Shree H. N. Shukla Institute ofPharmaceutical Education andResearch,Rajkot

B.Pharm

Semester

III

Subject Name: Pathophysiology

Subject Code: BP304TT

Basic principles of Cell injury and Adaptation

DEFINITION OF PATHOLOGY

The word 'Pathology' is derived from two Greek words—pathos meaning suffering, and logos meaning study. Pathology is, thus, scientific study of structure and function of the body in disease; or in other words, pathology consists of the abnormalities that occur in normal anatomy (including histology) and physiology owing to disease. Another commonly used term with reference to study of diseases is 'pathophysiology' comprised by two words: patho=suffering; physiology=study of normal function.

TERMINOLOGY IN PATHOLOGY

It is important for a beginner in pathology to be familiar with the language used in pathology:

Patient is the person affected by disease.

Lesions are the characteristic changes in tissues and cells produced by disease in an individual or experimental animal.

Pathologic changes or morphology consist of examination of diseased tissues.

Pathologic changes can be recognised with the naked eye (gross or macroscopic changes) or studied by microscopic examination of tissues.

Causal factors responsible for the lesions are included in etiology of disease (i.e. 'why' of disease).

Mechanism by which the lesions are produced is termed pathogenesis of disease (i.e. 'how' of disease).

Functional implications of the lesion felt by the patient are symptoms and those discovered by the clinician are the physical signs.

Clinical significance of the morphologic and functional changes together with results of other investigations help to arrive at an answer to what is wrong (diagnosis), what is going to happen (prognosis), what can be done about it (treatment), and finally what should be done to avoid complications and spread (prevention) (i.e. 'what' of disease).

ETIOLOGY OF CELL INJURY

The cells may be broadly injured by two major ways:

A. By genetic causes – genetically defect.

B. By acquired causes

The acquired causes of disease comprise vast majority of common diseases afflicting mankind. Based on underlying agent, the acquired causes of cell injury can be further categorised as under:

1. Hypoxia and ischaemia

2. Physical agents

3. Chemical agents and drugs

4. Microbial agents

5. Immunologic agents

6. Nutritional derangements

7. Aging

8. Psychogenic diseases

9. Iatrogenic factors

10. Idiopathic diseases.

1. HYPOXIA AND ISCHAEMIA.

Cells of different tissues essentially require oxygen to generate energy and perform metabolic functions. Deficiency of oxygen or hypoxia results in failure to carry out these activities by the cells.

Hypoxia is the most common cause of cell injury. Hypoxia may result from the following: The most common mechanism of hypoxic cell injury is by reduced supply of blood to cells due to interruption i.e. ischaemia.

However, hypoxia may result from other causes as well e.g. disorders of oxygen-carrying RBCs (e.g. anaemia, carbon monoxide poisoning), heart diseases, lung diseases and increased demand of tissues.

2. PHYSICAL AGENTS.

Physical agents in causation of disease are as under:

mechanical trauma (e.g. road accidents);

thermal trauma (e.g. by heat and cold);

electricity;

radiation (e.g. ultraviolet and ionising)

rapid changes in atmospheric pressure.

3. CHEMICALS AND DRUGS. An ever increasing list of chemical agents and drugs may cause cell injury. Important examples include the following: chemical poisons such as cyanide, arsenic, mercury; strong acids and alkalis; environmental pollutants; insecticides and pesticides; oxygen at high concentrations; hypertonic glucose and salt; social agents such as alcohol and narcotic drugs; and therapeutic administration of drugs.

4. MICROBIAL AGENTS. Injuries by microbes include infections caused by bacteria, rickettsiae, viruses, fungi, protozoa, metazoa, and other parasites.

5. IMMUNOLOGIC AGENTS. Immunity is a 'doubleedged sword'—it protects the host against various injurious agents but it may also turn lethal and cause cell injury e.g. hypersensitivity reactions; anaphylactic reactions; and autoimmune diseases.

6. NUTRITIONAL DERANGEMENTS. A deficiency or an excess of nutrients may result in nutritional imbalances. Nutritional deficiency diseases may be due to overall deficiency of nutrients (e.g. starvation), of protein calorie (e.g. marasmus, kwashiorkor), of minerals (e.g. anaemia), or of trace elements. Nutritional excess is a problem of affluent societies resulting in obesity, atherosclerosis, heart disease and hypertension.

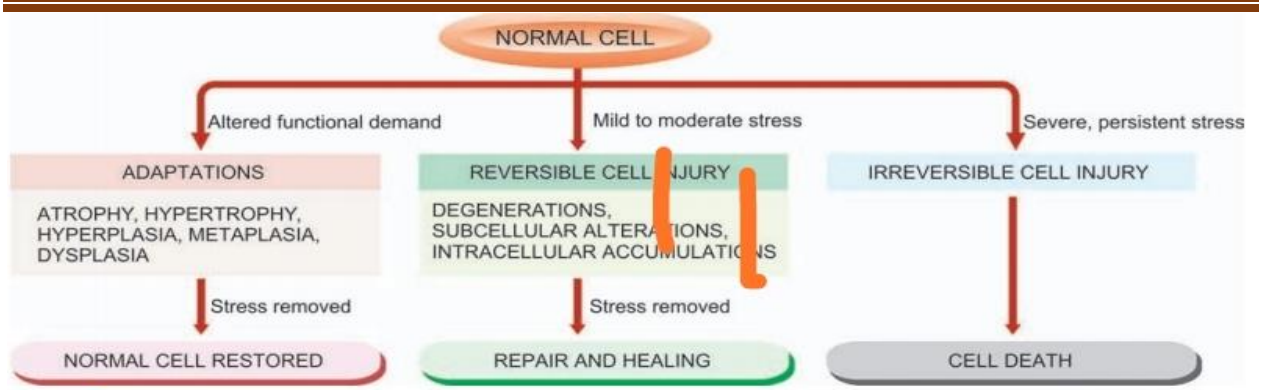
7.AGING. Cellular aging or senescence leads to impaired ability of the cells to undergo replication and repair, and ultimately lead to cell death culminating in death of the individual. This aspect is dealt at the end of this chapter.

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

9. IATROGENIC CAUSES. Although as per Hippocratic oath, every physician is bound not to do or administer anything that causes harm to the patient, there are some diseases as well as deaths attributed to iatrogenic causes (owing to physician). Examples include occurrence of disease or death due to error in judgment by the physician and untoward effects of administered therapy (drugs, radiation).

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

PATHOGENESIS OF CELL INJURY



ure 3.1 ◆ Cellular responses to cell injury.

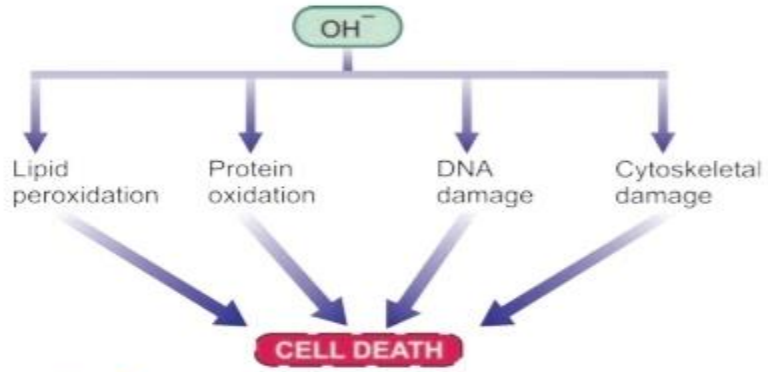
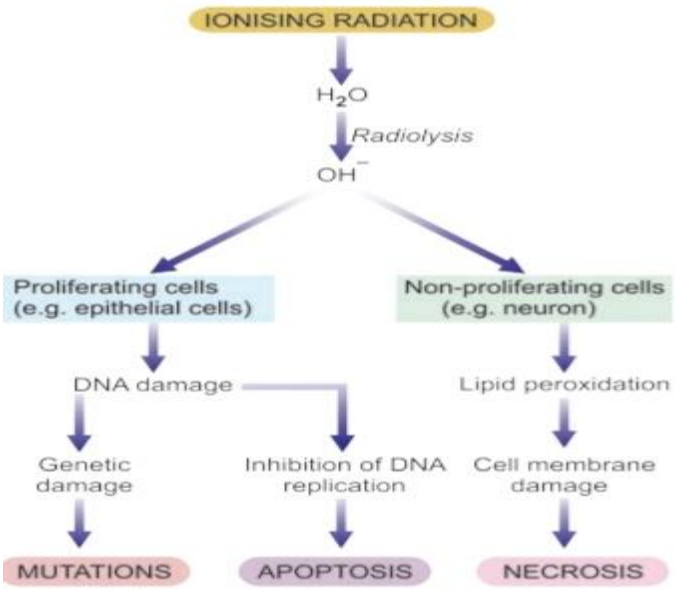


Figure 3.9 ◆ Mechanism of cell death by hydroxyl radical, the most reactive oxygen species.



3.10 ◆ Mechanisms of cell injury by ionising radiation.

Injury to the normal cell by one or more of the above listed etiologic agents may result in a state of reversible or irreversible cell injury. The underlying alterations in biochemical systems of cells for reversible and irreversible cell injury by various agents is complex and varied. However, in

general, the following principles apply in pathogenesis of most forms of cell injury by various agents:

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

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

3. Underlying intracellular phenomena: Irrespective of other factors, following essential biochemical phenomena underlie all forms of cell injury: i) Mitochondrial damage causing ATP depletion. ii) Cell membrane damage disturbing the metabolic and trans-membrane exchanges. iii). Release of toxic free radicals.

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

Pathogenesis of hypoxic and ischaemic cell injury is, therefore, described in detail below followed by brief discussion on pathogenesis of chemical and physical (ionising radiation) agents.

PATHOGENESIS OF ISCHAEMIC AND HYPOXIC INJURY

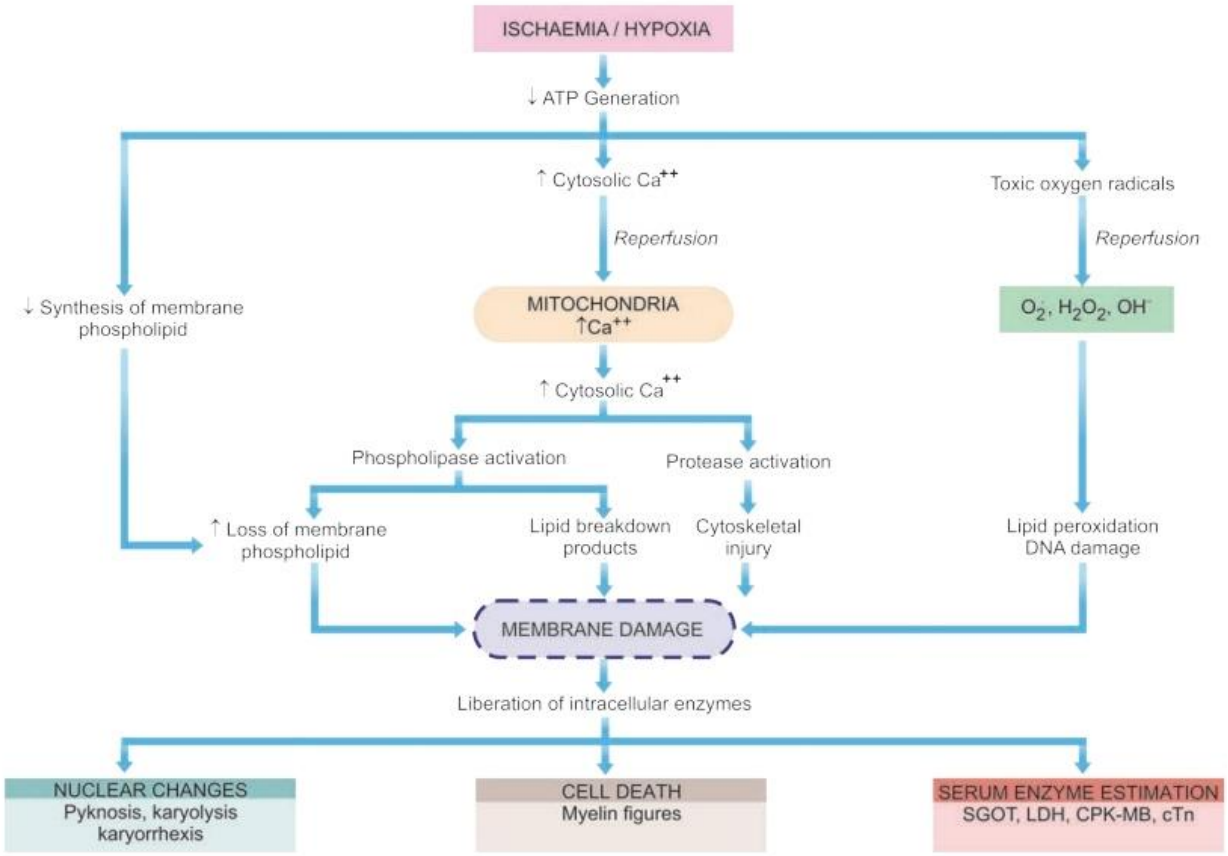
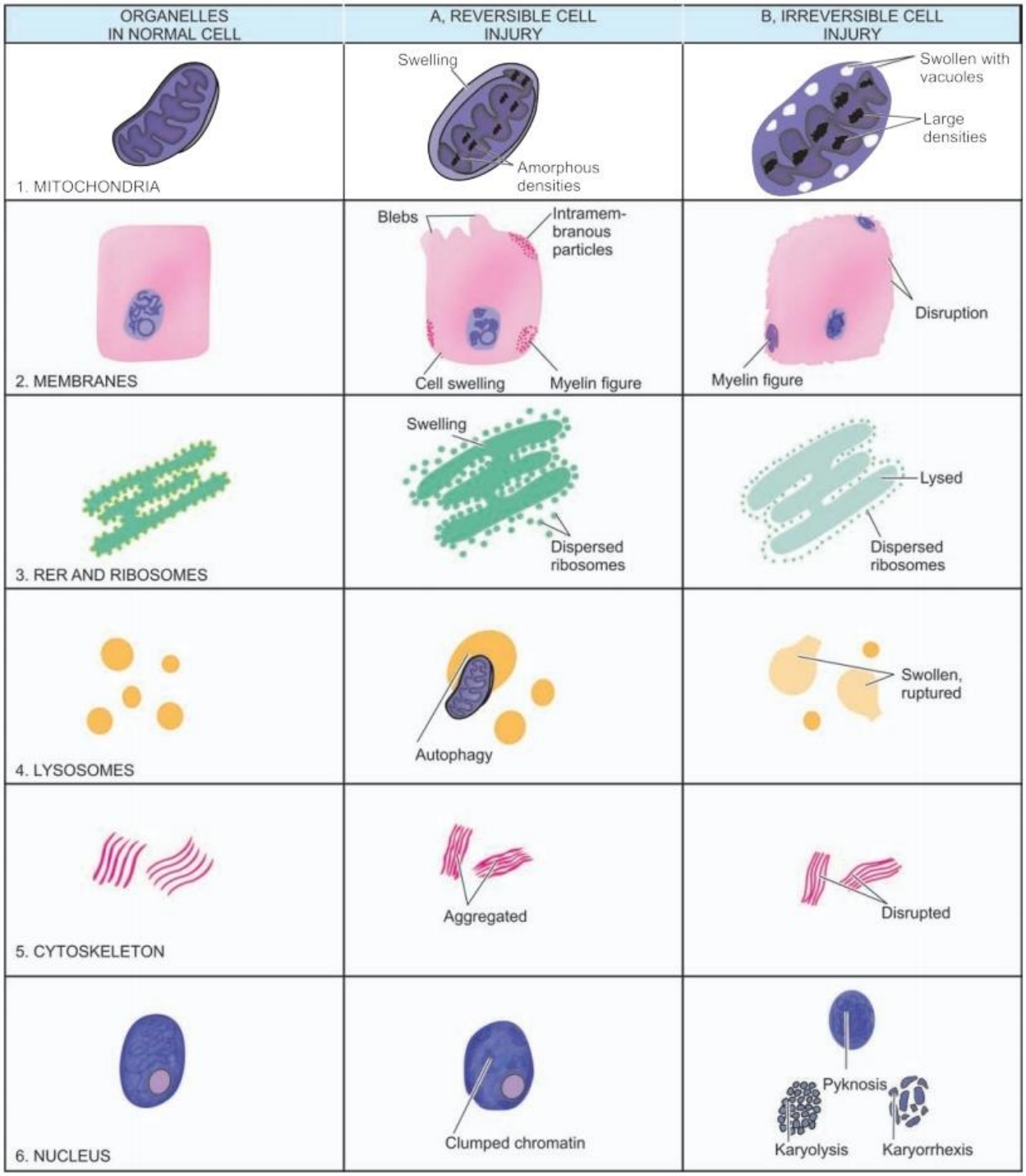


Figure 3.6 Sequence of events in the pathogenesis of reversible and irreversible cell injury caused by hypoxia/ischaemia.



igure 3.7 Ultrastructural changes during cell injury due to hypoxia-ischaemia.

REVERSIBLE CELL INJURY

1. Decreased generation of cellular ATP: Damage by ischaemia versushypoxia from other causes
2. Intracellular lactic acidosis:Nuclear clumping
3. Damage to plasma membrane pumps: Hydropic swelling and other membrane changes
4. Reduced protein synthesis: Dispersed ribosomes

IRREVERSIBLE CELL INJURY

1. Calcium influx: Mitochondrial damage.
   As a result of continued hypoxia, a large cytosolic influx of calcium ions occurs, especially after reperfusion of irreversibly injured cell. Excess intracellular calcium collects in the mitochondria disabling its function. Morphologically, mitochondrial changes are vacuoles in the mitochondria and deposits of amorphous calcium salts in the mitochondrial matrix.
2. Activated phospholipases: Membrane damage. Damage to membrane function in general, and plasma membrane in particular, is the most important event in irreversible cell injury in ischaemia. As a result of sustained ischaemia, there is increased cytosolic influx of calcium in the cell. Increased calcium activates endogenous phospholipases. These in turn degrade membrane phospholipids progressively which are the main constituent of the lipid bilayer membrane. Besides, there is also decreased replacement-synthesis of membrane phospholipids due to reduced ATP. Other lytic enzyme which is activated is ATPase which causes further depletion of ATP.
3. Intracellular proteases: Cytoskeletal damage. The normal cytoskeleton of the cell (microfilaments, microtubules and intermediate filaments) which anchors the cell membrane is damaged due to degradation by activated intracellular proteases or by physical effect of cell swelling producing irreversible cell membrane injury.
4. Activated endonucleases: Nuclear damage. The nucleoproteins are damaged by the activated lysosomal enzymes such as proteases and endonucleases. Irreversible damage to the nucleus can be in three forms:
   i) Pyknosis: Condensation and clumping of nucleus which becomes dark basophilic.
   ii) Karyorrhexis: Nuclear fragmentation in to small bits dispersed in the cytoplasm.
   iii) Karyolysis: Dissolution of the nucleus.
5. Lysosomal hydrolytic enzymes: Lysosomal damage, cell death and phagocytosis. The lysosomal membranes are damaged and result in escape of lysosomal hydrolytic enzymes. These enzymes are activated due to lack of oxygen in the cell and acidic pH. These hydrolytic enzymes include: hydrolase, RNAase, DNAase, protease, glycosidase, phosphatase, lipase, amylase, cathepsin etc) which on activation bring about enzymatic digestion of cellular components and hence cell death. The dead cell is eventually replaced by masses of phospholipids called myelin figures which are either phagocytosed by macrophages or there may be formation of calcium soaps.

MORPHOLOGY OF REVERSIBLE CELL INJURY

1. Hydropic change (cloudy swelling, or vacuolar degeneration)

2. Fatty change

3. Hyaline change

4. Mucoid change

Hydropic Change

Hydropic change means accumulation of water within the cytoplasm of the cell. Other synonyms used are cloudy swelling (for gross appearance of the affected organ) and vacuolar degeneration (due to cytoplasmic vacuolation). ETIOLOGY. This is the commonest and earliest form of cell injury from almost all causes. The common causes include acute and subacute cell injury from various etiologic agents such as bacterial toxins, chemicals, poisons, burns, high fever, intravenous administration of hypertonic glucose or saline etc.

PATHOGENESIS

Cloudy swelling results from impaired regulation of sodium and potassium at the level of cell membrane. This results in intracellular accumulation of sodium and escape of potassium. This, in turn, leads to rapid flow of water into the cell to maintain iso-osmotic conditions and hence cellular swelling occurs. In addition, influx of calcium too occurs. Hydropic swelling is an entirely reversible change upon removal of the injurious agent.

Hyaline Change

The word 'hyaline' means glassy (hyalos = glass). Hyaline is a descriptive histologic term for glassy, homogeneous, eosinophilic appearance of material in haematoxylin and eosin-stained sections and does not refer to any specific substance. Though fibrin and amyloid have hyaline appearance, they have distinctive features and staining reactions and can be distinguished from non-specific hyaline material. Hyaline change is associated with heterogeneous pathologic conditions. It may be intracellular or extracellular.

Mucoid Change

Mucus secreted by mucous glands is a combination of proteins complexed with mucopolysaccharides. Mucin, a glycoprotein, is its chief constituent. Mucin is normally produced by epithelial cells of mucous membranes and mucous glands, as well as by some connective tissues like in the umbilical cord. By convention, connective tissue mucin is termed myxoid (mucus like). Both types of mucin are stained by alcian blue. However, epithelial mucin stains positively with periodic acid-Schiff (PAS), while connective tissue mucin is PAS negative but is stained positively with colloidal iron.

EPITHELIAL MUCIN.

Following are some examples of functional excess of epithelial mucin:

1. Catarrhal inflammation of mucous membrane (e.g. of respiratory tract, alimentary tract, uterus).

2. Obstruction of duct leading to mucocele in the oral cavity and gallbladder.

3. Cystic fibrosis of the pancreas.

4. Mucin-secreting tumours (e.g. of ovary, stomach, large bowel etc)

CONNECTIVE TISSUE MUCIN.

A few examples of disturbances of connective tissue mucin are as under:

1. Mucoid or myxoid degeneration in some tumours e.g. myxomas, neurofibromas,fibroadenoma, soft tissue sarcomas etc.

2. Dissecting aneurysm of the aorta due to Erdheim's medial degeneration and Marfan's syndrome.

3. Myxomatous change in the dermis in myxoedema.

4. Myxoid change in the synovium in ganglion on the wrist.

INTRACELLULAR ACCUMULATIONS

Intracellular accumulation of substances in abnormal amounts can occur within the cytoplasm (especially lysosomes) or nucleus of the cell. This phenomenon was previously referred to as infiltration, implying thereby that something unusual has infiltrated the cell from outside which is not always the case. Intracellular accumulation of the substance in mild degree causes reversible cell injury while more severe damage results in irreversible cell injury. Such abnormal intracellular accumulations can be divided into 3 groups:

i) Accumulation of constituents of normal cell metabolism produced in excess e.g. accumulations of lipids (fatty change, cholesterol deposits), proteins and carbohydrates. In addition, deposits of amyloid and urate are discussed separately later.

ii) Accumulation of abnormal substances produced as a result of abnormal metabolism due to lack of some enzymes e.g. storage diseases or inborn errors of metabolism.

iii) Accumulation of pigments e.g. endogenous pigments under special circumstances, and exogenous pigments due to lack of enzymatic mechanisms to degrade the substances or transport them to other sites.

These pathologic states are discussed below.

FATTY CHANGE (STEATOSIS)

Fatty change, steatosis or fatty metamorphosis is the intracellular accumulation of neutral fat within parenchymal cells. It includes the older, now abandoned, terms of fatty degeneration and fatty infiltration because fatty change neither necessarily involves degeneration nor infiltration. The deposit is in the cytosol and represents an absolute increase in the intracellular lipids. It is especially common in the liver but may occur in other non-fatty tissues like the heart, skeletal muscle, kidneys (lipoid nephrosis or minimum change disease) and other organs.

Fatty Liver

Liver is the commonest site for accumulation of fat because it plays central role in fat metabolism. Depending upon the cause and amount of accumulation, fatty change may be mild and reversible, or severe producing irreversible cell injury and cell death.

ETIOLOGY.

1. Conditions with excess fat:

i) Obesity

ii) Diabetes mellitus

iii) Congenital hyperlipidaemia

2. Liver cell damage:

- Alcoholic liver disease (most common)
- Starvation
- Protein calorie malnutrition
- Chronic illnesses (e.g. tuberculosis)
- Acute fatty liver in late pregnancy
- Hypoxia (e.g. anaemia, cardiac failure)
- Hepatotoxins (e.g. carbon tetrachloride, chloroform, ether, aflatoxins and other poisons)
- Drug-induced liver cell injury (e.g. administration of methotrexate, steroids, CCl4, halothane anaesthetic, tetracycline etc)
- Reye's syndrome

Pigments of the Body.

A. ENDOGENOUS PIGMENTS

1. Melanin

2. Melanin-like pigment

- Alkaptonuria
- Dubin-Johnson syndrome

3. Haemoprotein-derived pigments

- Haemosiderin
- Acid haematin (Haemozoin)
- Bilirubin
- Porphyrins

4. Lipofuscin (Wear and tear pigment)

B. EXOGENOUS PIGMENTS

- Inhaled pigments
- Ingested pigments
- Injected pigments (Tattooing)

MORPHOLOGY OF IRREVERSIBLE CELL INJURY (CELL DEATH)

AUTOLYSIS

Autolysis (i.e. self-digestion) is disintegration of the cell by its own hydrolytic enzymes liberated from lysosomes. Autolysis can occur in the living body when it is surrounded by inflammatory reaction (vital reaction), but the term is generally used for postmortem change in which there is complete absence of surrounding inflammatory response. Autolysis is rapid in some tissues rich in hydrolytic enzymes such as in the pancreas, and gastric mucosa; intermediate in tissues like the heart, liver and kidney; and slow in fibrous tissue. Morphologically, autolysis is identified by homogeneous and eosinophilic cytoplasm with loss of cellular details and remains of cell as debris.

NECROSIS

Necrosis is defined as a localised area of death of tissue followed by degradation of tissue by hydrolytic enzymes liberated from dead cells; it is invariably accompanied by inflammatory reaction. Necrosis can be caused by various agents such as hypoxia, chemical and physical agents, microbial agents, immunological injury, etc. Two essential changes characterise irreversible cell injury in necrosis of all types

i) Cell digestion by lytic enzymes. Morphologically this change is identified as homogeneous and intensely eosinophilic cytoplasm. Occasionally, it may show cytoplasmic vacuolation or dystrophic calcification.
ii) Denaturation of proteins. This process is morphologically seen as characteristic nuclear changes in necrotic cell. These nuclear changes may include: condensation of nuclear chromatin (pyknosis) which may either undergo dissolution (karyolysis) or fragmentation into many granular clumps (karyorrhexis).

Types of Necrosis

1. COAGULATIVE NECROSIS. This is the most common type of necrosis caused by irreversible focal injury, mostly from sudden cessation of blood flow (ischaemia), and less often from bacterial and chemical agents. The organs commonly affected are the heart, kidney, and spleen.
2. LIQUEFACTION (COLLIQUATIVE) NECROSIS. Liquefaction or colliquative necrosis occurs commonly due to ischaemic injury and bacterial or fungal infections. It occurs due to degradation of tissue by the action of powerful hydrolytic enzymes. The common examples are infarct brain and abscess cavity.
3. CASEOUS NECROSIS. Caseous necrosis is found in the centre of foci of tuberculous infections. It combines features of both coagulative and liquefactive necrosis.

4. FAT NECROSIS. Fat necrosis is a special form of cell death occurring at two anatomically different locations but morphologically similar lesions. These are: following acute pancreatic necrosis, and traumatic fat necrosis commonly in breasts. In the case of pancreas, there is liberation of pancreatic lipases from injured or inflamed tissue that results in necrosis of the pancreas as well as of the fat depots throughout the peritoneal cavity, and sometimes, even affecting the extraabdominal adipose tissue.
5. FIBRINOID NECROSIS. Fibrinoid necrosis is characterised by deposition of fibrin-like material which has the staining properties of fibrin. It is encountered in various examples of immunologic tissue injury (e.g. in immune complex vasculitis, autoimmune diseases, Arthus reaction etc), arterioles in hypertension, peptic ulcer etc.

APOPTOSIS

Apoptosis is a form of 'coordinated and internally programmed cell death' having significance in a variety of physiologic and pathologic conditions (apoptosis is a Greek word meaning 'falling off' or 'dropping off'). The term was first introduced in 1972 as distinct from necrosis by being a form of cell death which is controlled and regulated by the rate of cell division; when the cell is not needed, pathway of cell death is activated ('cell suicide') and is unaccompanied by any inflammation and collateral tissue damage.

APOPTOSIS IN BIOLOGIC PROCESSES.

Apoptosis is responsible for mediating cell death in a wide variety of physiologic and pathologic processes as under:

Physiologic Processes:

1. Organised cell destruction in sculpting of tissues during development of embryo.

2. Physiologic involution of cells in hormone-dependent tissues e.g. endometrial shedding, regression of lactating breast after withdrawal of breast-feeding.

3. Normal cell destruction followed by replacement proliferation such as in intestinal epithelium.
4. Involution of the thymus in early age.

Pathologic Processes:

1. Cell death in tumours exposed to chemotherapeutic agents.

2. Cell death by cytotoxic T cells in immune mechanisms such as in graft-versus-host disease and rejection reactions.

3. Progressive depletion of CD4+T cells in the pathogenesis of AIDS.

4. Cell death in viral infections e.g. formation of Councilman bodies in viral hepatitis.

5. Pathologic atrophy of organs and tissues on withdrawal of stimuli e.g. prostatic atrophy after orchiectomy, atrophy of kidney or salivary gland on obstruction of ureter or ducts, respectively.

6. Cell death in response to injurious agents involved in causation of necrosis e.g. radiation, hypoxia and mild thermal injury.

7. In degenerative diseases of CNS e.g. in Alzheimer's disease, Parkinson's disease, and chronic infective dementias.

8. Heart diseases e.g. heart failure, acute myocardial infarction (20% necrosis and 80% apoptosis).

TABLE 3.4: Contrasting Features of Apoptosis and Necrosis.

	Feature	Apoptosis	Necrosis
1.	*Definition*	Programmed and coordinated cell death	Cell death along with degradation of tissue by hydrolytic enzymes
2.	*Causative agents*	Physiologic and pathologic processes	Hypoxia, toxins
3.	*Morphology*	i) No Inflammatory reaction ii) Death of single cells iii) Cell shrinkage iv) Cytoplasmic blebs on membrane v) Apoptotic bodies vi) Chromatin condensation vii) Phagocytosis of apoptotic bodies by macrophages	i) Inflammatory reaction always present ii) Death of many adjacent cells iii) Cell swelling initially iv) Membrane disruption v) Damaged organelles vi) Nuclear disruption vii) Phagocytosis of cell debris by macrophages
4.	*Molecular changes*	i) Lysosomes and other organelles intact ii) Genetic activation by proto-oncogenes and oncosuppressor genes, and cytotoxic T cell-mediated target cell killing iii) Initiation of apoptosis by intra- and extracellular stimuli, followed by activation of caspase pathway (FAS-R, BCL-2, p53)	i) Lysosomal breakdown with liberation of hydrolytic enzymes ii) Cell death by ATP depletion, membrane damage, free radical injury

GANGRENE

Gangrene is a form of necrosis of tissue with superadded putrefaction. The type of necrosis is usually coagulative due to ischaemia (e.g. in gangrene of the bowel, gangrene of limb). On the other hand, gangrenous or necrotising inflammation is characterised by primarily inflammation provoked by

virulent bacteria resulting in massive tissue necrosis. Thus, the end-result of necrotising inflammation and gangrene is the same but the way the two are produced, is different. The examples of necrotising inflammation are: gangrenous appendicitis, gangrenous stomatitis (noma, cancrum oris).

There are 2 main forms of gangrene—dry and wet, and a variant form of wet gangrene called gas gangrene. In all types of gangrene, necrosis undergoes liquefaction by the action of putrefactive bacteria.

Dry Gangrene

This form of gangrene begins in the distal part of a limb due to ischaemia. The typical example is the dry gangrene in the toes and feet of an old patient due to arteriosclerosis. Other causes of dry gangrene foot include thromboangiitis obliterans (Buerger's disease), Raynaud's disease, trauma, ergot poisoning.

It is usually initiated in one of the toes which is farthest from the blood supply, containing so little blood that even the invading bacteria find it hard to grow in the necrosed tissue. The gangrene

spreads slowly upwards until it reaches a point where the blood supply is adequate to keep the tissue viable. A line of separation is formed at this point between the gangrenous part and the viable part.

MORPHOLOGIC FEATURES.

Grossly, the affected part is dry, shrunken and dark black, resembling the foot of a mummy. It is black due to liberation of haemoglobin from haemolysed red blood cells which is acted upon by hydrogen disulfide (H2S) produced by bacteria resulting in formation of black iron sulfide. The line of separation usually brings about complete separation with eventual falling off of the gangrenous tissue if it is not removed surgically.

Wet Gangrene

Wet gangrene occurs in naturally moist tissues and organs such as the mouth, bowel, lung, cervix, vulva etc. Diabetic foot is another example of wet gangrene due to high sugar content in the necrosed tissue which favours growth of bacteria. Bed sores occurring in a bed-ridden patient due to pressure on sites like the sacrum, buttocks and heels are the other important clinical conditions included in wet gangrene. Wet gangrene usually develops rapidly due to blockage of

venous, and less commonly, arterial blood flow from thrombosis or embolism. The affected part is stuffed with blood which favours the rapid growth of putrefactive bacteria. The toxic products formed by bacteria are absorbed causing profound systemic manifestations of septicaemia, and finally death. The spreading wet gangrene generally lacks clear-cut line of demarcation and may spread to peritoneal cavity causing peritonitis.

GAS GANGRENE.

It is a special form of wet gangrene caused by gas-forming clostridia (gram-positive anaerobic bacteria) which gain entry into the tissues through open contaminated wounds, especially in the muscles, or as a complication of operation on colon which normally contains clostridia. Clostridia produce various toxins which produce necrosis and oedema locally and are also absorbed producing profound systemic manifestations.

CALCIFICATION

TABLE 3.6: Differences between Dystrophic and Metastatic Calcification.

	Feature	Dystrophic Calcification	Metastatic Calcification
1.	*Definition*	Deposits of calcium salts in dead and degenerated tissues	Deposits of calcium salts in normal tissues
2.	*Calcium metabolism*	Normal	Deranged
3.	*Serum calcium level*	Normal	Hypercalcaemia
4.	*Reversibility*	Generally irreversible	Reversible upon correction of metabolic disorder
5.	*Causes*	Necrosis (caseous, liquefactive, fat), infarcts, thrombi, haematomas, dead parasites, old scars, atheromas, Mönckeberg's sclerosis, certain tumours, cysts, calcinosis cutis	Hyperparathyroidism (due to adenoma, hyperplasia, CRF), bony destructive lesions (e.g. myeloma, metastatic carcinoma), prolonged immobilisation, hypervitaminosis D, milk-alkali syndrome, hypercalcaemia of infancy
6.	*Pathogenesis*	Increased binding of phosphates with necrotic and degenerative tissue, which in turn binds to calcium forming calcium phosphate precipitates	Increased precipitates of calcium phosphate due to hypercalcaemia at certain sites e.g. in lungs, stomach, blood vessels and cornea

Deposition of calcium salts in tissues other than osteoid or enamel is called pathologic or heterotopic calcification.

Two distinct types of pathologic calcification are recognised:

Dystrophic calcification, which is characterised by deposition of calcium salts in dead or degenerated tissues with normal calcium metabolism and normal serum calcium levels.

Metastatic calcification, on the other hand, occurs in apparently normal tissues and is associated with deranged calcium metabolism and hypercalcaemia.

Etiology and pathogenesis of the two are different but morphologically the deposits in both resemble normal minerals of the bone.

Etiopathogenesis

The two types of pathologic calcification result from distinctly different etiologies and mechanisms.

DYSTROPHIC CALCIFICATION.

As apparent from definition, dystrophic calcification may occur due to 2 types of causes: Calcification in dead tissue

Calcification of degenerated tissue.

Calcification in dead tissue

Calcification of degenerated tissue.

1. Caseous necrosis in tuberculosis is the most common site for dystrophic calcification. Living bacilli may be present even in calcified tuberculous lesions, lymph nodes, lungs, etc (Fig. 3.36).

2. Liquefaction necrosis in chronic abscesses may get calcified.

3. Fat necrosis following acute pancreatitis or traumatic fat necrosis in the breast results in deposition of calcium soaps.

4. Gamna-Gandy bodies in chronic venous congestion (CVC) of the spleen is characterised by calcific deposits admixed with haemosiderin on fibrous tissue.

5. Infarcts may sometimes undergo dystrophic calcification.

6. Thrombi, especially in the veins, may produce phleboliths.

7. Haematomas in the vicinity of bones may undergo dystrophic calcification.

8. Dead parasites like in hydatid cyst, Schistosoma eggs, and cysticercosis are some of the examples showing dystrophic calcification.

9. Calcification in breast cancer detected by mammography.

10. Congenital toxoplasmosis involving the central nervous system visualised by calcification in the infant brain.

Calcification in degenerated tissues

1. Dense old scars may undergo hyaline degeneration and subsequent calcification.

2. Atheromas in the aorta and coronaries frequently undergo calcification.

3. Mönckeberg's sclerosis shows calcification in the tunica

media of muscular arteries in elderly people.

4. Stroma of tumours such as uterine fibroids, breast cancer, thyroid adenoma, goitre etc show calcification.

5. Some tumours show characteristic spherules of calcification called psammoma bodies or calcospherites such as in meningioma, papillary serous cystadenocarcinoma of the ovary and papillary carcinoma of the thyroid.

6. Cysts which have been present for a long time may show calcification of their walls e.g. epidermal and pilar cysts.

7. Calcinosis cutis is a condition of unknown cause in which there are irregular nodular deposits of calcium salts in the skin and subcutaneous tissue.

8. Senile degenerative changes may be accompanied by dystrophic calcification such as in costal cartilages, tracheal or bronchial cartilages, and pineal gland in the brain etc.

METASTATIC CALCIFICATION.

Since metastatic calcification occurs in normal tissues due to hypercalcaemia, its causes would include one of the following two conditions:

- Excessive mobilisation of calcium from the bone.
- Excessive absorption of calcium from the gut.

Free radical induced injury

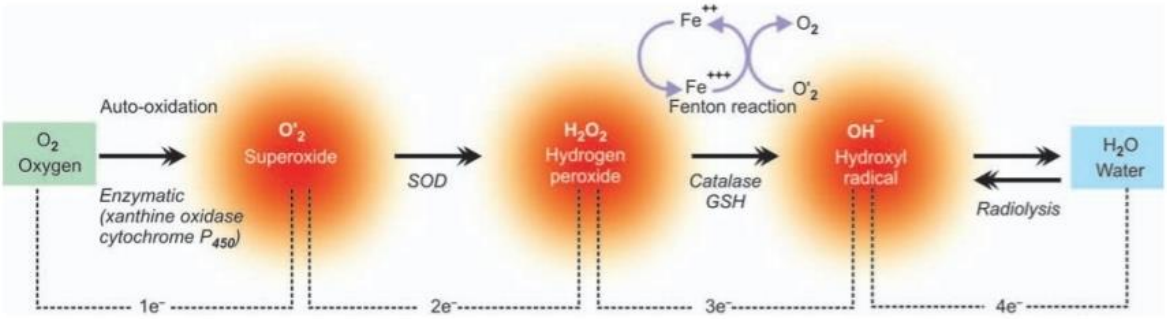
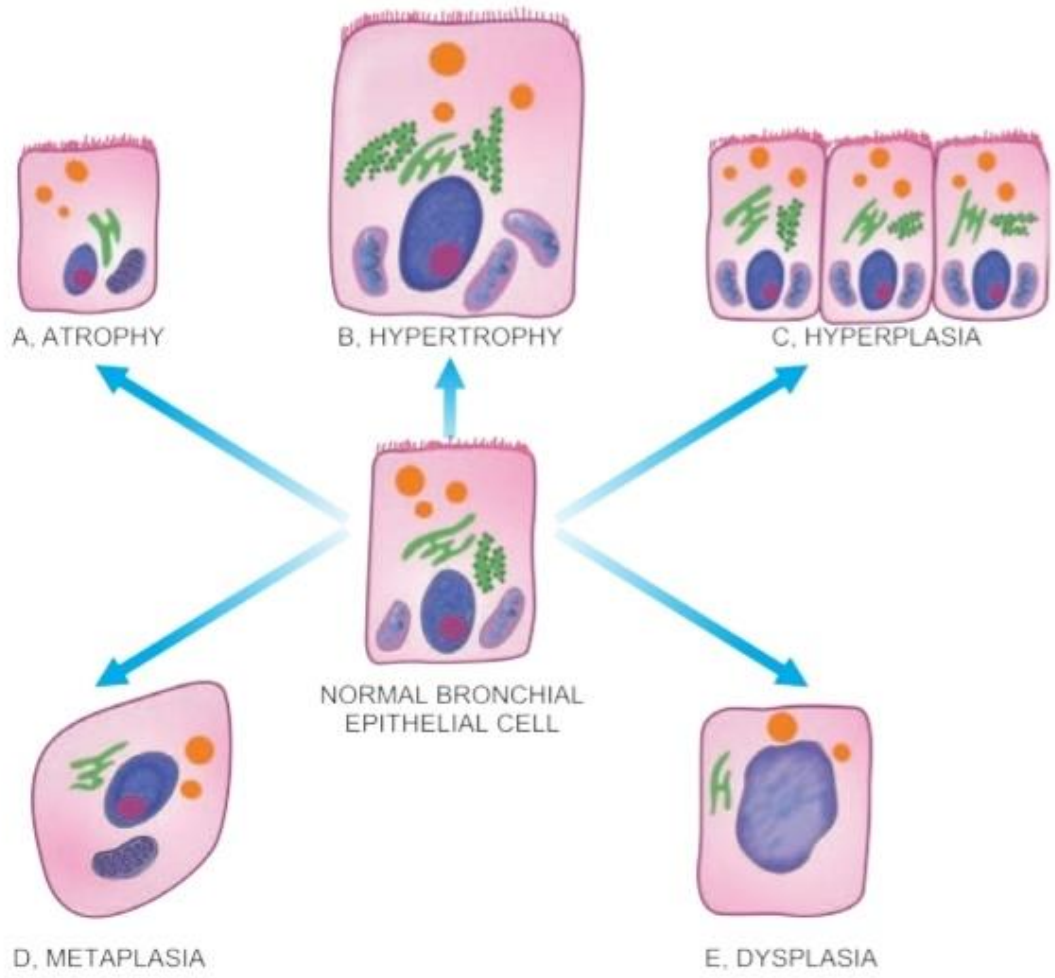


Figure 3.8 Mechanisms of generation of free radicals by four electron step reduction of oxygen. (SOD = *superoxide dismutase;* GSH = *glutathione peroxidase*).

CELLULAR ADAPTATIONS

For the sake of survival on exposure to stress, the cells make adjustments with the changes in their environment (i.e. adapt) to the physiologic needs (physiologic adaptation) and to non-lethal pathologic injury (pathologic adaptation). Broadly speaking, such physiologic and pathologic adaptations occur by following processes.



Adaptive disorders of growth.

ATROPHY

Reduction of the number and size of parenchymal cells of an organ or its parts which was once normal is called atrophy (compared from hypoplasia which is the term used for developmentally small size, and aplasia for extreme failure of development so that only rudimentary tissue is present).

CAUSES.

Atrophy may occur from physiologic or pathologic causes:

A. Physiologic atrophy. Atrophy is a normal process of aging in some tissues, which could be due to loss of endocrine stimulation or arteriosclerosis.

For example:

i) Atrophy of lymphoid tissue in lymph nodes, appendix and thymus.

ii) Atrophy of gonads after menopause.
iii) Atrophy of brain with aging.

B. Pathologic atrophy. The causes are as under:

1. Starvation atrophy. In starvation, there is first depletion of carbohydrate and fat stores followed by protein catabolism. There is general weakness, emaciation and anaemia referred to as cachexia seen in cancer and severely ill patients.

2. Ischaemic atrophy. Gradual diminution of blood supply due to atherosclerosis may result in shrinkage of the affected organ

e.g. i) Small atrophic kidney in atherosclerosis of renal artery.

ii) Atrophy of brain in cerebral atherosclerosis.

3. Disuse atrophy. Prolonged diminished functional activity is associated with disuse atrophy of the organ

e.g. i) Wasting of muscles of limb immobilised in cast.

ii) Atrophy of. the pancreas in obstruction of pancreatic duct.

4. Neuropathic atrophy. Interruption in nerve supply leads to wasting of muscles

e.g. Poliomyelitis, Motor neuron disease, Nerve section.

5. Endocrine atrophy. Loss of endocrine regulatory mechanism results in reduced metabolic activity of tissues and hence atrophy

e.g. i) Hypopituitarism may lead to atrophy of thyroid, adrenal and gonads.
ii) Hypothyroidism may cause atrophy of the skin and its adnexal structures.

6. Pressure atrophy. Prolonged pressure from benign tumours or cyst or aneurysm may cause compression and atrophy of the tissues

e.g. i) Erosion of spine by tumour in nerve root.

ii) Erosion of skull by meningioma arising from piaarachnoid.

iii) Erosion of sternum by aneurysm of arch of aorta.

7. Idiopathic atrophy. There are some examples of atrophy where no obvious cause is present
e.g. i) Myopathies.

ii) Testicular atrophy.

HYPERTROPHY

Hypertrophy is an increase in the size of parenchymal cells resulting in enlargement of the organ or tissue, without any change in the number of cells.

CAUSES. Hypertrophy may be physiologic or pathologic. In both cases, it is caused either by increased functional demand or by hormonal stimulation. Hypertrophy without accompanying hyperplasia affects mainly muscles. In nondividing cells too, only hypertrophy occurs.

A. Physiologic hypertrophy. Enlarged size of the uterus in pregnancy is an excellent example of physiologic hypertrophy as well as hyperplasia.

B. Pathologic hypertrophy. Examples of certain diseases associated with hypertrophy are as under:

1. Hypertrophy of cardiac muscle may occur in a number of cardiovascular diseases. A few conditions producing left ventricular hypertrophy are as under: i) Systemic hypertension ii) Aortic valve disease (stenosis and insufficiency) iii) Mitral insufficiency

2. Hypertrophy of smooth muscle e.g. i) Cardiac achalasia (in oesophagus) ii) Pyloric stenosis (in stomach) iii) Intestinal strictures iv) Muscular arteries in hypertension.

3. Hypertrophy of skeletal muscle e.g. hypertrophied muscles in athletes and manual labourers.

4. Compensatory hypertrophy may occur in an organ when the contralateral organ is removed e.g. i) Following nephrectomy on one side in a young patient, there is compensatory hypertrophy as well as hyperplasia of the nephrons of the other kidney. ii) Adrenal hyperplasia following removal of one adrenal gland.

HYPERPLASIA

Hyperplasia is an increase in the number of parenchymal cells resulting in enlargement of the organ or tissue. Quite often, both hyperplasia and hypertrophy occur together. Hyperplasia occurs due to increased recruitment of cells from G0 (resting) phase of the cell cycle to undergo mitosis, when stimulated.

CAUSES.

As with other non-neoplastic adaptive disorders of growth, hyperplasia has also been divided into physiologic and pathologic.

A. Physiologic hyperplasia. The two most common types are as follows:

1. Hormonal hyperplasia i.e. hyperplasia occurring under the influence of hormonal stimulation e.g. i) Hyperplasia of female breast at puberty, during pregnancy and lactation.

ii) Hyperplasia of pregnant uterus.

iii) Proliferative activity of normal endometrium after a normal menstrual cycle.

iv) Prostatic hyperplasia in old age.

2. Compensatory hyperplasia i.e. hyperplasia occurring following removal of part of an organ or a contralateral organ in paired organ e.g.

i) Regeneration of the liver following partial hepatectomy

ii) Regeneration of epidermis after skin abrasion

iii) Following nephrectomy on one side, there is hyperplasia of nephrons of the other kidney.

B. Pathologic hyperplasia. Most examples of pathologic hyperplasia are due to excessive stimulation of hormones or growth factors e.g.

i) Endometrial hyperplasia following oestrogen excess.

ii) In wound healing, there is formation of granulation tissue due to proliferation of fibroblasts and endothelial cells.

iii) Formation of skin warts from hyperplasia of epidermis due to human papilloma virus.

iv) Pseudocarcinomatous hyperplasia of the skin. v) Intraductal epithelial hyperplasia in the breast in fibrocystic breast disease.

METAPLASIA

Metaplasia is defined as a reversible change of one type of epithelial or mesenchymal adult cells to another type of adult epithelial or mesenchymal cells, usually in response to abnormal stimuli, and often reverts back to normal on removal of stimulus. However, if the stimulus persists for a long time, epithelial metaplasia may transform into cancer.

A. EPITHELIAL METAPLASIA. This is the more common type. The metaplastic change may be patchy or diffuse and usually results in replacement by stronger but less wellspecialised epithelium. However, the metaplastic epithelium being less well-specialised such as squamous type, results in deprivation of protective mucus secretion and hence more prone to infection. Depending upon the type epithelium transformed, two types of epithelial metaplasia are seen squamous and columnar:

1. Squamous metaplasia. This is more common. Various types of specialised epithelium are capable of undergoing squamous metaplastic change due to chronic irritation that may be mechanical, chemical or infective in origin. Some common examples of squamous metaplasia are seen at following sites:

i) In bronchus (normally lined by pseudostratified columnar ciliated epithelium) in chronic smokers.

ii) In uterine endocervix (normally lined by simple columnar epithelium) in prolapse of the uterus and in old age.

iii) In gallbladder (normally lined by simple columnar epithelium) in chronic cholecystitis with cholelithiasis.

iv) In prostate (ducts normally lined by simple columnar epithelium) in chronic prostatitis and oestrogen therapy.

v) In renal pelvis and urinary bladder (normally lined by transitional epithelium) in chronic infection and stones. vi) In vitamin A deficiency, apart from xerophthalmia, there is squamous metaplasia in the nose, bronchi, urinary tract, lacrimal and salivary glands.

2. Columnar metaplasia. There are some conditions in which there is transformation to columnar epithelium. For example:

i) Intestinal metaplasia in healed chronic gastric ulcer.

ii) Columnar metaplasia in Barrett's oesophagus, in which there is change of normal squamous epithelium to columnar epithelium.

B. MESENCHYMAL METAPLASIA. Less often, there is transformation of one adult type of mesenchymal tissue to another. The examples are as under:

1. Osseous metaplasia. Osseous metaplasia is formation of bone in fibrous tissue, cartilage and myxoid tissue. Examples of osseous metaplasia are as under:

i) In arterial wall in old age (Mönckeberg's medial calcific sclerosis)

ii) In soft tissues in myositis ossificans

iii) In cartilage of larynx and bronchi in elderly people iv) In scar of chronic inflammation of prolonged duration v) In the fibrous stroma of tumour.

2. Cartilaginous metaplasia. In healing of fractures, cartilaginous metaplasia may occur where there is undue mobility.

DYSPLASIA

Dysplasia means 'disordered cellular development', often accompanied with metaplasia and hyperplasia; it is therefore also referred to as atypical hyperplasia. Dysplasia occurs most often in epithelial cells. Epithelial dysplasia is characterised by cellular proliferation and cytologic changes. These changes include:

1. Increased number of layers of epithelial cells

2. Disorderly arrangement of cells from basal layer to the surface layer

3. Loss of basal polarity i.e. nuclei lying away from basement membrane

4. Cellular and nuclear pleomorphism

5. Increased nucleocytoplasmic ratio

6. Nuclear hyperchromatism

7. Increased mitotic activity.

The two most common examples of dysplastic changes are the uterine cervix and respiratory tract.

Dysplastic changes often occur due to chronic irritation or prolonged inflammation. On removal of the inciting stimulus, the changes may disappear. In a proportion of cases, however, dysplasia progresses into carcinoma in situ (cancer confined to layers superficial to basement membrane) or invasive cancer.

Metabolic Acidosis

A fall in the blood pH due to metabolic component is brought about by fall of bicarbonate level and excess of H+ ions in the blood. This occurs in the following situations:

- Production of large amounts of lactic acid (lactic acidosis)
- in vigorous exercise,
- shock.
- Uncontrolled diabetes mellitus (diabetic ketoacidosis).
- Starvation.
- Chronic renal failure.
- Therapeutic administration of ammonium chloride or acetazolamide (diamox).
- High blood levels of H+ ions in metabolic acidosis stimulate the respiratory centre so that the breathing is deep and rapid (air hunger or Kussmaul's respiration).
- There is fall in the plasma bicarbonate levels.

Metabolic Alkalosis

A rise in the blood pH due to rise in the bicarbonate levels of plasma and loss of H+ ions is called metabolic alkalosis. This is seen in the following conditions:

- Severe and prolonged vomitings.
- Administration of alkaline salts like sodium bicarbonate.
- Hypokalaemia such as in Cushing's syndrome, increased secretion of aldosterone

Clinically, metabolic alkalosis is characterised by depression of respiration, depressed renal function with uraemia and increased bicarbonate excretion in the urine. The blood level of bicarbonate is elevated.

Respiratory Acidosis

A fall in the blood pH occurring due to raised Pco2 consequent to underventilation of lungs (CO_2 retention) causes respiratory acidosis. This can occur in the following circumstances:

- Air obstruction as occurs in chronic bronchitis, emphysema, asthma.
- Restricted thoracic movement e.g. in pleural effusion, ascites, pregnancy, kyphoscoliosis.
- Impaired neuromuscular function e.g. in poliomyelitis, polyneuritis.

Clinically, there is peripheral vasodilatation and raised intracranial pressure. If there is severe CO_2 retention, patients may develop confusion, drowsiness and coma. The arterial Pco2 level is raised.

Respiratory Alkalosis

A rise in the blood pH occurring due to lowered Pco2 consequent to hyperventilation of the lungs (excess removal of CO2) is called respiratory alkalosis. This is encountered in the following conditions:

- Hysterical overbreathing
- Working at high temperature
- At high altitude
- Meningitis,
- encephalitis
- Salicylate intoxication

Clinically, the patients with respiratory alkalosis are characterized by peripheral vasoconstriction and consequent pallor, lightheadedness and tetany. The arterial Pco2 is lowered.

INFLAMMATION

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent, followed by removal of the necrosed cells and tissues.

The agents causing inflammation may be as under:

1. Infective agents like bacteria, viruses and their toxins, fungi, parasites.
2. Immunological agents like cell-mediated and antigenantibody reactions.
3. Physical agents like heat, cold, radiation, mechanical trauma.
4. Chemical agents like organic and inorganic poisons.
5. Inert materials such as foreign bodies.

SIGNS OF INFLAMMATION

The Roman writer Celsus in 1st century A.D. named the famous 4 cardinal signs of inflammation as:

rubor (redness);

tumor (swelling);

calor (heat); and

dolor (pain).

To these, fifth sign functio laesa (loss of function) was later added by Virchow.

TYPES OF INFLAMMATION.

Depending upon the defense capacity of the host and duration of response, inflammation can be classified as acute and chronic.

A. Acute inflammation is of short duration (lasting less than 2 weeks) and represents the early body reaction, resolves quickly and is usually followed by healing. The main features of acute inflammation are:

1. accumulation of fluid and plasma at the affected site;

2. intravascular activation of platelets; and

3. polymorphonuclear neutrophils as inflammatory cells. Sometimes, the acute inflammatory response may be quite severe and is termed as fulminant acute inflammation.

B. Chronic inflammation is of longer duration and occurs either after the causative agent of acute inflammation persists for a long time, or the stimulus is such that it induces chronic inflammation

from the beginning. A variant, chronic active inflammation, is the type of chronic inflammation in which during the course of disease there are acute exacerbations of activity.

ACUTE INFLAMMATION

Acute inflammatory response by the host to any agent is a continuous process but for the purpose of discussion, it can be divided into following two events:

I. Vascular events.
II. Cellular events.

1. VASCULAR EVENTS

Alteration in the microvasculature (arterioles, capillaries and venules) is the earliest response to tissue injury. These alterations include:

- haemodynamic changes
- changes in vascular permeability.

Haemodynamic Changes

The earliest features of inflammatory response result from changes in the vascular flow and calibre of small blood vessels in the injured tissue. The sequence of these changes is as under:

1. Irrespective of the type of injury, immediate vascular response is of transient vasoconstriction of arterioles. With mild Inflammation and Healing form of injury, the blood flow may be re-established in 3-5 seconds while with more severe injury the vasoconstriction may last for about 5 minutes.

2. Next follows persistent progressive vasodilatation which involves mainly the arterioles, but to a lesser extent, affects other components of the microcirculation like venules and capillaries. This change is obvious within half an hour of injury. Vasodilatation results in increased blood volume in microvascular bed of the area, which is responsible for redness and warmth at the site of acute inflammation.

3. Progressive vasodilatation, in turn, may elevate the local hydrostatic pressure resulting in transudation of fluid into the extracellular space. This is responsible for swelling at the local site of acute inflammation.

4. Slowing or stasis of microcirculation follows which causes increased concentration of red cells, and thus, raised blood viscosity.

5. Stasis or slowing is followed by leucocytic margination or peripheral orientation of leucocytes (mainly neutrophils) along the vascular endothelium. The leucocytes stick to the vascular endothelium briefly, and then move and migrate through the gaps between the endothelial cells into the extravascular space. This process is known as emigration.

changes in vascular permeability

PATHOGENESIS.

In and around the inflamed tissue, there is accumulation of oedema fluid in the interstitial compartment which comes from blood plasma by its escape through the endothelial wall of peripheral vascular bed. In the initial stage, the escape of fluid is due to vasodilatation and consequent elevation in hydrostatic pressure. This is transudate in nature.

But subsequently, the characteristic inflammatory oedema, exudate, appears by increased vascular permeability of microcirculation.

MECHANISMS OF INCREASED VASCULAR PERMEABILITY.

I. Contraction of endothelial cells. This is the most common mechanism of increased leakiness that affects venules exclusively while capillaries and arterioles remain unaffected. The endothelial cells develop temporary gaps between them due to their contraction resulting in vascular leakiness. It is mediated by the release of histamine, bradykinin and other chemical mediators. The response begins immediately after injury, is usually reversible, and is for short duration (15-30 minutes).

II. Retraction of endothelial cells. In this mechanism, there is structural re-organisation of the cytoskeleton of endothelial cells that causes reversible retraction at the intercellular junctions. This change too affects venules and is mediated by cytokines such as interleukin-1 (IL-1) and tumour necrosis factor (TNF)-α. The onset of response takes 4-6 hours after injury and lasts for 2-4 hours or more (somewhat delayed and prolonged leakage).

III. Direct injury to endothelial cells. Direct injury to the endothelium causes cell necrosis and appearance of physical gaps at the sites of detached endothelial cells. Process of thrombosis is initiated at the site of damaged endothelial cells. The change affects all levels of microvasculature (venules, capillaries and arterioles). The increased permeability may either appear immediately after injury and last for several hours or days (immediate sustained leakage), or may occur after a delay of 2-12 hours and last for hours or days (delayed prolonged leakage).

IV. iv) Endothelial injury mediated by leucocytes. Adherence of leucocytes to the endothelium at the site of inflammation may result in activation of leucocytes. The activated leucocytes release proteolytic enzymes and toxic oxygen species which may cause endothelial injury and increased vascular leakiness.

V. v) Leakiness in neovascularisation. In addition, the newly formed capillaries under the influence of vascular endothelial growth factor (VEGF) during the process of repair and in tumours are excessively leaky.

II. CELLULAR EVENTS

The cellular phase of inflammation consists of 2 processes:

1. exudation of leucocytes; and

2. phagocytosis.

1. Exudation of Leucocytes

The escape of leucocytes from the lumen of microvasculature to the interstitial tissue is the most important feature of inflammatory response.

The changes leading to migration of leucocytes are as follows:

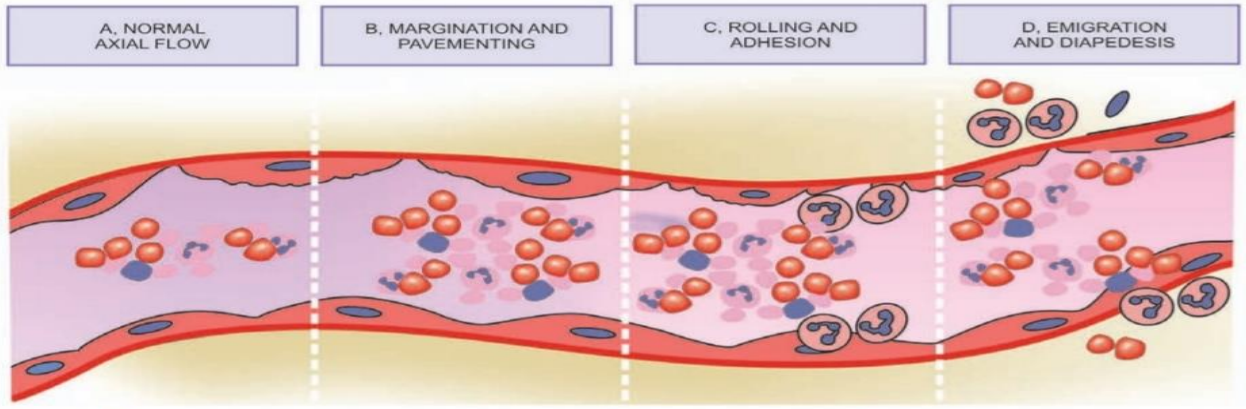


Figure 6.4 Sequence of changes in the exudation of leucocytes. A, Normal axial flow of blood with central column of cells and peripheral zone of cell-free plasma. B, Margination and pavementing of neutrophils with narrow plasmatic zone. C, Adhesion of neutrophils to endothelial cells with pseudopods in the intercellular junctions. D, Emigration of neutrophils and diapedesis with damaged basement membrane.

1. CHANGES IN THE FORMED ELEMENTS OF BLOOD. In the early stage of inflammation, the rate of flow of blood is increased due to vasodilatation. But subsequently, there is slowing or stasis of bloodstream. With stasis, changes in the normal axial flow of blood in the microcirculation take place. The normal axial flow consists of central stream of cells comprised by leucocytes and RBCs and peripheral cellfree layer of plasma close to vessel wall. Due to slowing and stasis, the central stream of cells widens and peripheral plasma zone becomes narrower because of loss of plasma by exudation. This phenomenon is known as margination. As a result of this redistribution, the neutrophils of the central column come close to the vessel wall; this is known as pavementing.
2. ROLLING AND ADHESION. Peripherally marginated and pavemented neutrophils slowly roll over the endothelial cells lining the vessel wall (rolling phase). This is followed by the transient bond between the leucocytes and endothelial cells becoming firmer (adhesion phase). The following molecules bring about rolling and adhesion phases:

   i) **Selectin**s are expressed on the surface of activated endothelial cells which recognise specific carbohydrate groups found on the surface of neutrophils, the most important of which is s-Lewis X molecule. While P-selectin (preformed and stored in endothelial cells and platelets) is involved in rolling, E-selectin (synthesised by cytokineactivated endothelial cells) is associated with both rolling and adhesion; L-selectin (expressed on the surface of lymphocytes and neutrophils) is responsible for homing of circulating lymphocytes to the endothelial cells in lymph nodes.

   ii**) Integrins** on the endothelial cell surface are activated during the process of loose and transient adhesions between endothelial cells and leucocytes. At the same time the receptors for integrins on the neutrophils are also stimulated. This process brings about firm adhesion between leucocyte and endothelium.

iv) **Immunoglobulin** gene superfamily adhesion molecule such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) allow a tighter adhesion and stabilise the interaction between leucocytes and endothelial cells. Platelet-endothelial cell adhesion molecule1 (PECAM-1) or CD31 may also be involved in leucocyte migration from the endothelial surface.

3. EMIGRATION. After sticking of neutrophils to endothelium, the former move along the endothelial surface till a suitable site between the endothelial cells is found where the neutrophils throw out cytoplasmic pseudopods.
4. CHEMOTAXIS. The chemotactic factor-mediated transmigration of leucocytes after crossing several barriers (endothelium, basement membrane, perivascular myofibroblasts and matrix) to reach the interstitial tissues is called chemotaxis. The concept of chemotaxis is well illustrated by Boyden's chamber experiment. In this, a millipore filter (3 µm pore size) separates the suspension of leucocytes from the test solution in tissue culture chamber. If the test solution contains chemotactic agent, the leucocytes migrate through the pores of filter towards the chemotactic agent (Fig. 6.5). The following agents act as potent chemotactic substances or chemokines for neutophils: i) Leukotriene B4 (LT-B4), a product of lipooxygenase pathway of arachidonic acid metabolites ii) Components of complement system (C5a and C3a in particular) iii) Cytokines (Interleukins, in particular IL-8) iv) Soluble bacterial products (such as formylated peptides).

Phagocytosis

Phagocytosis is defined as the process of engulfment of solid particulate material by the cells (cell-eating). The cells performing this function are called phagocytes. There are 2 main types of phagocytic cells:

i) Polymorphonuclear neutrophils (PMNs) which appear early in acute inflammatory response, sometimes called as microphages.
ii) Circulating monocytes and fixed tissue mononuclear phagocytes, commonly called as macrophages.

Neutrophils and macrophages on reaching the tissue spaces produce several proteolyitc enzymes—lysozyme, protease, collagenase, elastase, lipase, proteinase, gelatinase, and acid hydrolases. These enzymes degrade collagen and extracellular matrix. The microbe undergoes the process of phagocytosis by polymorphs and macrophages and involves the following 3 steps

1. Recognition and attachment

2. Engulfment

3. Killing and degradation

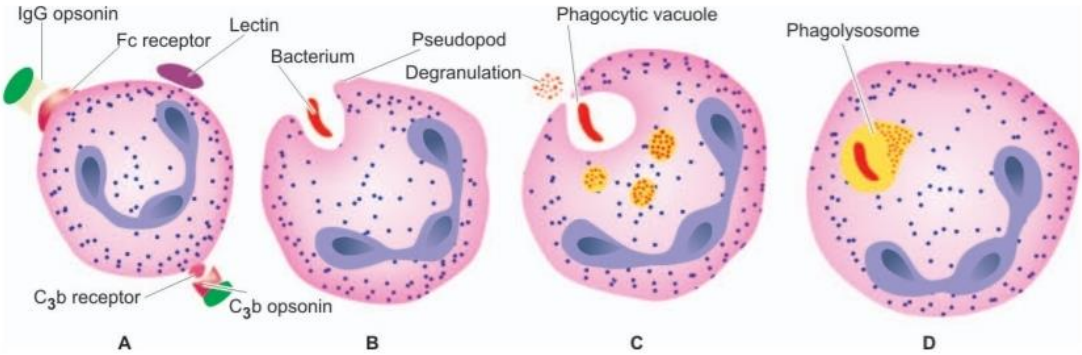


Figure 6.6 Stages in phagocytosis of a foreign particle. A, Opsonisation of the particle. B, Pseudopod engulfing the opsonised particle. C, Incorporation within the cell (phagocytic vacuole) and degranulation. D, Phagolysosome formation after fusion of lysosome of the cell.

1. RECOGNITION AND ATTACHMENT Phagocytosis is initiated by the expression of surface receptors on macrophages which recognise microorganisms: mannose receptor and scavenger receptor. The process of phagocytosis is further enhanced when the microorganisms are coated with specific proteins, opsonins, from the serum or they get opsonised. Opsonins establish a bond between bacteria and the cell membrane of phagocytic cell. The main opsonins present in the serum and their corresponding receptors on the surface of phagocytic cells (PMNs or macrophages) are as under:

i) IgG opsonin is the Fc fragment of immunoglobulin G; it is the naturally occurring antibody in the serum that coats the bacteria while the PMNs possess receptors for the same.

ii) C3b opsonin is the fragment generated by activation of complement pathway. It is strongly chemotactic for attracting PMNs to bacteria.

iii) Lectins are carbohydrate-binding proteins in the plasma which bind to bacterial cell wall.

2. ENGULFMENT The opsonised particle bound to the surface of phagocyte is ready to be engulfed. This is accomplished by formation of cytoplasmic pseudopods around the particle due to activation of actin filaments beneath cell wall, enveloping it in a phagocytic vacuole. Eventually, the plasma membrane enclosing the particle breaks from the cell surface so that membrane lined phagocytic vacuole or phagosome lies internalised and free in the cell cytoplasm. The phagosome fuses with one or more lysosomes of the cell and form bigger vacuole called phagolysosome.

3. KILLING AND DEGRADATION Next comes the stage of killing and degradation of microorganism to dispose it off justifying the function of phagocytes as scavanger cells. The microorganisms after being killed by antibacterial substances are degraded by hydrolytic enzymes. However, this mechanism fails to kill and degrade some bacteria like tubercle bacilli.

Disposal of microorganisms can proceed by following mechanisms:

A. Intracellular mechanisms:

i) Oxidative bactericidal mechanism by oxygen free radicals

a) MPO-dependent

b) MPO-independent

ii) Oxidative bactericidal mechanism by lysosomal granules

iii) Non-oxidative bactericidal mechanism

B. Extracellular mechanisms

CHEMICAL MEDIATORS OF INFLAMMATION

TABLE 6.2: Chemical Mediators of Acute Inflammation.

I. CELL-DERIVED MEDIATORS
1. Vasoactive amines (Histamine, 5-hydroxytryptamine, neuropeptides)
2. Arachidonic acid metabolites (Eicosanoids)
   i. Metabolites via cyclo-oxygenase pathway (prostaglandins, thromboxane A_2, prostacyclin, resolvins)
   ii. Metabolites via lipo-oxygenase pathway (5-HETE, leukotrienes, lipoxins)
3. Lysosomal components (from PMNs, macrophages)
4. Platelet activating factor
5. Cytokines (IL-1, TNF-α, TNF-β, IFN-γ, chemokines)
6. Free radicals (Oxygen metabolites, nitric oxide)

II. PLASMA-DERIVED MEDIATORS (PLASMA PROTEASES)
Products of:
1. The kinin system
2. The clotting system
3. The fibrinolytic system
4. The complement system

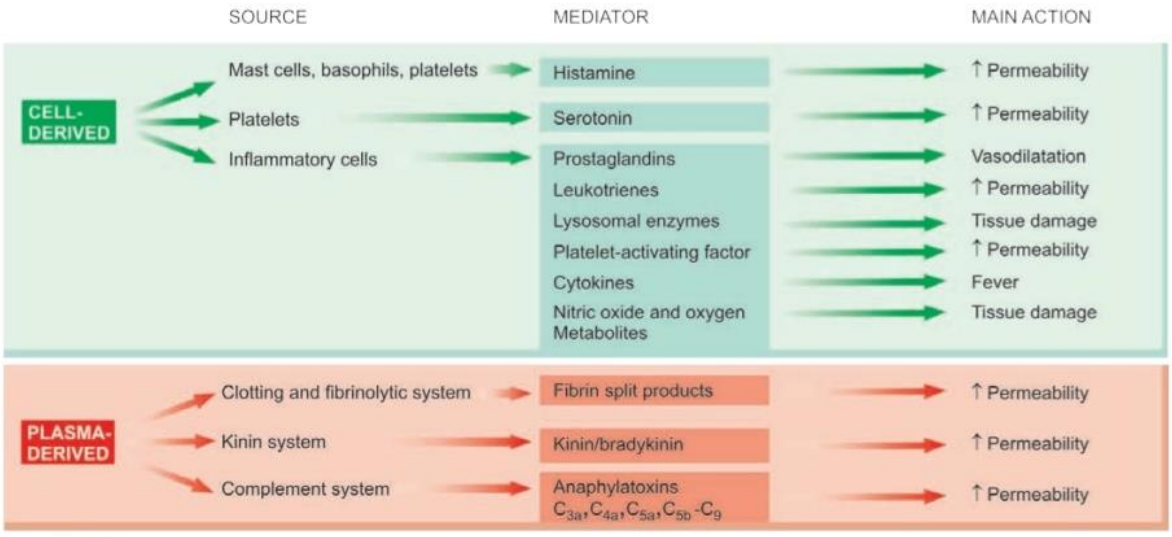


Figure 6.7 Chemical mediators of inflammation.

VASOACTIVE AMINES. Two important pharmacologically active amines that have role in the early inflammatory response (first one hour) are histamine and 5hydroxytryptamine (5-HT) or serotonin.

ARACHIDONIC ACID METABOLITES (EICOSANOIDS)

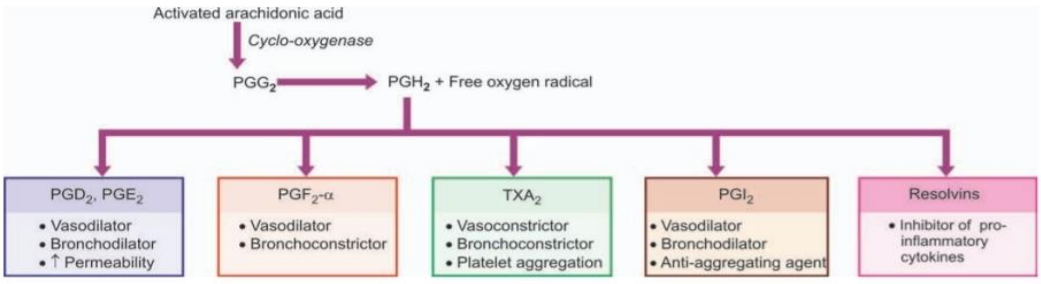


Figure 6.8 Arachidonic acid metabolites via cyclooxygenase pathway.

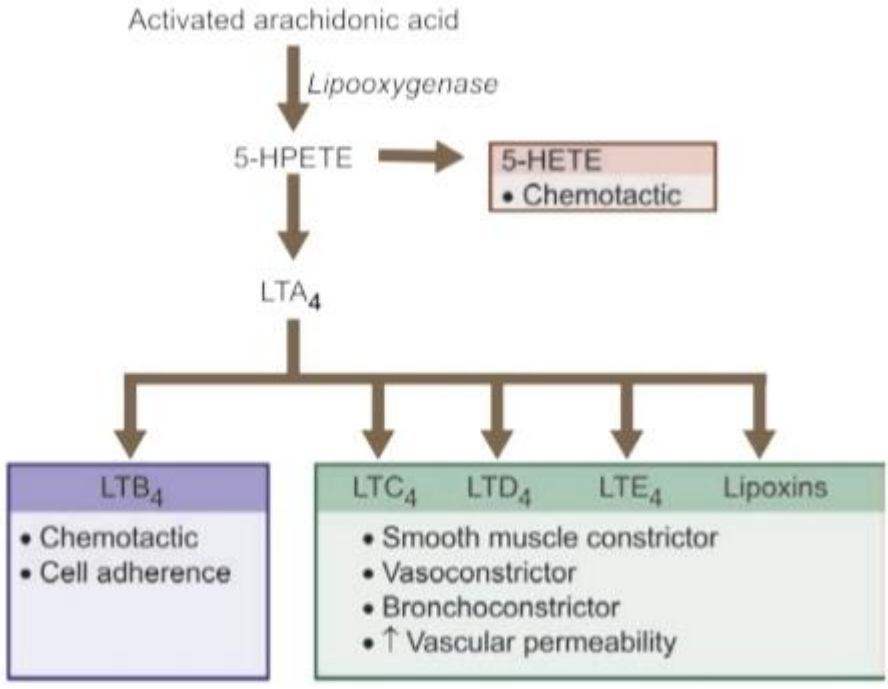


Figure 6.9 Arachidonic acid metabolites via lipooxygenase pathway.

LYSOSOMAL COMPONENTS The inflammatory cells—neutrophils and monocytes, contain lysosomal granules which on release elaborate a variety of mediators of inflammation.

PLATELET ACTIVATING FACTOR (PAF). It is released from IgE-sensitised basophils or mast cells, other leucocytes, endothelium and platelets. Apart from its action on platelet aggregation and release reaction, the actions of PAF as mediator of inflammation are:

- increased vascular permeability;
- vasodilatation in low concentration and vasoconstriction otherwise;
- bronchoconstriction;

- adhesion of leucocytes to endothelium; and
- chemotaxis.

CYTOKINES. Cytokines are polypeptide substances produced by activated lymphocytes (lymphokines) and activated monocytes (monokines). These agents may act on 'self' cells producing them or on other cells. Although over 200 cytokines have been described, major cytokines acting as mediators of inflammation are: interleukin-1 (IL-1), tumour necrosis factor (TNF)-α and β, interferon (IFN)-γ, and chemokines (IL-8, PF-4).

FREE RADICALS: OXYGEN METABOLITES AND NITRIC OXIDE. Free radicals act as potent mediator of inflammation: i) Oxygen-derived metabolites are released from activated neutrophils and macrophages and include superoxide oxygen (O'2), H2O2, OH' and toxic NO products. These oxygen-derived free radicals have the following action in inflammation:

- Endothelial cell damage and thereby increased vascular permeability.
- Activation of protease and inactivation of antiprotease causing tissue matrix damage.
- Damage to other cells.

II. Plasma-derived Mediators (Plasma Proteases)

1. THE KININ SYSTEM. This system on activation by factor Xlla generates bradykinin, so named because of the slow contraction of smooth muscle induced by it. First, kallikrein is formed from plasma prekallikrein by the action of prekallikrein activator which is a fragment of factor Xlla. Kallikrein then acts on high molecular weight kininogen to form bradykinin. Bradykinin acts in the early stage of inflammation and its effects include: smooth muscle contraction; vasodilatation; increased vascular permeability; and pain.

2. THE CLOTTING SYSTEM. Factor Xlla initiates the cascade of the clotting system resulting in formation of fibrinogen which is acted upon by thrombin to form fibrin and fibrinopeptides. The actions of fibrinopeptides in inflammation are: increased vascular permeability; chemotaxis for leucocyte; and anticoagulant activity.

3. THE FIBRINOLYTIC SYSTEM. This system is activated by plasminogen activator, the sources of which include kallikrein of the kinin system, endothelial cells and leucocytes. Plasminogen activator acts on plasminogen present as component of plasma proteins to form plasmin. Further breakdown of fibrin by plasmin forms fibrinopeptides or fibrin split products. The actions of plasmin in inflammation are as follows: activation of factor XII to form prekallikrein activator that stimulates the kinin system to generate bradykinin; splits off complement C3 to form C3a which is a permeability factor; and degrades fibrin to form fibrin split products which increase vascular permeability and are chemotactic to leucocytes.

4. THE COMPLEMENT SYSTEM. The activation of complement system can occur either: i) by classic pathway through antigen-antibody complexes; or ii) by alternate pathway via non-immunologic agents such as bacterial toxins, cobra venoms and IgA.

CHRONIC INFLAMMATION

DEFINITION AND CAUSES. Chronic inflammation is defined as prolonged process in which tissue destruction and inflammation occur at the same time. Chronic inflammation can be caused by one of the following 3 ways:

1. Chronic inflammation following acute inflammation. When the tissue destruction is extensive, or the bacteria survive and persist in small numbers at the site of acute inflammation e.g. in osteomyelitis, pneumonia terminating in lung abscess.

2. Recurrent attacks of acute inflammation. When repeated bouts of acute inflammation culminate in chronicity of the process e.g. in recurrent urinary tract infection leading to chronic pyelonephritis, repeated acute infection of gallbladder leading to chronic cholecystitis.

3. Chronic inflammation starting de novo. When the infection with organisms of low pathogenicity is chronic from the beginning e.g. infection with Mycobacterium tuberculosis.

GENERAL FEATURES OF CHRONIC INFLAMMATION

Though there may be differences in chronic inflammatory response depending upon the tissue involved and causative organisms, there are some basic similarities amongst various types of chronic inflammation. Following general features characterise any chronic inflammation:

1. MONONUCLEAR CELL INFILTRATION. Chronic inflammatory lesions are infiltrated by mononuclear inflammatory cells like phagocytes and lymphoid cells. Phagocytes are represented by circulating monocytes, tissue macrophages, epithelioid cells and sometimes, multinucleated giant cells. The macrophages comprise the most important cells in chronic inflammation. These may appear at the site of chronic inflammation from:

i) chemotactic factors and adhesion molecules for continued infiltration of macrophages;
ii) local proliferation of macrophages; and
iii) longer survival of macrophages at the site of inflammation.

2. TISSUE DESTRUCTION OR NECROSIS. Tissue destruction and necrosis are central features of most forms of chronic inflammatory lesions. This is brought about by activated macrophages which release a variety of biologically active substances e.g. protease, elastase, collagenase, lipase, reactive oxygen radicals, cytokines (IL-1, IL-8, TNF-α), nitric oxide, angiogenesis growth factor etc.

3. PROLIFERATIVE CHANGES. As a result of necrosis, proliferation of small blood vessels and fibroblasts is stimulated resulting in formation of inflammatory granulation tissue. Eventually, healing by fibrosis and collagen laying takes place.

SYSTEMIC EFFECTS OF CHRONIC INFLAMMATION

Chronic inflammation is associated with following systemic features:

1. Fever. Invariably there is mild fever, often with loss of weight and weakness.

2. Anaemia. chronic inflammation is accompanied by anaemia of varying degree.

3. Leucocytosis. As in acute inflammation, chronic inflammation also has leucocytosis but generally there is relative lymphocytosis in these cases.

4. ESR. ESR is elevated in all cases of chronic inflammation.

5. Amyloidosis. Long-term cases of chronic suppurative inflammation may develop secondary systemic (AA) amyloidosis.

TYPES OF CHRONIC INFLAMMATION

1. Non-specific, when the irritant substance produces a nonspecific chronic inflammatory reaction with formation of granulation tissue and healing by fibrosis e.g. chronic osteomyelitis, chronic ulcer.

2. Specific, when the injurious agent causes a characteristic histologic tissue response e.g. tuberculosis, leprosy, syphilis.

However, for a more descriptive classification, histological features are used for classifying chronic inflammation into 2 corresponding types:

1. Chronic non-specific inflammation. It is characterised by non-specific inflammatory cell infiltration e.g. chronic osteomyelitis, lung abscess. A variant of this type of chronic inflammatory response is chronic suppurative inflammation in which infiltration by polymorphs and abscess formation are additional features e.g. actinomycosis.

2. Chronic granulomatous inflammation. It is characterised by formation of granulomas e.g. tuberculosis, leprosy, syphilis, actinomycosis, sarcoidosis etc.

WOUND HEALING

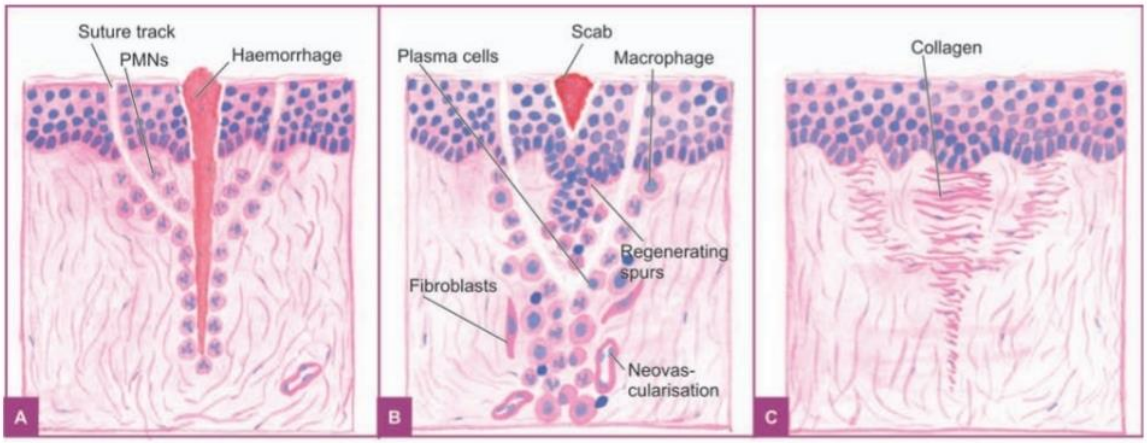
Healing of skin wounds provides a classical example of combination of regeneration and repair described above. Wound healing can be accomplished in one of the following two ways:

1. Healing by first intention (primary union)
2. Healing by second intention (secondary union).

1. Healing by first intention (primary union)

This is defined as healing of a wound which has the following characteristics:

- clean and uninfected;
- surgically incised;
- without much loss of cells and tissue; and
- edges of wound are approximated by surgical sutures.



igure 6.42 Primary union of skin wounds. A, The incised wound as well as suture track on either side are filled with blood clot and there is nflammatory response from the margins. B, Spurs of epidermal cells migrate along the incised margin on either side as well as around the suture rack. Formation of granulation tissue also begins from below. C, Removal of suture at around 7th day results in scar tissue at the sites of incision nd suture track.

The sequence of events in primary union is illustrated in Fig. and described below:

1. Initial haemorrhage. Immediately after injury, the space between the approximated surfaces of incised wound is filled with blood which then clots and seals the wound against dehydration and infection.

2. Acute inflammatory response. This occurs within 24 hours with appearance of polymorphs from the margins of incision. By 3rd day, polymorphs are replaced by macrophages.

3. Epithelial changes. The basal cells of epidermis from both the cut margins start proliferating and migrating towards incisional space in the form of epithelial spurs. A wellapproximated wound is covered by a layer of epithelium in 48 hours. The migrated epidermal cells separate the underlying viable dermis from the overlying necrotic material and clot, forming scab which is cast off. The basal cells from the margins continue to divide. By 5th day, a multilayered new epidermis is formed which is differentiated into superficial and deeper layers.

4. Organisation. By 3rd day, fibroblasts also invade the wound area. By 5th day, new collagen fibrils start forming which dominate till healing is completed. In 4 weeks, the scar tissue with scanty cellular and vascular elements, a few inflammatory cells and epithelialised surface is formed.

5. Suture tracks. Each suture track is a separate wound and incites the same phenomena as in healing of the primary wound i.e. filling the space with haemorrhage, some inflammatory cell reaction, epithelial cell proliferation along the suture track from both margins, fibroblastic proliferation and formation of young collagen. When sutures are removed around 7th day, much of epithelialised suture track is avulsed and the remaining epithelial tissue in the track is absorbed. However, sometimes the suture track gets infected (stitch abscess), or the epithelial cells may persist in the track (implantation or epidermal cysts).

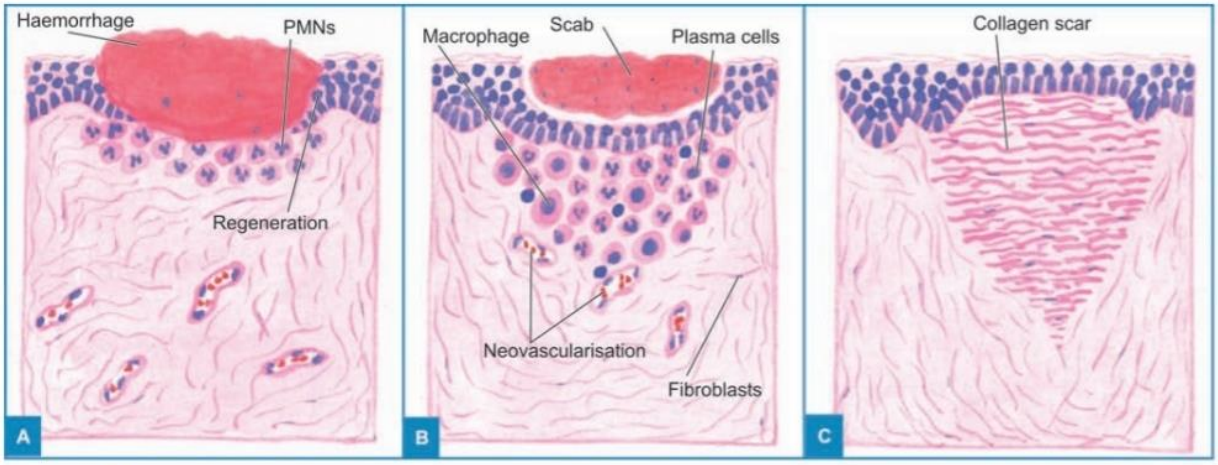
Thus, the scar formed in a sutured wound is neat due to close apposition of the margins of wound; the use of adhesive tapes avoids removal of stitches and its complications.

2.Healing by second intention (secondary union).

Healing by Second Intention (Secondary Union) This is defined as healing of a wound having the following characteristics:

- open with a large tissue defect, at times infected;
- having extensive loss of cells and tissues; and
- the wound is not approximated by surgical sutures but is left open.

The basic events in secondary union are similar to primary union but differ in having a larger tissue defect which has to be bridged. Hence healing takes place from the base upwards as well as from the margins inwards. The healing by second intention is slow and results in a large, at times ugly, scar as compared to rapid healing and neat scar of primary union. The sequence of events in secondary union is illustrated in Fig. 6.43 and described below:



igure 6.43 Secondary union of skin wounds. A, The open wound is filled with blood clot and there is inflammatory response at the junction of iable tissue. B, Epithelial spurs from the margins of wound meet in the middle to cover the gap and separate the underlying viable tissue from ecrotic tissue at the surface forming scab. C, After contraction of the wound, a scar smaller than the original wound is left.

1. Initial haemorrhage. As a result of injury, the wound space is filled with blood and fibrin clot which dries.

2. Inflammatory phase. There is an initial acute inflammatory response followed by appearance of macrophages which clear off the debris as in primary union.

3. Epithelial changes. As in primary healing, the epidermal cells from both the margins of wound proliferate and migrate into the wound in the form of epithelial spurs till they meet in the middle and re-epithelialise the gap completely. However, the proliferating epithelial cells do not cover the surface fully until granulation tissue from base has started filling the wound space. In this way, pre-existing viable connective tissue is separated from necrotic

material and clot on the surface, forming scab which is cast off. In time, the regenerated epidermis becomes stratified and keratinised.

4. Granulation tissue. Main bulk of secondary healing is by granulations. Granulation tissue is formed by proliferation of fibroblasts and neovascularisation from the adjoining viable elements. The newly-formed granulation tissue is deep red, granular and very fragile. With time, the scar on maturation becomes pale and white due to increase in collagen and decrease in vascularity. Specialised structures of the skin like hair follicles and sweat glands are not replaced unless their viable residues remain which may regenerate.

5. Wound contraction. Contraction of wound is an important feature of secondary healing, not seen in primary healing. Due to the action of myofibroblasts present in granulation tissue, the wound contracts to one-third to onefourth of its original size. Wound contraction occurs at a time when active granulation tissue is being formed.

6. Presence of infection. Bacterial contamination of an open wound delays the process of healing due to release of bacterial toxins that provoke necrosis, suppuration and thrombosis. Surgical removal of dead and necrosed tissue, debridement, helps in preventing the bacterial infection of open wounds.

Differences between primary and secondary union of wounds are given in Table.

TABLE 6.6: Differences between Primary and Secondary Union of Wounds.

Feature	Primary Union	Secondary Union
1. *Cleanliness of wound*	Clean	Unclean
2. *Infection*	Generally uninfected	May be infected
3. *Margins*	Surgical clean	Irregular
4. *Sutures*	Used	Not used
5. *Healing*	Scanty granulation tissue at the incised gap and along suture tracks	Exuberant granulation tissue to fill the gap
6. *Outcome*	Neat linear scar	Contracted irregular wound
7. *Complications*	Infrequent, epidermal inclusion cyst formation	Suppuration, may require debridement