

INFLAMMATION AND REPAIR

CHAPTER

2

LEARNING OBJECTIVES

After the completion of this chapter, you should be able to,

- ◆ *Explain the signs, types and causes of inflammation*
- ◆ *Describe the vascular and cellular events involved in the pathogenesis of acute inflammation*
- ◆ *Summarize the pathogenesis, aetiology and types of chronic inflammation*
- ◆ *Explain the various cell-derived and plasma-derived chemical mediators of inflammation*
- ◆ *Differentiate between acute and chronic inflammation*
- ◆ *Elaborate the process and complications of wound healing.*

1. INFLAMMATION

1.1 INTRODUCTION

Inflammation is defined as the fundamental protective (defensive) local response of the living tissues of mammals to any injury. Injury may be caused due to various exogenous and endogenous stimuli which in turn initiate protective response in the host cell.

The process of inflammation is useful to get rid of microbes, toxins and necrotic cells. It also helps to dilute, eliminate or limit the spread of injurious agents in the host. The distinctive characteristic of the inflammatory process is the accumulation of fluid and leucocytes (WBCs) in the extravascular tissues of the body. Although inflammation is considered to be harmful, it still helps in checking the infections because if the inflammation remains unchecked, the process of healing/repair would never occur making the injuries permanent.

Causes

The agents causing inflammation are divided into the following four categories,

1. Physical factors (E.g.s: Heat, cold, accidents etc.,)
2. Chemical factors (E.g: Poisonous substances)
3. Microbial agents (E.g.s: Bacteria, fungi, viruses etc.,)
4. Immunological reactions (E.g.s: T-cell and B-cell-mediated reactions).

Signs

1. Rubor (Redness)
2. Tumor (Swelling)
3. Calor (Heat)
4. Dolor (Pain)
5. Functio laesa (Loss of functional activities).

Types

Inflammation can be divided into acute and chronic types, based upon the defensive capacity of the host and the duration of response.

1. *Acute Inflammation*

Acute inflammation represents the early response to injury and is therefore rapid in onset but lasts only for shorter duration.

2. *Chronic Inflammation*

Chronic inflammation persists for longer duration and is relatively severe. It occurs either when the injurious stimuli responsible for acute inflammation persists for prolonged periods or when the stimuli is so severe that it directly induces chronic inflammation.

Basic Definitions

Inflammation is also associated with other features like exudate, transudate and oedema.

1. *Exudate*

It is the fluid which slowly gets filtered from the vascular system into the areas of inflammation by a process known as exudation. The basic composition of exudate include WBCs, plasma proteins, platelets, cellular debris etc.

2. *Transudate*

It is an ultrafiltrate of blood plasma but is not an inflammatory fluid. Transudation occurs due to osmotic and hydrostatic imbalances (disturbances) in the vascular endothelium, but not due to inflammation. It does not cause any increase in the vascular permeability.

3. *Oedema*

Oedema is the accumulation of excess fluid either in the interstitial or serous cavities. It may be exudate or transudate. It is of the following two types,

- Inflammatory Oedema* : Caused due to an increase in the vascular permeability.
- Non-inflammatory Oedema* : Caused due to changes in the haemodynamic (hydrostatic or osmotic pressure) forces in the walls of the capillaries.

1.2 ACUTE INFLAMMATION

Definition

Acute inflammation may be defined as an instant defensive response of tissue to an injurious stimuli. It basically involves the transportation of leucocytes and plasma proteins (essential components of the host defence system) to the injured site.

Acute inflammation can be induced by various agents.

- Physical agents such as burns, mechanical trauma etc.
- Chemical agents such as environmental chemicals, irradiation, toxins, etc.

- Microorganisms such as bacteria, viruses, parasites as well as microbial toxins.
- Other stimuli include immune reactions, tissue necrosis and presence of other foreign bodies such as sutures, splinters etc.

Systemic Effects of Acute Inflammation

Acute inflammation is generally associated with local tissue response, but it can also bring about systemic effects. The systemic effects of acute inflammation are as follows.

1. *Fever*

Fever occurs due to bacteraemia and is mediated through release of prostaglandins (PGs), interleukin-1 and TNF.

2. *Leucocytosis*

Increase in leucocyte (WBC) count is known as *leucocytosis* which lies in the range of 15,000 to 20,000/ μ l during acute inflammation. Leucocytes travel to the site of injury which decreases their systemic levels. This acts as a stimulant for increasing the systemic leucocyte count.

3. *Lymphangitis and Lymphadenitis*

Inflammation of lymphatic vessels (lymphangitis) and lymph nodes (lymphadenitis) is seen during acute inflammation. This represents either an immunological reaction in response to a foreign antigen or a non-specific reaction to the inflammatory mediators released from the site of injury.

4. *Shock*

The effects of inflammatory mediator like TNF- α released during acute inflammation include vasodilation, increased vascular permeability and decrease in intravascular blood volume resulting in hypotension and shock.

Outcomes of Acute Inflammation

Acute inflammation may have any one of the following outcomes.

1. *Complete Resolution*

Resolution refers to complete neutralization and elimination of the injurious stimuli and restoration of the injured site to normal. Resolution is possible when the stimuli persists for only shorter time period or when the tissue damage is not extensive.

2. *Healing by Fibrosis*

Healing by fibrosis occurs when tissues have been subjected to extensive destruction or when there has been excessive amounts of fibrin exudation.

3. *Progression to Chronic Inflammation*

Acute inflammation may progress to chronic inflammation when the former cannot be resolved either because the injurious stimuli persists for prolonged time period or due to certain interferences in the normal process of healing.

1.2.1 Pathogenesis of Acute Inflammation

The mechanism involved in acute inflammation can be studied under two headings, vascular events and cellular events.

Vascular Events

Vascular events during acute inflammation are confined only to the microvasculature (arterioles, venules and capillaries). These vascular events are categorized as follows.

1. Haemodynamic Changes

These changes result due to changes in the vascular flow and alterations in the diameter of smaller blood vessels. The sequential changes occurring in blood vessels are as follows.

(a) *Transient Vasoconstriction*

It is an immediate response characterized by vasoconstriction of only arterioles which decrease the amount of blood flowing through them. It may last for 3-5 seconds in minor injury and upto 5 minutes in case of severe injury.

(b) *Progressive Vasodilatation*

It is predominantly seen in arterioles and minorly in venules and capillaries within half an hour of injury. It results in increased blood flow which is the main reason behind redness (rubor) and warmth (calor) at the site of injury.

(c) *Changes in Hydrostatic Pressure*

Due to persistent vasodilation, local hydrostatic pressure within the blood vessels increases. This results in the escape of vascular fluid from the microvasculature into the interstitial spaces, thereby causing swelling (tumor) at the inflamed site.

(d) *Slowing of Microcirculation*

Due to persistent escape of exudate (protein-rich fluid) and transudate into the interstitial spaces, blood becomes more concentrated. This increases its viscosity which leads to slowing of microcirculation.

(e) *Leucocyte Margination and Emigration*

The leucocytes (mainly neutrophils) align themselves along the periphery of blood vessels i.e., along the vascular endothelium by a process known as *margination*. The leucocytes then stick to the endothelial lining of the blood vessels and move into the extravascular space through the gaps present between the endothelial cells by the process known as *emigration*.

Haemodynamic changes are responsible for inducing the four cardinal signs of inflammation i.e., redness, swelling, heat and pain.

2. Altered Vascular Permeability

The escape of blood plasma along with leucocytes from the gaps in the endothelial walls of the peripheral blood vessels results in the accumulation of oedematous fluid at the site of inflammation. Initially, only two factors i.e., vasodilation and elevated hydrostatic pressure are responsible for the escape of vascular fluid. However, later vascular permeability also increases causing the oedematous fluid to become exudate (rich in plasma proteins).

Under physiological conditions, the normal fluid balance is governed by two forces.

- Forces leading to outward movement of fluid from microcirculation i.e., intravascular hydrostatic pressure and osmotic pressure of interstitial fluid.
- Forces leading to inward movement of fluid into the circulation i.e., intravascular osmotic pressure hydrostatic pressure of interstitial fluid.

Under normal conditions, both inward and outward movement of the fluid is balanced. However, in case of inflammation, the intravascular osmotic pressure decrease while osmotic pressure of interstitial fluid increase thereby resulting in exudative inflammatory oedema due to outflow of excessive amounts of fluid into the interstitial spaces.

Mechanisms of Increased Vascular Permeability

Increased vascular permeability evident during acute inflammation may occur by one or more of the following mechanisms.

(a) *Contraction of Endothelial Cells*

This leads to increased intercellular gaps from which the fluid escapes.

Microvasculature - Mainly affects the venules.

Response type - Immediate but transient (15-30 min) and is reversible.

Pathogenesis - The chemical mediators (histamine, bradykinin and others) released due to inflammation, contract the endothelial cells resulting in the development of temporary gaps between them thereby leading to alterations in vascular permeability.

(b) *Reversible Retraction of Endothelial Cells*

It occurs due to structural reorganization of the cytoskeleton.

Microvasculature - Mainly affects the venules.

Response type - Delayed onset (takes 4-6 hours) and is prolonged (lasts for about 24 hours).

Pathogenesis - Cytokines (interleukin-1) and tumour necrosis factor (TNF) released, induce the structural reorganization of cytoskeletal structures of the endothelial cells, thereby bringing about reversible retraction of intercellular junctions.

(c) *Direct Endothelial Cell Injury*

It occurs due to direct injury to the endothelial cells.

Microvasculature - Affects arterioles, venules and capillaries.

Response type - The increase in vascular permeability may be,
 (i) Immediate sustained leakage, wherein the response is immediate but of longer duration (hours to days) or
 (ii) Delayed prolonged leakage, wherein the response is delayed (by 2-12 hrs) but persists for hours or days.

Pathogenesis - Direct injury causes necrosis of the endothelial cells. Formation of large physical gaps and intravascular clotting (thrombosis) occurs at the site of damaged endothelial cells.

(d) *Leucocytes Mediated Endothelial Cell Injury*

Microvasculature - Affects venules and capillaries.

Response type - Delayed response which persists for prolonged time period.

Pathogenesis - At the site of injury, leucocytes adhere to the endothelium and get activated. These activated leucocytes release proteolytic enzymes and toxic oxygen species which causes injury to the endothelial cells and alter the vascular permeability.

(e) *Neovascularization*

The new capillaries formed during the process of repair are extremely leaky as the endothelial gaps are not properly filled, altering the vascular permeability.

Microvasculature - Affects all the levels of microvasculature

Response type - Varies.

Cellular Events

The different cellular events occurring during acute inflammation are discussed below.

1. **Exudation of WBCs**

Exudation (escape) of WBCs (leucocytes) occurs from the lumen of microvasculature into the interstitial spaces. Polymorphonuclear neutrophils (PMNs) are the first to undergo exudation followed by monocytes and macrophages.

The process of exudation involves the following stages.

(a) *Margination and Pavementing*

Under normal physiological conditions, axial flow of blood is seen wherein the cellular components (WBCs and RBCs) are at the centre, while the plasma is present at the periphery. During inflammation, increased vascular permeability allows the exudation of plasma into the extravascular spaces. This concentrates the contents in the microvasculature peripheral layers to become thin and the central layers to become wider. Due to such redistribution, neutrophils (WBCs) migrate from the centre towards the periphery of the endothelium. This process is known as *pavementing*.

(b) *Rolling and Adhesion of WBCs*

The neutrophils at the periphery roll along the endothelial lining of the blood vessels. This is known as the *rolling phase*. These leucocytes then adhere to the endothelial lining by adhesion molecules which are,

- (i) **Selectins:** These help the PMNs to undergo the rolling phase.

Examples

P-selectin - These are preformed and stored in the endothelium and platelets.

E-selectin - Synthesized by those endothelial cells which have been activated by cytokines.

L-selectin - Expressed on the surface of leucocytes (neutrophils, lymphocytes).

- (ii) **Immunoglobulins:** Examples include intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM).
- (iii) **Integrins:** Present on the endothelial cell surface and brings about firm adhesion between WBCs and endothelial cells.

(c) **Emigration and Diapedesis**

The adhered neutrophils move along the endothelial cells until they come to a suitable site through which they can escape into the extravascular space. Initially, the neutrophils position themselves between the endothelial cells and the basement membrane by coming in contact with the basement membrane. The basement membrane is destroyed by collagenases released by the migrating leucocytes. The neutrophils then move into the extravascular space through the damaged basement membrane. This process is known as *emigration*. The damaged basement membrane gets repaired almost immediately.

Neutrophils are the first to migrate in the initial 24 hours, followed by monocytes and macrophages in the next 24-48 hours. This is an active process. Either due to the development of defects in the endothelial cells (caused by emigration of leucocytes) or due to an increase in hydrostatic pressure, RBCs also escape passively through the endothelial cell gaps. This is known as *diapedesis*.

(d) **Chemotaxis**

The process of migration of leucocytes from the extravascular space to the site of injury along a chemical gradient is known as *chemotaxis*.

The agents which act as chemoattractants for WBCs are called chemokines or chemotactic agents. These agents help the leucocytes to travel to the site of injury from the extravascular space. The various chemokines include,

- (i) Soluble bacterial products
- (ii) Components of complement system (C_3 , C_5)
- (iii) Products of lipoxygenase pathway of arachidonic acid metabolism

- (iv) Cytokines

- (v) Platelet factor 4 (PF_4)

- (vi) Monocyte chemoattractant protein (MCP-1)

- (vii) Chemotactic factor for $CD4^+$ T cells.

2. **Phagocytosis**

It is defined as the process of engulfment of solid particles and cell debris by the phagocytes. The phagocytic cells are of two types i.e., the polymorphonuclear neutrophils (PMNs) also called microphages which appear first during an inflammatory response and the circulating monocytes and fixed tissue mononuclear phagocytes called macrophages.

The process of cell eating is similar for both phagocytic cells (microphages and macrophages) and involves the following stages.

(a) **Recognition and Attachment Stage**

Initial step is the recognition of bacteria by the phagocytes due to chemotactic substances released by the bacterial products. The circulating phagocytes and bacteria are negatively charged, hence repel each other when they come in contact. This is overcome by coating the bacteria with opsonins (which are neutral proteins) present in the circulating serum. The corresponding receptors for opsonins are present on the surface of phagocytes.

These opsonins are of two types.

- (i) **IgG Opsonin:** It is the Fc fragment of immunoglobulin G. It coats the bacteria and its corresponding receptors are expressed on the PMNs.
- (ii) **C_{3b} Opsonin:** It is the fragment of complement pathway generated upon the activation of the same.

Phagocytes recognize the opsonin coated bacteria and bind to it by the corresponding phagocytic receptors.

(b) **Engulfment Stage**

Phagocytes engulf the opsonized bacterium by forming pseudopods around it. The pseudopods completely enclose the bacterium in the phagocytic vacuole. Then the cell membrane of the lysosome fuses with the cell membrane of the phagocytic vacuole to form phagolysosome or phagosome.

(c) **Secretory Stage**

In this stage the preformed granules which are stored in the PMN's are secreted into the phagolysosome as well as extracellularly. The predominant product secreted are the lysosomes. Apart from these, certain proteins (IL-2, IL-6, TNF), arachidonic acid metabolites, oxygen free radicals etc., synthesized by the mononuclear phagocytes are also secreted.

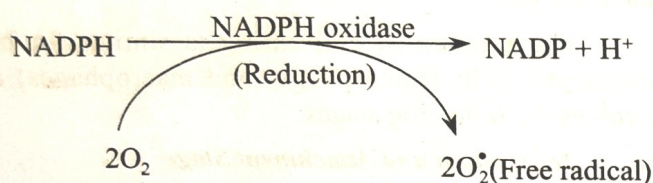
Degradation Stage

In this stage, phagocytes act as scavenger cells. In this, the engulfed bacteria is killed and degraded by antibacterial agents and hydrolytic enzymes respectively. However, certain microbes like the tubercle bacilli is difficult to kill and degrade.

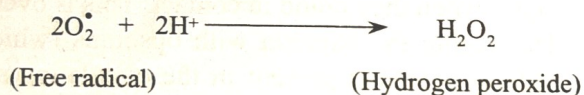
Basically, there are three mechanisms by which the antibacterial agents can exert their effect.

(i) *Oxygen - Dependent Mechanism*

This mechanism involves the production of reactive oxygen free radicals.

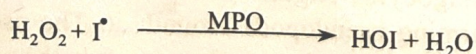
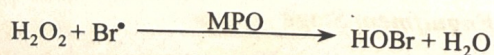
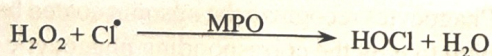


NADPH oxidase present in the phagosome reduces oxygen to form superoxide ion (free radical).



Antibacterial activity of H_2O_2 is elicited by the following two mechanisms.

1. H_2O_2 - MPO - Dependent Killing : Myeloperoxidase (MPO) acts on H_2O_2 in the presence of halides (chloride, bromide, iodide) and leads to the formation of hypohalous acids. These acids are relatively potent antibacterial agents when compared to H_2O_2 .



2. H_2O_2 - MPO - Independent Killing: Mature macrophages lack MPO and exert antibacterial activity by producing hydroxyl radicals ($\text{OH}^\bullet$) in the presence of superoxide anion (Haber-Weiss reaction) or ferrous ion (Fenton reaction).

The oxygen free radicals are extremely beneficial in combating microbes such as *M. tuberculosis* and *Histoplasma capsulatum* which grows within the phagocytic cells.

(ii) *Oxygen Independent Mechanisms*

The agents which can exert their antibacterial activity even in the absence of oxygen include, lysosomal enzymes, iron binding protein (E.g: Lactoferrin enzyme), phagocytin and defensins.

(iii) Nitric Oxide Mediated Killing

Nitric oxide produced by the endothelial cells and activated macrophages directly destroy the microorganisms. Although the antimicrobial role of NO has been established in animal models, it is yet to be elucidated in humans.

1.2.2 Morphological Forms of Acute Inflammation

Acute inflammation can assume various morphological forms which are described below.

1. Catarrhal/Mucosal Inflammation

This form of acute inflammation is characterized by excessive production of mucus from the mucous membranes. Such excessive production is evident in nasal passages, airways etc., and the causative factors include certain virulent microorganisms, foods, irritants, allergens (pollen) etc. Few examples of conditions exhibiting mucosal inflammation include flu and hay fever.

2. Haemorrhagic Inflammation

Haemorrhagic inflammation is an indicative of serious inflammation. It is characterized by an exudate which comprises of large number of erythrocytes along with leucocytes, fibrin, serum etc. The exudate due to the presence of erythrocytes appears brown in colour. The pathological condition which expresses haemorrhagic inflammation is gastroenteritis.

3. Pseudomembranous Inflammation

It results when the mucous membranes of the oral, respiratory or intestinal tract have been exposed to either gaseous irritants or toxins released by the causative organism of diphtheria (*Corynebacterium diphtheriae*). It is marked by plasma exudate and necrosis of the epithelial layer which eventually detaches from the underlying tissue. A false (pseudo) membrane gets formed by the union of necrosed and detached epithelium and the plasma exudate. Pseudomembranous inflammation is seen in necrotic enteritis.

4. Fibrinous Inflammation

Fibrinous inflammation is an indicative of chronic infection and severe systemic injuries. This increases the permeability of small vasculatures such that the fibrin can move into the extravascular tissue, where it can form fibrinous exudates. *Fusobacterium necrophorum* causes fibrinous inflammation.

5. Ulcers

Ulcers are inflammation-induced local deformities that appear on organ surfaces. Initially, increased blood flow along with leucocytic infiltration marks the acute stage of ulcers, while lymphocytic and macrophagic infiltration along with fibrosis and scarring are evident in persistent (long standing) ulcers.

6. Cellulitis

In cellulitis, the affected areas are deeper regions of the skin. It is mostly caused by β -haemolytic *Streptococci*, which releases hyaluronidase (an enzyme involved in converting polymeric hyaluronic acid into its monomeric forms). This increases the permeability of connective tissues.

7. Lymphocytic Inflammation

Lymphocytic inflammation involves tissue infiltration by lymphocytes accompanied with excessive supply of blood to a particular region (referred to as hyperaemia). These lymphocytes tend to accumulate along the circumference of the vasculature. This form of inflammation is mainly seen in highly contagious infections affecting the animals which may get transmitted to humans such as rabies, leptospirosis, toxoplasmosis etc.

8. Infections of Blood

Infections of blood are manifested by three conditions such as bacteraemia, septicaemia and pyaemia.

Table: Manifestations of Infections of Blood

Bacteraemia	Septicaemia	Pyaemia
1. Defined as the presence of small number of bacteria in blood which proliferate at a very slow rate.	Defined as the presence of highly proliferative bacteria in blood which are pathogenic and release potent toxins.	Defined as the presence of generalized thrombi which have been rendered putrid (wherein proteins are decomposed) by bacteria or their toxins.
2. These bacteria are detected by means of blood cultures as they are undetectable by direct microscopy.	Septicaemia occurs along with, (a) Toxaemia (b) Neutropenia (c) Widespread blood coagulation resulting in exhaustion of clotting factors, a condition termed disseminated intravascular coagulation (DIC).	These septic thrombi via blood get embedded at various sites and elicit severe effects.
3. Examples of bacteria causing bacteraemia include <i>Escherichia coli</i> , <i>Salmonella typhi</i> , and <i>Streptococcus viridans</i> .	Examples of bacteria causing septicaemia include <i>Yersinia pestis</i> , <i>Staphylococcus aureus</i> , and <i>Streptococcus haemolyticus</i> .	

9. Suppurative/Purulent Inflammation

This form of inflammation is characterized by the formation of pus in a cavity called abscess by a process termed as *suppuration*. It is a process which indicates that the infection has progressed to a great extent because the body's defence mechanisms have been incapable of preventing it. The suppuration process is facilitated by pyogenic bacteria.

E.g.s: *Escherichia coli*, *Staphylococcus aureus*, *S. albus* and *Streptococcus pyogenes*.

1.3 CHEMICAL MEDIATORS OF INFLAMMATION

Chemical mediators of inflammation are basically endogenous compounds which mediate increased vascular permeability and oedematous conditions (characteristic features of inflammation). Therefore, these are also termed as *permeability factors*. However, apart from increased vascular permeability, these endogenous substances are also involved in fever, pain, chemotaxis as well as tissue damage. These chemical mediators of inflammation can be released from the cells derived from plasma or even from the injured tissues. These are categorized as cell-derived and plasma derived mediators.

Cell-Derived Mediators

1. Vasoactive Amines

The two predominant vasoactive amines which take active part in the early inflammatory response are histamine and serotonin (5-HT).

(a) Histamine

(i) Histamine is stored in the granules of mast cells, basophils and platelets and is released from these cells in response to the following agents,

1. Substances which induce acute inflammation
E.g.s: Chemicals, heat, cold, mechanical injury, irradiation etc.
2. Anaphylatoxins (C3a and C5a fragments) which alter the vascular permeability and cause oedema
3. Neutrophils, monocytes and platelets
4. Neuropeptides (Substance P)
5. Interleukins (IL).

(ii) Actions of histamine in inflammatory process include,

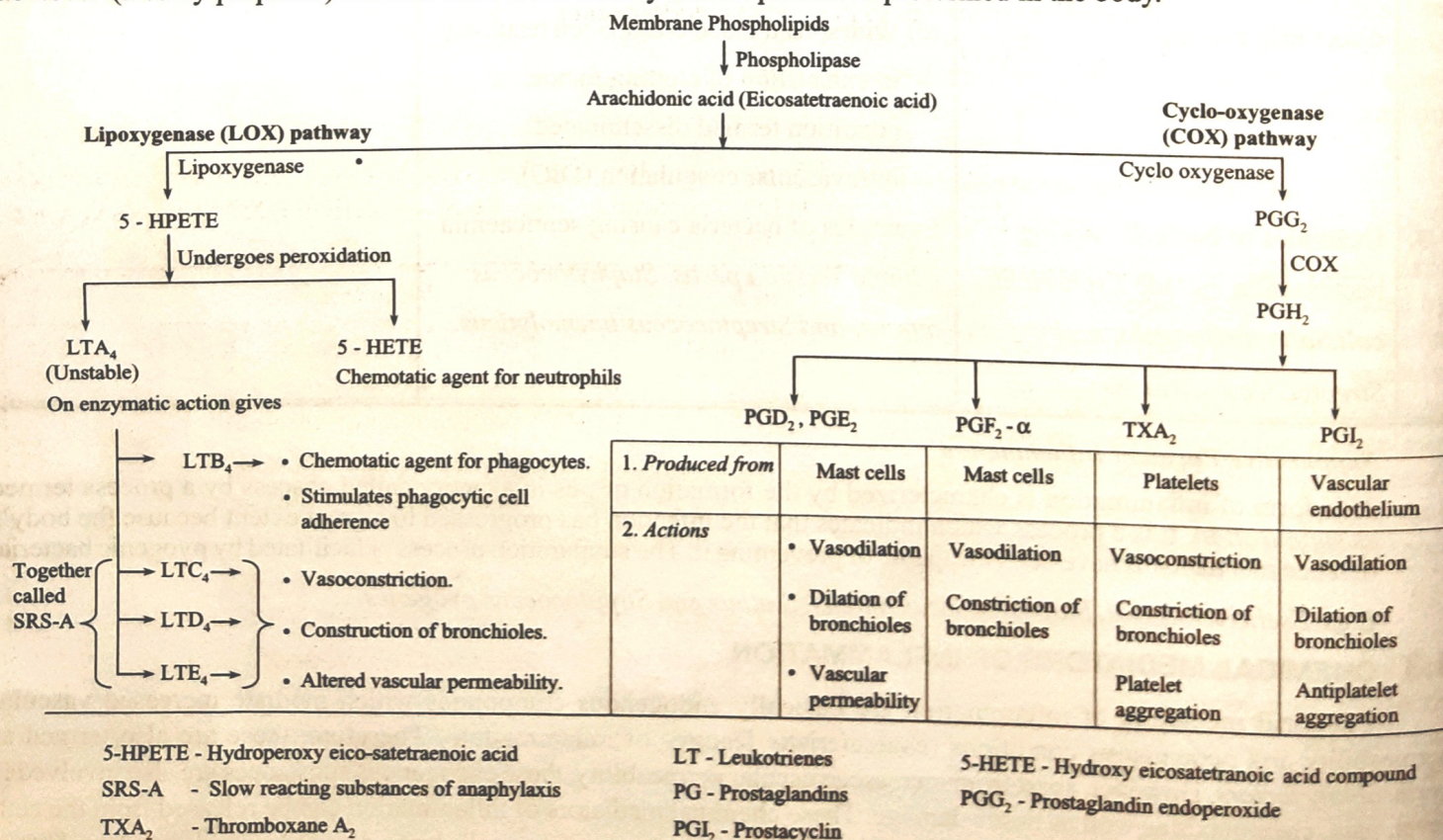
1. Vasodilation
2. Increased vascular permeability in venules
3. Itching and pain.

(b) Serotonin or 5-HT (5-Hydroxytryptamine)

Serotonin or 5-HT is present in chromaffin cells of GIT, spleen, nervous tissue, mast cells and platelets. The actions of 5-HT include altered vascular permeability and vasodilatation. These actions are however less potent than histamine.

2. Arachidonic Acid Metabolites

Arachidonic acid, an important cell membrane constituent gets activated in response to certain injurious stimuli and undergoes two metabolic pathways i.e., cyclo-oxygenase pathway and lipoxygenase pathway to give rise to eicosanoids. Eicosanoids constitute a large group of autacoids such as prostaglandins (PGs), leukotrienes (LTs), thromboxanes (TXs), hydroperoxy eicosatetraenoic acids (HPETEs) and hydroxy eicosatetraenoic acids (HETEs). These eicosanoids are considered as one of the most important regulators and intermediary substances involved in inflammation. They are synthesized 'de novo' (freshly prepared) and are short-lived as they are not present as preformed in the body.



3. Lysosomal Components

Two inflammatory leucocytes, neutrophils and monocytes contain lysosomal granules. These granules are released in response to injurious stimuli and are in turn responsible for releasing numerous mediators of inflammation.

(a) Neutrophilic Granules

The two main granules released from neutrophils have been tabulated below.

Table: Neutrophilic Granules

Azurophilic or primary granules		Specific or secondary granules
1.	They are larger in size.	They are smaller in size.
2.	Released in the phagosome under the influence of high concentrations of agonist.	Released extracellularly against low concentrations of agonist.
3.	These granules contain, (a) Myeloperoxidase (b) Acid hydrolases (c) Neutral proteases etc.	These granules contain, (a) Lactoferrin (b) Lysozyme (c) Alkaline phosphatases etc.

Each granule enzyme has a specific role to play. For example, acid proteases act within the phagolysosome (phagosome) and catalyze the destruction of engulfed bacteria. On contrary, neutral proteases exert their effect extracellularly on components such as basement membrane, fibrin, elastin, cartilage.

(b) Monocytic Granules

The mediators of inflammation released from the granules of monocytes and macrophages include acid proteases, collagenase, elastase, plasminase activator etc.

4. Platelet Activating Factor (PAF)

It is synthesized by the action of phospholipase A₂ on membrane phospholipids. It is released from basophils, mast cells, endothelium and platelets. Its major functions include,

1. Platelet aggregation and release
2. Increased vascular permeability
3. Vasodilation in low concentration and vasoconstriction in high concentration
4. Bronchoconstriction
5. Increased adhesion of leucocytes to the vascular endothelium
6. Acts as a chemotactic agent
7. Induces degranulation.

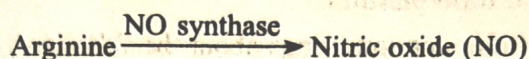
5. Cytokines

These are polypeptide substances produced by activated lymphocytes (lymphokines) and activated monocytes (monokines). These are also released from the endothelium, connective tissue cells, epithelium etc. Cytokines can act on the cells which have released them or can even act on other cells.

Cytokines		Source	Functions
1.	Interleukin-1 (IL-1) and tumour necrosis factor- α (TNF- α)	Activated macrophages	• $\uparrow$ leucocyte adherence • $\uparrow$ PGI synthesis • $\uparrow$ proliferation of fibroblasts • Acute phase reactions (fever, anorexia, neutrophilia, shock etc.)
2.	Tumour necrosis factor- β (TNF- β)	Activated T-cells	Same as above
3.	Interferon (IF)	Activated T-cells	• Activates macrophages and neutrophils • Generates NO synthase
4.	Chemokines (a) Interleukin-8 (IL-8) (b) Platelet factor-4 (PF-4)	Activated macrophages Activated platelets	• Acts as a chemoattractant for neutrophils • Acts as a chemoattractant for neutrophils, monocytes and eosinophils.

6. Nitric Oxide and Oxygen Metabolites

Nitric oxide is produced by activated macrophages by the action of nitric oxide synthase on the amino acid, arginine. The functions of nitric oxide during inflammation are vasodilation, antiplatelet activity and microbicidal action.



Exposure of leucocytes, especially macrophages and neutrophils to microbes, chemokines etc., results in the extracellular release of oxygen-derived metabolites such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\text{OH}^\cdot$). These radicals bring about the following inflammatory responses,

1. Increased vascular permeability by inducing endothelial damage
2. Activates proteases and inactivates antiproteases (which keep the proteases in check) and results in the destruction of tissue matrix
3. Damages other cells.

Plasma-Derived Mediators (Plasma Proteases)

These include various products derived from activation and interaction of four interlinked systems.

Activation of factor XII (Hageman factor or clot promotion factor) of the clotting system activates the clotting, fibrinolytic and kinin systems. The factors produced by the activation of these three systems in turn activate the 4th system i.e., the complement system. Such activation generates permeability factors which activate the clotting system.

1. Clotting System

It gets triggered by the activation of factor XII (synthesized in the liver) after which the intrinsic pathway of the clotting system initiates. The clotting system results in the formation of fibrinogen. This is acted upon by thrombin to form fibrin and fibrinopeptides. The latter mediates inflammatory responses such as increased vascular permeability, acts as a chemoattractant for leucocytes and possesses anticoagulant activity.

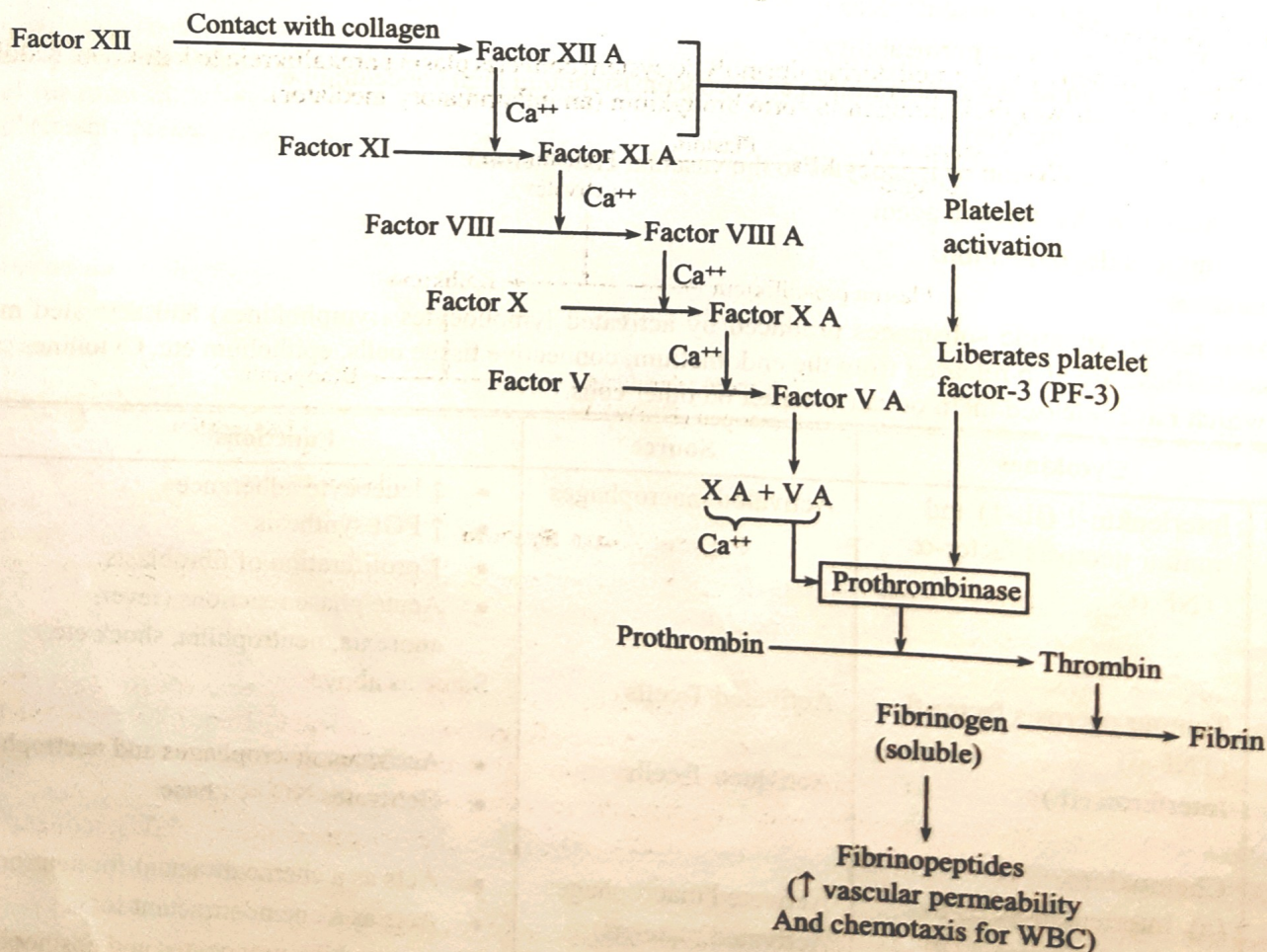


Figure: Clotting System

2. Fibrinolytic System

The mesh of fibrin threads (clot) formed during the process of clotting should undergo lysis. Fibrinolytic system counteracts clotting by causing cleavage of fibrin molecules. The system initiates with the activation of plasminogen activator, which cleaves plasminogen to form plasmin.

The plasmin so formed during inflammation brings about the following which causes vasodilation and increased vascular permeability.

- Cleavages complement C3 to C3a.
- Breakdown of fibrin threads into fibrin split products which also increase vascular permeability and act as a chemoattractant for WBCs.
- Aids in the formation of prekallikrein activator (from factor XIIa) which stimulates kinin system.

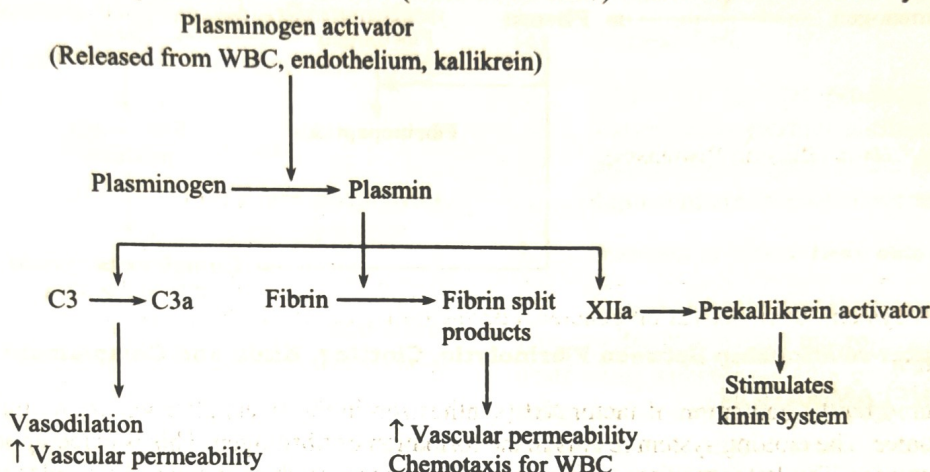


Figure: Fibrinolytic System

3. Kinin System

Prekallikrein activator formed during fibrinolytic system converts plasma prekallikrein to kallikrein. Kallikrein then acts on high molecular weight kininogen to form bradykinin (an inflammatory mediator).

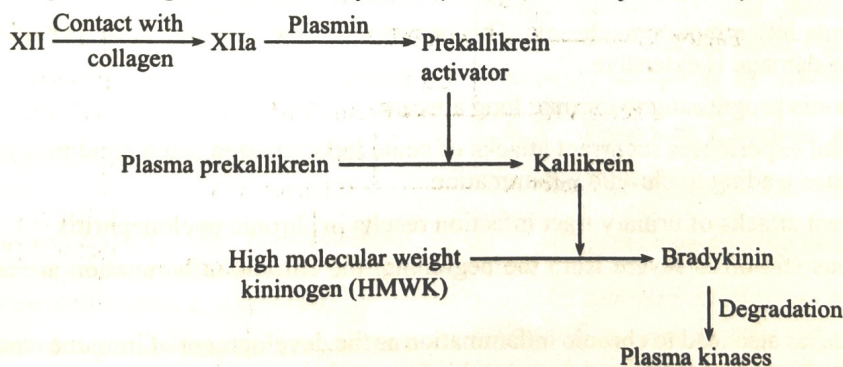


Figure: Kinin System

The actions of bradykinin are prominent during the early stages of inflammation and include contraction of smooth muscles, vasodilation, increased vascular permeability and pain.

4. Complement System

This system is activated by two pathways, the classical pathway and the alternate pathway. Classical pathway activates complement C1 which in turn activates C2 and C4 resulting in the formation of C2a and C2b and C4a and C4b respectively. Both C2a and C4b activate C3 into C3a and C3b. Such activation is also brought about by the alternate pathway. Both C3a and C3b are important activated fragments which mediate the following inflammatory responses.

- Along with C5b, C3a induces the mast cells to release histamine and other mediators of inflammation.
- C3b facilitates in phagocytosis.
- The system results in the formation of membrane attack complex (MAC).
- C3a, C4a and C5a increase vascular permeability and cause tissue oedema.

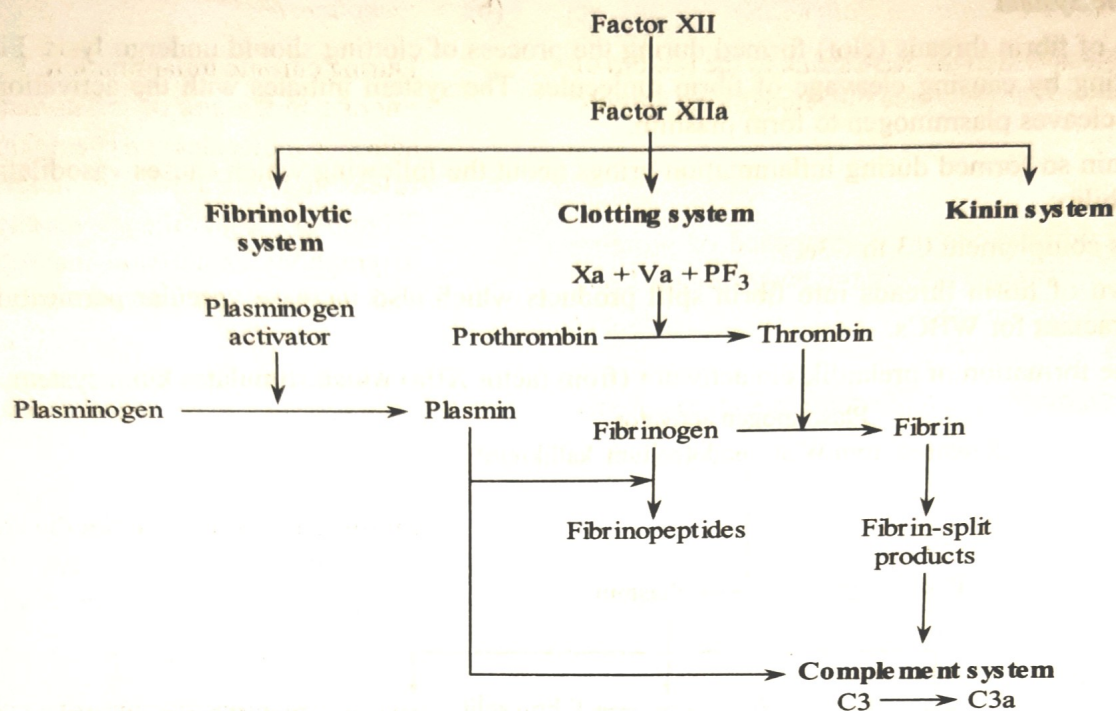


Figure: Inter-relationship Between Fibrinolytic, Clotting, Kinin and Complement Systems

1.4 CHRONIC INFLAMMATION

Definition

Chronic inflammation is defined as a process occurring for prolonged time period in which inflammation, tissue destruction and repair proceed simultaneously. Numerous diseases such as tuberculosis, rheumatoid arthritis and other chronic lung diseases are examples of chronic inflammation.

Aetiology

Chronic inflammation may arise in the following situations.

1. Progression of acute inflammation to chronic. This occurs when the injurious agent persists for longer time periods or when the tissue damage is extensive.

Example: Pneumonia progressing to chronic lung abscess.

2. When an individual experiences recurrent attacks of acute inflammation, such condition may enhance the chronic nature of the disease leading to chronic inflammation.

Example: Recurrent attacks of urinary tract infection results in chronic pyelonephritis.

3. When the injurious stimuli is severe from the beginning, the chronic inflammation arises directly without acute inflammation.

4. Autoimmune diseases also lead to chronic inflammation as the development of immune reactions against the body's own tissues results in extensive tissue damage and inflammation.

E.g.s: Rheumatoid arthritis, systemic lupus erythematosus (SLE) etc.

Systemic Effects of Chronic Inflammation

Systemic effects of chronic inflammation are as follows.

1. *Pyrexia or Fever*

Pyrexia is defined as body temperature above 98.6°F. It indicates an infection and is generally associated with weight loss and weakness.

2. *Anaemia*

It accompanies chronic inflammation and is characterized by decreased oxygen carrying capacity of blood.

3. *Leucocytosis*

It is a condition wherein the WBC count is less than 100,000/mm³. Leucocytosis is also associated with acute inflammation but in chronic inflammation, lymphocytosis (decreased count of lymphocytes) is predominant.

4. *Erythrocyte Sedimentation Rate (ESR)*

It is the rate at which the RBCs sink to the bottom of the test tube. ESR levels are elevated during chronic inflammation.

5. *Amyloidosis*

In this condition, amyloid composed of proteins and starch gets deposited in the body tissues. Long term cases of chronic inflammation lead to the development of systemic amyloidosis.

1.4.1 Pathogenesis of Chronic Inflammation

Chronic inflammation is characterized by the following morphological features.

1. *Infiltration with Mononuclear Cells*

Mononuclear inflammatory cells are phagocytes, lymphocytes, plasma cells, eosinophils and mast cells.

(a) *Phagocytes*

These include circulating monocytes and tissue macrophages like Kupffer cells in liver and alveolar macrophages in lungs.

Macrophages are the most vital cells of chronic inflammation. They arrive at the site of inflammation and remain there because of the following reasons,

1. Chemotactic factors and adhesion monocytes attract macrophages to the site of injury
2. Local proliferation of macrophages when they reach the site of injury
3. Immobilization of the macrophages at the site of injury
4. Antigen engulfment and presenting them to lymphocytes.

In chronic inflammation, the accumulated macrophages undergo immobilization to form a large multinucleate cell known as *giant cell*.

The macrophages also get activated in response to the following,

1. Cytokines secreted by the sensitized T lymphocytes
2. Bacterial endotoxins and
3. Fibronectin (a component of extracellular matrix).

Activated macrophages secrete various substances like acid and neutral proteases, complement components (C1-C5), coagulation factors (V, VIII and tissue factor) etc.

All these components are involved in tissue destruction and also in the process of repair which comprises fibrosis, neovascularization etc.

(b) *Lymphocytes*

During chronic inflammation, T and B lymphocytes migrate to the site of inflammation in response to adhesion molecules and chemokines. The substances released from activated macrophages activate T-lymphocytes, while the mediators released from T-lymphocytes activate macrophages forming a vicious circle. This process leads to excessive tissue destruction and continues until the irritating agent is completely removed.

(c) *Plasma Cells*

These are the end products of B cell activation. Plasma cells produce antibodies which are directed against the antigens at the site of inflammation or against altered tissue components.

2. *Necrosis or Tissue Destruction*

It is the characteristic feature of chronic inflammation. It occurs due to activated macrophages which release tissue destructing substances like acid and neutral proteases, complement components, coagulation factors, reactive oxygen species, nitric oxide, cytokines, tumor necrosis factor, growth factors and eicosanoids.

3. *Proliferative Changes*

In order to overcome the effects of necrosis, body initiates the proliferation of blood vessels (neovascularization) and fibroblasts. This results in the formation of inflammatory granulation tissue which eventually gets healed by collagen laying and fibrosis.

1.4.2 Types of Chronic Inflammation

Chronic inflammation is of the following types.

1. *Chronic Non-specific Inflammation*

In this type of chronic inflammation, the tissue is infiltrated by non-specific inflammatory cells. Diseases such as lung abscess, chronic osteomyelitis are examples of chronic non-specific inflammation.

2. *Chronic Granulomatous Inflammation*

Chronic granulomatous inflammation is characterized by the formation of granulomas which are small (about 1mm) circumscribed lesions mainly composed of modified macrophages called epithelioid cells at the centre and lymphoid cells at the periphery.

Epithelioid cells are modified macrophages or histiocytes bearing close resemblance to epithelial cells. These cells are elongated containing slipper-shaped nucleus and abundant cytoplasm.

Other Components of Granuloma1. **Giant Cells**

Granuloma also shows the presence of giant cells, necrosis and fibrosis. These are formed by the fusion of epithelioid cells and contain many nuclei (20 or more). Giant cells nuclei are arranged at the periphery of the granuloma either like a ring, a horse shoe or are clustered at two poles (called Langerhan's giant cells) or located centrally (called foreign body giant cells). Giant cells have weak phagocytic activity but produce certain secretory products which aid in expelling the foreign invading agents.

2. **Necrosis**

Necrosis is a common feature of certain granulomatous conditions like caseous necrosis of tuberculosis which shows cheese-like appearance.

3. **Fibrosis**

This results due to the proliferation of fibroblasts at the periphery of granulomas.

Factors Favouring Formation of Granulomas

1. Presence of irritants such as talc particles or organisms like *M. tuberculosis*, which cannot be digested or are poorly digestible by the macrophages.
2. Hypersensitivity i.e., cell-mediated immune response upon exposure to irritants.

The classical examples of diseases with granulomatous inflammation are tuberculosis, leprosy, syphilis, brucellosis, cryptococcosis etc.

Table: Differences Between Acute and Chronic Inflammation

Features	Acute inflammation	Chronic inflammation
Causative agents	Pathogens and injured tissues.	Persistent acute inflammation, presence of foreign bodies or autoimmune reactions.
Cells involved	Neutrophils and mononuclear cells (monocytes, macrophages).	Mononuclear cells (monocytes, macrophages, lymphocytes, plasma cells) and fibroblasts.
Mediators	Vasoactive amines and eicosanoids.	Cytokines, growth factors, IFN- γ , oxygen species.
Onset	Abrupt and well-defined	Vague
Characteristics	• Exudation of fluid and plasma proteins. • Emigration of WBC(polymorphonuclear neutrophils) as inflammatory cells. • Intravascular activation of platelets.	• Presence of lymphocytes, plasma cells and macrophages at the site of inflammation. • Proliferation of blood vessels and connective tissues.
Symptoms	Prominent	They are subdued and/or insidious
Duration	Few days	Months or years
Outcomes	Healing, abscess formation, chronic inflammation.	Tissue destruction and fibrosis.

2. HEALING**Definition**

Under normal conditions, body maintains homeostasis by maintaining a balance between cell proliferation and cell death (apoptosis). Occurrence of an injury or inflammation tends to disturb this balance. Hence, it is necessary to replace the dead/injured cells in order to maintain the normal conditions for the survival of the host.

Healing is defined as the highly complex, dynamic, interactive and well regulated physiological response of the body cells to injury to restore the normal structure and function of the damaged tissue.

Healing involves two processes, regeneration and repair.

1. **Regeneration:** It refers to the complete replacement of the injured tissue by the original tissue by proliferation of parenchymal cells.
2. **Repair:** It involves replacement of injured tissue by proliferation of connective tissue which results in fibrosis and scarring.

Fibrosis

Fibrosis refers to excess deposition of fibroblast-derived collagens and extracellular matrix (ECM) molecules in the tissues and organs. It occurs either as reversible part of physiological wound healing or irreversible part of pathological process. When connective tissue deposition occurs in response to injury, it is called *scarring*. The terms fibrosis and scarring are used interchangeably. However, the term fibrosis is most often used to reflect excess connective tissue deposition in chronic and inflammatory diseases. The normal tissue repair evolves into irreversible fibrogenesis if there is persistent injurious stimuli such as persistent infections, allergic reactions, autoimmune reactions, radiation and mechanical injury or if the wound healing response becomes dysregulated. Pathological deposition of ECM proteins results in thickening of the affected tissue which ultimately leads to organ dysfunction and even organ failure.

Fibrosis can develop in various organs and impair their functioning. Examples include in lungs (pulmonary fibrosis), in liver (cirrhosis) and in heart (cardiac fibrosis).

Wound healing involves the mechanisms mentioned under both regeneration or repair process. Healing of wounds associated with skin is referred to as *cutaneous wound healing*. It is a fibroproliferative response involving various growth factors and cytokines.

Factors Influencing Wound Healing

1. Local Factors

Among local factors, presence of infections, foreign agents, ionizing radiations, movement of injured area, poor blood supply to the injured area, large excisional wounds tend to delay the healing process. Ultraviolet (or) radiations, on the other hand, improves healing.

2. Systemic Factors

Various systemic factors that delay healing include age, glucocorticoids, uncontrolled diabetes, malignant disease, obesity, malnutrition, temperature, systemic infection, trauma, uraemia, vitamin deficiency, steroids, haematological disturbances, etc.

Mechanism of Wound Healing

Healing in case of cutaneous wound takes place in two ways.

1. Primary Union (Healing by First Intention)

Healing by primary union occurs in wounds that are,

- ❖ Not prone to any infection
- ❖ Result of a surgical incision
- ❖ Associated with less loss of tissues and cells and
- ❖ Approximated by surgical sutures.

An injury to the skin causes loss of continuity in the epithelial basement membrane and death of small portion of epithelial and connective tissue.

Healing by primary union involves the following steps.

(a) Haemorrhage

The first step in wound healing is haemorrhage (bleeding). The gaps between the incised wounds get filled with blood which ultimately clot. Formation of blood clot (containing fibrin and blood cells) is essential to protect the wound from infection as well as dehydration.

(b) Inflammation

This phase is characterized by the invasion of polymorphs/neutrophils (within 24 hours of injury) from the incised margins of the wound. At the end of the third day, the macrophages become active and replace the polymorphs.

(c) Epithelial Cell Proliferation

This phase is characterized by the formation of a continuous, thin epithelial cover over the wound in around 48 hours. The formation of this epithelial cover involves proliferation and migration of epidermal basal cells in the form of projections (spurs) from the margins of the incised area. As the epidermal cells migrate, there occurs deposition of components of basement membrane forming a continuous and thin epithelial layer called the scab. This scab differentiates the viable dermis from the clot formed during haemorrhage as well as from the necrotic material and later gets removed off. Within five days, there occurs formation of multi layered epidermis due to continuous division of the epidermal cells.

(d) Granulation Tissue Formation and Fibrous Union

In this phase the following events take place.

- (i) By the end of third day,
 1. Fibroblasts start invading the incised area.
 2. Presence of collagen fibrils at the incised margins.
 3. Thickening of epithelial layer of epidermis.

- (ii) By the end of fifth day,
 1. Filling of the incised area by granulation tissue
 2. Angiogenesis
 3. Bridging of the incised area with collagen fibrils
 4. Restoration of normal thickness of epidermis
 5. Keratinization and stratification of epidermis.
- (iii) By the end of second week,
 1. Replication of fibroblasts and deposition of collagen continues
 2. Disappearance of vascular channels.
- (iv) By the end of first month,
 1. Formation of scar filled with connective tissue
 2. Gradual increase in the wound strength.

Healing in case of wounds formed due to sutures include the same steps as in primary union, starting from haemorrhage, inflammation, epithelial cell proliferation and formation of granulation tissue and fibrous union. Removal of sutures, ruptures the epithelial layer. At times stitch abscess (infection of sutured area) and epidermal cysts/implantation (due to persistence of epithelial cells) may arise as complications which can be culminated by using adhesive tapes.

2. Secondary Union(Healing by Second Intension)

Healing by secondary union occurs in wounds that are,

- ❖ Infected and open
- ❖ Associated with extensive tissue loss
- ❖ Not approximated by surgical sutures.

Healing by secondary union differs from primary union in the following aspects.

- (a) Consumes longer time and healing occurs from both the sides i.e., from the margins as well as from the base upwards, unlike in primary union where healing occurs only from the margins.
- (b) Scar formed is usually large and ugly in appearance.
- (c) Extensive formation of granulation tissue.
- (d) Involves an additional step i.e., wound contraction.
- (e) Involves relatively intense inflammatory reaction.

Healing by secondary union involves the following steps.

(a) Haemorrhage

Filling of the injured area with blood followed by clotting.

(b) Inflammation

Invasion of polymorphs to the site of injury and their subsequent replacement by macrophages.

(c) Epithelial Cell Proliferation

Epithelial cells from the margin and from the base continue to divide and invade the injured area until the gap between the wound is filled. Meanwhile, the granulation tissue formation begins and starts filling the gap between the wound from the base. This tissue is separated from the blood clot by the scab which later gets removed. This phase ends with the stratification and keratinization of the newly formed epidermal layer.

(d) Granular Tissue Formation

Fibroblast proliferation and angiogenesis are the two events involved in the formation of granular tissue. Due to the presence of collagen, the granulation tissue which is actually deep red, granular and fragile in nature tends to appear white.

(e) Contraction of Wound

Contraction of wound is the characteristic feature of secondary union. Wound contraction reduces the space between the edges of the wound by upto $1/3^{\text{rd}}$ or $1/4^{\text{th}}$ level. The wound contracts due to the presence of myofibroblasts in the granulation tissue.

(f) Scar Formation

The final step is the formation of scar and thinning of epidermis.

Wound Strength

Replication of fibroblasts and myofibroblasts tends to increase the wound strength which depends on various factors like the site of injury, degree of incision etc. Initially the wound strength is 10% which increases over the next few weeks and may be upto 80% after which there is no further increase.

This increase (upto 80%) in the tensile strength of the wound is due to the synthesis of collagen and cross linking of collagen fibres.

Complications of Wound Healing

1. Infection

An infected wound initiates necrosis and thrombosis because of release of bacterial toxins. This delays the healing process in infected wounds.

2. Pigmentation

Sometimes the healed tissue exhibits characteristic rust-like colour due to the presence of haemosiderin which stains the tissue.

3. Granulation Tissue

Incomplete formation of the granulation tissue causes incomplete scar formation.

4. Epidermal Cyst Formation

Presence of epithelial cells even after healing results in implantation which delays wound healing.

5. *Excess Formation of Components of Repair*

Hypertrophied scars arise due to excessive deposition of collagen fibres within the borders of the wound. The keloid (which resembles a claw) also occurs due to excessive collagen deposition but here the ugly-looking keloid forms a tumour-like projection beyond the boundaries of the scar.

6. *Excessive Wound Contraction*

Excess contraction of wounds results in deformity of bones and the surrounding tissue (contracture) as well as loss of regeneration capacity (cicatrization).

7. *Neoplasia*

The site of formation of the scar may later become susceptible to the formation of carcinomas.

2.1 REGENERATION

The cells or tissues that are to be replaced by regeneration should be capable of undergoing replication or proliferation and have the ability to grow. Injury to some tissues destroys only a portion of the tissue without destroying their stem cells. Such tissues regenerate the lost tissue by self-renewal as its cells that are capable of undergoing cell division on their own.

E.g.s: Hematopoietic system, gastrointestinal tract and epithelium of the skin.

Some tissues cannot proliferate until a stimulus is received.

E.g.s: Tissues of liver and kidneys.

In general, the cell population can be controlled by maintaining a balance between cell proliferation, differentiation and cell death. Regeneration is a process which involves restoration of normal conditions by replacing the tissue components with tissues similar to those lost due to injury.

Cell Cycle

The cell cycle includes five different phases.

1. *Mitotic phase (M)*

It is the phase during which a cell undergoes replication (mitosis). It consists of 4 stages, prophase, metaphase, anaphase and telophase.

2. *Gap 0 (G_0) phase*

This is considered as the resting or dormant phase. The cells in this phase remain inactive.

3. *Presynthetic or Gap 1 (G_1) phase*

The daughter cells of mitosis now enter the G_1 phase and synthesize various organelles and cytoplasmic contents which are required for forthcoming cell division.

4. *Synthesis (S) phase*

As the name suggests, synthesis of nuclear DNA occurs during S-phase.

5. *Gap 2 (G_2) Phase*

During this phase, the enzymes and proteins necessary for the cell division are synthesized.

Cell replication is regulated by specific cellular genes called as *proto-oncogenes*. Any abnormality in these genes converts them into genes known as oncogenes. These oncogenes enable the cells to undergo rapid/abnormal proliferation which finally results in cancer. Hence, it is necessary that the cell cycle should be regulated.

Growth factors and the signals received from extracellular matrix components act as stimuli for cell proliferation. Cell cycle plays an important role in homeostasis and in growth processes such as regeneration and repair. Any disturbance in the cell cycle therefore produces abnormal growth.

Entry of inactive cells from G_0 into G_1 phase is the main step of cell cycle and this can happen in two ways:

- Cells may directly enter from the G_0 phase or
- Cells may enter after undergoing the mitotic (M) phase.

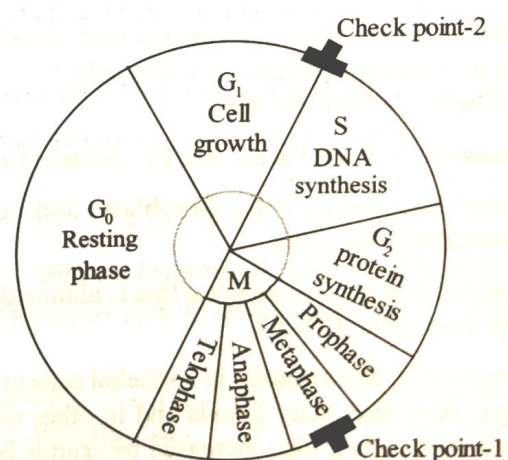


Figure: Different Phases of Cell Cycle

Some quality control checks referred to as check points are also present. These check points tend to remove the damaged DNA and chromosomes from undergoing further replication. Thus, any abnormality in these checkpoints causes neoplasia and tissue alterations. There are two such check points located in the cell cycle:

1. *G_1/S Checkpoint*

This checkpoint checks for any damage to the DNA before replication.

2. *G_2/M Checkpoint*

This checkpoint checks for any abnormalities in the DNA replication.

Growth Factors

Apart from exerting their effects on locomotion, contraction, differentiation and angiogenesis of a cell, growth factors also play a key role in cellular proliferation.

1. Epidermal Growth Factor (EGF)

Source: Tissue secretions like saliva, intestinal juices, urine and sweat. At the injured area, it is generated from cells like keratinocytes, macrophages etc.

Receptor: Epidermal growth factor binds to a receptor known as EGFR that possess intrinsic tyrosine kinase activity.

Function: It induces mitosis in keratinocytes and fibroblasts. It enhances the movement of keratinocytes and other inflammatory cells towards the injured area. It also stimulates granulation tissue formation.

2. Transforming Growth Factor- α (TGF- α)

Source: T-lymphocytes, keratinocytes and macrophages.

Receptor: Binds to a receptor known as EGFR and exerts almost similar effects as that of epidermal growth factor.

Function: Stimulates hepatocytes and endothelial cells to undergo replication. It also plays a role in endothelial cell proliferation.

3. Hepatocyte Growth Factor (HGF) / Scatter Factor

Source: Endothelial cells, fibroblasts and hepatic non-parenchymal cells.

Receptor: It binds to a receptor that is obtained from proto-oncogene, C-MET.

Functions: Induces mitosis in epithelial cells of liver, lungs, skin, mammary glands and in other tissues. Motility of cells is also increased by scatter factor.

4. Vascular Endothelial Growth Factor (VEGF)

The vascular endothelial growth factor is further divided into four different types as VEGF-A, VEGF-B, VEGF-C and VEGF-D. These factors are present in variety of adult tissues such as in podocytes of glomerulus and cardiac myocytes.

(a) VEGF-A

- ❖ Receptor: VEGF-A binds to tyrosine kinase based receptor VEGFR-2 (present on endothelial cells).
- ❖ Function: In case of wounds, tumours and in chronic inflammation, VEGF-A stimulates angiogenesis (growth of new blood vessels).

(b) VEGF-B

- ❖ Receptor: VEGF-B binds to VEGFR-1
- ❖ Function: Controls myocardial functionality.

(c) VEGF-C and VEGF-D

- ❖ Receptor: VEGF-C and VEGF-D bind to VEGFR-3 present in lymphatic endothelial cells.
- ❖ Function: They promote lymphangiogenesis (formation of new lymphatic vessels).

5. Platelet Derived Growth Factor (PDGF)

Platelet derived growth factor contains five different isoforms named PDGF-C, PDGF-D, PDGF-AA, PDGF-BB and PDGF-AB.

Source: Present in platelets, macrophages, smooth muscle cells, endothelial cells and tumour cells.

Receptor: Binds to cell surface receptors, PDGFR- α and PDGFR- β .

Functions: It promotes the movement of fibroblasts, monocytes and smooth muscle cells to the injured area. Promotes production of matrix-metalloproteinases, hyaluronic acid. It inhibits aggregation of platelets.

6. Fibroblast Growth Factor (FGF)

More than ten different growth factors have been identified of which the main ones are FGF-1 and FGF-2.

Source: Produced by macrophages, endothelial cells, fibroblasts, mast cells and T-lymphocytes.

Functions: It plays an important role in angiogenesis.

- (a) It promotes the movement of fibroblasts, macrophages and endothelial cells to the site of injury.
- (b) It also participates in the development of skeletal muscle tissues and lungs. FGF-6 promotes mitosis in myocytes and generates angioblasts.
- (c) Induces mitosis in fibroblasts and keratinocytes and plays a key role in wound contraction and matrix deposition.

7. Transforming Growth Factor- β (TGF- β) and Other Factors

The transforming growth factor- β (TGF- β) contains three different isoforms namely TGF- β_1 , TGF- β_2 and TGF- β_3 .

Source: Obtained from lymphocytes, macrophages, endothelial cells and platelets.

Receptor: Binds to cell surface receptors called type-I and type-II.

Functions

- (a) It inhibits the growth of epithelial cells and leucocytes.
- (b) Induces mitosis in fibroblasts and smooth muscle cells.
- (c) Induces production of collagen, proteoglycans, fibronectin and suppresses degradation of collagen. Hence it plays an important role in fibrosis.

Types of Cells

Based on their proliferative capacity, the body cells are divided into three types.

1. Labile/Continuously Dividing Cells

These cells are in a state of continuous division even under normal conditions because the mature cells are produced from stem cells.

Examples

- (a) Surface epithelia of skin, oral cavity, vagina and cervix.
- (b) Haematopoietic cells of bone marrow, cells of spleen and lymph nodes, mucosal lining of excretory ducts of the glands like pancreas, biliary tract and salivary glands.
- (c) Epithelium of gastrointestinal tract and uterus.

2. Stable/Quiescent Cells

Cells that remain inactive under normal physiological conditions but tend to undergo proliferation upon stimulation are referred as stable. The stable cells remain inactive in G_0 phase and upon stimulation, they enter G_1 phase where they undergo proliferation.

Examples

- (a) Parenchymal cells of liver, kidney and pancreas.
- (b) Mesenchymal cells like fibroblasts and smooth muscle cells.
- (c) Vascular endothelial cells, resting lymphocytes and leucocytes.

3. Permanent/Nondividing Cells

Cells that do not proliferate are referred to as non-dividing cells. Such cells do not undergo mitotic division and die if they encounter any injury. However, the tissues can be replaced with the help of adjacent supportive cells that are capable of proliferation.

Examples: Neurons, skeletal muscle cells and cells of cardiac muscle.

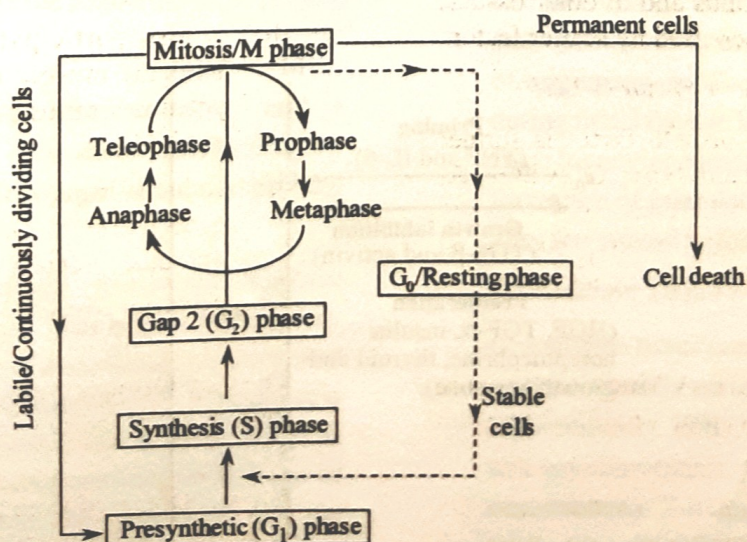


Figure: Various Cells in Different Phases of Cell Cycle

Mechanism of Regeneration

Regeneration is a process which involves restoration of normal conditions, by replacing the tissue components with tissues similar to those lost due to injury.

The basic steps involved in regeneration are as follows.

1. Upon injury, the cells of the damaged tissues begin to replicate and move from the margins of the damaged area into the spaces created by the injury.
2. The cells then undergo differentiation and maturation and thus replace the lost tissue.
3. Regeneration takes place by the following two different mechanisms.
 - (a) In labile tissues like epidermis, GIT epithelium and in haemopoietic system the cells are capable of self-renewal i.e., they are capable of replicating on their own. Regeneration in such tissues is referred to as true regeneration, where the damaged tissue is replaced by fresh tissue which resembles the lost tissue.
 - (b) In stable tissues like liver and kidney, regeneration is not entire, wherein that part of the tissue which has been effected during injury is replaced and not the entire tissue. Such regeneration is generally referred to as compensatory growth.

Hepatic Regeneration

The cells involved in regeneration of liver are hepatocytes which are stable cells, that remain inactive. They enter the G_1 phase after receiving the stimulus. The regeneration in case of partial hepatectomy involves the following three stages:

1. Priming

Cytokines like TNF, IL-6 and others send a stimulus to the hepatocytes. On receiving the stimulus, the hepatocytes from G_0 enter the G_1 phase and start replicating.

2. Proliferation

Formation of cyclin-CDK complex and phosphorylation of RB protein facilitates the hepatocytes to continue other phases of the cell cycle. Growth factors like HGF, TGF- α and others are involved in the continuation of the cell cycle. Several hormones like norepinephrine, insulin, thyroid hormone and growth hormone serve as adjuvants for replication.

3. Growth Inhibition

Termination of proliferation of hepatocytes after regeneration is due to the presence of growth inhibitors like TGF- β and activin.

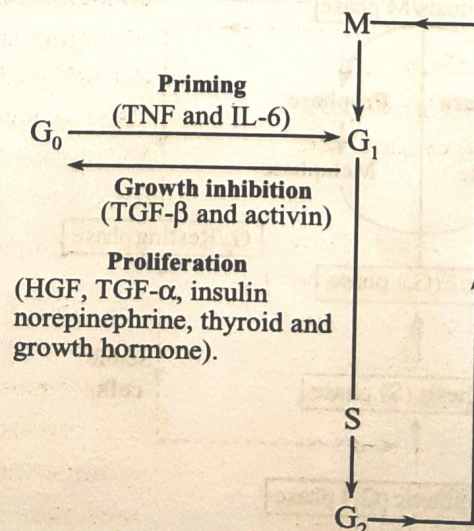


Figure: Steps involved in Regeneration of Partial Hepatectomy

2.2 REPAIR

Repair is a complex phenomenon involving the replacement of injured tissue with fibrous tissue. It involves the following,

- (a) Initiation of an inflammatory process immediately in response to an injury
- (b) Parenchymal and connective tissue cells proliferate and are recruited to the site of injury
- (c) Initiation of angiogenesis and formation of granulation tissue
- (d) Synthesis of extracellular matrix proteins and deposition of collagen
- (e) Contraction of wound
- (f) Tissue remodelling.

However, not all repair reactions involve the above mentioned processes.

Factors Influencing the Repair Process

1. Extent of tissue damage and environment of the tissue.
2. Intensity and duration of stimuli.
3. Inadequate blood supply, presence of foreign bodies and other factors which inhibit repair process.
4. Diseases such as diabetes which inhibit repair.
5. Treatment with steroids and other drugs which inhibit the repair process.

Restoration following an injury may be complete or partial, depending upon the tissue involved. In certain types of injuries, regeneration may not be possible. In such circumstances, repair is achieved by connective tissue rather than parenchymal cells thereby producing a scar.

Phases Involved in Repair Process

Repair process comprises the following three phases,

1. Inflammatory Phase

This phase occurs soon after an injury and is characterized by *haemostasis* (prevention of bleeding by coagulation and contraction of damaged blood vessels) and *inflammation*.

Immediately after injury, blood vessels are constricted to prevent blood loss. This is later followed by vasodilation due to the release of endothelial products and mast cell derived factors from damaged cells. Histamine is the major factor which is involved in vasodilation. It makes the blood vessels more permeable resulting in leakage of proteins and vascular fluid from the circulation into the extravascular space. This in turn contributes to enhanced osmolar load resulting in *oedema*.

Polymorphonuclear neutrophils appear at the site of injury after an hour and initiate the process of phagocytosis. They release free radicals which bring about bacterial lysis. They also secrete protease enzymes which help in breaking the damaged tissue and cleaning the wound.

Macrophages are very essential in the wound healing process. As the inflammation reduces, neutrophils and macrophages present at the wound site decrease in number indicating the termination of inflammatory phase.

2. Proliferative Phase

This phase occurs 2-3 days after injury. Presence of fibroblasts at wound site indicates the onset of this phase. The steps involved in proliferative phase are as follows,

(a) Epithelization

Epithelization involves migration of epithelial cells. If the basement membrane is intact, the epithelial cells undergo mitotic division and migrate upwards in a normal fashion to restore the damaged epidermis.

If the basement membrane is damaged, re-epithelization occurs from the peripheral normal cells or from skin appendages like hair follicles, sweat glands and oil glands. If the injury is very deep and no basement membrane or skin appendages are intact, then re-epithelization occurs from the edges of the wound.

(b) Angiogenesis

Angiogenesis is a complex process involving the formation of new blood vessels from pre-existing vessels to ensure supply of oxygen and nutrients to the newly formed granulation tissue. Production of fibroblast growth factor (FGF) and endothelial cell derived growth factor by macrophages and endothelial cells is mainly responsible for induction of angiogenesis. The former directs angiogenesis during first 3 days of healing, while the latter sets the stage for angiogenesis from 4-7 days (i.e., during the formation of granulation tissue). This process stops after the wound is filled with granulation tissue.

(c) Granulation Tissue Formation

Granulation tissue contains numerous capillaries and a support matrix enriched with collagen fibroblasts, inflammatory cells, endothelial cells, pericytes and myofibroblasts. Granulation tissue formation commences 2-4 days after injury. Chemical mediators released from macrophages during the inflammatory phase causes fibroblast activation.

Activated fibroblast releases proteolytic enzymes to facilitate its migration into the provisional matrix of the wound clot. Fibrin, fibrinectin and hyaluronic acid molecules present in the matrix contributes to the formation of granulation tissue by providing a scaffold for migration of cells. After migrating into the wound, fibroblast replaces the provisional matrix with collagenous matrix by releasing collagen, elastin and proteoglycans into it. Once large amount of collagenous matrix is formed, production of collagen comes to a halt.

(d) **Wound Contraction**

It is a complex interactive process involving extracellular matrix and cytokines. When the defect is covered with epithelial cells, fibroblast modifies into myofibroblasts. Since myofibroblasts are connected to each other and to the margins of wound, they draw the edges of the wound together and contract the complete granulating bed.

3. **Maturation Phase (Remodelling)**

This phase involves the transition of granulation tissue into a scar. During this phase, collagen deposited during granulation tissue formation stage undergoes rearrangement and cross-linking to attain more tensile strength. Such remodelling gradually replaces type-I collagen with type-III until normal skin ratio of 4:1 is reached. After 3 months, 80% of original tissue strength is restored. The remodelling process continues upto 1 year, however there is no further increase in tensile tissue strength.

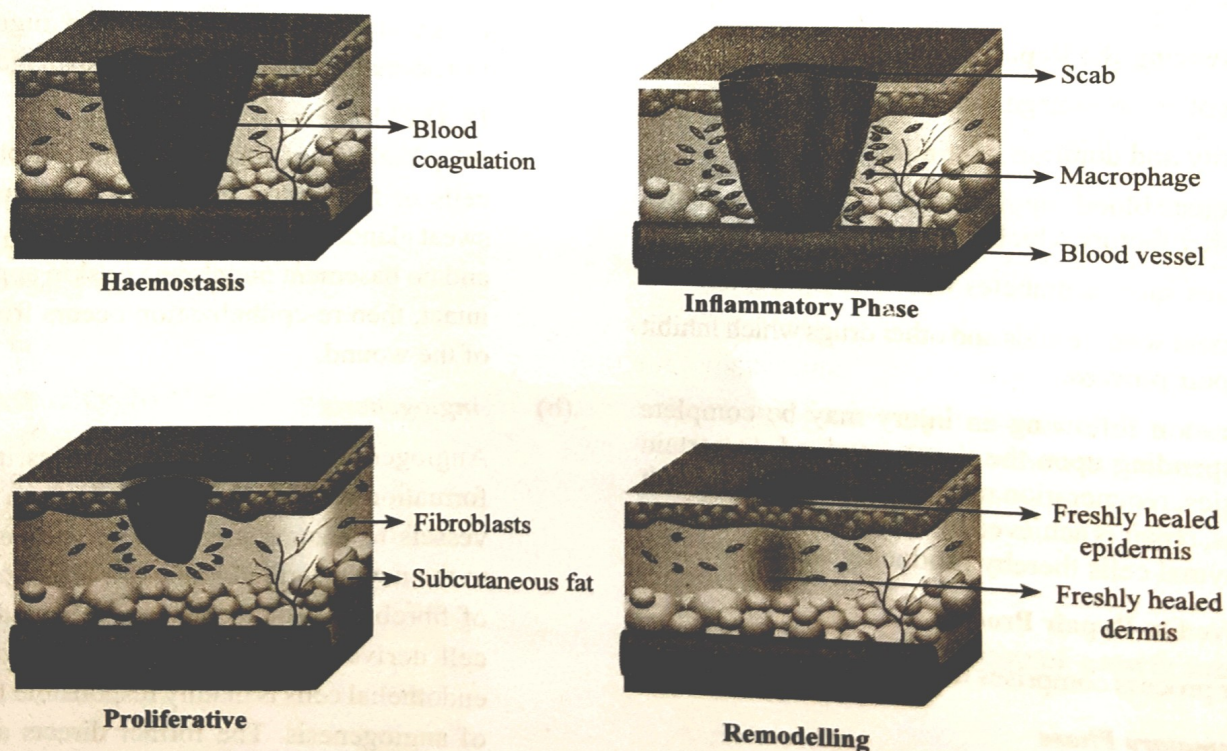


Figure: Phases Involved in Repair