CHAPTER TWO
CELLULAR ADAPTATIONS & INJURY

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Objectives:
- Understand the concepts of cellular growth, adaptations—Hyperplasia, Hypertrophy, Atrophy, Metaplasia
- List the factors of cell injury and death—O2, Physical, Chemical, Infection, Immunologic, Genetic, Nutritional
- Describe the pathologic mechanisms at the SUB-cellular level—ATP, Mitochondria, Ca++, Free Radicals, Membranes
- Compare and differentiate the concepts of APOPTOSIS and NECROSIS
- Identify common INTRA-cellular accumulations—Fat, Hyaline, CA++, Proteins, Glycogen, Pigments
- Understand aging and differentiate the concepts of preprogrammed death versus wear and tear.

CELLULAR RESPONSES TO HARMFUL STIMULI
Each cell in the body is designed to carry a specific function or functions, which is dependent on its machinery and metabolic pathways. These specificities are genetically determined.

Cells are continuously adjusting their structure and function, within a narrow range, to deal with the continually changing extra-cellular environment. This ability on the part of the cell to maintain a dynamically stable state is referred to as homeostasis.

Should the cells encounter more severe external changes (physiological or pathological); they can modify the homeostatic state and achieve a new steady state to counteract the noxious effects of these external stresses. These changes are referred to as adaptations. The aim behind these adaptations is to avoid cell injury & death.

The morphological & functional changes induced by the injury may be reversible, i.e. the cells return to a normal state on the removal of the offending agent, or irreversible i.e. there is no possibility of making a u-turn to normal. Irreversible changes ultimately eventuate in cell death.

The above mentioned possibilities can be exemplified by the myocardium that is subjected to persistently increased pressure load (hypertension); this adapts by undergoing hypertrophy i.e. an increase in the size of individual cells and ultimately the entire heart. This generates the required higher contractile force to counteract the effect of hypertension (Fig. 2-1). If the hypertension (an injurious external stress) is not relieved, the muscle cells may undergo injury. The injury may be reversible, if the hypertension is mild; otherwise irreversible injury (cell death) occurs.

CELLULAR ADAPTATIONS
Adaptations are reversible changes and are divided into physiologic & pathologic adaptations. Physiologic adaptations usually represent responses of cells to normal stimulations e.g., the hormone-induced enlargement of the breast and uterus during pregnancy. Pathologic adaptations, on the other hand, can take several distinct forms (see below).
Examples of adaptations include

**Hypertrophy:** this refers to an increase in the size of cells that results in enlargement of their relevant organ. Hypertrophy can be physiologic or pathologic and is caused either by increased functional demand or by specific hormonal stimulation. Examples of physiologic hypertrophy include that of skeletal muscles in athletes and mechanical workers & the massive physiologic enlargement of the uterus during pregnancy due to estrogen-stimulated smooth muscle hypertrophy (and hyperplasia) (Fig. 2-2). Pathologic hypertrophy is exemplified by cardiomegaly secondary to hypertension (Fig. 2-1). The stimuli of hypertrophy turn on signals that lead to the induction of a number of genes, which in turn stimulate synthesis of numerous contractile myofilaments per cell. This leads to improved performance to house the excessive demand imposed by the external burden. There is, however, a limit for the adaptation beyond which injury occurs; as for e.g. in the heart, where several degenerative changes occur in the myofilaments that culminate in their loss. This limitation of cardiac hypertrophy (an adaptation) may be related to the amount of available blood to the enlarged fibers. The net result of these regressive changes is ventricular dilation and ultimately cardiac failure. This means that an adaptation can progress to dysfunction if the stress is not relieved.

**Hyperplasia** refers to an increase in the number of cells. It takes place only if the cells are capable of replication; it may occur with hypertrophy and often in response to the same stimuli. Hyperplasia can be physiologic or pathologic.

**Physiologic hyperplasia:** this is of two types
1. Hormonal hyperplasia, exemplified by the proliferation of the glandular epithelium of the female breast at puberty and during pregnancy.
2. Compensatory hyperplasia, which occurs when a portion of the tissue is removed or diseased. For example, when a liver is partially resected, mitotic activity in the remaining cells begins that eventually restore the liver to its normal weight. The stimuli for hyperplasia in this setting are growth factors produced by remaining hepatocytes & other cells within the organ. After restoration of the liver mass, cell proliferation is "turned off" by various growth inhibitors.

**Pathologic hyperplasia:** is mostly caused by excessive hormonal or growth factor stimulation. Examples include
1. Endometrial hyperplasia: this results from persistent or excessive estrogen stimulation of the endometrium. This hyperplasia is a common cause of abnormal uterine bleeding. (Fig. 2-3)
2. Skin warts: these are caused by Papillomaviruses, and are composed of masses of hyperplastic epithelium. (Fig. 2-4) The growth factors responsible may be produced by the virus or by the infected cells.

In all the above situations, the hyperplastic process remains controlled; if hormonal or growth factors stimulation subsides, the hyperplasia disappears. It is this response to normal regulatory control mechanisms that distinguishes pathologic hyperplasias from cancer, in which the growth control mechanisms become ineffective. However, some types of pathologic hyperplasias may become a fertile soil for the development of carcinoma.

**Atrophy:** this refers to shrinkage in the size of the cell due to loss of its constituent substances. This situation is exactly opposite to hypertrophy. When a sufficient number of cells are involved, the entire tissue or organ diminishes in size i.e. becomes atrophic (Fig. 2-5).
Causes of atrophy include
1. A decreased workload, which is the most common form of atrophy; it follows reduced functional demand. For example, after immobilization of a limb in a cast as treatment for a bone fracture or after prolonged bed rest. In these situations the limb's muscle cells atrophy and muscular strength is reduced. When normal activity resumes, the muscle's size and function return.
2. Denervation of a limb as in poliomyelitis and traumatic spinal cord injury
3. Diminished blood supply e.g. decreased blood supply to a limb or brain due to narrowing of the lumina of the relevant artery (or arteries) by atherosclerosis.
4. Inadequate nutrition as in starvation and famines
5. Loss of endocrine stimulation as in postmenopausal endometrial atrophy (due to decrease in the levels of estrogen after menopause) and testicular atrophy (due to decrease in the production of LH & FSH as in hypopituitarism (Fig. 2-6)
6. Aging (senile atrophy).

Although some of these stimuli are physiologic (e.g., the loss of hormone stimulation in menopause) and others pathologic (e.g., denervation), the fundamental cellular changes in atrophy are identical. They represent a retreat by the cell to a smaller size at which survival is still possible; a new equilibrium is achieved between cell size and diminished blood supply, nutrition, or trophic stimulation. Atrophy results from decreased protein synthesis together with increased protein degradation in the affected cells. In many situations, atrophy is also accompanied by increased autophagy ("self-eating"), with resulting increases in the number of autophagic vacuoles. The starved cell eats its own components in an attempt to find nutrients and survive.

Metaplasia refers to a reversible change in which there is “replacement of normal mature epithelium at a given site by another mature benign epithelium inappropriate to that site.” In this type of cellular adaptation, cells sensitive to a particular stress are replaced by other cell types that are more capable of resisting the adverse environment. Metaplasia is thought to arise by genetic "reprogramming" of stem cells. Epithelial metaplasia is exemplified by the squamous change that occurs in the respiratory epithelium in habitual cigarette smokers. The normal ciliated columnar epithelial cells of the trachea and bronchi are focally or extensively replaced by stratified squamous epithelial cells. Although the metaplastic squamous epithelium is more resistant to the injurious environment, it has its adverse effects that include
1. Loss of protective mechanisms, such as mucus secretion and ciliary clearance of particles.
2. Predisposition to malignancy. In fact, squamous cell carcinoma of the bronchi often coexists with squamous metaplasia. Squamous metaplasia is also seen in the urinary bladder harboring Shistosomal ova. When there persistent regurgitation of the gastric contents in to the esophagus (chronic gastro-esophageal reflux disease [GERD]), the normal stratified squamous epithelium of the lower esophagus may be replaced by a metaplastic intestinal-type columnar epithelium. The latter is more resistant to the highly acidic regurgitating gastric contents (Fig. 2-7).
CELL INJURY AND CELL DEATH

Cell injury results when cells are exposed to
1. Persistent stress so that the affected cells are no longer able to adapt or
2. Inherently damaging agents. (Fig. 2-8)

Reversible cell injury occurs when the injurious agent is mild but persistent or severe but short lived. In this type of injury the functional and morphologic changes are reversible. With continuing damage, there is irreversible injury, at which time the cell cannot recover even with the removable of the injurious agent i.e. it dies.

Causes of Cell Injury include
1. Oxygen deprivation (Hypoxia) insufficient supply of oxygen interferes with aerobic oxidative respiration and is a common cause of cell injury and death.
   - Causes of hypoxia include
     a. Ischemia i.e. loss of blood supply in a tissue due to interference with arterial flow or reduced venous drainage. This is the most common cause of hypoxia
     b. Inadequate oxygenation of the blood, as in pneumonia or chronic bronchitis
     c. Reduction in the oxygen-carrying capacity of the blood, as in anemia or carbon monoxide (CO) poisoning.

2. Chemical agents: various poisons cause damage by affecting either membrane permeability, or the integrity of the cellular enzymes. Environmental toxins as pollutants, insecticides, CO, alcohol and drugs can cause cell injury.

3. Infectious agents including viruses, bacteria, rickettsiae, fungi and parasites.

4. Immunologic reactions; these are primarily defensive in nature but they can also result in cell and tissue injury. Examples include autoimmune diseases & allergic reactions.

5. Genetic defects including gross congenital malformations (as in Down syndrome) or point mutations (as in sickle cell anemia). Genetic defects may cause cell injury because of deficiency of enzymes in inborn errors of metabolism.

6. Nutritional imbalances: nutritional deficiencies are still a major cause of cell injury. Protein-calorie & vitamins insufficiencies are obvious example. Excesses of nutrition are also important causes of morbidity and mortality; for example, obesity markedly increases the risk for type 2 diabetes mellitus. Moreover, diets rich in animal fat are strongly implicated in the development of atherosclerosis as well as in increased vulnerability to cancer e.g. that of the colon.

7. Physical agents: trauma, extremes of temperatures, radiation, electric shock, and sudden changes in atmospheric pressure all are associated with cell injury.

8. Aging: this leads to impairment of replicative and repair abilities of individual cells that result in a diminished ability to respond to damage and, eventually, the death of cells and of the individual.
MECHANISMS OF CELL INJURY
The outcomes of the interaction between injurious agents & cells depend on
1. The injurious agent: its type, severity, and the duration of its application to the cells.
2. The cells exposed to the injury: its type, adaptability, and their genetic makeup.

The above are exemplified by the following facts
Low doses of toxins or a brief duration of ischemia may lead to reversible cell injury, whereas larger toxin doses or longer ischemic intervals may result in irreversible injury and cell death.

The same injury has different outcomes depending on the cell type; thus, striated skeletal muscles in the leg resist complete ischemia for 2 to 3 hours without irreversible injury (as in applying a tourniquet to stop severe uncontrollable bleeding from a limb trauma), whereas cardiac muscles die after only 20 to 30 minutes of severe acute ischemia. Individuals who inherit variants of the same gene that encodes an enzyme that degrades a particular toxin show differences in the speed (rate) of toxins degradation. This explains the different outcomes that may occur when different individuals are exposed to the same dose of a given toxin.

The most important targets of injurious agents are
1. Mitochondria (the sites of ATP generation)
2. Cell membranes, which influence the ionic and osmotic homeostasis of the cell
3. Protein synthesis (ribosomes)
4. The cytoskeleton (microtubules, and various filaments)
5. The genetic apparatus of the cell (nuclear DNA)

ATP Depletion
ATP, the energy fuel of cells, is produced mainly by oxidative phosphorylation of ADP within the mitochondria. In addition, the glycolytic pathway can generate ATP in the absence of oxygen using glucose derived either from the circulation or from the hydrolysis of intracellular glycogen (aerobic glycolysis).

The major causes of ATP depletion are
1. Reduced supply of oxygen and nutrients
2. Mitochondrial damage
3. The actions of some toxins (e.g., cyanide)

High-energy phosphate in the form of ATP is required for virtually all synthetic and degradative processes within the cell, including membrane transport, protein synthesis, phospholipid turnover, etc. Depletion of ATP to less than 5% to 10% of normal levels has widespread effects on many critical cellular systems.

a. The activity of the plasma membrane energy-dependent sodium pump is reduced, resulting in intracellular accumulation of sodium and efflux of potassium. The net gain of solute is accompanied by iso-osmotic gain of water, causing cell swelling.
b. There is a compensatory increase in anaerobic glycolysis in an attempt to maintain the cell's energy sources. As a consequence, intracellular glycogen stores are rapidly depleted, and lactic acid accumulates, leading to decreased intracellular pH and decreased activity of many cellular enzymes.
c. Failure of the Ca\(^{2+}\) pump leads to influx of Ca\(^{2+}\), with damaging effects on numerous cellular components (described below).
d. Structural disruption of the protein synthetic apparatus manifested as detachment of ribosomes from the rough endoplasmic reticulum (RER), with a consequent reduction in protein synthesis.
e. Ultimately, there is irreversible damage to mitochondrial and lysosomal membranes, and the cell undergoes necrosis.

Mitochondrial Damage
Mitochondria can be damaged by increases of cytosolic Ca$^{2+}$, reactive oxygen species, and oxygen deprivation, and so they are sensitive to virtually all types of injurious stimuli, including hypoxia and toxins. There are two major consequences of mitochondrial damage:

Failure of oxidative phosphorylation with progressive depletion of ATP, culminating in necrosis of the cell.

Leakage of cytochrome c that is capable of activating apoptotic pathways.

Influx of Calcium
Cytoplasmic free calcium is normally maintained by ATP-dependent calcium pump (transporter) at concentrations that are 10,000 times lower than the concentration of extra-cellular calcium or intracellular mitochondrial and ER calcium. Ischemia and certain toxins cause an increase in cytoplasmic calcium concentration, initially because of release of Ca$^{2+}$ from the intracellular stores, and later resulting from increased influx across the plasma membrane.

Increased cytosolic Ca$^{2+}$ leads to
1. Activates a number of enzymes including phospholipases (which cause membrane damage), proteases (which break down both membrane and cytoskeletal proteins), endonucleases (which are responsible for DNA and chromatin fragmentation), and ATPases (worsen ATP depletion).
2. Induction of apoptosis, by direct activation of certain enzymes called caspases.

Accumulation of Oxygen-Derived Free Radicals (Oxidative Stress)
These are designated as reactive oxygen species (ROS) & are units with a single unpaired electron in their outer orbit. When generated in cells they enthusiastically attack nucleic acids, cellular proteins and lipids. ROS are produced normally in cells during mitochondrial respiration and energy generation, but they are degraded and removed by cellular defense systems. When their production increases or the defense systems are ineffective, the result is an excess of these free radicals, leading to a condition called oxidative stress. Cell injury in many circumstances involves damage by free radicals; these include:

Reperfusion injury
Chemical and radiation injury
Toxicity from oxygen and other gases
Cellular aging
Inflammatory cells mediated tissue injury
DEFECTS IN MEMBRANE PERMEABILITY
Early loss of selective membrane permeability leading ultimately to overt membrane damage is a consistent feature of most forms of cell injury (except apoptosis). The plasma membrane can be damaged by ischemia, microbial toxins, complement components-mediated lysis, etc.

**Biochemical mechanisms contribute to membrane damage include:**
1. **Decreased phospholipid synthesis due to a fall in ATP levels.** This affects all cellular membranes including mitochondrial, which worsen the loss of ATP.
2. **Degradation of membrane phospholipids due to activation of intracellular phospholipases through increased levels of intracellular Ca\(^{2+}\).**
3. **Injury to cell membranes by Oxygen free radicals (ROS)**
4. **Damage to the cytoskeleton through activation of proteases by increased cytoplasmic Ca\(^{2+}\)**
5. **They detergent effect of free fatty acids on membranes.** These products result from phospholipid degradation.

The most important sites of membrane damage during cell injury are the mitochondrial membrane, the plasma membrane membranes of lysosomes.

**Damage to DNA & proteins**
Cells have mechanisms that repair damage to DNA, but if this damage is too severe to be corrected (e.g., after radiation injury or oxidative stress), the cell initiates its suicide program and dies by apoptosis. A similar reaction is triggered by improperly folded (configured) proteins (see unfolded protein response), which may be the result of inherited mutations or through free radicals. These mechanisms of cell injury typically cause apoptosis.

**Morphologic features of cell and tissue injury**
All harmful influences exert their effects first at the molecular (subcellular) or biochemical level. Function may be lost long before morphologic changes of cell injury become obvious (Fig. 2-9). For example, myocardial cells fail to contract after 1 to 2 minutes of ischemia, although they do not die until after 20 to 30 minutes of ischemia. These myocytes do not appear morphologically dead by electron microscopy until after 3 hours and by light microscopy after 6 to 12 hours. The cellular changes associated with reversible injury can be repaired once the injurious agent is removed. Changes associated with irreversible injury (as with persistent or excessively severe injury) are irreversible even with the removal of the injurious agent, i.e. their occurrence signals the point of no retrun, and the cell inevitably dies.

There are two changes that characterize irreversible injury (cell death)
1. Mitochondrial dysfunction manifested as lack of oxidative phosphorylation leading to ATP depletion
2. Membrane dysfunction including not only the outer cell membrane but also the membranes that surround intracytoplasmic lysosomes. This results in liberation of the harmful lysosomal enzymes into the cytoplasm, which in turn leads to dissolution of vital cellular structures.
Morphologic examples of reversible injury
1. Cellular swelling (hydropic change or vacuolar degeneration): this is due to paralysis of energy-dependent ion pumps of the plasma membrane. This leads to influx of sodium (with water) into the cell and departure of potassium out. It is the first manifestation of almost all forms of cell injury. Microscopically, there are clear vacuoles (of water) within the cytoplasm. (Fig. 2-10)
2. Fatty change: this is manifested by the appearance of lipid vacuoles in the cytoplasm. It is principally encountered in cells participating in fat metabolism such as hepatocytes; as in alcoholic liver disease, malnutrition & total parenteral nutrition.

Irreversible cell injury
There are two morphological types of cell death
1. Apoptosis
2. Necrosis

Apoptosis is an active, energy-dependent, regulated type of cell death. It serves many normal functions and is not necessarily pathological is the mode of cell death when
a. The cell is deprived of its growth factors or
b. The cell's DNA or proteins are damaged beyond repair

Necrosis is a reflection of the morphological changes that accompany cell death due to the digestion of cellular contents by the liberated degradative lysosomal enzymes. It is associated with an inflammatory repose (unlike apoptosis), due to leakage of the cellular contents through the damaged plasma membrane. The lysosomes of the inflammatory cells also contribute to the digestion of the dying cells. Necrosis is the major pathway of cell death in many commonly encountered injuries, such as those resulting from ischemia, exposure to toxins, & various infections.

Morphologic features of necrosis: these consists of cytoplasmic & nuclear changes
Cytoplasmic changes: the necrotic cells show increased cytoplasmic eosinophilia i.e. it appears deep pink in color than normal cells. This is attributable in part to increased binding of eosin to denatured cytoplasmic proteins and in part to loss of the basophilia that is normally imparted by the RNA in the cytoplasm (basophilia is the blue staining from the hematoxylin dye). The cell may have a more homogeneous appearance than viable cells, mostly because of the loss of glycogen particles. The latter is responsible in normal cells for the granularity of the cytoplasm.

Nuclear changes (Fig. 2-11): these assume one of three patterns, all due to breakdown of DNA and chromatin
a. Karyolysis i.e. the basophilia of the chromatin become pale secondary to deoxyribonuclease (DNase) activity.
b. Pyknosis characterized by nuclear shrinkage and increased basophilia; the DNA condenses into a solid shrunken mass.
c. Karyorrhexis, the pyknotic nucleus undergoes fragmentation.
Patterns of Tissue Necrosis
There are several morphologically patterns of tissue necrosis, which may provide clues about the underlying cause.

1. **Coagulative necrosis** is characterized grossly by firmness of the affected tissue due to denaturation of structural proteins and microscopically by loss of the cellular fine structural details but preservation of the basic tissue architecture. (Fig. 2-12) The necrotic cells show homogeneously eosinophilic cytoplasm and are devoid of nuclei. Ultimately the necrotic cells are removed through the degradative enzymes released from both the dead cells themselves as well as from the already present inflammatory cells. The latter also contribute by phagocytosing the cellular debris. Coagulative necrosis is characteristic of ischemic damage in all solid organs except the brain.

2. **Liquefactive necrosis** is characterized by complete digestion of the dead cells, resulting in transformation of the affected tissue into thick liquid mass (hence the name liquefactive). Eventually, the liquefied necrotic tissue is enclosed within a cystic cavity. This type of necrosis is seen in two situations
   a. Focal pyogenic bacterial infections. These bacteria stimulate the accumulation of inflammatory cells & the enzymes of leukocytes digest ("liquefy") the tissue. This process is associated acute suppurative inflammation (abscess); the liquefied material is frequently creamy yellow and is called pus. (Fig. 12-13)
   b. Ischemic destruction of the brain tissue: for unclear reasons, hypoxic death of cells within the central nervous system often induces liquefactive necrosis (Fig. 2-14).

3. **Gangrenous necrosis** (gangrene) is not a distinctive pattern of cell death; however, the term is still commonly used in clinical practice. It is usually applied to a limb, usually a leg that has lost its blood supply and has undergone coagulative necrosis involving multiple tissue layers (dry gangrene). When bacterial infection is superimposed, coagulative necrosis is modified by the liquefactive action of the bacteria and the attracted leukocytes (wet gangrene). (Fig. 2-15) Intestinal gangrene (the consequences of mesenteric vascular occlusion) and gangrenous appendix are also commonly used terms; they signify ischemic necrosis of these structures with superimposed bacterial infection.

4. **Caseous necrosis**, unlike coagulative necrosis, the tissue architecture is completely lost and cellular outlines cannot be detected. It is encountered most often in foci of tuberculous infection. The term "caseous" (cheese-like) is derived from the friable yellow-white appearance of the area of necrosis (Fig. 2-16). Microscopically, the necrotic focus appears as pinkish, and granular in appearance. Caseous necrosis is often bordered by a granulomatous inflammation.

5. **Fat necrosis** is typically seen in acute pancreatitis and results from release of activated pancreatic lipases into the pancreas and the peritoneal cavity. Pancreatic enzymes that have leaked out of acinar cells liquefy the membranes of fat cells in and outside the pancreas. Lipases split the liberated triglycerides into fatty acids that combine with calcium to produce grossly visible chalky white areas (Fig. 2-17). Microscopically, the foci of necrosis contain vague outlines of necrotic fat cells with bluish calcium deposits. The necrotic foci are surrounded by an inflammatory reaction. Another example of fat necrosis is seen in female breasts; at least some of these cases are preceded by a history of trauma (traumatic fat necrosis).
6. **Fibrinoid necrosis** is typically seen in immune reactions involving blood vessels. Deposits of immune complexes, together with fibrin that has leaked out of vessels result in a homogeneous bright pink appearance. This type is exemplified by the necrosis seen in polyarteritis nodosa (Fig. 2-18).

The leakage of intracellular proteins through the damaged cell membrane and ultimately into the blood provides means of detecting necrosis of specific tissues using blood or serum samples. The measurement of the levels of these specific enzymes in the serum is used clinically to assess damage to these tissues. Cardiac muscles contain a unique enzyme creatine kinase (CK) and the contractile protein troponin. The serum levels of both are elevated after acute myocardial infarction. Hepatocytes contain transaminases & these are elevated in the serum following an episode of hepatitis (viral or otherwise).

**SUBCELLULAR RESPONSES TO INJURY**

Some of these alterations occur in acute lethal injury, others in chronic cell injury, and still others are adaptive responses.

**Autophagy** refers to lysosomal digestion of the cell's own components. It is a survival mechanism whenever there is nutrient deprivation; the starved cell lives by eating its own contents. In this process, organelles are sequestered from the cytoplasm in an autophagic vacuole. The vacuole fuses with lysosomes to form phagolysosome, & the cellular components are digested by lysosomal enzymes. Lysosomes with undigested debris may persist within cells. Lipofuscin pigment is indigestible material; it is seen as brownish-yellow granules within parenchymal cells e.g. of the liver & heart in old age & in atrophy of these organs. Carbon particles inhaled from the atmosphere and inoculated pigment in tattoos can persist in phagolysosomes of macrophages for decades. (Fig. 2-19) In hereditary lysosomal storage diseases there are deficiencies of enzymes that degrade certain macromolecules; the result is an abnormal collection of these in the lysosomes of cells all over the body.

**Induction (hypertrophy) of smooth endoplasmic reticulum (SER):** the SER is involved in the metabolism of various chemicals including some drugs, and cells exposed to these chemicals show hypertrophy of the SER as an adaptive response that may have important functional consequences for e.g. induction of hepatic drug-metabolizing activity.

**Mitochondrial Alterations:** mitochondria may show
1. An increase in their number in cellular hypertrophy.
2. A decrease in number during cellular atrophy (probably via autophagy).
3. Extremely large and abnormal shapes (megamitochondria), e.g. in hepatocytes in association with nutritional deficiencies and alcoholic liver disease.

**Cytoskeletal Abnormalities:**

Cytoskeleton is the cellular scaffold, which is represented by myosin, intermediate filaments and microtubules

Examples of cytoskeletal abnormalities are seen in

1. Alcoholic liver disease: Mallory bodies are eosinophilic, collections of intermediate filaments that accumulate within the cytoplasm of hepatocytes.
2. Kartagener (immotile cilia) syndrome: due to disorganization of microtubules, which is associated with sterility due to inhibition of sperm motility? There are also chronic infections of the lung due to defective motility of cilia of the respiratory epithelium, and thus accumulation of the secreted mucus & impaired clearance of inhaled bacteria.
EXAMPLES OF CELL INJURY AND NECROSIS
Ischemic and Hypoxic Injury
Ischemia is the most common cause of cell injury in clinical medicine. Unlike hypoxia, in which energy generation by anaerobic glycolysis can continue, in ischemia the delivery of the substrates for glycolysis is also interfered with. Consequently, anaerobic energy generation ceases in ischemic tissues. Therefore, ischemia injures tissues faster than does hypoxia.

The fundamental biochemical abnormality in hypoxic cells that leads to cell injury is reduced intracellular generation of ATP, as a consequence of reduced supply of oxygen. Loss of ATP leads to the failure of many energy-dependent systems of the affected cell; these include:

1. Paralysis of ion pumps (leading to cell swelling, and influx of Ca^{2+})
2. Depletion of glycogen stores with accumulation of lactic acid (anaerobic glycolysis)
3. Reduction in protein synthesis

The functional consequences may be severe. For instance, heart muscle ceases to contract within 60 seconds of coronary occlusion. However, loss of contractility does not mean cell death. If hypoxia continues, worsening ATP depletion causes further deterioration, for e.g., in renal tubular epithelium, there is loss of microvilli and the formation of "blebs". (Fig. 2-20) At this time, the entire cell and its organelles (mitochondria, ER) are markedly swollen, with increased concentrations of water, sodium, and chloride and a decreased concentration of potassium. If oxygen is restored, all of these disturbances are reversible.

If ischemia persists, irreversible injury and necrosis ensue. Irreversible injury is associated with severe swelling of mitochondria, extensive damage to plasma membranes, and swelling of lysosomes. Massive influx of calcium into the cell may occur. The cell's components are progressively degraded, and there is widespread leakage of cellular enzymes into the extracellular space.

Reperfusion Injury
If cells are reversibly injured, the restoration of blood flow can result in cell recovery. However, under certain circumstances, the restoration of blood flow to reversibly ischemic tissues results in worsening the injury. This situation may contribute to tissue damage in myocardial and cerebral infarctions.

The additional damage may be initiated during the blood re-flow by:

1. Increased generation of reactive oxygen species from native parenchymal and endothelial cells, as well as the infiltrating inflammatory cells.
2. The cellular antioxidant defense mechanisms are already interfered with by ischemia.
3. Ischemic injury is associated with inflammation, which may increase with reperfusion. The products of activated leukocytes and activation of the complement system may cause additional tissue injury.
Chemical Injury

Chemicals induce cell injury by one of two general mechanisms
1. Direct cytotoxic effect through combination with a vital molecular component or cellular organelle. In HgCl$_2$ poisoning, mercury binds to various cell membrane proteins, causing inhibition of transport and increased membrane permeability. Many antineoplastic chemotherapeutic agents also induce cell damage by direct cytotoxic effects.
2. Formation of free radicals: many chemicals must be first converted to reactive toxic metabolites, which then act on target cells. Although the metabolites might cause membrane damage and cell injury by direct binding to protein and lipids, the most important mechanism of cell injury involves the formation of free radicals. Carbon tetrachloride (CCl$_4$, which was used widely in the dry cleaning industry but is now banned) and the analgesic paracetamol belong to this category.

APOPTOSIS

This form of cell death is a regulated suicide program in which the relevant cells activate enzymes capable of degrading the cells' own nuclear DNA and other nuclear and cytoplasmic proteins.
The plasma membrane of the apoptotic cell remains intact, but is altered in such a way that the cell becomes avid targets for phagocytes. The dead cell is rapidly cleared before its contents have leaked out, and therefore cell death by this pathway does not elicit an inflammatory reaction in the host.
Thus, apoptosis differs from necrosis; the latter is characterized by loss of membrane integrity, leakage of cellular contents, and frequently a host reaction.

Causes of Apoptosis

Apoptosis occurs in physiologic situations; it serves to eliminate potentially harmful cells and cells that are no longer useful to the wellbeing of the body.
It is also a pathologic event when cells are damaged beyond repair, especially when the damage affects the cell's DNA or proteins; in these situations, the irreparably damaged cell is eliminated.

Apoptosis in Physiologic Situations

Death by apoptosis is a normal phenomenon that serves to eliminate cells that are no longer needed. It is important in the following physiologic situations:
1. During embryogenesis (organogenesis and involution).
2. Involution of hormone-dependent tissues (hormone deprivation, as endometrial cell breakdown during the menstrual cycle, and regression of the lactating breast after weaning)
3. In proliferating cells, such as intestinal crypt epithelia (to maintain a constant number).
4. In cells that have served their useful purpose (as neutrophils in an acute inflammation).

Apoptosis in Pathologic Conditions

Apoptosis eliminates cells that are genetically altered or injured beyond repair without eliciting a host reaction, thus keeping the damage as restricted as possible.
Microscopic features (Fig. 2-21)
Apoptotic cells may appear as round or oval masses with intensely eosinophilic cytoplasm. Nuclei show chromatin condensation and, ultimately fragmentation (karyorrhexis). The cells rapidly shrink, and fragment into apoptotic bodies that are composed of membrane-bound vesicles of cytoplasm and organelles. These fragments are quickly extruded and phagocytosed without eliciting an inflammatory response.

The fundamental event in apoptosis is the activation of enzymes caspases that culminate in activation of nucleases with DNA degradation.

Examples of Apoptosis
1. Growth factor deprivation: hormone-sensitive cells deprived of the relevant hormone, lymphocytes that are not stimulated by antigens and cytokines, and neurons deprived of nerve growth factor die by apoptosis. These are attributable to activation of pro-apoptotic members of the Bcl-2 family.

2. DNA Damage: exposure of cells to radiation or cytotoxic anticancer chemotherapeutic agents & extremes of temperature induces DNA damage, and if this is too severe to be repaired it triggers apoptotic death. When DNA is damaged, the p53 protein accumulates in cells. It first arrests the cell cycle (at the G1 phase) to allow time for repair. However, if the damage is too great to be repaired successfully, p53 triggers apoptosis by stimulating synthesis of pro-apoptotic members of the Bcl-2 family. When p53 is mutated or absent (as it is in certain cancers), it is incapable of inducing apoptosis, so that cells with damaged DNA are allowed to survive. This enhances the possibility of mutations or translocations that lead to neoplastic transformation and subsequently providing the tumor cells with a growth advantage.

3. Accumulation of Misfolded Proteins
During normal protein synthesis, chaperones (escorters) in the ER control the proper folding of newly synthesized proteins, and misfolded polypeptides are targeted for proteolysis. If, however, unfolded or misfolded proteins accumulate in the ER because of inherited mutations or stresses, they induce "ER stress" that triggers a number of cellular responses, collectively called the unfolded protein response. This response activates signaling pathways that increase the production of chaperones and retard protein translation, thus reducing the levels of misfolded proteins in the cell. However, if this response is unable to cope with the accumulation of misfolded proteins, the result is the activation of caspases that lead to apoptosis. Intracellular accumulation of abnormally folded proteins is now recognized as a feature of a number of neurodegenerative diseases, including Alzheimer, Huntington, and Parkinson diseases, and possibly type II diabetes.

4. Apoptosis of Self-Reactive Lymphocytes: lymphocytes capable of recognizing self antigens are normally produced in all individuals. If these lymphocytes encounter self antigens, the cells die by apoptosis. Failure of apoptosis of self-reactive lymphocytes is one of the causes of autoimmune diseases.

5. Cytotoxic T Lymphocyte-Mediated Apoptosis: cytotoxic T lymphocytes (CTLs) recognize foreign antigens presented on the surface of infected host cells and tumor cells. Upon activation, CTL granule proteases called granzymes enter the target cells. Granzymes are able to activate cellular caspases. In this way, the CTL kills target cells by directly inducing the effector phase of apoptosis.
6. Cell injury in certain infections, particularly viral infections, in which loss of infected cells is largely due to apoptotic death that may be induced by the virus (as in HIV infection) or by the host immune response (as in viral hepatitis).
7. Pathologic atrophy in parenchymal organs after duct obstruction as occurs in the pancreas, parotid gland, and kidney.

**INTRACELLULAR ACCUMULATIONS**

Cells may accumulate abnormal amounts of various substances; these may be harmless or associated with injury. The locations of these substances are either cytoplasmic within organelles (typically lysosomes), or in the nucleus.

There are three main pathways of abnormal intracellular accumulations (Fig. 2-22):
1. Inadequate removal of a substance i.e. the metabolic rate of its removal is inadequate. An example of this type of process is fatty change in the liver.
2. Defective transport of a substance: endogenous substance accumulates because of genetic or acquired defects in its folding, packaging, transport, or secretion. Mutations may lead to accumulation of proteins (e.g., α₁-antitrypsin deficiency).
3. Failure to degrade a metabolite either because of:
   a. an inherited defect in an enzyme (as in storage diseases) or
   b. the cell has neither the enzymatic machinery to degrade an abnormal exogenous substance nor the ability to transport it to other sites. Accumulations of carbon or silica particles are examples of this type of alteration.

**Fatty Change (Steatosis)**

This refers to any abnormal accumulation of triglycerides within parenchymal cells. It is most often seen in the liver, since this is the major organ involved in fat metabolism, but it may also occur in the heart.

Causes of fatty change include:
1. Toxins including alcohol
2. Diabetes mellitus
3. Obesity
4. Protein malnutrition
5. Anoxia

Alcohol abuse and diabetes associated with obesity are the most common causes of fatty change in the liver (fatty liver) in industrialized nations.

Free fatty acids from adipose tissue or ingested food are normally transported into hepatocytes, where they are esterified to triglycerides, converted into cholesterol or phospholipids, or oxidized to ketone bodies. Triglycerides from the hepatocytes require the formation of complexes with apoproteins to form lipoproteins, which are able to enter the circulation. Excess accumulation of triglycerides may result from defects at any step from fatty acid entry to lipoprotein exit. Hepatotoxins (e.g., alcohol) alter mitochondrial and SER functions and thus inhibit fatty acid oxidation; CCL₄ and protein malnutrition decrease the synthesis of apoproteins; anoxia inhibits fatty acid oxidation; and starvation increases fatty acid mobilization from peripheral stores.

The significance of fatty change depends on the cause and severity of the accumulation. When mild it may have no effect. More severe fatty change may transiently impair cellular
function, but the change is reversible. In the severe form, fatty change may precede cell death.

**Gross features** (Fig. 2-23)
Fatty change is most commonly seen in the liver and the heart.
In the liver, mild fatty change may not affect the gross appearance.
With increasing accumulation, the organ enlarges and becomes progressively yellow until, in extreme cases, it may weigh 5 kg (3 times the normal weight) and appear bright yellow, soft, and greasy.

**Microscopic features**
Early fatty change is seen by light microscopy as small fat vacuoles in the cytoplasm around the nucleus.
In later stages, the vacuoles coalesce to create cleared spaces that displace the nucleus to the cell periphery.

**Cholesterol and Cholesteryl Esters accumulations**
Cellular cholesterol metabolism is tightly regulated to ensure normal cell membrane synthesis without significant intracellular accumulation. However, phagocytic cells may become overloaded with lipid (triglycerides, cholesterol, and cholesteryl esters) in several different pathologic processes.

1. Macrophages in contact with the lipid debris of necrotic cells or abnormal (e.g., oxidized) forms of lipoproteins may become stuffed with phagocytosed lipid. These macrophages may be filled with minute, membrane-bound vacuoles of lipid, imparting a foamy appearance to their cytoplasm (foam cells). In **atherosclerosis**, smooth muscle cells and macrophages are filled with lipid vacuoles composed of cholesterol and cholesteryl esters; these give atherosclerotic plaques their characteristic yellow color and contribute to the pathogenesis of the lesion. (Fig. 2-24)

2. In hereditary and acquired hyperlipidemic syndromes, macrophages accumulate intracellular cholesterol; when present in the subepithelial connective tissue of skin or in tendons, clusters of these foamy macrophages form masses called **xanthomas**. (Fig. 2-25)

**Protein accumulations**
Morphologically visible protein accumulations may occur because excesses of proteins are presented to the cells or because the cells synthesize proteins in excessive amounts. In renal disorders with heavy protein leakage across the glomerular filter (nephrotic syndrome), there is a much larger reabsorption of the protein. Pinocytic vesicles containing this protein fuse with lysosomes, resulting in the histologic appearance of pink, hyaline cytoplasmic droplets. Another example is the marked accumulation of newly synthesized immunoglobulins that occurs in the rough endoplasmic reticulum of some plasma cells, forming rounded, eosinophilic Russell bodies.
Accumulations of intracellular proteins are also seen in certain types of cell injury. For example, the Mallory body (alcoholic hyaline) is an eosinophilic cytoplasmic inclusion in liver cells that is highly characteristic of alcoholic liver disease. Such inclusions are composed predominantly of aggregated intermediate filaments that resist degradation. The neurofibrillary tangle found in the brain of Alzheimer disease is an aggregated protein that and neurofilaments, a reflection of a disrupted neuronal cytoskeleton.
Glycogen accumulations
Excessive intracellular deposits of glycogen are associated with abnormalities in the metabolism of either glucose or glycogen. In poorly controlled diabetes mellitus, the prime example of abnormal glucose metabolism, glycogen accumulates in renal tubular epithelium, cardiac myocytes, and β cells of the islets of Langerhans. Glycogen also accumulates within cells in a group of closely related genetic disorders collectively referred to as glycogen storage diseases. In these diseases, enzymatic defects in the synthesis or breakdown of glycogen result in massive accumulations, with secondary injury and cell death.

Pigments
Pigments are colored substances that are exogenous, coming from outside the body, or endogenous, synthesized within the body itself.
Exogenous pigments; the most common of these is carbon (an example is coal dust), a ubiquitous air pollutant of urban life. When inhaled, it is phagocytosed by alveolar macrophages and transported through lymphatic channels to the regional tracheobronchial lymph nodes. Aggregates of the pigment blacken the draining lymph nodes and pulmonary parenchyma (anthracosis). Heavy accumulations may induce fibroblastic reaction that can result in a serious lung disease called coal workers’ pneumoconiosis (Fig. 2-28).
Endogenous pigments include lipofuscin, melanin, and certain derivatives of hemoglobin. Lipofuscin, or "wear-and-tear pigment," is an insoluble brownish-yellow granular intracellular material that accumulates in a variety of tissues (particularly the heart, liver, and brain) as a function of age or atrophy (Fig. 2-29). Lipofuscin represents complexes of lipid and protein that derive from peroxidation of polyunsaturated lipids of subcellular membranes. It is not injurious to the cell but is important as a marker of past free-radical injury. The brown pigment, when present in large amounts, imparts an appearance to the tissue that is called brown atrophy.
Melanin is an endogenous, brown-black pigment produced in melanocytes following the tyrosinase-catalyzed oxidation of tyrosine to dihydroxyphenylalanine. It is synthesized exclusively by melanocytes located in the epidermis and acts as a screen against harmful ultraviolet radiation. Although melanocytes are the only source of melanin, adjacent basal keratinocytes in the skin can accumulate the pigment (e.g., in freckles), as can dermal macrophages.

Hemosiderin is a hemoglobin-derived granular pigment that is golden yellow to brown and accumulates in tissues when there is a local or systemic excess of iron. Hemosiderin pigment represents large aggregates of iron, readily visualized by light microscopy; the iron can be identified by the Prussian blue histochemical reaction (Fig. 2-30). Local excesses of iron, and consequently of hemosiderin, result from hemorrhage. The best example is the common bruise. After lysis of the erythrocytes at the site of hemorrhage, the red cell debris is phagocytosed by macrophages; the hemoglobin content is then catabolized by lysosomes with accumulation of the heme iron in hemosiderin. The array of colors through which the bruise passes reflects these transformations. The original red-blue color of hemoglobin is transformed to varying shades of green-blue by the local formation of biliverdin (green bile) and bilirubin (red bile) from the heme moiety; the iron ions of hemoglobin accumulate as golden-yellow hemosiderin. Whenever there is systemic overload of iron, hemosiderin is deposited in many organs and tissues including their macrophages.
This condition is called **hemosiderosis**. With progressive accumulation, parenchymal cells throughout the body (but principally the liver, pancreas, heart, and endocrine organs) become "bronzed" with accumulating pigment. Hemosiderosis occurs in the setting of
1. Increased absorption of dietary iron
2. Impaired utilization of iron
3. Hemolytic anemias, and
4. Transfusions (the transfused red cells constitute an exogenous load of iron).

In most instances of systemic hemosiderosis, the iron pigment does not damage the parenchymal cells or impair organ function despite an impressive accumulation. However, more extensive accumulations of iron are seen in **hereditary hemochromatosis**, with tissue injury including liver fibrosis, heart failure, and diabetes mellitus. *(Fig. 2-31)*

**Bilirubin**
This is a normal major pigment of bile, which is derived from Hb (but unlike hemosiderin contains no iron). The conversion to bile occurs within hepatocytes. Jaundice results from excess bilirubin pigment that is distributed throughout all tissues and body fluids. In the liver, particularly when there is obstruction to the bile flow (e.g. obstruction of the common bile duct by a stone or atresia) bilirubin is seen within bile canaliculi, kupffer cells and hepatocytes as green-brown globular deposits. This imparts greenish color to the liver grossly. *(Fig. 2-32)*

**PATHOLOGIC CALCIFICATION**
Pathologic calcification is a common process in a wide variety of disease states; it implies the abnormal deposition of calcium salts. When the deposition occurs in dead or dying tissues, it is called **dystrophic calcification**; it occurs in the absence of calcium metabolic derangements (i.e., with normal serum levels of calcium). In contrast, the deposition of calcium salts in normal tissues is known as **metastatic calcification** and almost always reflects some derangement in calcium metabolism (hypercalcemia). It should be noted that while hypercalcemia is not a prerequisite for dystrophic calcification, it can exacerbate it.

**Dystrophic Calcification**
Dystrophic calcification is encountered in
- Areas of necrosis (of any type as coagulative, caseous, etc.)
- Advanced atherosclerosis (as in the aorta and coronaries)
- Aging or damaged heart valves resulting in severely impaired valve motion. Dystrophic calcification of the aortic valves is an important cause of aortic stenosis in the elderly *(Fig. 2-33).*

Regardless of the site, calcium salts are grossly seen as fine white granules or clumps, often felt as gritty deposits. Sometimes a tuberculous lymph node is essentially converted to radiopaque stone. *Microscopically,* calcification appears as intracellular and/or extracellular basophilic (bluish) deposits. In time, metplastic bone may be formed in the focus of calcification.

**Metastatic Calcification**
This is seen in cases of hypercalcemia of any cause. The four major causes of hypercalcemia are
1. Increased secretion of parathyroid hormone, (primary parathyroid tumors or production of parathyroid hormone-related protein by other malignant tumors)
2. Destruction of bone (effects of accelerated turnover as in Paget disease, immobilization, or tumors due to increased bone catabolism associated with multiple myeloma, leukemia, or diffuse skeletal metastases
3. vitamin D-related disorders including vitamin D intoxication and sarcoidosis (in which macrophages activate a vitamin D precursor)
4. Renal failure, in which phosphate retention leads to secondary hyperparathyroidism. Metastatic calcification can occur widely throughout the body but principally affects the interstitial tissues of the vessels, kidneys, lungs, and gastric mucosa. The calcium deposits morphologically resemble those described in dystrophic calcification. Although they do not generally cause clinical dysfunction, extensive calcifications in the lungs may produce remarkable radiographs and respiratory deficits, and massive deposits in the kidney (nephrocalcinosis) can cause renal damage.

Note: the text in green color and Italian style is for self study.