

Unit-I

Basic principles of cell injury and Adaptation:

* Pathophysiology: study of disease is comprise by 2 words: 'Patho' meaning 'suffering' and 'physiology' is study of normal function.

Pathophysiology include study disordered function or breakdown of homeostasis.

* Disease: A defined pathological process having a characteristic set of sign and symptoms i.e., disease.

* Disorder: A derangement (decrement) or abnormality of function. e.g., A morbid physical or mental state.

* Homeostasis: (Homeo= Unchanging and stasis = standing). It refers to existence of stable internal environment.

• Mechanisms involved in homeostasis regulation:

A) Autoregulation: eg, Nitric oxide \rightarrow vasodilation \rightarrow Blood flow \downarrow fulfill O₂ demand

B) Extrinsic regulation: Nervous system and endocrine system help to regulate homeostasis.

* Component of feedback system: The regulatory mechanism consists of 3 components:

- Receptor
- control centre
- Effector

A) Receptor: A sensor i.e., sensitive to a particular environmental change or a stimulus.

B) control centre / Integration centre: It receives and processes the information supplied by the receptor. e.g., hypothalamus, pituitary gland (master gland).

C) Effector: A cell or organ that responds to the commands of control centre and whose activity oppose or enhances the stimulus.

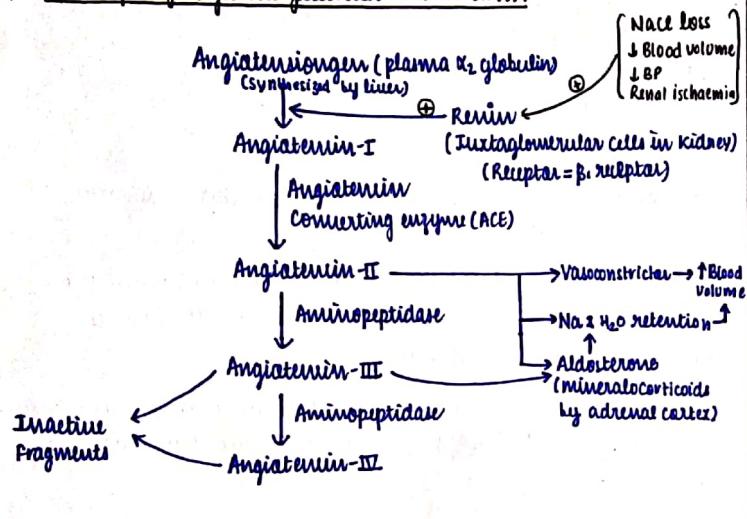
* Types of feedback system: 2 types:

- Negative feedback system
- Positive feedback system.

1) Negative feedback system: It is a primary mechanism of homeostasis regulation and it provides long-term control over internal conditions and systems. They maintain a normal range rather than a fixed value. e.g., Regulation of BP.

2) Positive feedback system: e.g., process of child birth and blood clotting must be completed quickly to limit stress that can be dangerous to both mother and infants.

Ex. Example of negative feedback mechanism:



∴ Receptor = β_1 receptor
central centre = hypothalamus, pituitary gland
Effector = Adrenal cortex

∴ Ischaemia = ↓ Blood Flow
Renal ischaemia = ↓ Blood Flow to kidney

Fig: Regulation of electrolyte balance, plasma volume & blood pressure by Renin-angiotensin system

* Cell injury: It is defined as the functional and morphologic effects of a variety of stresses on the cell from various aetiologic agents which results in changes in its internal and external environment.

- The cellular response to stress may vary depending upon following 2 types of factors:

- A) Host factors i.e., type of cell, nutritional status of cell etc.
- B) Factors pertaining to injurious agent i.e., its type, dose etc.

• Accordingly, various forms of cellular responses to injurious agents may be as follows: (show in fig, cellular responses to cell injury).

A) When there is altered functional demand (increased or decreased), cells may adapt to changes that expressed morphologically, which then revert back to normal after the stress is removed; these are termed as cellular adaptation.

B) 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).

C) 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).

• Aetiology / causes of cell injury: There are:

- 1) Hypoxia and ischaemia: Hypoxia may result from following 2 pathways:

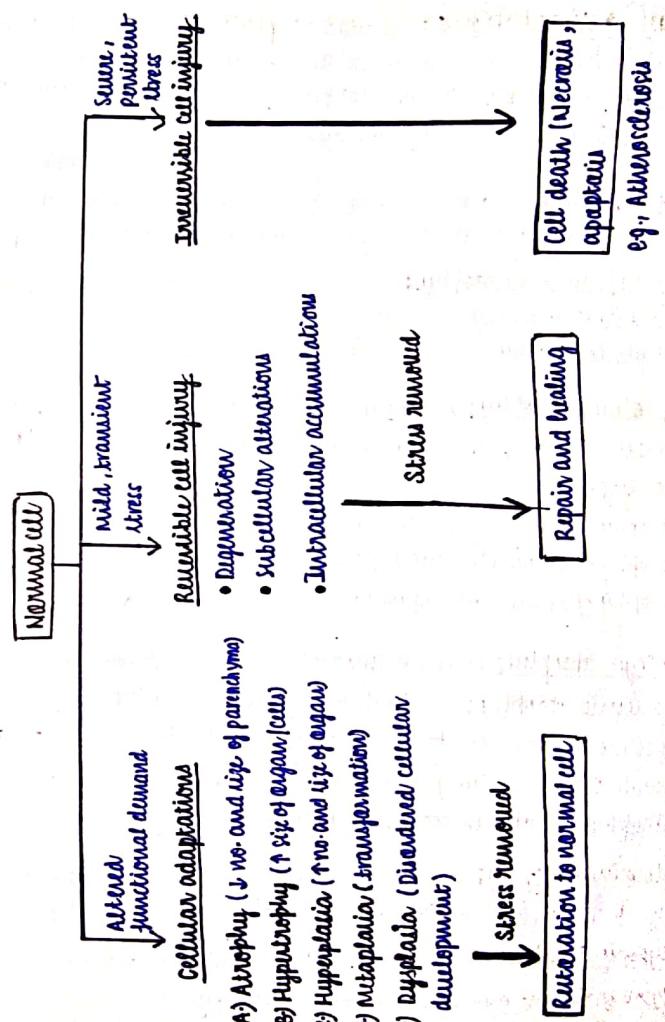
- a) By reduced blood supply to the cells due to interruption i.e., ischaemia
- b) Hypoxia may also result from impaired blood supply from causes other than interruption e.g., disorders of oxygen-carrying RBCs (e.g., anaemia, carbon monoxide poisoning), heart disease, lung disease etc.

- 2) Physical agents: e.g., Mechanical trauma (road accidents), thermal trauma, electricity and radiation etc.
- 3) chemicals and drugs: e.g., chemical poisons such as cyanide, arsenic and mercury, insecticides, pesticides, alcohol and Narcotic drugs.
- 4) Microbial agents: Microbial infection.
- 5) Immunologic causes: e.g., hypersensitivity rxn, anaphylactic rxn & autoimmune disease.
- 6) Nutritional derangement:- overall deficiency of nutrition e.g., starvation, deficiency of protein e.g., Marasmus, Kwashiorkor, deficiency of minerals e.g., Anaemia.
- Nutritional excess leads to obesity, atherosclerosis, hypertension.
- 7) Ageing: cellular ageing leads to impaired ability of the cell to undergoes replication and repair.
- 8) Psychogenic causes
- 9) Iatrogenic causes
- 10) Idiopathic disease or unknown cause.

Factors variables of cell injury:

- A) Type of cell and tissue involved
- B) Extent and type of cell injury.

** Note: Neoplasia differs from hyperplasia in having hyperplastic growth with loss of growth regulatory mechanism due to change in the genetic composition of the cell. whereas hyperplasia persists so long as stimulus is present.



* **Cellular Adaptations:** cellular adaptations 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).

A) **Atrophy:** Reduction of the no. and size of parenchymal cells of an organ or its parts which was once normal is called atrophy.

• **Aetiology / causes of atrophy:**

- a) Physiologic atrophy
- b) Pathologic atrophy

a) **Physiologic atrophy:** Atrophy is a normal process of ageing in some tissues, due to loss of endocrine stimulation or from senile arteriosclerosis - e.g.,

- Atrophy of thymus in adult life.
- Atrophy of gonads after menopause.
- Atrophy of brain with ageing.

b) **Pathologic atrophy:** causes of this are:

i) **Ischaemic atrophy:** gradual reduction of blood supply due to atherosclerosis may result in shrinkage of the affected organ - e.g.,
- small atrophic kidney in atherosclerosis of renal artery.
- Atrophy of brain in cerebral atherosclerosis.

ii) **Endocrine atrophy:** loss of endocrine regulatory mechanism results in reduced metabolic activity of tissues and hence atrophy e.g.,
- Hypopituitarism may lead to atrophy of thyroid, adrenal & gonads
- Hypothyroidism may cause atrophy of the skin.

iii) **Pressure atrophy:** Prolonged pressure from benign tumours (or mild tumours) may cause compression and atrophy of the tissue - e.g., Erosion of the spine by tumour in nerve root.

• **Morphologic features:** due to atrophy:

The organ is often shrunk the cells becomes smaller in size but are not dead cells - e.g., Testicular atrophy (shrinkage in cell size is due to reduction in cell organelles mainly mitochondria, myofilament and endoplasmic reticulum).

B) **Hypertrophy:** It is an increase in size of parenchymal cells resulting in an enlargement of the organ/tissue without any change in no. of the cells.

• **Aetiology / cause of hypertrophy:** Hypertrophy is caused by either red functional demand or by hormonal stimulation. (Hypertrophy without accompanied hyperplasia affects mainly muscles).

- a) Physiologic hypertrophy
- b) Pathologic hypertrophy

a) **Physiologic hypertrophy:** Enlarged size of the uterus in pregnancy under estrogenic stimulation is an example of physiologic hypertrophy (as well as hyperplasia).

b) **Pathologic hypertrophy:** For example:

- i) **Hypertrophy of cardiac muscle:** It leads to congestive heart failure (CHF), systemic hypertension.
- A myocardial fibre subjected to persistent ↑ ed (in hypertension) adapt by undergoing hypertrophy: an ↑ in size of left ventricle but is insufficient to pump blood against an ↑ load.

- Systemic hypertension is related with mitral valve insufficiency results in left ventricular hypertrophy.

ii) Hypertrophy of smooth muscles: e.g., pyloric stenosis (in stomach)

iii) Hypertrophy of skeletal muscles: e.g., Hypertrophied muscles in athletes and manual labour.

iv) Compensatory hypertrophy: It may occurs in an organ when the contralateral organ (organ in pair) is removed e.g., Nephrectomy on one side in a young patient, there is compensatory hypertrophy as well as hyperplasia of the nephrons of the other kidney.

- Adrenal hyperplasia followed by removal of one adrenal gland.

Morphological features of hypertrophy:

- At the ultrastructural level, there is increased protein synthesis, increased number of myofilaments, and ↑ no. of organelles such as mitochondria, endoplasmic reticulum and myofibrils.

- A hypertrophied heart of a patient with systemic hypertension may weigh 700 to 800 gm as compared to average normal adult weight of 200 to 250 gm.

c) Hyperplasia: It is an increase in the no. of parenchymal cells resulting in enlargement of an organ or tissue.

• All the 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 muscles) have little or no capacity for regenerative hyperplastic growth.

• Aetiology/causes of hyperplasia: Hyperplasia may also be:

a) Physiologic hyperplasia

b) Pathologic hyperplasia

a) physiologic hyperplasia: It is classified into:

1) Hormonal hyperplasia

2) compensatory hyperplasia.

1) Hormonal hyperplasia: For e.g.:

- Hyperplasia of pregnant uterus.

- Proliferative activity of normal endometrium after a normal menstrual cycle.

- Prostatic hyperplasia in old age.

2) Compensatory hyperplasia: For e.g.:

- Regeneration of liver after partial hepatectomy (cutting of liver).

- Following Nephrectomy (cutting of nephron) on one side, there is hyperplasia of nephron in other kidney.

b) Pathologic hyperplasia: For e.g.:

- Endometrial hyperplasia following estrogen excess.

- In wounds healing, there is formation of granulation tissue due to proliferation of fibroblast and endothelial cell.

• Morphologic features of hyperplasia: There is enlargement of the affected organ and ↑ in no. of cell.

e.g., Sequential changes in uterine cervix from normal epithelium to development of carcinoma (cancer) by squamous metaplasia and dysplastic change. This is due to ↑ rate of DNA synthesis and therefore ↑ mitosis of the cell.

D) Metaplasia: It is defined as reversible change of one type of mature differentiated epithelial or mesenchymal adult cell to another type of mature (adult) epithelial or mesenchymal cells, usually in response to abnormal stimuli and often revert back to normal on removal of stimulus.

• If stimulus persist for a long time, epithelial metaplasia may transform into cancer.

• Types of metaplasia: Metaplasia is broadly divided into 2 types:

- a) Epithelial metaplasia
- b) Mesenchymal metaplasia

a) Epithelial metaplasia: It is more common metaplasia. The metaplastic changes due to patchy or diffuse, usually results in replacement by stronger but less well-specialised epithelium. However, metaplastic epithelium being less well-specialised such as squamous type, results in deprivation of protective mucous secretion and hence more prone to infection. Epithelial metaplasia is of 2 types:

- i) Squamous metaplasia
- ii) Columnar metaplasia

i) Squamous metaplasia: Various types of specialised epithelium are capable of undergoing squamous metaplastic change due to chronic irritants as stimuli which may be mechanical, chemical or infective in origin. For e.g.,

- In bronchus (normally lined by pseudostratified ciliated epithelium) in chronic smokers changes into squamous epithelium.
- In uterine endocervix (normally lined by simple columnar epithelium) in prolapse of uterus and in old age. e.g., squamous

metaplasia of uterine cervix

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

ii) Columnar epithelial metaplasia: There are some conditions in which cell is transform to columnar epithelium. For e.g.,

- Intestinal metaplasia in healed chronic gastric ulcer
- Conversion of pseudostratified ciliated columnar epithelium in chronic bronchitis and bronchiectasis (abnormal dilation of bronchus like balloon) to columnar type.

- In cervical erosion (congenital and adult type), there is variable area of endocervical glandular mucosa into vagina.

b) Mesenchymal metaplasia: Less often, there is transformation of the mature (adult) differentiated type of mesenchymal tissue to another. For e.g.,

- Ossification metaplasia is formation of bone in fibrous tissues cartilage.
e.g., 1) In soft tissue myositis ossificans occurring following haematoma
2) In cartilage of larynx and bronchi especially in older people
3) In old scar of chronic inflammation bcoz of prolonged duration
4) In the fibrous stroma of tumours. e.g., in leiomyoma.

- Cartilaginous metaplasia: In healing of fractures; cartilaginous metaplasia may occur where there is undue mobility.

E) Dysplasia: Dysplasia means 'disordered cellular development' often accompanied with metaplasia and hyperplasia. So, such a condition is also atypical hyperplasia (includes metaplasia, hyperplasia alongwith dysplasia).

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| <p>② Dysplasia occurs most often in epithelial cells so, called epithelial dysplasia. Epithelial dysplasia is characterized by cellular proliferation and cytologic changes. These changes includes:</p> <ol style="list-style-type: none"> 1) Increased no. of epithelial cell layer 2) Disorderly arrangement of cells from basal layer to the surface layer 3) Loss of basal polarity i.e., nuclei lying away from basement membrane. 4) Cellular and nuclear pleomorphism 5) Increased nucleocytoplasmic ratio. 6) Nuclear hyperchromatism 7) ↑ Ed mitotic activity due to accelerated cell proliferation. <p>• Examples: The 2 most common examples of dysplastic changes are the uterine cervix and respiratory tract.</p> <p>• Dysplastic changes often occur due to chronic irritation or prolonged inflammation. On removal of the stimulus, mild and moderate dysplastic changes may disappear.</p> | <p>b) <u>Pathologic hypertrophy</u>: eg, hypertrophy of smooth muscle in pyloric stenosis of stomach.</p> <p>- compensatory hypertrophy of contralateral organ after removal of our kidney.</p> <p>3) Morphology</p> <p>GA: Affected organ enlarged and heavy. ME: Enlarged muscle fibres as well as nucleomegaly. EM: ↑ DNA synthesis, RNA, myofibrillar, ↑ no. of organelles.</p> <p>4) Molecular pathogenesis</p> <p>Increased protein synthesis by increased growth factors acting on cell surface and causing activation of signal transduction pathways.</p> <p>5) Natural history</p> <p>Reversible on withdrawal of stimulus.</p> | <p>b) <u>Compensatory hypertrophy</u> in hepatomegaly of liver.</p> <p>b) <u>Pathological hyperplasia</u>: eg, Endometrial hyperplasia due to oestrogen excess during menstrual cycle.</p> <p>GA: Affected organ enlarged and heavy. ME: Increased no. of cells. EM: Ted DNA/RNA regular mitoses.</p> <p>Increased proliferation of cells by increased growth factors acting on cell surface receptors and stimulating signal transduction pathways.</p> <p>May regress on removal of inciting stimulus; persistence of stimulus may lead to dysplasia or neoplasia.</p> |
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Differences between metaplasia and dysplasia:

| Features | Metaplasia | Dysplasia |
|------------------------------|---|--|
| 1) Definition | Change of an adult type of epithelial or mesenchymal cell to another type of adult epithelial or mesenchymal cell; often in response to inciting stimulus. | Disordered cellular development, may be accompanied with hyperplasia or metaplasia; often in response to inciting stimulus. |
| 2) Types and common examples | <ul style="list-style-type: none"> Squamous metaplasia: E.g.; Bronchus, uterine cervix. Columnar metaplasia: E.g., Osseous metaplasia, Myositis ossificans. | <ul style="list-style-type: none"> Epithelial only e.g.,: e.g., uterine cervix and respiratory tract cell dysplasia. |
| 3) Morphology | Mature cellular development from specialised to less well-specialised and resistant cells. | Disorderly cellular development (few layers, loss of polarity, pleomorphism, nuclear hyperchromia, mitoses). |
| 4) Molecular Pathogenesis | Reprogramming of precursor stem cells triggered by exogenous stimuli to another pathway. | Abnormal cell growth by mutations in genes as in neoplasia. |
| 5) Natural history | Reversible on withdrawal of stimulus; persistence of stimuli may cause progression to dysplasia. | Mild and moderate grade regress on removal of inciting stimulus; severe grade and persistence of stimuli may cause progression to carcinoma in situ and invasive cancer. |

* **Pathogenesis of cell injury:** The following general features characterize most forms of cell injury by various agents:

1) **Factors pertaining to etiologic agent and host:** Following parameters pertaining to etiologic agent or host determine the outcome of cell injury:

- Type, duration and severity of injurious agent.
- Type, status and adaptability of target cell.

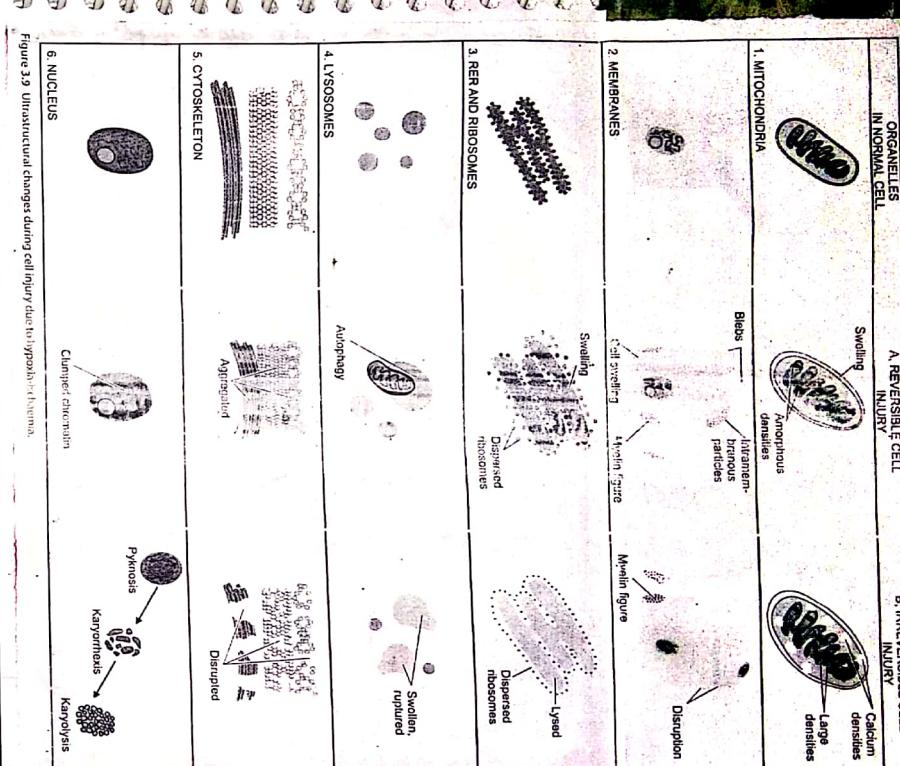
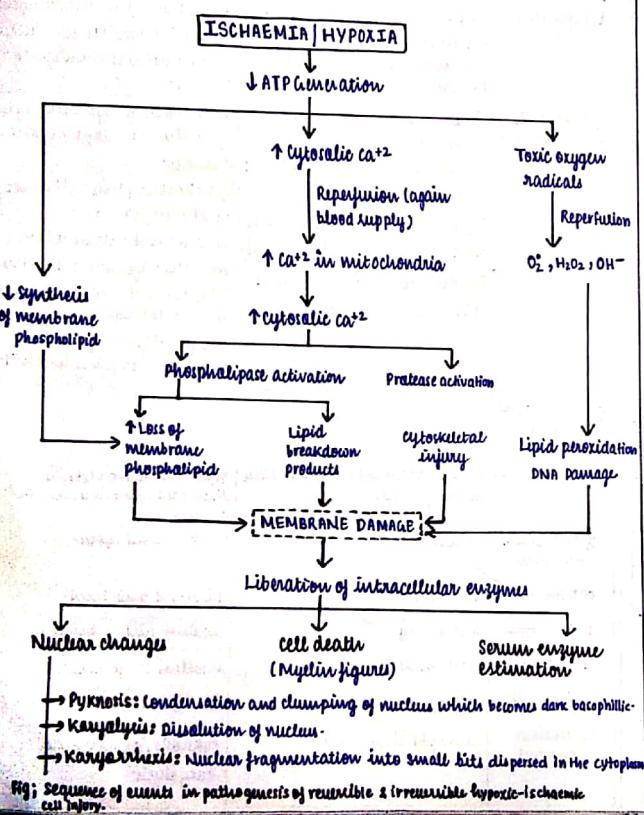
2) **General underlying mechanisms:** Following general intracellular biochemical phenomena underlie most forms of cell injury:

- Mitochondrial damage causing ATP depletion.
- Cell membrane damage disturbing the metabolic and trans-membrane exchanges.
- Release of reactive oxygen-derived free radicals.
- Reduced protein synthesis and nuclear damage.

3) **Usual morphologic changes:** The morphologic changes of reversible cell injury (e.g., hydropic swelling) appear earlier while morphologic alteration of cell death occur later (e.g., in myocardial infarction).

4) **Functional implications and disease outcome:** 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 being. The other cause of cell injury are: chemical and physical (i.e., ionizing radiation) agents:

A) Pathogenesis of ischaemic-hypoxic injury:



* * Note: Morphological consequences (All forms of biochemical change underlying cell injury are expressed in terms of morphological changes).

• Common enzyme markers of cell death:

| Enzyme | Disease |
|--|---|
| 1) Aspartate aminotransferase (AST, SGOT) | Diffuse liver cell necrosis e.g., viral hepatitis, alcoholic liver disease. |
| Serum glutamicoxaloacetic transaminase (SGOT) | Acute myocardial infarction |
| 2) Alanine aminotransferase (ALT) | Diffuse liver cell damage, viral hepatitis. |
| Serum glutamic pyruvate transaminase (SGPT) is a more specific for diffuse liver cell damage than AST & ALT. | |
| 3) Creatine kinase - MB (CK-MB) | Acute myocardial infarction, myocarditis, skeletal muscle injury. |
| 4) Lipase | More specific for acute pancreatitis |
| 5) Amylase | Acute pancreatitis, Sialadenitis |
| 6) Lactic dehydrogenase (LDH) | Acute myocardial infarction, myocarditis, skeletal muscle injury |
| 7) Cardiac troponin (cTn) | Specific for acute myocardial infarction |

| Feature | Reversible cell injury | Irreversible cell injury |
|---|--|--|
| 1) Definition | Exposure to injurious agent for short duration; its removal reverts the cell back to normal. | Persistence of injurious agent; causes irreversible structural and functional damage to the cell. |
| 2) Biochemical and molecular mechanisms | i) Decreased cellular ATP generation. ii) Intracellular lactic acidosis: nuclear clumping. iii) Damage to plasma membrane Na-K ⁺ , Ca pumps: hydroptic swelling iv) Reduced protein synthesis: dispersal of ribosomes. | i) Continued cytosolic influx of calcium: mitochondrial damage. ii) Activated phospholipases: membrane damage. iii) Activated intracellular proteases: cytoskeletal damage. iv) Activated endonucleases: nuclear damage. v) Liberation of lysosomal hydrolytic enzymes in blood. |
| 3) Morphological changes in cell organelles | | |
| A) Cell membrane | Blebs, intramembranous particles, myelin figures. | More prominent blebs, or disrupted membrane, myelin figures. |
| B) Endoplasmic reticulum | Swollen | Swollen and lysed. |
| C) Ribosomes | Dispersed | Dispersed and lysed. |
| D) Lysosomes | Autophagy | Swollen and ruptured. |
| E) Mitochondria | Swollen, amorphous densities | Swollen, large densities. |
| F) Cytoskeleton | Aggregated | Disrupted. |
| G) Nuclear changes | Clumped chromatin | Pyknosis, karyorrhexis, karyolysis. |

• Ultrastructural changes during cell injury due to hypoxia-ischaemia:
and difference b/w reversible & irreversible cell injury:

Ischaemic-reperfusion and free radical mediated cell injury: Ischaemic-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 3 aspects are involved:

- calcium overload.
- Excessive generation of free radicals (superoxide, H_2O_2 , hydroxyl radical, pernitrate).
- Subsequent inflammatory reaction.

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:** Free radicals may produce membrane damage by the following mechanisms:

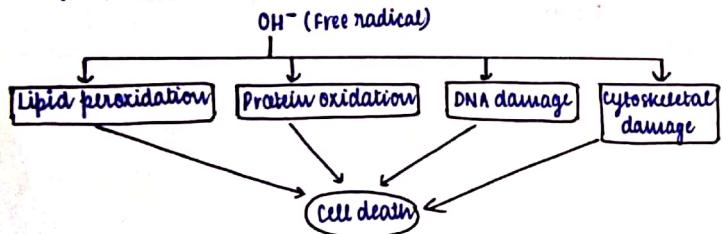


Fig: Mechanism of cell death by hydroxyl radical, the most reactive oxygen species

- c) **Free radicals**
- Oxygen free radical (O_2^- , H_2O_2 , OH^-)
 - Nitric oxide (NO) and peroxynitrite ($ONOO^-$)
 - Halide reagent (chlorine & chloride) e.g., $HClO$.

c) **Inflammatory reaction:** Ischaemic-reperfusion event is followed by inflammatory reaction. Incoming activated neutrophils utilise oxygen quickly (oxygen burst) and further 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.

B.) **Pathogenesis of chemical injury:** chemicals induce cell injury by one of the two mechanisms:

- By direct cytotoxicity
- By conversion of chemical into reactive metabolites.

a) **By direct cytotoxicity effect:** e.g., Mercuric chloride poisoning (greatest damage occurs to cells of the GIT when it is absorbed and the kidney when it is excreted), Cyanide poisoning (affects mitochondrial cytochrome oxidase thus blocks oxidative phosphorylation), Chemotherapeutic agent used in treatment of cancer, toxic heavy metals (Hg, Pb and Cd).

b) **By conversion of reactive toxic metabolites:** e.g., cell injury by conversion of reactive metabolites is toxic liver necrosis caused by CCl₄, acetaminophen (antipyretic & analgesic) and bromobenzene.

c) **Pathogenesis of physical injury:** Physical injury may be due to the changes in atmospheric pressure (e.g., decompression sickness), ionising radiation (Radiation injury) etc.

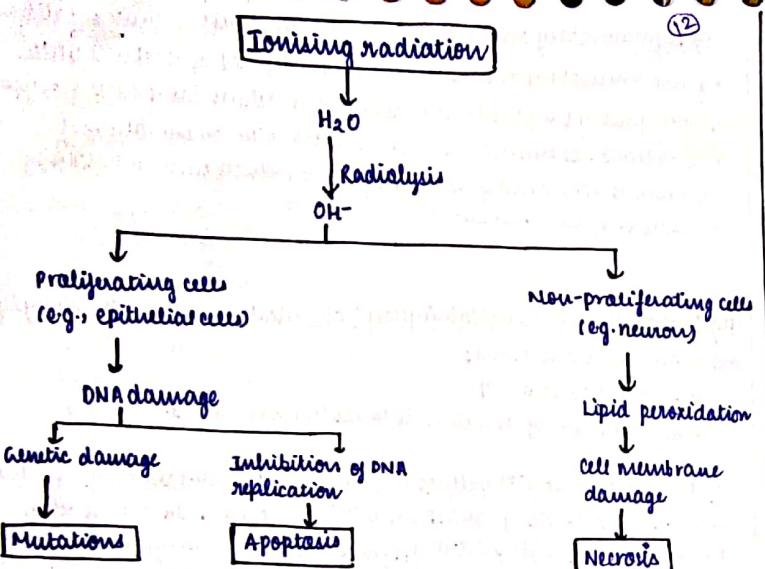


Fig: Mechanism of cell injury by ionising radiation

• Difference b/w apoptosis and necrosis:

| Feature | Apoptosis | Necrosis |
|--------------------|---|--|
| 1) Definition | Programmed and coordinated cell death. | cell death alongwith degradation of tissue by hydrolytic enzymes. |
| 2) Causative agent | Physiologic and pathologic process | Hypoxia, toxins. |
| 3) Morphology | a) No inflammatory rxn. b) Mortality of single cells. c) cell shrinkage d) cytoplasmic blebs on membrane e) Apoptotic bodies. | a) Inflammatory rxn always present. b) Mortality of many adjacent cells. c) cell swelling initially. |

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| 1) Direct effect: | e) chromatin condensation g) phagocytosis of apoptotic bodies by macrophages. h) lysosomes & other organelles intact (attach) | d) Membrane disruption e) Damaged organelles f) Nuclear disruption g) Phagocytosis of cell debris by macrophages. h) lysosomal breakdown |
| 2) Molecular change | a) genetic activation by proto-oncogenes and oncogene-suppressor genes and cytotoxic T-cell mediated target cell killing. b) Initiation of apoptosis by intra and extracellular stimuli followed by activation of caspase pathway. | a) cell death by ATP depletion, membrane damage free radical injury. |
| 3) Fate | a) Once of variable inflammatory rxn. b) phagocytosis of necrotic tissue. | a) No inflammatory rxn. b) brisk phagocytosis of apoptotic bodies. |

Reversible and irreversible cell injury:

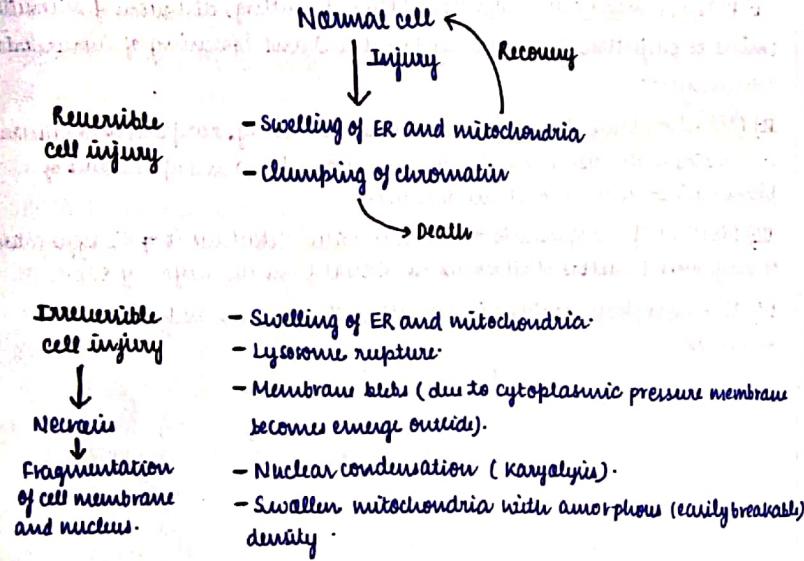
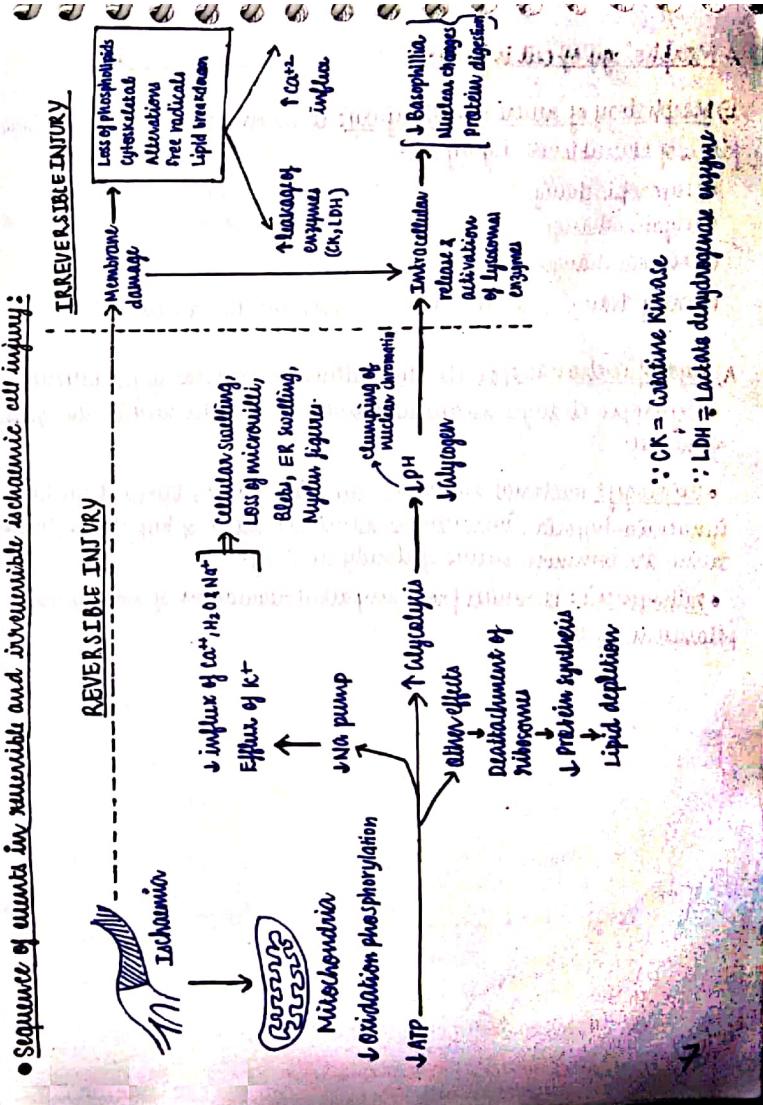


Fig: Morphological changes in cell organelles in reversible and irreversible cell injury.

Blebs: Emerging out of plasma membrane.

Myelin figures are intercellular structures formed when certain surfactants swell in excess water. These are scroll (ring)-like arrangement of lipid bilayer within a cell.



* Cell Swelling (or cloudy swelling | hydropic swelling):

- Cell swelling is the first evidence of almost all forms of injury to the cell.
- Hydropic change is accumulation of water within the cell.
- Vacuolar degeneration occurs due to cytoplasmic vacuolation.
- Cell swelling affects liver, kidney, pancreas or heart muscle. It causes:
 - Paller
 - Turgor
 - ↑ weight of organs

Paller is the unhealthy pale (yellowish) appearance.

Turgor means normal rigidity of the cell due to pressure against plasma membrane from within by the cell contents.

• Example of non-lethal injury is swelling of renal-tubular epithelial cell.

• Aetiology/ cause of cell swelling: Bacterial toxins, chemicals, poisons, burns, high fever, ischaemia-hypoxia, intravenous administration of hypertonic glucose/saline are common cause of cellular swelling.

- swelling results from impaired regulation of cellular volume especially for sodium (Na^+). This regulation is operative at 3 levels:

I) At the plasma membrane itself.

II) At the sodium (Na^+) pump on the plasma membrane.

III) At the supply of ATP.

• pathogenesis: Injurious agent may interfere with the above mentioned regulatory mechanisms and results in accumulation of Na^+ in the cell which consequently leads to inflow of water to maintain iso-osmotic condition & hence cellular swelling occurs. In addition, influx of calcium too occurs.

* Ultrastructural changes of reversible cell injury in hydropic swelling:

It includes the following:

- I) Plasma membrane changes: Blebbing, blunting, distortion of microvilli (minute projections from the surface of cell) and loosening of intracellular attachment.
- II) Mitochondrial changes: Mitochondrial swelling, rarefaction (reduction in density of the tissue especially nervous and bone) and appearance of phospholipid rich amorphous densities.
- III) Dilatation of endoplasmic reticulum with detachment & disaggregation of polyribosomes (clusters of ribosomes on mRNA) from the surface of RER.
- IV) Nuclear changes with disaggregation of granular and fibrillar elements.

* **Intracellular accumulations:** Intracellular accumulation is a cellular response to injury. It occurs due to metabolic change, genetic or acquired stimulus.

• **Mechanism of intracellular accumulation:**

1) Abnormal metabolism in fatty change in liver. The overload of parenchymal liver cells by triglycerides (TGs) is termed as fatty change (steatosis).

2) Mutations (genetic change) cause alterations in protein folding and transport. e.g., α_1 -antitrypsin deficiency. α_1 -antitrypsin protein is synthesized by liver and it protects lungs.

3) Deficiency of critical enzymes prevents breakdown of substances that accumulate in lysosomes as in lysosomal storage disease.

4) Inability to degrade phagocytosed particles as in haemosiderosis (overload of iron) and carbon pigment accumulation. (Haemosiderosis is a form of iron overload disorder, resulting in the accumulation of haemosiderin i.e., iron storage complex).

• **Types of process:**

1) A normal endogenous substance is produced at a normal rate as the increased rate, but the rate of metabolism is inadequate to remove it. e.g., Fatty change in liver due to triglycerides (TGs) accumulation, induced by alcohol abuse, protein malnutrition, diabetes mellitus.

2) A normal or abnormal endogenous substance accumulates because it can't be metabolized. The most common cause is lack of enzyme that blocks a specific metabolic pathway so that some particular metabolite can't be used. (Storage disease = OT-NOT)

3) An abnormal exogenous substance is deposited and accumulated. e.g., Accumulation of carbon particles and such mettals non-metabolizable particles and chemicals like silica.

• The metabolic derangements in cells may be associated with a ~~metabolic~~ accumulation of a number of substances like:

1. Lipids i.e., triglycerides, cholesterol
2. Proteins
3. Glycogen
4. Complex lipid and carbohydrates.
5. Pigments
 - Endogenous pigment
 - Exogenous pigment.

1) **Lipids:** Phagocytic cells may become over-loaded with lipid (triglyceride, cholesterol, and cholesteroyl ester) when there is hypercholesterolemia. Scavenger macrophages, whenever in contact with the lipid debris (content) of necrotic cells/abnormal forms of plasma lipids may becomes stuffed with lipid because of their phagocytic activity.

- Macrophages become filled with minute vacuoles of lipid, so as to impart a foamy appearance to their cytoplasm - thus, macrophages are turned into foam cells. The examples are as follows:

- A) Fibrofatty plaques of atherosclerosis.
- B) Clusters of foam cells in tumour-like masses of a Xanthoma & Xanthelasma.
- C) Cholesterosis is focal presence of cholesterol-laden macrophages in the lamina propria of gall bladder in cholelithiasis.

2) **Proteins:** Protein accumulations may be encountered in the cells because excessive proteins are present into the cell or cell synthesizes excessive amount of protein. e.g., albumen (Normally a trace amount of albumin filtered via glomerulus are absorbed in PCT).

- When pinocytic vesicles in epithelial cells fuses with lysosomes, hyaline cytoplasmic droplets, which appear pink in Hematoxylin & eosin, stains are formed.

- Russell bodies are eosinophilic, large homogeneous immunoglobulin containing inclusions. Usually found in plasma cells undergoing excessive synthesis of immunoglobulin. Russell body is characteristic of distended endoplasmic reticulum. They are found in peripheral areas of tumour. Accumulation of immunoglobulin synthesized in the cisternae of RER may create rounded acidophilic Russell bodies.

3) Glycogen: Excessive intracellular deposits of glycogen are seen in the patients with an abnormality in either glucose or glycogen metabolism. The glycogen masses appear as a vacuole within the cell. e.g.,

- Diabetes mellitus is the prime example of glucose metabolism disorder and a metabolic disorder. Glycogen found in renal tubular epithelium as well as in liver cells and heart muscles.

- Glycogen storage disease, Abnormal or normal forms of glycogen can't be metabolized and consequently results secondary injury and cell mortality.

4) Complex lipids and carbohydrates: In certain forms of storage diseases resulting from inborn (congenital) errors of metabolism, abnormal complexes of carbohydrates and lipids accumulate that can't be metabolized normally. These substances collect within the cell throughout the body mainly in reticulo-endothelial system (RES) causing hepatomegaly (hypertrophy of liver), splenomegaly (hypertrophy of spleen).

5) Pigments: Pigments can either be exogenous (coming from outside of the body) and endogenous (synthesized within the body).

I) Exogenous pigments: It includes carbon or coal dust, silica, iron or iron oxide, lead poisoning and tattooing etc:

A) Anthracosis (i.e., deposition of carbon particles): carbon or coal-dust, exogenous pigment is an air pollutant. When inhaled it is picked up by alveolar macrophages & transported via lymphatic channels to the regional tracheobronchial lymph nodes.

B) Emphysema: Aggregates of coal dust may induce fibroblast axis or emphysema (accumulation of pus in a cavity of body mainly the chest).

C) Pneumoconiosis (lung infection), Silicosis, asbestosis etc. provoke low grade inflammation, fibrosis and impaired respiratory function.

D) Chronic lead poisoning may produce the characteristic blue lines on teeth at the gumline.

E) Tattooing (injected pigments): Pigments like India ink, cinnamon and carbon are introduced into the dermis in the process of tattooing where the pigment is taken up by macrophages and lies permanently in the connective tissue.

II) Endogenous pigments: Eg. Melanin, alkaptonuria, Dubin-Johnson syndrome, Haemosiderin, Bilirubin etc.

A) Melanin: Melanin is the brown-black, non-haemoglobin-derived pigment normally present in the hair, skin, mucus at some places (e.g., oral cavity, oesophagus, anal canal), choroid of the eye, meninges and adrenal medulla. In skin, it is synthesized in the melanocytes and dendritic cells, both of which are present in the basal layer of the epidermis & is stored in the form of cytoplasmic granules in the phagocytic cells of the melanophores, but in the underlying upper dermis.

- Various disorders of melanin pigmentation cause generalized and localized hyperpigmentation and hypopigmentation:

a) In Addison's disease, there is generalized hyperpigmentation of the skin, especially in areas exposed to light, and of buccal mucosa.

- b) Albinism is an extreme degree of generalised hypopigmentation in which tyrosinase enzyme is genetically deficient and no melanin is formed in the melanocytes.
- c) Leucoderma is an autoimmune condition with localised loss of pigmentation of the skin.
- d) Vitiligo is also local hypopigmentation of the skin and is more common. It may have familial tendency.
- B) Alkaptonuria: This is a rare autosomal recessive disorder in which there is deficiency of an oxidase enzyme required for breakdown of homogentisic acid; then accumulate in the tissues and excreted in the urine (homogentisicaciduria). The urine turns black in color due to oxidation of homogentisic acid.
- C) Dubin-Johnson syndrome: An autosomal recessive form of hereditary conjugated hyperbilirubinaemia, contain melanin-like pigment in the cytoplasm.
- D) Haemosiderin: It is a haemoglobin derivative golden yellow to brown granular or crystalline pigment in which stored iron is stored in the cells.
 - Haemosiderin pigment represents aggregates of ferritin during breakdown of red blood cells (RBCs). Haemosiderin is deposited in reticulo-endothelial cells, mononuclear phagocytes of the liver, bone marrow, spleen and lymph nodes. This condition is known as Haemosiderosis (overload of iron).
- E) Bilirubin: Bilirubin is the normal non-iron containing pigment present in the bile. It is derived from porphyrin ring of the haem moiety of haemoglobin. Normal level of bilirubin in blood is <1mg/dl. Excess of bilirubin or hyperbilirubinemia leads to the Jaundice.

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* Calcification:

Pathologic calcification is a common process that implies the abnormal deposition of calcium salt, together with smaller amounts of Fe, Mg and other mineral salts.

• Classification of calcification: Calcification can be divided into 2 types:

- A) Dystrophic calcification
- B) Metastatic calcification.

A) Dystrophic calcification: Dystrophic calcification is characterized by deposition of calcium salts in necrotic or degenerated tissues with normal calcium metabolism and normal serum calcium level.

- Aetiology: As dystrophic calcification may occur in either dead or degenerated tissues:

a) Calcification in dead tissue:

i) Caseous necrosis in tuberculosis is the most common site for dystrophic calcification.

ii) Liquefaction necrosis in chronic abscesses.

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

iv) Damaged heart valves may undergo dystrophic calcification.

v) Microcalcification in breast cancer detected by mammography.

b) Calcification in degenerated tissue:

i) Atherosclerosis which are focal intimal injury in the aorta and large arteries that are characterized by accumulation of lipids.

The calcium salts appear microscopically as fine white granules or often felt gritty deposits (gritty represents small hard particles).

ii) Monckeberg's sclerosis show calcification in the degenerated tunica media of muscular arteries in elderly people

iii) Nodular goitre of the thyroid.

-Pathogenesis: 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 precipitate of calcium phosphate. It involves phases of initiation and propagation leading ultimately to the formation of crystalline calcium phosphate 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. There is excess uptake of calcium by injured mitochondria in degeneration and necrosis. Thus, calcium and phosphate forms precipitates of calcium phosphate.

b) Propagation: Simultaneously, some structural changes occur in calcium and phosphate groups which results in further propagating deposits and form mineral crystals.

B) Metastatic calcification: It occurs in apparently normal tissues and is associated with deranged calcium metabolism and hypercalcemia.

- Common sites of metastatic calcification: Kidneys (Basement membrane of tubular epithelium), Lungs (alveolar walls), Stomach (acid-secreting fundal glands), Blood vessels (internal elastic lamina), cornea, Synovium of joints.

- Aetiology: It occurs due to:

- a) Excessive mobilisation of calcium from the bone
- b) Excessive absorption of calcium from the gut.

a) Excessive mobilisation of calcium from the bone: These include:

- i) Hyperparathyroidism
- ii) Bony destructive lesions e.g., multiple myeloma, metastatic carcinoma.
- iii) Hypercalcaemia

b) Excessive absorption of calcium from the gut: These causes are:

- i) Hypervitaminosis-D e.g., excessive intake, sarcoidosis.
- ii) Addison's disease means adrenocortical insufficiency.
- iii) Renal causes such as in renal tubular acidosis.

- Pathogenesis: 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 \downarrow one of acid secretion or rapid change in pH level at these sites. Metastatic calcification is reversible upon correction of underlying metabolic disorder.

• Consequences: Hypercalcemia results nephropathy. The resulting phosphate retention results secondary hyperparathyroidism (\uparrow level of parathyroid hormone in blood).

- Metastatic calcification affects the interstitial tissue of blood vessels, kidney, lungs and gastric mucosa.

- Nephrocalcinosis causes renal damage.

• Difference b/w dystrophic and metastatic calcification:

| Feature | Amyotrophic calcification | Metastatic calcification |
|------------------------|--|--|
| 1) Definition | Deposits of calcium salts in dead and degenerated tissue. | 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) Common causes | Necrosis (caseous, liquefactive, fat, old infarcts), thrombi, haematomas, dead parasites, old scars, atheromas, Monckeberg's sclerosis. | Hyperparathyroidism (due to adenoma, hyperplasia), bony destructive lesions (e.g. myeloma, metastatic carcinoma), prolonged immobilization, hypervitaminosis-D, hypercalcaemia of infancy. |
| 6) Pathogenesis | Increased binding of phosphate 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. |
| 7) Common sites | a) Tuberculous lymphadenitis b) Advanced atherosclerosis c) Medial calcification of uterine arteries in multigravida d) Chama-Handy bodies in cecum, spleen | Along the epithelial lining of intact normal tissues - e.g. a) Basement membrane of tubules in kidney. b) Alveolar lining in lungs. c) FUNDIC mucosa of stomach. d) Internal elastic lamina of blood vessels. e) Band keratopathy in cornea |

* **Acidosis:** It is the reduction in pH due to the presence of excess H^+ ion. It is a disorder that lowers cell or tissue pH to < 7.35 .

Note: Acidemia refers to an arterial pH less than 7.3.

• Examples of acidosis:

1) **Respiratory acidosis:** It is a medical emergency in which decreased ventilation causes ↑ red blood CO_2 concentration and ultimately leads to ↓ in the pH level during alveolar hypoventilation. There is an ↑ in CO_2 and consequently results to the increased partial pressure of CO_2 .

- **Type of respiratory acidosis:** → 2 types i.e., acute and chronic.

A) **Acute respiratory acidosis:** Partial pressure of CO_2 (P_{CO_2}) is elevated above the limit of reference range (i.e., $> 45 \text{ mmHg}$) with an accompanied acidemia ($pH < 7.3$). It occurs when an abrupt failure of ventilation. Failure in ventilation may be caused by depression of central respiratory centre, inability to ventilate adequately due to neuromuscular disease or airway obstruction related to asthma or chronic obstructive pulmonary disease (COPD e.g. Asthma, bronchitis).

In acute respiratory acidosis, compensation occurs into 2 steps:

I) The initial step is cellular buffering that occurs over minutes to hours. Cellular buffering elevates plasma HCO_3^- ions only slightly approximately 1 mEq/L for each 10 mmHg increase in partial pressure of CO_2 .

II) The second step is renal compensation that occurs over 3 to 5 days. With renal compensation renal excretion of carbonic acid is ↑ and bicarbonate reabsorption is ↑.

B) **Chronic respiratory acidosis:** It may also be secondary to neuromuscular disorder and severe restrictive ventilatory defect as observed in interstitial fibrosis and thoracic deformities.

2) Metabolic acidosis: It is a condition that occurs when the body produces the excessive quantities of acids or when the kidneys are not removing enough acid from the body. If untreated it leads to the acidemia.

- The consequences are so serious leading to coma and death. Metabolic acidosis is characterized by primary reduction in serum bicarbonate concentration. The secondary decrease in the arterial pressure of CO_2 of mmHg for every 1 mmol/litre fall in serum bicarbonate ion concentration and a reduction in blood pH.

- Acute form last for minutes to days while chronic form last for week to year of the disorder can occurs.

- Acute forms of metabolic acidosis most frequently results from the over production of organic acids such as ketoacids or lactic acids, by contrast chronic metabolic acidosis often reflects bicarbonate wasting (impaired renal acidification).

• Sign and symptoms of acidosis: symptoms are not specific. Symptoms may be palpitation (irregular heart beat), chest pain, headache, altered mental status (anxiety due to hypoxia), sed visual acuity, nausea, vomiting, abdominal pain, altered appetite and weight gain, muscle weakness, bone pain and joint pain; extreme acidemia leads to neurological and cardiac complications.

* Alkalosis: Alkalosis is excessive blood alkalinity caused by an over abundance of bicarbonate ions in blood or loss of acid from the blood (metabolic alkalosis) or by a low level of CO_2 in the blood that results rapid / deep breathing (respiratory alkalosis). [Alkalosis means increased alkaline content in blood ($\text{pH} > 7.45$)]

• Examples of alkalosis:

1) Respiratory alkalosis: It is a medical condition in which fast respiration elevates blood pH beyond the normal range (7.35 to 7.45) with a concurrent reduction in arterial level of CO_2 . This occurs in the following conditions:

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

- clinically, the patients with respiratory alkalosis are characterized by peripheral vasoconstriction and consequent pallor, lightheadedness & tetany. The arterial PCO_2 is lowered.

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

- Severe and prolonged vomiting.
- Administration of alkaline salts like sodium bicarbonate.
- Hypoalkalemia such as in cushing's syndrome, increased secretion of aldosterone.

- clinically, metabolic alkalosis is characterized by depression of the

respiration, depressed renal function with uraemia and increased bicarbonate excretion in the urine. The blood level of bicarbonate is elevated.

* **Electrolyte imbalance:** The concentration of electrolytes within the cell and in the plasma is different. Intracellular compartment has higher concentration of potassium, magnesium and phosphate ions than the blood, while the extracellular fluid (including serum) has higher concentration of sodium, chloride, calcium and bicarbonate ions.

- For electrolyte homeostasis, the concentration of electrolytes in both these compartments should be within normal limits. Normal serum levels of electrolytes are maintained in the body by a careful balance of 4 processes: their intake, absorption, distribution and excretion. Disturbance in any of these processes in disease pathophysiological states may cause electrolyte imbalance.

- Blood electrolyte imbalance chart is shown on next page:

Table; Blood electrolyte imbalances:

(22)

| | DEFICIENCY | | EXCESS | |
|--|--|---|--|---|
| Electrolyte | Name and causes | Sign & Symptoms | Name and cause | Signs and symptoms |
| 1) Sodium (Na^+) 136-148 mEq/L | <u>Hyponatremia</u> may be due to decreased sodium intake; fed sodium loss through vomiting, diarrhoea, aldosterone deficiency, or taking certain diuretics, & excessive water intake. | Muscular weakness, dizziness, headache, and hypertension; tachycardia & shock, mental confusion, stupor and coma. | <u>Hypernatremia</u> may occur with dehydration, water deprivation, or excessive sodium in diet or intravenous fluids; causes hyperosmolarity of ECF, which pulls water out of body cells into ECF, causing cellular dehydration. | Intense thirst, hypertension, edema, agitation and convulsions. |
| 2) Chloride (Cl^-) 95-105 mEq/L | <u>Hypochloraemia</u> may be due to excessive vomiting, overhydration, aldosterone deficiency, congestive heart failure, and therapy with certain diuretics such as Lasix. | Muscle spasms, metabolic alkalosis, shallow respirations, hypertension, and tetany. | <u>Hyperchloraemia</u> may result from dehydration due to water loss or water deprivation, excessive chloride intake; or severe renal failure, hyperaldosteronism, certain types of acidosis and some drugs. | Lethargy, weakness, metabolic acidosis, and rapid, deep breathing. |
| 3) Potassium (K^+) 3.5 to 5.0 mEq/L | <u>Hypokalemia</u> may result from excessive loss due to vomiting or diarrhoea, fed K^+ intake, hyperaldosteronism, kidney disease, and therapy with some diuretics. | Muscle fatigue, flaccid paralysis, mental confusion, fed urine output, shallow respirations, and changes in ECG including flattening of T-waves. | <u>Hyperkalemia</u> may be due to excessive potassium intake, renal failure, aldosterone deficiency, crushing injuries to body tissues, or transfusion of hemolyzed blood. | Irritability, nausea, vomiting, diarrhoea, muscular weakness, can cause death by inducing ventricular fibrillation. |
| 4) Calcium (Ca^{++}) Total = 9-10.5 mg/dL Ionized = 4.8-5.5 mg/dL | <u>Hypocalcemia</u> may be due to ↑ Ca^{++} loss, reduced calcium intake, elevated levels of phosphate, or hypoparathyroidism. | Numbness and tingling of fingers, hyperactive reflexes, muscle cramps, tetany, and convulsions, bone fractures, spasms of laryngeal muscles and that can cause death by asphyxiation. | <u>Hypocalcemia</u> may result from hyperparathyroidism, some cancers, excessive intake of vitamin D, and Paget's disease of bone. | Lethargy, weakness, anorexia, nausea, vomiting, bone pain, depression, confusion, paresthesia, stupor and coma. |
| 5) Phosphate (HPO_4^{2-}) 1.7-2.6 mEq/L | <u>Hypophosphatemia</u> may occur through increased urinary losses, fed intestinal absorption or increased utilization. | Confusion, seizures, coma, chest and muscle pain, numbness and tingling of the fingers, decreased coordination, memory loss and lethargy. | <u>Hyperphosphatemia</u> occurs when the kidneys fail to excrete excess phosphate, as happens in renal failure; can also result from increased intake of phosphates or destruction of body cells, which releases phosphate into the blood. | Anorexia, nausea, vomiting, muscular weakness, hyperactive reflexes, tetany and tachycardia. |
| 6) Magnesium (Mg^{++}) 1.3 to 2.8 mEq/L | <u>Hypomagnesemia</u> may be due to inadequate intake or excessive loss in urine or feces; also occurs in alcoholism, malnutrition, diabetes mellitus, and diabetic therapy. | weakness, irritability, tetany, delirium, convulsions, confusion, anorexia, nausea, vomiting, and cardiac arrhythmias. | <u>Hypermagnesemia</u> occurs in renal failure or due to fed intake of Mg^{++} , such as Mg^{++} containing antacids, also occurs in aldosterone deficiency and hypothyroidism. | Hypotension, muscular weakness or paralysis, nausea, vomiting, and altered mental functioning. |

#Basic mechanism involved in the process of inflammation and repair:

* Inflammation: (The word inflammation means burning).

Inflammation is defined as local responses of living mammalian tissues to injury from an agent. It is a body defense reaction/mechanism in order to eliminate or limit the spread of injurious agent, followed by removal of the necrotic necrode cells and tissues.

• Causative agents: The agents causing inflammation may be as under:

- Injective agents like bacteria, viruses and their toxins, parasite & fungi.
- Immunological agents like cell-mediated and antigen-antibody reactions.
- Physical agents like heat, cold, radiation and mechanical trauma leads to fracture, bleeding and infection.
- Chemical agents like organic and inorganic poisons.
- Inert materials such as foreign bodies, dirt, sutures etc.

* Clinical signs of inflammation: There are 5 cardinal signs of the inflammation:

- Ruber (Redness)
- Tumor (swelling)
- Calor (Heat)
- Dolor (Pain)
- Functio laesa (Loss of function).

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

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

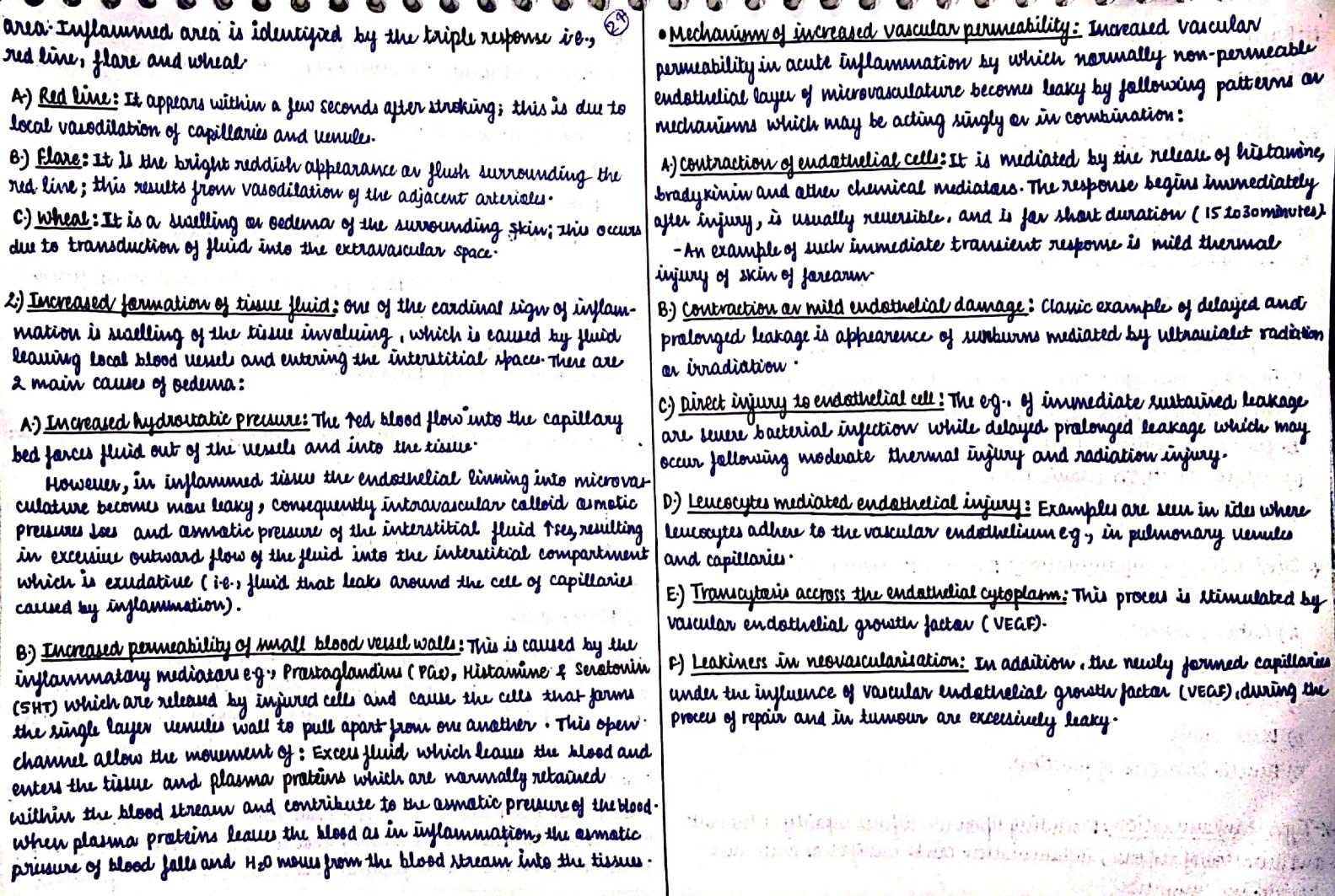
- Accumulation of the fluid and plasma at the affected site.
 - Intravascular activation of platelets.
 - Polymorphonuclear neutrophils as inflammatory cells.
- 2) Chronic inflammation: It is of longer duration and occurs after a delay, either when the causative agent of acute inflammation persists for a long time, or the stimulus is such that it induces chronic inflammation from the beginning. e.g., Tuberculosis.

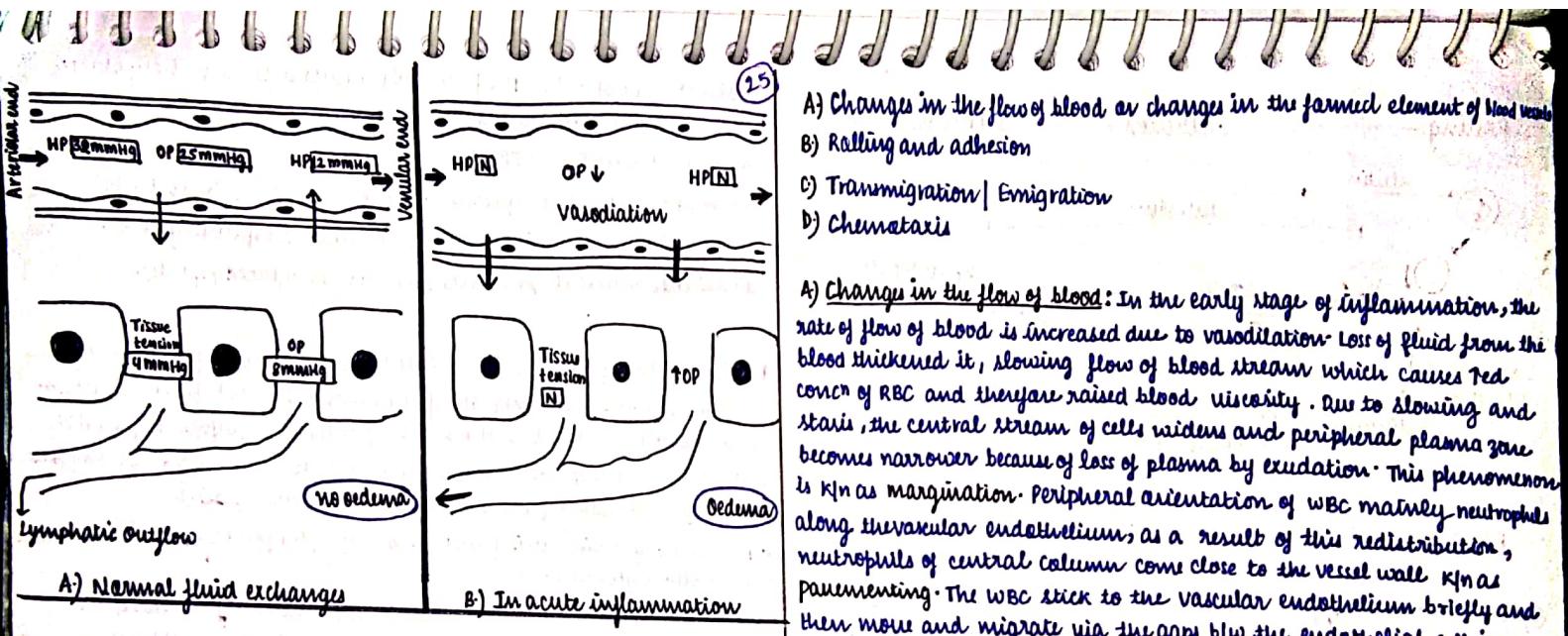
* Mechanism involved in acute inflammation: There are following stages:

- Increased blood flow
- Increased formation of tissue fluid
- Increased vascular permeability
- Migration of leucocytes (WBCs) / Exudation of leucocytes
- Phagocytosis

1) Increased blood flow: Following injury, both arterioles supplying the damaged area and the local capillaries dilates i.e., increased blood flow to the site. This is caused by mainly release of a no. of chemical mediators released from damaged cells e.g., histamine, serotonin (5-HT or 5-hydroxy tryptamine).

Increased blood flow to the area of tissue damage provides new oxygen and nutrients for the red cellular activity that accompanies inflammation. Red blood flow cause the red temperature and reddening of an inflamed





Fig; Fluid interchange b/w blood and extracellular fluid (ECF).

• HP = Hydrostatic pressure

OP = Osmotic pressure

N = Normal

3) **Migration of leucocytes (WBC):** 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 by phagocytes (monocytes and macrophages). The changes leading to migration of leucocytes are as follows:

- 25
 A) Changes in the flow of blood or changes in the formed element of blood
 B) Rolling and adhesion
 C) Transmigration / Emigration
 D) Chemotaxis

A) Change in the flow of blood: In the early stage of inflammation, the rate of flow of blood is increased due to vasodilation. Loss of fluid from the blood thickens it, slowing flow of blood stream which causes red concn of RBC and therefore raised blood viscosity. 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. Peripheral orientation of WBC mainly neutrophils along the vascular endothelium; as a result of this redistribution, neutrophils of central column come close to the vessel wall known as pavementing. The WBC stick to the vascular endothelium briefly and then move and migrate via the gaps b/w the endothelial cells into the vascular space, the process is known as emigration.

B) Rolling and adhesion: Peripherally marginated and pavemented neutrophils slowly roll over the endothelial cells lining the vessel wall (rolling phase). This is followed by transient bond / sticking b/w the leucocytes and endothelial cells becoming firm (adhesion phase). The process of rolling is facilitated by following cell adhesion molecules (CAMs) or adhesion receptors (ARs):

- selectins: i.e., P-selectin, E-selectin and L-selectin.
- Integrins
- Immunoglobulins

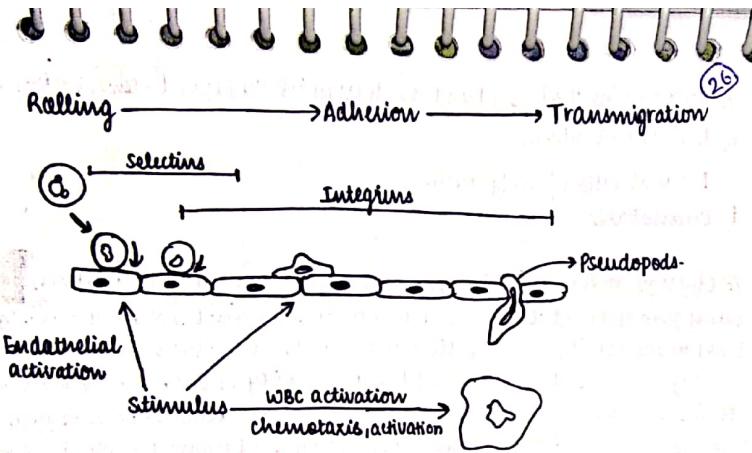


Fig: Sequence of events in WBC migration in inflammation

C) Transmigration / Emigration: After sticking of neutrophils to endothelium, the neutrophils move along the endothelial surface till a suitable site b/w the endothelial cell is found where the neutrophils throw out cytoplasmic pseudopods. Neutrophils are dominant cells in acute inflammatory exudate in first 24 hours and monocyte, macrophages appears in next 24 to 48 hrs. However, neutrophils are short lived (24 to 48 hrs) while monocytes, macrophages survive much longer.

D) Chemotaxis: The transmigration of leucocyte after crossing several barriers (endothelium, Basement membrane, perivascular myofibroblast, matrix) to reach the interstitial tissue is a Chemotactic factor mediated process of chemotaxis (chemical attraction of leucocytes to an area of inflammation is known as chemotaxis). Chemo-attractants acts to retain passing leucocytes in inflamed area rather than attracting them from

distant area of body. The following agents acts as a chemotactic substance for neutrophils:

- a) Leukotriene B₄ (LTB₄)
- b) components of complement system (e.g., C5a and C3a).
- c) cytokines / chemokines (e.g. Interleukins especially IL-8).
- d) soluble bacterial products (such as farnylated peptide).

4) Phagocytosis: After the site of infection has been infiltrated by leucocytes, process of clearing off of the microbial agent is set in action. Phagocytosis is defined as the process of cellular engulfment of a solid particulate material (e.g., microbes, foreign particulate material). Phagocytosis is also called cell-eating (while cell-drinking is pinocytosis).

The cells performing this function are called phagocytes. There are 2 main types of phagocytic cells:

- A) Polymorphonuclear neutrophils (PMNs) which appear early in acute inflammatory response, sometimes called as macrophages.
 - B) Circulating monocytes and fixed tissue mononuclear phagocytes, commonly called macrophages.
- Phagocytes at site of infection produce several proteolytic enzymes i.e., lysozyme, protease, proteinase, lipase and hydrolases etc. These enzymes degrade collagen and extracellular matrix. Phagocytosis of the microbe by polymorphs and macrophages involved the following 3 steps:

- I) Recognition and attachment.
- II) Envelopment
- III) Killing and degradation

* Mediators of inflammation: A chemical mediator is any messenger that acts on blood vessels, inflammatory cells or other cells to contribute to an inflammatory response.

• Two main groups of substance acting as chemical mediators of inflammation — released from the cells and those from the plasma proteins as shown below:

I) Cell-derived mediators:

| Substance | Made by / source | Trigger of release | Pro-inflammatory action |
|--|---|---|--|
| 1) Vasoactive amines | | | |
| A) Histamine | mast cells, basophils, platelets. stored in cytoplasmic granules. | binding of antibody to mast cell and basophils. | vasodilation, itching, vascular permeability increased, degranulation, smooth muscle contraction, bronchoconstriction. |
| B) Serotonin (5-hydroxytryptamine or 5-HT). | platelets, mast cells, basophils, it also acts as a neurotransmitter. | when platelets are activated and when mast cell & basophil degranulate. | vasoconstriction, ↑ vascular permeability. |
| C) Neuropeptide (e.g., tachykinin, substance P, neuropeptide A & somatostatin) | central and peripheral nervous system. | | ↑ vascular permeability, transmission of pain stimuli & mast cell degranulation |
| 2) Arachidonic acid metabolites (Eicosanoids) | | | |

| | | | |
|--|---|---|--|
| A) Prostaglandins (PGs) | Nearly all cells, not stored, but made from cell membrane as required. | many different stimuli e.g. drugs, toxins, other inflammatory mediators, hormones, trauma | Diverse; sometimes opposing, e.g. fever, pain, vasodilation & vasoconstriction, ↑ vascular permeability. |
| B) Leukotrienes (LTs) | leucocytes | chimotactic for phagocytic cells. | contraction of smooth muscles, ↑ vasoconstriction, bronchoconstriction, ↑ vascular permeability, stimulate phagocytic cell adherence. |
| 3) Heparin | Liver, mast cells, basophils (stored in cytoplasmic granules) | Released when cells degranulated. | Anti-coagulant (prevents blood clotting), when maintains blood supply (nutrients, O ₂) to injured tissue & washes away necrotic and waste. |
| 4) Cytokines and chemokines | | | Acts as chemoattractants. |
| A) Interleukins (IL-1, IL-6, IL-8, IL-12, IL-13) | Monocytes, B & T cells, Macrophages, dendritic cells, neutrophils, fibroblasts. | virus-infected cell releases interferon. | Role in fever & shock, differentiation & growth of T and B cells, migration of neutrophils and monocytes. |
| B) Tumour necrosis factor (TNF-α & TNF-β) | Monocytes/macrophages, mast cells, eosinophils, B cells, T cells, NK cells. | Released in response to lipopolysaccharide, bacterial products & IL-1. | Fever, shock, anaesthesia, expression of endothelial adhesion molecules, enhanced leucocyte cytotoxicity. |
| C) Interferon (IFN) | T cells, NK cells. | Response to the presence of viruses. | Activation of macrophages and NK cells, stimulates to secrete IgG by B cells, differentiation of T-cells. |

| | | | |
|--------------------------------|--|--|---|
| 5) Platelet activating Factor. | IgE-sensitized basophils or mast cells, other leukocytes, endothelium and platelets. | IgE-sensitized basophils. | ↑ vascular permeability, vasodilation or vasoconstriction, bronchoconstriction, adhesion of leucocytes to endothelium, chemotaxis |
| 6) Free radicals | A) Oxygen intermediates (O_2^-, H_2O_2 or OH^-) | Activated neutrophils and macrophages. | Endothelial cell damage ↑ vascular permeability, activation of protease, tissue damage |
| B) Nitric oxide (NO) | Endothelial cell. | vasoconstriction | Vasodilation, Anti-platelet activating agent, possibly microbicidal action. |

• Classification of chemical mediators on the basis of their effects:

| Effect | Mediators |
|----------------------------|--|
| 1) Vasodilation | Histamine, serotonin, NO, PGs. |
| 2) ↑ vascular permeability | Histamine, Complement (C _{3a} & C _{5a}), Bradykinin, oxygen metabolites, leukotrienes (LTC ₄ , LTD ₄ , LTE ₄) and platelet activating factor (PAF). |
| 3) Chemotaxis | Complement C _{5a} , Leukotrienes (LTC ₄ , LTD ₄). |
| 4) Chemokines | TNF- α , IL-1, IL-8 (Interleukin), Bacterial products - e.g., lipopolysaccharides. |
| 5) Fever | IL-1, TNF- α & IL-6, Prostaglandins (PGs). |
| 6) Pain | Bradykinin & substance P, PAF 2- α . |
| 7) Tissue damage | Oxygen metabolites, NO, lysosomal enzymes. |

II) Plasma protein-derived mediators (plasma proteases)

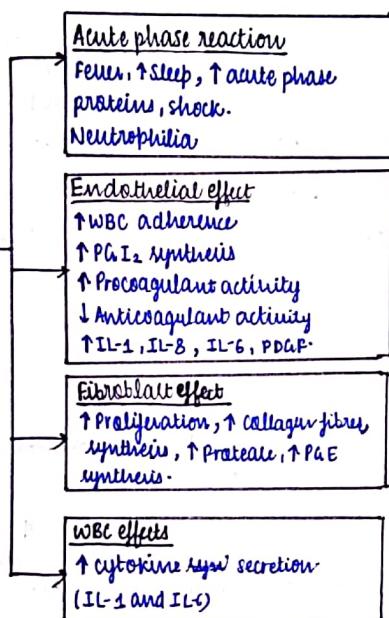
| Substance | Made by / source | Trigger of release | Pro-inflammatory action. |
|---|---------------------------------|---|--|
| 1) Bradykinin | Tissue & blood | when blood clots, in trauma & inflammation. | Pain, vasodilation. |
| 2) Fibrinopeptides | Blood plasma, platelets. | blood clots. | ↑ vascular permeability, chemotaxis for leucocytes, anticoagulant activity. |
| 3) Fibrin split products. | clotting & fibrinolytic system. | activated by plaminogen activator. | ↑ vascular permeability and are chemotactic to leucocytes. |
| 4) Anaphylatoxins (C _{3a} , C _{4a} , C _{5a} , C _{6b} and C ₉) | complement system | due to bacterial infection. | ↑ vascular permeability, activate mast cells & basophils to release histamine. |

Effect of IL-1 and TNF- α in inflammation:

Bacterial products,
immune complexes, toxins,
physical injury, cytokines

↓
Macrophage activation

IL-1, TNF- α



* Basic principles of wound healing in the skin: wound healing can be accomplished in one of the following 2 ways:

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

1) Healing by first intention (primary union): This is defined as healing of a wound which has the following characteristics:

- Clean and uninfected,
- Surgically incised,
- without much loss of cells and tissues, and
- edges of wound are approximated by surgical sutures.

• The incision cause mortality of a limited no. of epithelial cells and connective tissue cells as well as disruption of epithelial basement membrane continuity. The narrow incisional space immediately fill with clotted blood containing fibrin and blood cells, dehydration of surface clots forms the wet K/n scab that covers the wound.

• Within 24 hours, neutrophils appears at the margin of the incision, moving towards the fibrin clot. The epidermis at its cut edges thickens as a result of mitotic activity of basal cells and within 24 to 48 hrs, spurs of epithelial cells from the edge both migrate and grow along the cut margins of dermis, depositing basement membrane components as they move. They fuse in the midline under the surface scab, thus producing a continuous but thin epithelial layer.

• By the day 3, the neutrophils have been largely displaced / replaced by macrophages. Granulation tissue progressively invades the incision space. Collagen fibres are now present in the margin of the incision but at the first they are vertically oriented and don't bridge the

- incision. Epithelial cell proliferation continues thickening the epidermal covering layer.
- By day 5, the incisional space is filled with granulation tissue. New vessels originate by budding or sprouting of pre-existing vessels, a process called Angiogenesis (Formation of new blood vessels). Vascularisation is maximal collagen fibrils becomes more abundant and begin to bridge the incision. The epidermis recovers its normal thickness and differentiation of surface cells is a mature epidermal architecture with surface keratinization.
 - During the 2nd week, there is continuous accumulation of collagen and proliferation of fibroblast. The leucocytes infiltrate oedema and red vascular supply have largely disappeared. At this time, long process of balance begins, accomplished by the red accumulation of collagen within the incisional scar, accompanied by regression of vascular channel.
 - By the end of 1 month, the scar comprises a cellular connective tissue devoid of inflammatory infiltrate covered by intact epidermis. The dermal appendages that have been destroyed in the line of incision are permanently lost. Tensile strength of the wound rises, thereafter it may take months to attain its maximal strength.
 - When there is more extensive loss of cells and tissues as occurs in infarction, inflammatory ulceration, abscess formation (T&U) and surface wound that creates large defects. The reparative process is more complicated.
There is a large tissue defect that must be filled in these situations. Granulation tissue grows in the form of margin to complete the repair.
- 2) Healing by second intention (Secondary Union): Secondary healing differs from the primary healing in several respects:
- Large tissue defects initially have more fibrin and more necrotic debris and exudate that must be removed; consequently the inflammatory reaction becomes more intense.
 - much larger amount of granulation tissue are formed.
- c) wound contraction: Wound contraction occurs in large surface wound. The wound starts contracting after 2 to 3 days and the process is completed by the second week. During this period, the wound is reduced by approximately 80% of its original size. Contracted wound results in rapid healing since, lesser surface area of injured tissue has to be replaced.
- The following factors have been proposed to explain the mechanism of wound contraction:
- a) Dehydration
 - b) Contraction of collagen
 - c) Myofibroblast cells.
- a) Dehydration: Dehydration occurs as a result of removal of fluid by drying of wound.
- b) Contraction of collagen: It is responsible for contraction but wound contraction proceed at a stage when the collagen content of granulation tissue is very small.
- c) Myofibroblast cells: These cells migrate into the wound area and their active contraction uses the size of defects. Myofibroblast cells have intermediate features b/w fibroblasts and smooth muscle cells.

* Pathophysiology of atherosclerosis:

(3)

- Atherosclerosis:** Atherosclerosis is a 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.

- The term atherosclerosis is derived from 'atheros' means soft lipid-rich material in the centre of 'atheroma' (fibrofatty plaques) and 'sclerosis' (scarring) referring to connective tissue in the plaques.

- Hence, atherosclerosis occurs due to deposition of fibrofatty plaques (lipids) onto the portion of blood vessels such as aorta, coronary & cerebral arteries leads to Athero-thrombotic disease such as:

a.) Abdominal aortic aneurism (abnormal dilation) induced massive fatal bleeding.

b.) Myocardial infarction (severe ischaemic heart disease) in which coronary artery lesion is complicated by thrombosis.

c.) Stroke (cerebral ischaemia).

- Pathogenesis of atherosclerosis:** Progressive change in artery affect the intima, media and the elastic lamina:

A) Intimal thickening

B) Medial fibrosis

C) Elastic degeneration.

A) **Intimal thickening:** Intimal thickening in coronary artery, abdominal aorta, and large artery of lower limb due to accumulation of droplets in the intima is noted.

B) **Medial fibrosis:** There is ↑ amount of collagen and ground substance at

the expense of smooth muscle fibres of the media from an early age onwards. e.g., Muscular arteries of viscera and arterioles.

C) **Elastic degeneration:** Degeneration of elastic tissue is present in elastic lamina and media. Inelastic content and calcium salt in the arterial wall are↑ with progression of age.

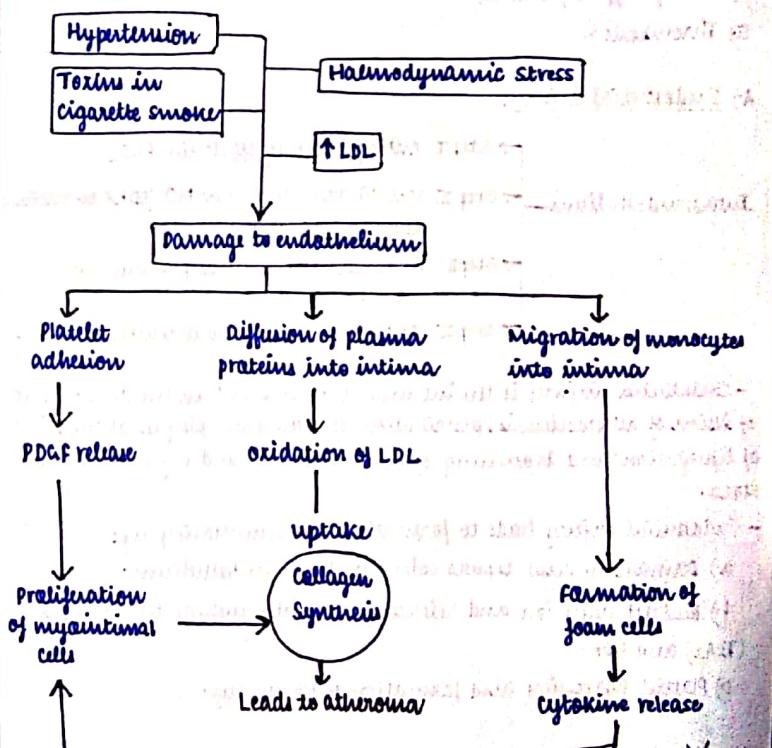


Fig: Flow chart of pathogenesis of atherosclerosis

Pathophysiology of atherosclerosis:

- A) Endothelial injury
- B) Intimal smooth muscle cell proliferation
- C) Role of macrophages
- D) Role of hyperlipidemia
- E) Thrombosis.

A) Endothelial injury:

- Intact endothelium →
- Step I Antiaggregatory effect via PGI₂
 - Step II vasodilatory effect via NO (EDRF or endothelial derived relaxin factor)
 - Step III Fibrinolytic via tissue plasminogen activator.
 - Step IV Antithrombotic via thrombomodulin.

- Endothelial injury is the initial trigger event in the development of lesion of atherosclerosis; distribution of atheroma plaques at the point of bifurcation and branching of blood vessels is under great shearing stress.

- Endothelial injury leads to formation of Haemostatic plug:

- a) damage to vessel exposes collagen of sub-endothelium.
- b) Platelet adhesion and release of granules such as thromboxane A₂ (Tx A₂) and ADP.
- c) Platelet aggregation and formation of fibrin plug.

B) Intimal smooth muscle cell proliferation:

Endothelial smooth muscle injury causes adherence, aggregation and platelet release rxn at the

site of exposed sub-endothelial connective tissue. Proliferation of smooth muscle cell is stimulated by following factors:

a) Platelet derived growth factor (PDGF)

b) Fibroblast growth factor (FGF).

c) Transforming growth factor (TGF) (TGF- α).

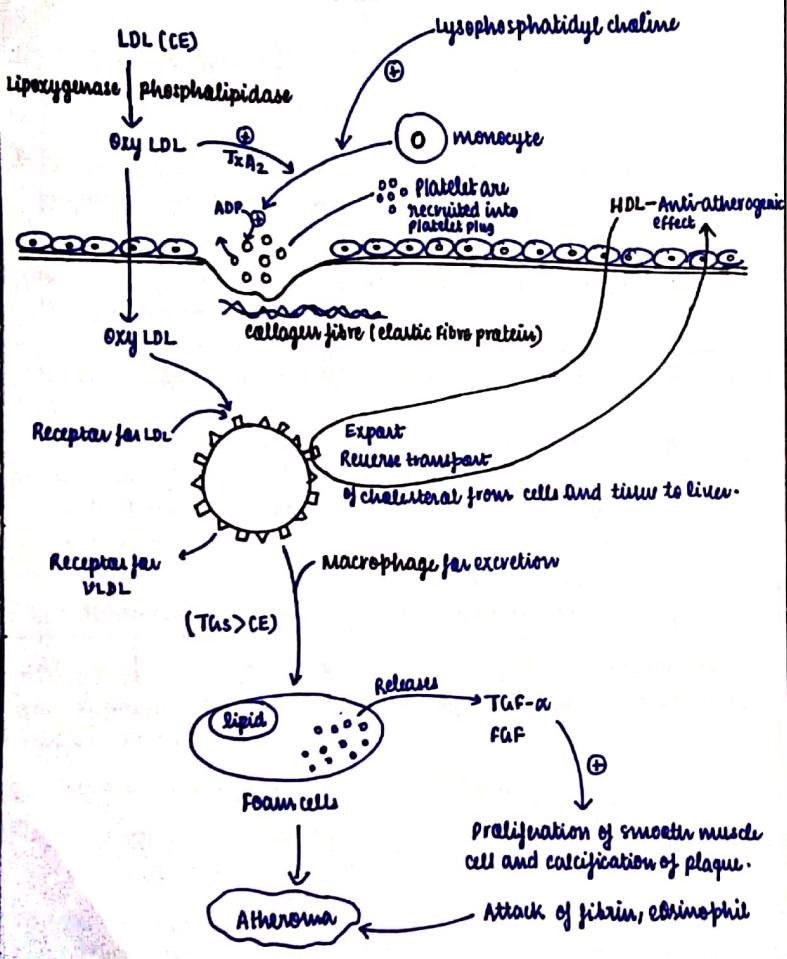
- Loss of growth factor: Intimal proliferation of smooth cells is accompanied by synthesis of matrix protein: collagen, elastic fibre proteins and proteoglycans.

c) Role of macrophage: Blood monocytes adhere to the injured endothelium, enters into the intima and release the macrophage derived growth factor which leads to proliferation of smooth muscle cells. Beside macrophages possess receptor for VLDL and LDL. Therefore, their is accumulation of intracellular lipids and consequently results foam cells (lipids containing macrophages) and atheroma.

d) Role of hyperlipidemia: chronic hyperlipidemia ↑ in vascular permeability and leads to endothelium injury and its disjunction. ↑ serum concentration of LDL and VLDL, promotes formation of foam cells while serum concⁿ of HDL (good lipid) has anti-atherogenic effect.

e) Thrombosis: Endothelial injury expose sub-endothelial connective tissue resulting in formation of small platelets aggregate at the site which cause proliferation of smooth muscle cells and mild inflammatory rxn. As a result foam cell is incorporated into atherosomatous plaque. The lesions enlarge by attacking fibrin and blood cells, that thrombin becomes a part of atherosomatous plaque.

∴ CE = cholesteryl esters



Fig; Cholesterol round trip through vascular endothelium and intima

②

-Thromboxane A₂ (Tx A₂) and ADP are the chemical mediators released by platelets. Macrophages consumes excess modified (oxidised lipoproteins or oxy LDL) becoming foam cells.

Foam cells accumulate, release growth factor that stimulates proliferation of smooth muscle and calcification of plaque.

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