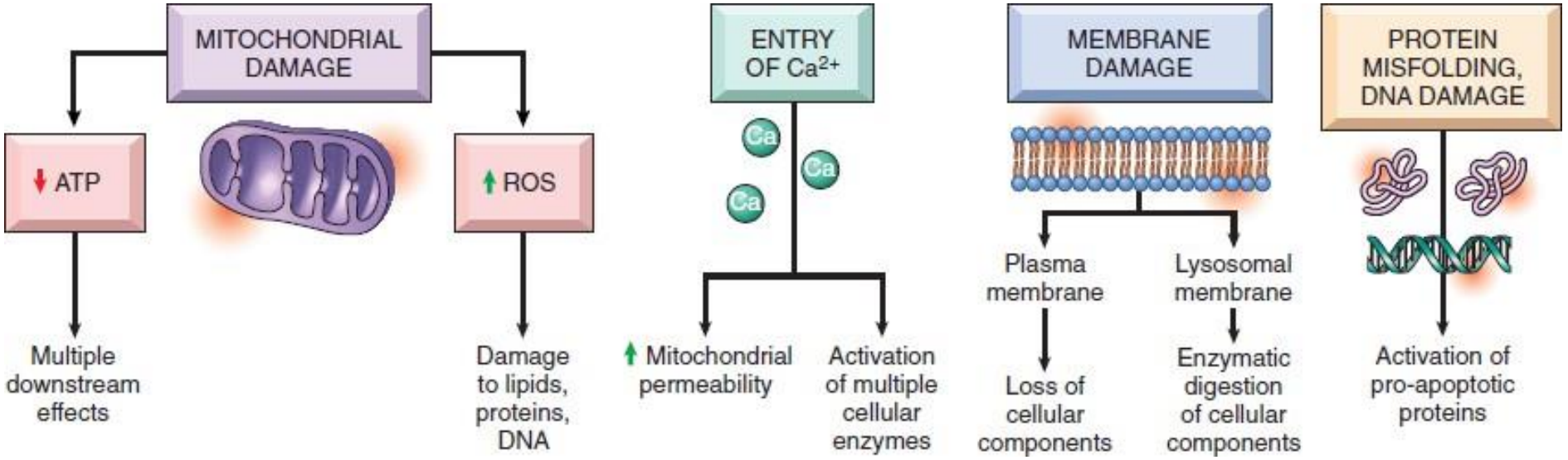
# Tissue sensitivity to hypoxia

| SENSITIVITY | CELL TYPE | TIME (to irreversible cell injury) |
|---|---|---|
| HIGH | Neurons | ~ 3 to 5 min |
| INTERMEDIATE | Cardiac myocyte<br>Hepatocyte<br>Renal epithelium | ~ 30 min to 1 hrs |
| LOW | Fibroblasts<br>Keratinocytes<br>Skeletal muscle | many hrs |

## ❸ Cell injury results from functional and biochemical abnormalities in one or more of several essential cellular components

- The principal targets and biochemical mechanisms of cell injury are
  1) Mitochondria and their ability to generate ATP and ROS under pathologic conditions
  2) Disturbance in calcium homeostasis
  3) Damage to cellular (plasma and lysosomal) membranes; and
  4) Damage to DNA and misfolding of proteins.

# Common Biochemical mechanism of cell injury

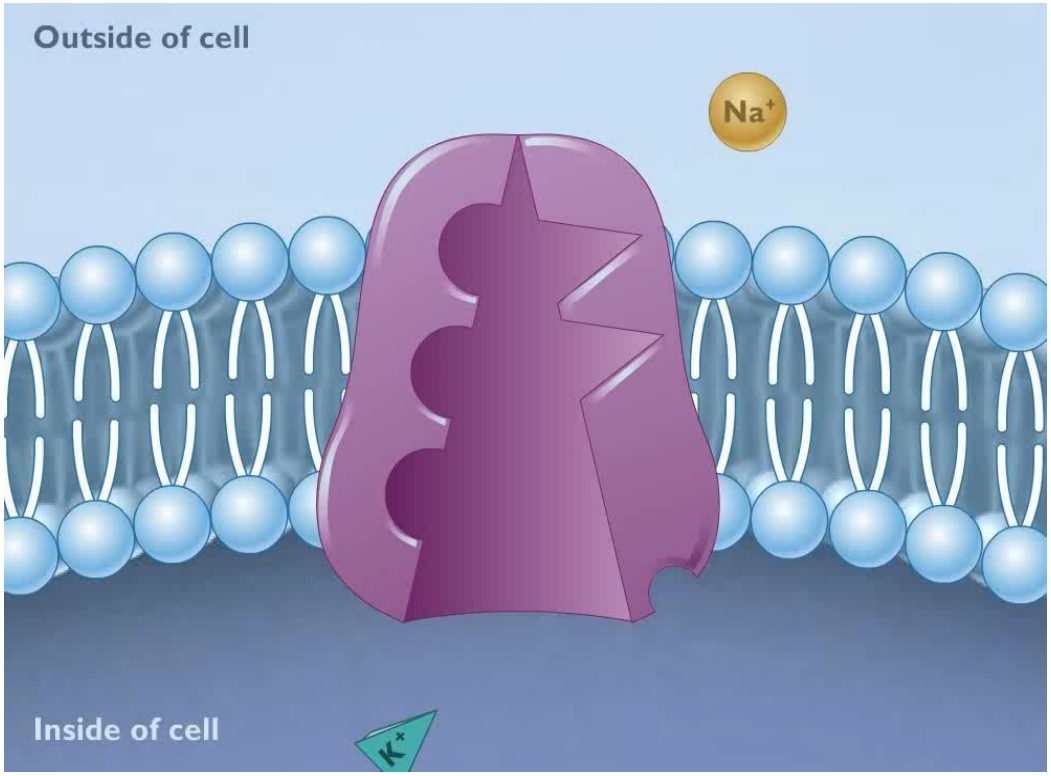


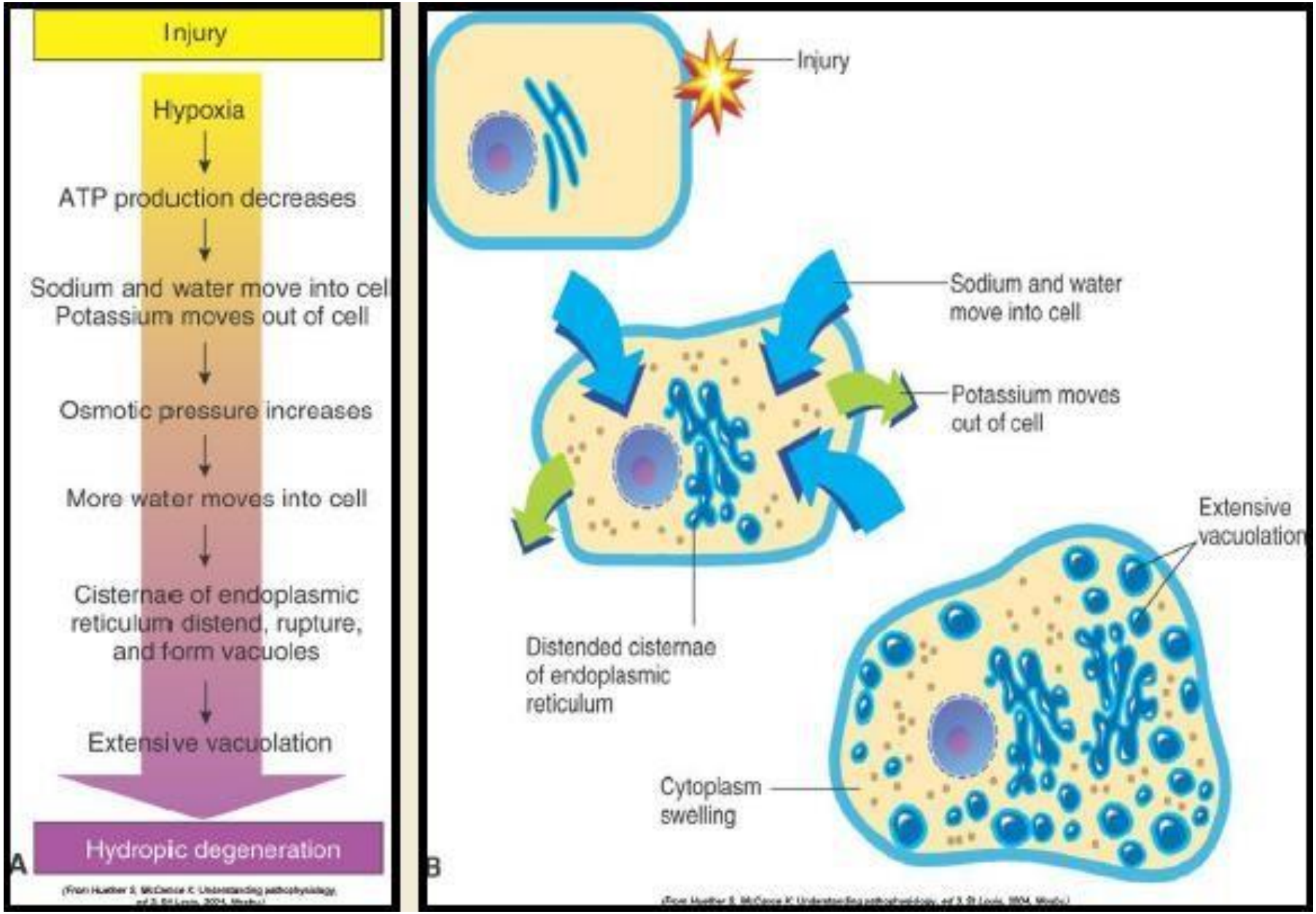
# ❶ Depletion of ATP

- ATP produced mainly by
  - Oxidative phosphorylation in mitochondria
  - Glycolytic pathway
- Major causes of ATP depletion are
  - Reduced supply of oxygen and nutrients
  - Mitochondrial damage
  - Actions of some toxins (e.g., cyanide)

# For your information only

- $Na^+$- high in Extra cellular fluid - outside the cells
- $K^+$ high in cytoplasm – Inside the cells
- Exchange occurs by $Na^+$- $K^+$ ion pumps
- ATP drives drive Na + out of the cell in exchange for K + moving into the cell.
- For each molecule of ATP used, the pump moves three Na + out of the cell and two K + into the cell.




Injury
Hypoxia
ATP production decreases
Sodium and water move into cell
Potassium moves out of cell
Osmotic pressure increases
More water moves into cell
Cisternae of endoplasmic reticulum distend, rupture, and form vacuoles
Extensive vacuolation
Hydropic degeneration
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Distended cisternae of endoplasmic reticulum
Cytoplasm swelling
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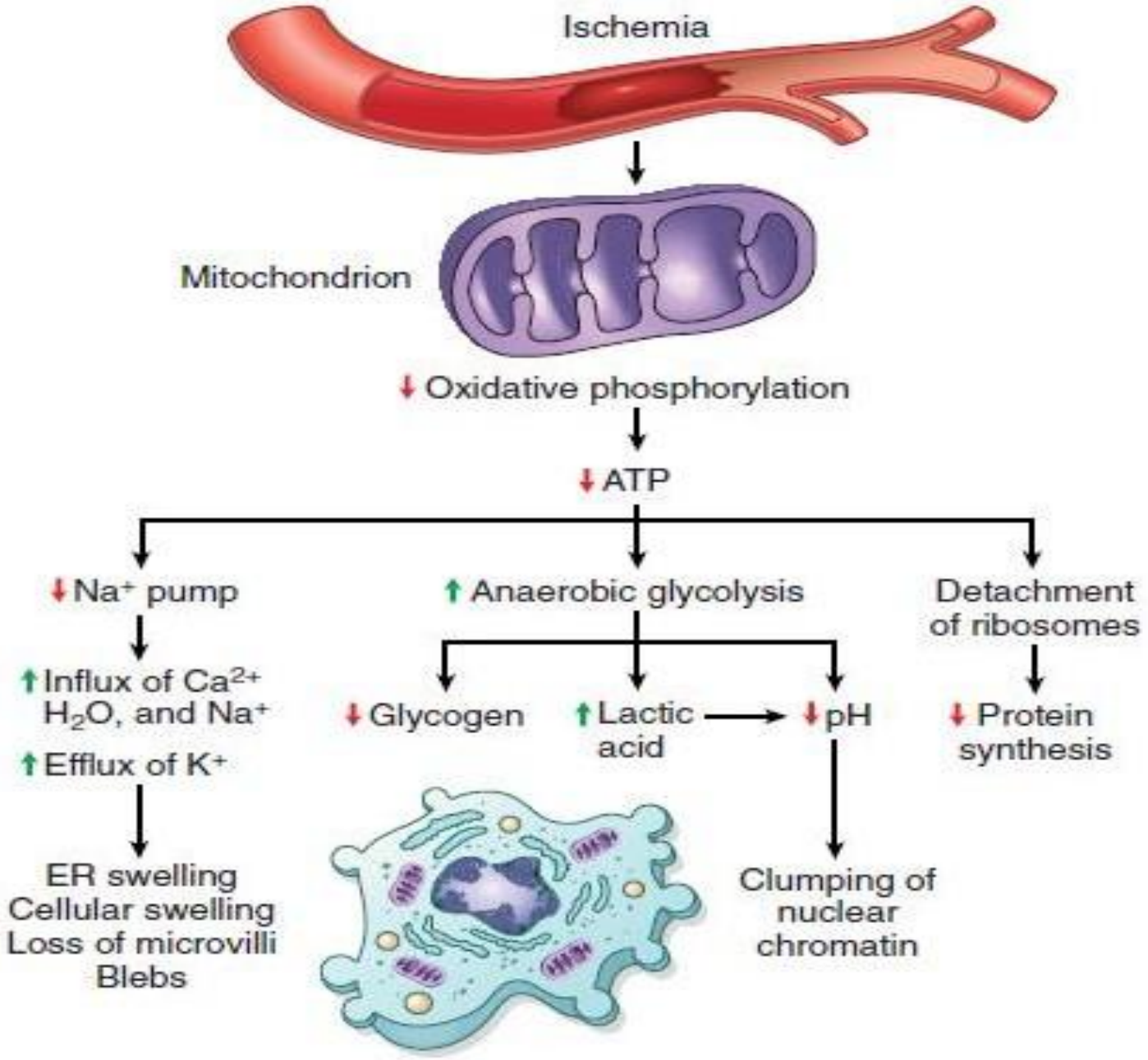
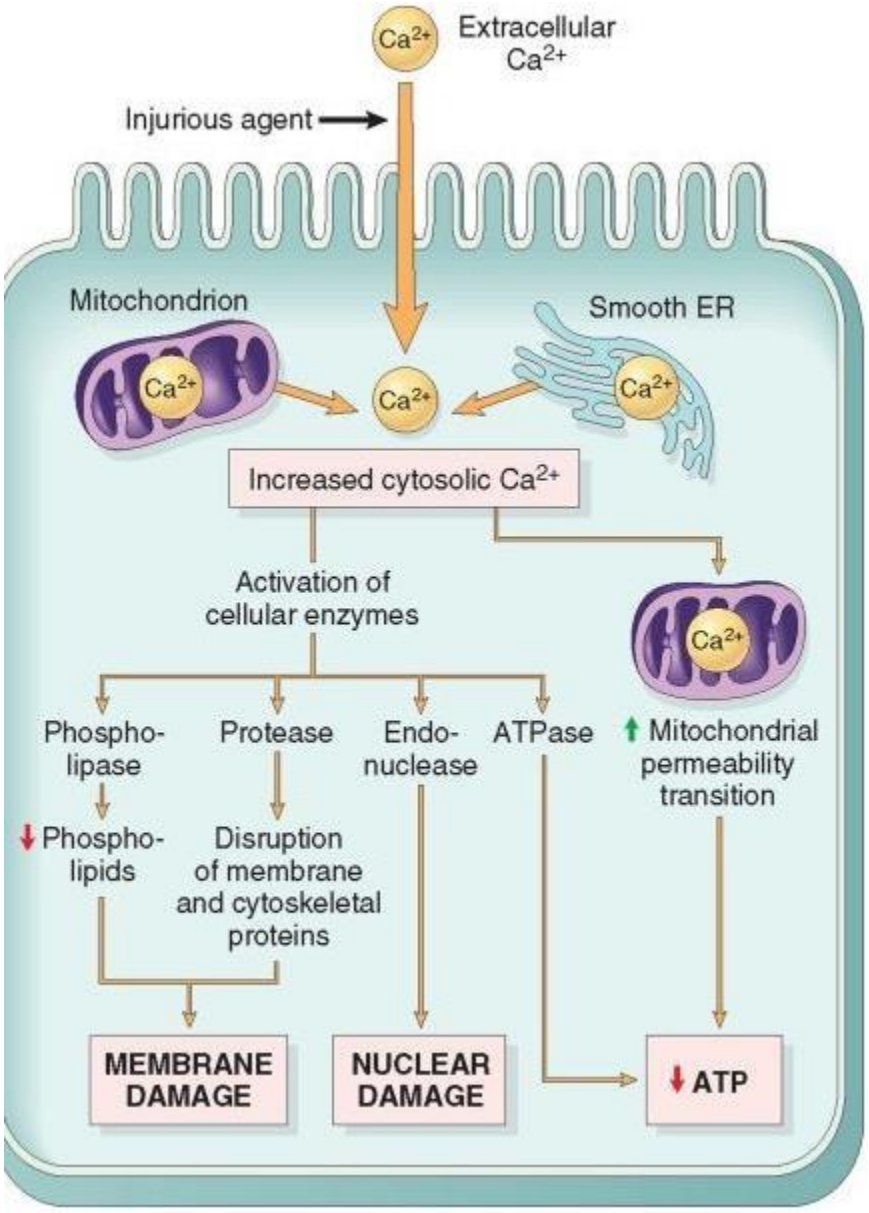
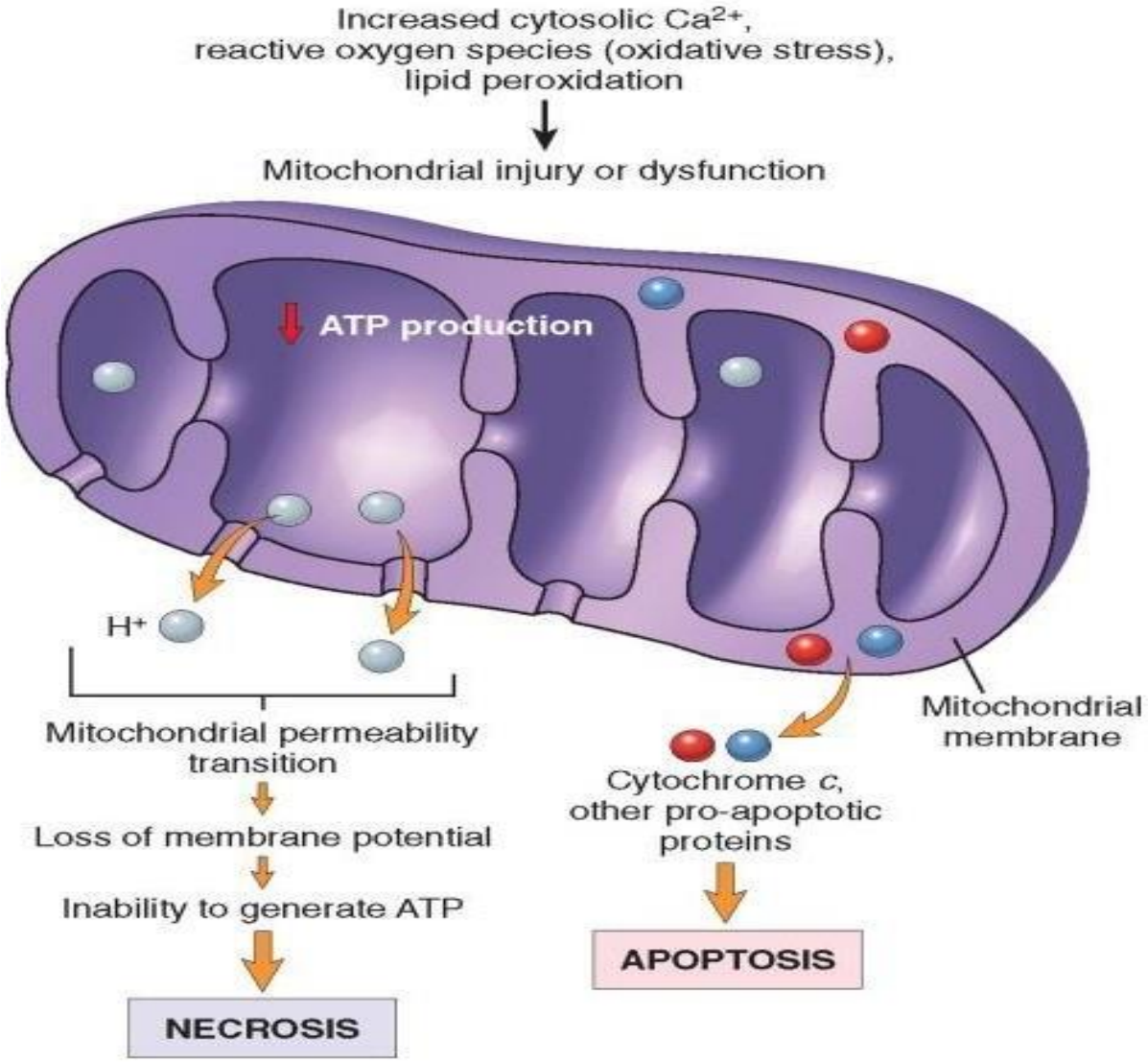

Ischemia
Mitochondrion
↓ Oxidative phosphorylation
↓ ATP
↓ Na+ pump
↑ Influx of Ca2+ H2O, and Na+
↑ Efflux of K+
ER swelling
Cellular swelling
Loss of microvilli
Blebs
↑ Anaerobic glycolysis
↓ Glycogen
↑ Lactic acid
↓ pH
Clumping of nuclear chromatin
Detachment of ribosomes
↓ Protein synthesis

# 2 Influx of calcium and loss of calcium homeostasis

- Cytosolic free calcium is normally maintained by ATP-dependent calcium transporters at concentrations as much as 10,000 times lower than the concentration of extracellular calcium
- Intracellular Ca sequestered in mitochondria and ER.
- Ca-Mg – ATPase pump damage or increased membrane permeability
- Intracellular Calcium results in enzyme activation



# 3 Mitochondrial Damage and Dysfunction



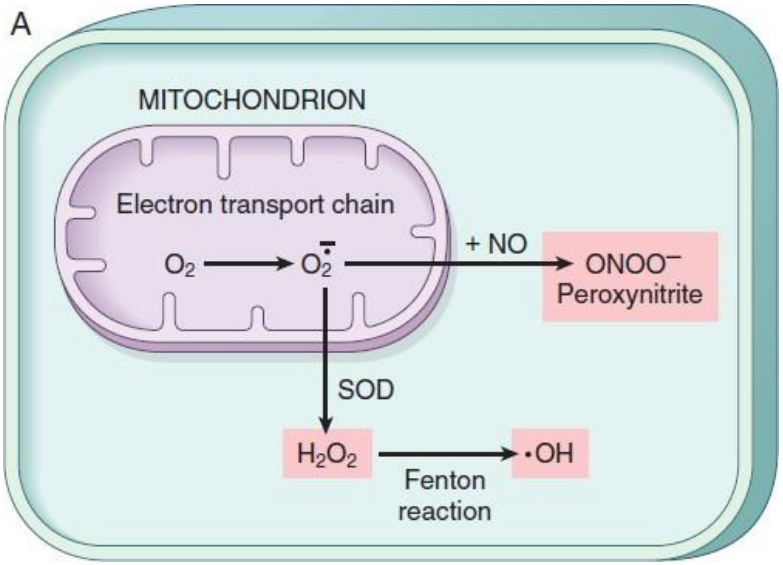
# ❹Accumulation of oxygen-derived free radicals (oxidative stress)

- **Free radicals are chemical species with a single unpaired electron in an outer orbital.**
- Extremely unstable
- Readily react with inorganic and organic chemical and injured the cells
- In addition, free radicals initiate reactions in which molecules that react with free radicals are themselves converted into other types of free radicals, thereby propagating the chain of damage.
- **Reactive oxygen species (ROS)** are a type of oxygen derived free radical

# Production of ROS / free radicles

1. ROS are produced normally in small amounts in all cells during the reduction-oxidation (redox) reactions that occur during mitochondrial respiration and energy generation

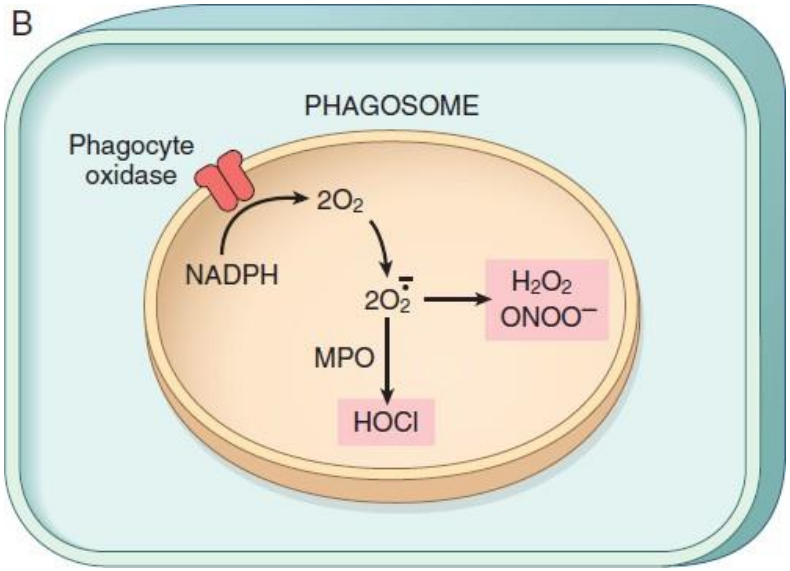
- Superoxide ($\bar{O}_2$) $\xrightarrow{superoxide\ dismutase}$
- Hydrogen peroxide ($H_2O_2$) $\xrightarrow[e^-]{Fe^{2+}\ Fenton\ reaction}$ highly reactive hydroxyl radical (•OH)




In all cells, $\bar{O}_2$ is generated during mitochondrial respiration by the electron transport chain and may be converted to H2O2 and the •OH free radical or to peroxynitrite ($ONOO^-$). NO, nitric oxide; SOD, superoxide dismutase.

❷ ROS are produced in phagocytic leukocytes, mainly neutrophils and macrophages, as a weapon for destroying ingested microbes and other substances during inflammation and host defense

- In leukocytes the phagocyte oxidase enzyme in the phagosome membrane generates $\bar{O}2$, which can be converted to other free radicals. Myeloperoxidase (MPO) in phagosomes also generates hypochlorite from ROS.



❸ The absorption of radiant energy (e.g., ultraviolet light, x-rays). Ionizing radiation can hydrolyze water into hydroxyl (•OH) and hydrogen (H•) free radicals.

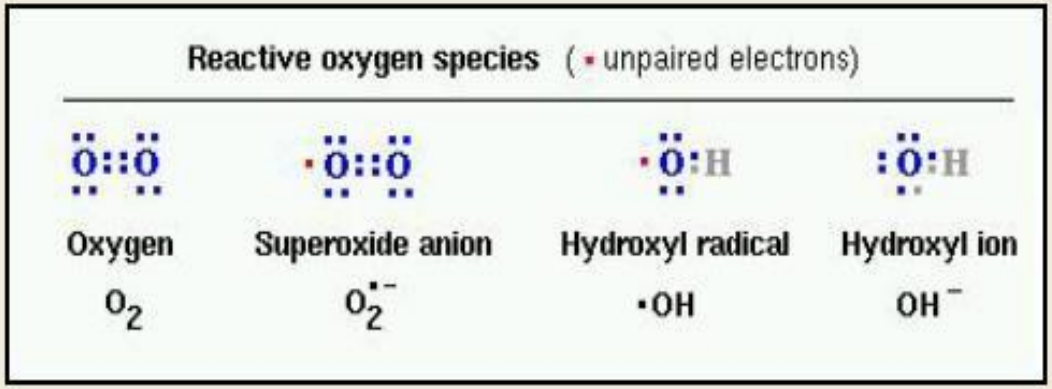
- $H_2O \rightarrow$ radiant energy hydrolysis $\rightarrow$ $^{\bullet}OH$ & $H^{\bullet}$

❹ The enzymatic metabolism of exogenous chemicals

- Antineoplastic drugs, such as doxorubicin, generate oxygen radicals that cause significant injury to cardiac myocytes.

# Important Reactants

- ROS → superoxide anions, hydroxyl radical and hydrogen peroxide




- $H_2O_2$ generates $OH^{\cdot}$ radicals from reactions with **Cu** or **Fe** ions

$$Fe^{2+} + H_2O_2 \rightarrow OH^{\cdot} + OH^- + Fe^{3+}$$

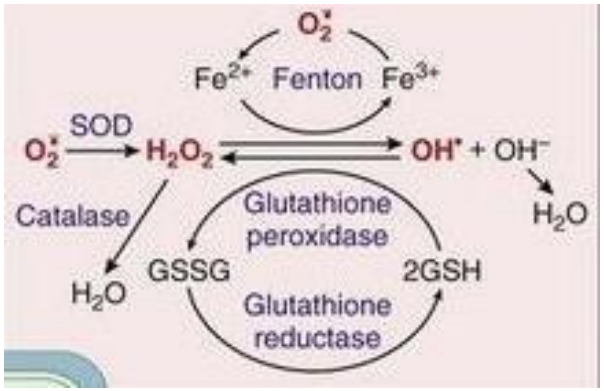
(Fenton reaction)

- $Fe^{3+}$ often reduced by superoxide anions $[Fe^{3+} + O_2^{-\cdot} \rightarrow Fe^{2+}]$

# Mechanisms to remove free radicals

- **Superoxide dismutases (SODs)**
  - SOD converts highly toxic superoxide to less toxic hydrogen peroxide
  - Superoxide dismutases (SODs) found in cytoplasm of many cell types.
- **Glutathione (GSH) peroxidases**
  - Found in cytoplasm of cells
  - It catalyzes the breakdown of $H_2O_2$ by the reaction

    **2 GSH (glutathione) + $H_2O_2$ → GS-SG + 2$H_2O$**
  - GSH – Reduced glutathione
  - GSSG – Oxidized glutathione
- Glutathione reductase convert GSSG →GSH



- **Catalase**
  - Present in peroxisomes
  - Catalyzes the decomposition of hydrogen peroxide ($2\ H_2O_2 \rightarrow\ O2 + 2H2O$).
  - It is one of the most active enzymes known, capable of degrading millions of molecules of $H_2O_2$ per second.

- **Endogenous or exogenous antioxidants**
  - Vitamins E, A, and C and β-carotene
  - May either block the formation of free radicals or scavenge them once they have

# ROS cause cell injury by three main reactions

## Lipid peroxidation of membranes

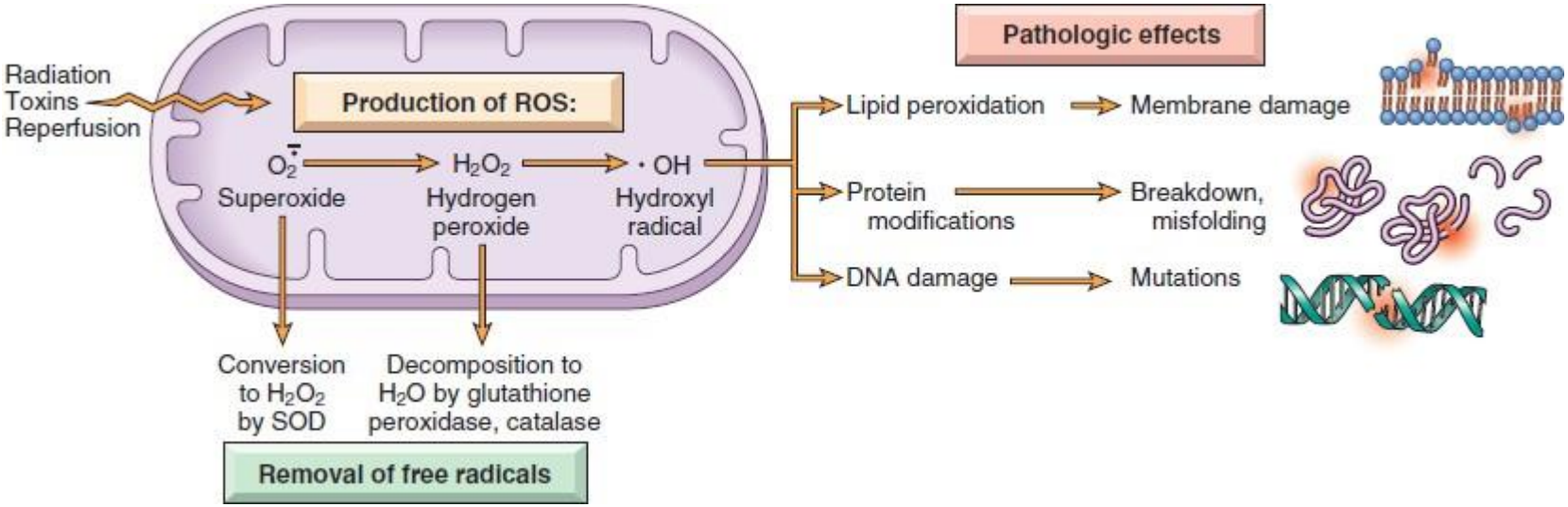
- Free radicals react with polyunsaturated lipids → lipid–radical interactions yield peroxides
- Which are themselves unstable and reactive, and an autocatalytic chain reaction ensues.

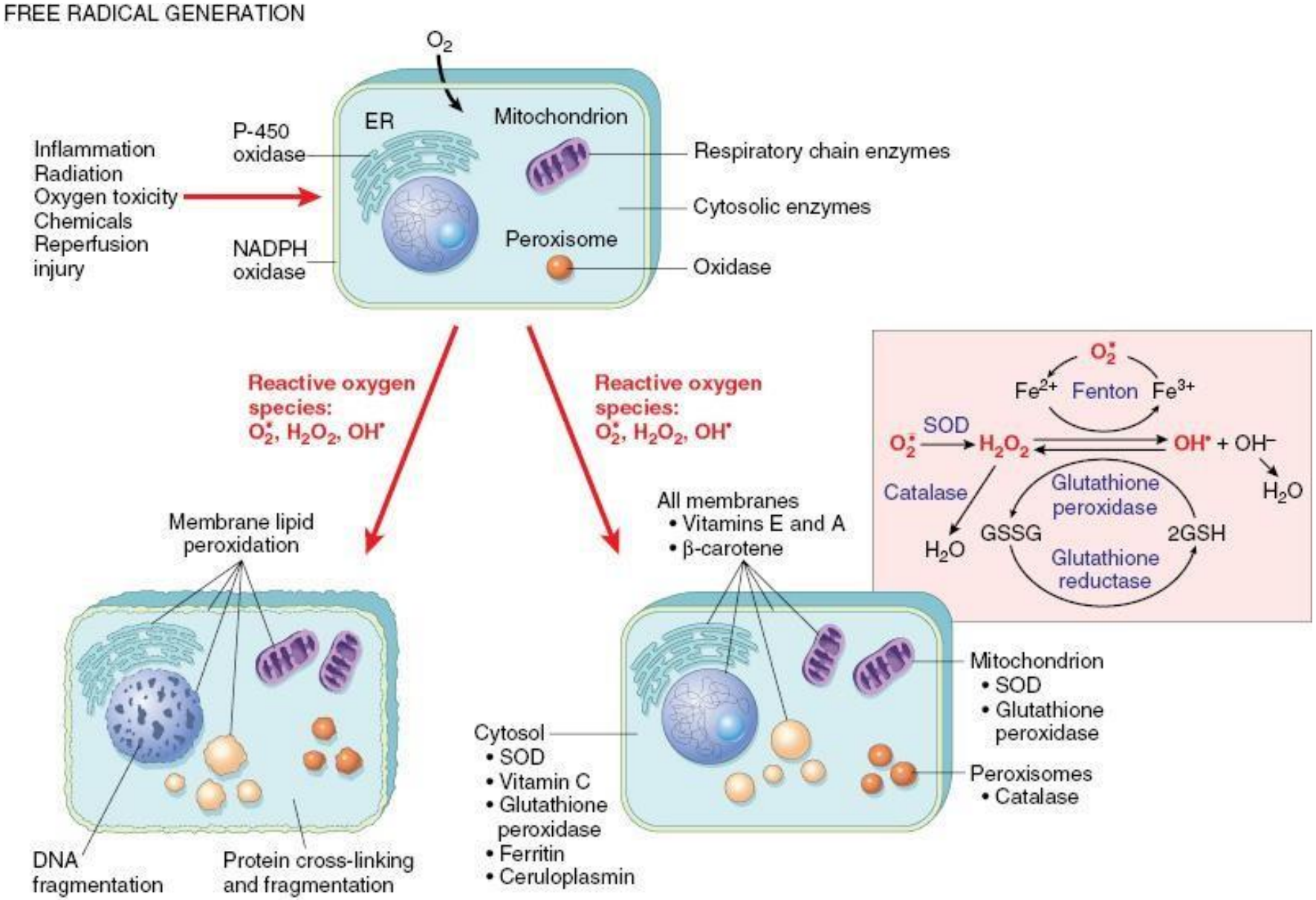
## Cross-linking and other changes in proteins.

- Free radicals promote sulfhydryl-mediated protein cross-linking, resulting in enhanced degradation or loss of enzymatic activity.
- Free radical cause polypeptide fragmentation.

## DNA damage.

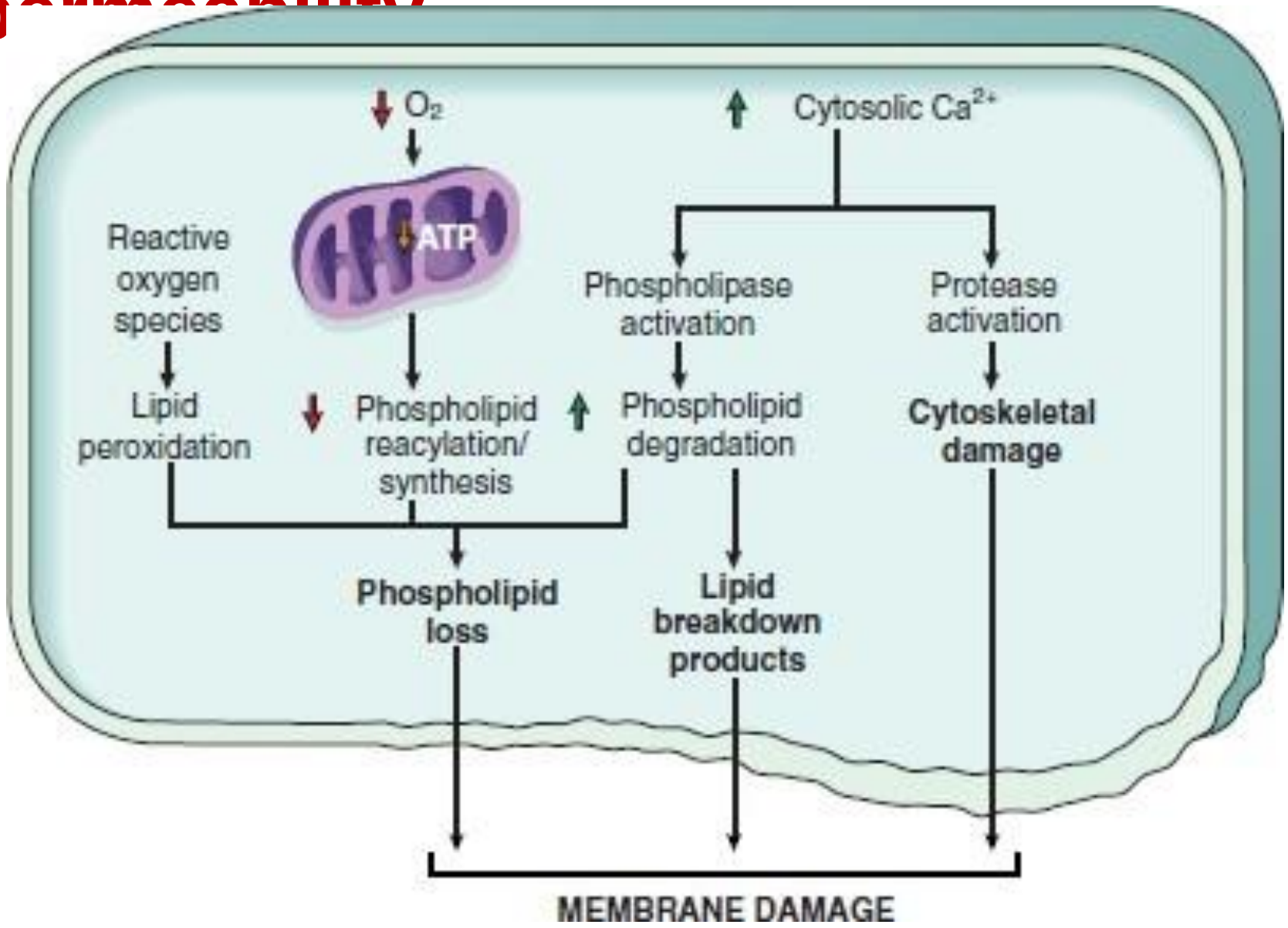
- Free radical reactions with thymine DNA → produce single-strand breaks.
- Cell death, aging, and cancer


Radiation
Toxins
Reperfusion
Production of ROS:
$O_2^{\cdot-}$
Superoxide
$H_2O_2$
Hydrogen peroxide
· OH
Hydroxyl radical
Conversion to $H_2O_2$ by SOD
Decomposition to $H_2O$ by glutathione peroxidase, catalase
Removal of free radicals
Pathologic effects
Lipid peroxidation
Membrane damage
Protein modifications
Breakdown, misfolding
DNA damage
Mutations




(From Kumar V, Abbas A, Fausto N: *Robbins & Cotran pathologic basis of disease*, ed 7, Philadelphia, 2005, Saunders.)
Zachary and McGavin: Pathologic Basis of Veterinary Disease, 5th edition.

# 5 Defects in membrane permeability



## MOST IMPORTANT SITES

- ***Mitochondrial membrane damage*** ↓ *ATP production*

- ***Plasma membrane damage*** influx of fluids and ions

- ***Injury to lysosomal membranes:*** *leakage of their* enzymes into the cytoplasm
  - Ribonucleases (RNases)
  - DNases, proteases
  - Glucosidases
  - Other enzymes
  - – Activation of these enzymes leads to enzymatic digestion of cell components, and the cells die by necrosis

# 6 Damage to DNA and Proteins

- Cell initiates its suicide program and dies by apoptosis whenever
  - DNA damage is too severe to be corrected (e.g., after radiation injury or oxidative stress
  - Accumulation of improperly folded proteins, which may result from inherited mutations or external triggers such as free radicals.

# SUMMARY - Mechanisms of Cell Injury

***ATP depletion:*** *failure of energy-dependent functions* → reversible injury → necrosis

***Mitochondrial damage:*** *ATP depletion → failure of energy dependent* cellular functions → ultimately, necrosis; under some conditions, leakage of mitochondrial proteins that cause apoptosis

***Influx of calcium:*** *activation of enzymes that damage cellular* components and may also trigger apoptosis

***Accumulation of reactive oxygen species:*** *covalent modification* of cellular proteins, lipids, nucleic acids

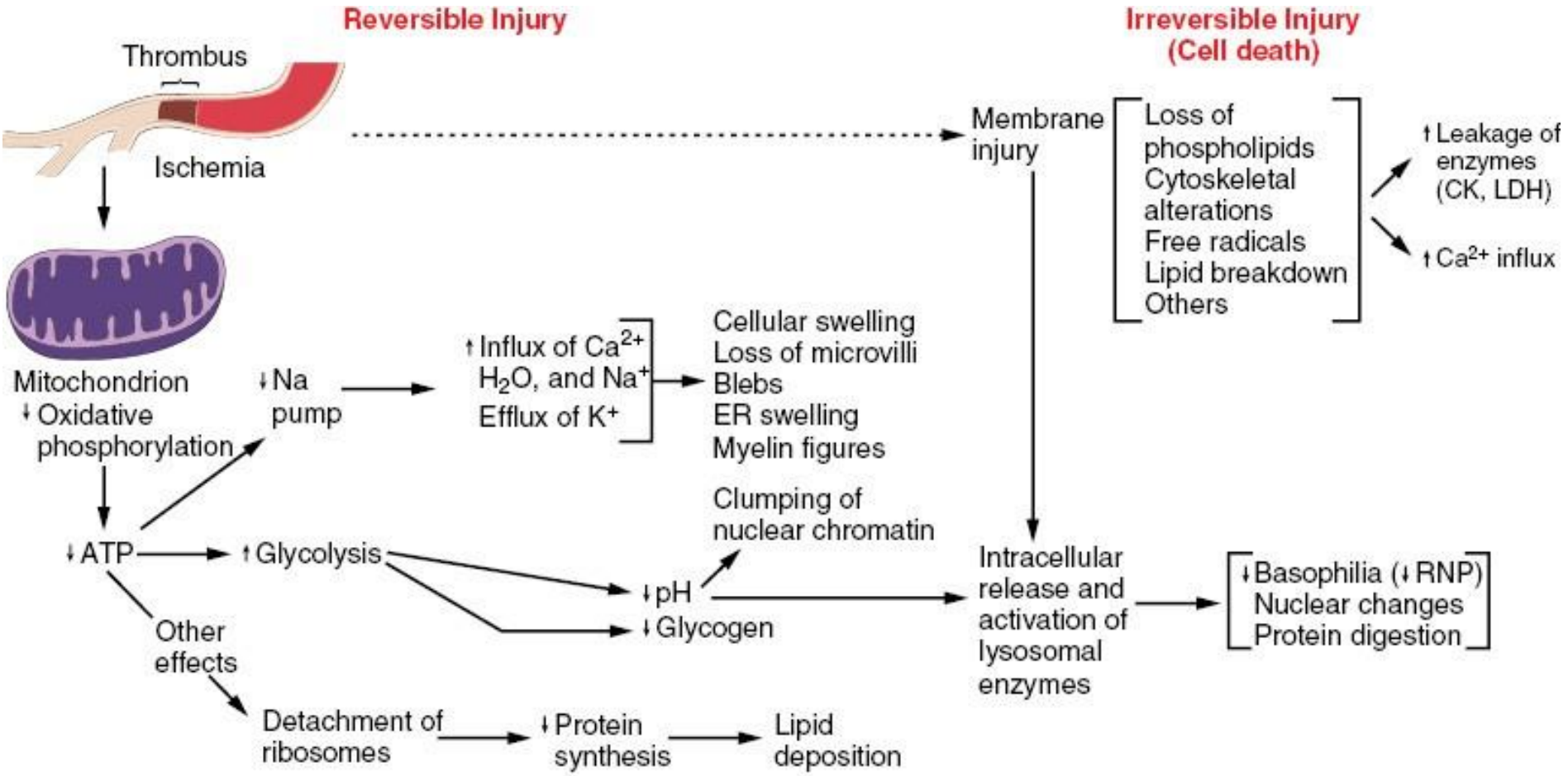
***Increased permeability of cellular membranes:*** *may affect* plasma membrane, lysosomal membranes, mitochondrial membranes; typically culminates in necrosis

***Accumulation of damaged DNA and misfolded proteins****:*

# Ischemic and hypoxic cell injury

- a common type of cell injury in clinical veterinary medicine
- In hypoxia - anaerobic glycolysis can continue
- In ischemia
  - Compromises the delivery of substrates for glycolysis
  - Anaerobic glycolysis can ceases
  - Accumulation of metabolites

***Ischemia injures tissues faster and usually more severely than does hypoxia.***

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## Two phenomena consistently characterize irreversibility

- **The inability to correct mitochondrial dysfunction**
  - Lack of oxidative phosphorylation and ATP generation
- **Profound disturbances in membrane function**.
  - Injury to lysosomal membranes results in the enzymatic dissolution of the injured cell, which is the culmination of injury progressing to necrosis.

# THE MORPHOLOGY OF CELL AND TISSUE INJURY

# Reponses of cell injury

- First changes occurs at molecular or biochemical level
  - Loss of cellular function
  - Changes in organelles at ultrastructure level - electron microscopy required to detect the change
  - If irreversible injury – cell death
  - Cell death – detected with electron microscopy
  - Cell death – detected with light microscopy
  - Cell death – detected grossly

# Reponses of cell injury

- For example,
  - Myocardial cells become noncontractile after 1 to 2 minutes of ischemia (loss of cell function)
  - Die after ~ 40 minutes of ischemia
  - These myocytes may not appear dead by electron microscopy for 2 to 3 hours
  - 
  - These myocytes may not appear dead by light microscopy for 6 to 12 hours.

# Reversible Injury

- The two main morphologic changes of reversible cell injury are

## ❶ Cellular swelling

- Failure of energy-dependent ion pumps in the plasma membrane, leading to an inability to maintain ionic and fluid homeostasis

## ❷ Fatty change

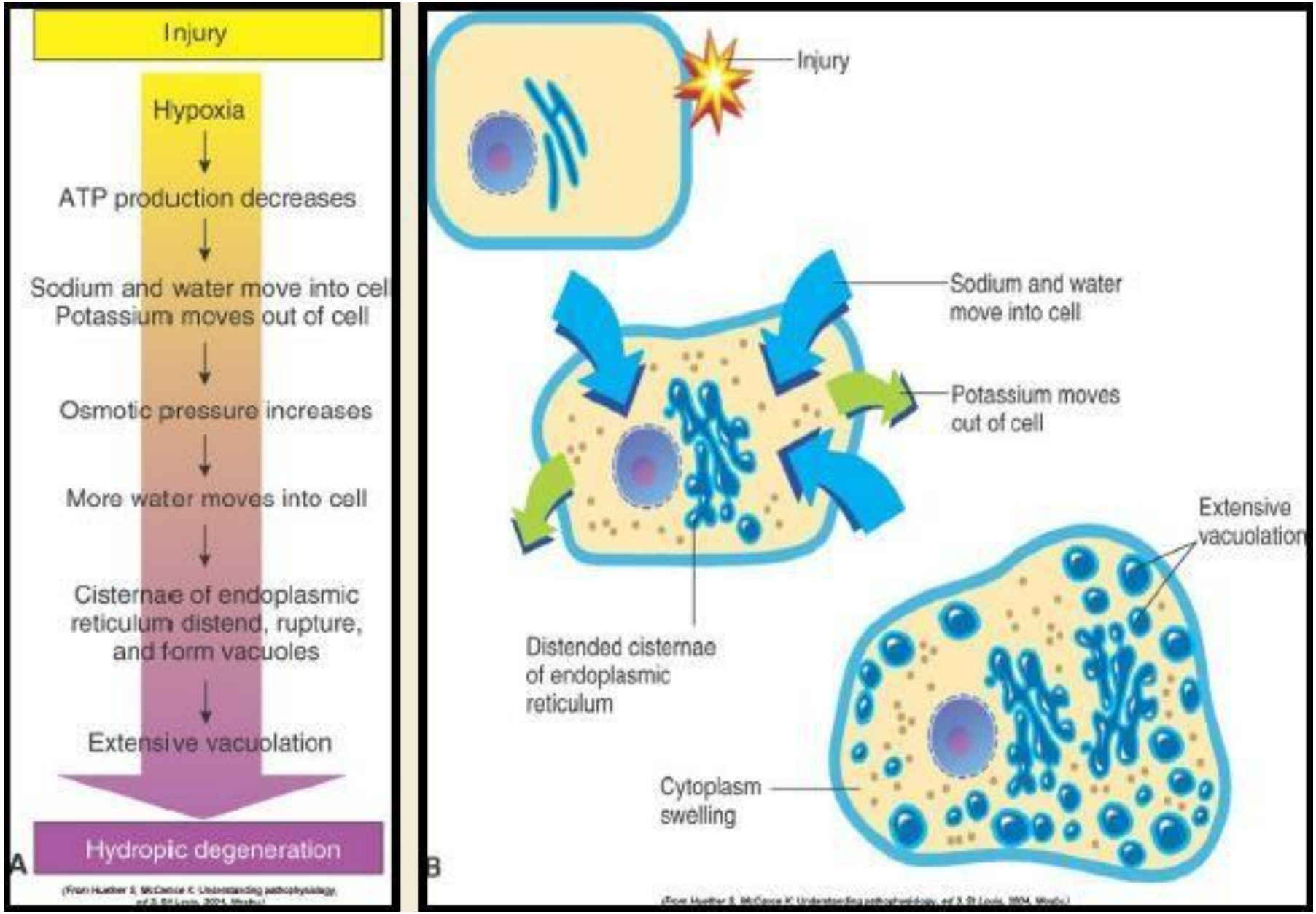
- Occurs in hypoxic injury and in various forms of toxic or metabolic injury and is manifested by the appearance of small or large lipid vacuoles in the cytoplasm

# Cellular swelling

- **First manifestation of almost all forms of injury to cells**
- Also known as
  - Hydropic degeneration
  - Vacuolar degeneration
  - Cloudy swelling
  - Parenchymatous degeneration
  - Albuminous degeneration
  - Cytotoxic edema in the central nervous system
  - Ballooning degeneration in the epidermis (skin)

# Etiology

- Hypoxia:- probably the most important fundamental cause
- Bacterial toxins
- Fever
- Metabolic diseases (diabetes and acetonaemia)
- Organic or inorganic poisons
- Circulatory disturbances
  - Anaemia, infarction, passive hyperaemia, and haemorrhage) when insufficient oxygen is brought to the cell.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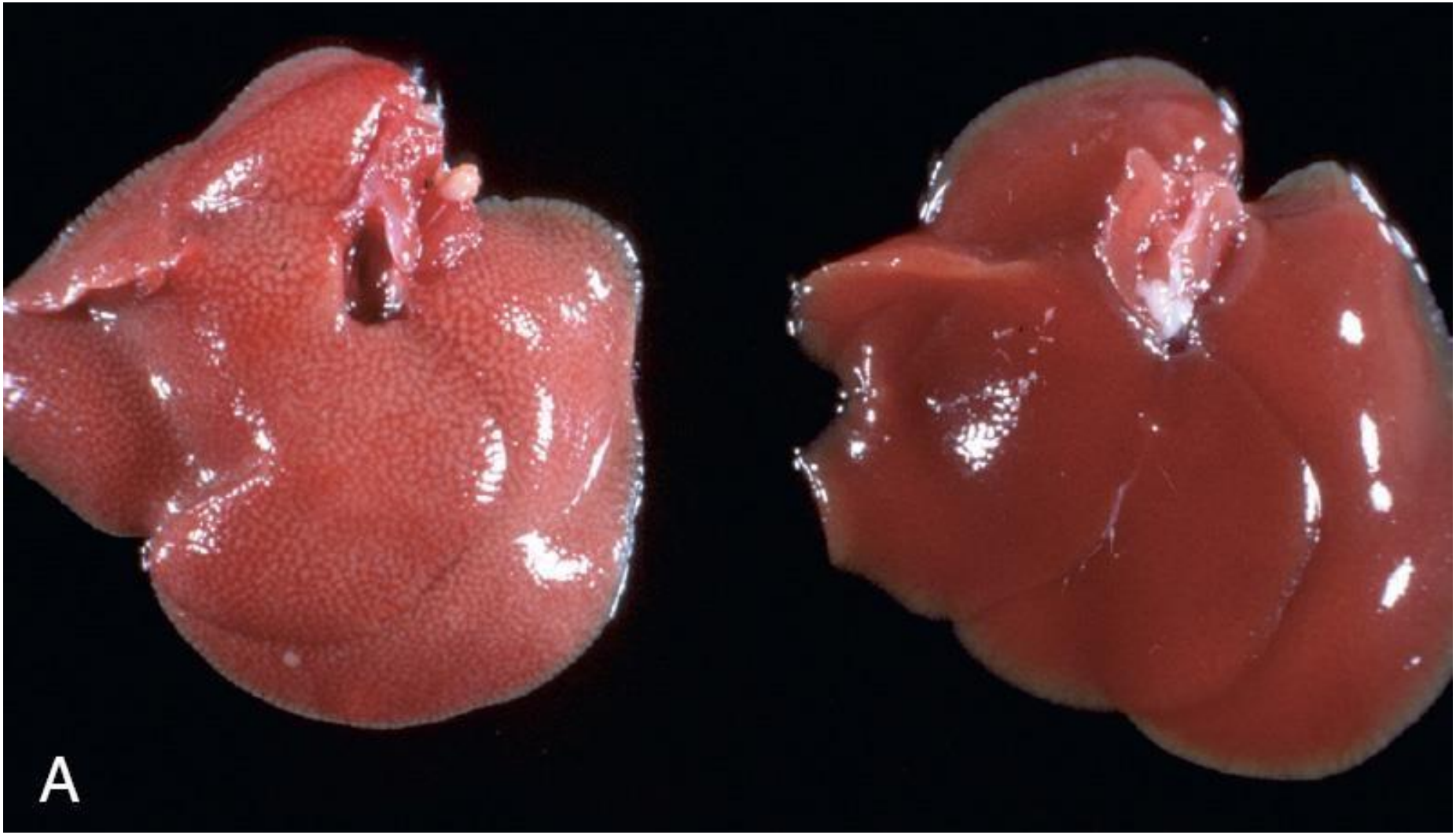
Extensive vacuolation
Hydropic degeneration
A
Injury
Sodium and water move into cell
Potassium moves out of cell
Extensive vacuolation
Distended cisternae of endoplasmic reticulum
Cytoplasm swelling
B

# Gross Pathology

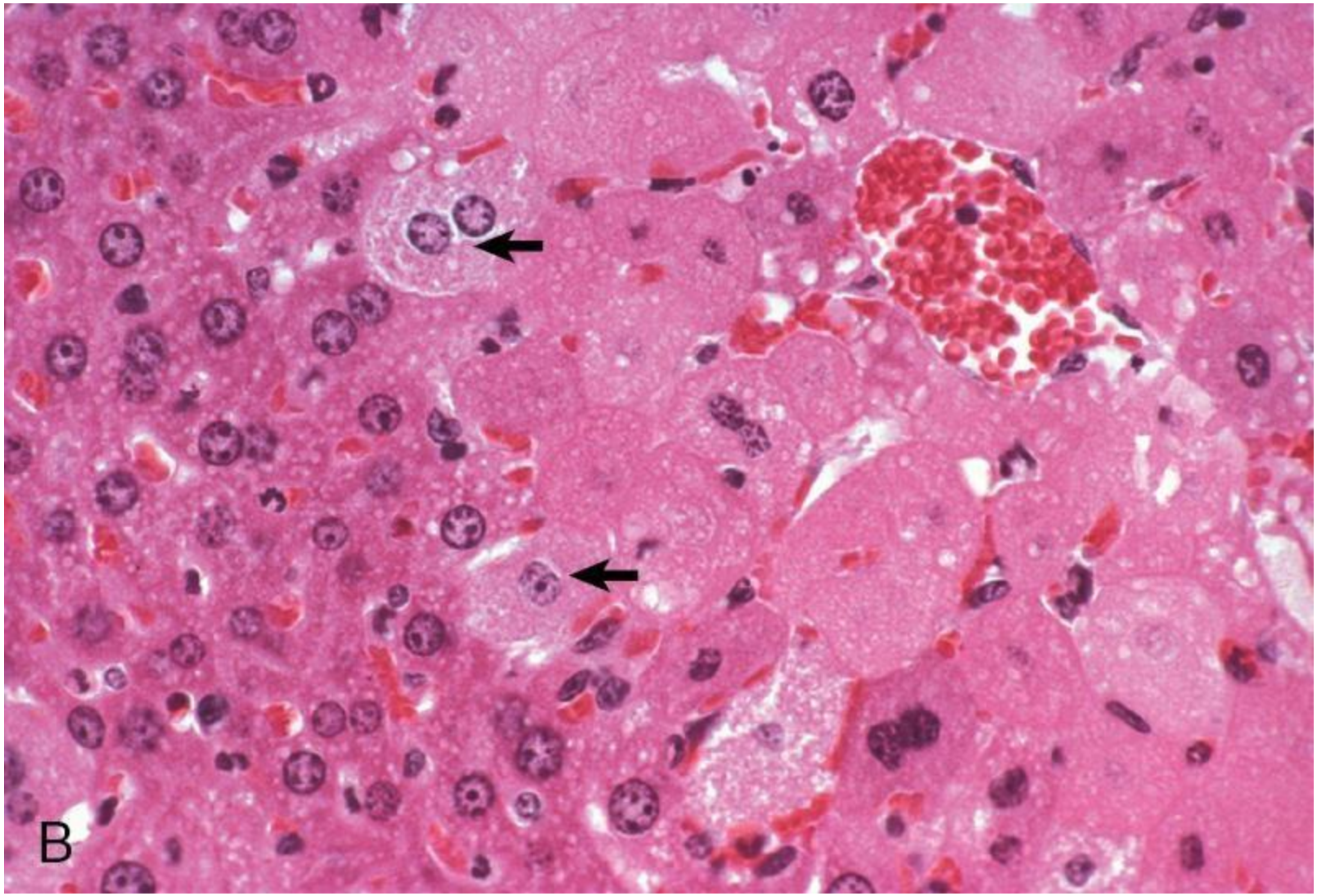
- Organ become
  - Pale in color
  - Enlarged
  - Heavier than normal
- Decreased specific gravity
- When incised, the cut surface bulges and its capsule draws back slightly



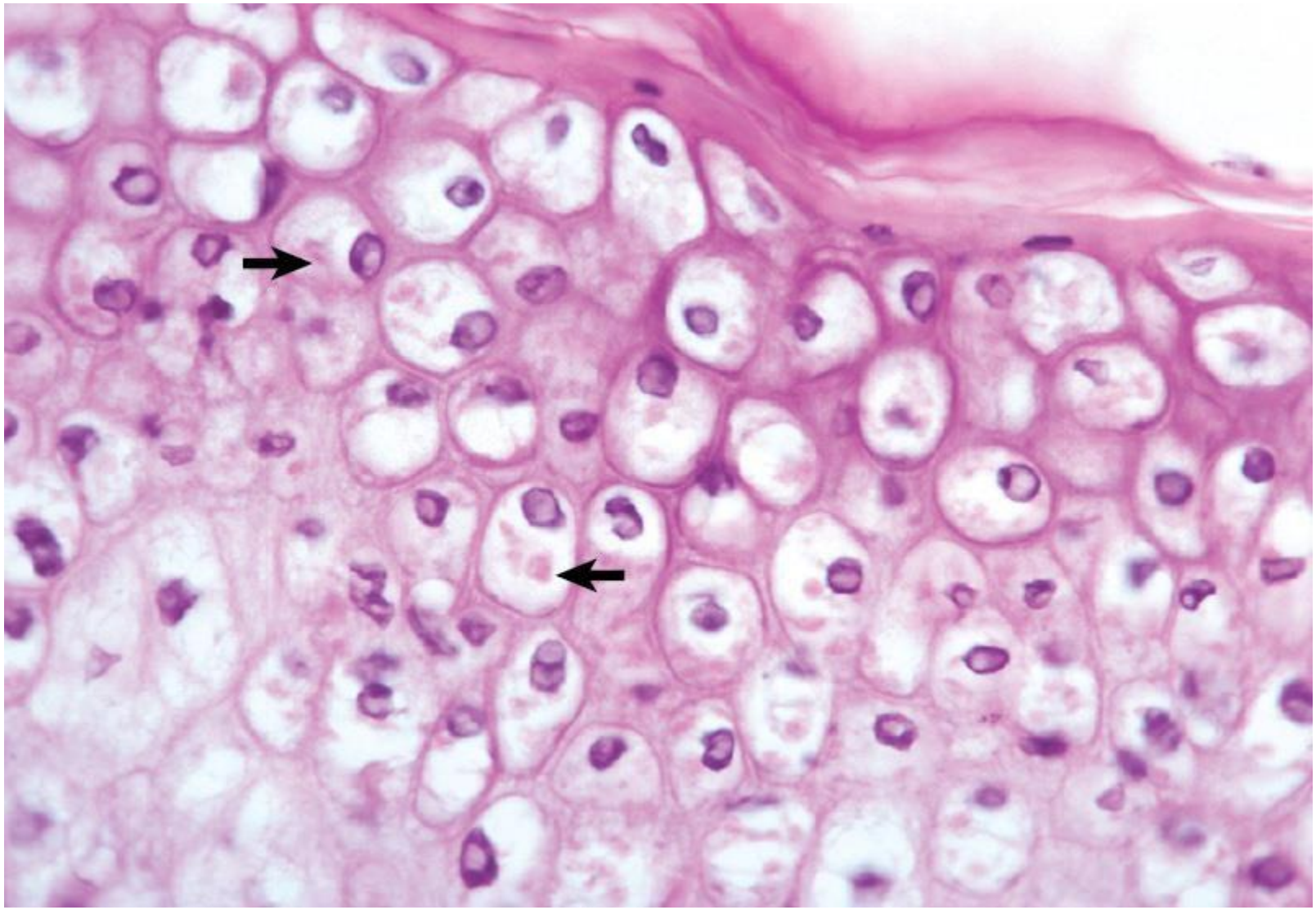

Acute cell swelling, liver, mouse. A, Hepatic swelling in a mouse exposed to chloroform 24 hours previously. The accentuated lobular pattern and slight pallor in the liver on the left are the result of acute cell swelling (hydropic degeneration) and necrosis of centrilobular hepatocytes. The right liver is normal

# Microscopic Appearance

- Cellular swelling is best observed in the liver, the convoluted tubules of the kidney, or in skeletal and cardiac muscle
- Cells become pale, finely vacuolated appearance (cloudy swelling)
- These vacuoles represent swollen mitochondria and dilated cisternae of the Golgi and ER.
- Ballooning degeneration : Seen in epidermal cells
  - Cells are greatly enlarged by cytoplasmic clear space
  - Seen in cells infected by epitheliotropic viruses (e.g., poxvirus)



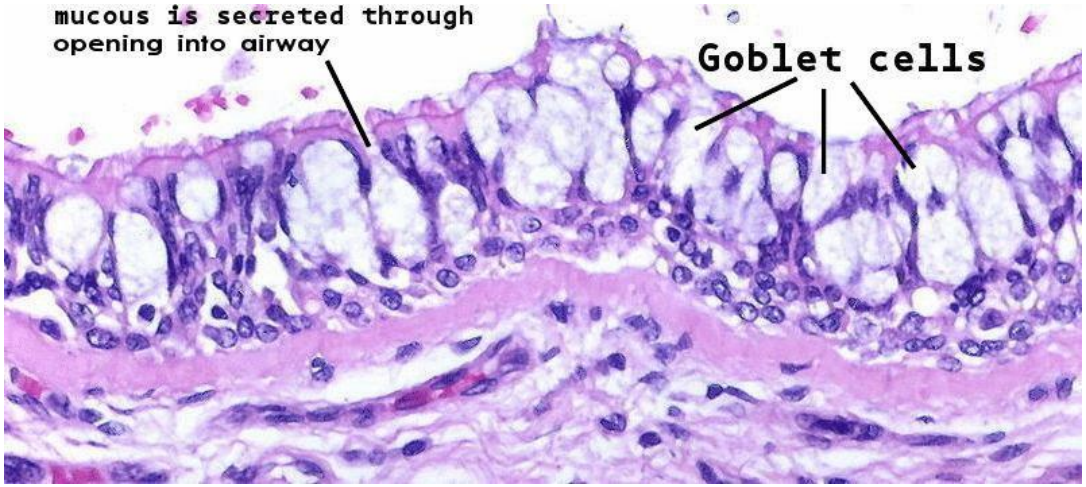

Liver from a mouse with chloroform toxicosis. While many hepatocytes in the centrilobular areas (at right) are necrotic, several cells at the interface of normal and necrotic (arrows) are still undergoing acute cell swelling (hydropic degeneration). H&E stain.



Ballooning degeneration, papular stomatitis, oral mucosa, cow. Cells infected by some types of virus, such as papular stomatitis virus, are unable to regulate their volume and swell at certain stages of the infection. These cells may become very large (ballooning degeneration) and eventually rupture. Some of the cells have viral inclusion bodies (arrows)

# Mucinous or Mucous Degeneration

- Excessive accumulation of mucin in degenerating epithelial cells.
- Mucin – Glycoprotein
- Produced by goblet cells
  - Present in columnar and cuboidal epithelial cells.
- When mucin is mixed with water or tissue fluid, it is known as mucus.



# Etiology

- Mild mechanical injury to a mucous membrane
- Mild chemicals such as disinfectants and soaps used on mucous membranes during obstetrical procedures
- Irritating effect of moderate heat and cold
- Infectious agents, especially viral (canine distemper, viral diarrhoea of cattle)
- Neoplasms involving columnar epithelium, e.g., adenocarcinoma of the cattle stomach.

# Gross Pathology

- Mucous membrane is covered with a clear, white transparent material, which is stringy and slimy in consistency.
- The mucous membrane is usually hyperemic.

# Microscopic Appearance

- Increased number of goblet cells
- Mucosa may be congested or hyperemic

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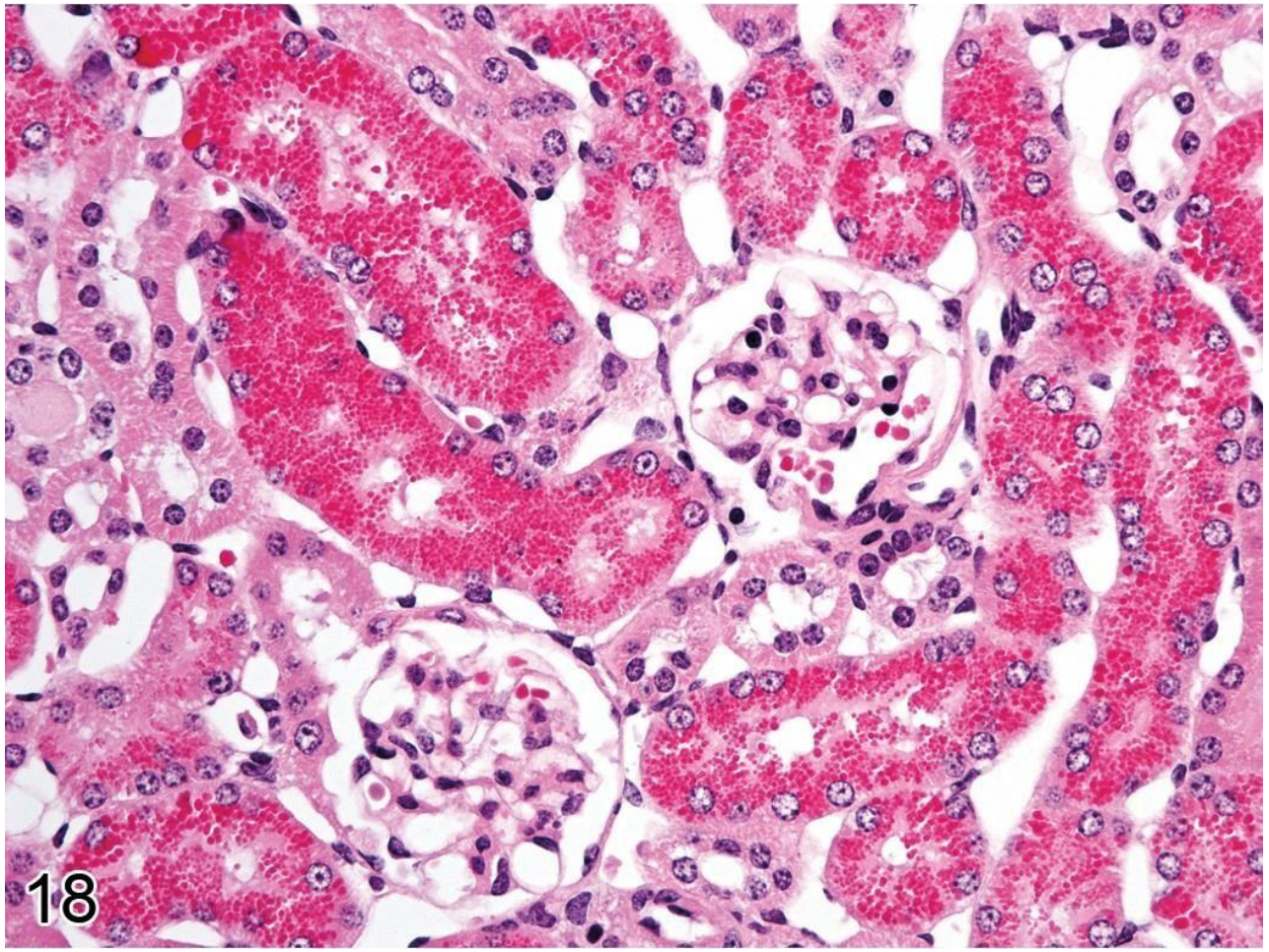
- Increased number of goblet cells
- Mucosa may be congested or hyperemic
-

# Hyaline Change

- Hyalin – Glassy and transparent
- Histologically:- Homogeneous, eosinophilic, and glassy (translucent) appearance
- Purely descriptive term
  - and rather loosely applied to a variety of changes, none of which is a true cellular degeneration.
- May be intracellular or extracellular

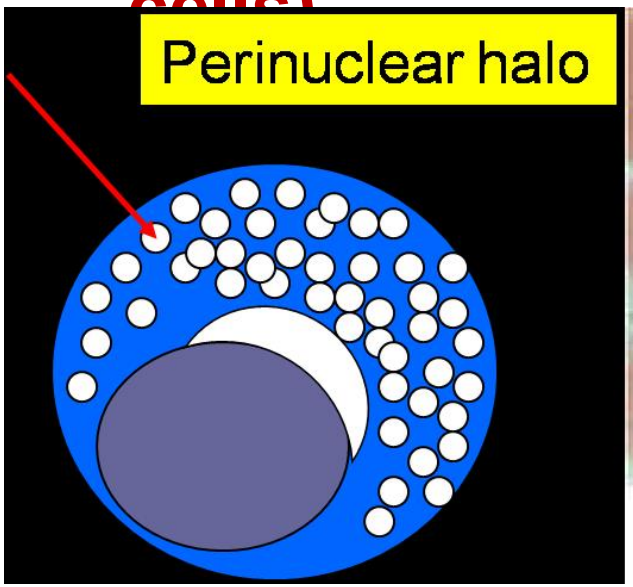
# Example - Intracellular

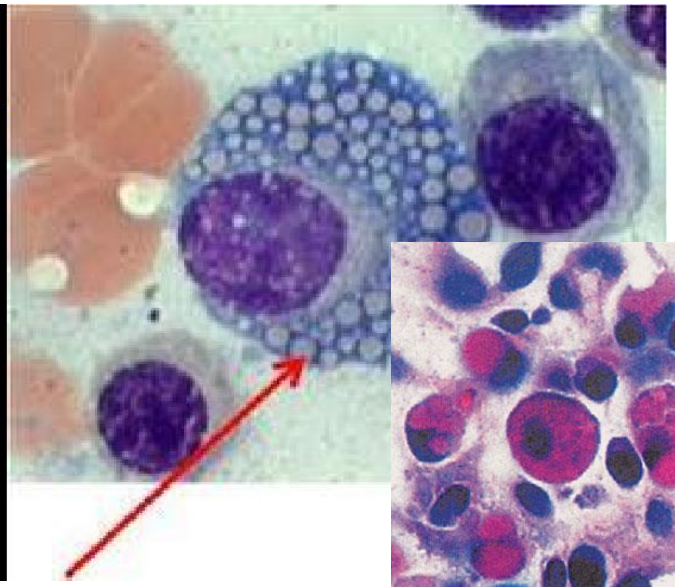
- Resorption droplets in the epithelial cells of renal proximal tubules

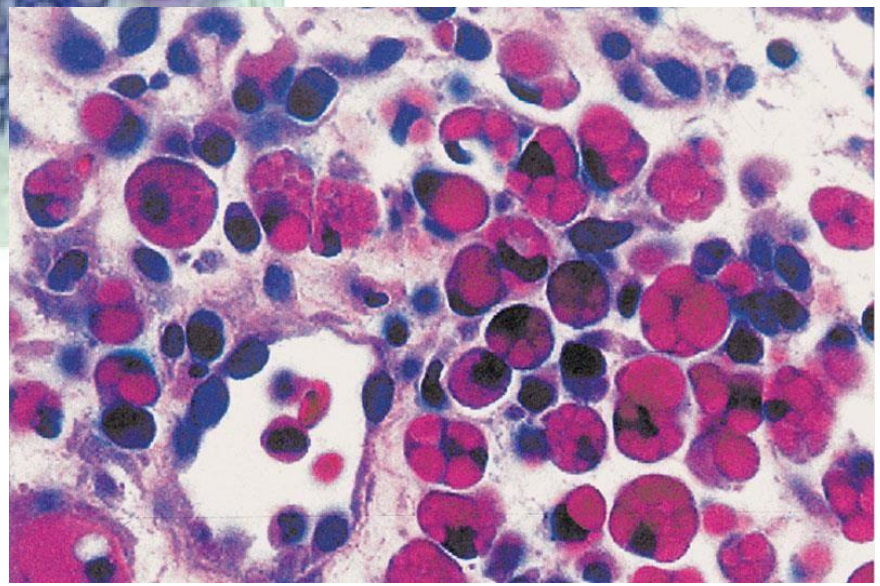


# Example - Intracellular

- Excessive production of normal protein:
- Hyaline bodies called Russell bodies are seen in the cytoplasm of some plasma cells **(Mott cells)**





Mott cells: Russell bodies laden plasma cells

# Example - Extracellular

- Hyaline casts in the lumens of renal tubules in a proteinuria.
- Old scars.
  - With age, the number of nuclei in collagen deposits decreases as the result of cell senescence, and the collagen fibers condense and become hyalinized
- Hyaline microthrombus
- Amyloid
- Corpora amylacea:- Observed in the prostate, alveoli of the lungs in the mammary glands and ventricles of the brain and central canal of the spinal cord (brain sand)

# Thank you