Cell Injury:

Cell injury results when cells are exposed to :
1-severe stress so that they are no longer adapt or
2-inherently damaging agents.

Reversible cell injury. Initially, injury is manifested as functional and morphologic changes that are reversible if the damaging stimulus is removed. The hallmarks of reversible injury are reduced oxidative phosphorylation, adenosine triphosphate (ATP) depletion, and cellular swelling caused by changes in ion concentrations and water influx.
Irreversible injury and cell death. With continuing damage, the injury becomes irreversible, at which time the cell cannot recover

There are two principal patterns of cell death:

1. Necrosis: Occurs after exposure to noxious conditions, and characterized by cell swelling, protein denaturation and organellar breakdown.
2. Apoptosis: Is a programmed cell death occurring in the normal or physiologic conditions.

Causes of Cell Injury:

1. Hypoxia: Impinges on ærobic oxidative respiration. It should be distinguished from ischæmia, which also results in hypoxic cell injury. It occurs in inadequate oxygenation of the blood.
2. Physical agents: Including trauma, extremes of temperatures, radiation, electric shock, and sudden changes in atmospheric pressure.
3. Chemicals and drugs: Any chemical agent may cause cell injury by altering membrane permeability, osmotic homeostasis or the integrity of the enzyme cofactor.
4. Microbiologic agents: ranging from viruses to tapeworms.
5. Immunologic reactions: The immune system of the body may cause cell injury, e.g. anaphylactic reaction.
6. Genetic defects: e.g. Down's syndrome, sickle cell anæmia.
7. Nutritional imbalances: Protein-calorie insufficiency, vitamin deficiencies. Diets rich in animal fat has been strongly implicated in the pathogenesis of atherosclerosis.
8. Aging.

Mechanisms of Cell Injury:

Cell response to injurious stimuli depends on the type of injury, its duration and its severity. The consequences of an injurious stimulus is also dependent on the type of cell injured, its current status of health and its adaptability. Four intracellular systems are vulnerable to injury:

1. Cell membrane integrity, with changing cell ionic and osmotic homeostasis.
2. Ærobic respiration.
3. Protein synthesis.
4. The genetic apparatus.

Cytosolic free calcium is maintained at extremely low levels by ATP-dependent calcium transporters. Mitochondria and endoplasmic reticulum contain a higher concentration of calcium. Ischæmia or toxins allow an influx of calcium from the

extracellular space with a release of mitochondrial calcium, resulting in activation of various enzymes including phospholipases (degrade membranes), proteases (catabolizing structural proteins), ATPases (resulting in ATP depletion) and endonucleases (fragmenting the DNA).

The generation of oxygen free radicals are important mediators of cell death.

Ischæmic And Hypoxic Injury:

Reversible injury: The first effect of hypoxia is on the ærobic respiration (oxidative phosphorylation by mitochondria), consequently resulting in reduced intracellular ATP which in turn results in influx of extracellular calcium and reduction of the plasma membrane sodium pump with accumulation of intracellular sodium and diffusion of potassium out of the cell, with consequent gain in isosmotic water producing *acute cellular swelling*. This is associated with accumulation of other metabolites like inorganic phosphates, lactic acid and purine nucleotides.

Decreased ATP and AMP results in stimulation of phosphofructokinase and an increased rate of anærobic glycolysis. As a result, glycogen is rapidly depleted, with accumulation of lactic acid and inorganic phosphates and *reduced intracellular pH, seen under the light microscope as cytoplasmic eosinophilia.*

Detachment of ribosomes from RER occurs with consequent *reduction of protein synthesis*. If hypoxia continues, the cytoskeleton disappears resulting in *loss of ultrastructural features like microvilli and formation of cell surface blebs*.

Irreversible Injury: Is seen as 1. Severe vacuolization of mitochondria and accumulation of calcium particles. 2. Extensive damage of plasma membrane. 3. Swelling of lysosomes. 3. Reperfusion of oxygen results in calcium mediated injury. 4. Continued loss of proteins, coenzymes and RNA from the hyperpermeable membranes, with leak of lysosomal enzymes into the cytoplasm, where they are activated by the reduced pH starting degrading the cytoplasmic components. 5. Dead cells may be replaced by whorled masses of phospholipids (myelin figures).

Mechanisms of Irreversible Injury:

1. Progressive loss of membrane phospholipids.
2. Cytoskeletal abnormalities: Activation of proteases and increased calcium may result in detachment of the cell membrane.
3. Toxic oxygen radicals: generated after reperfusion of the ischæmic area released by influxed neutrophils.
4. Lipid breakdown products: have detergent effects.

Free Radical Mediation of Cell Injury: Are implicated in:

1. Chemical mediated injury.
2. Radiation mediated injury.
3. Oxygen toxicity.
4. Cellular aging.
5. Microbial killing.
6. Inflammatory damage.
7. Tumour killing.

Free radicals are chemical species with a single unpaired electron in an outer orbital; they are extremely unstable and readily react with organic and inorganic chemicals. They may be generated within the cell by:

1. Absorption of radiant energy: e.g. hydrolysis of water into $OH^{\cdot}$ and $H^{\cdot}$.
2. The reduction-oxidation (redox) reactions, occur during normal physiologic conditions. O_2 is reduced by the addition of four electrons to generate water, with the generation of small amounts of toxic intermediate species including superoxide radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and $OH^{\cdot -}$. Some intracellular enzymes like xanthine oxidase generate superoxide radicals. Copper and iron accept or donate electrons with free radical formation.

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

3. Enzymatic catabolism of oxygenous chemicals e.g.

$CCl_4 \longrightarrow CCL_3^{\cdot}$ in the liver with generation of an autocatalytic membrane phospholipids peroxidation.

Oxygen free radicals react with three main cell components:

1. Lipid peroxidation of plasma membranes.
2. Deoxyribonucleic acid (DNA): react with thymine.
3. Cross linking of proteins.

Cells have developed multiple mechanisms to remove free radicals and thereby minimize injury:

- *Antioxidants* either block the initiation of free radical formation or inactivate free radicals and terminate radical damage. Examples are the lipid-soluble vitamins E and A as well as ascorbic acid and glutathione in the cytosol.
- As we have seen, *iron* and *copper* can catalyze the formation of reactive oxygen species. The levels of these reactive forms are minimized by binding of the ions to storage and transport proteins (e.g., transferrin, ferritin, lactoferrin, and ceruloplasmin), thereby minimizing OH formation.
- A series of *enzymes* acts as free radical-scavenging systems and break down hydrogen peroxide and superoxide anion. These enzymes are located near the sites of generation of these oxidants and include the following:
    - *Catalase*, present in peroxisomes, which decomposes H_2O_2 ($2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$).
    - *Superoxide dismutases* are found in many cell types and convert superoxide to H_2O_2 ($2\ O_2^- + 2\ H \rightarrow H_2O_2 + O_2$).[23] This group includes both manganese-superoxide dismutase, which is localized in mitochondria, and copper-zinc-superoxide dismutase, which is found in the cytosol.
    - *Glutathione peroxidase* also protects against injury by catalyzing free radical breakdown ($H_2O_2 + 2$ GSH $\rightarrow$ GSSG [glutathione homodimer] $+ 2\ H_2O$, or 2 OH $+ 2$ GSH $\rightarrow$ GSSG $+ 2\ H_2O$). The intracellular ratio of oxidized glutathione (GSSG) to reduced glutathione (GSH) is a reflection of the oxidative state of the cell and is an important aspect of the cell's ability to detoxify reactive oxygen species.

Chemical Injury:

Two main mechanisms of chemical injury is identified:

1. By combining with a critical molecular component or cellular organelle: e.g. mercury binds to sulfhydryl groups of the cell membrane and other proteins causing inhibition of ATP dependent transport.
2. Other chemicals are not toxic by themselves and must be converted to reactive toxic metabolites, usually happens by the P-450 oxidases in the SER. Carbon tetrachloride (CCl_4) is converted to the toxic free radical $CCl_3^{\cdot}$ in the liver this will cause autocatalytic membrane peroxidation with rapid breakdown of endoplasmic reticulum in less than 30 min. Within 2 hours, swelling of SER and dissociation of ribosomes from the RER will occur, with resultant reduced lipid export from hepatocytes and fatty liver change.

Patterns of Acute Cell Injury:

Reversible Cell Injury: Light microscopic changes:

1. Cell swelling: Is difficult to appreciate on light microscope, but small clear vacuoles may be seen within the cytoplasm (hydropic changes or vacuolar degeneration).
2. Cytoplasmic eosinophilia due to cytoplasmic acidosis and loss of ribosomes.
3. Fatty change; seen in hypoxic and chemical injury, in the liver and myocardial cells.

Ultrastructural changes (EM):

1. Plasma membrane: Blebing, distortion of microvilli and loosening of intercellular attachments.
2. Mitochondrial changes: Swelling and appearance of phospholipids rich amorphous densities.
3. Dilatation of endoplasmic reticulum, detachment of ribosomes and dissociation of polysomes.
4. Nuclear alterations: Disaggregation of granular elements.

Necrosis:

Refers to a sequence of morphologic changes that follow cell death in living tissue, and is the gross and the histologic terms of cell death occurring in the setting of irreversible exogenous injury. The morphologic appearances of necrosis is the result of two processes: enzymatic digestion of the cell and denaturation of proteins. The hydrolytic enzymes may be derived from the dead cells themselves (autolysis) or from lysosomes of the infiltrating leukocytes (heterolysis).

- Cytoplasmic changes: Eosinophilia and glassy appearance due to loss of glycogen, cytoplasmic vacuolation and calcification.
- Nuclear changes: 1. Karyolysis: due to digestion of DNA. 2. Pyknosis: nuclear shrinkage and increased basophilia, seen mainly in apoptosis. 3. Karyorrhexis: The pyknotic nucleus fragments.

Types of Necrosis:

1. Coagulative necrosis: Preservation of the structural outlines of the coagulated cell or tissue for days. The injury and acidosis denatures the enzymes that block cellular hydrolysis. The prime example is myocardial infarction appearing as acidophilic coagulated anucleated cells. The necrotic cells are removed by

fragmentation and phagocytosis by leukocytes. Coagulative necrosis is characteristic of hypoxic death in all tissues except in the brain.

2. Liquefactive necrosis: Caused by focal bacterial or fungal infection with accumulation of white cells. Hypoxic cell death in the CNS also results in liquefactive necrosis.

3. Gangrenous necrosis: is not a distinctive pattern of necrosis, but is still being used in surgical practice, referring to ischæmic coagulative necrosis with superimposed infection and liquifactive necrosis, called "wet gangrene".

4. Caseous necrosis: Seen in tuberculous infection, derived from the cheesy, white gross appearance of the central necrotic area. Microscopically, it is composed of structureless amorphous granular debris within granulomatous inflammation.

5. Fat necrosis: It describes focal areas of fat destruction following acute pancreatitis, resulting from release of activated pancreatic enzymes with resultant hydrolysis of triglyceride esters within fat cells of the peritoneal cavity.

6. Fibrinoid necrosis : seen in immune reactions involving blood vessels ,deposition of immune complex with fibrin that has leaked out of vessels results in bright pink & amorphous appearance in H&E stains as necrosis in polyarteritis nodosa

Apoptosis:

Is responsible for the programmed cell death in physiologic and pathologic conditions .

It is important in the following physiologic situations:

1. . *The programmed destruction of cells during embryogenesis*, including implantation, organogenesis, developmental involution, and metamorphosis.
2. *Hormone-dependent involution in the adult*, such as endometrial cell breakdown during the menstrual cycle, ovarian follicular atresia in the menopause, the regression of the lactating breast after weaning, and prostatic atrophy after castration.
3. *Cell deletion in proliferating cell populations*, such as intestinal crypt epithelia, in order to maintain a constant number.
4. Death of host cells that have served their useful purpose, such as neutrophils in an *acute inflammatory response*, and lymphocytes at the end of an *immune response*. In these situations, cells undergo apoptosis because they are deprived of necessary survival signals, such as growth factors.
5. *Elimination of potentially harmful self-reactive lymphocytes*, either before or after they have completed their maturation.
6. *Cell death induced by cytotoxic T cells*, a defense mechanism against viruses and tumors that serves to eliminate virus-infected and neoplastic cells. The same mechanism is responsible for cellular rejection of transplants).

Death by apoptosis is also responsible for loss of cells in a variety of pathologic states:

- *Cell death produced by a variety of injurious stimuli*. For instance, radiation and cytotoxic anticancer drugs damage DNA, and if repair mechanisms cannot cope with the injury the cell kills itself by apoptosis. In these situations, elimination of the cell may be a better alternative than risking mutations and translocations in the damaged DNA, which may result in malignant transformation. These injurious stimuli, as well as heat and hypoxia, can induce apoptosis if the insult is mild, but large doses of the same stimuli result

in necrotic cell death. Endoplasmic reticulum (ER) stress, which is induced by the accumulation of unfolded proteins, also triggers apoptotic death of cells .

- *Cell injury in certain viral diseases*, such as viral hepatitis, in which loss of infected cells is largely because of apoptotic death.
- *Pathologic atrophy in parenchymal organs after duct obstruction*, such as occurs in the pancreas, parotid gland, and kidney.
- *Cell death in tumors*, most frequently during regression but also in actively growing tumors.

Apoptosis usually involves single cells or clusters of cells appear on H&E stained sections as round masses with intensely eosinophilic cytoplasm. The nuclear chromatin is condensed aggregating peripherally under the nuclear membrane into well delimited masses of various shapes and sizes. The karyorrhexis occurs by the activation of endonucleases. The cell shrinks, form cytoplasmic buds and fragment into apoptotic bodies. Apoptosis does not elicit an inflammatory response.

Apoptosis is initiated by:

1. Withdrawal of growth factors or hormones.
2. Engagement of specific receptors (e.g. FAS, and TNF).
3. Injury by radiation, toxins and free radicals.
4. Intrinsic protease activation (e.g. in embryogenesis).

All these stimuli will lead to activation of intracellular proteases including calpain I and interleukin 1β converting enzyme (*Ice),* culminating in endonuclease activation, catabolism of cytoskeleton and formation of apoptotic bodies with identification by phagocytic cell receptors.

Subcellular Responses to Injury:

- **Autophagy:** Autophagy refers to lysosomal digestion of the cell's own components. In this process, intracellular organelles and portions of cytosol are first sequestered from the cytoplasm in an *autophagic vacuole* formed from ribosome-free regions of the rough endoplasmic reticulum. The vacuole fuses with lysosomes or Golgi elements to form an *autophagolysosome*. .
- **INDUCTION (HYPERTROPHY) OF SMOOTH ENDOPLASMIC RETICULUM** : cells exposed to chemicals show hypertrophy of the ER as an adaptive response.
- **MITOCHONDRIAL ALTERATIONS :** plays an important role in cell injury and apoptosis, For example, in cell hypertrophy and atrophy, there is an increase and decrease, respectively, in the number of mitochondria in cells.
- CYTOSKELETAL ABNORMALITIES:cytoskeleton consists of actin &myosin filaments , microtubules & various intermediate filaments , all these act as cellular scaffold . Cytoskeletal abnormalities may be reflected by: (1) defects in cell function, such as cell locomotion and intracellular organelle movements, and (2) in some instances by intracellular accumulations of fibrillar material.abnormalities of cytoskeleton may be manifested as abnormal appearance and function of cells as Mallory bodies in alcoholic liver disease .In kartagener syndrome there are both infertility and chronic infection of lung ,sterility due to disorganization of microtubulesthat inhibit sperm motility , respiratory infections result from immobilize the cilia of respiratory epithelium, causing interference with the ability of this epithelium to clear inhaled bacteria & leading to bronchiectasis .

Intracellular Accumulations:

Normal cells may accumulate abnormal substances in various circumstances, either transiently or permanently, being harmful or injurious, and may locate in the cytoplasm or within the nucleus. It may be synthesized by the affected cell or produced elsewhere. Intracellular accumulation can be subdivided into three categories:

1. A normal endogenous substance produced at a normal or increased rate with an inadequate rate of metabolism. E.g. fatty change of the liver.
2. A normal or abnormal endogenous substance which can not be metabolized by genetic enzymatic defect, these diseases are called storage diseases.
3. An abnormal exogenous substance deposit because the cell has neither the enzymatic machinery nor the ability to transport it to other sites.

Fatty Change (Steatosis):

Is an abnormal accumulation of triglycerides within parenchymal cells. Fatty change is most often seen in the liver, and is reversible, but it may also occur in the heart, skeletal muscle, kidney and other organs. It may be caused by toxins, diabetes mellitus, protein malnutrition, obesity and anoxia. Excess acuumulation of triglycerides may result from defects at any step from fatty acid entry to synthesis of lipoproteins. Hepatotoxins (e.g. alcohol) alter mitochondrial and SER function, CCl4 and protein malnutrition decrease synthesis of apoproteins, anoxia inhibits fatty acid oxidation and starvation increases fatty acid mobilization from peripheral stores.

When fatty change is mild, it may have no effect on cellular function, more severe changes may transiently impair cellular function. Grossly the liver enlarges and become progressively yellow. It is first seen by light microscope as small vacuoles in the cytoplasm around the nucleus, these vacuoles coalesce to create clear spaces displacing the nucleus to the periphery.

Cholesterol And Cholesterol Esters:

Macrophages in contact with lipid debris of necrotic cells may become stuffed with lipid because their phagocytic activities, appearing as foamy cells. In atherosclerosis, the smooth muscle cells and macrophages are filled with lipid vacuoles composed of cholesterol and cholesterol esters. Xanthomas are accumulation of fat within macrophages of subcutaneous connective tissues, appearing as white nodules.

Proteins:

Are less commonly seen, e.g. in glomerular diseases with proteinuria, accumulating in proximal convoluted tubules.

Glycogen:

Seen in cases of abnormal metabolism of glucose or glycogen, and appear under the light microscope as vacuoles.

Pigments:

Are colored substances either exogenous or endogenous. Endogenous pigments as: Melanin accumulate in basal cells of the epidermis resulting in freckles

or in dermal macrophages ,Hæmosiderin is a hæmoglobin-derived granular pigment, golden brown, accumulates in tissues when there is local or systemic excess iron. Bilirubin is derived from hemoglobin but contains no iron. Its normal formation and excretion are vital to health, and jaundice is a common clinical disorder caused by excesses of this pigment within cells and tissues . *Lipofuscin* is an insoluble pigment, also known as lipochrome and wear-and-tear or aging pigment. Lipofuscin is not injurious to the cell or its functions. Its importance lies in its being the telltale sign of free radical injury and lipid peroxidation , is particularly prominent in the liver and heart of aging patients or patients with severe malnutrition and cancer cachexia.

Pathologic Cacification:

It is an abnormal accumulation of calcium salts, with smaller amounts of iron, magnesium and other minerals. When deposition occurs in dead or dying tissues it is called *dystrophic calcification*, despite normal serum levels of calcium and in the absence of calcium metabolic derangement. It is encountered in areas of necrosis anywhere, seen in atheromas of advanced atherosclerosis on areas of intimal injuries of large arteries. It is also seen in aging and in aortic valves. It appears as intracellular or extracellular basophilic deposits, sometimes heterotopic bone may be formed.

Metastatic calcification: may occur in normal tissues whenever there is hypercalcæmia. Causes of hypercalcæmia include:

1. Primary endocrine dysfunction (e.g. hyperparathyroidism).
2. Tumours associated with increased bone catabolism (e.g. multiple myeloma, metastatic cancer and leukæmia).
3. Ingested exogenous substances resulting in vitamin D intoxication or milk alkali syndrome.
4. Sarcoidosis.
5. Advanced renal failure where the resulting phosphate retention leads to secondary hyperparathyroidism.

Metastatic calcification may occur widely in tissues principally in the interstitial tissues, kidneys, lungs and gastric mucosa. They usually do not cause significant impairment of organ function, but in extensive nephrocalcinosis, some impairment may occur.

Cellular Adaptations of Growth And Differentiation:

Physiologic adaptations usually represent responses of cells to normal stimulations by hormones or endogenous chemicals (induction of breast growth and lactation). Pathologic adaptations often share the same underlying mechanisms, but they allow the cells to modulate their environment and hopefully escape injury.

Atrophy:

Is shrinkage in the size of the cell by loss of cell substance, it may involve the entire organ. Atrophic cells have diminished function but are not dead. Apoptotic death may also be induced by the same signals that cause atrophy. Causes:

1. Decreased work load.
2. Loss of innervation.
3. Diminished blood supply.
4. Inadequate nutrition.

5. Loss of endocrine stimulation.
6. Aging.

Cells become smaller in size at which survival is possible with a new equilibrium between cell size and diminished blood supply, nutrition or trophic stimulation. Biochemically, there is decreased synthesis, increased catabolism or both.

Hypertrophy:

Is an increase in the size of cells by increased synthesis of the structural proteins and organelles with an increase in the size of the organ. It can be physiologic or pathologic, caused by increased functional demand or specific hormonal stimulation (e.g. hypertrophy of smooth muscles of the uterus during pregnancy).pathological hypertrophy as cardiomegaly secondary to hypertension .

Hyperplasia:

Is an increase in the number of cells in an organ or tissue. Hypertrophy and hyperplasia are closely related and often develop concurrently in tissues. Hyperplasia can be physiologic or pathologic. Physiologic hyperplasia is divided into: 1. Hormonal hyperplasia. 2. Compensatory hyperplasia, occurs when a portion of tissue is removed or diseased.

Most cases of pathologic hyperplasia are due to excessive hormonal or growth factor stimulation.

Metaplasia:

Is a reversible change in which one adult cell type is replaced by another adult cell type, another cellular adaptation where cells sensitive to a particular stress are replaced by other cell types able to withstand the adverse environment.