CELL INJURY AND DEATH

Somphong narkpinit, M.D.

Department of Pathobiology
Faculty of Science
Mahidol University

somphong.nar@mahidol.ac.th
Objectives:

after learning, student should be able to

- Describe cell injury and cell death
- Describe intracellular accumulations
- Describe pathologic calcification
Stages in the cellular response

NORMAL CELL (homeostasis) -> ADAPTATION

Stress -> Inability to adapt

Injurious stimulus

REVERSIBLE INJURY

Mild, transient

CELL INJURY

Inability to adapt

Severe, progressive

IRREVERSIBLE INJURY

NERCROSIS

CELL DEATH

APOPTOSIS
Cell Injury, Definitions

• When the cell is exposed to an injurious agent or stress, a sequence of events follows that is loosely termed *cell injury*.

• Cell injury is *reversible* up to a certain point.

• If the stimulus persists or is severe enough from the beginning, the cell reaches a point of no return and suffers *irreversible* cell injury and ultimately *cell death*.

• *Cell death*, is the ultimate result of cell injury.
Cell injury

• Results when cells are stressed so severely that they are no longer able to adapt or when cells are exposed to damaging agents.

• Cell injury can be reversible or irreversible.
Reversible cell injury

- Functional and morphologic changes are reversible if the damaging stimulus is removed.

- The features are: decreased oxidative phosphorylation, ATP depletion and cellular swelling.
Irreversible injury and cell death.

- With continuing damage, injury becomes irreversible.

- Cells undergo morphologic changes recognizable as cell death.

- Cell death is of 2 types—necrosis and apoptosis.
Effect of Duration of Injury

- Reversible
  - Cell Function
  - EM Changes
- Irreversible
  - Cell death
  - EM Changes
  - LM Changes
  - Gross Changes
Duration of Injury

- Reversible cell injury
- Irreversible cell injury
- Biochemical alterations → cell death
- Ultrastructural changes
- Light microscopic changes
- Gross morphologic changes

Copyright © 2009 by Saunders, an imprint of Elsevier, Inc. All rights reserved.
Reversible and irreversible injury

Figure 5-8 Outcomes of cell injury: reversible cell injury, apoptosis and programmed cell removal, cell death and necrosis.
Reversible and Irreversible Cell Injury

- **NORMAL**
  - Normal cell

- **REVERSIBLE CELL INJURY**
  - Injury
  - Swelling of endoplasmic reticulum and mitochondria
  - Clumping of chromatin
  - Recovery

- **IRRREVERSIBLE CELL INJURY -> NECROSIS**
  - Death
  - Swelling of endoplasmic reticulum and loss of ribosomes
  - Lysosome rupture
  - Membrane blebs
  - Myelin figures
  - Swollen mitochondria with amorphous densities
  - Fragmentation of cell membrane and nucleus
  - Necrosis
  - Nuclear condensation

© Elsevier 2005
Earliest changes in reversible cell injury are:

• Decreased generation of ATP.

• Loss of cell membrane integrity.

• Defects of protein synthesis.

• Cytoskeletal damage.

• DNA damage.

• Within limits, the cell can compensate for these derangements. Persistent or excessive injury leads to irreversible injury.
Ischemia

- Cell oxygen tension
  - Reduced ATP
  - Incr. Glycolysis
  - Decr. Protein synthesis
  - Decr glycogen
  - Lipid deposition

- Membrane injury
  - Loss of phospholipids
  - Increase of free radicals
  - Lipid breakdown
  - Leakage of cell enzymes
  - Calcium influx

- Intracellular lysosomal enzyme release

**Reversible injury:**
- cell swelling
- microvilli loss
- blebs
- ER swelling
- myelin figures
- chromatin clumping

**Irreversible injury**
- Reduced basophilia
- Nuclear changes
- Protein digestion
Characteristic phenomena of irreversibility:

• Inability to reverse mitochondrial dysfunction.

• Development of profound disturbances in membrane function.

• Therefore, in cardiac muscle death there is leakage of CKMB & troponin.

• In injury to bile duct epithelium & liver, serum alkaline phosphatase is raised.

• In hepatocyte injury, transaminases are raised.
Light microscopic patterns of reversible cell injury:

• Cellular swelling & fatty change.

• Morphology in cellular swelling-
  Gross: Pallor, increased turgor & increase in organ weight.
  Micro: small clear vacuoles seen within cytoplasm.

• Fatty change-seen in injured myocardial cells and hepatocytes. There is appearance of small or large lipid vacuoles in the cytoplasm.
Reversible cellular changes & accumulations

**Hydropic degeneration** (hydropic change)
- Only the cytoplasm is involved
- Water accumulates & the cell swells
  - Large vacuoles in the cytoplasm
- Light microscopy
  - Cytoplasm is pink & granular
- Electron microscopy (ultrastructural)
  - Organelles are swollen
  - Ribosomes displaced
  - Lysosomal activity very apparent
Hydropic Change

Kidney - microscopic

Source: TUSDM
Hydropic Change - Leukoedema

Source: TUSDM

Oral epithelium - microscopic

Source: TUSDM
Intracellular Accumulations

Intracellular accumulation of abnormal amounts of various substances.

(1) a *normal cellular constituent* accumulated in excess, such as water, lipids, proteins, and carbohydrates

(2) an *abnormal substance*, either exogenous, such as a mineral or products of infectious agents, or endogenous, such as a product of abnormal synthesis or metabolism

(3) a *pigment*. 
Intracellular Accumulations

- The substance may be either the cytoplasm or the nucleus.
- In some instances, the cell may be producing the abnormal substance, and
- In others it may be merely storing products of pathologic processes occurring elsewhere in the body.
Reversible cellular changes & accumulations

fatty change (steatosis, fatty metamorphosis)

- Characterized by accumulation of intracellular parenchymal triglycerides, nucleus is displaced & the cells swells.
- Observed frequently in liver, heart, & kidney.
- Ex. In liver secondary to alcoholism, diabetes mellitus, malnutrition, obesity, poisoning
- Results from imbalance among the uptake, utilization & secretion of fat.
- Increased transport of triglycerides (fatty acids) to affected cells
- Decreased mobilization of fat from cells
- Most often due to decreased production for transport
- Decreased use of fat by cells.
- Overproduction of fat in cells.
Fatty Change

Liver

Source: TUSDM
Fatty Change - Liver
Fatty Change - Liver

Source: TUSDM
Hyaline change

- Homogenous, glassy, eosinophilic appearance in H&E stained tissue sections

- Caused most often by nonspecific accumulations of proteinaceous material

- Ex. Glomeruli tufts in diabetic glomerulosclerosis
Reversible cellular changes & accumulations

**Accumulation of exogenous pigments**

- Naturally colored substances not requiring tissue stain to be seen
  1. Pulmonary accumulations of carbon, silica, & iron dust
  2. Plumbism (lead poisoning)
  3. Algeria (silver poisoning)

- May cause a permanent gray discoloration of the skin & conjunctiva
Accumulation of endogenous pigments

- **Melanin**:
  - Most common; brown pigment
  - Formed from tyrosine via tyrosinase
  - Synthesized in melanosomes of melanocytes within the basement membrane of the epidermis & choroid of the eye.
  - Transferred by melanocytes to adjacent clusters of keratinocytes & macrophages (melanophores) in the subjacent dermis
  - Seen also in neoplasm
    - Ex. Melanocytic nevus, melanotic macule
    - Ex. Melanoma
Labial Melanotic Macule - Focal Melanosis
Melanin Pigmentation – Labial Melanotic Macule
- **Bilirubin**
  
  - Catabolic product of the heme moiety of hemoglobin & myoglobin
  - In pathologic conditions, accumulates & stains the blood, sclera, mucosae, & internal organs producing a yellow discoloration (jaundice)
  - Hemolytic jaundice
  - Destruction of red blood cells.
  - Obstructive jaundice
    - intra or extrahepatic obstruction of the biliary tract.
    - Hepatocellular jaundice  Ex. Parenchyma liver damage
- **Hemosiderin**

- Iron – containing pigment, aggregates of ferritin
  - In tissue appears as golden – brown amorphous aggregates.
    - Prussian blue dye – positive blue color stain reaction.
  - Exists normally in small amounts as physiologic iron stores within tissue macrophages of the bone marrow, liver, & spleen.
Hemosiderin

Melanin
Hemosiderin – Lung Alveoli

Source: TUSDM
- **Haemosiderin**
  - found in
    1. Week – old haemorrhage
    2. Hemolysis
    3. Inborn errors of metabolism affecting transport & absorption as in the liver & pancreas
  - Accumulates pathologically in tissue in excess amounts (sometimes massive)

- **Hemosiderosis** vs. **hemochromatosis**
Hemosiderosis

- Accumulation of hemosiderin, primarily within tissue macrophages, without associated tissue organ damage

- **Local** – most often from hemorrhage into tissue; derived from breakdown of hemoglobin

- **Systemic** – generalized; from hemorrhage, multiple blood transfusions, hemolysis, excessive dietary intake, often accompanied by alcohol consumption.
Lipofuscin

- yellowish to light brown, fat-soluble pigment; end product of membrane lipid peroxidation
- “Wear & tear” pigment
- Commonly accumulates in elderly patients
  - Found most often within hepatocytes & at the poles of nuclei of myocardial cells.

Brown atrophy:
- accumulation of lipofuscin & atrophy of organs
Lipofuscin – Striated Muscle and Liver

Cardiac muscle

Liver
- **Pathologic calcifications**
  - Abnormal deposition of calcium salts in soft tissue
  - Deep blue-purple in nondecalcified H&E stained tissue
  - May stimulate further bone deposition

- **Metastatic calcification**: caused by hypercalcemia
  - Most often from hyperparathyroidism
  - Osteolytic tumours with mobilization of Ca$^{2+}$ & Po$^{4-}$
  - Hypervitaminosis D
  - Excess calcium intake
Metastatic Calcification

Hypercalcemia - Lung
Dystrophic calcifications:

- Intracellular or extracellular; gritty
- Deposition of calcium in tissue altered by injury
  1- Areas of old trauma
  2- Tuberculosis lesions
  3- Affects crucial organs, heart valves, vessels
    - Scarred heart valves
    - Atherosclerosis
- Not caused by hypercalcemia but calcium attracted by released membrane phosphates.
  4- Serum calcium concentration normal
AMYLOIDOSIS

• Amyloid is a pathologic proteinaceous substance, deposited between cells in various tissues and organs of the body in a wide variety of clinical settings.

• Light microscope: amyloid appears as amorphous, eosinophilic, hyaline, extracellular substance that gradually encroaches on and produces pressure atrophy of adjacent cells.
<table>
<thead>
<tr>
<th>Clinicopathologic Category</th>
<th>Associated Diseases</th>
<th>Major Fibril Protein</th>
<th>Chemically Related Precursor Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systemic (Generalized) Amyloidosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunocyte dyscrasias with amyloidosis (primary amyloidosis)</td>
<td>Multiple myeloma and other monoclonal B-cell proliferations</td>
<td>AL</td>
<td>Immunoglobulin light chains, chiefly λ type</td>
</tr>
<tr>
<td>Reactive systemic amyloidosis (secondary amyloidosis)</td>
<td>Chronic inflammatory conditions</td>
<td>AA</td>
<td>SAA</td>
</tr>
<tr>
<td>Hemodialysis-associated amyloidosis</td>
<td>Chronic renal failure</td>
<td>Aβ₂ m</td>
<td>β₂-microglobulin</td>
</tr>
<tr>
<td>Hereditary amyloidosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial Mediterranean fever</td>
<td>-</td>
<td>AA</td>
<td>SAA</td>
</tr>
<tr>
<td>Familial amyloidotic neuropathies (several types)</td>
<td>-</td>
<td>ATTR</td>
<td>Transthyretin</td>
</tr>
<tr>
<td>Systemic senile amyloidosis</td>
<td>-</td>
<td>ATTR</td>
<td>Transthyretin</td>
</tr>
</tbody>
</table>
**Localized Amyloidosis**

<table>
<thead>
<tr>
<th>Localized Amyloidosis</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Senile cerebral</td>
<td>Alzheimer disease</td>
<td>Aβ</td>
<td>APP</td>
</tr>
<tr>
<td>Endocrine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medullary carcinoma of thyroid</td>
<td>-</td>
<td>A Cal</td>
<td>Calcitonin</td>
</tr>
<tr>
<td>Islet of Langerhans</td>
<td>Type II diabetes</td>
<td>AIAPP</td>
<td>Islet amyloid peptide</td>
</tr>
<tr>
<td>Isolated atrial amyloidosis</td>
<td>-</td>
<td>AANF</td>
<td>Atrial natriuretic factor</td>
</tr>
<tr>
<td>Prion diseases</td>
<td>Various prion diseases of the CNS</td>
<td>Misfolded prion protein (PrP$^{sc}$)</td>
<td>Normal prion protein PrP</td>
</tr>
</tbody>
</table>
Necrosis

• There is denaturation of intracellular proteins and enzymatic digestion of the cell.

• The enzymes are derived either from the lysosomes of the dead cells themselves, in which case the enzymatic digestion is referred to as autolysis, or from the lysosomes of immigrant leukocytes, during inflammatory reactions.
Necrosis

- A spectrum of morphologic changes that follow cell death in living tissue, due to progressive degradative action of enzymes on the lethally injured cells.

- Leaked out contents of necrotic cells may elicit inflammation in the surrounding tissue.

- The morphologic appearance is due to denaturation of proteins and enzymatic digestion.

- The enzymes are derived from lysosomes of the dead cells themselves - AUTOLYSIS.
Nuclear changes in necrotic cells:

- **Karyolysis**- basophilia of chromatin may fade.
- **Pyknosis**- nuclear shrinkage and increased basophilia.
- **Karyorrhexis**- nuclear fragmentation.
- **Disappearance of nucleus.**
Normal cell

Reversible cell injury with cytoplasmic & organelle swelling, blebbing & ribosome detachment

Irreversible cell injury with rupture of membrane & organelles, & nuclear pyknosis

Karyorrhexis

Karyolysis
Morphology of necrosis.

Necrotic cells show

- increased eosinophilia with a glassy homogeneous appearance.

- The cytoplasm becomes vacuolated and appears moth-eaten.

- Finally, calcification of the dead cells may occur.
Morphology of necrosis

By electron microscopy, necrotic cells are characterized by:

• overt discontinuities in plasma membrane,
• marked dilation of mitochondria with the appearance of large amorphous densities,
• intracytoplasmic myelin figures,
• amorphous osmiophilic debris, and
• aggregates of fluffy material probably representing denatured protein
Necrosis

• Definition
  • Death of groups of contiguous cells in tissue or organ

• Patterns
  • Coagulative
  • Liquefactive
  • Caseous
  • Fat necrosis
  • (gangrene)
  • (Infarct)
    • Red/haemorrhagic
    • White
Coagulative necrosis

• Cells have died but the basic shape and architecture of the tissue endures
• Most common manifestation of ischaemic necrosis in tissues.
• Affected tissue maintains solid consistency.
• In most cases the necrotic cells are ultimately removed by inflammatory cells.
• The dead cells may be replaced by regeneration from neighboring cells, or by scar (fibrosis).
Coagulative necrosis

Normal

Necrosis
Coagulative necrosis
Liquefactive necrosis

- Complete dissolution of necrotic tissue.
- Most commonly due to massive infiltration by neutrophils (abscess formation).
  - Release of reactive oxygen species and proteases
- Liquefaction is also characteristic of ischaemic necrosis in the brain.
Liquefactive necrosis
Caseous necrosis

• Accumulation of amorphous (no structure) debris within an area of necrosis.

• Tissue architecture is abolished and viable cells are no longer recognizable.

• Characteristically associated with the granulomatous inflammation of tuberculosis. Also seen in some fungal infections.
Caseous necrosis
Caseous necrosis
Fat necrosis

• Results from the action of lipases released into adipose tissue.
  • pancreatitis, trauma.

• Free fatty acids accumulate and precipitate as calcium soaps (saponification).
  • These precipitates are grossly visible as pale yellow/white nodules

• Microscopically, the digested fat loses its cellular outlines. There is often local inflammation
Fat necrosis
Fibrinoid necrosis

- Special form of necrosis, visible by light microscopy
- Fibrin-like--- *in immune reactions*
Gangrene ("gangrenous necrosis")

• Not a separate kind of necrosis at all, but a term for necrosis that is advanced and visible grossly.
  
  • If there's mostly coagulation necrosis, (i.e., the typical blackening, desiccating foot which dried up before the bacteria could overgrow), we call it dry gangrene.
  • If there's mostly liquefactive necrosis (i.e., the typical foul-smelling, oozing foot infected with several different kinds of bacteria), or if it's in a wet body cavity, we call it wet gangrene.
Gangrenous necrosis
Apoptosis

• Programmed cell death.

• Noxious stimuli that damage DNA result in nuclear dissolution without complete loss of cell membrane integrity.

• Can be physiologic or pathologic.
Apoptosis - basics

• is a distinct reaction pattern which represents programmed single-cell suicide.

• Cells actually expend energy in order to die.

• Derived from Greek "falling off" (as for autumn leaves)

• Apoptosis is "the physiological way for a cell to die", seen in a variety of normal situations.
Apoptosis - morphology

- **Necrosis:**
  - pathological response to cellular injury.
  - Chromatin clumps, mitochondria swell and rupture, membrane lyses, cell contents spill, inflammatory response triggered

- **Apoptosis**
  - DNA cleaved at specific sites - 200 bp fragments.
  - Cytoplasm shrinks without membrane rupture
  - Blebbing of plasma and nuclear membranes
  - Cell contents in membrane bounded bodies, no inflammation
Apoptosis - normal

A stain for apoptotic cells in the developing paw of a foetal mouse.
Apoptosis - pathological

Graft-versus-host disease in colonic mucosa
Apoptosis - triggers

• Withdrawal of growth stimuli
  E.g. growth factors

• Death signals
  E.g. TNF and Fas

• DNA damage
  p53 plays an important role
Apoptosis - mechanisms

- Extrinsic factors
  E.g. by members of the TNF family
- Intrinsic mechanisms
  E.g. hormone withdrawal
Intrinsic pathway
Extrinsic pathway

(Robbin 2005)
Mechanism of apoptosis

Kumar et al: Robbins & Cotran Pathologic Basis of Disease, 8th Edition. Copyright © 2009 by Saunders, an imprint of Elsevier, Inc. All rights reserved.
Patterns Of Cell Death

There are two principal patterns of cell death:

1- Necrosis and

2- Apoptosis.
Cell Injury and Cell Death

- **Reversible injury**: Normal cell恢复
- **Progressive injury**: Myelin figure, membrane blebs, swelling of endoplasmic reticulum and mitochondria
- **Necrosis**: Amorphous densities in mitochondria, inflammation
- **Apoptosis**: Condensation of chromatin, membrane blebs, cellular fragmentation, apoptotic body, phagocytosis of apoptotic cells and fragments

Kumar et al: Robbins & Cotran Pathologic Basis of Disease, 8th Edition. Copyright © 2009 by Saunders, an imprint of Elsevier, Inc. All rights reserved.
<table>
<thead>
<tr>
<th>Feature</th>
<th>Necrosis</th>
<th>Apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size</td>
<td>Enlarged (swelling)</td>
<td>Reduced (shrinkage)</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Pyknosis → karyorrhexis → karyolysis</td>
<td>Fragmentation into nucleosome size fragments</td>
</tr>
<tr>
<td>Plasma membrane</td>
<td>Disrupted</td>
<td>Intact; altered structure, especially orientation of lipids</td>
</tr>
<tr>
<td>Cellular contents</td>
<td>Enzymatic digestion; may leak out of cell</td>
<td>Intact; may be released in apoptotic bodies</td>
</tr>
<tr>
<td>Adjacent inflammation</td>
<td>Frequent</td>
<td>No</td>
</tr>
<tr>
<td>Physiologic or pathologic role</td>
<td>Invariably pathologic (culmination of irreversible cell injury)</td>
<td>Often physiologic, means of eliminating unwanted cells; may be pathologic after some forms of cell injury, especially DNA damage</td>
</tr>
</tbody>
</table>
Causes of cell injury

- Oxygen deprivation.
- **Physical agents** eg: mechanical trauma, burns, deep cold, barotrauma, electric shock.
- **Chemical agents & drugs** eg: poisons, environmental pollutants, CO, asbestos, alcohol, narcotic drugs etc.
- **Infectious** agents-viruses, rickettsiae, bacteria, fungi, protozoa and helminths.
- **Immunologic** reactions-anaphylaxis, autoimmune disorders.
- **Genetic derangements**.
- **Nutritional imbalances**-PEM, obesity, specific vitamin deficiencies etc.
Causes of Cell Injury

1) Oxygen Deprivation (*Hypoxia*). It is a common cause of cell injury and cell death.

- *Hypoxia* can be due to:
  
  A- inadequate oxygenation of the blood due to Cardiorespiratory failure
  
  B- loss of the oxygen-carrying capacity of the blood, as in anemia or carbon monoxide poisoning.

Depending on the severity of the hypoxic state, cells may adapt, undergo injury, or die.
2) **Physical Agents**:  
- Mechanical trauma,  
- Burns,  
- Deep cold  
- Sudden changes in atmospheric pressure,  
- Radiation, and electric shock
Causes of Cell Injury cont.

3) **Chemical Agents and Drugs**
   - oxygen, in high concentrations
   - *poisons*, such as arsenic, cyanide, or mercuric salts
   - environmental and air pollutants
   - insecticides, herbicides, industrial and occupational hazards
   - alcohol and narcotic drugs and therapeutic drugs
Causes of Cell Injury cont.

4) Infectious Agents e.g. bacteria, fungi, viruses and parasites.
5) Immunologic Reactions.
6) Genetic Derangements.
7) Nutritional Imbalances
Mechanisms of cell injury

• **Depletion of ATP**-affects activity of Na, K-ATPase pump. This results in anaerobic glycolysis.

• **Mitochondrial damage**-leakage of cytochrome-C into cytosol, resulting in apoptosis.

• **Influx of Ca & loss of Ca homeostasis**, leading to activation of ATPases, phospholipases, proteases & endonucleases.
MECHANISM OF CELL INJURY

1. DEPLETION OF ATP:

- ATP depletion and decreased ATP synthesis are associated with both hypoxic and chemical (toxic) injury.

- ATP is required for many synthetic and degradative processes within the cell.
ATP is produced in two ways.

A- The major pathway is oxidative phosphorylation of adenosine diphosphate.

B- The second is the glycolytic pathway, which generate ATP in absence of oxygen using glucose derived from body fluids or from glycogen
Effects of depleted ATP

a) The activity of the plasma membrane energy-dependent sodium pump is reduced. It causes sodium to accumulate intracellularly and potassium to diffuse out of the cell causing cell swelling, and dilation of the endoplasmic reticulum.
MECHANISM OF CELL INJURY cont.

b) If oxygen supply to cells is reduced, as in ischemia, oxidative phosphorylation ceases and cells rely on glycolysis for energy production (anaerobic metabolism) resulting in depletion of glycogen stores. Glycolysis results in the accumulation of lactic acid which reduces the intracellular pH, resulting in decreased activity of many cellular enzymes.
MECHANISM OF CELL INJURY cont.

c) Failure of the Ca$^{2+}$ pump leads to influx of Ca$^{2+}$, with damaging effects on numerous cellular components

d) Ribosomes detach from the RER and polysomes breakdown into monosomes, leading to reduction in protein synthesis. Ultimately, irreversible damage to mitochondrial and lysosomal membranes occurs, and cell undergoes necrosis
e) In cells deprived of oxygen or glucose, proteins may become misfolded, and trigger the unfolded protein response leading to cell injury and even death.
Functional and morphologic consequences of decreased intracellular ATP during cell injury.
MECHANISM OF CELL INJURY cont.

2- Mitochondrial Damage:
Mitochondria are important targets for all types of injury, including hypoxia and toxins.
MECHANISM OF CELL INJURY cont.

Mitochondria can be damaged by:
A- Increases of cytosolic Ca2+
B- Oxidative stress
C- Breakdown of phospholipids, and by
D- Lipid breakdown products.
MECHANISM OF CELL INJURY cont.

- Mitochondrial damage results in the formation of a high-conductance channel, called mitochondrial permeability transition, present in the inner mitochondrial membrane. In the initial phase it is reversible but once mitochondrial permeability transition is irreversible it becomes a deathblow to the cell.

- Mitochondrial damage can also be associated with leakage of cytochrome c into the cytosol.
Mitochondrial damage and dysfunction
3. INFLUX OF INTRACELLULAR CALCIUM & LOSS OF CALCIUM HOMEOSTASIS.

- Ischemia causes an increase in cytosolic calcium concentration. Increased Ca\(^{2+}\) in turn activates a number of enzymes, e.g.
  - ATPases (thereby hastening ATP depletion),
  - Phospholipases (which cause membrane damage),
  - Proteases (which break down both membrane and cytoskeletal proteins), and
  - Endonucleases (which are responsible for DNA and chromatin fragmentation).
Influx of intracellular calcium
4. ACCUMULATION OF OXYGEN-DERIVED FREE RADICALS (OXIDATIVE STRESS)
- Small amounts of partially reduced reactive oxygen forms are produced as a byproduct of mitochondrial respiration.
- Some of these free radicals can damage lipids, proteins, and nucleic acids.
- They are referred to as reactive oxygen species.
Free radical injury

- Oxygen derived.

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

- Energy created by this unstable configuration is released through reactions with adjacent molecules.

- They initiate autocatalytic reactions.
Free radicals are initiated by:

- Absorption of radiant energy (u-v or ionising):
  Water is hydrolysed to (OH) & (H) free radicals.

- Enzymatic metabolism of exogenous chemicals or drugs, eg: CCl$_4$ converted to CCl$_3$.

- Redox reactions in the cell: (O$_2$), (H$_2$O$_2$) & (OH).

- Transition metals like iron and copper.

- Nitrous oxide.
Effects of free radicals:

- Lipid peroxidation of membranes.
- Oxidative modification of proteins.
- Lesions in DNA.
Accumulation of oxygen-derived free radicals

PATHOLOGIC EFFECTS OF ROS: CELL INJURY AND DEATH
ROS react with:
• Fatty acids → oxidation → generation of lipid peroxides → disruption of plasma membrane, organelles
• Proteins → oxidation → loss of enzymatic activity, abnormal folding
• DNA → oxidation → mutations, breaks

REMOVAL OF FREE RADICALS
Antioxidant mechanisms:
• SOD (in mitochondria) converts $O_2^-$ $\rightarrow$ $H_2O_2$
• Glutathione peroxidase (in mitochondria) converts $\cdot OH$ $\rightarrow$ $H_2O_2$ $\rightarrow$ $H_2O + O_2$
• Catalase (in peroxisomes) converts $H_2O_2$ $\rightarrow$ $H_2O + O_2$

Kumar et al: Robbins & Cotran Pathologic Basis of Disease, 8th Edition. Copyright © 2009 by Saunders, an imprint of Elsevier, Inc. All rights reserved.
5. Defects In Membrane Permeability:
   - In ischemic cells, membrane damage may be the result of ATP depletion and calcium-modulated activation of phospholipases.
   - It can also be damaged directly by certain bacterial toxins, viral proteins etc.
The biochemical mechanisms which contribute to membrane damage are:

- Mitochondrial dysfunction
- Cytoskeletal abnormalities
- Reactive oxygen species
- Lipid breakdown products
Defects in membrane permeability

- Reactive oxygen species
- Lipid peroxidation
- ATP
- Phospholipid reacylation/synthesis
- Phospholipid degradation
- Lipid breakdown products
- Cytoskeletal damage
- Protease activation
- Cytosolic Ca²⁺
- O₂

Kumar et al: Robbins & Cotran Pathologic Basis of Disease, 8th Edition. Copyright © 2009 by Saunders, an imprint of Elsevier, Inc. All rights reserved.
Cellular and biochemical sites of damage in cell injury.