Cell Injury and Cellular Adaptations

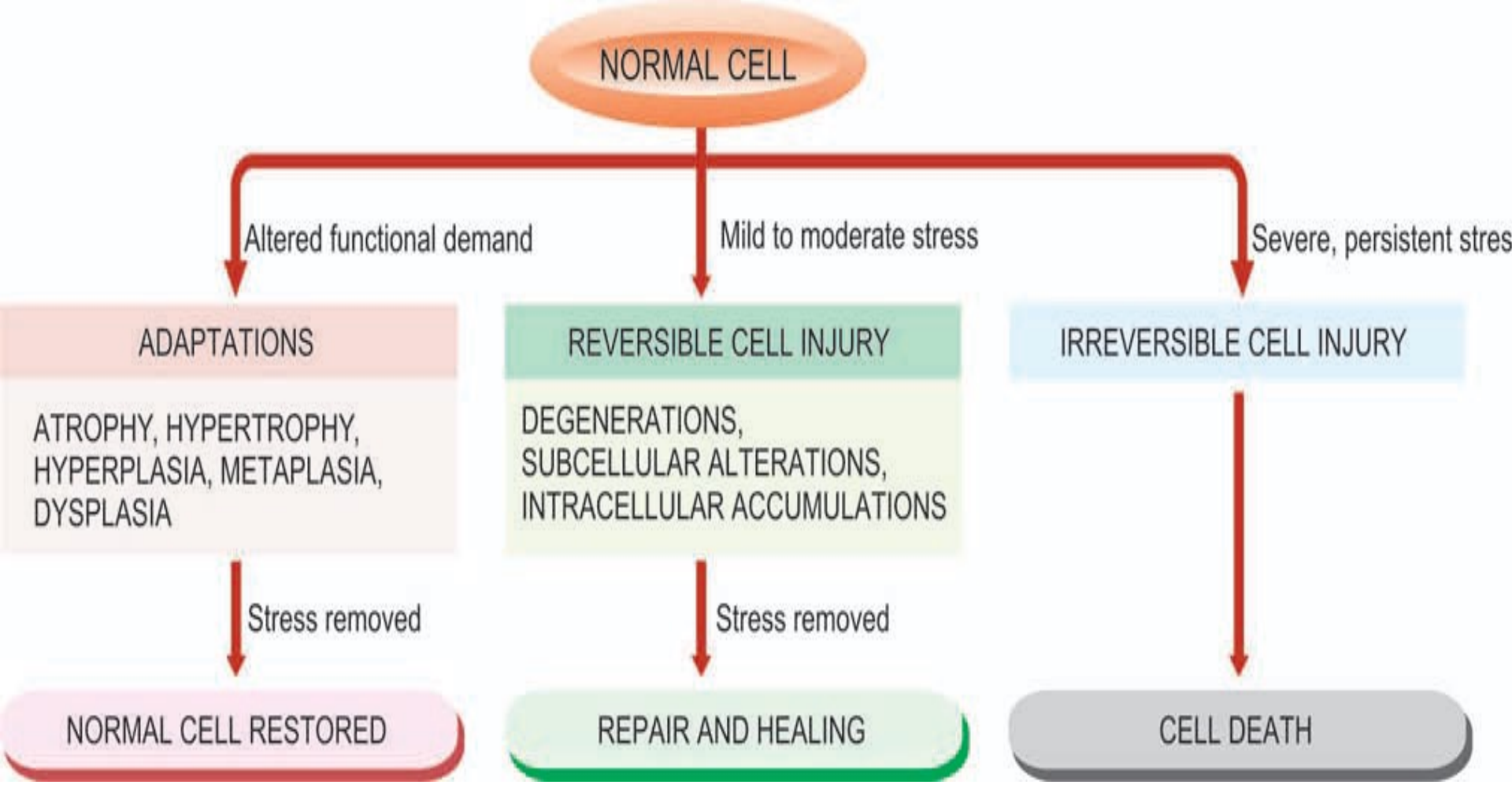
Cell Injury and Cellular Adaptations

- Most forms of diseases begin with cell injury followed by consequent loss of cellular function. **Cell injury** is defined as a **variety of stresses a cell encounters as a result of changes in its internal and external environment**.
- In general, cells of the body have inbuilt mechanism to deal with changes in environment to an extent. The cellular response to stress may vary and depends upon the following variables:
- **1- The type of cell and tissue involved**.
- **2- Extent and type of cell injury.**

Cellular responses to cell injury.

- Various forms of cellular responses to cell injury may be
- as follows :
- **1.** When there is **increased functional demand**, the cell may adapt to the changes which are expressed morphologically and then revert back to normal after the stress is removed (cellular adaptations).
- **2.** When the stress is **mild to moderate**, the injured cell may recover (**reversible cell injury**), while when the injury is persistent cell death may occur (**irreversible cell injury**).
- **3.** The 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).

Cellular responses to cell injury



Etiology of Cell Injury

- The cells may be broadly injured by two major ways:
- **A. By genetic causes**
- **B. By acquired causes**
- **The genetic causes:**

➢ **Chromosomal abnormalities**

➢ **Genetic mutation**

Acquired causes

- The acquired causes of cell injury can be further categorized as under:
- **1**. Hypoxia and ischaemia
- **2**. Physical agents
- **3**. Chemical agents and drugs
- **4.** Microbial agents
- **5**. Immunologic agents
- **6**. Nutritional derangements
- **7**. Aging
- **8**. Psychogenic diseases
- **9**. Iatrogenic factors
- **10**. Idiopathic diseases.

1. Hypoxia and Ischaemia.

- Cells of different tissues essentially require oxygen to generate energy and perform metabolic functions. Deficiency of oxygen or hypoxia results in failure to carry out these activities by the cells. Hypoxia is the most common cause of cell injury. Hypoxia may result from the following:
- **I-**The most common mechanism of hypoxic cell injury is by **reduced supply of blood** to cells due to interruption i.e. ischaemia.
- **II-**However, hypoxia may result from other causes as well e.g. **disorders of oxygen-carrying** RBCs (e.g. anaemia, carbon monoxide poisoning), heart diseases, lung diseases and increased demand of tissues.

2. physical agents.

- Physical agents in causation of disease are as under:
- Mechanical trauma (e.g. road accidents)
- Thermal trauma (e.g. by heat and cold);
- Electricity;
- Radiation (e.g. ultraviolet and ionising); and
- Rapid changes in atmospheric pressure.

3. Chemicals and Drugs.

- Important chemical agents and drugs that causes cell injury includes the following:
- Chemical poisons such as cyanide, arsenic, mercury;
- Strong acids and alkalis;
- Environmental pollutants;
- Insecticides and pesticides;
- Oxygen at high concentrations;
- Hypertonic glucose and salt;
- Social agents such as alcohol and narcotic drugs; and therapeutic administration of drugs.

- **4. Microbial agents.**
- Injuries by microbes include infections caused by bacteria, viruses, fungi, protozoa, metazoa, and other parasites.
- **5. Immunologic agents**.
- Including autoimmune diseases and cell injury following responses to infection, hypersensitivity reaction to some drugs like penicillin

6. Nutritional imbalances.

- Including protein–calorie deficiency or lack of specific vitamins, as well as nutritional excesses(obesity).

- **7.Aging**.
- Cellular aging or senescence leads to impaired ability of the cells to undergo replication and repair, and ultimately lead to cell death culminating in death of the individual.

8. Psychogenic diseases.

- There are no specific biochemical or morphologic changes in common acquired mental diseases due to mental stress, strain, anxiety, overwork and frustration e.g. depression, schizophrenia. However, problems of drug addiction, alcoholism, and smoking result in various organic diseases such as liver damage, chronic bronchitis, lung cancer, peptic ulcer, hypertension, ischaemic heart disease etc.

9. Iatrogenic causes

- There are some diseases as well as deaths attributed to iatrogenic causes (owing to physician).
- Examples include occurrence of disease or death due to error in judgment by the physician and untoward effects of administered therapy (drugs, radiation).

10. Idiopathic diseases

- Idiopathic means “of unknown cause”. Finally, although so much is known about the etiology of diseases, there still remain many diseases for which exact cause is undetermined. For example, most common form of hypertension (90%) is idiopathic (or essential) hypertension. Similarly, exact etiology of many cancers is still incompletely known.

Types of cell injury:

- **1-Reversible Cell injury** the cells return back to their stable baseline state after removal the cause of cell injury. This is called **Degeneration.**

- **2- Irreversible Cell injury** cells cannot return to their baseline state after removal the cause of cell injury. This is called **Cell death & Necrosis**

Pathogenesis of cell injury

- Injury to the normal cell by one or more of the etiologic agents may result in a state of **reversible** or **irreversible** cell injury.
- The underlying alterations in biochemical systems of cells for reversible and irreversible cell injury by various agents is complex and varied. However, in general, the following principles apply in pathogenesis of most forms of cell injury by various agents:

1. Type, duration and severity of injurious agent:

- The extent of cellular injury depends upon type, duration and severity of the stimulus e.g. small dose of chemical toxin or short duration of ischaemia cause reversible cell injury while large dose of the same chemical agent or persistent ischaemia cause cell death.

2. Type, status and adaptability of target cell:

- The type of cell as regards its susceptibility to injury, its nutritional and metabolic status, and adaptation of the cell to hostile environment determine the extent of cell injury e.g. skeletal muscle can withstand hypoxic injury for long-time while cardiac muscle suffers irreversible cell injury after 20-30 minutes of persistent ischaemia.

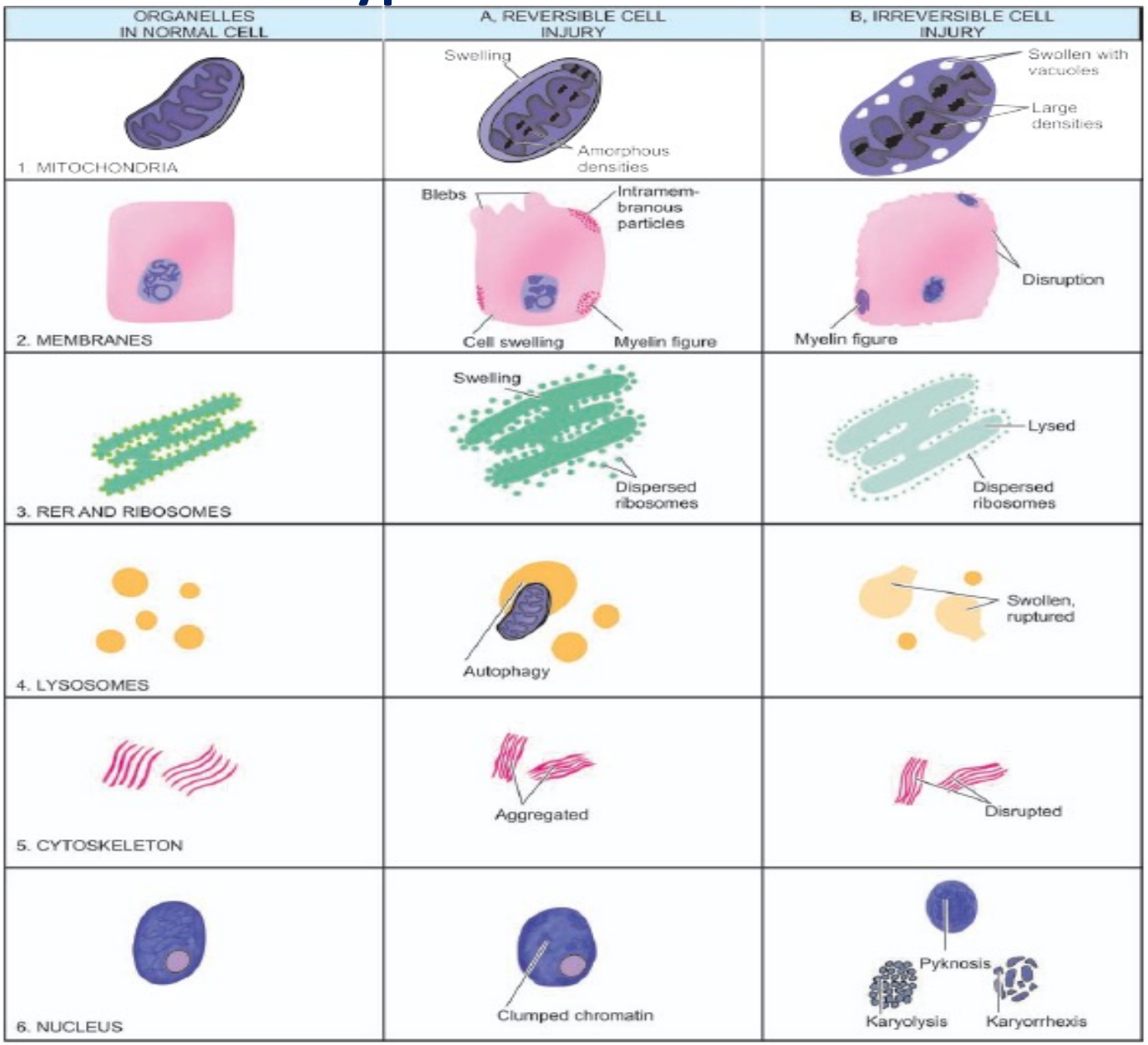
4. Morphologic consequences:

- All forms of biochemical changes underlying cell injury are expressed in terms of morphologic changes.
- . The morphologic changes of reversible cell injury (e.g. hydropic swelling) appear earlier than morphologic alterations in cell death (e.g. in myocardial infarction). The interruption of blood supply (i.e. ischaemia) and impaired oxygen supply to the tissues (i.e. hypoxia) are most common form of cell injury in human beings.

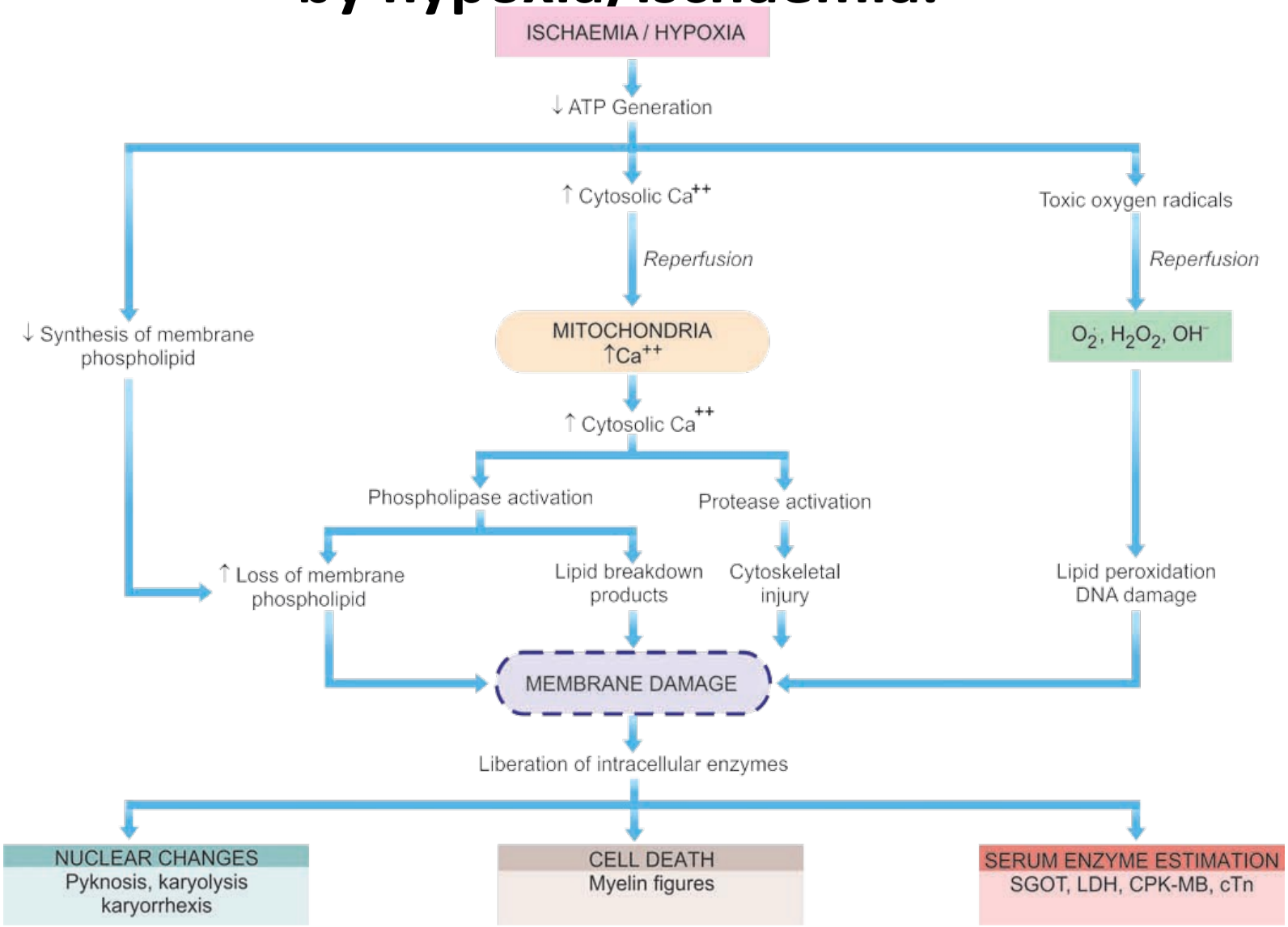
Pathogenesis of ischaemic and hypoxic injury

- **Reversible cell injury**. If the ischaemia or hypoxia is of short duration, the effects may be reversible on rapid restoration of circulation e.g. in coronary artery occlusion, myocardial contractility, metabolism and ultrastructure are reversed if the circulation is quickly restored. The sequential biochemical and ultrastructural changes in reversible cell injury are as under :

Ultrastructural changes during Cell injury due to hypoxia-ischaemia



Sequence of events in the pathogenesis of reversible and irreversible cell injury caused by hypoxia/ischaemia.



1.Decreased generation of cellular ATP:

- Damage by ischaemia versus hypoxia from other causes. All living cells require continuous supply of oxygen to produce **ATP** which is essentially required for a variety of cellular functions (e.g. Membrane transport, protein synthesis, lipid synthesis and phospholipid metabolism).
- **ATP** in human cell is derived from 2 sources:
- **Firstly,** by aerobic respiration or oxidative phosphorylation (which requires oxygen) in the mitochondria, and
- **Secondly**, cells may switch over to anaerobic glycolytic oxidation to maintain constant supply of ATP (in which ATP is generated from glucose/glycogen in the absence of oxygen).

- Ischaemia due to interruption in blood supply as well as hypoxia from other causes limit the supply of oxygen to the cells, thus causing decreased ATP generation from ADP:
- **In ischaemia**, aerobic respiration as well as glucose availability are both compromised resulting in more severe and faster effects of cell injury. Ischaemic cell injury also causes accumulation of metabolic waste products in the cells.
- **In hypoxia** from other causes (RBC disorders, heart disease, lung disease), anaerobic glycolytic ATP generation continues, and thus cell injury is less severe.

- However, highly specialized cells such as myocardium, proximal tubular cells of the kidney, and neurons of the CNS are dependent solely on aerobic respiration for ATP generation and thus these tissues suffer from ill-effects of ischaemia more severely and rapidly.

2. Intracellular lactic acidosis: Nuclear clumping

- Due to low oxygen supply to the cell, aerobic respiration by mitochondria fails first. This is followed by switch to anaerobic glycolytic pathway for the requirement of energy (i.e. ATP).
- This results in rapid depletion of glycogen and accumulation of lactic acid lowering the intracellular pH. Early fall in intracellular pH (i.e. intracellular lactic acidosis) results in clumping of nuclear chromatin.

3. Damage to plasma membrane pumps: Hydropic swelling and other membrane changes.

- Lack of ATP interferes in generation of phospholipids from the cellular fatty acids which are required for continuous repair of membranes.
- This results in damage to membrane pumps operating for regulation of sodium and calcium as under:

3. Damage to plasma membrane pumps:

- **i) Failure of sodium-potassium pump.**
- Normally, the energy (ATP)-dependent sodium pump (Na^+-K^+ ATPase) operating at the plasma membrane allows active transport of sodium out of the cell and diffusion of potassium into the cell. Lowered ATP in the cell and consequent increased ATPase activity interfere with this membrane-regulated process. This results in intracellular accumulation of sodium and diffusion of potassium out of cell. The accumulation of sodium in the cell leads to increase in intracellular water to maintain iso-osmotic conditions (i.e. hydropic swelling occurs).
- **ii) Failure of calcium pump**.
- Membrane damage causes disturbance in the calcium ion exchange across the cell membrane. Excess of calcium moves into the cell (i.e. calcium influx), particularly in the mitochondria, causing its swelling and deposition of phospholipid-rich amorphous densities.

4. Reduced protein synthesis: Dispersed ribosomes

- As a result of continued hypoxia, membranes of endoplasmicreticulum and Golgi apparatus swell up. Ribosomes are detached from granular endoplasmic reticulum and polysomes are degraded to monosomes, thus dispersing ribosomes in the cytoplasm and inactivating their function.
- Similar reduced protein synthesis occurs in Golgi apparatus. Up to this point, withdrawal of acute stress that resulted in reversible cell injury can restore the cell to normal state.

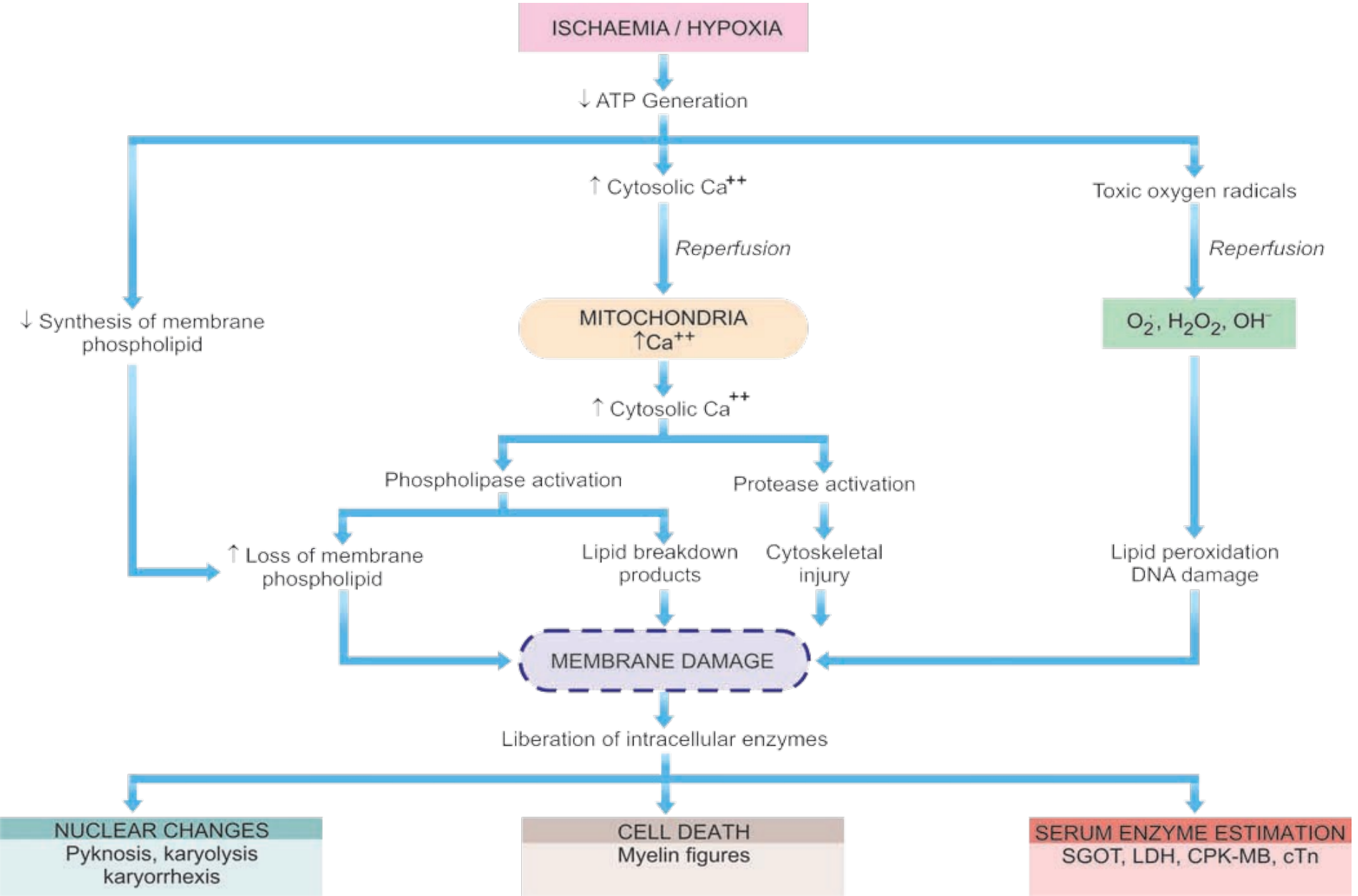
Irreversible cell injury.

- Persistence of ischaemia or hypoxia results in irreversible damage to the structure and function of the cell (cell death). The stage at which this point of no return or irreversibility is reached from reversible cell injury is unclear but the sequence of events is a continuation of reversibly injured cell.
- **Two** essential phenomena always distinguish irreversible from reversible cell injury:
- **Inability of the cell to reverse** mitochondrial dysfunction on reperfusion or reoxygenation.
- **Disturbance in cell membrane function** in general, and in plasma membrane in particular.

Irreversible cell injury

- In addition, there is further reduction in ATP, continued depletion of proteins, reduced intracellular pH, and leakage of lysosomal enzymes into the cytoplasm. These biochemical changes have effects on the ultrastructural components of the cell

Sequence of events in the pathogenesis of reversible and irreversible cell injury caused by hypoxia/ischaemia.



1. Calcium influx: Mitochondrial damage

- As a result of continued hypoxia, a large cytosolic influx of calcium ions occurs, especially after reperfusion of irreversibly injured cell. Excess intracellular calcium collects in the mitochondria disabling its function. Morphologically, mitochondrial changes are vacuoles in the mitochondria and deposits of amorphous calcium salts in the mitochondrial matrix.

2. Activated phospholipases: Membrane damage

- Damage to membrane function in general, and plasma membrane in particular, is the most important event in irreversible cell injury in ischaemia. As a result of sustained ischaemia, there is increased cytosolic influx of calcium in the cell. Increased calcium activates endogenous phospholipases. These in turn degrade membrane phospholipids progressively which are the main constituent of the lipid bilayer membrane. Besides, there is also decreased replacement-synthesis of membrane phospholipids due to reduced ATP. Other lytic enzyme which is activated is ATPase which causes further depletion of ATP.

3. Intracellular proteases: Cytoskeletal damage

- . The normal cytoskeleton of the cell (microfilaments, microtubules and intermediate filaments) which anchors the cell membrane is damaged due to degradation by activated intracellular proteases or by physical effect of cell swelling producing irreversible cell membrane injury.

4. Activated endonucleases: Nuclear damage

- The nucleoproteins are damaged by the activated lysosomal enzymes such as proteases and endonucleases. Irreversible damage to the nucleus can be in three forms:
- **i) Pyknosis:** Condensation and clumping of nucleus which becomes dark basophilic.
- **ii) Karyorrhexis**: Nuclear fragmentation in to small bits dispersed in the cytoplasm.
- **iii) Karyolysis:** Dissolution of the nucleus.

5. Lysosomal hydrolytic enzymes: Lysosomal damage, cell death and phagocytosis

- The lysosomal membranes are damaged and result in escape of lysosomal hydrolytic enzymes. These enzymes are activated due to lack of oxygen in the cell and acidic pH. These hydrolytic enzymes include:
- Hydrolase, RNAase, DNAase, protease, glycosidase, phosphatase, lipase, amylase, cathepsin etc) which on activation bring about enzymatic digestion of cellular components and hence cell death. The dead cell is eventually replaced by masses of phospholipids called myelin figures which are either phagocytosed by macrophages or there may be formation of calcium soaps.

- Liberated enzymes leak across the abnormally permeable cell membrane into the serum, the estimation of which may be used as clinical parameters of cell death.
- For example, in myocardial infarction, estimation of elevated serum glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), isoenzyme of creatine kinase (CK-MB), and more recently cardiac troponins (cTn) are useful guides for death of heart muscle.

TABLE 3.1: Common Enzyme Markers of Cell Death.

Enzyme	Disease
1. *Aspartate aminotransferase (AST, SGOT)*	Diffuse liver cell necrosis e.g. viral hepatitis, alcoholic liver disease Acute myocardial infarction
2. *Alanine aminotransferase (ALT, SGPT)*	More specific for diffuse liver cell damage than AST e.g. viral hepatitis
3. *Creatine kinase-MB (CK-MB)*	Acute myocardial infarction, myocarditis Skeletal muscle injury
4. *Lipase*	More specific for acute pancreatitis
5. *Amylase*	Acute pancreatitis Sialadenitis
6. *Lactic dehydrogenase (LDH)*	Acute myocardial infarction Myocarditis Skeletal muscle injury
7. *Cardiac troponin (CTn)*	Specific for acute myocardial infarction

Ischaemia-Reperfusion Injury and Free Radical-Mediated Cell Injury

- Depending upon the duration of ischaemia/hypoxia,
- restoration of blood flow may result in the following 3 different consequences:

- **1.** From ischaemia to reversible injury.
- **2.** From ischaemia to reperfusion injury.
- **3.** From ischaemia to irreversible injury.

1. From ischaemia to reversible injury.

- When the period of ischaemia is of short duration, reperfusion with resupply of oxygen restores the structural and functional state of the injured cell i.e. reversible cell injury.
- **2. From ischaemia to reperfusion injury.**
- When ischaemia is for longer duration, then rather than restoration of structure and function of the cell, reperfusion paradoxically deteriorates the already injured cell. This is termed ischaemia-reperfusion injury.

3. From ischaemia to irreversible injury.

- Much longer period of ischaemia may produce irreversible cell injury during ischaemia itself when so much time has elapsed that neither blood flow restoration is helpful nor reperfusion injury can develop. Cell death in such cases is not attributed to formation of activated oxygen species. But instead, on reperfusion there is further marked intracellular excess of sodium and calcium ions due to persistent cell membrane damage.

- The underlying mechanism of reperfusion injury and free radical mediated injury is complex but following three main components are involved in it:

- **1**. Calcium overload.
- **2.** Generation of reactive oxygen radicals (superoxide, H_2O_2, hydroxyl radicals).
- **3.** Subsequent inflammatory reaction.

1. Calcium overload

- Upon restoration of blood supply, the ischaemic cell is further bathed by the blood fluid that has more calcium ions at a time when the ATP stores of the cell are low.

- This results in further calcium overload on the already injured cells, triggering lipid peroxidation of the membrane causing further membrane damage.

2. Generation of reactive oxygen radicals.

- Although oxygen is the lifeline of all cells and tissues, its molecular forms as reactive oxygen radicals or reactive oxygen species can be most devastating for the cells. In recent times, free radical-mediated cell injury has been extensively
- studied and a brief account is given below.

Cytotoxicity of oxygen free radicals.

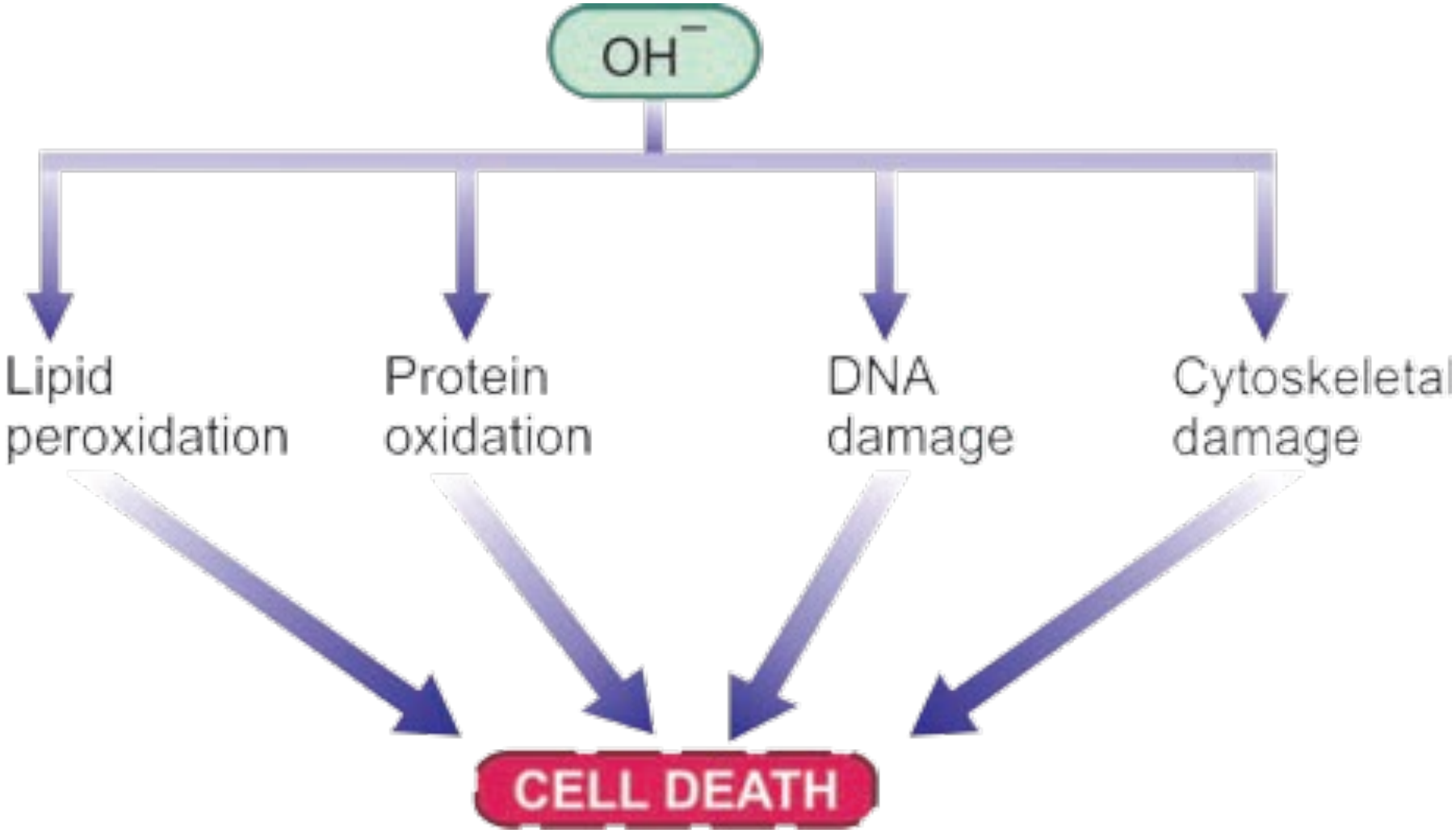
- Free radicals are formed in physiologic as well as pathologic processes. Basically, oxygen radicals are unstable and are destroyed spontaneously.
- The rate of spontaneous destruction is determined
- by catalytic action of certain enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase. The net effect of free radical injury in physiologic and disease states, therefore, depends upon the rate of free radical
- formation and rate of their elimination.

Cytotoxicity of oxygen free radicals

- However, if not degraded, then free radicals are highly destructive to the cell since they have electron-free residue and thus bind to all molecules of the cell; this is termed oxidative stress. Out of various free radicals, hydroxyl radical is the most reactive species. Free radicals may produce membrane damage by the following mechanisms.

- **i) Lipid peroxidation.**
- **ii) Oxidation of proteins.**
- **iii) DNA damage.**
- **iv) Cytoskeletal damage**

Mechanism of cell death by hydroxyl radical



Conditions with free radical injury.

- Currently, oxygen derived free radicals have been known to play an important role in many forms of cell injury:
- **i) Ischaemic reperfusion injury**
- **ii) Ionizing radiation by causing radiolysis of water**
- **iii) Chemical toxicity**
- **iv) Chemical carcinogenesis**
- **v) Hyperoxia (toxicity due to oxygen therapy)**
- **vi) Cellular aging**
- **vii) Killing of microbial agents**
- **viii) Inflammatory damage**
- **ix) Destruction of tumour cells**
- **x) Atherosclerosis.**

3. Subsequent inflammatory reaction

- Ischaemia-reperfusion event is followed by inflammatory reaction. Incoming activated neutrophils utilize oxygen quickly (oxygen burst) and release a lot of oxygen free radicals.
- Ischaemia is also associated with accumulation of precursors of ATP, namely ADP and pyruvate, which further build-up generation of free radicals.