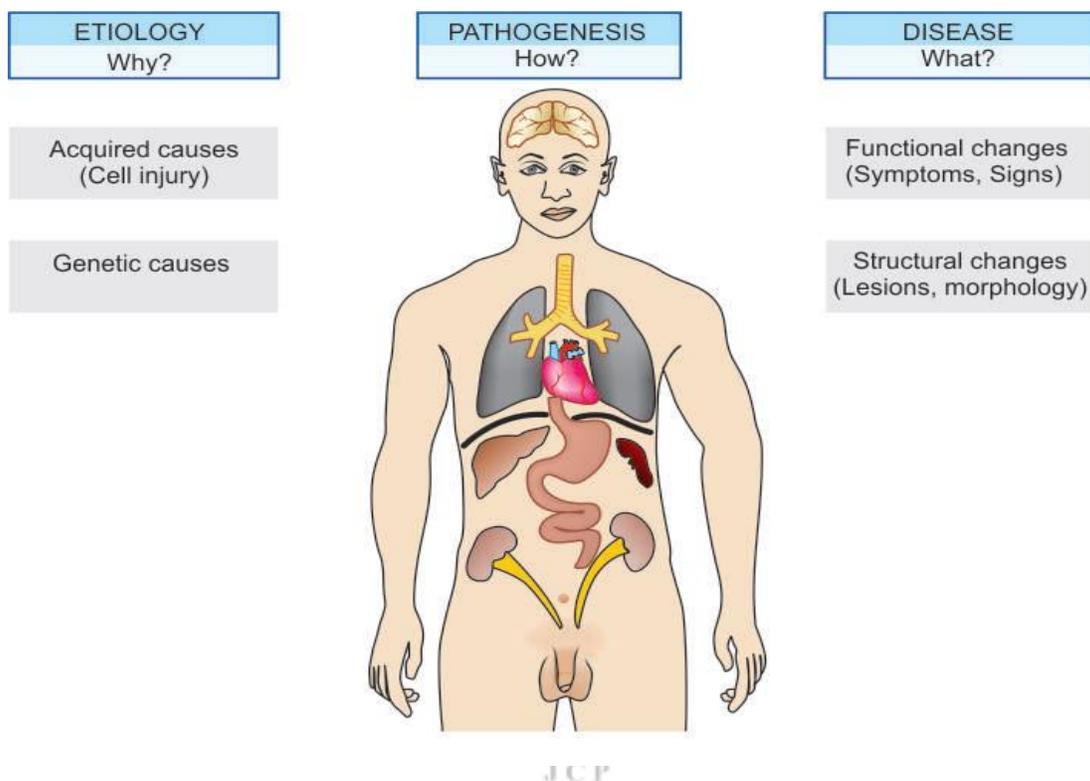


PATHOPHYSIOLOGY

Patho=suffering, Physiology=study of normal function

DEFINITION OF PATHOPHYSIOLOGY:-

Study of diseases is 'pathophysiology'. Pathophysiology, thus, includes study of disordered function (i.e. physiological changes) and breakdown of homeostasis in diseases (i.e. biochemical changes).



1. CELL INJURY

1.1 INTRODUCTION:-

Study of abnormalities in structure and function of cells in disease has remained the focus of attention in understanding of diseases. Thus, most forms of diseases begin with cell injury followed by consequent loss of cellular function.

1.2 DEFINITION:-

Cell injury is defined as the effect of a variety of stresses due to etiologic agents a cell encounters resulting in changes in its internal and external environment. The cellular response to stress may vary and depends upon following two variables:

- i) Host factors i.e. the type of cell and tissue involved.
- ii) Factors pertaining to injurious agent i.e. extent and type of cell injury.

Various forms of cellular responses to cell injury may be as follows (Fig. 2.1):

1. When there is increased functional demand, the cell may adapt to the changes which are expressed morphologically, which then revert back to normal after the stress is removed (cellular adaptations).
2. When the stress is mild to moderate, the injured cell may recover (reversible cell injury), while persistent and severe form of cell injury may cause cell death (irreversible cell injury).
3. The residual effects of reversible cell injury may persist in the cell as evidence of cell injury at subcellular level (subcellular changes), or metabolites may accumulate within the cell (intracellular accumulations).

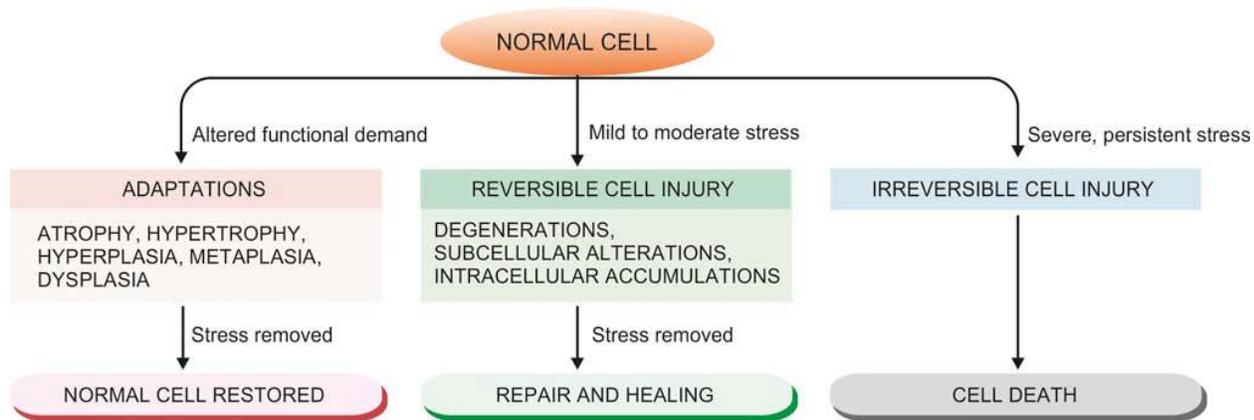


Figure 1.2 Cellular responses to cell injury.

1.3 ETIOLOGY OF CELL INJURY:-

The cells may be broadly injured by two major ways:

- A. Genetic causes
- B. Acquired causes

The genetic causes of various diseases are discussed in

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:

- Hypoxia and ischaemia
- Physical agents
- Chemical agents and drugs
- Microbial agents
- Immunologic agents
- Nutritional derangements
- Ageing
- Psychogenic diseases
- Iatrogenic factors
- Idiopathic diseases.

1.3.1 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 2 ways:

- The most common mechanism of hypoxic cell injury is by reduced supply of blood to cells due to interruption i.e.

ischaemia.

□ Hypoxia may also result from impaired blood supply from causes other than interruption e.g. disorders of oxygen carrying RBCs (e.g. anaemia, carbon monoxide poisoning), heart diseases, lung diseases and increased demand of tissues.

1.3.2 Physical Agents

Physical agents in causation of disease are as under:

- i) mechanical trauma (e.g. road accidents);
- ii) thermal trauma (e.g. by heat and cold);
- iii) electricity;
- iv) radiation (e.g. ultraviolet and ionising); and
- v) rapid changes in atmospheric pressure.

1.3.3 Chemicals And Drugs

An ever-increasing list of chemical agents and drugs may cause cell injury. Important examples include the following:

- i) chemical poisons such as cyanide, arsenic, mercury;
- ii) strong acids and alkalis;
- iii) environmental pollutants;
- iv) insecticides and pesticides;
- v) oxygen at high concentrations;
- vi) hypertonic glucose and salt;
- vii) social agents such as alcohol and narcotic drugs; and
- viii) therapeutic administration of drugs.

1.3.4 Microbial Agents

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

1.3.5 Immunologic Agents

Immunity is a 'double-edged sword'—it protects the host against various injurious agents but it may also turn lethal and cause cell injury e.g.

- i) hypersensitivity reactions;
- ii) anaphylactic reactions; and
- iii) autoimmune diseases.

1.3.6 Nutritional Derangements

A deficiency or an excess of nutrients may result in nutritional imbalances.

□ Nutritional deficiency diseases may be due to over all 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.

1.3.7 Ageing

Cellular ageing 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.

1.3.8 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.

1.3.9 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 judgement by the physician and untoward effects of administered therapy (drugs, radiation).

1.3.10 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.

1.4 PATHOGENESIS OF CELL INJURY

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 are complex and varied. However, in general, irrespective of the type, following common scheme applies to most forms of cell injury by various agents:

1.4.1 Factors pertaining to etiologic agent and host

As mentioned above, factors pertaining to host cells and etiologic agent determine the outcome of cell injury:

- i) 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.
- ii) 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 persistent ischaemia due to total coronary occlusion >20 minutes.

1.4.2 Common underlying mechanisms

Irrespective of other factors, following essential intracellular biochemical phenomena underlie all forms of cell injury:

- i) Cell membrane damage disturbing the metabolic and trans-membrane exchanges.
- ii) Release of toxic free radicals.

1.4.3 Usual morphologic changes

Biochemical and molecular changes underlying cell injury from various agents become apparent first, and are associated with appearance of ultrastructural changes in the injured cell. However, eventually, gross and light microscopic changes in morphology of organ and cells appear. The morphologic changes of reversible cell injury (e.g. hydropic swelling) appear earlier while later morphologic alterations of cell death are seen (e.g. in myocardial infarction).

1.4.4 Functional implications and disease outcome

Eventually, cell injury affects cellular function adversely which has bearing on the body. Consequently, clinical features in the form of symptoms and signs would appear. Further course or prognosis will depend upon the response to treatment versus the biologic behaviour of disease. 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 (principally ionising radiation) agents.

1.5 PATHOGENESIS OF ISCHAEMIC AND HYPOXIC INJURY

Ischaemia and hypoxia are the most common forms of cell injury. Although underlying intracellular mechanisms and ultrastructural changes seen in reversible and irreversible cell injury by hypoxia-ischaemia (depending upon extent of hypoxia and type of cells involved) are a continuation of the process, these mechanisms are discussed separately below and illustrated diagrammatically in **Figs. 1.3 and 1.4**:

1.5.1 Reversible Cell Injury

If the ischaemia or hypoxia is of short duration, the effects may be reversible on rapid restoration of circulation e.g. in coronary artery occlusion, myocardial contractility, metabolism and ultrastructure are reversed if the circulation is quickly restored. The sequential biochemical and ultrastructural changes in reversible cell injury are as under (**Fig. 1.4**):

1. Decreased generation of cellular ATP: Damage by ischaemia from interruption versus hypoxia from other causes

All living cells require continuous supply of oxygen to produce ATP which is essentially required for a variety of cellular functions (e.g. membrane transport, protein synthesis, lipid synthesis and phospholipid metabolism). ATP in human cell is derived from 2 sources:

- Firstly, by aerobic respiration or oxidative phosphorylation (which requires oxygen) in the mitochondria.
- Secondly, cells may subsequently switch over to anaerobic glycolytic oxidation to maintain constant supply of ATP (in which ATP is generated from glucose/glycogen in the absence of oxygen).
- Ischaemia due to interruption in blood supply as well as hypoxia from other causes limit the supply of oxygen to the cells, thus causing decreased ATP generation from ADP:
- In ischaemia from interruption of blood supply, aerobic respiration as well as glucose availability are both compromised resulting in more severe and faster effects of cell injury. Ischaemic cell injury also causes accumulation of metabolic waste products in the cells.

- On the other hand, in hypoxia from other causes (RBC disorders, heart disease, lung disease), anaerobic glycolytic ATP generation continues, and thus cell injury is less severe. However, highly specialised cells such as myocardium, proximal tubular cells of the kidney, and neurons of the CNS are dependent solely on aerobic respiration for ATP generation and thus these tissues suffer from ill-effects of ischaemia more severely and rapidly.

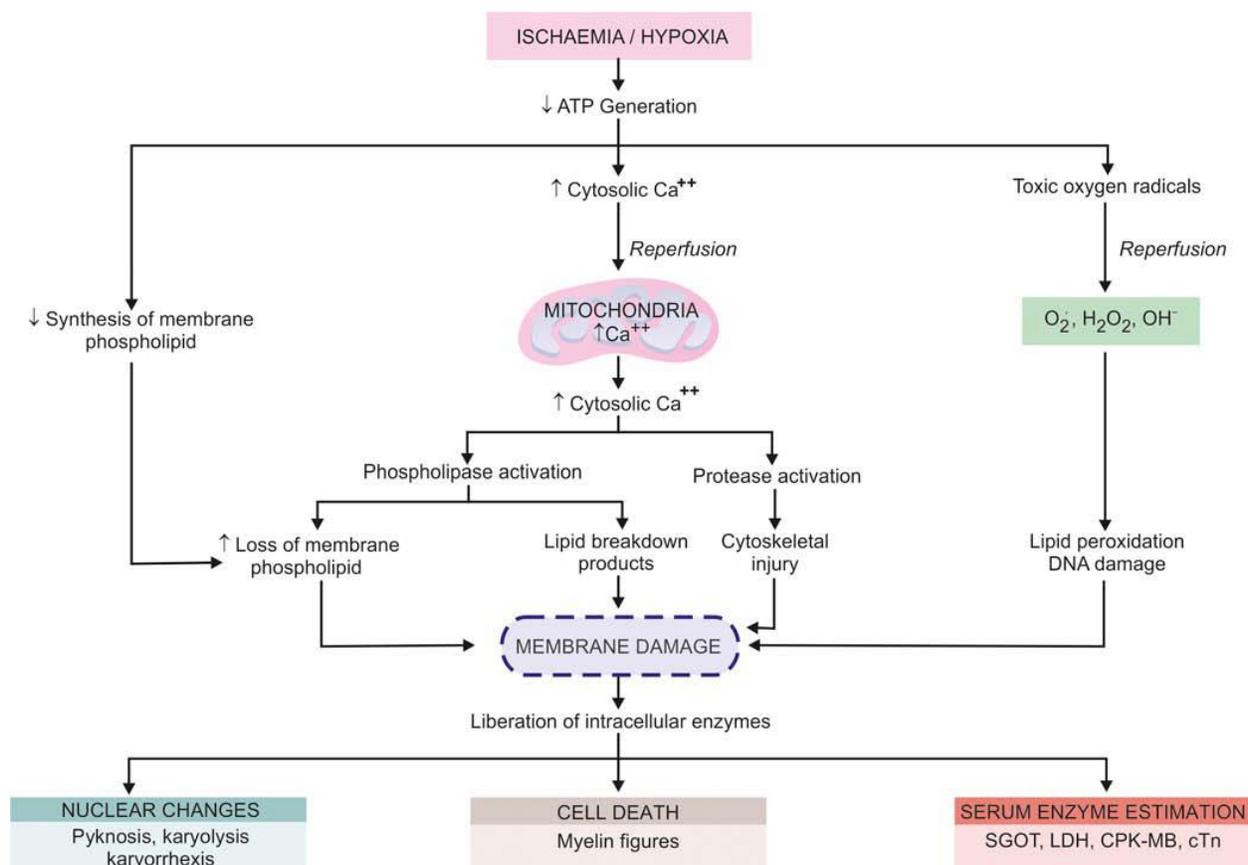


Figure 1.3 Sequence of events in the pathogenesis of reversible and irreversible cell injury caused by hypoxia/ischaemia.

2. Intracellular lactic acidosis: Nuclear clumping

Due to low oxygen supply to the cell, aerobic respiration by mitochondria fails first. This is followed by switch to anaerobic glycolytic pathway for the requirement of energy (i.e. ATP). This results in rapid depletion of glycogen and accumulation of lactic acid lowering the intracellular pH. Early fall in intracellular pH (i.e. intracellular lactic acidosis) results in clumping of nuclear chromatin.

3. Damage to plasma membrane pumps: Hydropic swelling and other membrane changes

Lack of ATP interferes in generation of phospholipids from the cellular fatty acids which are required for continuous repair of membranes. This results in damage to membrane pumps operating for regulation of sodium-potassium and calcium as under:

i) Failure of sodium-potassium pump

Normally, the energy (ATP)-dependent sodium pump (also called Na⁺-K⁺ ATPase) operating at the plasma membrane allows active transport of sodium out of the cell and diffusion of

potassium into the cell. Lowered ATP in the cell lowers the activity of sodium pump and consequently interferes with this membrane-regulated process. This results in intracellular accumulation of sodium and diffusion of potassium out of the cell. The accumulation of sodium in the cell leads to increase in intracellular water to maintain iso-osmotic conditions (i.e. hydropic swelling occurs).

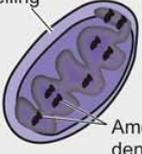
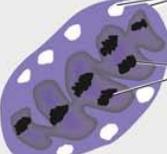
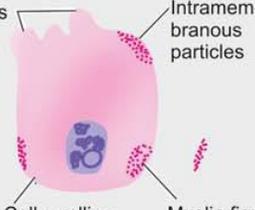
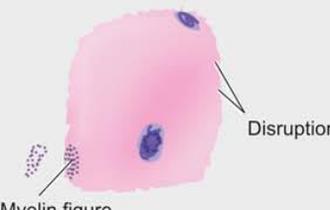
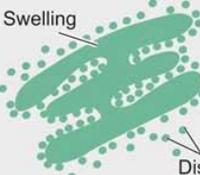
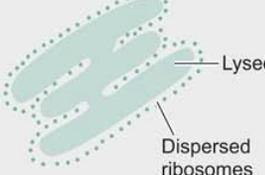
ORGANELLES IN NORMAL CELL	A, REVERSIBLE CELL INJURY	B, IRREVERSIBLE CELL INJURY
1. MITOCHONDRIA 	Swelling  Amorphous densities	Swollen with vacuoles  Large densities
2. MEMBRANES 	Blebs  Intramembranous particles Cell swelling Myelin figure	Disruption  Myelin figure
3. RER AND RIBOSOMES 	Swelling  Dispersed ribosomes	Lysed  Dispersed ribosomes
4. LYSOSOMES 	Autophagy 	Swollen, ruptured 
5. CYTOSKELETON 	Aggregated 	Disrupted 
6. NUCLEUS 	Clumped chromatin 	Pyknosis  Karyorrhexis  Karyolysis 

Figure 1.4 Ultrastructural changes during cell injury due to hypoxia-ischaemia.

ii) Failure of calcium pump

Membrane damage causes disturbance in the calcium ion exchange across the cell membrane. Excess of calcium moves into the cell (i.e. calcium influx), particularly in the mitochondria, causing its swelling and deposition of phospho lipid-rich amorphous densities.

4. Reduced protein synthesis: Dispersed ribosomes

As a result of continued hypoxia, membranes of endoplasmic reticulum and Golgi apparatus swell up. Ribosomes are detached from granular (rough) endoplasmic reticulum and polysomes are degraded to monosomes, thus dispersing ribosomes in the cytoplasm and inactivating their function. Similar reduced protein synthesis occurs in Golgi apparatus. Ultrastructural evidence of reversible cell membrane damage is seen in the form of loss of microvilli, intramembranous particles and focal projections of the cytoplasm (blebs). Myelin figures may be seen lying in the cytoplasm or present outside the cell; these are derived from membranes (plasma or organellar) enclosing water and dissociated lipoproteins between the lamellae of injured membranes. Up to this point, withdrawal of acute stress that resulted in reversible cell injury can restore the cell to normal state.

1.5.2 Irreversible Cell Injury

Persistence of ischaemia or hypoxia results in irreversible damage to the structure and function of the cell (cell death). The stage at which this point of no return or irreversibility is reached from reversible cell injury is unclear but the sequence of events is a continuation of reversibly injured cell. Two essential phenomena always distinguish irreversible from reversible cell injury (**Fig. 1.3**):

- Inability of the cell to reverse mitochondrial dysfunction on reperfusion or reoxygenation.
 - Disturbance in cell membrane function in general, and in plasma membrane in particular.
- In addition, there is further reduction in ATP, continued depletion of proteins, reduced intracellular pH, and leakage of lysosomal enzymes into the plasma. These biochemical changes have effects on the ultrastructural components of the cell (**Fig. 1.4**).

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. Morphological changes are in the form of 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. Increased cytosolic influx of calcium in the cell 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, micro tubules 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

DNA or 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. Damaged DNA activates proapoptotic proteins leading the cell to death.

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: (e.g. hydrolase, RNAase, DNAase, protease, glycosidase, phosphatase, lipase, amylase, cathepsin etc) 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. Liberated enzymes just mentioned leak across the abnormally permeable cell membrane into the serum, the estimation of which may be used as clinical parameters of cell death. For example, in myocardial infarction, estimation of elevated serum glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), isoenzyme of creatine kinase (CK-MB), and cardiac troponins (cTn) are useful guides for death of heart muscle. While cell damage from oxygen deprivation by above mechanisms develops slowly, taking several minutes to hours, the cell injury may be accentuated after restoration of blood supply and subsequent events termed ischaemic-reperfusion injury and liberation of toxic free radicals (or reactive oxygen species), discussed below.

1.6 ISCHAEMIA-REPERFUSION INJURY AND FREE RADICAL-MEDIATED CELL INJURY

Depending upon the duration of ischaemia/hypoxia, restoration of blood flow may result in the following 3 different consequences:

1.6.1 From ischaemia to reversible injury

When the period of ischaemia is of short duration, reperfusion with resupply of oxygen restores the structural and functional state of the injured cell i.e. reversible cell injury.

1.6.2 From ischaemia to irreversible injury

Another extreme is when much longer period of ischaemia has resulted in irreversible cell injury during ischaemia itself i.e. when so much time has elapsed that neither blood

flow restoration is helpful nor reperfusion injury can develop. Cell death in such cases is not attributed to formation of activated oxygen species. But instead, on reperfusion there is further marked intracellular excess of sodium and calcium ions due to persistent cell membrane damage.

1.6.3 From ischaemia to reperfusion injury

When ischaemia is for somewhat longer duration, then restoration of blood supply to injured but viable cells (i.e. reperfusion), rather than restoring structure and function of the cell, paradoxically deteriorates the already injured cell and leads it to cell death. This is termed ischaemia-reperfusion injury. The examples of such forms of cell injury are irreversible cell injury in myocardial and cerebral ischaemia.

Ischaemia-reperfusion injury occurs due to excessive accumulation of free radicals or reactive oxygen species. The mechanism of reperfusion injury by free radicals is complex but following three aspects are involved:

- a) Calcium overload.
- b) Excessive generation of free radicals (superoxide, H₂O₂, hydroxyl radical, pernitrite).
- c) Subsequent inflammatory reaction.

These are discussed below:

a) Calcium Overload

Upon restoration of blood supply, the ischaemic cell is further bathed by the blood fluid that has more calcium ions at a time when the ATP stores of the cell are low. This results in further calcium overload on the already injured cells, triggering lipid peroxidation of the membrane causing further membrane damage.

b) Excessive Generation Of Free Radicals

Although oxygen is the lifeline of all cells and tissues, its molecular forms as reactive oxygen radicals or reactive oxygen species can be most devastating for the cells.

c) Subsequent Inflammatory Reaction

Ischaemia reperfusion event is followed by inflammatory reaction. Incoming activated neutrophils utilise oxygen quickly (oxygen burst) and release large excess of oxygen free radicals. Ischaemia is also associated with accumulation of precursors of ATP, namely ADP and pyruvate, which further build-up generation of free radicals.

1.6.4 Stress Proteins in Cell Injury

When cells are exposed to stress of any type, a protective response by the cell is by release of proteins that move molecules within the cell cytoplasm; these are called stress protein. There are 2 types of stress-related proteins: heat shock proteins (HSP) and ubiquitin (so named due to its universal presence in the cells of the body).

HSPs These are a variety of intracellular carrier proteins present in most cells of the body, especially in renal tubular epithelial cells. They normally perform the role of molecular chaperones (house-keeping) i.e. they direct and guide metabolic molecules to the sites of metabolic activity e.g. protein folding,

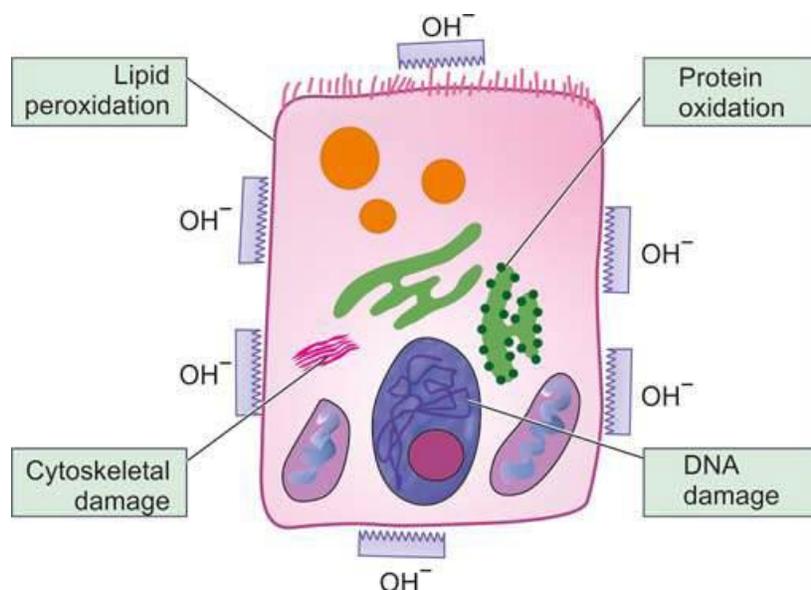
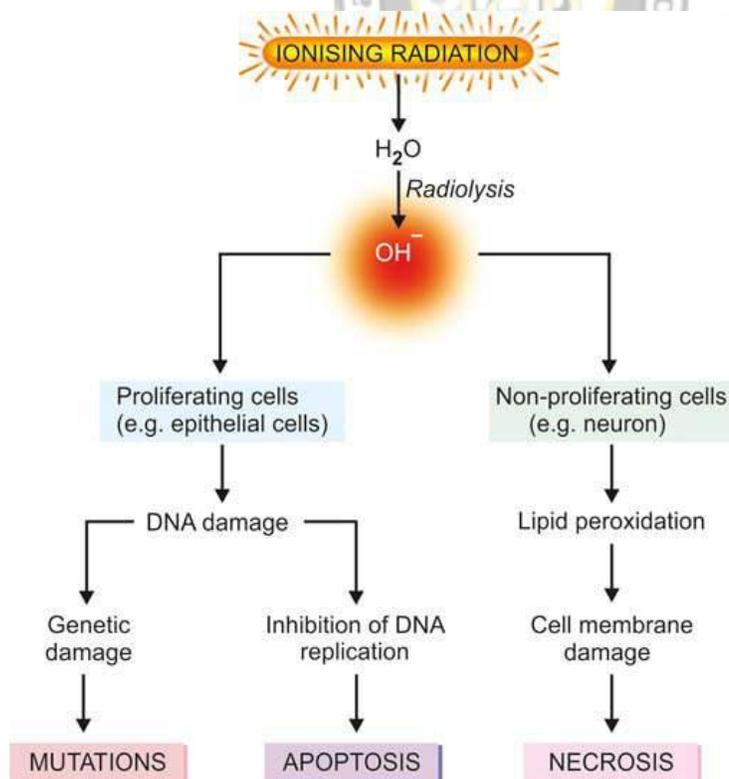


Figure 1.5 Mechanism of cell death by hydroxyl radical, the most reactive oxygen species.

disaggregation of protein-protein complexes and transport of proteins into various intracellular organelles (protein kinesis). However, in response to stresses of various types (e.g. toxins, drugs, poisons, ischaemia), their level goes up, both inside the cell and also they leak out into the plasma, and hence the name stress proteins. In experimental studies HSPs have been shown to limit tissue necrosis in ischaemic reperfusion injury in myocardial infarcts. In addition, they have also been shown to have a central role in protein aggregation in amyloidosis.



2. HOMEOSTASIS

Homeostasis → homeo = same; stasis = standing

‘Homeostasis’ refers to the maintenance of constant internal environment of the body. **Homeostasis** is the state of steady internal, physical, and chemical conditions maintained by living systems.

This dynamic state of equilibrium is the condition of optimal functioning for the organism and includes many variables, such as body temperature and fluid balance, being kept within certain pre-set limits (homeostatic range). Other variables include the pH of extracellular fluid, the concentrations of sodium, potassium and calcium ions, as well as that of the blood sugar level, and these need to be regulated despite changes in the environment, diet, or level of activity. Each of these variables is controlled by one or more regulators or homeostatic mechanisms, which together maintain life.

For the functioning of homeostatic mechanism, the body must recognize the deviation of any physiological activity from the normal limits. Fortunately, body is provided with appropriate **detectors** or **sensors**, which recognize the deviation. These detectors sense the deviation and alert the **integrating center**. The integrating center immediately sends information to the concerned **effectors** to either accelerate or inhibit the activity so that the normalcy is restored.

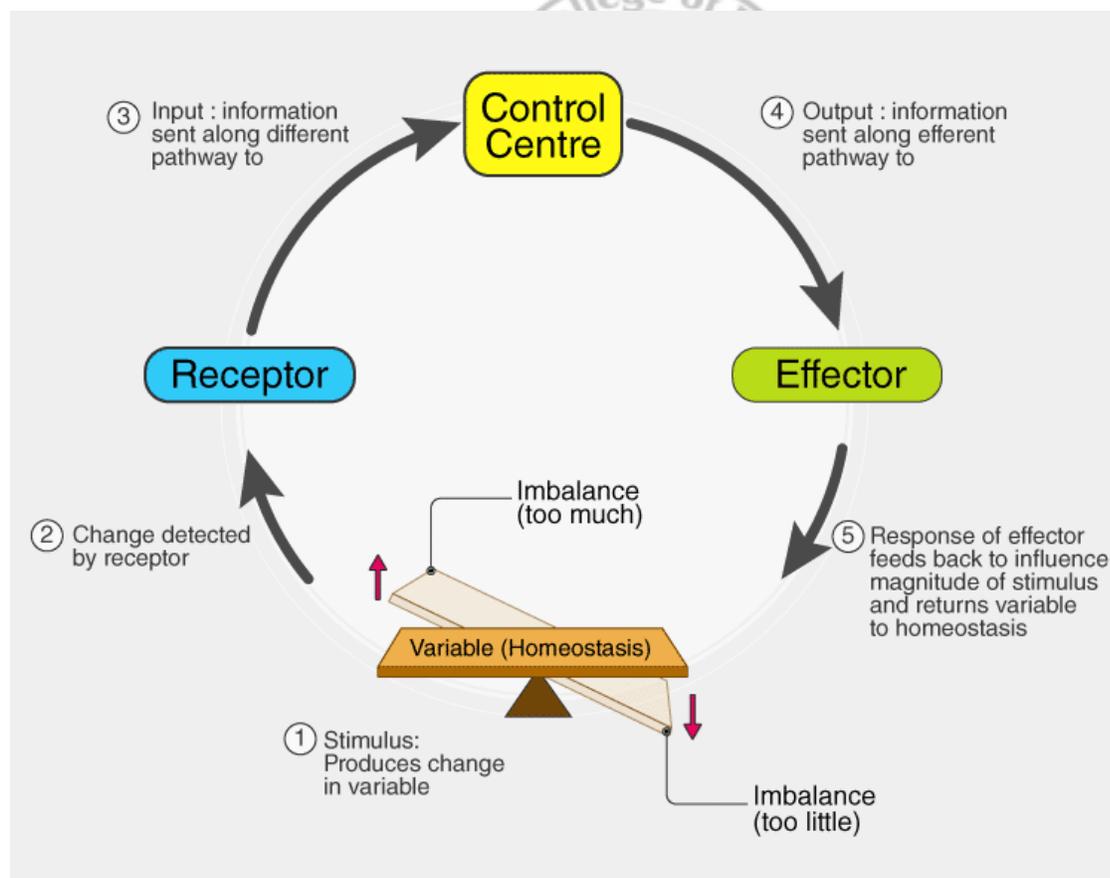


Figure 2.1 Homeostasis

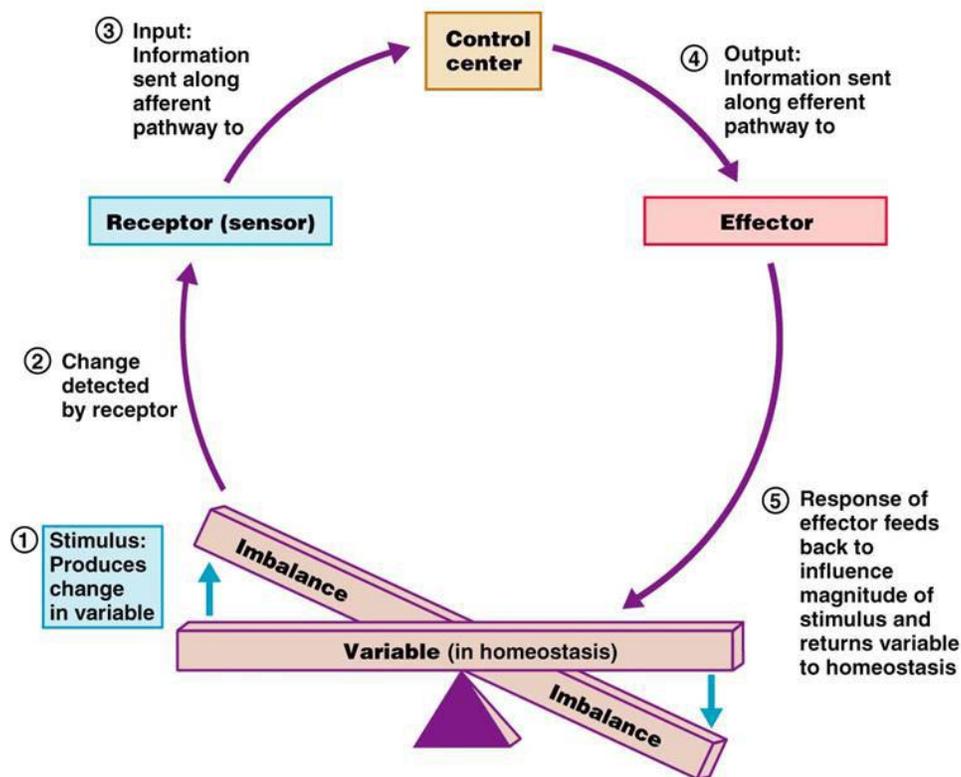


Figure 2.2 Homeostasis

2.1 ROLE OF VARIOUS SYSTEMS OF THE BODY IN HOMEOSTASIS

One or more systems are involved in homeostatic mechanism of each function. Some of the functions in which the homeostatic mechanism is well established are given below:

1. The pH of the ECF has to be maintained at the critical value of 7.4. The tissues cannot survive if it is altered. Thus, the decrease in pH (acidosis) or increase in pH (alkalosis) affects the tissues markedly. The respiratory system, blood and kidney help in the regulation of pH.
2. Body temperature must be maintained at 37.5°C. Increase or decrease in temperature alters the metabolic activities of the cells. The skin, respiratory system, digestive system, excretory system, skeletal muscles and nervous system are involved in maintaining the temperature within normal limits.
3. Adequate amount of nutrients must be supplied to the cells. Nutrients are essential for various activities of the cell and growth of the tissues. These substances also form the source of energy required for various activities of the cells. Nutrients must be digested, absorbed into the blood and supplied to the cells. Digestive system and circulatory system play major roles in the supply of nutrients.
4. Adequate amount of oxygen should be made available to the cells for the metabolism of the

nutrients. Simultaneously, the carbon dioxide and other metabolic end products must be removed. Respiratory system is concerned with the supply of oxygen and removal of carbon dioxide. Kidneys and other excretory organs are involved in the excretion of waste products.

5. Many hormones are essential for the metabolism of nutrients and other substances necessary for the cells. Hormones are to be synthesized and released from the endocrine glands in appropriate quantities and these hormones must act on the body cells appropriately. Otherwise, it leads to abnormal signs and symptoms.

6. Water and electrolyte balance should be maintained optimally. Otherwise it leads to dehydration or water toxicity and alteration in the osmolality of the body fluids. Kidneys, skin, salivary glands and gastrointestinal tract take care of this.

7. For all these functions, the blood, which forms the major part of internal environment, must be normal. It should contain required number of normal red blood cells and adequate amount of plasma with normal composition. Only then, it can transport the nutritive substances, respiratory gases, metabolic and other waste products.

8. Skeletal muscles are also involved in homeostasis. This system helps the organism to move around in search of food. It also helps to protect the organism from adverse surroundings, thus preventing damage or destruction.

9. Central nervous system, which includes brain and spinal cord also, plays an important role in homeostasis. Sensory system detects the state of the body or surroundings. Brain integrates and interprets the pros and cons of these information and commands the body to act accordingly through motor system so that, the body can avoid the damage.

10. Autonomic nervous system regulates all the vegetative functions of the body essential for homeostasis.

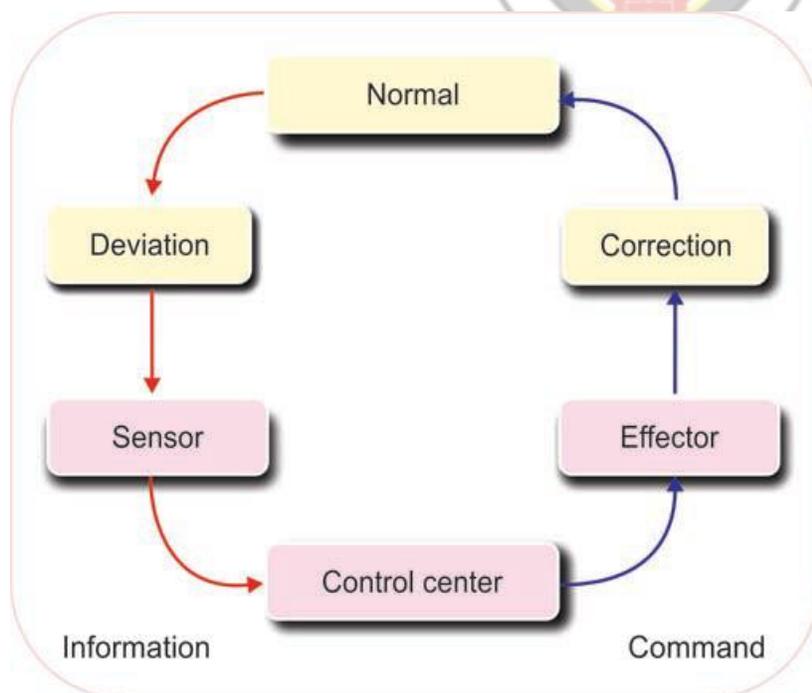


FIGURE 2.3: Components of homeostatic system

2.2 COMPONENTS OF HOMEOSTATIC SYSTEM

Homeostatic system in the body acts through self regulating devices, which operate in a cyclic manner (Fig. 4.1). This cycle includes four components:

- **Sensors** or **detectors**, which recognize the deviation
- Transmission of this message to a **control center**
- Transmission of information from the control center to the effectors for correcting the deviation Transmission of the message or information may be an electrical process in the form of impulses through nerves or a chemical process mainly in the form of hormones through blood and body fluids.
- **Effectors**, which correct the deviation.

2.3 MECHANISM OF ACTION OF HOMEOSTATIC SYSTEM

Homeostatic mechanism in the body is responsible for maintaining the normalcy of various body systems. Whenever there is any change in behavioral pattern of any system, the effectors bring back the normalcy either by inhibiting and reversing the change or by supporting and accelerating the change depending upon requirement of the situation. This is achieved by means of **feedback signals**.

Feedback is a process in which some proportion of the output signal of a system is fed (passed) back to the input. This is done more often intentionally in order to control the behavior pattern of the system. Whenever any change occurs, system receives and reacts to two types of feedback:

1. Negative feedback
2. Positive feedback.

2.3.1 NEGATIVE FEEDBACK

Negative feedback is the one to which the system reacts in such a way as to arrest the change or reverse the direction of change. After receiving a message, effectors send negative feedback signals back to the system. Now, the system stabilizes its own function and makes an attempt to maintain homeostasis.

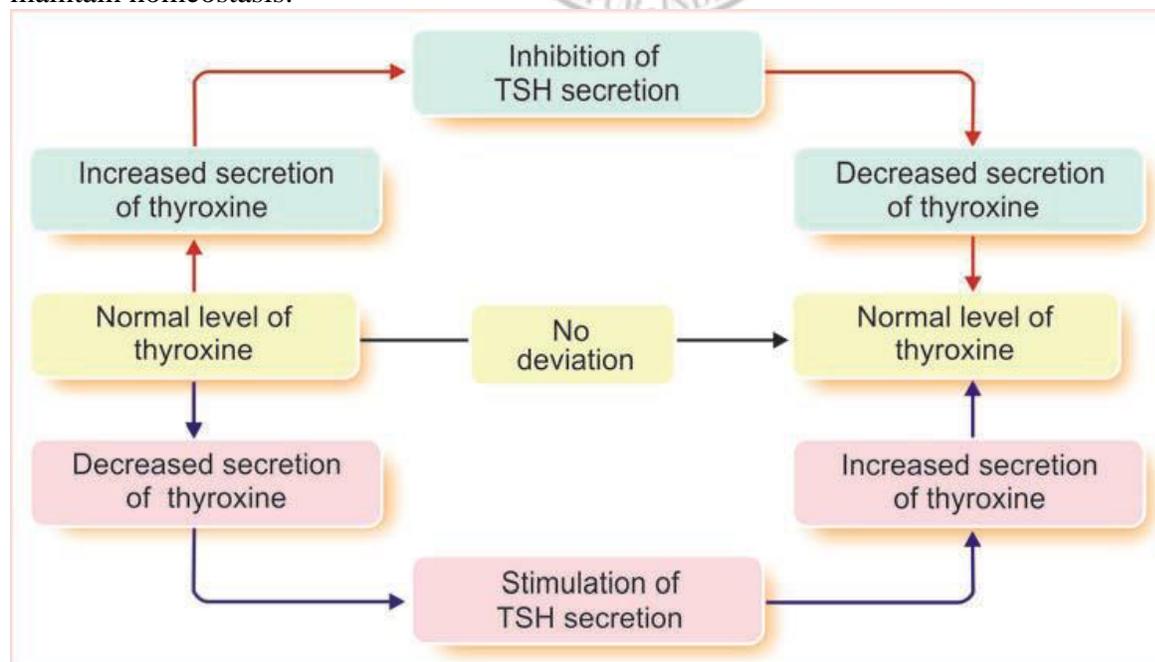


FIGURE 2.4: Negative feedback mechanism – secretion of thyroxine.

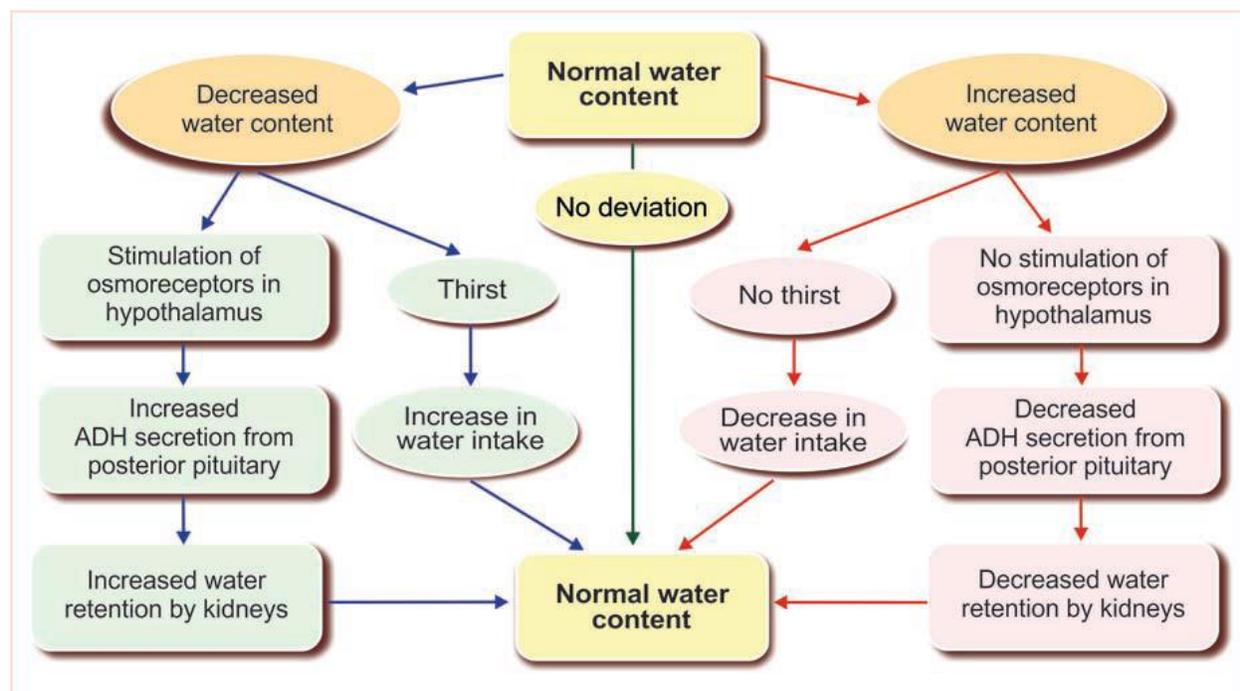


FIGURE 2.4: Negative feedback mechanism – maintenance of water balance.
ADH = Antidiuretic hormone.

Many homeostatic mechanisms in the body function through negative feedback. For example, thyroid-stimulating hormone (TSH) released from pituitary gland stimulates thyroid gland to secrete thyroxine. When thyroxine level increases in blood, it inhibits the secretion of TSH from pituitary so that, the secretion of thyroxine from thyroid gland decreases (Fig. 2.3). On the other hand, if thyroxine secretion is less, its low blood level induces pituitary gland to release TSH. Now, TSH stimulates thyroid gland to secrete thyroxine. Another example for negative feedback mechanism is maintenance of water balance in the body (Fig. 2.4).

2.3.2 POSITIVE FEEDBACK

Positive feedback is the one to which the system reacts in such a way as to increase the intensity of the change in the same direction. Positive feedback is less common than the negative feedback. However, it has its own significance particularly during emergency conditions. One of the positive feedbacks occurs during the blood clotting. Blood clotting is necessary to arrest bleeding during injury and it occurs in three stages.

The three stages are:

- i. Formation of prothrombin activator
- ii. Conversion of prothrombin into thrombin
- iii. Conversion of fibrinogen into fibrin.

Thrombin formed in the second stage stimulates the formation of more prothrombin activator in addition to converting fibrinogen into fibrin (Fig. 2.5). It causes formation of more and more amount of prothrombin activator so that the blood clotting process is accelerated and blood loss is prevented quickly. Other processes where positive feedback occurs are milk ejection reflex and parturition (Fig. 2.6) and both the processes involve oxytocin secretion.

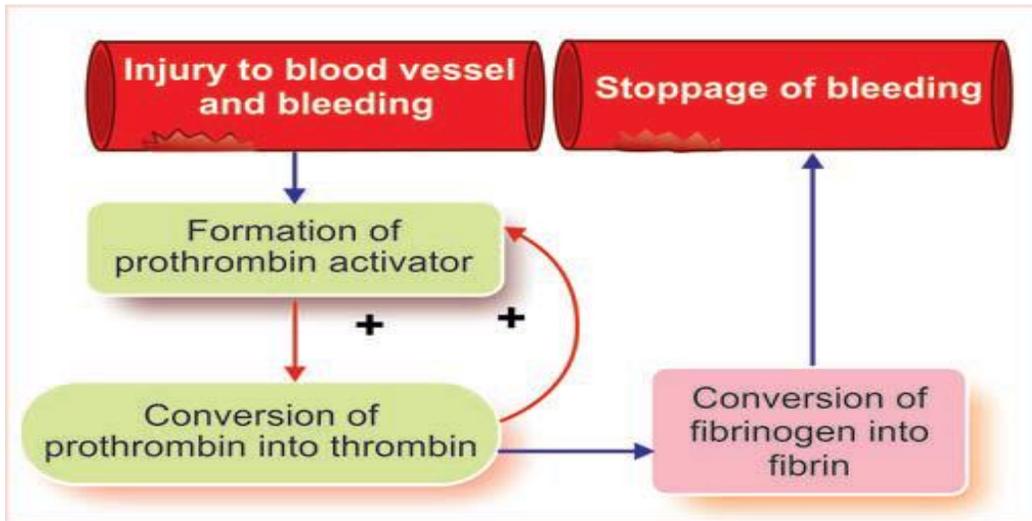


FIGURE 2.5: Positive feedback mechanism – coagulation of blood. Once formed, thrombin induces the formation of more prothrombin activator.

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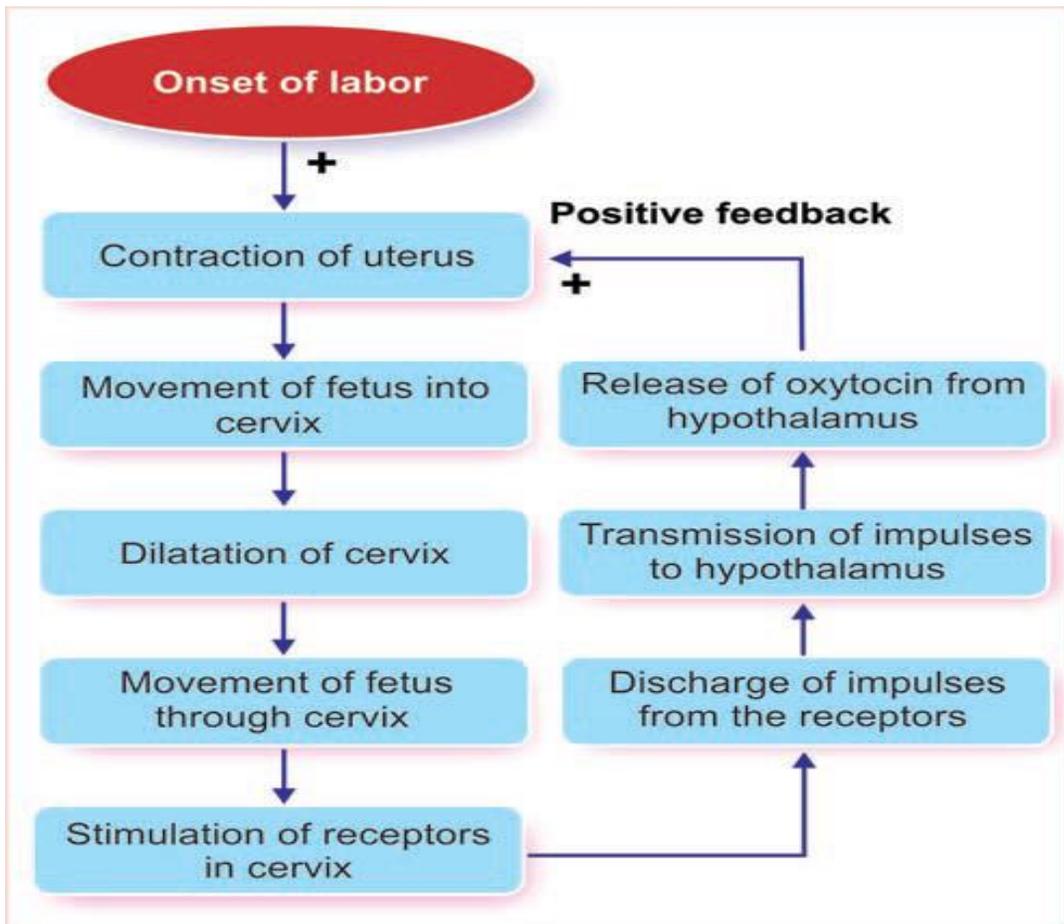


FIGURE 2.6: Positive feedback mechanism – parturition

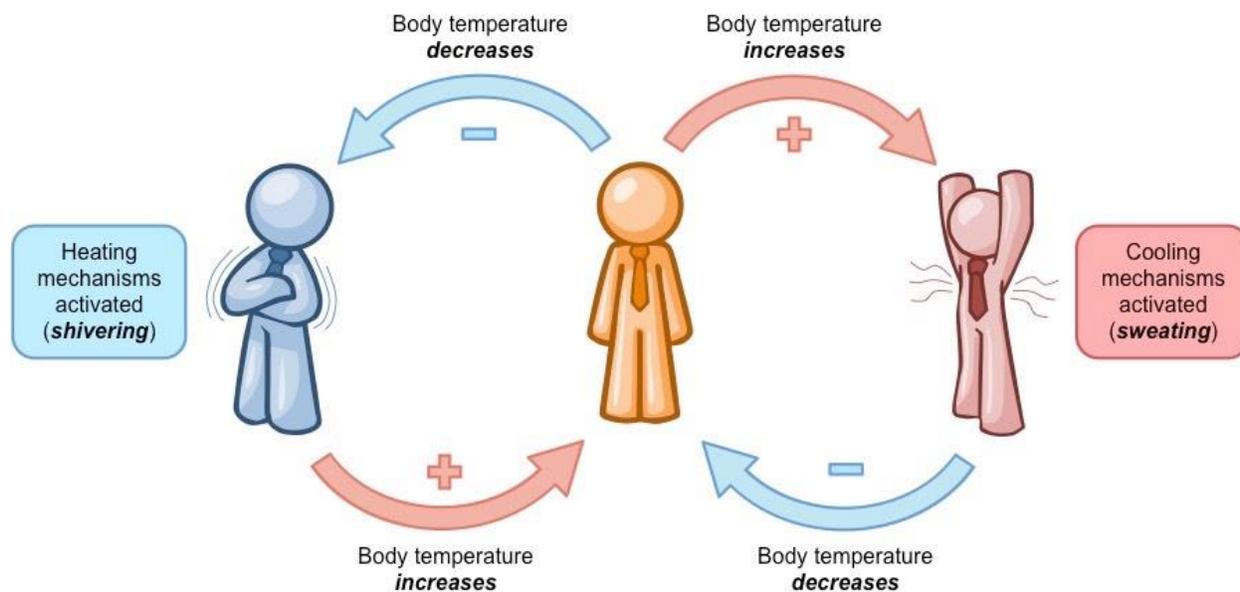


Fig.2.7 Negative feedback & Positive feedback

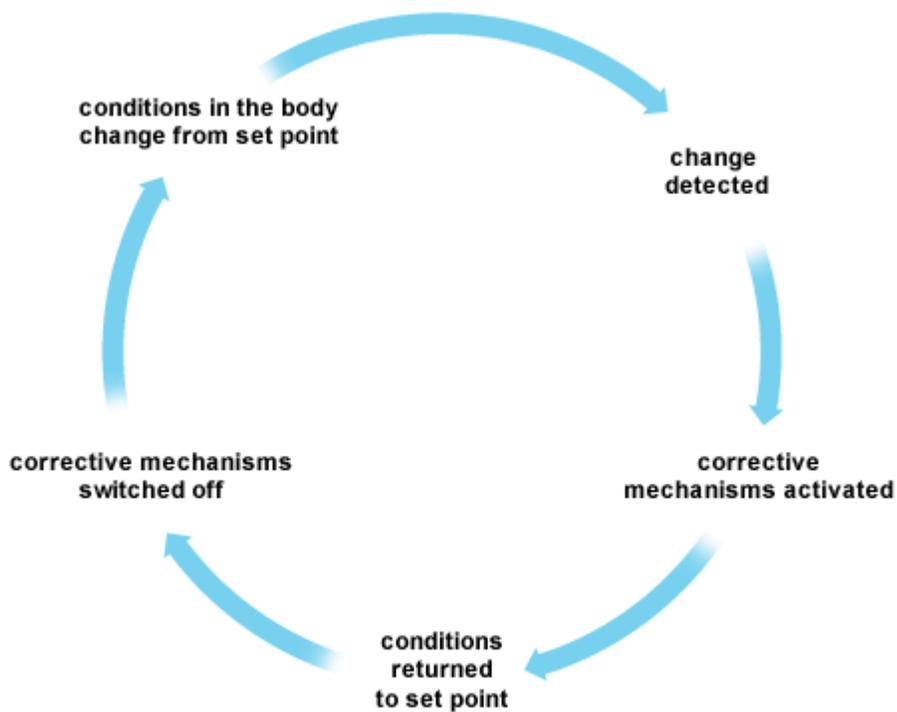


Fig.2.8 Positive feedback

1. MORPHOLOGY OF REVERSIBLE CELL INJURY

After having discussed the molecular and biochemical mechanisms of various forms of cell injury, we now turn to morphologic changes of reversible and irreversible cell injury.

Morphologic terms used in cell injury of varying intensity and from different mechanisms are given in **Table 1.1** and are discussed below. In older literature, the term degeneration was commonly used to denote morphology of reversible cell injury. However, since this term does not provide any information on the nature of underlying changes, currently the term retrogressive changes or simply reversible cell injury are applied to non-lethal cell injury. Common examples of morphologic forms of reversible cell injury are as under:

1. Hydropic change
2. Hyaline change
3. Mucoïd change
4. Fatty change (discussed under intracellular accumulations)

1.1 HYDROPIIC 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). Hydropic swelling is an entirely reversible change upon removal of the injurious agent.

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, is accompanied with rapid flow of water into the cell to maintain iso-osmotic conditions and hence cellular swelling occurs. In addition, influx of calcium too occurs.

Table 1.1 Classification of morphologic forms of cell injury.

MECHANISM OF CELL INJURY	NOMENCLATURE
1. Reversible cell injury	Retrogressive changes (older term: degenerations)
2. Irreversible cell injury	Cell death—necrosis
3. Programmed cell death	Apoptosis
4. Deranged cell metabolism	Intracellular accumulation of lipid, protein, carbohydrate
5. After-effects of necrosis	Gangrene, pathologic calcification

1.2 HYALINE CHANGE

The word 'hyaline' or 'hyalin' means glassy (hyalos = glass). Hyalinisation is a common descriptive histologic term for glassy, homogeneous, eosinophilic appearance of proteinaceous 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 seen in heterogeneous pathologic conditions and may be intracellular or extracellular.

Intracellular Hyaline

Intracellular hyaline is mainly seen in epithelial cells. A few examples are as follows:

1. Hyaline droplets in the proximal tubular epithelial cells due to excessive reabsorption of plasma proteins in proteinuria.
2. Hyaline degeneration of rectus abdominalis muscle called Zenker's degeneration, occurring in typhoid fever. The muscle loses its fibrillar staining and becomes glassy and hyaline.
3. Mallory's hyaline represents aggregates of intermediate filaments in the hepatocytes in alcoholic liver cell injury.
4. Nuclear or cytoplasmic hyaline inclusions seen in some viral infections.
5. Russell's bodies representing excessive immunoglobulins in the rough endoplasmic reticulum of the plasma cells.

Extracellular Hyaline

Extracellular hyaline commonly termed hyalinisation is seen in connective tissues. A few examples of extracellular hyaline change are as under:

1. Hyaline degeneration in leiomyomas of the uterus.
2. Hyalinised old scar of fibrocollagenous tissues.
3. Hyaline arteriosclerosis in renal vessels in hyper tension and diabetes mellitus.
4. Hyalinised glomeruli in chronic glomerulonephritis.
5. Corpora amylacea seen as rounded masses of concentric hyaline laminae in the enlarged prostate in the elderly, in the brain and in the spinal cord in old age, and in old infarcts of the lung.

1.3 MUCOID CHANGE

Mucoid means mucus-like. Mucus is the secretory product of mucous glands and 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 such as ground substance in the umbilical cord. By convention, connective tissue mucin is termed myxoid. Both epithelial and connective tissue 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, instead, stained positively with colloidal iron.

a) Epithelial Mucin

Following are some examples of functional excess of epithelial mucin:

- Catarrhal inflammation of mucous membrane (e.g. of respiratory tract, alimentary tract, uterus).
- Obstruction of duct leading to mucocele in the oral cavity and gallbladder.
- Cystic fibrosis of the pancreas.
- Mucin-secreting tumours (e.g. of ovary, stomach, large bowel etc).

b) Connective Tissue Mucin

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

1. Muroid or myxoid change 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.

BOX:- Morphology of Reversible Cell Injury

- Degenerations or reversible cell injury depict light microscopic changes occurring at ultrastructural level.
- Hydropic swelling is the earliest form of cell injury from various etiologies and its main features are cellular swelling due to cytoplasmic vacuoles.
- Hyaline change is intra- and extracellular deposition of pink, proteinaceous material.
- Muroid change is deposition of mucinous material in epithelial and connective tissues in excessive amounts.

MORPHOLOGY OF IRREVERSIBLE CELL INJURY (CELL DEATH)

Cell death is a state of irreversible injury. It may occur in the living body as a local or focal change (i.e. autolysis, necrosis and apoptosis) and the changes that follow it (i.e. gangrene and pathologic calcification), or result in end of the life (somatic death).

2. CELL DEATH

Cell death occurs by two distinct processes:

1. Apoptosis
2. Necrosis.

2.1 APOPTOSIS

Apoptosis is defined as the natural or **programed death** of the cell under genetic control. Originally, apoptosis refers to the process by which the leaves fall from trees in autumn (In Greek, apoptosis means 'falling leaves'). It is also called '**cell suicide**' since the genes of the cell play a major role in the death. This type of programmed cell death is a normal phenomenon and it is essential for normal development of the body. In contrast to necrosis, apoptosis usually does not produce inflammatory reactions in the neighboring tissues.

Functional Significance of Apoptosis:

The purpose of apoptosis is to remove unwanted cells without causing any stress or damage to the neighboring cells. The functional significance of apoptosis:

1. Plays a vital role in cellular homeostasis. About 10 million cells are produced everyday in human body by mitosis. An equal number of cells die by apoptosis. This helps in cellular homeostasis
2. Useful for removal of a cell that is damaged beyond repair by a virus or a toxin
3. An essential event during the development and in adult stage.

Examples:

- i. A large number of neurons are produced during the development of central nervous system. But up to 50% of the neurons are removed by apoptosis during the formation of synapses between neurons
- ii. Apoptosis is responsible for the removal of tissues of webs between fingers and toes during developmental stage in fetus
- iii. It is necessary for regression and disappearance of duct systems during sex differentiation in fetus.
- iv. The cell that loses the contact with neighboring cells or basal lamina in the epithelial tissue dies by apoptosis. This is essential for the death of old enterocytes that shed into the lumen of intestinal glands.
- v. It plays an important role in the cyclic sloughing of the inner layer of endometrium, resulting in menstruation.
- vi. Apoptosis removes the autoaggressive T cells and prevents autoimmune diseases.

Activation of Apoptosis

Apoptosis is activated by either withdrawal of positive signals (survival factors) or arrival of negative signals. Withdrawal of positive signals Positive signals are the signals which are necessary for the long-time survival of most of the cells. The positive signals are continuously produced by other cells or some chemical stimulants. Best examples of chemical stimulants are:

- i. Nerve growth factors (for neurons)
- ii. Interleukin-2 (for cells like lymphocytes).

The absence or withdrawal of the positive signals activates apoptosis. Arrival of negative signals Negative signals are the external or internal stimuli which initiate apoptosis. The negative signals are produced during various events like:

1. Normal developmental procedures
2. Cellular stress
3. Increase in the concentration of intracellular oxidants
4. Viral infection
5. Damage of DNA
6. Exposure to agents like chemotherapeutic drugs, X-rays, ultraviolet rays and the death-receptor ligands. Death-receptor ligands and death receptors Death receptor ligands are the substances which bind with specific cell membrane receptors and initiate the process of apoptosis. The common death-receptor ligands are tumor necrosis factors (TNF α , TNF β) and Fas ligand (which binds to the receptor called Fas). Death receptors are the cell membrane receptors which receive the death-receptor ligands. Well-characterized death receptors are TNF receptor-1 (TNFR1) and TNF-related apoptosis inducing ligand (TRAIL) receptors called DR4 and DR5. Role of mitochondria in apoptosis External or internal stimuli initiate apoptosis by activating the proteases called **caspases** (cysteiny-dependent aspartate specific proteases). Normally, caspases are suppressed by the inhibitor protein called **apoptosis inhibiting factor (AIF)**. When the cells receive the apoptotic stimulus, mitochondria releases two protein materials. First one is **Cytochrome C** and the second protein is called second mitochondria-derived activator of caspases (SMAC) or its homolog diablo. SMAC/diablo inactivates AIF so that the inhibitor is inhibited. During this process, SMAC/diablo and AIF aggregate to form **apoptosome** which activates caspases. Cytochrome C also facilitates caspase activation.

Apoptotic Process

Cell shows sequence of characteristic morphological changes during apoptosis, viz.:

- i) Activated caspases digest the proteins of cytoskeleton and the cell shrinks and becomes round
- ii) Because of shrinkage, the cell losses the contact with neighboring cells or surrounding matrix
- iii) Chromatin in the nucleus undergoes degradation and condensation
- iv) Nuclear membrane becomes discontinuous and the DNA inside nucleus is cleaved into small fragments
- v) Following the degradation of DNA, the nucleus breaks into many discrete nucleosomal units, which are also called chromatin bodies
- vi) Cell membrane breaks and shows bubbled appearance
- vii) Finally, the cell breaks into several fragments containing intracellular materials including chromatin bodies and organelles of the cell. Such cellular fragments are called vesicles or apoptotic bodies
- viii) Apoptotic bodies are engulfed by phagocytes and dendritic cells.

Abnormal Apoptosis

Apoptosis within normal limits is beneficial for the body. However, too much or too little apoptosis leads to abnormal conditions.

Common abnormalities due to too much apoptosis:

- 1) Ischemic related injuries
- 2) Autoimmune diseases like:
 - i. Hemolytic anemia
 - ii. Thrombocytopenia

- iii. Acquired immunodeficiency syndrome (AIDS)
- 3) Neurodegenerative diseases like Alzheimer's disease.

Common abnormalities due to too little apoptosis:

1. Cancer
2. Autoimmune lymphoproliferative syndrome (ALPS).

2.2 NECROSIS

Necrosis (means 'dead' in Greek) is the uncontrolled and **unprogramed death** of cells due to unexpected and accidental damage. It is also called '**cell murder**' because the cell is killed by extracellular or external events. After necrosis, the harmful chemical substances released from the dead cells cause damage and inflammation of neighboring tissues.

Causes for Necrosis

Common causes of necrosis are injury, infection, inflammation, infarction and cancer. Necrosis is induced by both physical and chemical events such as heat, radiation, trauma, hypoxia due to lack of blood flow and exposure to toxins.

Necrotic Process

Necrosis results in lethal disruption of cell structure and activity. The cell undergoes a series of characteristic changes during necrotic process, viz.

- i) Cell swells causing damage of the cell membrane and appearance of many holes in the membrane
- ii) Intracellular contents leak out into the surrounding environment
- iii) Intracellular environment is altered
- iv) Simultaneously, large amount of calcium ions are released by the damaged mitochondria and other organelles
- v) Presence of calcium ions drastically affects the organization and activities of proteins in the intracellular components
- vi) Calcium ions also induce release of toxic materials that activate the lysosomal enzymes
- vii) Lysosomal enzymes cause degradation of cellular components and the cell is totally disassembled resulting in death
- viii) Products broken down from the disassembled cell are ingested by neighboring cells.

Reaction of Neighboring Tissues after Necrosis

Tissues surrounding the necrotic cells react to the breakdown products of the dead cells, particularly the derivatives of membrane phospholipids like the arachidonic acid. Along with other materials, arachidonic acid causes the following inflammatory reactions in the surrounding tissues:

- Dilatation of capillaries in the region and thereby increasing local blood flow
- Increase in the temperature leading to reddening of the tissues
- Release of histamine from these tissues which induces pain in the affected area
- Migration of leukocytes and macrophages from blood to the affected area because of increased
- capillary permeability
- Movement of water from blood into the tissues causing local edema

- Engulfing and digestion of cellular debris and foreign materials like bacteria by the leukocytes and macrophages
- Activation of immune system resulting in the removal of foreign materials
- Formation of pus by the dead leukocytes during this process
- Finally, tissue growth in the area and wound healing.

3. 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, meaning 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.

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.
- 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 an infiltration. The deposit is in the cytosol and represents an absolute increase in the intracellular lipids. Fatty change is particularly common in the liver but may occur in other non-fatty tissues as well e.g. in 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

Fatty change in the liver may result from one of the two types of causes:

1. Conditions with excess fat

These are conditions in which the capacity of the liver to metabolise fat is exceeded e.g.

- i) Obesity
- ii) Diabetes mellitus
- iii) Congenital hyperlipidaemia

2. Liver cell damage

These are conditions in which fat cannot be metabolised due to liver cell injury e.g.

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

PATHOGENESIS

Mechanism of fatty liver depends upon the stage at which the etiologic agent acts in the normal fat transport and metabolism. Hence, pathogenesis of fatty liver is best understood in the light of normal fat metabolism in the liver.

Lipids as free fatty acids enter the liver cell from either of the following 2 sources:

- From diet as chylomicrons (containing triglycerides and phospholipids) and as free fatty acids.
- From adipose tissue as free fatty acids.

Normally, besides above two sources, a small part of fatty acids is also synthesised from acetate in the liver cells. Most of free fatty acid is esterified to triglycerides by the action of □-glycerophosphate and only a small part is changed into cholesterol, phospholipids and ketone bodies. While cholesterol, phospholipids and ketones are used in the body, intracellular triglycerides are converted into lipoproteins, which require 'lipid acceptor protein'. Lipoproteins are released from the liver cells into circulation as plasma lipoproteins (LDL, VLDL).

In fatty liver, intracellular accumulation of triglycerides occurs due to defect at one or more of the following 6 steps in the normal fat metabolism

- Increased entry of free fatty acids into the liver.
- Increased synthesis of fatty acids by the liver.
- Decreased conversion of fatty acids into ketone bodies resulting in increased esterification of fatty acids to triglycerides.
- Increased □-glycerophosphate causing increased esterification of fatty acids to triglycerides.
- Decreased synthesis of 'lipid acceptor protein' resulting in decreased formation of lipoprotein from triglycerides.
- Block in the excretion of lipoprotein from the liver into plasma.

In most cases of fatty liver, one of the above mechanisms is operating. But liver cell injury from chronic alcoholism is multifactorial as follows:

- a) Increased lipolysis
- b) Increased free fatty acid synthesis
- c) Decreased triglyceride utilisation
- d) Decreased fatty acid oxidation to ketone bodies
- e) Block in lipoprotein excretion

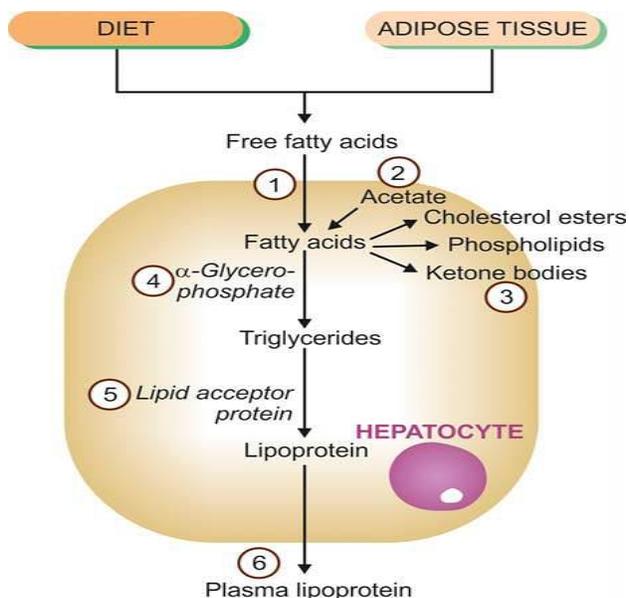


Figure 3.1 Lipid metabolism in the pathogenesis of fatty liver. Defects in any of the six numbered steps (corresponding to the description in the text) can produce fatty liver by different etiologic agents.

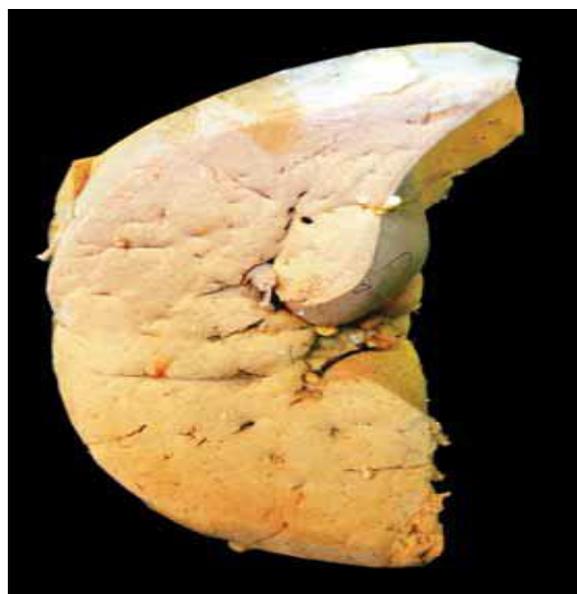


Figure 3.2 Fatty liver. Sectioned slice of the liver shows pale yellow parenchyma with rounded borders.

Even a severe form of fatty liver may be reversible if the liver is given time to regenerate and progressive fibrosis has not developed. For example, intermittent drinking is less harmful because the liver cells get time to recover; similarly a chronic alcoholic who becomes teetotaler the enlarged fatty liver may return to normal if fibrosis has not developed.

Cholesterol Deposits

Intracellular deposits of cholesterol and its esters in macrophages may occur when there is hypercholesterolaemia. This turns macrophages into foam cells. The examples are as follows:

1. Fibrofatty plaques of atherosclerosis.
2. Clusters of foam cells in tumour-like masses called xanthomas and xanthelasma.

Stromal Fatty Infiltration

This form of lipid accumulation is quite different from parenchymal fatty change just described. Stromal fatty infiltration is the deposition of mature adipose cells in the stromal connective tissue in contrast to intracellular deposition of fat in the parenchymal cells in fatty change. The condition occurs most often in patients with obesity. The two commonly affected organs are the heart and the pancreas. Thus, heart can be the site for intramyocardial fatty change as well as epicardial (stromal) fatty infiltration. The presence of mature adipose cells in the stroma generally does not produce any dysfunction.

INTRACELLULAR ACCUMULATION OF PROTEINS

Pathologic accumulation of proteins in the cytoplasm of cells may occur in the following conditions:

1. In proteinuria, there is excessive renal tubular re absorption of proteins by the proximal tubular epithelial cells which show pink hyaline droplets in their cytoplasm. The change is reversible; with control of proteinuria the protein droplets disappear.
2. The cytoplasm of actively functioning plasma cells shows pink hyaline inclusions called Russell's bodies representing synthesised immunoglobulins.
3. In α 1-antitrypsin deficiency, the cytoplasm of hepatocytes shows eosinophilic globular deposits of a mutant protein.
4. Mallory's body or alcoholic hyaline in the hepatocytes is intracellular accumulation of intermediate filaments of cytokeratin and appear as amorphous pink masses.

INTRACELLULAR ACCUMULATION OF GLYCOGEN

Conditions associated with excessive accumulation of intracellular glycogen are as under:

- In diabetes mellitus, there is intracellular accumulation of glycogen in different tissues because normal cellular uptake of glucose is impaired. Glycogen deposits in diabetes mellitus are seen in epithelium of distal portion of proximal convoluted tubule and descending loop of Henle, in the hepatocytes, in beta cells of pancreatic islets, and in cardiac muscle cells. In routine H & E stained sections, deposits of glycogen produce clear vacuoles in the cytoplasm of the affected cells. Best's carmine and periodic acid-Schiff (PAS) staining may be employed to confirm the presence of glycogen in the cells.
- In glycogen storage diseases or glycogenosis, there is defective metabolism of glycogen due to genetic disorders.

BOX: Intracellular Accumulations

- Intracellular accumulations may occur from normal constituents of cell metabolism (e.g. fats, proteins, carbohydrates), or accumulation of abnormal substances due to either absence of some metabolic enzymes or due to pigments.
- Fatty change is deposition of fat in the parenchymal cells or organs such as liver, kidneys, muscle, pancreas etc.
- Fatty liver is more common and occurs from various etiologies, most often from alcoholic liver disease; others are obesity, diabetes, starvation, pregnancy, drugs etc.
- Mechanism for fatty liver is due to excess of free fatty acids, either from diet or from adipose tissues, resulting in intracellular accumulation of triglycerides in the hepatocytes.
- Fatty liver is characterised by enlarged pale-yellow liver, having cytoplasmic vacuoles (microvesicles or macrovesicles) in the hepatocytes.

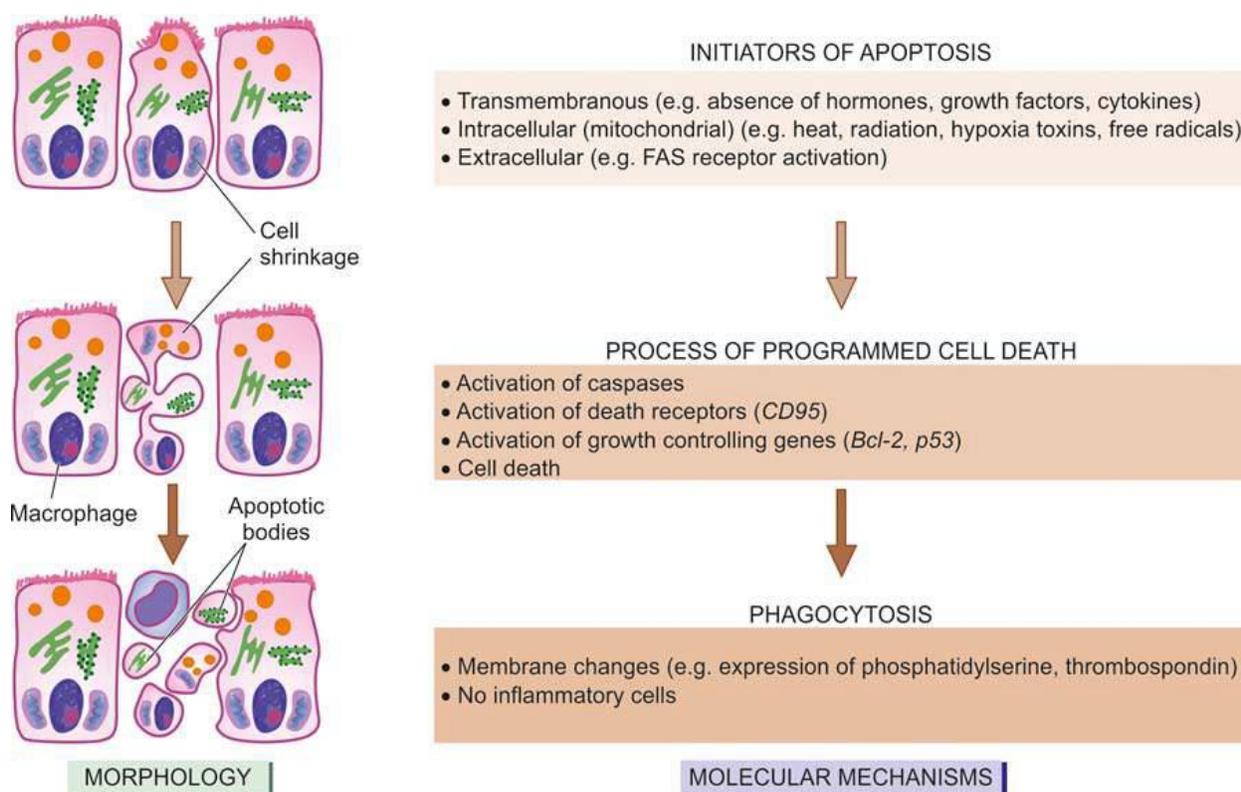


Figure 3.3 Molecular mechanism of apoptosis contrasted with sequence of morphologic changes.

4. PATHOLOGIC CALCIFICATION

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 is characterised by deposition of calcium salts in dead or degenerated tissues with normal calcium metabolism and normal serum calcium level.
- 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.

4.1 DYSTROPHIC CALCIFICATION

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

- 1) Calcification in dead tissue.
- 2) Calcification of degenerated tissue.

1) Calcification in dead tissue

- a) 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.
- b) Liquefaction necrosis in chronic abscesses may get calcified.
- c) Fat necrosis following acute pancreatitis or traumatic fat necrosis in the breast results in deposition of calcium soaps.
- d) Gamna-Gandy bodies in chronic venous congestion (CVC) of the spleen is characterised by calcific deposits admixed with haemosiderin on fibrous tissue.
- e) Infarcts may sometimes undergo dystrophic calcification.
- f) Thrombi, especially in the veins, may produce phleboliths.
- g) Haematomas in the vicinity of bones may undergo dystrophic calcification.
- h) Dead parasites like in hydatid cyst, Schistosoma eggs, and cysticercosis are some of the examples showing dystrophic calcification.
- i) Microcalcification in breast cancer detected by mammography.
- j) Congenital toxoplasmosis involving the central nervous system visualised by calcification in the infant brain.

2) Calcification in degenerated tissues

- a) Dense old scars may undergo hyaline degeneration and subsequent calcification.
- b) Atheromas in the aorta and coronaries frequently undergo calcification.
- c) Mönckeberg's sclerosis shows calcification in the degenerated tunica media of muscular arteries in elderly people.
- d) Stroma of tumours such as uterine fibroids, breast cancer, thyroid adenoma, goitre etc show calcification.
- e) Goitre of the thyroid may show presence of calcification in areas of degeneration.
- f) 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.
- g) Cysts which have been present for a long time may show calcification of their walls e.g. epidermal and pilar cysts.
- h) Calcinosis cutis is a condition of unknown cause in which there are irregular nodular deposits of calcium salts in the skin and subcutaneous tissue.
- i) 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.

Pathogenesis of dystrophic calcification

The process of dystrophic calcification has been likened to the formation of normal hydroxyapatite of bone i.e. binding of phosphate ions with calcium ions to form precipitates of calcium phosphate. It involves phases of initiation and propagation as follows:

- a) Initiation: Following cell injury (i.e. degeneration or necrosis), there is membrane damage and release of membrane phospholipids. Phosphatases associated with phospholipids generate phosphate ions. It is also known that there is excess uptake of calcium by injured mitochondria in degeneration and necrosis. Thus, calcium and phosphate so generated from these mechanisms form precipitates of calcium phosphate.
- b) Propagation: Simultaneously, some structural changes occur in calcium and phosphate groups which result in further propagation of deposits and form mineral crystals.

4.2 METASTATIC CALCIFICATION

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

- 1) Excessive mobilisation of calcium from the bone.
- 2) Excessive absorption of calcium from the gut.

1) Excessive mobilisation of calcium from the bone

These causes are more common and include the following:

- a) Hyperparathyroidism which may be primary such as due to parathyroid adenoma, or secondary such as from parathyroid hyperplasia, chronic renal failure etc.
- b) Bony destructive lesions such as multiple myeloma, metastatic carcinoma.
- c) Hypercalcaemia as a part of paraneoplastic syndrome e.g. in breast cancer.
- d) Prolonged immobilisation of a patient results in disuse atrophy of the bones and hypercalcaemia.

2) Excessive absorption of calcium from the gut

Less often, excess calcium may be absorbed from the gut causing hypercalcaemia and metastatic calcification. These causes are as under:

- a) Hypervitaminosis D from excessive intake or in sarcoidosis.
- b) Milk-alkali syndrome caused by excessive oral intake of calcium in the form of milk and administration of calcium carbonate in the treatment of peptic ulcer.
- c) Idiopathic hypercalcaemia of infancy (Williams's syndrome).
- d) Renal causes such as in renal tubular acidosis.

Sites of metastatic calcification

Metastatic calcification may occur in any normal tissue of the body but preferentially affects the following organs and tissues:

1. Kidneys, especially at the basement membrane of tubular epithelium and in the tubular lumina causing nephrocalcinosis.
2. Lungs, especially in the alveolar walls.
3. Stomach, on the acid-secreting fundal glands.
4. Blood vessels, especially on the internal elastic lamina.
5. Cornea is another site affected by metastatic calcification.
6. Synovium of the joint causing pain and dysfunction.

Pathogenesis of metastatic calcification

Metastatic calcification occurs due to excessive binding of inorganic phosphate ions with elevated calcium ions due to underlying metabolic derangement. This leads to precipitates of calcium phosphate at the preferential sites, due to presence of acid secretions or rapid changes in pH levels at these sites. Metastatic calcification is reversible upon correction of underlying metabolic disorder.

The distinguishing features between the two types of pathologic calcification are summarised in **Table 4.1.**

Table 4.1 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

5. ADAPTIVE DISORDERS

Adaptive disorders are the adjustments which the cells make in response to stresses which may be for physiologic needs (physiologic adaptation) or a response to non-lethal pathologic injury (pathologic adaptation). Broadly speaking, such physiologic and pathologic adaptations occur by following processes (**Fig. 5.1**):

- Decreasing or increasing their size i.e. atrophy and hypertrophy respectively, or by increasing their number i.e. hyperplasia (postfix word -trophy means nourishment; -plasia means growth of new cells).
- Changing the pathway of phenotypic differentiation of cells i.e. metaplasia and dysplasia (prefix word meta- means transformation; dys- means bad development).

In general, the adaptive responses are reversible on withdrawal of stimulus. However, if the irritant stimulus persists for long time, the cell may not be able to survive and may either die or progress further e.g. cell death may occur in sustained atrophy; dysplasia may progress into carcinoma in situ. Thus, the concept of evolution 'survival of the fittest' holds true for adaptation as 'survival of the adaptable'.

Various mechanisms which may be involved in adaptive cellular responses include the following:

1. Altered cell surface receptor binding.
2. Alterations in signal for protein synthesis.
3. Synthesis of new proteins by the target cell such as heat shock proteins (HSPs).

Common forms of cellular adaptive responses along with examples of physiologic and pathologic adaptations are briefly discussed here.

5.1 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).

5.1.1 CAUSES

Atrophy may occur from physiologic or pathologic causes:

A. Physiologic atrophy

Atrophy is a normal process of ageing in some tissues, which could be due to loss of endocrine stimulation or arteriosclerosis. For example:

- i) Atrophy of lymphoid tissue with age.
- ii) Atrophy of thymus in adult life.
- iii) Atrophy of gonads after menopause.
- iv) Atrophy of brain with ageing.
- v) Osteoporosis with reduction in size of bony trabeculae due to ageing.

B. Pathologic atrophy

The causes are as under:

- a) 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.
- b) 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 the brain in cerebral atherosclerosis.
- c) 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.
- d) Neuropathic atrophy Interruption in nerve supply leads to wasting of muscles e.g.
 - i) Poliomyelitis
 - ii) Motor neuron disease
 - iii) Nerve section.
- e) 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.
- f) Pressure atrophy Prolonged pressure from benign tumours or cyst or aneurysm may cause compression and atrophy of the tissues e.g.

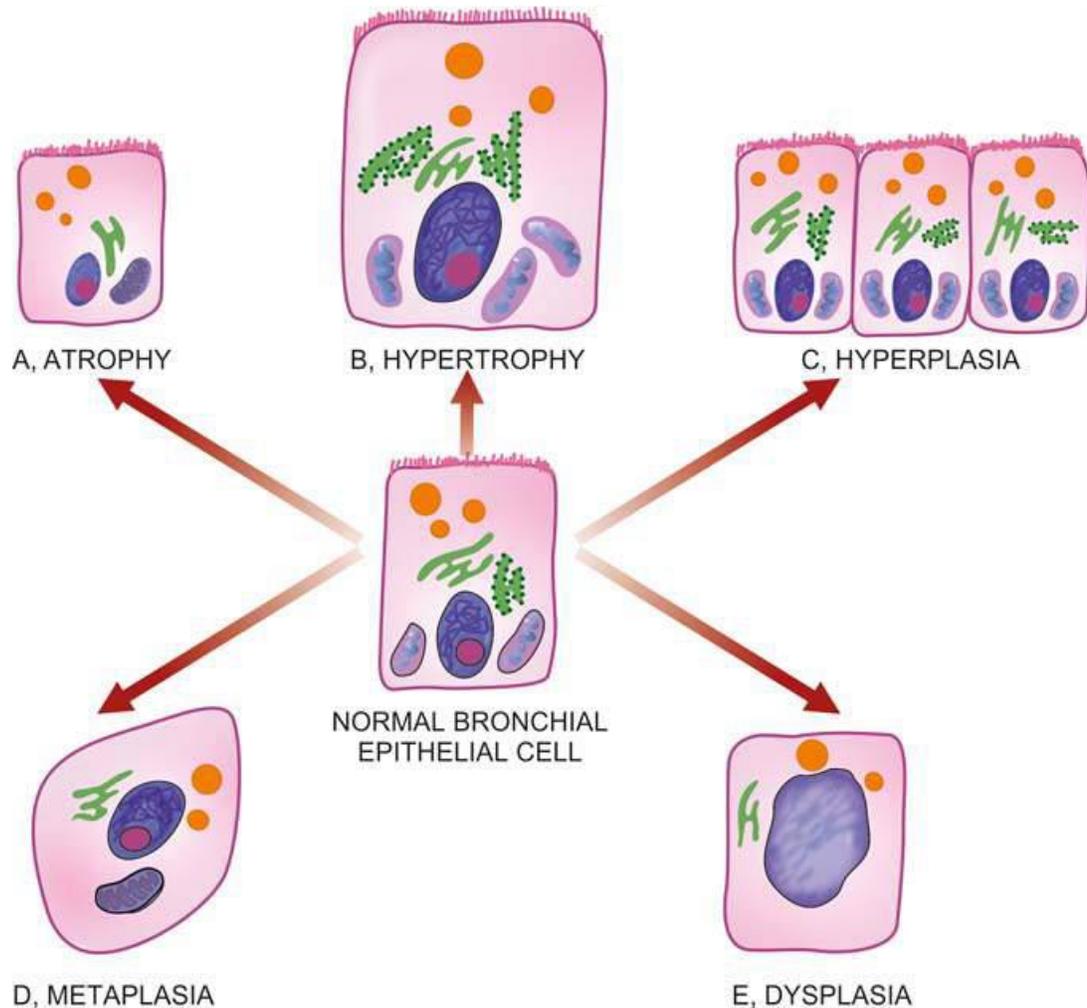


Figure 5.1 Adaptive disorders of growth.

- i) Erosion of the spine by tumour in nerve root.
- ii) Erosion of the skull by meningioma arising from piaarachnoid.
- iii) Erosion of the sternum by aneurysm of arch of aorta.
- g) Idiopathic atrophy There are some examples of atrophy where no obvious cause is present e.g.
 - i) Myopathies.
 - ii) Testicular atrophy.

5.2 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.

5.2.1 CAUSES

Hypertrophy may be physiologic or pathologic. In either case, it is caused by increased functional demand or by hormonal stimulation. Hypertrophy without accompanying hyperplasia affects mainly muscles. In non-dividing cells too, only hypertrophy occurs.

A. Physiologic hypertrophy

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

B. Pathologic hypertrophy

Examples of certain diseases associated with hypertrophy are as under:

- a) 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
- b) Hypertrophy of smooth muscle e.g.
 - i) Cardiac achalasia (in oesophagus)
 - ii) Pyloric stenosis (in stomach)
 - iii) Intestinal strictures
 - iv) Muscular arteries in hypertension.
- c) Hypertrophy of skeletal muscle e.g. hypertrophied muscles in athletes and manual labourers.
- d) 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.

5.3 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. All body cells do not possess hyperplastic growth potential. Labile cells (e.g. epithelial cells of the skin and mucous membranes, cells of the bone marrow and lymph nodes) and stable cells (e.g. parenchymal cells of the liver, pancreas, kidney, adrenal, and thyroid) can undergo hyperplasia, while permanent cells (e.g. neurons, cardiac and skeletal

muscle) have little or no capacity for regenerative hyperplastic growth. Neoplasia differs from hyperplasia in having hyperplastic growth with loss of growth-regulatory mechanism due to change in genetic composition of the cell, while hyperplasia persists so long as stimulus is present.

5.3.1 CAUSES

As with other adaptive disorders of growth, hyperplasia may also be physiologic and pathologic.

A. Physiologic hyperplasia

The two most common types are hormonal and compensatory:

- a) 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.
- b) Compensatory hyperplasia i.e. hyperplasia occurring following removal of part of an organ or in the 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 occurring at the margin of a non-healing ulcer.
- v) Intraductal epithelial hyperplasia in fibrocystic change in the breast.

5.4 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 progress to dysplasia and further into cancer (**Fig. 5.2**).

Metaplasia is broadly divided into 2 types: epithelial and mesenchymal.

5.4.1 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 well specialized 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 of epithelium transformed, two types of epithelial metaplasia are seen:
squamous and columnar.

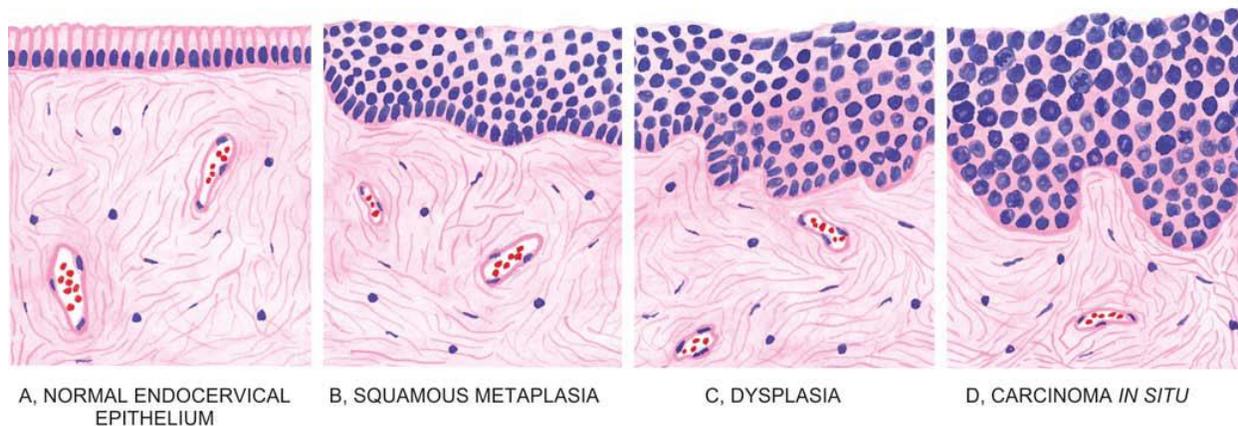


Figure 5.2 Schematic diagram showing sequential changes in uterine cervix from normal epithelium to development of carcinoma in situ.

a) 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.

b) 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.
- iii) Conversion of pseudostratified ciliated columnar epithelium in chronic bronchitis and bronchiectasis to columnar type.
- iv) In cervical erosion (congenital and adult type), there is variable area of endocervical glandular mucosa eroded into the vagina.

5.4.2 Mesenchymal Metaplasia

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

a) 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 e.g. in leiomyoma.

b) Cartilaginous metaplasia

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

5.5 DYSPLASIA

Dysplasia means 'disordered cellular development', often preceded or 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 as under:

- Increased number of layers of epithelial cells
- Disorderly arrangement of cells from basal layer to the surface layer
- Loss of basal polarity i.e. nuclei lying away from basement membrane
- Cellular and nuclear pleomorphism
- Increased nucleocytoplasmic ratio
- Nuclear hyperchromatism
- 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 may progress into carcinoma in situ (cancer confined to layers superficial to basement membrane) or invasive cancer. The differences between dysplasia and metaplasia are contrasted in **Table 5.1**.

BOX: Adaptive Disorders

- Atrophy is reduction of the number and size of parenchymal cells of an organ or its parts which was once normal.
- 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.
- Hyperplasia is an increase in the number of parenchymal cells resulting in enlargement of the organ or tissue.
- 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.

Table 5.1 Differences between metaplasia and dysplasia.

FEATURE	METAPLASIA	DYSPLASIA
i) Definition	Change of one type of epithelial or mesenchymal cell to another type of adult epithelial or mesenchymal cell	Disordered cellular development, may be accompanied with hyperplasia or metaplasia
ii) Types	Epithelial (squamous, columnar) and mesenchymal (osseous, cartilaginous)	Epithelial only
iii) Tissues affected	Most commonly affects bronchial mucosa, uterine endocervix; others mesenchymal tissues (cartilage, arteries)	Uterine cervix, bronchial mucosa
iv) Cellular changes	Mature cellular development	Disordered cellular development (pleomorphism, nuclear hyperchromasia, mitosis, loss of polarity)
v) Natural history	Reversible on withdrawal of stimulus	May regress on removal of inciting stimulus, or may progress to higher grades of dysplasia or carcinoma in situ

6. DISTURBANCES OF ACID-BASE STATUS

ACIDOSIS

Acidosis is the reduction in pH (increase in H⁺ concentration) below normal range.

Acidosis is produced by:

1. Increase in partial pressure of CO₂ in the body fluids particularly in arterial blood
2. Decrease in HCO₃⁻ concentration.

ALKALOSIS

Alkalosis is the increase in pH (decrease in H⁺ concentration) above the normal range.

Alkalosis is produced by:

1. Decrease in partial pressure of CO₂ in the arterial blood
2. Increase in HCO₃⁻ concentration.

Since the partial pressure of CO₂ (pCO₂) in arterial blood is controlled by lungs, the acid-base disturbances produced by the change in arterial pCO₂ are called the respiratory disturbances.

On the other hand, the disturbances in acid-base status produced by the change in HCO₃⁻ concentration are generally called the metabolic disturbances.

Thus the acid-base disturbances are:

1. Respiratory acidosis
2. Respiratory alkalosis
3. Metabolic acidosis
4. Metabolic alkalosis.

6.1 RESPIRATORY ACIDOSIS

Respiratory acidosis is the acidosis that is caused by alveolar hypoventilation. During hypoventilation the lungs fail to expel CO₂, which is produced in the tissues. CO₂ is the major end product of oxidation of carbohydrates, proteins and fats.

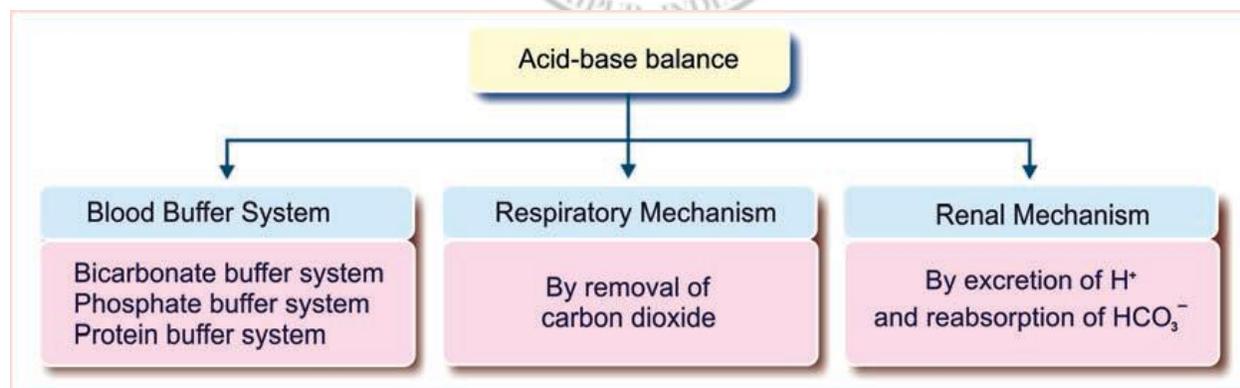


FIGURE 6.1: Regulation of acid-base balance

CO₂ accumulates in blood where it reacts with water to form carbonic acid, which is called **respiratory acid**. Carbonic acid dissociates into H⁺ and HCO₃⁻. The increased H⁺ concentration in blood leads to decrease in pH and acidosis. Normal partial pressure of CO₂ in arterial blood is about 40 mm Hg. When it increases above 60 mm Hg acidosis occurs.

Causes of Excess CO₂ in the Body

Hypoventilation (decreased ventilation) is the primary cause for excess CO₂ in the body. Some of the conditions when increase in pCO₂ and respiratory acidosis occur due to hypoventilation

6.2 RESPIRATORY ALKALOSIS

Respiratory alkalosis is the alkalosis that is caused by alveolar hyperventilation. Hyperventilation causes excess loss of CO₂ from the body. Loss of CO₂ leads to decreased formation of carbonic acid and decreased release of H⁺. Decreased H⁺ concentration increases the pH leading to respiratory alkalosis. When the partial pressure of CO₂ in arterial blood decreases below 20 mm Hg, alkalosis occurs.

Causes of Decrease in CO₂ in the Body

Hyperventilation is primary cause for loss of excess CO₂ from the body because during hyperventilation, lot of CO₂ is expired through respiratory tract leading to decreased pCO₂. Some of the conditions when decreased pCO₂ and respiratory alkalosis occur due to hyperventilation

6.3 METABOLIC ACIDOSIS

Metabolic acidosis is the acid-base imbalance characterized by excess accumulation of **organic acids** in the body, which is caused by abnormal metabolic processes. Organic acids such as lactic acid, ketoacids and uric acid are formed by normal metabolism. The quantity of these acids increases due to abnormality in the metabolism.

Causes of Metabolic Acidosis

Lactic acid

The amount of lactic acid increases during anaerobic glycolysis in some abnormal conditions such as circulatory shock.

Ketoacids

The amount of ketoacids increases because of insulin deficiency as in the case of diabetes mellitus. In diabetes mellitus, glucose is not utilized due to lack of insulin. So, lipids are utilized for liberation of energy resulting in production of excess acetoacetic acid and beta hydroxybutyric acid.

Uric acid

The amount of uric acid increases in the body due to the failure of excretion. Normally uric acid is excreted by kidneys. But in renal diseases, the kidneys fail to excrete the uric acid. Some of the conditions when the metabolic acids increase in the body resulting in metabolic acidosis.

6.4 METABOLIC ALKALOSIS

Metabolic alkalosis is the acid-base imbalance caused by loss of excess H⁺ resulting in increased HCO₃⁻ concentration. Some of the endocrine disorders, renal tubular disorders, etc. cause metabolic disorders leading to loss of H⁺. It increases HCO₃⁻ and pH in the body leading to metabolic alkalosis. Some of the conditions when excess H⁺ is lost and HCO₃ content increases leading to metabolic alkalosis.

ELECTROLYTE IMBALANCE

Electrolyte imbalance, or **water-electrolyte imbalance**, is an abnormality in the concentration of electrolytes in the body. Electrolytes play a vital role in maintaining homeostasis in the body. They help to regulate heart and neurological function, fluid balance, oxygen delivery, acid–base balance and much more. Electrolyte imbalances can develop by consuming too little or too much electrolyte as well as excreting too little or too much electrolyte.

Electrolyte disturbances are involved in many disease processes, and are an important part of patient management in medicine. The causes, severity, treatment, and outcomes of these disturbances can vastly differ depending on the implicated electrolyte. The most serious electrolyte disturbances involve abnormalities in the levels of sodium, potassium or calcium. Other electrolyte imbalances are less common and often occur in conjunction with major electrolyte changes. The kidney is the most important organ in maintaining appropriate fluid and electrolyte balance, but other factors such as hormonal changes and physiological stress play a role.

Chronic laxative abuse or severe diarrhea or vomiting can lead to dehydration and electrolyte imbalance. People suffering from malnutrition are at especially high risk for an electrolyte imbalance. Severe electrolyte imbalances must be treated carefully as there are risks with overcorrecting too quickly, which can result in arrhythmias, brain herniation, or refeeding syndrome depending on the cause of imbalance.

GENERAL FUNCTION

Electrolytes are important because they are what cells (especially nerve, heart and muscle cells) use to maintain voltages across their cell membranes. Electrolytes have different functions, and an important one is to carry electrical impulses between cells. Kidneys work to keep the electrolyte concentrations in blood constant despite changes in the body. For example, during heavy exercise, electrolytes are lost in sweat, particularly in the form of sodium and potassium. The kidneys can also generate dilute urine to balance sodium levels. These electrolytes must be replaced to keep the electrolyte concentrations of the body fluids constant. Hyponatremia, or low sodium, is the most commonly seen type of electrolyte imbalance.

Treatment of electrolyte imbalance depends on the specific electrolyte involved and whether the levels are too high or too low. The level of aggressiveness of treatment and choice of treatment may change depending on the severity of the disturbance. If the levels of an electrolyte are too low, a common response to electrolyte imbalance may be to prescribe supplementation. However, if the electrolyte involved is sodium, the issue is not a deficiency of sodium, but rather a water excess, causing the imbalance. Supplementation for these people may correct the electrolyte imbalance but at the expense of volume overload, which can be dangerous particularly for neonates. Because each individual electrolyte affects physiological function differently, they must be considered separately when discussing causes, treatment, and complications.

SODIUM

Sodium is the most abundant electrolyte in the blood. Sodium and its homeostasis in the human body is highly dependent on fluids. The human body is approximately 60% water, a percentage which is also known as total body water. The total body water can be divided into two compartments called extracellular fluid (ECF) and intracellular fluid (ICF). The majority of the

sodium in the body stays in the extracellular fluid compartment. This compartment consists of the fluid surrounding the cells and the fluid inside the blood vessels. ECF has a sodium concentration of approximately 140 mEq/L. Because cells membranes are permeable to water and not sodium, the movement of water across membranes impacts the concentration of sodium in the blood. Sodium acts as a force that pulls water across membranes, and water moves from places with lower sodium concentration to places with higher sodium concentration. This happens through a process called osmosis. When evaluating sodium imbalances, both total body water and total body sodium must be considered.

I) Hypernatremia

Hypernatremia means that the concentration of sodium in the blood is too high. An individual is considered to be having high sodium at levels above 145 mEq/L of sodium. Hypernatremia is not common in individuals with no other health concerns. Most individuals with this disorder have either experienced loss of water from diarrhea, altered sense of thirst, inability to consume water, inability of kidneys to make concentrated urine, or increased salt intake.

■ Causes

There are three types of hypernatremia each with different causes. The first is dehydration along with low total body sodium. This is most commonly caused by heatstroke, burns, extreme sweating, vomiting, and diarrhea. The second is low total body water with normal body sodium. This can be caused by diabetes insipidus, renal disease, hypothalamic dysfunction, sickle cell disease, and certain drugs. The third is increased total body sodium which is caused by increased ingestion, Conn's syndrome, or Cushing's syndrome.

■ Symptoms

Symptoms of hypernatremia may vary depending on type and how quickly the electrolyte disturbance developed. Common symptoms are dehydration, nausea, vomiting, fatigue, weakness, increased thirst, excess urination. Patients may be on medications that caused the imbalance such as diuretics or nonsteroidal anti-inflammatory drugs. Some patients may have no obvious symptoms at all.

■ Treatment

It is crucial to first assess the stability of the patient. If there are any signs of shock such as tachycardia or hypotension, these must be treated immediately with IV saline infusion. Once the patient is stable, it is important to identify the underlying cause of hypernatremia as that may affect the treatment plan. The final step in treatment is to calculate the patients free water deficit, and to replace it at a steady rate using a combination of oral or IV fluids. The rate of replacement of fluids varies depending on how long the patient has been hypernatremic. Lowering the sodium level too quickly can cause cerebral edema.

II) Hyponatremia

Hyponatremia means that the concentration of sodium in the blood is too low. It is generally defined as a concentration lower than 135 mEq/L. This relatively common electrolyte disorder can indicate the presence of a disease process, but in the hospital setting is more often due to administration of Hypotonic fluids. The majority of hospitalized patients only experience mild hyponatremia, with levels above 130 mEq/L. Only 1-4% of patients experience levels lower than 130 mEq/L.

■ Causes

Hyponatremia has many causes including heart failure, chronic kidney disease, liver disease, treatment with thiazide diuretics, psychogenic polydipsia, syndrome of inappropriate antidiuretic hormone secretion. It can also be found in the postoperative state, and in the setting of accidental water intoxication as can be seen with intense exercise. Common causes in pediatric patients may be diarrheal illness, frequent feedings with dilute formula, water intoxication via excessive consumption, and enemas. Pseudohyponatremia is a false low sodium reading that can be caused by high levels of fats or proteins in the blood. Dilutional hyponatremia can happen in diabetics as high glucose levels pull water into the blood stream causing the sodium concentration to be lower. Diagnosis of the cause of hyponatremia relies on three factors: volume status, plasma osmolality, urine sodium levels and urine osmolality.

■ Symptoms

Many individuals with mild hyponatremia will not experience symptoms. Severity of symptoms is directly correlated with severity of hyponatremia and rapidness of onset. General symptoms include loss of appetite, nausea, vomiting, confusion, agitation, and weakness. More concerning symptoms involve the central nervous system and include seizures, coma, and death due to brain herniation. These usually do not occur until sodium levels fall below 120 mEq/L.

■ Treatment

Considerations for treatment include symptom severity, time to onset, volume status, underlying cause, and sodium levels. If the sodium level is <120 mEq/L, the person can be treated with hypertonic saline as extremely low levels are associated with severe neurological symptoms. In non-emergent situations, it is important to correct the sodium slowly to minimize risk of osmotic demyelination syndrome. If a person has low total body water and low sodium they are typically given fluids. If a person has high total body water (such as due to heart failure or kidney disease) they may be placed on fluid restriction, salt restriction, and treated with a diuretic. If a person has a normal volume of total body water, they may be placed on fluid restriction alone.

POTASSIUM

Potassium resides mainly inside the cells of the body, so its concentration in the blood can range anywhere from 3.5 mEq/L to 5 mEq/L. The kidneys are responsible for excreting the majority of potassium from the body. This means their function is crucial for maintaining a proper balance of potassium in the blood stream.

I) Hyperkalemia

Hyperkalemia means the concentration of potassium in the blood is too high. This occurs when the concentration of potassium is >5 mEq/L. It can lead to cardiac arrhythmias and even death. As such it is considered to be the most dangerous electrolyte disturbance.

■ Causes

Hyperkalemia is typically caused by decreased excretion by the kidneys, shift of potassium to the extracellular space, or increased consumption of potassium rich foods in patients with kidney failure. The most common cause of hyperkalemia is lab error due to potassium released as blood cells from the sample break down. Other common causes are kidney disease, cell death, acidosis, and drugs that affect kidney function.

■ Symptoms

Part of the danger of hyperkalemia is that it is often asymptomatic, and only detected during normal lab work done by primary care physicians. As potassium levels get higher, individuals may begin to experience nausea, vomiting, and diarrhea. Patients with severe hyperkalemia, defined by levels above 7 mEq/L, may experience muscle cramps, numbness, tingling, absence of reflexes, and paralysis. Patients may experience arrhythmias that can result in death.

■ Treatment

There are three mainstays of treatment of hyperkalemia. These are stabilization of cardiac cells, shift of potassium into the cells, and removal of potassium from the body. Stabilization of cardiac muscle cells is done by administering calcium intravenously.^[3] Shift of potassium into the cells is done using both insulin and albuterol inhalers. Excretion of potassium from the body is done using either hemodialysis, loop diuretics, or a resin that causes potassium to be excreted in the fecal matter.

II) Hypokalemia

The most common electrolyte disturbance, hypokalemia means that the concentration of potassium is <3.5 mEq/L. It often occurs concurrently with low magnesium levels.

■ Causes

Low potassium is caused by increased excretion of potassium, decreased consumption of potassium rich foods, movement of potassium into the cells, or certain endocrine diseases. Excretion is the most common cause of hypokalemia and can be caused by diuretic use, metabolic acidosis, diabetic ketoacidosis, hyperaldosteronism, and renal tubular acidosis. Potassium can also be lost through vomiting and diarrhea.

■ Symptoms

Hypokalemia is often asymptomatic, and symptoms may not appear until potassium concentration is <2.5 mEq/L. Typical symptoms consist of muscle weakness and cramping. Low potassium can also cause cardiac arrhythmias.

■ Treatment

Hypokalemia is treated by replacing the body's potassium. This can occur either orally or intravenously. Because low potassium is usually accompanied by low magnesium, patients are often given magnesium alongside potassium.

CALCIUM

Though calcium is the most plentiful electrolyte in the body, a large percentage of it is used to form the bones. It is mainly absorbed and excreted through the GI system. The majority of calcium resides extracellularly, and it is crucial for the function of neurons, muscle cells, function of enzymes, and coagulation. The normal range for calcium concentration in the body is 8.5 - 10.5 mg/dL. The parathyroid gland is responsible for sensing changes in calcium concentration and regulating the electrolyte with parathyroid hormone.

I) Hypercalcemia

Hypercalcemia describes when the concentration of calcium in the blood is too high. This occurs above 10.5 mg/dL.

■ Causes

The most common causes of hypercalcemia are certain types of cancer, hyperparathyroidism, hyperthyroidism, pheochromocytoma, excessive ingestion of

vitamin D, sarcoidosis, and tuberculosis. Hyperparathyroidism and malignancy are the predominate causes. It can also be caused by muscle cell breakdown, prolonged immobilization, dehydration.

■ Symptoms

The predominant symptoms of hypercalcemia are abdominal pain, constipation, kidney stones, extreme thirst, excessive urination, nausea and vomiting.^{[3][10]} In severe cases where the calcium concentration is >14 mg/dL, individuals may experience confusion, altered mental status, coma, and seizure.

■ Treatment

Primary treatment of hypercalcemia consists of administering IV fluids. If the hypercalcemia is severe and/or associated with cancer, it may be treated with bisphosphonates. For very severe cases, hemodialysis may be considered for rapid removal of calcium from the blood.

II) Hypocalcemia

Hypocalcemia describes when calcium levels are too low in the blood, usually less than 8.5 mg/dL.

■ Causes

Hypoparathyroidism and vitamin D deficiency are common causes of hypocalcemia.^[3] It can also be caused by malnutrition, blood transfusion, ethylene glycol intoxication, and pancreatitis.

■ Symptoms

Neurological and cardiovascular symptoms are the most common manifestations of hypocalcemia. Patients may experience muscle cramping or twitching, and numbness around the mouth and fingers. They may also have shortness of breath, low blood pressure, and cardiac arrhythmias.

■ Treatment

Patients with hypocalcemia may be treated with either oral or IV calcium. Typically, IV calcium is reserved for patients with severe hypocalcemia. It is also important to check magnesium levels in patients with hypocalcemia and to replace magnesium if it is low.

MAGNESIUM

Magnesium is mostly found in the bones and within cells. Approximately 1% of total magnesium in the body is found in the blood. Magnesium is important in control of metabolism and is involved in numerous enzyme reactions. A normal range is 0.70 - 1.10 mmol/L. The kidney is responsible for maintaining the magnesium levels in this narrow range.

I) Hypermagnesemia

Hypermagnesemia, or abnormally high levels of magnesium in the blood, is relatively rare in individuals with normal kidney function. This is defined by a magnesium concentration >2.5 mg/dL.

■ Causes

Hypermagnesemia typically occurs in individuals with abnormal kidney function. This imbalance can also occur with use of antacids or laxatives that contain magnesium. Most cases of hypermagnesemia can be prevented by avoiding magnesium-containing medications.

■ Symptoms

Mild symptoms include nausea, flushing, tiredness. Neurologic symptoms are seen most commonly including decreased deep tendon reflexes. Severe symptoms include paralysis, respiratory failure, and bradycardia progressing to cardiac arrest.

■ Treatment

If kidney function is normal, stopping the source of magnesium intake is sufficient. Diuretics can help increase magnesium excretion in the urine. Severe symptoms may be treated with dialysis to directly remove magnesium from the blood.

II) Hypomagnesemia: (Magnesium deficiency)

Hypomagnesemia, or low magnesium levels in the blood, can occur in up to 12% of hospitalized patients. Symptoms or effects of hypomagnesemia can occur after relatively small deficits.

■ Causes

Major causes of hypomagnesemia are from gastrointestinal losses such as vomiting and diarrhea. Another major cause is from kidney losses from diuretics, alcohol use, hypercalcemia, and genetic disorders. Low dietary intake can also contribute to magnesium deficiency.

■ Symptoms

Hypomagnesemia is typically associated with other electrolyte abnormalities, such as hypokalemia and hypocalcemia. For this reason, there may be overlap in symptoms seen in these other electrolyte deficiencies. Severe symptoms include arrhythmias, seizures, or tetany.

■ Treatment

The first step in treatment is determining whether the deficiency is caused by a gastrointestinal or kidney problem. People with no or minimal symptoms are given oral magnesium; however, many people experience diarrhea and other gastrointestinal discomfort. Those who cannot tolerate or receive magnesium, or those with severe symptoms can receive intravenous magnesium.

Hypomagnesemia may prevent the normalization of other electrolyte deficiencies. If other electrolyte deficiencies are associated, normalizing magnesium levels may be necessary to treat the other deficiencies.

CHLORIDE

Chloride, after sodium, is the second most abundant electrolyte in the blood, and most abundant in the extracellular fluid. Most of the chloride in the body is from salt (NaCl) in the diet. Chloride is part of gastric acid (HCl), which plays a role in absorption of electrolytes, activating enzymes, and killing bacteria. The levels of chloride in the blood can help determine if there are underlying metabolic disorders. Generally, chloride has an inverse relationship with bicarbonate, an electrolyte that indicates acid-base status. Overall, treatment of chloride imbalances involve addressing the underlying cause rather than supplementing or avoiding chloride.

I) Hyperchloremia: (Excessive chloride)**■ Causes**

Hyperchloremia, or high chloride levels, is usually associated with excess chloride intake (e.g., saltwater drowning), fluid loss (e.g., diarrhea, sweating), and metabolic acidosis.

■ Symptoms

Patients are usually asymptomatic with mild hyperchloremia. Symptoms associated with hyperchloremia are usually caused by the underlying cause of this electrolyte imbalance.

■ Treatment

Treat the underlying cause, which commonly includes increasing fluid intake.

II) Hypochloremia: (Chloride Deficiency)**■ Causes**

Hypochloremia, or low chloride levels, are commonly associated with gastrointestinal (e.g., vomiting) and kidney (e.g., diuretics) losses. Greater water or sodium intake relative to chloride also can contribute to hypochloremia.

■ Symptoms

Patients are usually asymptomatic with mild hypochloremia. Symptoms associated with hypochloremia are usually caused by the underlying cause of this electrolyte imbalance.

■ Treatment

Treat the underlying cause, which commonly includes increasing fluid intake.

DIETARY SOURCES

Diet significantly contributes to our electrolyte stores and blood levels. Below are a list of foods that are associated with higher levels of these electrolytes.

I) Sodium

It is recommended that an individual consumes less than 2,300 mg of sodium daily as part of a healthy diet. A significant portion of our sodium intake comes from a just a few types of food, which can be surprising as large sources of sodium may not taste salty.

- Breads, rolls
- Soups
- Cured meats and cold cuts
- Cheese
- Savory snacks (e.g., chips, crackers, pretzels)

II) Potassium

Good sources of potassium are found in a variety of fruits and vegetables. Recommend potassium intake for adults ranges from 2,300 mg to 3,400 mg depending on age and gender.

- Beans and lentils
- Dark leafy greens (e.g., spinach, kale)
- Apples
- Apricots
- Potatoes
- Squash

III) Calcium

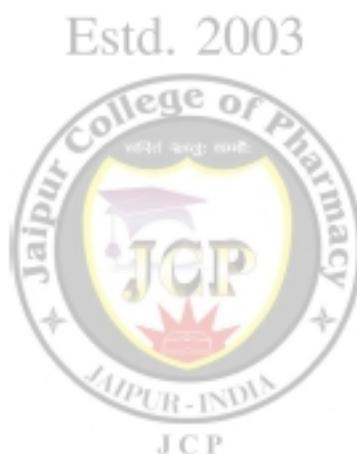
Dairy is a major contributor of calcium to diet in the United States. The recommended calcium intake for adults range from 1,000 mg to 1,300 mg depending on age and gender.

- Yogurt
- Cheese
- Milk
- Tofu
- Canned sardines

IV) Magnesium

Magnesium is found in a variety of vegetables, meats, and grains. Foods high in fiber generally are a source of magnesium. The recommended magnesium intake for adults range from 360 mg to 420 mg depending on age and gender.

- Epsom salt
- Nuts and seeds (e.g., pumpkin seeds, almonds, peanuts)
- Dark leafy greens (e.g., spinach)
- Beans
- Fortified cereals



1. INFLAMMATION

1.1 INTRODUCTION

1.1.1 Definition and Causes

Inflammation is defined as the local response of living mammalian tissues to injury from 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 injurious 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 antigen antibody 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.

Inflammation is a protective response by the body to variety of etiologic agents (infectious or non-infectious), while infection is invasion into the body by harmful microbes and their resultant ill-effects by toxins. Inflammation involves 2 basic processes with some overlapping.

1.2 SIGNS OF INFLAMMATION

4 cardinal signs of inflammation as:

- i) *rubor* (redness);
- ii) *tumor* (swelling);
- iii) *calor* (heat); and
- iv) *dolor* (pain).

To these, fifth sign *functio laesa* (loss of function) was later added

1.3 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 after delay, 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.

The characteristic feature of chronic inflammation is presence of chronic inflammatory cells such as lymphocytes, plasma cells and macrophages, granulation tissue formation, and in specific situations as granulomatous inflammation.

In some instances, the term *subacute inflammation* is used for the state of inflammation between acute and chronic.

1.3.1 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

I. VASCULAR EVENTS

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

- haemodynamic changes and
- changes in vascular permeability.

i) 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 cell injury, immediate vascular response is of **transient vasoconstriction** of arterioles. With mild 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*.

▪ Triple Response

The features of haemodynamic changes in inflammation are best demonstrated by the **Lewis experiment**. Lewis induced the changes in the skin of inner aspect of forearm by firm stroking with a blunt point. The reaction so elicited is known as *triple response* or *red line response* consisting of the following:

- i) *Red line* appears within a few seconds after stroking and is due to local vasodilatation of capillaries and venules.
- ii) *Flare* is the bright reddish appearance or flush surrounding the red line and results from vasodilatation of the adjacent arterioles.
- iii) *Wheal* is the swelling or oedema of the surrounding skin occurring due to transudation of fluid into the extravascular space.

These features, thus, elicit the classical signs of inflammation—

redness, heat and swelling, to which fourth feature, pain, has been added.

ii) Altered 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. The differences between transudate and exudate. The appearance of inflammatory oedema due to increased vascular permeability of microvascular bed is explained on the basis of **Starling's hypothesis**. According to this, normally the fluid balance is maintained by two opposing sets of forces:

i) Forces that cause **outward movement** of fluid from microcirculation: These are *intravascular hydrostatic pressure* and *colloid osmotic pressure of interstitial fluid*.

ii) Forces that cause **inward movement** of interstitial fluid into circulation: These are *intravascular colloid osmotic pressure* and *hydrostatic pressure of interstitial fluid*.

Whatever little fluid is left in the interstitial compartment is drained away by lymphatics and, thus, no oedema results normally (**Fig. 1.1 A**).

However, in inflamed tissues, the endothelial lining of microvasculature becomes more leaky. Consequently, intravascular colloid osmotic pressure decreases and osmotic pressure of the interstitial fluid increases resulting in excessive outward flow of fluid into the interstitial compartment which is exudative inflammatory oedema (**Fig. 1.1,B**).

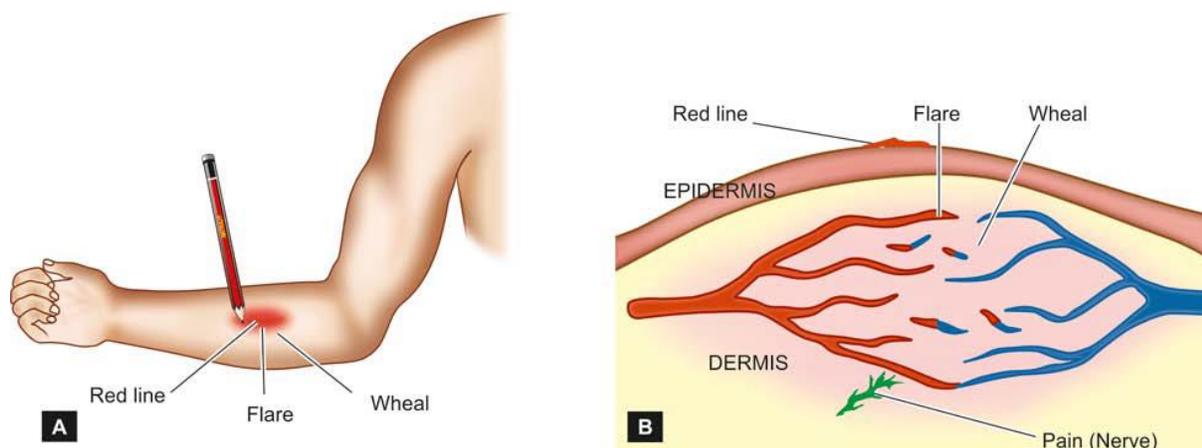


Figure 1.1 A, 'Triple response' elicited by firm stroking of skin of forearm with a pencil. **B**, Diagrammatic view of microscopic features of triple response of the skin.

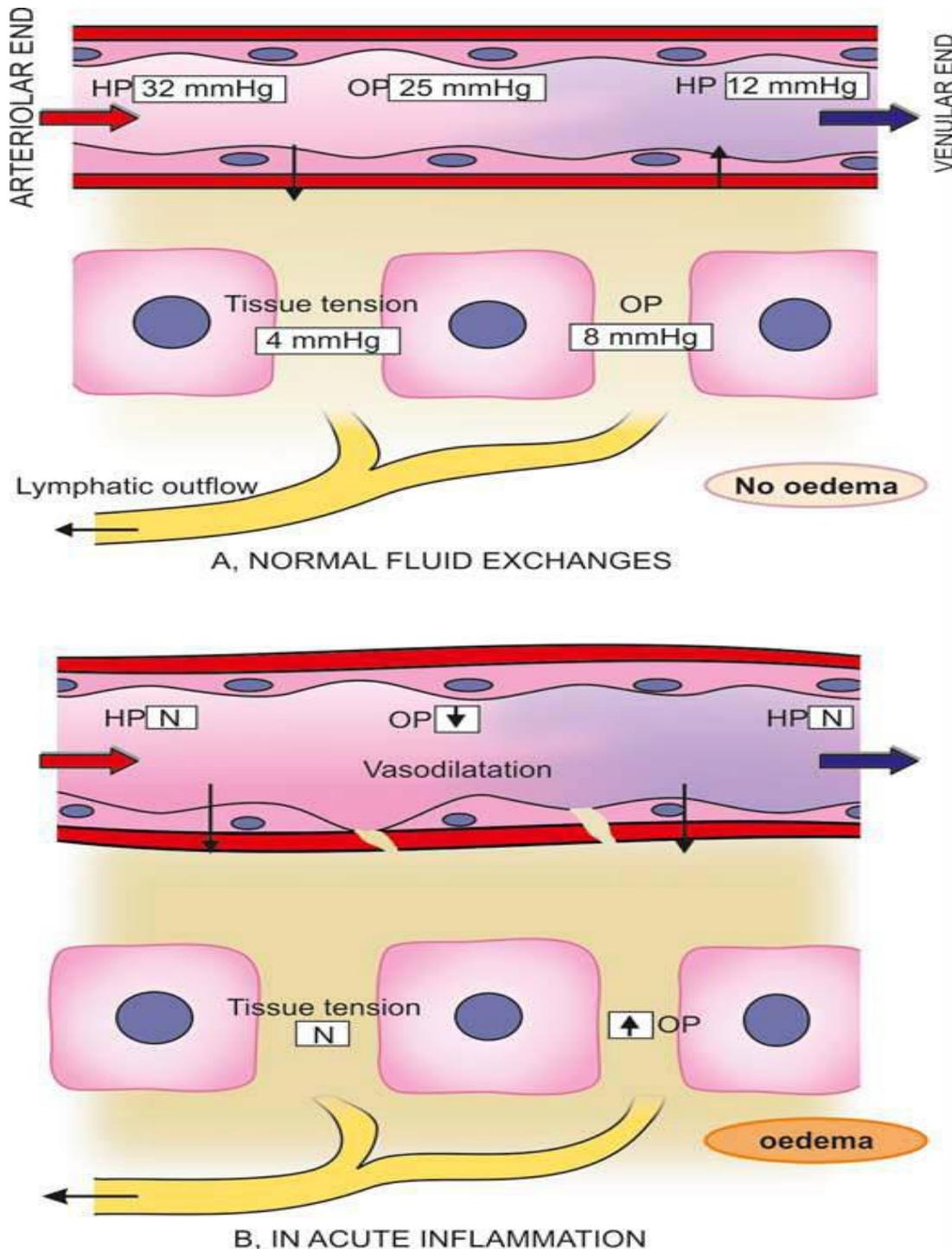


Figure 1.2 Fluid interchange between blood and extracellular fluid (ECF). (HP = hydrostatic pressure, OP = osmotic pressure).

PATTERNS OF INCREASED VASCULAR PERMEABILITY

Increased vascular permeability in acute inflammation by which normally non-permeable endothelial layer of microvasculature becomes leaky can have following patterns and mechanisms which may be acting singly or more often in combination (**Fig. 1.3**):

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).

An example of such *immediate transient response* is mild thermal injury of skin of forearm.

ii) Contraction or mild endothelial damage

In this mechanism, there is structural re-organisation of the cytoskeleton of endothelial cells that causes reversible retraction at the intercellular junctions or mild form of endothelial damage. This change affects venules and capillaries and is mediated by cytokines such as interleukin- 1 (IL-1) and tumour necrosis factor (TNF)-a. The onset of response occurs after delay of 4-6 hours following injury and lasts for several hours to days. Classic example of *delayed and prolonged leakage* is appearance of sunburns mediated by ultraviolet radiation.

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 involving platelets and fibrin 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*). The examples of *immediate sustained leakage* are severe bacterial infections while delayed prolonged leakage may occur following moderate thermal injury and radiation injury.

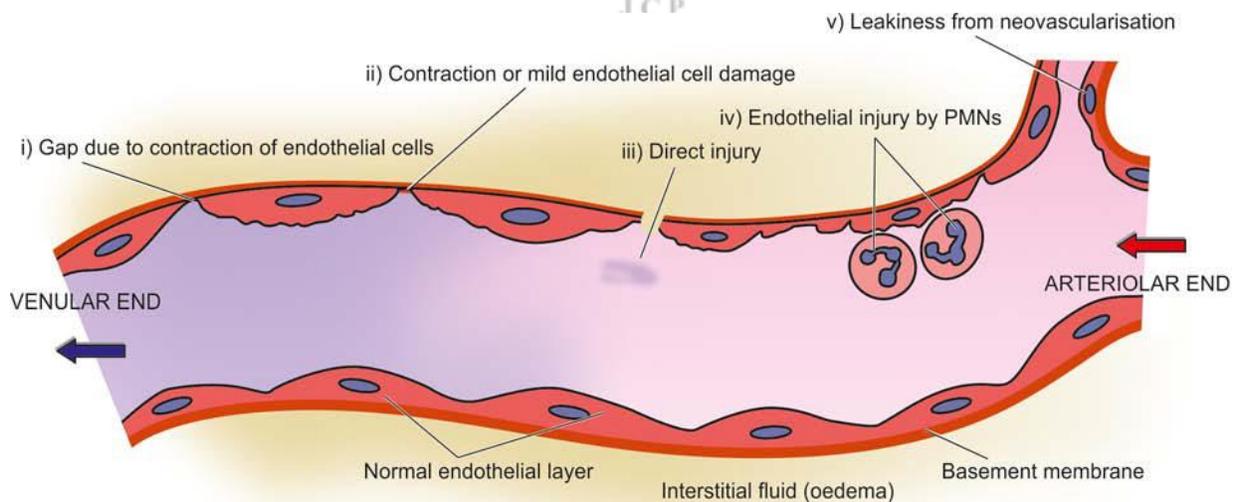


Figure 1.3 Schematic illustration of pathogenesis of increased vascular permeability in acute inflammation. The serial numbers in the figure correspond to five numbers described in the text.

iv) Leucocyte-mediated endothelial injury

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. This form of increased vascular leakiness affects mostly venules and is a *late response*.

The examples are seen in sites where leucocytes adhere to the vascular endothelium e.g. in pulmonary venules and capillaries.

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:

- a) exudation of leucocytes; and
- b) phagocytosis.

a. Exudation of Leucocytes

The escape of leucocytes from the lumen of microvasculature to the interstitial tissue is the most important feature of inflammatory response. In acute inflammation, polymorphonuclear neutrophils (PMNs) comprise the first line of body defense, followed later by monocytes and macrophages. The changes leading to migration of leucocytes are as follows (Fig. 1.4):

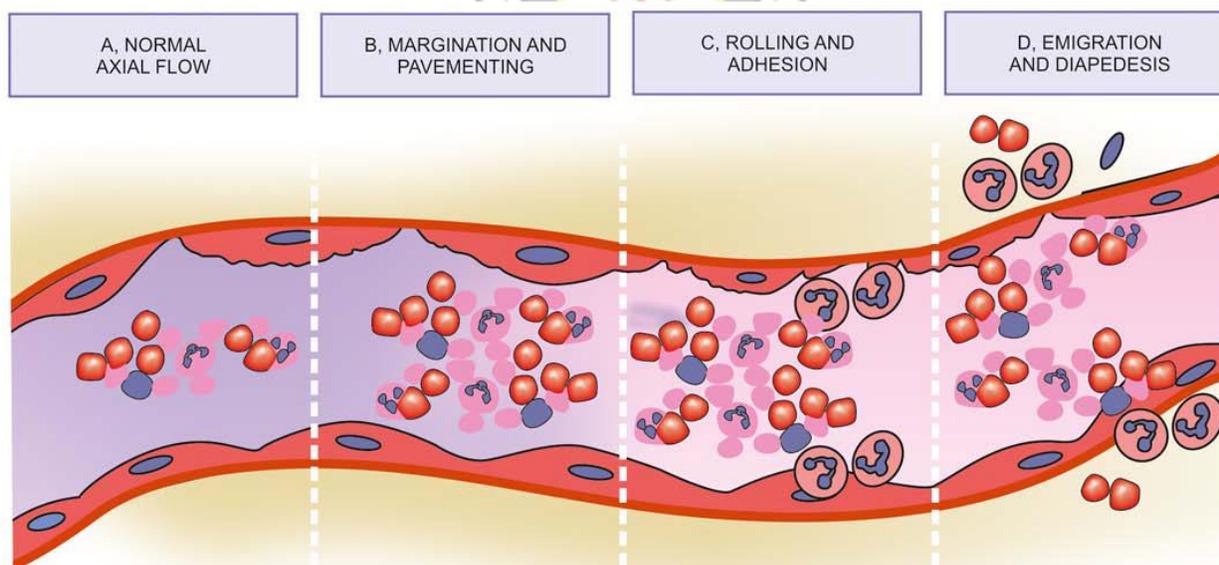


Figure 1.4 Sequence of changes in the exudation of leucocytes. A, Normal axial flow of blood with central column of cells and peripheral zone of cell-free plasma. B, Margination and paving of neutrophils with narrow plasmatic zone. C, Adhesion of neutrophils to endothelial cells with pseudopods in the intercellular junctions. D, Emigration of neutrophils and diapedesis with damaged basement membrane.

□ **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 cell-free 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, neutrophils of the central column come close to the vessel wall; this is known as *pavementing*.

□ **Rolling and Adhesion**

Peripherally margined and paved neutrophils slowly roll over the endothelial cells lining the vessel wall (*rolling phase*). This is followed by transient bond between the leucocytes and endothelial cells becoming firmer (*adhesion phase*). The following cell adhesion molecules (CAMs) bring about rolling and adhesion phases:

❖ **Selectins**

These are a group of CAMs expressed on the surface of activated endothelial cells and are structurally composed of lectins or lectin-like protein molecules the most important of which is s-Lewis X molecule. Their role is to recognise and bind to glycoproteins and glycolipids on the cell surface of neutrophils. There are 3 types of selectins:

- *P-selectin* (preformed and stored in endothelial cells and platelets, also called CD62) is involved in rolling.
- *E-selectin* (synthesised by cytokine-activated endothelial cells, also named ECAM) is associated with both rolling and adhesion.
- *L-selectin* (expressed on the surface of lymphocytes and neutrophils, also called LCAM) is responsible for homing of circulating lymphocytes to the endothelial cells in lymph nodes.

❖ **Integrins**

These are a family of endothelial cell surface proteins having alpha (or CD11) and beta (CD18) subunits, which 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.

❖ **Immunoglobulin gene superfamily adhesion molecules**

This group consists of a variety of immunoglobulin molecules present on most cells of the body. These partake in cell-to-cell contact through various other CAMs and cytokines. They have a major role in recognition and binding of immune competent cells as under:

- Intercellular adhesion molecule-1 (ICAM-1, also called CD54) and vascular cell adhesion molecule-1 (VCAM-1, also named CD106) allow a tighter adhesion and stabilise the interaction between leucocytes and endothelial cells.
- Platelet-endothelial cell adhesion molecule-1 (PECAM-1) or CD31 is involved in leucocyte migration from the endothelial surface.

□ 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. Subsequently, the neutrophils lodged between the endothelial cells and basement membrane cross the basement membrane by damaging it locally with secreted collagenases and escape out into the extravascular space; this is known as *emigration*.

The damaged basement membrane is repaired almost immediately. As already mentioned, neutrophils are the dominant cells in acute inflammatory exudate in the first 24 hours, and monocyte-macrophages appear in the next 24-48 hours. However, neutrophils are short-lived (24-48 hours) while monocyte-macrophages survive much longer. Simultaneous to emigration of leucocytes, escape of red cells through gaps between the endothelial cells, *diapedesis*, takes place. It is a passive phenomenon—RBCs being forced out either by raised hydrostatic pressure or may escape through the endothelial defects left after emigration of leucocytes. Diapedesis gives haemorrhagic appearance to the inflammatory exudate.

□ Chemotaxis

The transmigration of leucocytes after crossing several barriers (endothelium, basement membrane, perivascular myofibroblasts and matrix) to reach the interstitial tissues is a chemotactic factor-mediated process 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.

The following agents act as potent chemotactic substances for neutrophils:

- Leukotriene B₄ (LT-B₄), a product of lipooxygenase pathway of arachidonic acid metabolites
- Components of complement system (C5a and C3a in particular)
- Cytokines (Interleukins, in particular IL-8)
- Soluble bacterial products (such as formylated peptides).

In addition to neutrophils, other inflammatory cells too respond and partake in inflammation having specific chemokines, e.g. monocyte chemoattractant protein (MCP-1), eotaxin chemotactic for eosinophils, NK cells for recognizing virally infected cells etc.

b. 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 proteolytic enzymes—lysozyme, protease, collagenase, elastase, lipase, proteinase, gelatinase, and acid hydrolases. These enzymes degrade collagen and extracellular matrix. Phagocytosis of the microbe by polymorphs and macrophages involves the following 3 steps (Fig. 1.5):

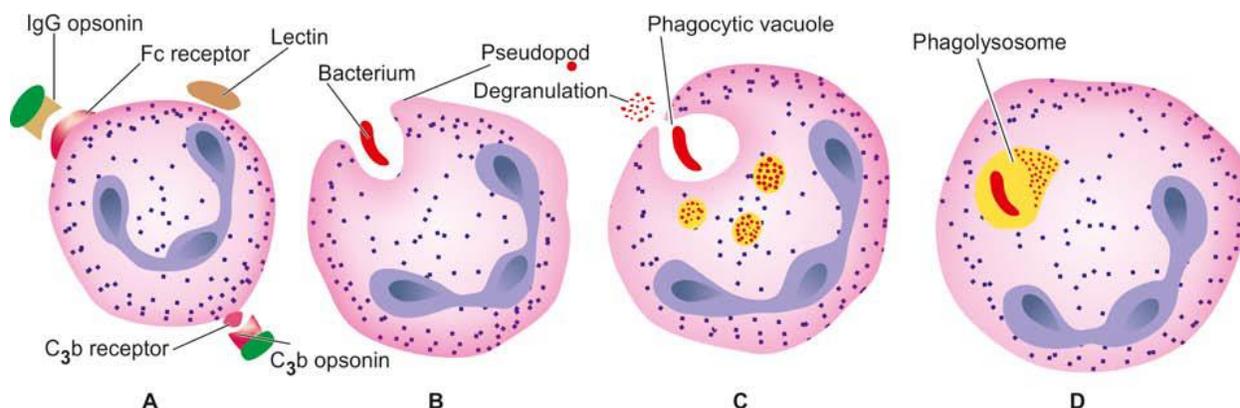


Figure 1.5 Stages in phagocytosis of a foreign particle. A, Opsonisation of the particle. B, Pseudopod engulfing the opsonised particle. C, Incorporation within the cell (phagocytic vacuole) and degranulation. D, Phagolysosome formation after fusion of lysosome of the cell.

- i) Recognition and attachment
- ii) Engulfment
- iii) Killing and degradation

i) **Recognition And Attachment**

Phagocytosis is initiated by the expression of cell 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 and the process is called opsonisation (meaning preparing for eating). 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:

- ❖ *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.
- ❖ *C3b opsonin* is the fragment generated by activation of complement pathway. It is strongly chemotactic for attracting PMNs to bacteria.
- ❖ *Lectins* are carbohydrate-binding proteins in the plasma which bind to bacterial cell wall.

ii) **Engulfment**

The opsonised particle or microbe 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, plasma membrane enclosing the particle breaks from the cell surface so that membrane-lined phagocytic vacuole or phagosome becomes internalised in the cell and lies free in the cell cytoplasm. The phagosome fuses with one or more lysosomes of the cell and form bigger vacuole called phagolysosome.

iii) Killing And Degradation

Next comes the stage of killing and degradation of microorganism to dispose it off which is the major function of phagocytes as scavenger 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. In general, following mechanisms are involved in disposal of microorganisms:

➤ *Intracellular mechanisms:*

- Oxidative bactericidal mechanism by oxygen free radicals
 - a) MPO-dependent
 - b) MPO-independent
- Oxidative bactericidal mechanism by lysosomal granules
- Non-oxidative bactericidal mechanism

➤ *Extracellular mechanisms:*

1.3.2 CHRONIC INFLAMMATION

I. Definition and Causes

Chronic inflammation is defined as prolonged process in which tissue destruction and inflammation occur at the same time.

Chronic inflammation may occur by one of the following 3 ways:

a. **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.

b. **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.

c. **Chronic inflammation starting *de novo***

When the infection with organisms of low pathogenicity is chronic from the beginning e.g. infection with *Mycobacterium tuberculosis*.

1.3.2.1 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:

a. **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 inflammation from:

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

The blood monocytes on reaching the extravascular space transform into tissue macrophages. Besides the role of macrophages in phagocytosis, they may get activated in response to stimuli such as cytokines (lymphokines) and bacterial endotoxins. On activation, macrophages release several biologically active substances e.g. acid and neutral proteases, oxygen-derived reactive metabolites and cytokines.

These products bring about tissue destruction, neovascularization and fibrosis. Other chronic inflammatory cells include lymphocytes, plasma cells, eosinophils and mast cells. In chronic inflammation, lymphocytes and macrophages influence each other and release mediators of inflammation.

b. **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.

c. **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.

1.3.2.2 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.

1.3.2.3 Types of Chronic Inflammation

Conventionally, chronic inflammation is subdivided into 2 types:

a. **Chronic non-specific inflammation**

When the irritant substance produces a non-specific chronic inflammatory reaction with formation of granulation tissue and healing by fibrosis, it is called chronic non-specific inflammation e.g. chronic osteomyelitis, chronic ulcer and lung abscess. A variant of this type of chronic inflammatory response is chronic suppurative inflammation in which infiltration by polymorphs and abscess formation (which are seen in acute inflammation) are additional features e.g. actinomycosis.

b. **Chronic granulomatous inflammation**

In this, the injurious agent causes a characteristic histologic tissue response by formation of granulomas e.g. tuberculosis, leprosy, syphilis, actinomycosis, sarcoidosis etc.

Table1.1 Major differences between acute and chronic inflammation.

FEATURE	ACUTE INFLAMMATION	CHRONIC INFLAMMATION
1. <i>Onset and Duration</i>	<ul style="list-style-type: none"> • Within short time • Lasts for short duration 	<ul style="list-style-type: none"> • After delay • Lasts longer
2. <i>Cardinal Signs</i>	Invariably present	Generally imperceptible
3. <i>Pathogenesis</i>	<ul style="list-style-type: none"> • Vascular events: haemodynamic changes, increased vascular permeability) • Cellular events: exudation of leucocytes, Phagocytosis • Role of chemical mediators and regulators 	<ul style="list-style-type: none"> • Following acute inflammation • Recurrent attacks of acute inflammation • Chronic inflammation from beginning
4. <i>Main Inflammatory Cells</i>	<ul style="list-style-type: none"> • Neutrophils • Eosinophils • Lymphomononuclear cells (late) • Pus cells 	<ul style="list-style-type: none"> • Lymphocytes • Plasma cells • Monocytes/macrophages (epithelioid cells in granulomas) • Giant cells (foreign body, Langhans')
5. <i>Plasma Exudation</i>	Present	May or may not be present
6. <i>Systemic Effects</i>	<ul style="list-style-type: none"> • Fever: high grade • Leucocytosis (neutrophilic, eosinophilic) • Lymphadenitis-lymphangiitis • Septic shock (in severe acute infection) 	<ul style="list-style-type: none"> • Fever: mild • Leucocytosis (lymphocytic, monocytic) • Lymphadenitis-lymphangiitis • Raised ESR • Anaemia • Amyloidosis (in long-term cases)
7. <i>Main morphology</i>	<ul style="list-style-type: none"> • Abscesses (suppuration) • Ulcers • Through blood (Bacteraemia, septicaemia, pyaemia) 	<ul style="list-style-type: none"> • Chronic non-specific inflammation (infectious, others) • Granulomatous inflammation (tuberculosis, leprosy, sarcoidosis, syphilis, actinomycosis, Crohn's disease etc)
8. <i>Fate</i>	<ul style="list-style-type: none"> • Resolution • Healing (regeneration, fibrosis) • Chronicity 	<ul style="list-style-type: none"> • Resolution • Healing (regeneration, fibrosis) • Dystrophic calcification
9. <i>Common Examples</i>	Pyogenic abscess, cellulitis, bacterial pneumonia, pyaemia	Granulation tissue, granulomatous inflammation (tuberculosis, leprosy etc), chronic osteomyelitis

1. MEDIATORS OF INFLAMMATION

Table 1.1 Mediators of 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 A₂, 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, IL-6, IL-8, IL-12, IIL-17, TNF-a, TNF-b, IFN-g, chemokines)
6. Free radicals (Oxygen metabolites, nitric oxide)

II. PLASMA PROTEIN-DERIVED MEDIATORS (PLASMA PROTEASES)

Products of:

1. The kinin system
2. The clotting system
3. The fibrinolytic system
4. The complement system

These are a large and increasing number of endogenous chemical substances which mediate the process of acute inflammation.

Mediators of inflammation have some common properties as under:

- a) These mediators are released either *from the cells or are derived from plasma proteins*:
 - Cell-derived mediators are released either from their storage in the cell granules or are synthesised in the cells.
 - The most common site of synthesis of plasma-derived mediators is the liver. After their release from the liver, these mediators require activation.
- b) All mediators are *released in response to certain stimuli*. These stimuli may be a variety of injurious agents, dead and damaged tissues, or even one mediator stimulating release of another. The latter are called secondary mediators which may perform the function of the initial mediator or may have opposing action.
- c) Mediators *act on different targets*. They may have similar action on different target cells or differ in their action on different target cells. They may act on cells which formed them or on other body cells.
- d) *Range of actions* of different mediators are: increased vascular permeability, vasodilatation, chemotaxis, fever, pain and tissue damage.
- e) Mediators have *short lifespan* after their release. After release, they are rapidly removed from the body by various mechanisms e.g. by enzymatic inactivation, antioxidants, regulatory proteins or may even decay spontaneously.

Two main groups of substances acting as chemical mediators of inflammation are those released from their range of actions in acute inflammation are shown in Fig. 1.1.

1.1 CELL-DERIVED MEDIATORS

1.1.1 Vasoactive Amines

Two important pharmacologically active amines that have role in the early inflammatory response (first one hour) are histamine and 5-hydroxytryptamine (5-HT) or serotonin; another addition to this group is neuropeptides.

- i) **Histamine** It is stored in the granules of mast cells, basophils and platelets. Histamine is released from these cells by various agents as under:
- Stimuli or substances inducing acute inflammation e.g. heat, cold, irradiation, trauma, irritant chemicals, immunologic reactions etc.
 - Anaphylatoxins like fragments of complement C3a, and C5a, which increase vascular permeability and cause oedema in tissues.
 - Histamine-releasing factors from neutrophils, monocytes and platelets.
 - Interleukins.

The main *actions* of histamine are: vasodilatation, increased vascular (venular) permeability, itching and pain. Stimulation of mast cells and basophils also releases products of arachidonic acid metabolism including the release of *slow reacting substances of anaphylaxis (SRS-As)*. The SRS-As consist of various leukotrienes (LTC₄, LTD₄ and LTE₄).

- ii) **5-Hydroxytryptamine (5-HT or serotonin)** It is present in tissues like chromaffin cells of GIT, spleen, nervous tissue, mast cells and platelets. The actions of 5-HT are similar to histamine but it is a less potent mediator of increased vascular permeability and vasodilatation than histamine. It may be mentioned here that carcinoid tumour is a serotonin-secreting tumour.

- iii) **Neuropeptides** Another class of vasoactive amines is tachykinin neuropeptides such as substance P, neurokinin A, vasoactive intestinal polypeptide (VIP) and somatostatin. These small peptides are produced in the central and peripheral nervous systems.

The major proinflammatory *actions* of these neuropeptides are as follows:

- Increased vascular permeability.
- Transmission of pain stimuli.
- Mast cell degranulation.

1.1.2. ARACHIDONIC ACID METABOLITES (EICOSANOIDS)

Arachidonic acid metabolites or eicosanoids are the most potent mediators of inflammation, much more than oxygen free radicals. Arachidonic acid is a fatty acid, eicosatetraenoic acid; Greek word '*eikosa*' means 'twenty' because of 20 carbon atom composition of this fatty acid. Arachidonic acid is a constituent of the phospholipid cell membrane, besides its presence in some constituents of diet. Arachidonic acid is released from the cell membrane by phospholipases.

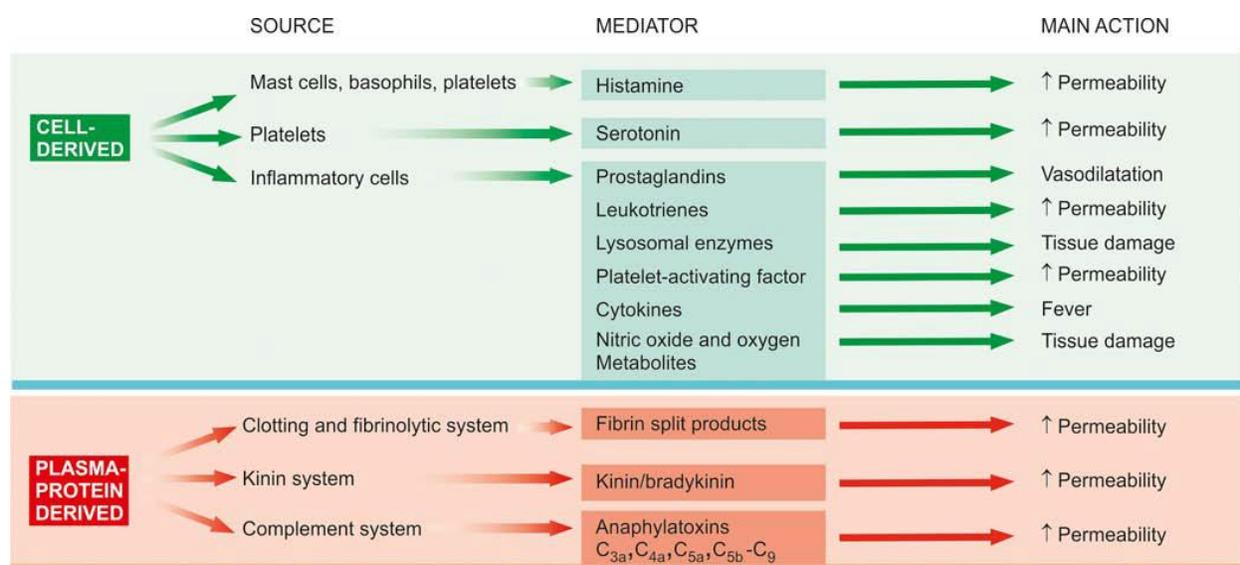


Figure 1.1 Mediators of inflammation.

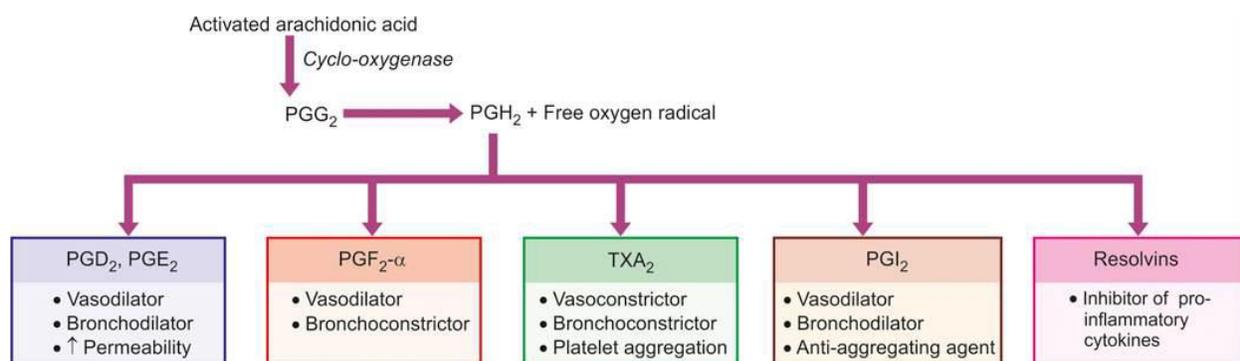


Figure 1.2 Arachidonic acid metabolites via cyclooxygenase pathway.

It is then activated to form arachidonic acid metabolites or eicosanoids by one of the following 2 pathways: via cyclo-oxygenase pathway or via lipoxygenase pathway:

i) Metabolites via cyclo-oxygenase pathway: Prostaglandins, thromboxane A₂, prostacyclin

The name 'prostaglandin' was first given to a substance found in human seminal fluid but now the same substance has been isolated from a number of other body cells. Prostaglandins and related compounds are also called *autocoids* because these substances are mainly autocrine or paracrine agents. The terminology used for prostaglandins is abbreviation as PG followed by suffix of an alphabet and a serial number e.g. PGG₂, PGE₂ etc. Cyclo-oxygenase (COX), a fatty acid enzyme present as COX-1 and COX-2, acts on activated arachidonic acid to form prostaglandin endoperoxide (PGG₂). PGG₂ is enzymatically transformed into PGH₂ with generation of free radical of oxygen. PGH₂ is further acted upon by enzymes and results in formation of the following 3 metabolites (Fig. 1.2):

- a) *Prostaglandins (PGD₂, PGE₂ and PGF₂-a)*. PGD₂ and PGE₂ act on blood vessels and cause increased venular permeability, vasodilatation and bronchodilatation and inhibit inflammatory cell function. PGF₂-a induces vasodilatation and bronchoconstriction.

- b) *Thromboxane A2 (TXA2)*. Platelets contain the enzyme thromboxane synthetase and hence the metabolite, thromboxane A2, formed is active in platelet aggregation, besides its role as a vasoconstrictor and broncho-constrictor.
 - c) *Prostacyclin (PGI2)*. PGI2 induces vasodilatation, bronchodilatation and inhibits platelet aggregation.
 - d) *Resolvins* are another derivative of COX pathway which act by inhibiting production of pro-inflammatory cytokines. Thus, resolvins are actually helpful—drugs such as aspirin act by inhibiting COX activity and stimulate production of resolvins. It may be mentioned here that some of the major anti-inflammatory drugs act by inhibiting activity of the enzyme COX; e.g. non-steroidal anti-inflammatory drugs (NSAIDs), COX-2 inhibitors.
- ii) **Metabolites via lipo-oxygenase pathway: 5-HETE, leukotrienes, lipoxins** The enzyme, lipo-oxygenase, a predominant enzyme in neutrophils, acts on activated arachidonic acid to form hydroperoxy eicosatetraenoic acid (5-HPETE) which on further peroxidation forms following 2 metabolites (Fig. 1.3):
- a) *5-HETE* (hydroxy compound), an intermediate product, is a potent chemotactic agent for neutrophils.
 - b) *Leukotrienes (LT)* are so named as they were first isolated from leucocytes. Firstly, unstable leukotriene A4 (LTA4) is formed which is acted upon by enzymes to form LTB4 (chemotactic for phagocytic cells and stimulates phagocytic cell adherence) while LTC4, LTD4 and LTE4 have common actions by causing smooth muscle contraction and thereby induce vasoconstriction, bronchoconstriction and increased vascular permeability; hence they are also called as slowreacting substances of anaphylaxis (SRS-As).
 - c) *Lipoxins (LX)* act to regulate and counterbalance actions of leukotrienes. Lipoxygenase-12 present in platelets acts on LTA4 derived from neutrophils and forms LXA4 and LXB4.

1.1.3. Lysosomal Components

The inflammatory cells— neutrophils and monocytes, contain lysosomal granules which on release elaborate a variety of mediators of inflammation. These are as under:

- i) **Granules of neutrophils** Neutrophils have 3 types of granules: primary or azurophil, secondary or specific, and tertiary.
 - a) *Primary or azurophil granules* are large azurophil granules which contain functionally active enzymes. These are myeloperoxidase, acid hydrolases, acid phosphatase, lysozyme, defensin (cationic protein), phospholipase, cathepsin G, elastase, and protease.
 - b) *Secondary or specific granules* contain alkaline phosphatase, lactoferrin, gelatinase, collagenase, lysozyme, vitamin-B12 binding proteins, plasminogen activator.
 - c) *Tertiary granules or C particles* contain gelatinase and acid hydrolases. Myeloperoxidase causes oxidative lysis by generation of oxygen free radicals, acid hydrolases act within the cell to cause destruction of bacteria in phagolysosome while proteases attack on the extracellular constituents such as basement membrane, collagen, elastin, cartilage etc. However, degradation of extracellular components like collagen, basement membrane, fibrin and cartilage by proteases results in harmful tissue destruction which is kept in check by presence of antiproteases like α 1-antitrypsin and α 2- macroglobulin.

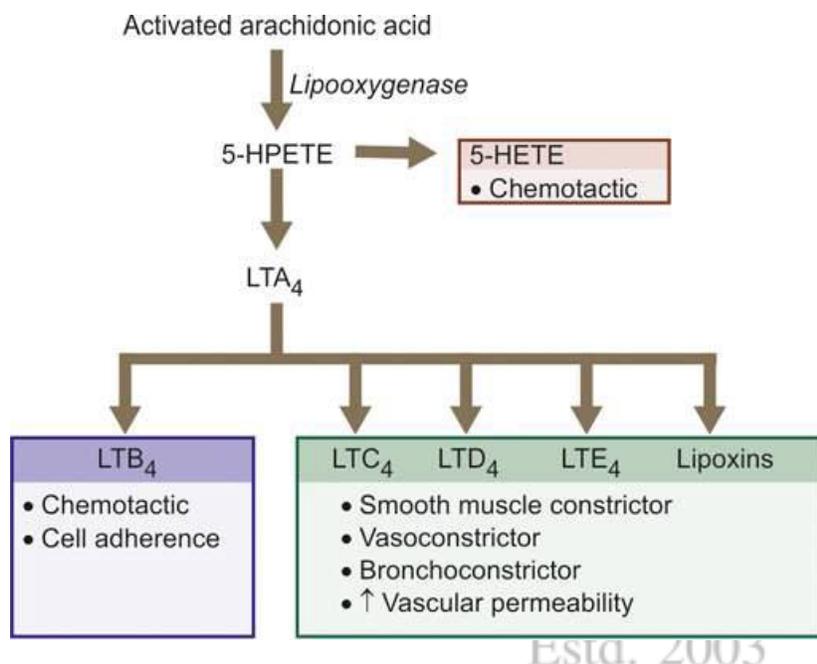


Figure: 1.3

ii) **Granules of monocytes and tissue macrophages** These cells on degranulation also release mediators of inflammation like acid proteases, collagenase, elastase and plasminogen activator. However, they are more active in chronic inflammation than acting as mediators of acute inflammation.

1.1.4 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:

- i) increased vascular permeability;
- ii) vasodilatation in low concentration and vasoconstriction otherwise;
- iii) bronchoconstriction;
- iv) adhesion of leucocytes to endothelium; and
- v) chemotaxis.

1.1.5 Cytokines

Cytokines are polypeptide substances produced by activated lymphocytes (*lymphokines*) and activated monocytes (*monokines*). The term *chemokine* is used for a family of substances which act as chemoattractants for inflammatory cells. All these agents may act on 'self' cells which produced them or on other cells.

1.1.6 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^-), H_2O_2 , OH^\cdot and toxic NO products. These oxygen-derived free radicals have the following actions in inflammation:
 - a) Endothelial cell damage and thereby increased vascular permeability.

- a) Activation of protease and inactivation of antiprotease causing tissue matrix damage.
- b) Damage to other cells.

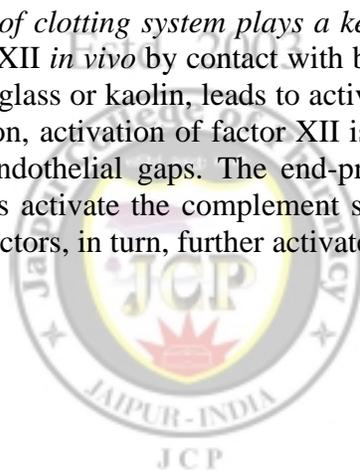
The actions of free radicals are counteracted by antioxidants present in tissues and serum which play a protective role.

- ii) **Nitric oxide (NO)** was originally described as vascular relaxation factor produced by endothelial cells. Now it is known that NO is formed by activated macrophages during the oxidation of arginine by the action of enzyme, NO synthase. NO plays the following roles in mediating inflammation:
 - a) Vasodilatation
 - b) Anti-platelet activating agent
 - c) Possibly microbicidal action.

1.2 PLASMA PROTEIN-DERIVED MEDIATORS (PLASMA PROTEASES)

These include various products derived from activation and interaction of 4 interlinked systems: kinin, clotting, fibrinolytic and complement. Each of these systems has its inhibitors and accelerators in plasma with negative and positive feedback mechanisms respectively.

Hageman factor (factor XII) of clotting system plays a key role in interactions of the four systems. Activation of factor XII *in vivo* by contact with basement membrane and bacterial endotoxins, and *in vitro* with glass or kaolin, leads to activation of clotting, fibrinolytic and kinin systems. In inflammation, activation of factor XII is brought about by contact of the factor leaking through the endothelial gaps. The end-products of the activated clotting, fibrinolytic and kinin systems activate the complement system that generate permeability factors. These permeability factors, in turn, further activate clotting system.



2. HEALING OF SKIN WOUNDS

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:

- Healing by first intention (*primary union*)
- Healing by second intention (*secondary union*).

2.1 HEALING BY FIRST INTENTION (PRIMARY UNION)

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

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

The sequence of events in primary union is described below:

2.1.1 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.

2.1.2 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.

2.1.3 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 well approximated 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.

2.1.4 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.

2.1.5 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 epithelialized 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 or metal clips avoids removal of stitches and its complications.

2.2 HEALING BY SECOND INTENTION (SECONDARY UNION)

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

- i) open with a large tissue defect, at times infected;
- ii) having extensive loss of cells and tissues; and
- iii) 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 upward and also from the margins inwards. 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. 2.1** and described below:

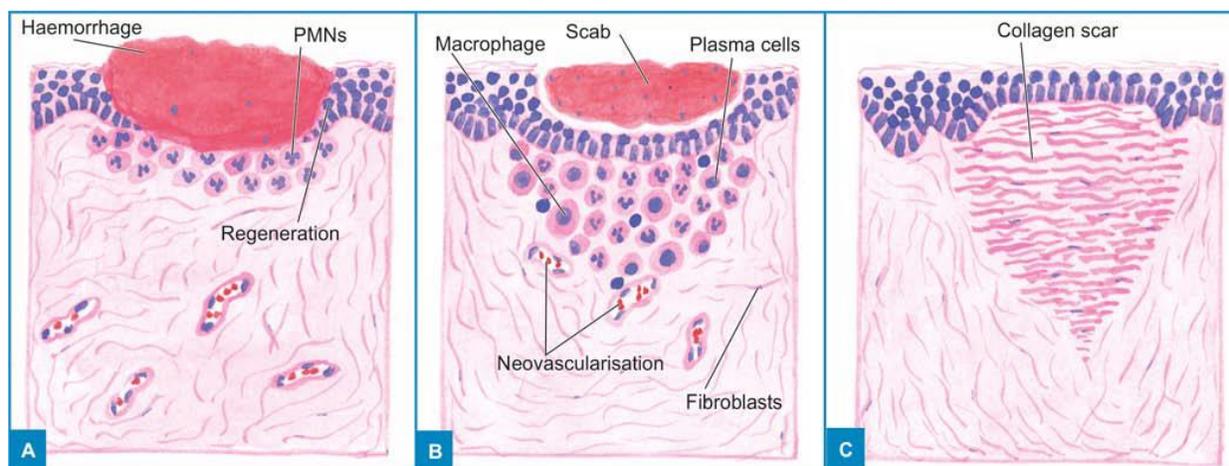


Figure 2.1 Secondary union of skin wounds. A, The open wound is filled with blood clot and there is inflammatory response at the junction of viable tissue. B, Epithelial spurs from the margins of wound meet in the middle to cover the gap and separate the underlying viable tissue from necrotic tissue at the surface forming scab. C, After contraction of the wound, a scar smaller than the original wound is left.

2.2.1 Initial haemorrhage

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

2.2.2 Inflammatory phase

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

2.2.3 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.

2.2.4 Granulation tissue

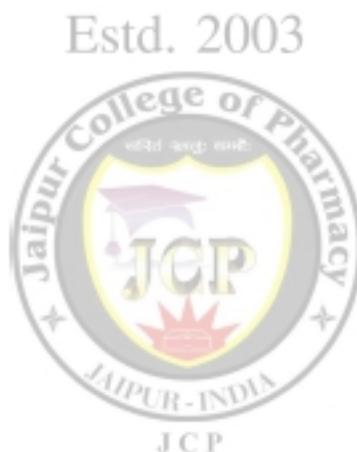
Main bulk of secondary healing is by granulations. Granulation tissue is formed by proliferation of fibroblasts and neovascularisation from the adjoining viable elements. The newly-formed granulation tissue is deep red, granular and very fragile. With time, the scar on maturation becomes pale and white 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.

2.2.5 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 one fourth of its original size.

2.2.6 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.



3. ATHEROSCLEROSIS

Atherosclerosis → *athero*-(meaning porridge) referring to the soft lipid-rich material in the centre of atheroma + *sclerosis* (scarring) referring to connective tissue in the plaques.

3.1 DEFINITION

Atherosclerosis is an thickening and hardening of large and medium-sized muscular arteries, primarily due to involvement of tunica intima and is characterised by fibrofatty plaques or atheromas.

3.2 PATHOGENESIS

As stated above, atherosclerosis is not caused by a single etiologic factor but is a multifactorial process whose exact pathogenesis is still not known. Since the times of Virchow, a number of theories have been proposed.

○ **Insudation hypothesis**

The concept hypothesised by *Virchow in 1856* that atherosclerosis is a form of cellular proliferation of the intimal cells resulting from increased imbibing of lipids from the blood came to be called the '*lipid theory*'. Modified form of this theory is currently known as '*response to injury hypothesis*' and is now-a-days the most widely accepted theory.

○ **Encrustation hypothesis**

The proposal put forth by *Rokitansky in 1852* that atheroma represented a form of encrustation on the arterial wall from the components in the blood forming thrombi composed of platelets, fibrin and leucocytes, was named as '*encrustation theory*' or '*thrombogenic theory*'. Since currently it is believed that encrustation or thrombosis is not the sole factor in atherogenesis but the components of thrombus (platelets, fibrin and leucocytes) have a role in atheromatous lesions, this theory has now been incorporated into the response-to-injury hypothesis mentioned above.

There are four key factors—arterial smooth muscle cells, endothelial cells, blood monocytes and dyslipidaemia, which participate in atherosclerosis process.

Pathogenesis of atherosclerosis is explained on the basis of the following two theories:

- 1). *Reaction-to-injury hypothesis*
- 2). *Monoclonal theory*: based on neoplastic proliferation of smooth muscle cells

3.2.1. Reaction-to-Injury Hypothesis

This theory is most widely accepted and incorporates aspects of two older historical theories of atherosclerosis—the lipid theory of Virchow and thrombogenic (encrustation) theory of Rokitansky.

- The *original response to injury theory* : according to which the initial event in atherogenesis was considered to be endothelial injury followed by smooth muscle cell proliferation so that the early lesions, according to this theory, consist of smooth muscle cells mainly.
- The *modified response-to-injury hypothesis* implicates lipoprotein entry into the intima as the initial event followed by lipid accumulation in the macrophages (foam cells now) which according to modified theory, are believed to be the dominant cells in early lesions.

following is the generally accepted role of key components involved in atherogenesis, diagrammatically illustrated in **Fig. 3.1**.

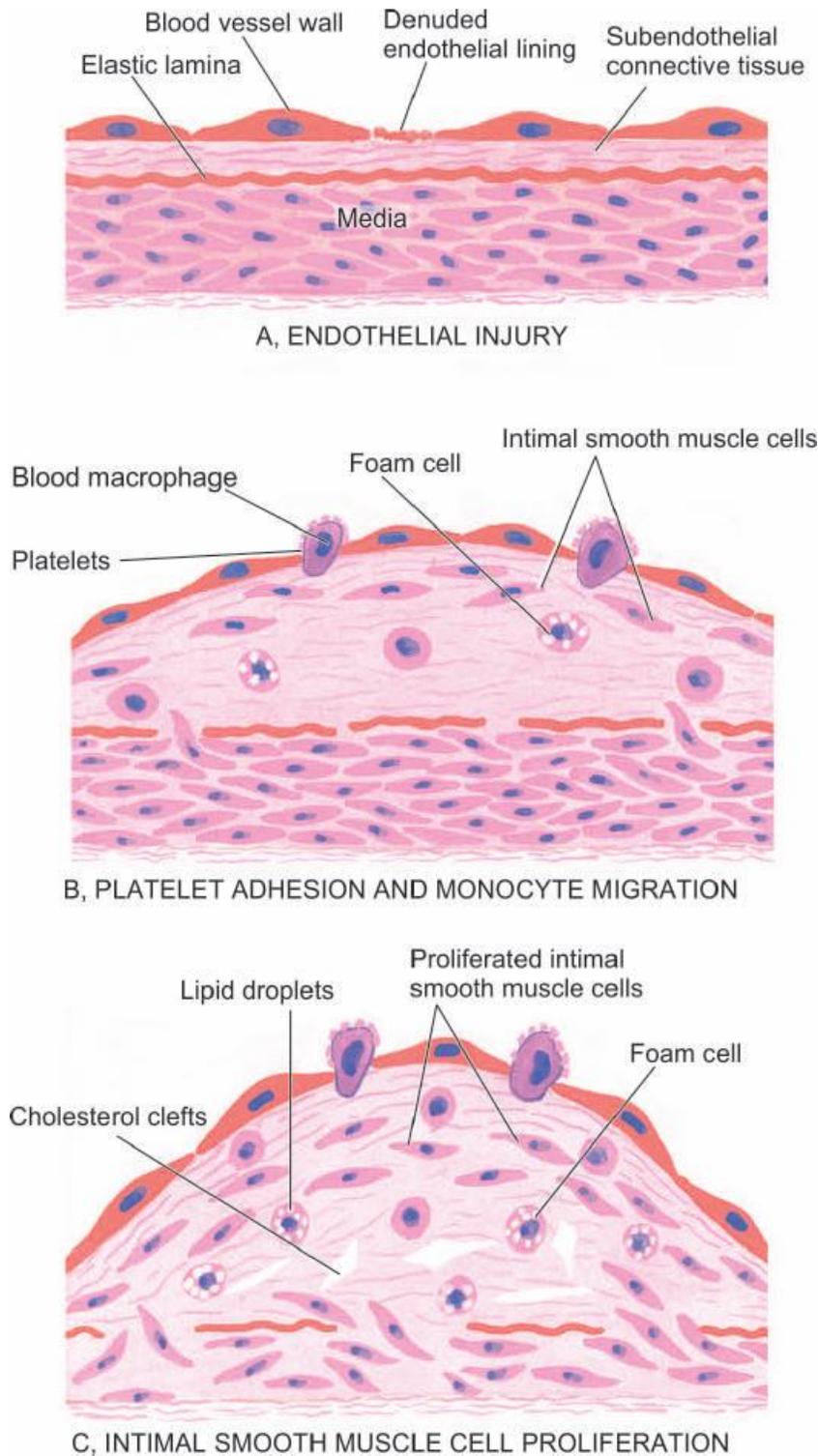


Figure 3.1 Diagrammatic representation of pathogenesis of atherosclerosis as explained by 'reaction-to-injury' hypothesis. A, Endothelial injury. B, Adhesion of platelets and migration of

blood monocytes from blood stream. C, Smooth muscle cell proliferation into the intima and in growth of new blood vessels.

i) Endothelial injury

Endothelial injury is the initial triggering event in the development of lesions of atherosclerosis. Actual endothelial denudation is not an essential requirement, but endothelial dysfunction may initiate the sequence of events. Numerous causes ascribed to endothelial injury in experimental animals are: mechanical trauma, haemodynamic forces, immunological and chemical mechanisms, metabolic agent as chronic dyslipidaemia, homocysteine, circulating toxins from systemic infections, viruses, hypoxia, radiation, carbon monoxide and tobacco products.

In humans, two of the major risk factors which act together to produce endothelial injury are: *haemodynamic stress from hypertension and chronic dyslipidaemia*. The role of haemodynamic forces in causing endothelial injury is further supported by the distribution of atheromatous plaques at points of bifurcation or branching of blood vessels which are under greatest shear stress.

ii) Intimal smooth muscle cell proliferation

Endothelial injury causes adherence, aggregation and platelet release reaction at the site of exposed subendothelial connective tissue and infiltration by inflammatory cells. Proliferation of intimal smooth muscle cells and production of extracellular matrix are stimulated by various cytokines such as IL-1 and TNF- α released from invading monocyte-macrophages and by activated platelets at the site of endothelial injury. These cytokines lead to local synthesis of following growth factors having distinct roles in plaque evolution:

Platelet-derived growth factor (*PDGF*) and fibroblast growth factor (*FGF*) stimulate proliferation and migration of smooth muscle cells from their usual location in the media into the intima which regulate the synthesis of collagen by smooth muscle cells. Smooth muscle cell proliferation is also facilitated by biomolecules such as nitric oxide and *endothelin* released from endothelial cells. Intimal proliferation of smooth muscle cells is accompanied by synthesis of matrix proteins—collagen, elastic fibre proteins and proteoglycans.

iii) Role of blood monocytes

Though blood monocytes do not possess receptors for normal LDL, LDL does appear in the monocyte cytoplasm to form foam cell by mechanism illustrated in **Fig. 13.5**. Plasma LDL on entry into the intima undergoes oxidation. The 'oxidised LDL' formed in the intima performs the following all-important functions on monocytes and endothelium:

- a) **For monocytes:** Oxidised LDL acts to attract, proliferate, immobilise and activate them as well as is readily taken up by scavenger receptor on the monocyte to transform it to a lipid laden foam cell.
- b) **For endothelium:** Oxidised LDL is cytotoxic. Death of foam cell by apoptosis releases lipid to form lipid core of plaque.

iv) **Role of dyslipidaemia**

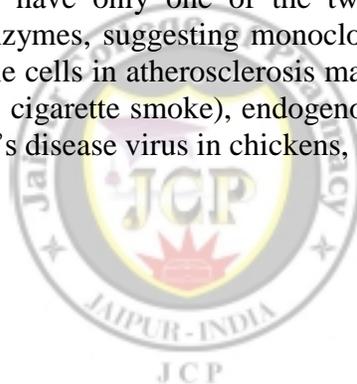
As stated already, chronic dyslipidaemia in itself may initiate endothelial injury and dysfunction by causing increased permeability. In particular, hypercholesterolaemia with increased serum concentration of LDL promotes formation of foam cells, while high serum concentration of HDL has anti-atherogenic effect.

v) **Thrombosis**

As apparent from the foregoing, endothelial injury exposes subendothelial connective tissue resulting in formation of small platelet aggregates at the site and causing proliferation of smooth muscle cells. This causes mild inflammatory reaction which together with foam cells is incorporated into the atheromatous plaque. The lesions enlarge by attaching fibrin and cells from the blood so that thrombus becomes a part of atheromatous plaque.

3.2.2 Monoclonal Hypothesis

This hypothesis is based on the postulate that proliferation of smooth muscle cells is the primary event and that this proliferation is monoclonal in origin similar to cellular proliferation in neoplasms (e.g. in uterine leiomyoma). The evidence cited in support of monoclonal hypothesis is the observation on proliferated smooth muscle cells in atheromatous plaques which have only one of the two forms of glucose-6-phosphate dehydrogenase (G6PD) isoenzymes, suggesting monoclonality in origin. The monoclonal proliferation of smooth muscle cells in atherosclerosis may be initiated by mutation caused by exogenous chemicals (e.g. cigarette smoke), endogenous metabolites (e.g. lipoproteins) and some viruses (e.g. Marek's disease virus in chickens, herpesvirus).



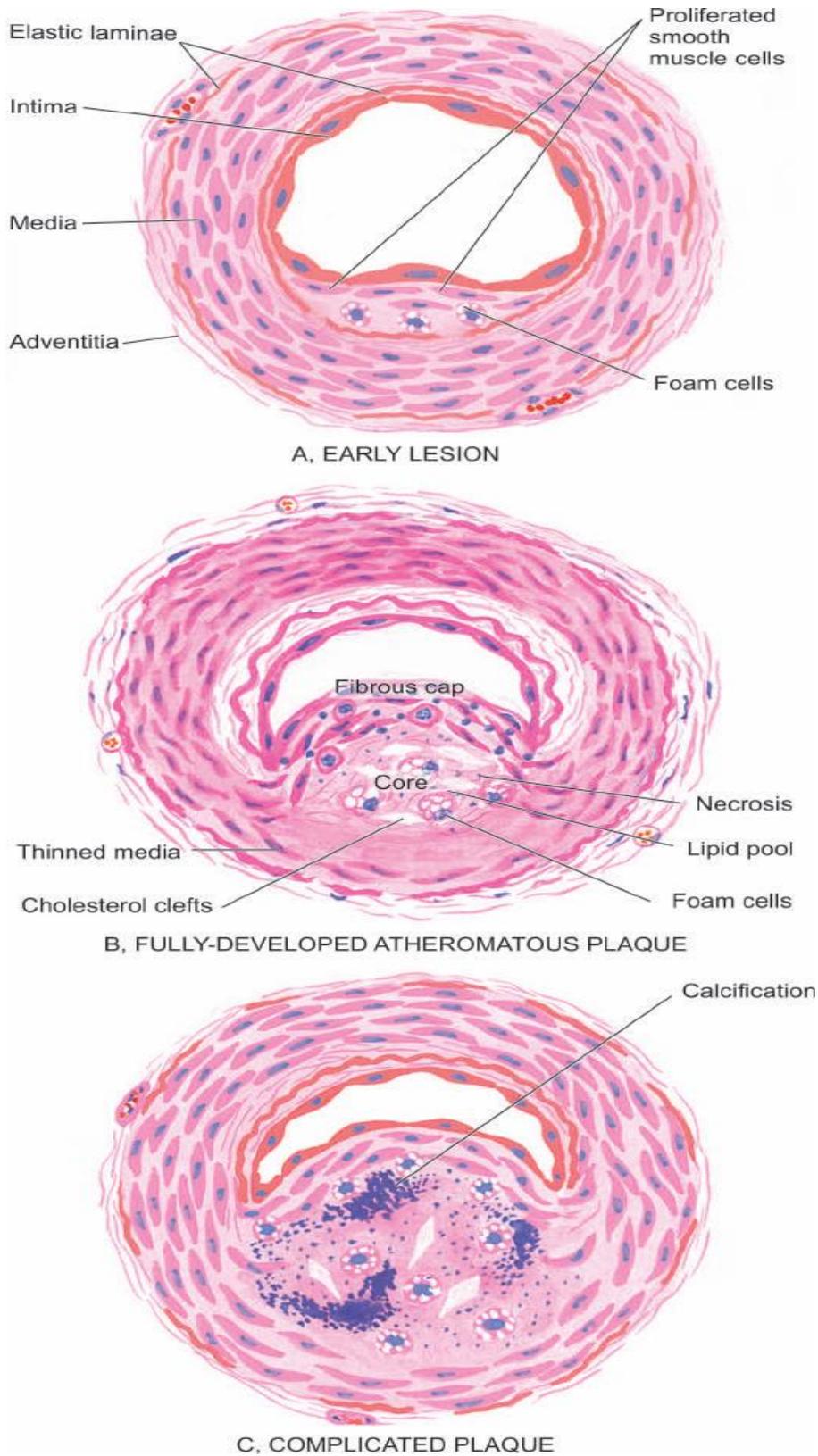


Figure 3.2 Schematic evolution of lesions in atherosclerosis.