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Clinical Pathology Hematology & Blood Banking

(For DMLT Students)





Highlights

- Based on latest syllabus from universities and Paramedical Council
- Concepts are supported with flowcharts and figures for better learning and remembrance
- Simple and lucid language makes it easy to understand

Nanda Maheshwari

4th Edition

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2
CHAPTER

Cellular Pathology

Learning Objectives

- Cells are the basic structural and functional unit of the body. This chapter describes detailed structure of cells and its components
- Most of the diseases of mankind could be understood in terms of cellular injury. This chapter overviews cellular injury, cellular adaptation to injury, causes of cell injury, morphology and types of cell injury
- It also discusses types of cell death

Keywords
Cell organelles, Atrophy, Hypertrophy, Hyperplasia, Metaplasia, Hypoxia, Steatosis, Cholesterolosis, Lipofuscin, Melanin, Hemosiderin, Bilirubin, Hyalinosis, Amyloidosis, Apoptosis, Necrosis

INTRODUCTION

Animal cells are eukaryotic. Animal cells have outer boundary known as the plasma membrane. The nucleus and the organelles of the cell are bound by a membrane. The genetic material (DNA) in animal cells is within the nucleus that is bound by a double membrane. The cell organelles have a vast range of functions to perform like hormone and enzyme production for providing energy for the cells.

CELL ORGANELLES

Animal cell contains membrane-bound nucleus, it also contains other membrane-bound cellular organelles. These cellular organelles carry out specific functions that are necessary for the normal functioning of the cell. Animal cells lack cell wall, a large vacuole and plastids. Due to the lack of the cell wall, the shape and size of the animal cells are mostly irregular.

The cell organelles are centrioles, endoplasmic reticulum, golgi apparatus, lysosomes, microfilaments, microtubules, mitochondria, nucleus, peroxisomes, plasma membrane and ribosomes **(Fig. 2.1)**.

Cell Membrane

- A typical cell membrane is 75 Å in thickness.
- It is a semipermeable barrier, allowing only a few molecules to move across it.
- An electron microscopic study of cell membrane shows the lipid bilayer model of the plasma membrane; it also known as the fluid mosaic model.
- The cell membrane is made up of phospholipids which has polar (hydrophilic) heads and nonpolar (hydrophobic) tails
- It encloses cytoplasm in which various cell organelles are present.

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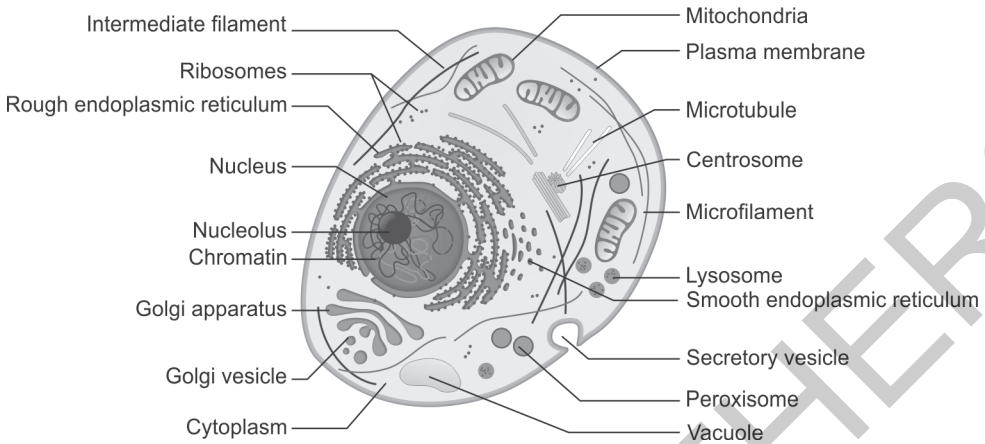

Fig. 2.1: Typical human cell.
(For color version See Color Plate 1)

Cytoplasm

- The fluid matrix that fills the cell is the cytoplasm.
- The cellular organelles are suspended in this matrix of the cytoplasm
- This matrix maintains the pressure of the cell, ensures the cell does not shrink or burst.

Nucleus

- It is a dense spherical structure present in the center of the cell. It is covered with a double-layered membrane called nuclear envelope
- A fluid present within nuclear envelope is called nucleoplasm
- Nucleus is the house for most of the cells genetic material—the DNA and RNA
- The RNA moves in/out of the nucleus through these pores
- Proteins needed by the nucleus enter through the nuclear pores
- The RNA helps in protein synthesis through transcription process
- The nucleus controls the activity of the cell and is known as the control center
- The nucleolus is the dark spot in the nucleus, and it is the location for ribosome formation.

Ribosomes

- Ribosome is the site for protein synthesis where the translation of the RNA takes place
- As protein synthesis is very important to the cell, ribosomes are found in large number in all cells
- Ribosomes are found freely suspended in the cytoplasm and also are attached to the endoplasmic reticulum.

Endoplasmic Reticulum

- Within cytoplasm of the cell is an extensive network of membrane arranged in plates and tubules, collectively called as endoplasmic reticulum (ER)
- ER is the transport system of the cell. It transports molecules that need certain changes and also molecules to their destination
- ER is of two types, rough and smooth
- ER bound to the ribosomes appears rough and is the rough endoplasmic reticulum, while the smooth ER does not have the ribosomes.

Lysosomes

- It is the digestive system of the cell
- They have digestive enzymes helps in break down the waste molecules and also help in detoxification of the cell

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- If the lysosomes were not membrane bound the cell could not have used the destructive enzymes
- They are referred to as the suicide bags of the cell. They have digestive enzymes and are involved in clearing the unwanted waste materials from the cell. They also engulf damaged materials like the damaged cells, and invading microorganisms and digest food particles.

Centrosomes

- It is located near the nucleus of the cell and is known as the 'microtubule organizing center' of the cell
- Microtubules are made in the centrosome
- During mitosis the centrosome aids in dividing of the cell and moving of the chromosome to the opposite sides of the cell.

Vacuoles

- They are bound by single membrane and small organelles
- In many organisms vacuoles are storage organelles. They store excess food or water
- Vesicles are smaller vacuoles which function for transport in/out of the cell.

Golgi Bodies

- Golgi bodies are compact and consist of parallel membrane plates and tubule
- It is the site for enzyme secretion. It participates in formation of lysosomes
- The Golgi bodies modify the molecules from the rough ER by dividing them into smaller units with membrane known as vesicles
- It is involved with processing and packaging of the molecules that are synthesized by the cells. The crude proteins that are passed on by the ER to the apparatus are developed by the Golgi apparatus into primary, secondary, and tertiary proteins.

Mitochondria

- Mitochondrion is the main energy source of the cell
- They are called the power house of the cell because energy (ATP) is created here
- All enzymes which are present in Krebs' cycle are present in mitochondria
- Mitochondria consist of inner and outer membrane. It is composed of largely proteins and lipids
- Each mitochondrian is composed of tubular or paired lamellae called cristae
- Its main function is to produce energy for cell by the process of cellular respiration
- It is spherical or rod-shaped organelle.
- It is an organelle which is independent, as it has its own hereditary material.

Peroxisomes

- Peroxisomes are single membrane bound organelle that contain oxidative enzymes that are digestive in function
- They help in digesting long chains of fatty acids and amino acids and help in synthesis of cholesterol.

Cytoskeleton

- It is the network of microtubules and microfilament fibers.
- They give structural support and maintain the shape of the cell.

FUNCTIONS OF CELL

The cells perform variety of activities by the aid of the cellular organelles. These cells function as a unit and the cells together form tissues. A group of tissues with similar function form an organ and a group of organs of specific function to perform become an organ system. Thus, the microscopic cells form the basic unit

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for the activities and coordination and help survival of the organism.

CELL PATHOLOGY

Rudolf Virchow (1821–1902) was a German physician. He is best known as Father of modern Pathology, as he is the founder of cellular pathology.

He pioneered the modern concept of pathological processes by his application of the cell theory to explain the effects of disease in the organs and tissues of the body. He emphasized that diseases arose, not in organs or tissues in general, but primarily in their individual cells. Most of the diseases of mankind could be understood in terms of the dysfunction of cells. Cellular dysfunction leads to organ dysfunction which is further diagnosed by clinical expression.

OVERVIEW OF CELL INJURY

- *Homeostasis:* Cells actively control the composition of their immediate environment and intracellular structure within a narrow range of physiological parameters. This is called as homeostasis
- *Cell adaption:* Under physiological stresses or pathological stimuli ("*injury*"), cells can undergo adaptation to achieve a new steady state that would be compatible to their viability in the new environment
- *Cell death:* If the injury is too severe ("*irreversible injury*"), the affected cells die.

Cellular Adaptation to Injury

Cellular adaptations can be induced and/or regulated at any of the number of regulatory steps, including receptor binding, signal transduction, gene transcription or protein synthesis.

The most common morphologically apparent adaptive changes are:

1. Atrophy (decrease in cell size)
2. Hypertrophy (increase in cell size)
3. Hyperplasia (increase in cell number)
4. Metaplasia (change in cell type).

Atrophy

Atrophy is the shrinkage in cell size by loss of cellular substance. With the involvement of a sufficient number of cells, an entire organ can become atrophic.

Mechanisms of atrophy are not specific, but atrophic cells usually contain increased autophagic vacuoles with persistent residual bodies, such as lipofuscin.

Causes and Examples of Atrophy

Decreased workload (disuse atrophy), loss of innervation (denervation atrophy), diminished blood supply (ischemia), inadequate nutrition (marasmus, cachexia), loss of endocrine stimulation (menopause), aging (senile atrophy), and pressure (enlarging benign tumor).

Hypertrophy

Hypertrophy is an increase in cell size by gain of cellular substance. With the involvement of a sufficient number of cells, an entire organ can become hypertrophic. Not only the size, but also the phenotype of individual cells can be altered in hypertrophy. With increasing demand, hypertrophy can reach a limit beyond which degenerative changes and organ failure can occur.

Causes and Examples of Hypertrophy

Increased workload (skeletal muscle of body builders), cardiac muscle hypertrophy, hormone-induced hypertrophy (in pregnant woman).

Hyperplasia

Hyperplasia constitutes an increase in the number of indigenous cells in an organ or tissue. This results in increase in size of the organ.

Pathological hyperplasia is typically the result of excessive endocrine stimulation.

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Hyperplasia is often a predisposing condition to neoplasia.

Causes and Examples of Hyperplasia

Hormonal hyperplasia (female breast at the time of puberty and pregnancy), compensatory hyperplasia (unilateral nephrectomy—at the loss of one kidney, other kidney becomes hyperplasic), erythroid hyperplasia of bone marrow in chronic hypoxia (such as in mountain climbers), excessive hormone stimulation (endometrial hyperplasia is common during menopause), prostatic hyperplasia, viral infections, (such as in papilloma virus—warts).

Metaplasia

Metaplasia is a "reversible" change in which one adult cell type is replaced by another adult cell type.

Metaplasia is a cellular adaptation in which indigenous cells are replaced by cells that are better suited to tolerate a specific abnormal environment.

Because of metaplasia, normal protective mechanisms may be lost. Persistence of signals that result in metaplasia often lead to neoplasia.

Causes and Examples of Metaplasia

It usually occurs in response to stress or chronic irritation. Tobacco smoke (Squamous metaplasia in the respiratory tract is most common), gastric acid reflux (gastric metaplasia of distal esophagus), repeated skeletal muscle injury with hemorrhage—muscle replaced by bone (myositis ossificans).

Cellular Injury

Cell injury is change in morphology and function of cell in response to stress. Cell injury occurs when the limits of cell adaptation have been exceeded or if the cells are not capable to adapt. Cells are complex interconnected systems, and single local injuries can result in multiple secondary and tertiary effects.

Cell function is lost far before biochemical and subsequently morphological manifestations of injury become detectable.

Effect of cell injury depends upon:

A. Type, duration and severity of injury.
B. On the type, status, adaptability and genetic make-up of the injured cell, e. g. brain tissue is very sensitive to hypoxia (2–5 min), skeletal muscles can adapt hypoxia for 2–6 hours.

Causes of Cell Injury

Oxygen Deprivation (Hypoxia or Ischemia)

It occurs usually as a result of ischemia (= loss of blood supply), which occurs; for example, when arterial flow suffers from atherosclerosis or thrombotic occlusion of arteries—most common cause of hypoxia.

Hypoxia is due to inadequate oxygenation, for example, in cardiorespiratory failure. It is caused by loss of oxygen-carrying capacity of the blood, either due to anemia (decreased capacity of the blood for oxygen), or after poisoning with carbon monoxide (CO)= loss of the carrying capacity of the blood depending on the severity of hypoxia—the cell may undergo adaptation, injury or cell death.

Example: If femoral artery is narrowed (due to atherosclerotic or atherothrombotic reduction of the lumen of the affected vessel), skeletal muscles of the leg decrease in size—atrophy. This reduction in size of cell mass may achieve a new balance on the lower level, but severe and prolonged hypoxia will induce severe injury and cell death.

Physical Agents

Many forms of physical energy may give rise to cell and tissue injury, such as mechanical trauma, extremes of temperature, sudden changes in atmosphere pressure, electromagnetic energy, radiation, electric shock and hyperthermia (burns).

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Chemical Agents

The list of chemicals that may cause cell and tissue injury includes poisons arsenic, cyanide, mercuric salts, etc. It also includes air pollutants, insecticides and herbicides, alcohol, narcotic drugs ,variety of therapeutic drugs and even oxygen in high concentrations.

Chemicals induce cell injury by one of two major mechanisms:

1. Some chemicals act directly by chemical bindings with some critical molecules or cellular organelles; for example, mercuric chloride poisoning (mercury binds directly to sulfhydryl groups of cell membranes)—GIT and kidney or anticancer drugs and some antibiotic drugs also induce cell damage by direct cytotoxic effects.
2. Other chemicals are not biologically active but convert into reactive toxic metabolites (role of free radicals for example).

Infectious Agents

These agents range from the submicroscopic viruses, Rickettsiae to bacteria, fungi and higher forms of parasites.

Viruses induce cellular changes by two general mechanisms:

1. Cytolytic and cytopathic viruses cause various degrees of cellular injury and cell death.
2. Oncogenic viruses stimulate host cell to proliferate and may induce tumors.

Cytopathic effects of viruses cause injury by two major mechanisms:

First is direct cytopathic effect, in which rapidly replicating virus particles interfere with some aspects of cell metabolism and cause cellular damage.

Second mechanism involves the induction of an immunological response-destruction of the cell by either antibody or cell-mediated reactions, for example the damage and death of hepatocytes caused by hepatitis B viruses are mediated by cytolysis induced by T-lymphocytes.

Immunologic Reactions

Immune system works in a defense against biologic agents. Immune reactions may, however, can cause cell injury; for example, anaphylactic reaction—to a foreign protein or drug and reactions to endogenous self-antigens are responsible for a number of autoimmune diseases.

Genetic Derangements

Genetic defects cause a number of diseases. Genetic injury may result in severe defects and congenital malformations (Down syndrome) or in mild derangements and errors of metabolism (lack of a distinctive enzyme), etc.

Nutritional Imbalances

Even now nutritional errors continue to be major cause of cell injury. Protein-calorie deficiencies chiefly among underprivileged population (starvation). Deficiencies of specific vitamins, nutritional excesses have become important in cell injury among overprivileged population (excess in lipids—predisposes to atherosclerosis, causes obesity, influences diabetes mellitus).

Morphology of Cell Injury

Ultrastructural changes include:

- Cellular swelling—it is near-universal manifestation of cell injury
- Formation of cytoplasmic blebs and distortion of microvilli
- Deterioration of cell attachments.

Mitochondrial changes—occur very rapidly in ischemic injury, but are delayed in some types of chemical injury. Early after ischemia—swelling of mitochondria is due to changes in ions. Small to large size amorphous densities in mitochondria—these consist of lipids or lipid-protein complexes—dense granules rich in calcium (appear early after reperfusion).

Endoplasmic reticulum—changes of ER occur early after injury due to changes in ion and water regulation. Detachment of ribosomes and disaggregation of polysomes—result

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in decrease of proteosynthesis. Progressive fragmentation of ER—results in formation of myelin figures - derived from plasma and organelle membranes (lipoproteins).

Alterations of lysosomes in cell injury—generally appear later.Lysosomes become swollen, and after the onset of lethal injury lysosomes rupture and this event causes leakage of the lysosomal enzymes at this stage it is irreversible cell injury.

General Biochemical Mechanisms of Cell Injury

1. *Loss of energy (ATP depletion, O_2 depletion)*: ATP depletion and decreased ATP synthesis are common with both hypoxic and toxic (or chemical) injury. As a result, Na^+, K^+- ATPase pump activity is reduced. Cellular energy metabolism is changed.
   There is failure of Ca^{++} pump as result calcium cannot get out of the cell. There is reduced protein synthesis.
2. *Mitochondrial damage:* Mitochondria is concerned with cell respiration and the production of ATP, which is responsible for vital functions of cell, including cellular osmolarity (Na/K), protein synthesis and membrane transport process. Loss of membrane potential results in failed oxidative phosphorylation and loss of ATP. Membrane damage leads to leakage of cytochrome C and other proteins which activate apoptotic pathways.
3. *Loss of calcium homeostasis:* Intracellular Ca^{++} is low and is sequestered in mitochondria and endoplasmic reticulum. Extracellular Ca^{++} is high. These gradients are maintained by Ca^{++} Mg^{++} ATPases. Increased cytosolic Ca^{++} activates enzymes: ATPases, phospholipases, proteases, and endonucleases. This starts breaking apart ATP, lipid membrane, cellular protein and DNA, respectively.
4. *Defects in plasma membrane permeability:* Mitochondrial membrane damage causes increased cytosolic Ca^{++}, oxidative stress, lipid peroxidation, phospholipase activity, loss of membrane potential, leakage of cytochrome C.
   Plasma membrane damage causes loss of osmotic balance, loss of proteins, enzymes and nucleic acids. Injury to lysosome membranes causes leakage of enzymes with destruction of cellular components. This may lead to cell death.
5. Generation of reactive oxygen species (O_2, H_2O_2, OH) and other free radicals. Free radicals are chemical species with a single unpaired electron in an outer orbital. Free radicals are chemically unstable and therefore readily react with other molecules, resulting in chemical damage.
   Free radicals initiate autocatalytic reactions; molecules that react with free radicals are in turn converted to free radicals.
   If not adequately neutralized, free radicals can damage cells by three basic mechanisms:
   1. *Lipid peroxidation of membranes:* Double bonds in polyunsaturated membrane lipids are vulnerable to attack by oxygen free radicals.
   2. *DNA fragmentation:* Free radicals react with thymine in nuclear and mitochondrial DNA to produce single strand breaks.
   3. *Protein cross-linking:* Free radicals promote sulfhydryl-mediated protein cross-linking, resulting in increased degradation or loss of activity.

Types of Cell Injury

Depending on the intensity of the factor causing injury and length of its action, cell injury may be reversible and irreversible, details of which are discussed below and shown in **Flowchart 2.1**.

1. Acute reversible changes
   A. Cellular swelling (hydropic change or vacuolar degeneration)
2. Chronic reversible changes
   A. By intracellular accumulation of lipids:

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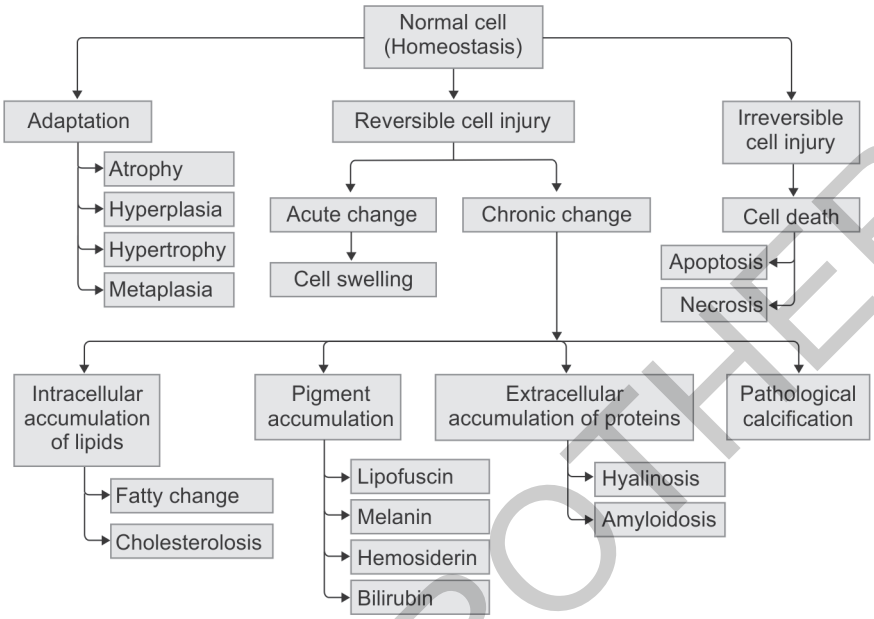
Flowchart 2.1: Types of cell injury.



   a. Fatty change (steatosis)
   b. Cholesterolosis
B. Pigment accumulation:
   a. Lipofuscin
   b. Melanin
   c. Hemosiderin
   d. Bilirubin
C. Extracellular accumulation of proteins:
   a. Hyalinosis
   b. Amyloidosis
D. Pathological calcification (metastatic and dystrophic).

3. Irreversible cell changes (cell death)
   a. Apoptosis
   b. Necrosis

Cellular Swelling

Cellular swelling (synonyms: hydropic change, vacuolar degeneration, cellular edema) is an acute reversible change resulting as a response to nonlethal injuries. It is an intracytoplasmic accumulation of water due to incapacity of the cells to maintain the ionic and fluid homeostasis. It is easy to be observed in parenchymal organs: liver (hepatitis, hypoxia), kidney (shock), myocardium (hypoxia, phosphates intoxication). It may be local or diffuse, affecting the whole organ.

In cellular swelling, at gross examination, the affected organ is enlarged, pale and soft. Microscopically, the cells are enlarged, with a clear cytoplasm (due to the presence of small clear or pale vacuoles, with indistinct shape and limits) and a normal nucleus in central position; blood capillaries are compressed, explaining the organ's pallor.

Fatty Change (Steatosis)

Fatty change or steatosis represents the intracytoplasmic accumulation of triglyceride (neutral fats) of parenchymal organs, such as: liver, myocardium and kidney.

Mechanism: Increase of free fatty acids (starvation, diabetes and chronic ethylism/alcoholism), reduction of free fatty acids oxidation (hypoxia, toxins, chronic ethylism/alcoholism), increase of esterification of free fatty acids into triglycerides (due to increased

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free fatty acids or reduction of their oxidation, chronic ethylism/alcoholism) and reduced export of triglycerides due to deficiency of lipid binding apoprotein (starvation/malnutrition, toxins). Initially, fatty change does not impair the cells function, being reversible.

At the beginning, the hepatocytes present small fat vacuoles in the vicinity of the endoplasmic reticulum (liposomes). In the late stages, the size of the vacuoles increases pushing the nucleus to the periphery of the cell—macrovesicular fatty change. These vesicles are well delineated and optically "empty" because fat solves during tissue processing (paraffin embedding). Large vacuoles may coalesce, producing fatty cysts, which are irreversible lesions.

Cholesterolosis

Cholesterolosis is an intracellular accumulation of lipids (cholesterol). When cholesterol is in excess in the bile, it passes into the lamina propria where is phagocytized by macrophages. These macrophages become larger and polygonal, with foamy cytoplasm and small, hyperchromatic, central nucleus (xantic cells). Aggregation of xantic cells may enlarge the mucosal folds, producing a polypoid appearance of the mucosal surface. The gallbladder may be affected in a patchy localized form or in a diffuse form. The diffuse form macroscopically appears as a bright red mucosa with yellow mottling (due to lipid), hence the term strawberry gallbladder.

Sometimes, cholesterolosis is associated with inflammation of the gallbladder (cholecystitis) or with gallstones (cholelithiasis).

Lipofuscin

It is insoluble pigment—fine intracytoplasmic granules, yellow-brown called aging pigment, because it is seen mainly in the cells that are undergoing slow regressive changes. Lipofuscin is composed of complex lipids; it is derived of peroxidation of lipids, mostly from cellular membranes in cell injury. Lipofuscin is often associated with atrophy—brown atrophy. There is prominent accumulation of lipofuscin in liver and heart of aging patients, or in patients with severe malnutrition; for example, in cancer cachexia (usually accompanied by shrinkage of the entire organ). Lipofuscin represents indigestible residues of autophagic vacuoles. Lipofuscin itself is not injurious to the cell.

Melanin

It is brown-black in colored pigment, produced in melanocytes. Melanin is derived from tyrosine; the pigment is formed when enzyme tyrosinase catalyzes the oxidation of tyrosine to dihydroxyphenylalanine. The function of melanin is to block harmful UV rays from the epidermal nuclei.

Melanin may accumulate in excessive quantities in non-malignant and malignant melanocytic lesions. In inflammatory skin lesions, melanin may be released from injured basal cells and taken up by dermal macrophages. This give rise to post-inflammatory pigmentation of the skin.

Hemosiderin

It is a golden-yellow to brown granular pigment found in lysosomes within the cell cytoplasm.

Hemosiderin is hemoglobin-derived pigment. It is composed of aggregates of partially degraded ferritin.

Iron metabolism is normally regulated so that the total amount of iron in the body is maintained within relatively narrow range. The body has no effective mechanism for elimination of excess iron. Excess of iron then accumulates in macrophages and parenchymal cell in the form of hemosiderin.

Deposition of hemosiderin in tissue macrophages is termed hemosiderosis.

Localized Hemosiderosis

It is common and results from gross hemorrhages, ruptures of small vessels or from severe

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vascular congestion, etc.; in this case, hemoglobin is broken down and its iron is deposited locally as hemosiderin. There is no clinical significance; its presence only indicates a site of hemorrhage. A change in color is due to subcutaneous hemorrhage. The tissue affected by hemorrhage is first red-blue (due to lysis of erythrocytes), then becomes green-blue (due to formation of biliverdin and bilirubin). Finally, it appears golden-yellow (due to transformation to hemosiderin). Hemosiderin is picked up by macrophages and deposited in the tissue.

Generalized Hemosiderosis

It is less common, occurs in those conditions when there is an excess iron in the body like following multiple tranfusions, following excessive dietary iron and in some hemolytic anemias. Hemosiderin is deposited in many organs (liver, bone marrow, spleen, lymph nodes) first in macrophages, later also in parenchymal cells. It has usually no clinical significance, except of being an indication of iron overload.

Hemochromatosis

It is uncommon inherited or idiopathic disease characterized by deposits of hemosiderin throughout the body. The mostly affected organs are the liver (cirrhosis), pancreas (diabetes mellitus), and the skin (brown color). Primary defect lies in mucosal cells of the small intestine. These cells usually absorb only limited amount of iron from the food. In hemochromatosis, this control is lost and large amounts of iron are absorbed. Iron is toxic to the tissues and leads to fibrosis and cirrhosis of the liver and fibrosis of pancreas with destruction of Langerhans islands, leading to diabetes mellitus.

Bilirubin

Accumulation of bilirubin is called jaundice (icterus)—yellowish discoloration of skin and sclera—occurs when bilirubin is elevated in the blood and deposited in tissues.

Bilirubin is a bile pigment that represents an end product of hemoglobin molecule destruction; it does not contain iron. Normally majority of bilirubin is formed in the cells of RES, where erythrocytes are destroyed (spleen), minor part of bilirubin is formed in bone marrow and liver then bilirubin is transported into the liver in an unconjugated form (as an indirect bilirubin)—bound to albumin.

In the liver, bilirubin is conjugated with glucuronide to form soluble (direct) bilirubin, which is excreted by liver cells to the bile and then to intestine, where it is changed to urobilinogen (then absorbed by portal blood and returned to the liver or excreted in urine). *Jaundice*—common clinical disorder due to excess of bilirubin within cells and tissues.

Causes of jaundice—may result from three distinct mechanisms:

- Increased production of bilirubin
- Decreased excretion by the liver
- Bile duct obstruction.

Hemolytic Jaundice (Increased Production)

Increased destruction of erythrocytes (for example due to hemolytic anemia) overhelms the capacity of the liver to conjugate bilirubin. This leads to an accumulation of unconjugated (indirect) bilirubin in serum-complexed to albumin, cannot be excreted in the urine—this BR is toxic to brain—soluble in lipids.

Hepatocellular jaundice (decreased uptake, conjugation and excretion): Usually both conjugated and unconjugated bilirubin levels are elevated. Urine levels of bilirubin and urobilinogen are elevated.

Obstructive Jaundice

Biliary tract obstruction results in accumulation of bilirubin in the liver—cholestasis. Bilirubin cannot reach the intestine—this result in a failure of lipid substances to be absorbed. Deposition in connective tissue (skin, sclera, internal organs) result in yellow color typical

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of jaundice. Deposition in parenchymal cells most important in basal ganglia (so called kernicterus).

Hyalinosis

Hyalin refers to any alteration within the cell or extracellular space which gives a homogenous glassy eosinophilic appearance-hyaline change does not represent specific alteration.

- Intracellular hyaline are hyaline droplets in proximal tubular epithelial cells of kidney that represent reabsorption of proteins which passed through damaged glomerular membrane into primitive urine.
  Alcoholic hyaline are aggregates of cytokeratin intermediate filaments in cytoplasm of hepatocytes in alcoholic liver disease.
- Extracellular hyaline are hyaline of extracellular spaces. Like hyalinization of collagenous fibrous tissue means regressive change that is represented by a decrease of a vascularity and an increase of thickness of collagen fibers occurs for example, in old scars.

Amyloidosis

Amyloidosis is a group of rare but serious conditions caused by deposits of abnormal protein, called amyloid, in tissues and organs throughout the body.

Proteins begin as a string of amino acids that fold themselves into a three-dimensional shape. This 'protein folding' allows them to perform useful functions within our cells.

Amyloid is a description of proteins which have folded abnormally and then collected together. In this form they do not break down as easily as normal proteins and can build up in tissues and organs.

If this build-up causes the tissues or organs to stop working properly, the resulting conditions are called amyloidosis.

The amyloid deposits occasionally only affect one part of the body (localized amyloidosis), but more often several different part of the body are affected (systemic amyloidosis), such as the heart, kidneys, liver, or nerves. Without treatment to address the underlying cause, the amyloid deposits can eventually lead to organ failure and death, sometimes within only a year or two.

There are around 30 different proteins that can misfold and form amyloid, which is why there are many different types of amyloidosis.

TYPES OF CELL DEATH

There are two types of cell death—necrosis and apoptosis.

Apoptosis

Apoptosis is a word from Greek language, which originally refers to falling of leaves from trees in the autumn. It is programmed cell death, (cell suicide), since it is active process, it requires energy and protein synthesis. It is an important mechanism for removal of cells.

Apoptosis is dependent on gene activation and new protein synthesis. It is thought that the process is regulated by a number of apoptosis-associated genes. These include bcl-2 protein, which inhibits apoptosis and therefore extends cell survival, p-53 protein which stimulates apoptosis.

Sequence of Events in Apoptosis

1. Elevation of cellular calcium and rapid reduction of volume of the cell (cell shrinkage).
2. Activation of calcium-dependent enzyme endonuclease which cleaves DNA.
3. Fragmentation of DNA is regular with fix intervals (180bp).
4. There is marked condensation of both nucleus and cytoplasm, followed by Membrane bleeping.
5. Formation of apoptotic bodies—small apoptotic bodies are composed of fragments of nuclei with condensed chromatin, larger apoptotic bodies are composed

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of both fragments of nuclei and condensed cytoplasm with preserved organelles.
6. Apoptotic bodies are rapidly phagocytosed by epithelial cells in Neighborhood or by macrophages—cell dying by apoptosis are recognized and phagocytosed soon after initiation of apoptosis.

Morphologic Changes in Apoptosis

- Rapid volume reduction and formation of cytoplasmic blebs
- Loss of cell–cell contacts
- Formation of apoptotic bodies
- Phagocytosis of apoptotic bodies by macrophages, within which they undergo hydrolytic phagocytic degradation.

This type of cell death occurs in physiological, embryological and also in pathological processes.

- *Physiological apoptosis:* It occurs in a number of situations
  - Is involved in normal tissue turnover
  - In hormone-induced atrophy (endometrium in menstrual cycle, mammary gland in menopause)
  - In developing tissues.
- *Embryological apoptosis:*
  - Formation of digits (by apoptosis of interdigital tissue).
- *Pathological apoptosis:* It is involved in response to pathologic stimuli, like inflammation and cancer, in an attempt by the body to arrest cell proliferation and tissue damage.
  - Such as viral infection (for example, Councilman bodies in liver cells in viral hepatitis)
  - Tumor regression induced by chemotherapy
  - At times there is involvement of apoptosis in tumor growth.

Necrosis

Necrosis is a word from Greek language, which originally refers to death, the stage of dying, or dead. It is a form of cell injury which results in unprogrammed (premature) death of cells in living tissue.

In contrast with apoptosis, cleanup of cell debris by phagocytes of the immune system is generally more difficult, as the disorderly death generally does not send cell signals which tell nearby phagocytes to engulf the dying cell. This lack of signaling makes it harder for the immune system to locate and recycle dead cells which have died through necrosis. Rather various receptors are activated, and result in the loss of cell membrane integrity and an uncontrolled release of products of cell death into the extracellular space. This initiates in the surrounding tissue an inflammatory response which spread the necrosis and also prevents nearby phagocytes from locating and eliminating the dead cells by phagocytosis. These enzymes are derived either from dying cells themselves. This is called as autolysis or from lysosomal enzymes of leukocytes, referred to as heterolysis.

For this reason, it is often necessary to remove necrotic tissue surgically, a procedure known as debridement. Untreated necrosis results in a build-up of decomposing dead tissue and cell debris at or near the site of the cell death. A classic example of it is gangrene.

Cellular Changes

Cell membrane disappears. Cytoplasm is swollen, mitochondria is rupture, forms myelin figures and may be calcified.

The nuclear changes in necrosis are characteristics by manner in which its DNA breaks down:

- *Karyolysis:* Chromatin of the nucleus fades due to the loss of the DNA by degradation.
- *Pyknosis:* Nucleus shrinks and the chromatin condenses.
- *Karyorrhexis:* The shrunken nucleus fragments to complete dispersal.

Cause of Necrosis

There may be external or internal factor responsible for necrosis.

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External factors may involve mechanical trauma (physical damage to the body which causes cellular breakdown), damage to blood vessels (which may disrupt blood supply to associated tissue), and ischemia. Thermal effects (extremely high or low temperature) can result in necrosis due to the disruption of cells like frostbite.

Internal factors causing necrosis include trophoneurotic disorders; injury and paralysis of nerve cells. Pancreatic enzymes (lipases) are the major cause of fat necrosis.

Morphological Types of Necrosis

Coagulative Necrosis

It is most common pattern of necrosis. It is characteristic of hypoxic cell death.

Macroscopic appearance: Firm consistency, yellowish color, dry appearance of the cut section.

This pattern of necrosis, most commonly results from sudden severe ischemia which is encountered mostly in solid organs, such as kidney, heart, spleen and adrenal gland.

Pathogenesis: Nucleus usually disappears, but the shape of cell is preserved. Presumably, the pattern of coagulative necrosis results from severe intracellular acidosis which denaturates not only of structural proteins, but also enzymes (this block rapid proteolysis of the cell). Finally, the necrotic cell breaks into fragments.

The best example of coagulative necrosis is myocardial infarction. Gross appearance of acute myocardial infarct changes with time.

Early infarct can be visualized by various histochemic reactions that may show depletion of oxidative enzymes from infarcted area.

Microscopic appearance: In early acute infarction, cell swelling, pyknosis, eosinophilia of cytoplasm.

Lesser than 8–10 hours, ischemic area is slightly paler, hardly discernable. By 18–24 hours, the infarct becomes apparent grossly - pale, more sharply circumscribed, hyperemic border.

Microscopically, total necrosis with loss of nuclei, heavy infiltrate of leukocytes, and macrophages. By 3–7 days, more apparent hyperemia at the border of infarct, yellow-brown color, soft consistency. At the end of 1st week, infarct becomes circumscribed by highly vascularized scar tissue. Microscopically, there is a prominent fibrovascular reaction in margins.

6th week by total replacement by scar now termed as myofibrosis.

Liquefactive Necrosis

Liquefactive necrosis (or colliquative necrosis) is characterized by the digestion of dead cells to form a viscous liquid by autolysis and heterolysis. This is typical of bacterial, or sometimes fungal, infections because of their ability to stimulate an inflammatory response. The necrotic liquid mass is frequently creamy yellow due to the presence of dead leukocytes and is commonly known as pus. Typically seen in an abscess where there are large numbers of neutrophils present, which release hydrolytic enzymes that break down the dead cells so rapidly that pus forms. Pus is the liquefied remnants of dead cells, including dead neutrophils.

Hypoxic infarcts in the brain presents as this type of necrosis, because the brain contains little connective tissue but high amounts of digestive enzymes and lipids, and cells therefore can be readily digested by their own enzymes.

Gross morphology: Necrotic area becomes very soft and fluidy. These changes are first detectable at about 12 hours.

Within 2–3 day softening and discoloration become more apparent.

In large infarcts, there is a marked swelling, tissue liquefaction result in subsequent pseudocystic degeneration. No fibrous scar is formed, necrotic area changes into postmalatic pseudocyst (postnecrotic pseudocyst)

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where cystic space is filled with debris and fluid.

Fat Necrosis

This refers to necrosis in adipose tissue due to action of activated lipases. When lipases are released into adipose tissue, triglycerides are cleaved into fatty acids, which bind and precipitate calcium magnesium or sodium, ions forming insoluble salts. These salts look chalky white on gross examination, basophilic in histological sections stained with H and E and are visible on radiographic examinations.

Most common is acute pancreatic necrosis, in which active pancreatic enzymes cause focal necrosis of the pancreas and the adipose tissue throughout the abdomen. Here, lipases are activated and released and destroy not only pancreatic tissue itself but also fat cells in the pancreas and also fat cells throughout the peritoneal cavity.

Caseous Necrosis

It is another distinctive type of necrosis that is a combination of coagulative and liquefactive necrosis. This pattern of cell injury occurs with granulomatous inflammation in response to certain microorganisms (tuberculosis or fungal). The host response to the organisms is a chronic inflammatory response and in the center of the caseating granuloma there is an area of cellular debris with the appearance grossly as soft, friable, whitish-gray debris resembling cheesy material—hence the term caseous necrosis.

Gross morphology: This appearance has been attributed to the specific lipopolysaccharides of the capsule of the agent, tuberculous bacillus *(Mycobacterium tuberculosis):* The exact interactions with dead cells are not completely understood.

Microscopy: Histologically, caseous necrosis appears as amorphous eosinophilic material with cell debris.

Fibrinoid Necrosis

This is a type of connective tissue necrosis that occurs in the wall of arteries in cases of vasculitis. There is necrosis of smooth muscle cells of the tunica media and endothelial damage which allows plasma proteins, (primarily fibrin) to be deposited in the area of medial necrosis. Fibrinoid necrosis is a special form of necrosis usually caused by immune-mediated vascular damage. It is marked by complexes of antigen and antibodies, sometimes referred to as "immune complexes" deposited within arterial walls together with fibrin. Fibrinoid necrosis is characterized by loss of normal structure of collagen fibers.

Gangrenous Necrosis

It is not a distinctive type of necrosis, it is a necrosis secondary modified usually by the attack of bacterial agents. The term gangrene is commonly used in clinical practice to describe a condition when extensive tissue necrosis is complicated by bacterial infection. The necrotic tissue appears as white and friable, like clumped cheese. Dead cells disintegrate but are not completely digested, leaving granular particles. There are three major types of gangrene that are as follows:

1. *Dry gangrene:* Commonly it occurs in extremities as a result of ischemic coagulative necrosis due to arterial obstruction necrotic tissue. It appears black and dry and is sharply demarcated from viable tissue.
2. *Wet gangrene:* It results from severe bacterial infection of necrotic area. It occurs in the extremities due to arterial obstruction, also in the internal organs, such as intestine—most common example—in acute suppurative appendicitis. Grossly, tissue is swollen, reddish-black with extensive liquefaction, wet gangrene is severe complication associated with high mortality rate.
3. *Gas gangrene:* It is a wound infection caused by *Clostridium perfringens* and other types of clostridia.

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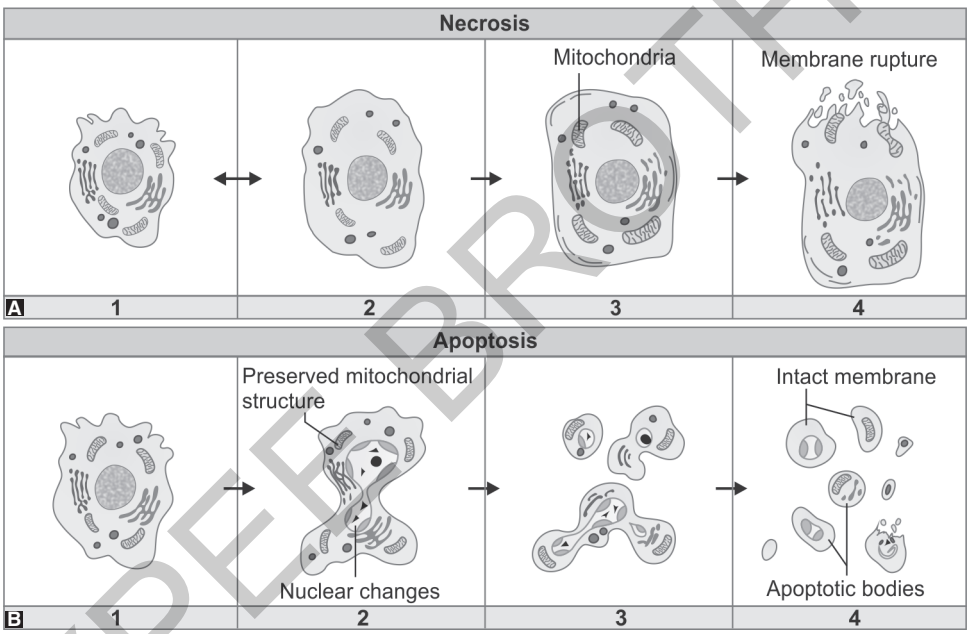
TABLE 2.1: Differentiation between necrosis and apoptosis.

Characteristics	*Necrosis*	*Apoptosis*
Cause	Not programmed (Damage/trauma)	Programmed
Cell size	Cellular swelling	Cellular shrinkage
Cell numbers	Many cells affected	One cell affected
Cell membrane	Loss of cell membrane integrity (cell lysis)	Membrane blebbing but integrity maintained (Apoptopic bodies are formed)
Requires ATP	No	Yes
Inflammation	Yes	No
Cell contents	Ingested by macrophages	Ingested by neighboring cells
Stimuli	Pathologic (hypoxia, toxins)	Physiologic and pathologic



Figs. 2.2A and B: Diagrammatic representation: (A) Necrosis—(1) Normal, (2) Reversible injury, (3) Cell swelling, (4) Lysed necrotic cell; (B) Apoptosis—(1) Normal, (2) Cell condensation (blebbing), (3) Cell fragmentation, (4) Apoptopic bodies.

It is characterized by extensive necrosis and tissue destruction and production of gas by fermentative action of bacteria. Grossly-appearance similar as in wet gangrene with additional presence of gas in tissues.

Table 2.1 and **Figures 2.2A and B** provide differentiating characteristics of necrosis and apoptosis.

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