FACULTY OF MEDICINE

DEPARTMENT OF PATHOLOGY

FOUNDATION BLOCK
CELL INJURY LECTURES

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OBJECTIVES

The students should:

A] Understand the concept of cells and tissue adaptation to environmental stress including the meaning of hypertrophy, hyperplasia, aplasia, atrophy, hypoplasia and metaplasia with their clinical manifestations.

B] Is aware of the concept of hypoxic cell injury and its major causes.

C] Understand the definitions and mechanisms of free radical injury.

D] Knows the definition of apoptosis, tissue necrosis and its various types with clinical examples.

E] Able to differentiate between necrosis and apoptosis.

F] Understand the causes of and pathologic changes occurring in fatty change (steatosis), accumulations of exogenous and endogenous pigments (carbon, silica, iron, melanin, bilirubin and lipofuscin).

G] Understand the causes of and differences between dystrophic and metastatic calcifications.

Lecture One : **Adaptation to environmental stress**: hypertrophy, hyperplasia, aplasia, hypoplasia, atrophy, squamous metaplasia, osseous metaplasia and myeloid metaplasia.

Hypoxic cell injury and its causes (ischaemia, anaemia, carbon monoxide poisoning, decreased perfusion of tissues by oxygen, carrying blood and poor oxygenation of blood).

- **Free radical injury**: definition of free radicals, mechanisms that generate free radicals, mechanisms that degrade free radicals.

Lecture Two : **Types of necrosis** : Coagulative, Liquefactive, Caseous, gangrenous, fibrinoid and fat necrosis.

- **Apoptosis** : definition, morphologic features, regulation of apoptosis and comparison between necrosis and apoptosis.

Lecture Three : **Reversible cellular changes and accumulations:** fatty change, hyaline change, accumulations of exogenous pigments (carbon, silica, iron dust, lead and argyria).

- Accumulations of endogenous pigments: melanin, bilirubin, haemosiderin (haemosiderosis and haemochromatosis), lipofuscin.

- **Pathologic calcifications**: metastatic calcification, dystrophic calcification.

CELL INJURY

Definition: Cell injury is best defined as the cellular changes which are caused by stresses which exceed the cell's adaptive capability. In other words, if the cell adaptive capability is exceeded (overtaxed) then cell injury develops.

I. **INTRODUCTION**. Cells are in homeostasis with the extracellular fluid and respond to changes in their environment.

A. **Adaptation** is the cell's response to prolonged stress.

B. **Cell injury.** If the cell's ability to adapt is overtaxed (exceeded), cellular injury results. Initially, cellular injury may be reversible (referred to as **hydropic change**), but in the face of prolonged or severe stress, the damage becomes **irreversible** (referred to as **necrosis**).

C. **Cell death.** There are two forms of cell death: **necrosis**, which is the ultimate result of irreversible cell injury, and **apoptosis** which is referred to as programmed cell death and could be due to physiologic or pathologic events.

II. **CELL INJURY**

A. Causes of cell injury include:

1. **Oxygen deficiency** or **ischemia** which causes cellular hypoxia/anoxia.
2. **Free radicals**, especially oxygen radicals [e.g., superoxide (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH)].
3. **Chemical** or **physical agents.**

Cellular hypoxia/anoxia is caused by arterial obstruction, decreased oxygenation of blood (lung disease), decreased oxygen-carrying capacity (anemia, CO poisoning) and inadequate tissue perfusion (heart failure, hypotension).

The mechanism of ischemic cell injury:

This type of cellular injury is due to increased intracellular calcium. The progression can be detailed as follows:

1. Hypoxia ⟶ decreased oxidative phosphorylation ⟶ decreased ATP ⟶ decreased membrane integrity and pump failure ⟶ loss of ion gradients ⟶ Ca influx into the cell.
2. Decreased oxidative phosphorylation ⟶ anaerobic metabolism ⟶ lactic acid production. This further results in nuclear chromatin, organelle and membrane damage which leads to calcium release from mitochondria and also Ca influx from the extracellular spaces.

A **free radical** has an unpaired electron in its outer orbit, which makes it unstable and highly reactive. The free radical initiates protein crosslinking, lipid peroxidation and amino acid oxidation in propagated chain reactions. This damages cell and organelle membranes and causes cell injury.

The sources of free radicals are:

1. Normal metabolism
2. Chemical toxicity
3. Reperfusion injury
4. Ionizing radiation
5. O_2 therapy
6. Immune response/inflammation (PMN leukocytes oxidative burst)

There are 3 free-radical scavengers which inactivate free radicals and they are: **superoxide dismutase**, **catalase** and **mannitol**. They bind and inactivate free radicals.

Ion gradient: is the equilibrium of ions like calcium, potassium and sodium between the intracellular and extracellular spaces.

Ultrastructural signs of cell injury:

1. **Seen in both reversible and irreversible cell injury**
    a. **Cellular swelling.** Diminished activity of the sodium pump in the cell membrane causes an influx of sodium (leading to an isosmotic gain of water and swelling of the cell) and an efflux of potassium.
    b. **Mitochondrial swelling** results in reduced aerobic respiration.
    c. **Dilatation and degranulation of the rough endoplasmic reticulum** results in cessation of protein synthesis.
    d. **Autophagocytosis** is the ingestion of damaged organelles by lysosomes.
2. **Seen only in irreversible cell injury**
    a. **Cell membrane rupture**
    b. **Nuclear changes,** including **pyknosis** (nuclear condensation), **karyolysis** (loss of nuclear chromatin) and **karyorrhexis** (nuclear fragmentation).

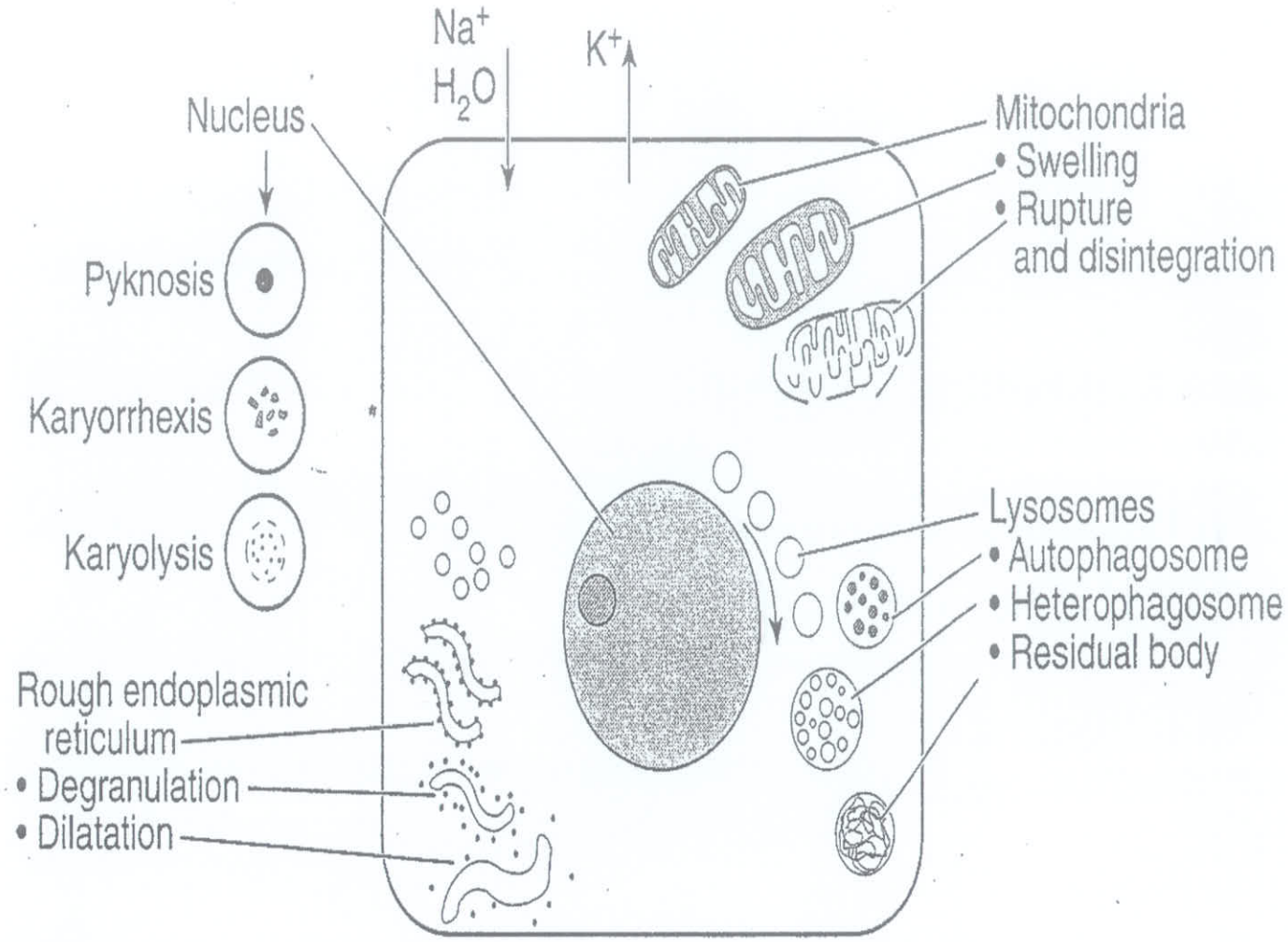

Figure 1. Ultrastructural signs of cell injury. Changes are seen in the mitochondria, lysosomes, rough endoplasmic reticulum and nucleus. Nuclear changes usually indicate irreversible cell injury. H_2o = water; K^+ = potassium; Na^+ = sodium.

III. CELL DEATH

A. **Necrosis** is a morphologic sign of cell death in a living tissue. Several forms of necrosis are recognized and these are:

1. **Coagulative necrosis,** typically caused by ischemia (infarct), is the most common form of necrosis. The necrotic tissue appears pale and firm and retains its normal shape because no enzymatic lysis occurs as the -enzymes, like all other proteins, have been "coagulated" (i.e., inactivated).
2. **Liquefactive necrosis** is typically found in the brain or in an abscess (i.e., a pus-filled cavity). Tissue is softened ("liquefied") through the action of enzymes released from brain cells or, in the case of an abscess, polymorphonuclear neutrophils (PMNs).
3. **Caseous necrosis** is typically seen in tuberculosis and certain fungal granulomas. The tissue appears cheesy; histologically, it consists of granular material surrounded by epithelioid and multinucleated giant cells.
4. **Fat necrosis** may be caused by trauma to adipose cells, or induced by lipolytic enzymes released during disease states (e.g., lipase release in acute pancreatitis). Free fatty acids released from fat cells bind with calcium to form white specks or streaks composed of calcium soaps.
5. **Fibrinoid necrosis** is typically seen in arteries, arterioles or glomerular capillaries damaged by autoimmune diseases. Blood vessels are impregnated by fibrin and other serum proteins and appear magenta-red in histologic sections.

> "Wet gangrene" is a clinical term for ischemic necrosis accompanied by bacterial decomposition, which leads to partial liquefaction of the tissue. "Dry gangrene" ("mummification") refers to noninfected ischemic necrosis accompanied by drying of the tissues.

B. **Apoptosis** is programmed cell death and is based on activation of specific "death pathway genes." Apoptosis may be physiologic or pathologic. The control of apoptosis is important in the process of carcinogenesis as some genes involved in cancer formation like the bcl2 oncogenes switch off apoptosis, thus allowing the neoplastic cells to live indefinitely.

1. Examples of **physiologic apoptosis include the:**

   a. Programmed death of embryonic cells in the limb buds (leading to the formation of fingers and toes).
   b. Predetermined death of cells on the surface of the intestinal mucosa.
   c. Hormone-induced cell death of endometrial cells at the end of the menstrual cycle.

2. Examples of **pathologic apoptosis** include:

   a. Hepatitis virus-induced liver cell apoptosis ("acidophlic bodies").
   b. Immune injury-related skin keratinocytes ("Civatte bodies").
   c. Corticosteroid-induced atrophy of the neonatal thymus.

   The main differences between apoptosis and necrosis are:

      1) Apoptosis is NOT associated with inflammatory reaction while necrosis can provoke an inflammatory reaction.
      2) Apoptosis can be physiologic or pathologic while necrosis is almost always pathologic.
      3) Apoptosis may occur as a single cell or in groups while necrosis is almost always seen in groups of cells.

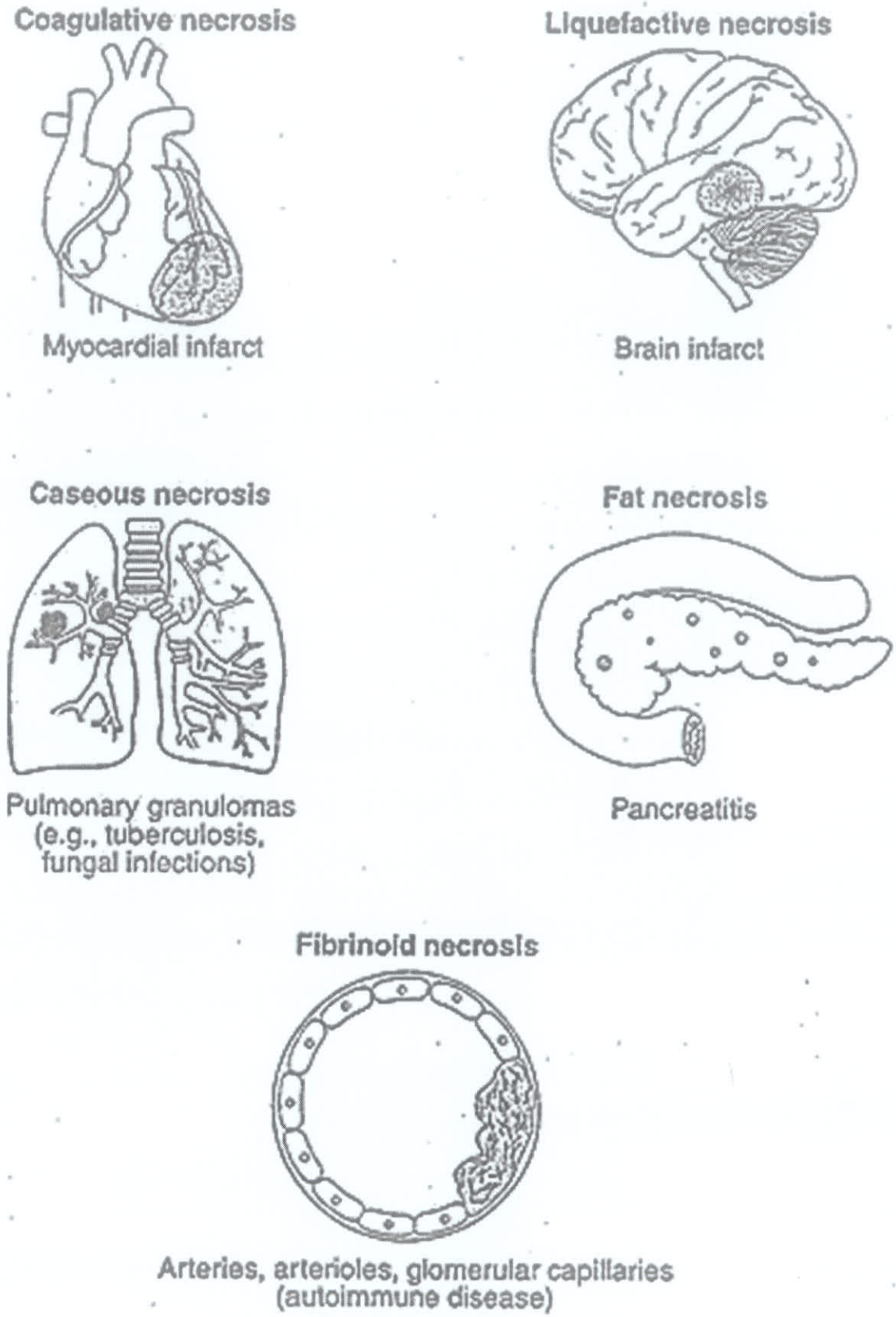

Figure 2. Forms of necrosis.

IV. ADAPTATION

Cellular adaptation to injurious agents include:

A. **Atrophy** is a reduction in the size of an organ or tissue owing to either cell loss or a reduction in the size of cells. Typical examples are atrophy of the brain in Alzheimer disease or thinning of the bones in osteoporosis.

B. **Hypertrophy** is an increase in the size of an organ or tissue owing to enlargement of constituent cells. Typical examples include the response of the heart and skeletal muscles to prolonged effort.

C. **Hyperplasia** is an increase in the size of an organ owing to an increased number of cells. Hyperplasia can be induced by hormones (e.g., endometrial hyperplasia induced by estrogen).

> In many instances, hypertrophy and hyperplasia occur coincidentally (e.g., benign prostatic hyperplasia, hypertrophy of the urinary bladder secondary to urethral obstruction). In clinical practice, such changes are designated as either hypertrophy or hyperplasia; these designations reflect time-honored terminology.

D. **Metaplasia** is the transformation of one tissue cell type into another. Examples include squamous metaplasia of the bronchial epithelium as a result of cigarette smoking and metaplasia of the squamous epithelium of the esophagus into intestinal or gastric epithelium owing to reflux of gastric juice (Barrett esophagus).

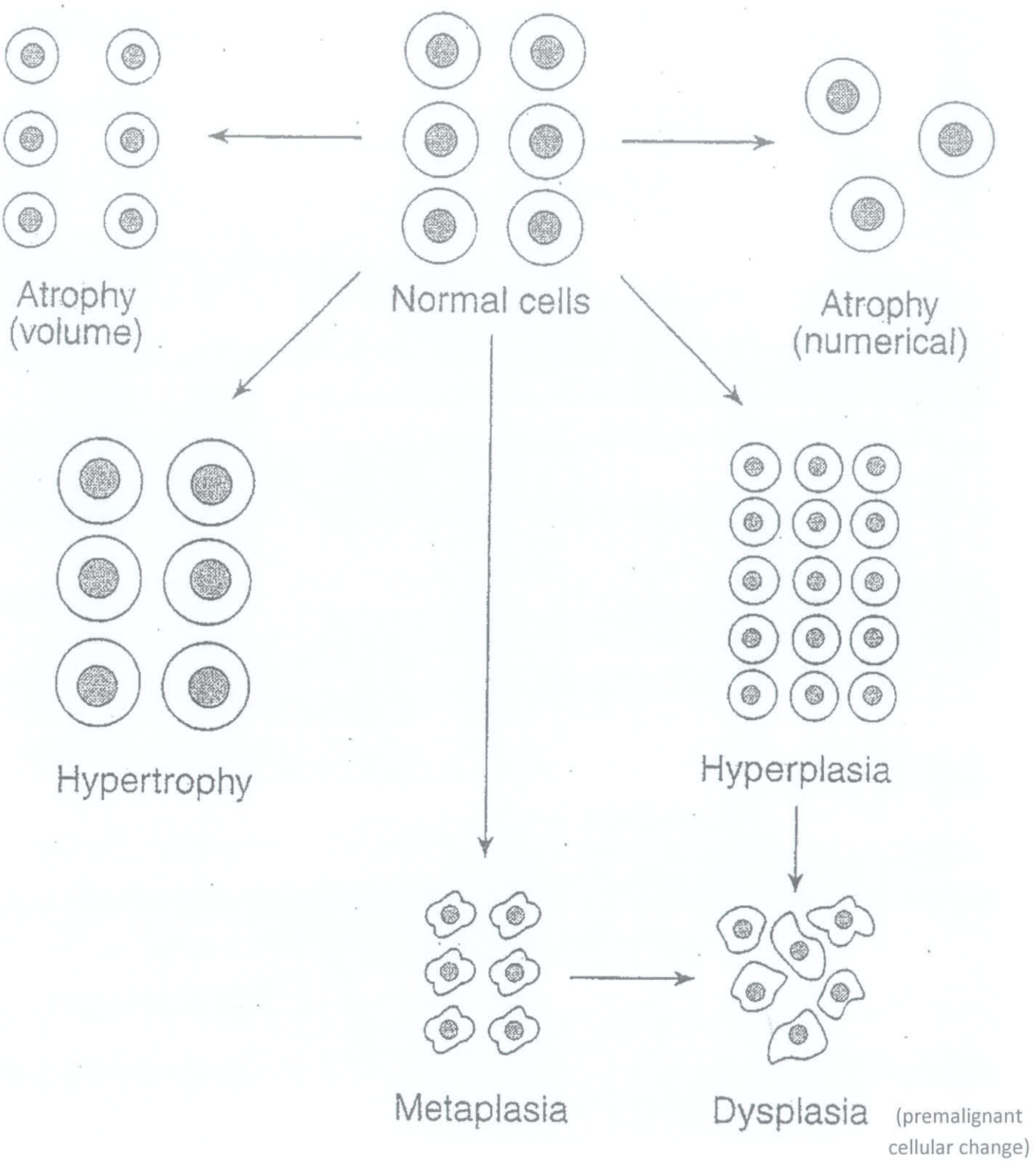

Figure 3. Adaptations of the cell caused by chronic stress or injury include atrophy, hypertrophy, hyperplasia, metaplasia and dysplasia.

V. **ACCUMULATIONS AND DEPOSITS.** Chronic cell injury or metabolic disorders can lead to the accumulation of substances in cells and the extracellular matrix.

A. **Intracellular accumulations**

1. **Glycogen** accumulates in the liver, muscles or kidneys in patients with inborn errors of glycogen metabolism or diabetes mellitus.
2. **Fat** accumulates in the liver in obese patients with chronic alcoholism.
3. **Protein** accumulates in the proximal renal tubules in patients with proteinuria.
4. **Pigments** that accumulate in various cells include **lipofuscin** (i.e., the brown pigment formed in the lysosomes of elderly people), **melanin** (i.e., the brown pigment typically found in melanocytes and melanomas) and **hemosiderin** (i.e., the iron-rich brown pigment derived from hemolyzed red blood cells).

> Hemochromatosis is a genetic disorder or iron absorption characterized by the deposition of hemosiderin in the spleen, liver and bone marrow. Patients with cirrhosis, diabetes and skin discoloration ("bronzed diabetes").

B. **Calcification.** Deposits of calcium salts in the cells and extracellular matrix can be classified as dystrophic or metastatic.

1. **Dystrophic calcification** involves damaged or dead tissue (e.g., calcification of atherosclerotic blood vessels and scarred aortic valves).
2. **Metastatic calcification** is secondary to hypercalcemia and is typically associated with hyperparathyroidism, hypervitaminosis D or end-stage renal disease. Metastatic calcification is most often seen in the kidneys, lungs or stomach.

C. **Amyloid deposition.** The deposition of amyloid, a proteinaceous substance, between the cells of various tissues leads to a group of clinical conditions collectively known as "amyloidosis".

1. **Histologic appearance of amyloid.** Amyloid is extracellular fibrillar material formed from a variety of polypeptides.
    a. By light microscopy, amyloid appears like hyaline (homogenous eosinophilic material).
    b. Although biochemically heterogenous, all forms of amyloid have the following common features:
        1) B-Pleated sheet structure on x-ray crystallography and infrared spectroscopy
        2) Beaded fibrillar appearance when stained with Congo red dye and examined under polarized light
2. **Important clinical forms of amyloidosis**
    a. **Primary amyloidosis**, a typical feature of multiple myeloma, is characterized by deposits of AL amyloid, which is derived from the immunoglobulin light chain. AL amyloid deposits are found in the kidneys, blood vessels and heart.
    b. **Secondary amyloidosis** is characterized by deposits of AA amyloid, which is derived from serum amyloid-associated protein. Serum amyloid-associated protein is produced by the liver in chronic inflammatory or autoimmune diseases (like chronic osteomyelitis, tuberculosis and rheumatoid arthritis). AA amyloid deposits are found in the kidneys, liver and spleen.

> The diagnosis of amyloidosis can be made only by biopsy; kidney biopsy in patients who have renal symptoms, or a gingival, rectal or subcutaneous fat tissue biopsy in others.

REVIEW QUESTIONS

Name 4 adaptive responses to stress.	Hyperplasia, hypertrophy, atrophy, metaplasia
Are adaptive responses to stress reversible?	Yes
What is hyperplasia?	Enlargement of a tissue secondary to an increase in the number of cells
Give examples of 2 types of hyperplasia.	Endometrium in the menstrual cycle (caused by physiologic stimuli) and female breast tissue during lactation
What is hypertrophy?	Increase in cell size and functional capacity
Give examples of 2 types of hypertrophy.	Skeletal muscle and myocardium (increased functional demand) and lactating breast (trophic hormones)
What is atrophy?	Decrease in cell size and function
What causes caseous necrosis?	Granulomatous inflammation caused by mycobacteria (tuberculosis)
What are the 2 types of gangrene?	1. "Wet gangrene" (liquefaction necrosis) 2. "Dry gangrene" (coagulation necrosis)
What are typical sites of gangrene?	Bowel wall and lower limbs (secondary to acute ischemia

What is fibrinoid necrosis?	Deposition of fibrinous material in damaged arterial walls
Under what circumstances does fat necrosis occur?	1. Hemorrhagic pancreatitis – liberated enzymes digest pancreatic fat 2. Trauma to fatty tissue in organs with large fat contents (e.g., breast)
Name 2 free radicals.	Superoxide O_2 and hydroxyl radical OH
Name 3 free-radical scavengers.	Superoxide dismutase, catalase, mannitol
Name 4 anti-oxidants.	Glutathione, vitamin E, transferrin, ceruloplasmin
How long can hepatocytes and myocardial cells survive ischemia?	1-2 hours
What is hydropic change?	Abnormal accumulation of water in cells
What is lipofuscin?	Breakdown product of lipids. It builds up in atrophic cells of elderly people.
What are the nuclear changes in necrosis?	1. Pyknosis (chromatin clumps) 2. Karyorrhexis (nucleus fragments) 3. Karyolysis (chromatin dissolves and fades) 4. Nuclear loss

What happens to cytoplasm in necrotic cells?	Increased eosinophilia
What is Barrett esophagus an example of?	Metaplasia: squamous epithelium transforms into gastric glandular mucosa secondary to acid reflux
What is the harbinger (main cause) of irreversible cell injury?	Massive intracellular buildup of calcium
How does autolysis accelerate cell death?	It doesn't. Autolysis takes place when a dead cell's own enzymes digest it (digest the cell itself!)
What kind of necrosis takes place in an abscess?	Liquefaction necrosis
What is pus?	Product of liquefaction necrosis: dead cell debris, PMNs, monocytes, lysosomal enzymes in an exudative and purulent thick material
What type of necrosis is seen in tuberculous granulomata?	Caseous necrosis
What type of necrosis might cause a hard breast mass after a car accident?	Fat necrosis saponification
What adaptive cellular change takes place in the heart of a chronically hypertensive man?	Hypertrophy
In which type of necrosis is tissue architecture	Coagulation necrosis

preserved?	
What type of cell death prevents us from having webbed fingers and toes?	Apoptosis
What type of adaptive response do foot cells have to uncomfortable shoes?	Hyperplasia (corns)
Give examples of 4 types of atrophy.	Muscle (denervation), vaginal mucosa (decreased trophic hormones), kidneys (ischemia) and brain (aging)
Give examples of 2 types of metaplasia.	Bronchial mucosa replaced by squamous epithelium (smoking) and cervical columnar epithelium replaced by squamous epithelium (cervicitis)
What are 4 signs of early hypoxic injury?	1. Hydropic change: pale, distended cytoplasm secondary to increased H_2O content (due to failure of plasma membrane barrier) 2. Swelling of endoplasmic reticulum 3. Swelling of mitochondria (dissipated energy gradient)

4. Ribosomal disaggregation (leads to failed protein synthesis)

What are signs of late, but still reversible, hypoxic injury?

1. Cell blebs: bubbles in cell membrane

2. Myelin figures: swirled blobs of denuded membrane

What is the point at which the progression of Ischemic cell injury becomes irreversible?

Massive calcium influx leading to mitochondrial and membrane damage

How long does it take for ischemic injury to become irreversible in the heart, liver, brain and skeletal muscle?

Myocardial cells and hepatocytes: 1-2 hours

Neurons: 3-5 minutes (especially hippocampus and Purkinje cells)

Skeletal muscles: variable (2-8 hours)

CELLULAR ACCUMULATIONS

What causes materials to accumulate abnormally in cells?

1. Failure of the mechanism involved in removal or metabolism of normal substance

2. Inability to remove or metabolize abnormal substance

What causes fatty change in the liver?

Imbalance of production, utilization and mobilization of fats:

1. ↑ entry of fatty acids into cells
2. ↑ synthesis of fatty acids
3. ↓ use of fatty acids
4. ↓ mobilization of fatty acids [↓apoprotein Synthesis (e.g., in CCl_4 toxicity or malnutrition)]

What are the terms for abnormal cellular accumulation of the following?

Bilirubin	Kernicterus-jaundice
Iron	Hemosiderosis (macrophages), hemochromatosis (parenchymal cells)
Melanin	Suntan (change in skin color because of ultraviolet rays in sun)
Silver	Argyria
Water	Hydropic change

What is the "wear-and-tear" pigment?

Lipofuscin. The breakdown product of lipids which accumulate in atrophic cells of elderly people as "brown atrophy."

Name 2 types of abnormal calcification.

1. Metastatic calcification – hypercalcemia

Resulting in deposition of calcium in living tissue.

2. Dystrophic calcification – deposition of calcium in damaged tissue.

NECROSIS

What is necrosis?

Death and degradation of cells from severe environmental insult

What is apoptosis?

Programmed, energy-dependent cell death

Give 2 examples of apoptosis.

Embryogenesis (excess tissue is killed off and removed during apoptosis); shedding of endometrium during menstrual cycle

What is autolysis?

Degradation of cells by their own lysosomal enzymes

How is autolysis different from necrosis?

Autolysis follows cell death; autolysis and heterolysis help to dissolve necrotic tissue

What cytoplasmic change occurs in necrosis?

Increased eosinophilia (and decreased basophilia) secondary to loss of RNA and denaturation of proteins by ↓ pH.

What nuclear changes take place in necrosis?

1. Pyknosis: condensation of chromatin (ball sitting in nucleus)

2. Karyorrhexis: fragmentation of nucleus

	3. Karyolysis: fading of chromatin secondary to dissolution by proteolytic enzymes 4. Nuclear loss
What are the 6 types of necrosis?	Coagulation, caseous, liquefaction, gangrenous, fibrinoid and fat
What does early coagulation necrosis look like?	Tissue architecture is preserved, cytoplasm is eosinophilic
Where and when does coagulation necrosis occur?	Heart, lung, kidney or spleen, usually after infarction
How does liquefaction necrosis differ from coagulation necrosis?	Loss of architecture occurs in liquefaction necrosis: tissue is softened and liquefied by autolysis
What is a typical site of liquefaction necrosis?	CNS (autolysis) and suppurative infection (abscess)
What is caseous necrosis?	A combination of coagulation and liquefaction necrosis. Caseous means "cheese like."